pH Control in Recirculating Aquaculture Systems for Pāua (*Haliotis iris*)

By

Jonathan P. Wright

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Abstract

In high intensity recirculated aquaculture systems (RAS), metabolic carbon dioxide can accumulate quickly and have a significant impact on the pH of the culture water. A reduction in growth rate and increased shell deformation have been observed in farmed abalone that has been attributed to reduced pH levels that occur in RAS due to accumulation of CO_2 in the culture water. The overall aim of this research programme was to assess two methods of pH control (physical vs. chemical) used in land-based aquaculture systems for the culture of the New Zealand abalone, pāua.

In the first study the efficiency of physical carbon dioxide removal from seawater using a cascade column degassing unit was considered. Hydraulic loading, counter current air flow, packing media height, and water temperature were manipulated with the goal of identifying the most effective column configuration for degassing. Three influent water treatments were tested between a range of pH 7.4 to 7.8 (~3.2 to 1.2 mg L^{-1} CO_2 respectively). For all influent CO_2 concentrations the resulting pH change between influent and effluent water (immediately post column) were very low, the most effective configuration removed enough CO_2 to produce a net gain of only 0.2 of a pH unit. Manipulating water flow, counter current air flow and packing media height in the cascade column had only minor effects on removal efficiency when working in the range of pH 7.4 – 7.8.

A secondary study was undertaken to examine the effects on pāua growth of adding chemicals to increase alkalinity. Industrial grade calcium hydroxide ($Ca(OH)_2$) is currently used to raise pH in commercial pāua RAS, however it is unknown if the addition of buffering chemicals affects pāua growth. Replicate pāua tanks were fed with seawater buffered with either sodium hydroxide, food grade $Ca(OH)_2$ or industrial grade $Ca(OH)_2$, with the aim of identifying the effects of buffered seawater on the growth of juvenile pāua (~30 mm shell length). Growth rate (μm/day) was not significantly affected by the addition of buffering chemicals into the culture water, and the continued use of industrial grade $Ca(OH)_2$ is recommended for the commercial production of pāua in RAS.

Shell dissolution is observed in cultured pāua reared in low pH conditions, however there is limited information surrounding the direct effect of lowered pH on the rate of biomineralisation and shell dissolution in abalone. A preliminary investigation was undertaken to examine shell mineralogy, the rate of biomineralisation and shell dissolution of pāua grown at pH 7.6 and 7.9 to determine their sensitivity to lowered pH. It was found that the upper prismatic layer of juvenile pāua shell (~40 mm) was composed almost exclusively of the relatively stable polymorph calcite, that suggests pāua are relatively tolerant to a low pH environment, compared to other abalone species that have proportionately more soluble aragonite in their prismatic layer. Regardless of shell composition, significant shell dissolution was observed in pāua reared in water of pH 7.6. Over the duration of the trial, the rate of mineralisation (μm/day) was significantly different between pāua reared in pH 7.6 and in pH 7.9 water. However, after a period of acclimation, low pH did not appear to effect rate of mineralisation in pāua.

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LIST OF ABBREVIATIONS

T	Tonnes (metric 1000 kg)
ITQ	Individual transferable quota
QMS	Quota management system
Mfish	The Ministry of Fisheries
QMA	Quota management area
SL	Shell length
TACC	Total allowable commercial catch
GABA	Gamma-amino-butyric-acid
FCR	Food conversion ratio
RAS	Recirculating aquaculture systems
UV	Ultra violet radiation
BOD	Biochemical oxygen demand
DO	Dissolved oxygen
NIWA	National Institute of Water and Atmosphere
TAN	Total ammonia nitrogen
EPS	Extrapallial space
XRD	X-ray diffraction

Chapter 1

General Introduction

1.1 Overview

The success of a commercial aquaculture operation requires a thorough understanding of the biology of the target species and tight management of culture environment. Much is known about the biology and culture of the New Zealand abalone *Haliotis iris* (pāua) and is summarised in this chapter. This chapter will also introduce the fundamental principles behind land-based recirculating aquaculture systems, and provide background information on pH and the influence of carbon dioxide and alkalinity on the chemistry of seawater. Finally, a summary of the objectives and aims of the research are listed.

Note: Photos that have not been credited have been taken by the author.

1.2 Pāua fisheries and aquaculture: A brief history

1.2.1 Wild fishery

The black foot abalone *Haliotis iris,* commonly referred to by its Māori name *pāua,* has significant commercial, recreational and cultural value to the New Zealand people. *H. iris* (henceforth referred to as pāua) is an endemic species found inhabiting shallow reefs in sub tidal coastal water throughout New Zealand.

Pāua historically has been a very valuable resource for iwi (tribes) across the country. Since before European settlement, pāua meat has been a staple of the traditional Māori seafood diet. Pāua were dislodged from the rocks using a long slender tool made from wood or bone called a *ripi*, and collected in flax kit bags. The flesh of pāua is tough, and the catch was often buried or soaked in freshwater for a period until it softened suitably for eating (Best, 1977). Such was the value of kai moana (seafood) to Māori, traditional enhancement techniques that involved the translocation

of shellfish into areas where food and space were abundant, were used by iwi to promote faster growth and extend the natural range of pāua (Booth and Cox, 2003).
Pāua has an iconic status in New Zealand. The attractive iridescent shell is universally recognised by many New Zealanders as coming from abalone. Māori use the shell extensively, incorporating the shell into carvings, artwork and traditional fishing lures (Phillips, 1935). The attractive shell, and its use as a decorative medium, justified the initial development of a commercial pāua fishing industry.

A commercial fishery opened in the mid 1940s following World War II. At this time the animals were harvested only for the shells. Total pāua landings before meat harvest were small, estimated to be up to 40 Tonnes (T) per year, and there was very little intensive fishing effort as a large proportion of the shell was gathered from beaches (Pritchard, 1982). At this time, the meat was discarded because no market existed and as a consequence it had little financial value. The shell however, was manufactured into a range of products including jewellery and trinkets (Schiel, 1992).

In the late 1960s, the industry moved beyond harvesting for shells, and new export markets for canned pāua were developed. The interest in pāua for meat triggered an uncontrolled expansion of fishing effort between 1968 and 1971 that led to intensive fishing of pāua beds in the Wellington, Wairarapa, Picton, Blenheim and Stewart Island regions (Murray, 1982). The increase in fishing pressure over this period was immediately followed by a regular decline in reported landings. This decline, particularly in areas that had been productive in past fishing years, was seen to be symptomatic of an eroding fishery, and provoked legislative action from the government eager to preserve a valuable fisheries asset. Beginning in 1973, a series of export restrictions were introduced to limit harvest volumes, and to allow time for the pāua beds to recover (Murray, 1982).

Since the introduction of export restrictions in the early 1970s, a strict regulatory environment has existed in New Zealand to prevent the commercial extinction of this valuable fishing resource. The quota management system (QMS) was introduced in 1986 by the Ministry of Fisheries (Mfish), and individual transferable quota (ITQ) (effectively a transferable property right), were allocated to fisherman based on their catch history. The premise of New Zealand's fisheries management system is based

on monitoring and regulation of catch volumes to ensure stocks are fished sustainably. Under the QMS, commercial species are monitored, and quota limits are revised and set by the government before each fishing season. Each species is subdivided into separate stocks defined by geographical location termed quota management areas (QMA). These areas are managed independently. This division is particularly important for pāua, as different areas of the country such as in the south of the South Island and the Chatham Islands, are more productive and support larger fisheries.

Even with fisheries regulations and the implementation of the stock management scheme, pāua remain acutely sensitive to fishing pressure. This is born from several key factors pertaining to the biology and life history of pāua. Typically, mature pāua form large aggregations on rocky reef habitat in very shallow water (5 to 20 m depth). These populations are easily targeted by divers who are able to remove a large number over a short period of time. These aggregations can take a long time to return, as abalone are slow growing animals with a relatively long life expectancy. On average pāua take 5 to 10 years to reach a commercial size of 125 mm, but in some areas where conditions are less favourable, they never attain this size (Moss et al., 2004). Irregularity in reproductive behaviour is commonly observed in abalone around the world. Similar variability in natural breeding cycles, and inconsistent recruitment of juveniles make pāua populations difficult to manage as a commercial fishery. Irrespective of their sensitive biology, ease of capture coupled with substantial market demand ensures that there is considerable illegal interest in pāua stocks in New Zealand. The influence of poaching and illegal take continues to be a problem for the pāua fishery in New Zealand. It has been estimated that in the lower North Island, considered to be one of the hotspots for illegal fishing, as much pāua has been removed illegally as has been harvested commercially (K Michael, pers. comm., Feb 2011).

Pāua is a valued commodity in the customary and recreational fishing sectors of New Zealand. Under the current regulatory regime, recreational fisherman can harvest up to a maximum of 10 pāua per day over the minimum size limit of 125 mm (shell length, SL). Harvesting pāua using SCUBA is prohibited. All commercial, recreational and customary catch must be obtained by free diving. The sensitive biology of abalone and the influence of illegal catch have made the pāua sector

difficult to manage, and has ensured that commercial harvest volumes remain relatively low. Total allowable commercial catch (TACC) over all pāua QMA has been static around 1000 T since 2002[1]. A large proportion of TACC has been allocated to the Chatham Islands and the Nelson/Marlborough regions (Mfish, 2010). The TACC of pāua (~1000 T) had an export value of $36.6 M NZD in 2009 (Mfish, 2010).

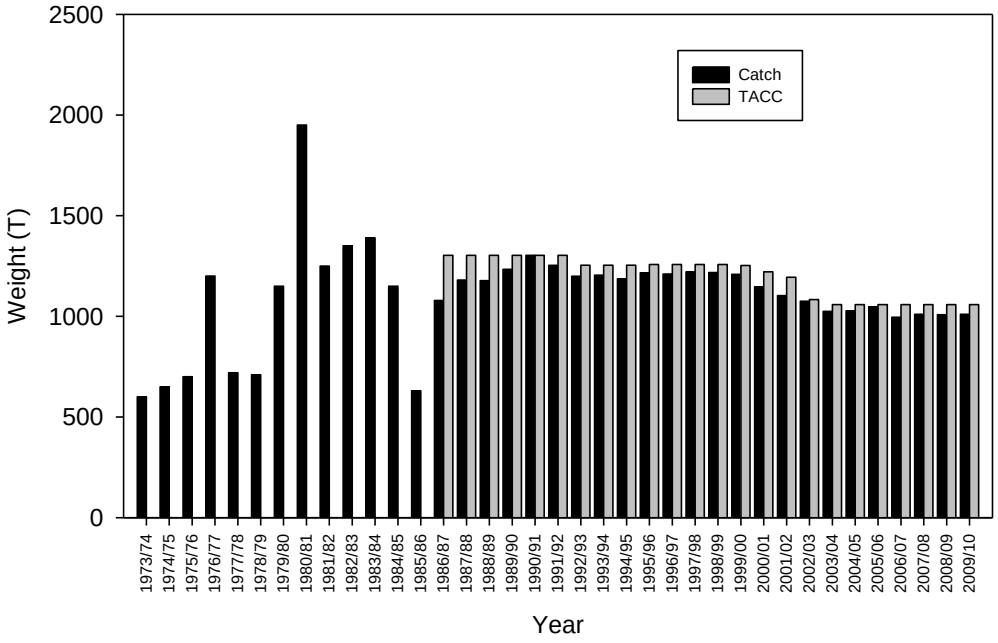


Figure 1.1 Total commercial catch of pāua (*H. iris*) in New Zealand. Catch data from 1973/74 to 1988/89 was adapted from Shiel (1992). Data from 1989/90 to 2010 was sourced from stock assessment plenary reports published by the Ministry of Fisheries (Mfish, 2011).

Global catch rates of abalone have declined over the last 20 years from approximately 18,000 T to 10,000 T (Fishtech, 2010). However, the global demand for abalone is still steadily increasing. The growing shortfall in supply is currently being met by farmed abalone. Wild populations have been exploited at a rate beyond that which is sustainable, and given the slow recovery time of natural populations, cultured abalone production will likely grow and continue to meet rising demand into the future.

[1] The current TACC for Hoki, New Zealand's largest fishery export, is 120,000 T.

1.2.2 Pāua farming

The decline in the pāua fishery in the 1970s was the catalyst to explore alternative means of fishery management. Enhancement programmes, where hatchery reared juveniles are reseeded back into the ocean to boost wild populations, were being used in Japan reportedly with good success. In the 1970s, abalone culture was relatively advanced in Japan, and by 1978 numerous laboratories and research institutes had produced over 10,000,000 juvenile abalone for reseeding back into the wild (Hahn, 1989a). Fishery researchers in New Zealand were eager to adopt these techniques and adapt a similar approach to develop a sustainable pāua fishery. In the late 1970s, the New Zealand government, through the Ministry of Agriculture and Fisheries, funded research into controlling the reproductive cycle and rearing larvae of pāua at the Mahanga Bay shellfish hatchery in Wellington. Built on the work of international abalone researchers, pāua were successfully spawned under controlled conditions in 1981. The early success of these trials was encouraging for researchers, and much of the 1980s was spent developing hatchery methodology and technology to produce and on grow abalone economically. All areas of pāua culture were explored. Broodstock maintenance, spawning procedure, egg handling, larval culture and diatom production (as larval food) were carefully examined and baseline hatchery protocols were established during this period (Tong and Moss, 1989). Researchers at the Mahanga Bay shellfish hatchery had proven that the aquaculture of pāua was biologically feasible, and laid the foundation for abalone farming in New Zealand.

One of the primary justifications for research into pāua culture was fisheries enhancement through reseeding. However the potential of land-based grow out operations was not ignored. Slow natural growth rates, and variability in environmental carrying capacity would ultimately limit the success of the reseeding programmes. Despite this, publicity from the advances made at Mahanga Bay generated significant interest in growing juvenile seed to a saleable size, and forging new markets for a farmed product. The first commercial pāua farming enterprise 'Crystal Park Marine Farms,' was established on the Wairarapa coast (southeast of the North Island) in 1987. Crystal Park was a simple land-based operation, its culture tanks were supplied by a flow through sea water system, and macroalgae was harvested from the beaches to use as food. From the beginning expectation was high,

however over the first 13 months of operation growth rates from farm reared pāua compared to those observed in the wild was disappointingly low (Henriques et al., 1988). The initial challenges of low growth rate, high cost of production, and marketing problems led to the subsequent closure of the pioneering Crystal Park pāua farming venture (G Moss, pers. comm., Nov 2010). This closure highlighted the difficulty in culturing a species that has never been farmed before.

The fledgling pāua industry suffered due to a lack of knowledge surrounding optimum culture conditions. By 2000 there were over 40 pāua farming permits issued by the Ministry of Fisheries, however the annual production of pāua for export was estimated to be less than 5 T (G Moss, pers. comm., Nov 2010). It was now apparent that farming pāua effectively and economically was a difficult process. The farming industry in New Zealand has been dominated by small scale operations. Only since the opening of OceaNZ Blue limited in 2002 at Ruakaka in the north-east of the North Island, did pāua farming have a flagship operation of necessary scale to compete with international abalone producers. OceaNZ Blue produces approximately 80 T of 87 mm to 102 mm pāua a year. The majority of their product is exported canned or frozen to overseas buyers, with live product being traded in small quantities in local markets (primarily in the Auckland region) (R Roberts, pers. comm., Oct 2010). It is estimated that OceaNZ Blue contributes over 90% of farmed pāua production in New Zealand (G Moss, pers. comm., Nov 2010).

Global economics and the value of New Zealand currency have hindered the industry in recent years. Abalone is primarily traded in US dollars. The steady weakening of the US dollar against the NZ dollar in the last decade has made significant impact on the profitability of export businesses in this country. The global financial crisis in 2008 has reduced the international demand for abalone. Competition from large abalone producers in China and Korea[2], has meant the gains in production efficiency made by advances in the research and development sector, were largely lost to movement in global economics. Due to the limited capacity of local markets, the

[2] In China, total farmed abalone production increased from approximately 20 000 T in 2006 to over 42 000 T in 2009. This increase in production has been credited to establishment of new farming areas, and development of a fast growing hybrid species.

tough international market for abalone is one of the major reasons why small scale operators struggle to establish a profitable business (M Tait, pers. comm., Mar 2011).

1.3 Biology

1.3.1 General

Abalone are large herbivorous marine snails that belong to the invertebrate class Gastropoda, under the phylum Mollusca. Abalone belong to the family Haliotidae, under the genus *Haliotis*[3], a genus that hosts approximately 210 taxa of abalone worldwide (Geiger, 2003). They are one of the most primitive gastropods in form and structure, and are immediately recognised by a characteristic low profile whoorling shell. They have a global distribution and are found in the coastal waters of every continent. The majority of larger abalone, and often the most commercially important species, are found at temperate latitudes. Relatively smaller species are commonly found in tropical and polar regions (Hahn, 1989e).

The New Zealand mainland and its satellite islands host 3 endemic species of abalone, *Haliotis iris* (pāua), *H. australis* (yellowfoot pāua) and *H. virginea* (virgin pāua). *H. virginea* has four sub species that are broadly separated by region. Collectively, these subspecies have a wide distribution. Their range covers the entire mainland, the Chatham Islands, and extends as far south as the sub-Antarctic Auckland Islands. All species inhabit rocky reef habitat close to the shore, where water motion is high and there is macroalgae available for food.

Pāua is the largest endemic species, and grows to approximately 180 mm SL. Mature pāua generally live in dense aggregations in open boulder habitat. This is in contrast to yellowfoot and virgin pāua that are cryptic by nature, and prefer to live in cracks and crevices and under boulders. Yellowfoot pāua reach a size of approximately 110 mm SL and coexist with pāua in areas that extend from the intertidal zone down to approximately 15 m depth. Virgin pāua are small by comparison and grow to approximately 80 mm SL.

[3] The latin name *Haliotis* means ‘sea ear’ in reference to oval shape of abalone.

Figure 1.2 Shells of *H. iris* (A), *H. australis* (B) and *H. virginea* (C). 'Foot' colour differs dramatically between the three species (D). Pāua has a dark foot (left), *H. australis* a striking yellow colouration (top right). *H. virginea* (botton right) tends to be relatively pale by comparison, and has an off white foot. Photos: G. Moss (NIWA).

1.3.2 Reproduction in wild abalone

Abalone have separate sexes, and gender cannot be distinguished without examining the gonad that is protected within the soft tissue. In pāua, the gonad colour reflects the colour of the gametes, the testis is a creamy white, and the ovary a grey-green. The gonad can be seen by shucking the pāua and removing the shell. However, live pāua can be readily sexed by gently pulling back the epipodium to expose the gonad.

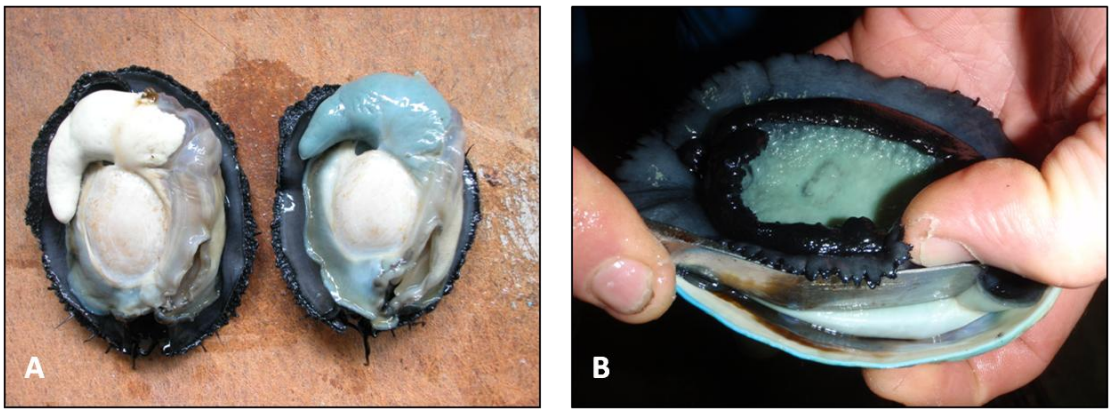


Figure 1.3 (A) Dorsal view of pāua with the shell removed. Sex is differentiated by gonad colour. Male is on the left, female on the right. (B) A common method used to determine sex and assess spawning condition.

Most temperate species of abalone have a seasonal reproductive cycle, with a primary spawning event in late summer to early autumn. In New Zealand, Poore (1973) observed variability in the annual spawning cycle of pāua at two sites on the central east coast of the South Island. He observed a typical late summer, early autumn spawn in the first year and then no spawning activity the following year (Poore, 1973). Variable spawning patterns of pāua were confirmed by Sainsbury (1982), who observed spawning in two successive years followed by two years of reproductive dormancy. Regional variation has also been observed. Wilson and Schiel (1995) measured an additional winter-spring spawn at a study site in the Otago region, south eastern coast of the South Island (Wilson and Schiel, 1995). In addition, in the warmer waters of Leigh, in the north east of the North Island, three discrete spawning events were recorded over a calendar year (Hooker and Creese, 1995).

Abalone are broadcast spawners, whereby they release their gametes into the surrounding seawater where fertilisation occurs. The fecundity of abalone (total egg production) differs between species. In general the Haliotids are a relatively fecund organism, and are capable of producing millions of eggs every spawning season. Although there has been considerable variability of fecundity observed between mature abalone (Sainsbury, 1982), there is a general trend of fecundity rapidly increasing with shell length (Ault, 1985). Gonad histology analyses indicate a sharp rise in egg numbers in mature females > 100 mm SL, and in field studies large female pāua (140 - 150 mm) have been observed holding approximately seven million eggs

(Poore, 1973; Wilson and Schiel, 1995). However, spent or empty pāua were not observed during post spawning periods in early field studies by Poore in 1972 (Poore, 1973). It is likely that only a proportion of total eggs are released during the short spawning season, and the remaining eggs are retained for a secondary spawning or resorbed into the gonad lumen.

Gamete release is dependent on many interacting abiotic and biotic factors. In some years, conditions such as food availability or water temperature may not permit (or trigger) spawning in a particular area (Rogers-Bennett et al., 2010). In the wild, gamete release can be variable, and populations may fail to reach reproductive potential if conditions do not favour spawning. The full potential of abalone reproductive capacity can be realised in the hatchery, where conditions are controlled. Egg releases of up to 2 million are common in hatchery conditioned adults (Moss et al., 1995), and can be as high as 5 million (Tong et al., 1992).

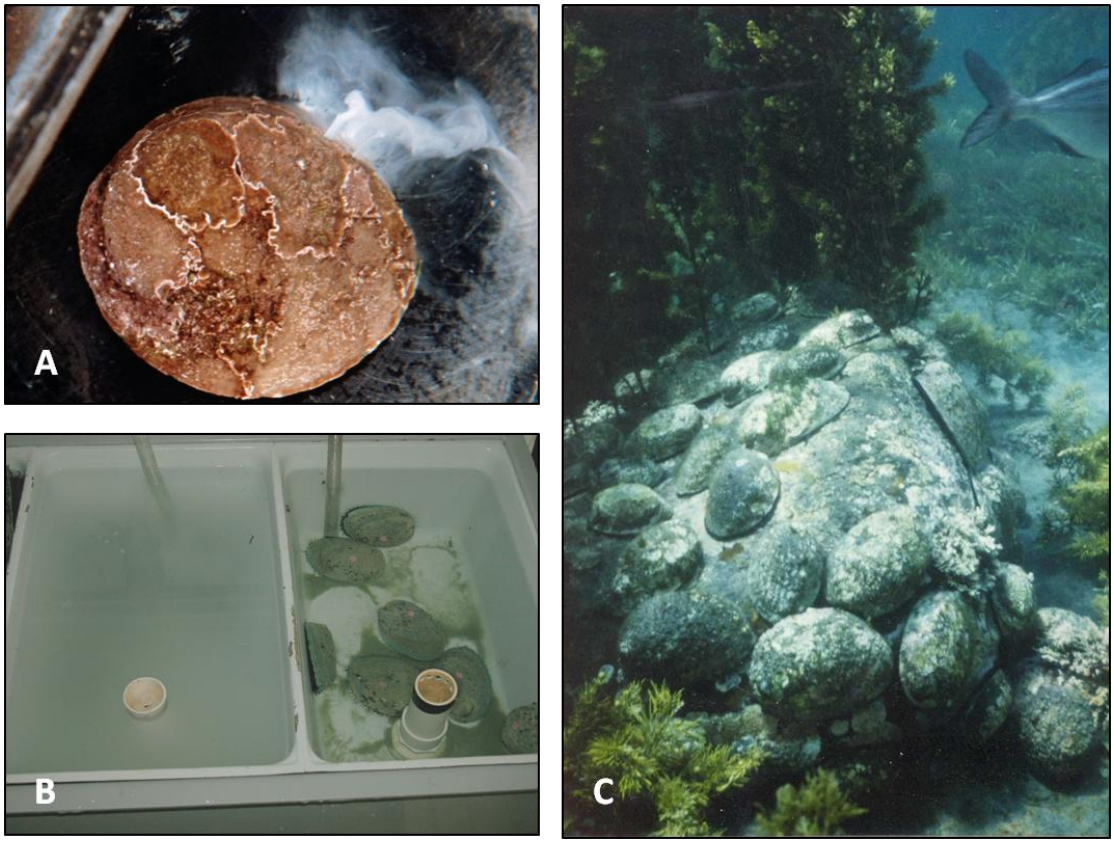


Figure 1.4 A male pāua releasing sperm through the respiratory pores (A). The release of gametes is carefully controlled in the hatchery (B). The males (left) and females (right) are usually separated during spawning, so fertilisation can be controlled. (C) The aggregating behaviour of wild adult pāua increases the chance of successful fertilisation by adjacent individuals. Photos A & C: G. Moss (NIWA). Photo C: S. Mercer (NIWA).

Variability in spawning events between localities and years are consistent with other reproductive studies of Haliotids from around the world (Boolootian et al., 1962; Shepherd and Laws, 1974). This variability has made abalone extremely difficult to manage as a commercial fishery. The effect of fishing on the reproductive capacity of an abalone population is acute. Divers target the largest, most fecund animals. A mature spawning population in an area can be quickly removed, and a population severely compromised for many years following. The impact of unregulated fishing and uncertainty in reproductive output make recruitment and population dynamics of abalone difficult to model.

1.3.3 Life cycle of pāua

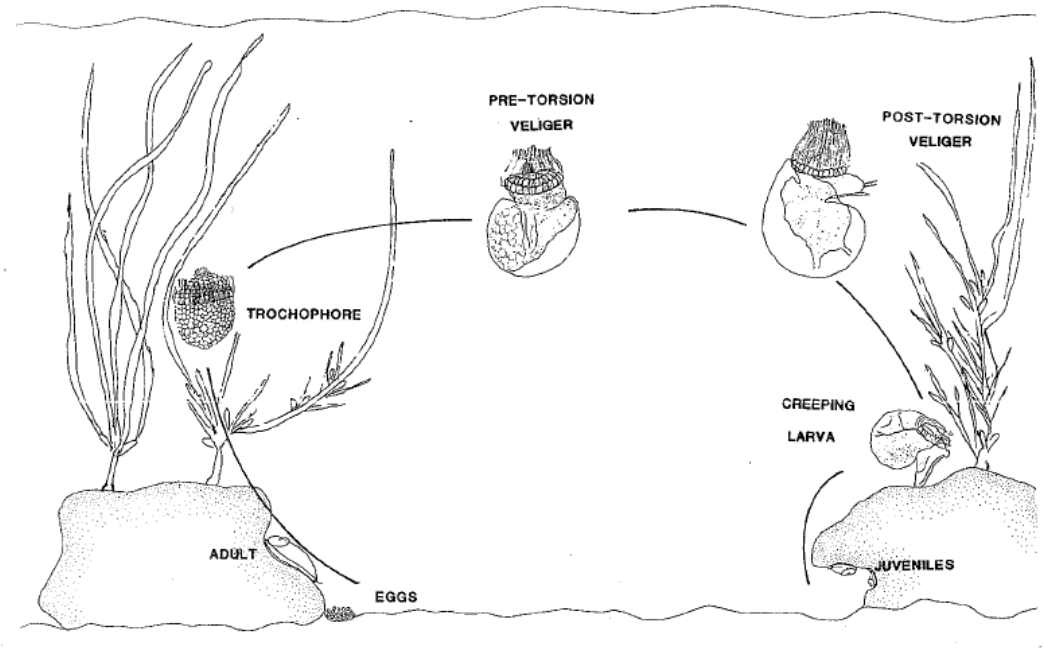


Figure 1.5 The larval life cycle of abalone. Source: This diagram was taken directly from McShane (1992).

1.3.3.1 Larval phase

Once the gametes fuse and the egg becomes fertilised, the cells divide and develop over 24 hours into the first stage of the larval life phase, the upward swimming trochophore. Trochophores are negatively geo-trophic and will swim by beating rows of cilia and move against the force of gravity (G Moss, pers. comm., Feb 2011). This behaviour ensures that the larvae have the opportunity to disperse, and potentially avoid predation by benthic filter feeders (Crisp, 1974). The trochophore will then quickly develop over a period of approximately 24 hours (dependent primarily on temperature) into the shelled veliger stage. Abalone larvae are lecithotrophic[4] and only absorb dissolved organics from the seawater during their development (Manahan and Jaeckle, 1992). Abalone larvae spend approximately 6 to 14 days in the motile veliger stage. This stage is characterised by the larvae undergoing torsion, the development of eye spots, and the formation of a rudimentary foot (Tong, 1982). It was commonly assumed that the veliger stage was primarily a pelagic mode, where

[4] Lecithotrophic larvae are largely or completely non-feeding, living on stored yolk.

larval spent development time high in the water column to optimise dispersal. However Prince (1987) observed very little movement of recruits (or juveniles) from the parent animals, and hypothesised that abalone larvae assumed a demersal rather than a pelagic existence in an effort to minimise dispersal distance. For abalone, constant transport of larvae away from the rocky coasts would likely cause high mortality rates, as the chance of encountering suitable reef habitat to colonise in the open sea is relatively slim. Local dispersal is favourable for reef dwellers as it increases the probability of settlement in suitable areas. However long range dispersal does occur and is ecologically important, as it contributes to the gene flow between populations (McShane, 1992).

1.3.3.2 Settlement

Abalone larvae are motile, but movement is passive, and effectively controlled by local hydrodynamics. When developmentally competent veliger larvae come into contact with a suitable substrate, the settlement phase (defined by metamorphosis from a free swimming form into a benthic form) is initiated. In the absence of suitable settling habitat, larvae can postpone settlement until the yolk supply is exhausted (McShane, 1992; Morse and Morse, 1984). Settlement appears to be triggered by specific cues and in the wild commonly occurs on crustose coralline algae (*Lithothamnion* sp.) (Tong, 1982).

The apparent affinity of abalone larvae to coralline algae is attributed to a subtle chemical interaction between the two (Morse and Morse, 1984). Coralline algae produce a neurotransmitter called gamma-amino-butyric-acid (GABA). GABA is known to immobilise larvae by inhibiting the ciliary functions of the veliger larvae. Corallines promote the beginning of the settlement phase by retaining free swimming larvae (Barlow, 1990). Mucous trials have also been identified in the laboratory as a potential settlement cue for larvae (Roberts and Watts, 2010). In gastropods, GABA is produced by epithelial cells in the foot, and is shed with the mucous trial as the animal moves. It has also been proposed that additional biochemical components of the mucous may be involved in selecting for specific species (Laimek et al., 2008).

1.3.3.3 Post larvae into adulthood

Newly settled abalone are nutritionally vulnerable, and need to begin feeding immediately. In this period, settlement to post settlement, there is a large spike in larval mortality where losses are estimated to exceed 95% in the first week (Heasman and Savva, 2007). The primary cause of mortality are attributed to predation. Active predators, including nematode and annelid worms, target zooplankton for food. Post-larvae are also vulnerable to accidental ingestion by reef surface grazers, particularly urchins, turban shells (cats eyes) and other marine snails (Shepherd and Breen, 1992).

First food for post-larvae will generally consist of diatoms and bacteria and their extracellular secretions, components of a biofilm community that cover rocks and organisms as a slime layer. The buccal cavity of post larvae is small, and as a result they can only physically ingest small species of benthic diatoms. Post-larvae browse selectively for benthic diatoms of the correct size (Moss et al., 2004). Trials examining the effects of various species of diatoms and microalgae on the growth and survival of post-larvae pāua reveal that an absence of an appropriate small diatom species at this vulnerable stage results in high mortality (G Moss, pers. comm., Feb 2011). Post larvae and small juveniles will graze on diatoms until they reach approximately 5 mm SL. At this stage they will begin spending daylight hours in shelter under rocks and only emerge at night to forage for food.

In pāua, the juvenile stage lasts from 3 to 5 years depending on growth rate. They will spend this time actively foraging for macroalgae in the intertidal zone, primarily small palatable red seaweeds including *Hymenocladia* sp., *Polysiphonia* sp. and *Pterocladia* sp. (Poore, 1972a). Juveniles are deemed to be adult once they reach sexual maturity. This is defined by their capability to produce viable gametes. The transition into adulthood (commonly at 70 – 90 mm SL) often coincides with a shift in behaviour, where young adults emerge out from their cryptic juvenile habitat into the open, where water movement is high and drift algae is relatively abundant (Moss, 2006).

1.3.4 Hatchery reproduction

Control over the reproductive cycle is of critical importance, because an abalone hatchery would not be operating efficiently if it lay idle outside of a natural spawning season.

Various mechanisms involved with regulating the reproductive cycle in the wild have been identified, including seawater temperature, physical disturbance, food supply, lunar cycles, and hormonal factors (Jebreen et al., 2000; Orton, 1920; Shepherd et al., 1985; Tutschulte and Connell, 1981; Webber and Geise, 1969). Of these, water temperature is accepted to play a key role for temperate species. Several studies have shown a correlation between water temperature and reproductive cycles and concluded that change in water temperature is an important cue in reproduction (Kikuchi and Uki, 1974; Tomita, 1967). Maintaining optimal water temperature in the hatchery is a key step to conditioning (inducing gonad ripeness) abalone to promote spawning out of season (Hahn, 1989b). In pāua culture, captive brood stock maintain good reproductive condition if they are kept in water temperatures between 14 -16 °C, fed a mixed diet of macroalgae (and/or a high protein artificial diet), and supplied with ample clean water. Maintaining parent stock in good reproductive condition allows operators to artificially induce spawning and produce gametes as required. Care of broodstock is particularly important in large commercial operations, where artificial spawning can be initiated as often as every two weeks (G Tutt, pers. comm., Aug 2010). The scope to manipulate water temperature in the hatchery ensures that the operator is not restricted by ambient seasonal water temperatures, and can develop a spawning regime to optimise production.

1.4 Growth

1.4.1 General

Growth rate between individuals within an abalone population is seldom uniform. Genetic factors greatly determine the overall potential of growth, however differences in interacting biotic and abiotic factors will ultimately determine the rate of growth of individual abalone (Heath and Moss, 2009). Growth, and understanding the factors

that influence growth rate, are of extreme importance in abalone aquaculture, as culture costs are effectively reduced by fast growing animals. Growth rates in natural pāua populations are often slow (5 to 10 years to 125 mm SL) (Poore, 1972b; Sainsbury, 1982) and variable (McShane and Naylor, 1995; McShane et al., 1994). On well managed farms cultured pāua reach a marketable size (80 – 90 mm SL) in three to four years (G Moss, pers. comm., Mar 2011). This has been achieved primarily by manipulation of environmental factors that influence growth, and the development of a suitable food and dietary regime.

It is helpful to examine the dynamics of abalone growth through a simplified energy budget, which is a balance sheet assessment of incoming energy versus energy spent. Incoming food energy is diverted into a number of metabolic pathways including somatic growth, respiration, reproduction, shell production and mucous production. In the wild, the proportion of food energy invested into each energetic pathway varies between season and habitat. To optimise the 'growth' pathway (i.e. a greater proportion of total food energy is allocated to growth), as is important in an aquaculture situation, energy channelled into other 'maintenance' pathways must be minimised. Laboratory evidence suggests there are four primary factors that influence growth rate in wild abalone. These factors are; water temperature, the quality and quantity of food, and reproductive state.

1.4.2 Temperature

Abalone, like all marine invertebrates, are thermo conformers. Their body temperature will match that of the surrounding environment. As most biochemical and physiological processes are sensitive to changes in temperature, water temperature has been considered one of the most important environmental factors controlling food utilization at all levels and all stages of growth in poikilothermic aquatic animals (Lovell, 1989). Cool water temperatures are known to slow the growth of abalone in the laboratory (Chen, 1984), and is often cited as the primary reason for slow growth in natural populations over winter. Some species of abalone (including pāua) when reared in elevated constant water temperatures show dramatic increases in growth rate (Leighton, 1974; Leighton et al., 1981; Tong, 1982). The influence of temperature on growth is an important consideration in abalone

aquaculture. If abalone are to be reared in or near ambient water temperatures, a careful assessment of the seasonal temperature dynamic is required to determine if the temperature range is appropriate for fast growth.

Further investigation into grow out conditions have shown temperature optima for growth is not constant with pāua size (Moss et al., 2008; Searle et al., 2006). Younger, smaller pāua (<60 mm) prefer warm water, and have been shown to grow quickly in water temperatures ranging from 18 – 21°C. Larger pāua prefer cooler water; the optimum growing temperature for 85 mm pāua is approximately 16°C (see Fig 1.6).

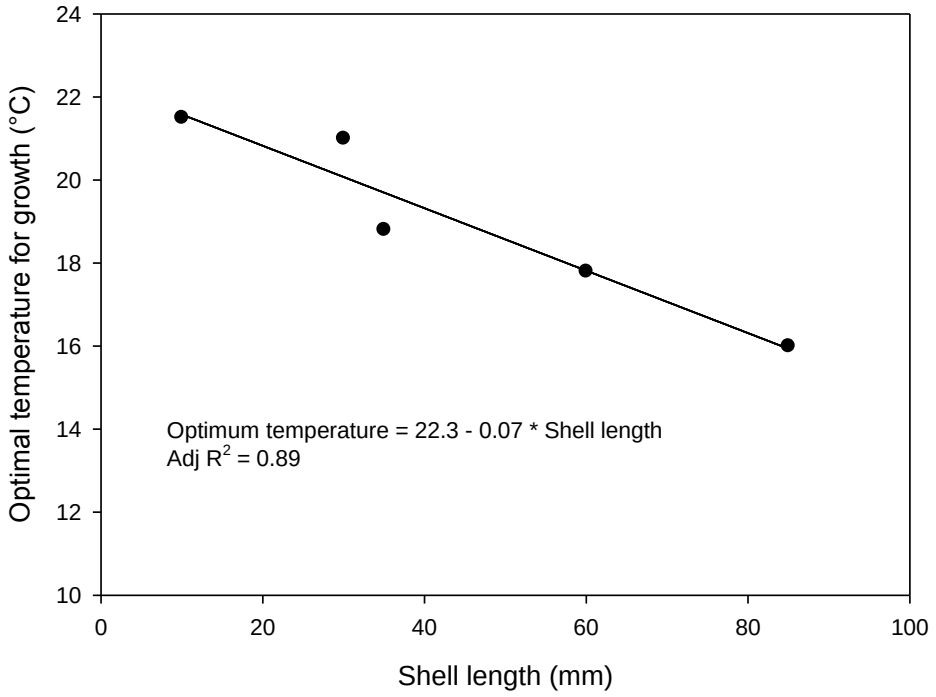
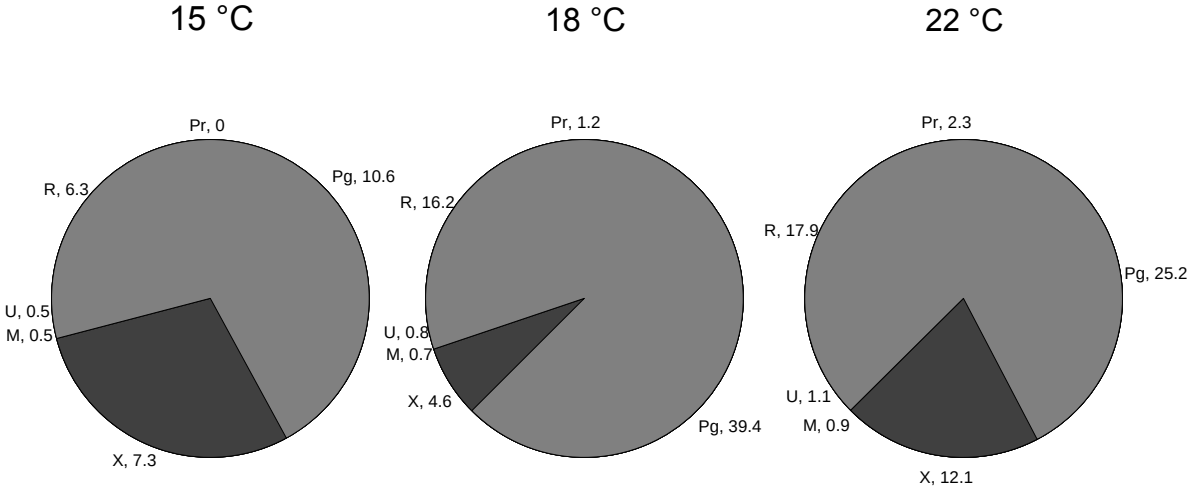


Figure 1.6 Optimal temperature for maximal growth of different size pāua (*H. iris*). Source: adapted from Moss *et al.* (2008) and Searle *et al.* (2006).

Temperature influences the rate of resting metabolic processes in poikilothermic animals. In cellular respiration, cells breakdown organic molecules to produce energy. This process is driven by oxygen. Measuring oxygen consumption is a useful and sensitive measure of energy expenditure (McBride et al., 2001), and is a method commonly used in bioenergetic assessments in a range of aquatic animals. Oxygen consumption (respiration) in abalone is directly influenced by water temperature, where the amount of oxygen consumed increases proportionally with water

temperature (FAO, 1990; Moss et al., 2008). In pāua, respiration rate increases up to approximately 24°C where one thermal maximum is reached and temperature begins to have a lethal effect on the animal (G Moss, pers. comm., Nov 2010).

The influence of temperature is a factor hypothesised to contribute to the size distribution of pāua in New Zealand (Naylor et al., 2006; Wells et al., 1998). There is a general trend of larger pāua occurring in cool southern regions, where temperatures suit the growth of larger animals. Smaller individuals occur in warm northern waters, in conditions that promote fast juvenile growth, but do not suit larger animals (McShane et al., 1994). In aquaculture, if abalone are cultured beyond their optimal temperature range there is an energetic cost to the animal, and consequently a financial cost to the operator. Optimal temperature for growth varies between species and size, however abalone that live outside their optimal temperature range use more energy for maintenance, and as a consequence invest less into growth (see below Fig. 1.7) (Lopez and Tyler, 2006; McBride et al., 2001).



Somatic growth (Pg), reproductive investment (Pr), respiration (R), ammonia excretion (U), mucous production (M), and unutilised energy (X).

Figure 1.7 Mean energy expenditure of juvenile (16.4 mm SL) *H. tuberculata* fed an artificial fish meal based diet and grown at three different temperature regimes. Each numeric value on the sector labels represents calories per animal per day. This figure was produced from data presented in Lopez and Tyler (2006). These charts show that *H. tuburculata* is able to use food more efficiently when cultured at 18 °C, as proportionally more energy is channelled into growth and less into maintenance pathways. Other studies of *H. tuberculata* have shown maximal growth at temperatures close to 20 °C (Shpigel et al., 1996).

In an aquaculture situation, manipulating water temperature to promote growth reduces the energetic cost of cellular maintenance. Food conversion ratio[5] (FCR) is an important parameter in aquaculture. Improvements of food conversion is vital to the cost effective production of pāua, as food is the second highest cost after labour at OceaNZ Blue Ltd (R Roberts, pers. comm., Oct 2010). Abalone have higher FCR and lower growth rates, when grown in temperatures outside of their optimal growth range (Britz et al., 1997; García-Esquivel et al., 2007). Even a very small improvement in FCR can equate to considerable savings in feed costs, and increase the profitability of an operation significantly. Because of the potential to reduce culture costs, food conversion is an area of considerable interest in aquaculture across all aquatic species.

1.4.3 Food

Like many temperate species, seasonal variation in growth is observed in pāua where more growth is observed in the warmer months (Hooker et al., 1997; Poore, 1972b; Sainsbury, 1982). However, the underlying cause may not be directly attributable to water temperature. The importance of diet in abalone growth is well documented (Hahn, 1989d). The availability and abundance of preferred algal species is often cited as a primary driver of growth (Day and Fleming, 1992b). Each macroalgal species has a different nutritive value to abalone, and therefore has the capacity to promote different growth rates. Uki et al. (1986) compared the growth of *H. discus hannai* fed a monospecific diet of 56 species of algae. They observed a range of growth rates, and confirmed some algal species promoted faster growth than others.

In abalone there appear to be broad dietary patterns on a global scale. Species from the northern hemisphere and South Africa prefer to feed on brown algae, and those from South East Asia and Australia prefer red algae. Food preference of abalone is an area that has been well studied, in part due to the effort to enhance culture techniques by understanding dietary needs. Pāua are largely opportunistic feeders and will eat a range of macroalgal species largely determined by availability. Mature pāua consume

[5] Food conversion ratio is a measure of an animal's efficiency in converting feed mass into body mass. Currently farmed Atlantic salmon have an FCR of approximatly 1.1 (1.1 kg of feed to produce 1 kg of body mass). Mature pāua in commercial operations have an average FCR of 2.0 (includes uneaten food and wastage). A low FCR is good, meaning less food is converted into body mass.

a high proportion of large brown seaweeds including *Lessonia variegata*, *Macrocystis pyrifera* and the introduced *Undaria pinnitifida*, a preference primarily driven by abundance. However, Poore (1972a) showed in laboratory selection experiments that pāua preferred the flat red alga *Hymenocladia lanceolata* to the large brown seaweeds, illustrating that food selection can be influenced by the presence of preferred food species. Physical characteristics of algae can also influence selection. Consumption rates in three endemic species of Australian abalone were shown to be negatively effected by both algal toughness and high levels of phenolic compounds[6] (Shepherd and Steinberg, 1992).

Foraging behaviour influences growth rate, as mobile animals invest more energy into movement (respiration, mucous production) and less into growth. Adult pāua foraging behaviour is not uniform. Some groups actively browse the substrate searching for food and are relatively mobile, where as other sedentary groups are found in areas of high water movement and capture passing drift algae. Capturing drift algae is energetically advantageous as movement is minimised, however a potential disadvantage to this feeding strategy is pāua are not able to selectively browse for preferred species of algae. Given the variable nutritional value of different species of algae, the composition of the drift may ultimately effect growth. Laboratory observations showed pāua from a site where drift algae was rare were more active during feeding periods, demonstrating a relationship between foraging behaviour and food availability (Poore, 1972a).

[6] In plant biology phenolic compounds are not directly involved with primary metabolic processes of growth and development but function in chemical defence against herbivory.

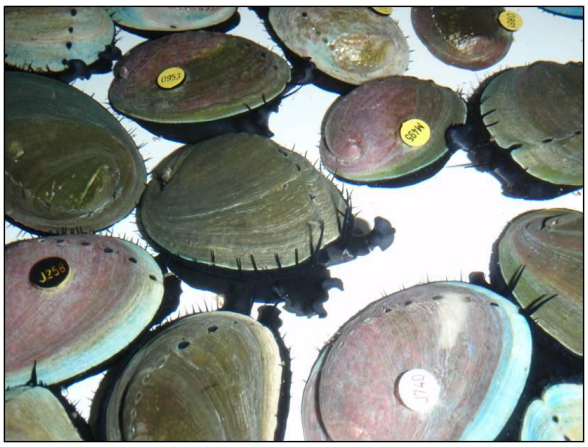


Figure 1.8 Pāua with its foot extended. A behavioural adaptation for collecting drift seaweed.

1.4.3.1 Formulated food

Large abalone production facilities require large amounts of food. Using macroalgae as a principle food source can be problematic, because of high labour costs (country specific) and the difficulties in managing the huge quantities that would be required for daily feeds. The development of formulated foods for abalone has been a significant advancement in abalone culture in New Zealand. Formulated food in dried pellet form is easy to store, easy to handle, and as a consequence is less expensive (in New Zealand) than macroalgae. Because it has a high protein content and low water content compared to macroalgae, formulated diets can be fed out at much lower rates. Abalone that would typically consume 10 to 30% of whole body wet weight per day in macroalgae, need only a fraction in formulated food to the match daily protein requirements of the algae (Hahn, 1989d). Commercial feeding rates in pāua being fed formulated diet range from 0.3 to 0.6% of body weight per day depending on water temperature (R Roberts, pers. comm., Oct 2010). A key advantage of formulated diets is the scope to manipulate ingredients to promote growth. This is common practice in aquaculture, as specifically formulated diets are developed to match the nutritional requirements of the target species. Although globally there is still a heavy reliance on natural seaweeds for farmed abalone food, the expansion of the industry in New Zealand is very much reliant on the use and continued development of formulated diets.

1.4.4 Reproduction

The energetic cost of reproduction is of primary importance in selecting an appropriate species for aquaculture. If an animal matures within the time it takes to culture that species to a marketable size, energy is channelled into reproduction and away from somatic growth. For this reason the production of sterile animals or artificial delays in gonad maturation are areas of focus in the aquaculture industry[7].

In general, pāua mature within the culture window and therefore a portion of food energy is unavoidably lost to reproduction. The energetic cost of reproduction can be clearly seen in wild *H. discus hannai* in Japan, by the presence of a distinct mark on the shell that is formed in September (Sakai, 1960). This annual mark is a thickening of the shell produced by a temporary halt in shell extension as a result of energy being diverted into gonad maturation and spawning. The growth ring is common among abalone species found in Japan, and makes it possible to determine age in a similar way to counting rings of a tree. This phenomenon has also been observed in some areas in New Zealand (Naylor, 2010). However, it is hypothesised that the abundance of available food may override the influence of the reproductive cycle on growth. In pāua, Poore (1972b) observed that growth of adults and juveniles at two study sites followed the same seasonal pattern suggesting the energetic requirements of gonad maturation for spawning has a relatively minor influence on overall growth in that area.

In wild pāua populations, there is a general pattern of decreasing size at maturity with increasing water temperature. New Zealand spans 10° of latitude and is subject to a wide range of water temperatures. Naylor et al. (2006) examined multiple adult pāua populations and although they had no age data to directly compare developmental differences between regions, there appeared to be a skewed allocation of energy resources into reproduction in warmer waters where conditions are less favourable for somatic growth. Lopez and Tyler (2006) evaluated this phenomenon in an energy budget assessment in the laboratory, and showed the relative proportion of energy channelled into reproduction can be influenced by external factors such as water

[7] Unless the gonad is an important part of the product, as in sea urchin culture or caviar production.

temperature and quality of diet. This has important implications in abalone culture, as the energetic cost of reproduction could be minimised by manipulating environmental conditions and food quality.

1.4.5 Growth summary

In aquaculture, there is a considerable amount of time and work needed to determine the optimal growing conditions for a particular species at a particular location. Observations of growth patterns in the wild provide a starting point, however identifying optimal conditions for growth through research and development are critical steps to developing a strong pāua farming industry in New Zealand.

Abalone are naturally slow growing animals. Although farmed abalone are produced faster, the time it takes to culture pāua is a bottleneck to expansion of the industry. Based on current understandings of culture conditions, it takes three to four years to produce standard commercial size pāua of 85 to 90 mm. There are many associated costs coupled to production time. Producing a commercial size abalone in the shortest possible time through further refinement of culture conditions will ultimately make pāua farming a more profitable industry.

Having control of the environment is a critical step for maximising growth. New Zealand has an ample supply of fresh clean seawater, however the wide fluctuation in water temperature between summer and winter can make it difficult to provide optimal growing conditions all year round. Due to the dynamic optima of pāua growth in relation to water temperature, a high degree of control over culture conditions is required to rear pāua quickly.

1.5 Recirculation aquaculture

1.5.1 General

In modern aquaculture there is a focus on sustainability. Wild fisheries are no longer able to meet current global demand for fish products, and aquaculture production has expanded rapidly to meet the growing shortfall in supply (FAO, 2008). Increasingly

there is disapproval of the damage done by wild capture fisheries, which has not only depleted targeted fish stocks but also affected the environment around them (Worm et al., 2006). The aquaculture sector has not been immune to criticism. There has been considerable negative press linked to environmental impact of aquaculture, through the direct impacts of nutrient deposition on the benthos, indirect ecosystem effects, disease propagation, and gene exchange with wild and farmed animals[8] (Jonsson and Jonsson, 2006; Krkošek et al., 2005; Naylor et al., 2000; Papageorgiou et al., 2010). Our understanding of anthropogenic effects on the marine environment has grown considerably in the last few decades. As a result, environmental sustainability is a major consideration in the establishment of new aquaculture operations and has been given considerable attention by aquaculture regulatory authorities in countries such as New Zealand, Australia, the United States of America and Norway. Sustainable growth in the aquaculture sector is dependent on industry and government cooperation. The development of new technologies to improve production efficiency, and strategies to mitigate the effect of aquaculture on the environment are paramount to the continued expansion of the industry. Land-based water reuse aquaculture is becoming more and more prominent as a method for producing aquatic food products. Land-based aquaculture offers a greater degree of control, and is touted as a necessary step toward a sustainable increase in production to meet the expanding global demand for seafood.

1.5.2 Recirculating aquaculture

Land-based recirculating aquaculture is a method used to farm aquatic organisms by recycling the water used in production. In principle, recirculating aquaculture systems (RAS) can be adapted to grow most species in aquaculture such as shrimp, fish, clams or abalone. Over the last 25 years significant advances have been made in understanding the management and design of RAS, and there are many operations that rely (fully or partially) on the principles of RAS.

[8] Interbreeding of cultured and wild stocks can dilute the natural gene pool of wild populations, and potentially result in a reduction of fitness in wild populations.

1.5.3 The fundamental recirculating aquaculture system

The recirculating aquaculture system is essentially a closed system. It is necessary to treat the water continuously to remove waste products that are produced by the cultured animals. This is achieved by a series of mechanical and biological filtration steps, which remove suspended solids and ammonia (NH_3) from the culture water. A degassing step is also necessary, where water is reoxygenated and carbon dioxide is stripped from the water. There are several other components that are commonly incorporated into RAS that include UV and/or ozone disinfection, oxygen enrichment, alkalinity dosing, heat exchange, and denitrification that may be added to meet the exact requirements of the cultured species.

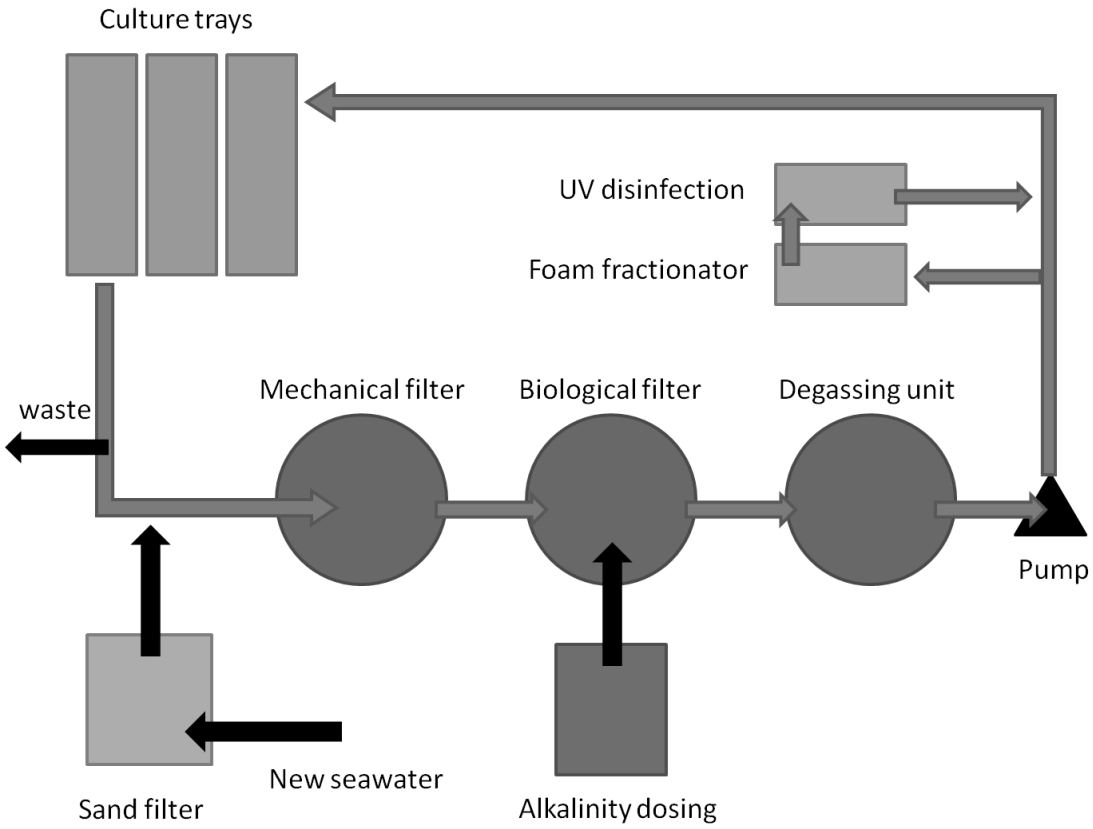


Figure 1.9: A simplified RAS system.

1.5.4 Solids removal

Solids removal is the process where the solid waste products produced by the cultured animals are removed from the culture water. Solids removal is a very important part of RAS as excess organic waste in the culture water can promote the growth of unwanted (potentially pathogenic) microorganisms in the system. Heterotrophic microorganisms use the organic carbon present in solids as an energy source for growth, a process that consumes dissolved oxygen. High biological activity in the water creates an extra demand for dissolved oxygen (refered to as biochemical oxygen demand or BOD), and can result in lowered oxygen concentrations in the culture tanks.

Additionally, accumulation of solids within the system can be problematic. Anoxic 'dead zones' can form and promote the growth of anaerobic bacteria that produce hydrogen sulphide (H_2S). Most aquatic species are susceptible to low concentrations of sulphides in the water, and suphide poisoning has been linked to mortality and health problems observed in commercial fish farms (Kiemer et al., 1995). Solids may also accumulate over gas exchange surfaces (biofouling) and influence gas transfer efficiencies (Colt and Bouck, 1984). It is therefore important to remove solid waste as quickly as possible from the system, as suspended solids negatively influence all aspects of a RAS (Timmons et al., 2007d).

Solids are faeces (or undigested food), biofloc (dead or living bacteria) and uneaten food. Generally there is a broad range of sizes of suspended solids particles present in a closed system, ranging from large settleable solids (> 100 μm), fine suspended solids (1 – 100 μm), through to a dissolved fraction (Timmons et al., 2007d). There are many different methods for removing solids in RAS. The first stage generally involves sedimentation of the larger particles into a settlement chamber, that removes the largest proportion of solids by weight. This is generally followed by a microscreen filter that strains out any particles larger than the screen mesh size. Typically the mesh size microscreen filters range from 40 – 100 μm, in this range, 30 to 80% of total suspended solids (solids > 1 μm) can be removed (Timmons et al., 2007d).

Fine suspended solids contribute to the BOD of the culture water, and can be particularly detrimental to fish health (Timmons et al., 2007d). Fine solids are often removed by microscreen filters (Fig. 1.10, B & C), granular media filters (common in swimming pool or display aquarium applications where water clarity is important) or commonly by foam fractionation[9] in seawater applications. Solids management is important in abalone culture as abalone are known to be sensitive to fine solids. Abalone possess adaptations to protect the delicate gills from solids contact. These include ciliated lamellae, and a mucous layer covering the gill to trap particles and transport them away from the delicate gills. However, these adaptations only provide moderate protection from suspended solids, and gill function can become compromised by smothering of the lamellae (Litved and Cripps, 1999). Although tolerance of suspended solids will differ between species, in general it is recommended that suspended solids load in the water supplied to abalone culture tanks are maintained at less than 20 mg/L (Heath and Tait, 2006a).

[9] Foam fractionation is a process by which a large number of small bubbles are injected into the culture water. Charged organic molecules are attracted to the air water interphase, and bind to the bubbles. The bubbles rise to the surface with other small particles and bacteria as foam. The foam is then skimmed from the surface and disposed.

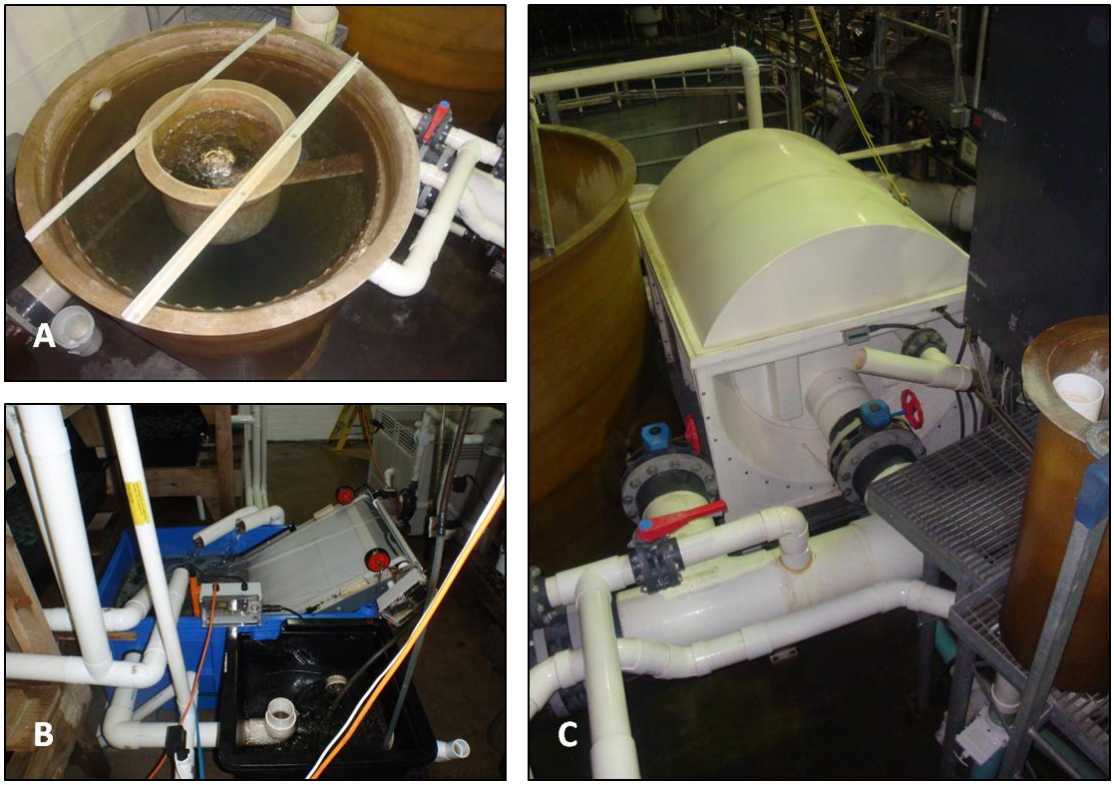


Figure 1.10 A radial flow settlement chamber (A). A settlement trap is generally the first stage of filtration exiting the culture units, and removes the largest particles by sedimentation. The belt and drum filters (B & C respectively) are microscreen filters that offer excellent solids removal capability. These units require minimal labour and remove the captured solids from the process flow before leaching can occur.

1.5.5 Biological filtration

Aquatic organisms expel nitrogenous waste compounds by various routes. These include gill diffusion, in urine and faeces, and in many invertebrates across the whole body surface (Campbell and Reece, 2002). Because ammonia is very soluble and toxic at low concentrations, most aquatic animals excrete nitrogenous waste continuously as ammonia. Ammonia is produced as the major end product of protein catabolism and is excreted as unionised ammonia across the gills (Timmons et al., 2007a). The removal or decomposition of nitrogenous wastes is of primary importance in RAS because ammonia is acutely toxic, and can build up to a lethal concentration in the culture water very quickly. While abalone appear to be more resistant to the effects of ammonia than fish, it is still recommended that exposure to ammonia should be kept under concentrations of 1 mg/L (at pH 8.0) (Heath and Tait, 2006a).

Biological filtration is the process of converting ammonia into less toxic forms in the recycled water. Biological filtration is a natural process that is amplified in RAS by incorporating a biofilter into the system. The biofilter is a packed bed of inert media that provides a high surface area for colonisation by communities of naturally occurring nitrifying bacteria[10]. Nitrification is a two step process. Toxic ammonia is first converted into less toxic nitrite (NO_2^-) and then into the least toxic nitrate (NO_3^-). Although high concentrations of nitrate are still harmful to aquatic organisms[11], nitrate accumulation is controlled by the addition of new water into the system that avoids toxicity by dilution. Nitrate can also be removed by the addition of a denitrification step into the system, in which anaerobic bacteria convert nitrate into nitrogen gas. This step can be particularly beneficial in areas where source water is limited, as it reduces make up water requirements and opens up opportunities for inland RAS away from source water.

Figure 1.11 A commercially produced inert biofilter material (A) commonly used in pāua RAS. Injecting air into the biofilter (B) provides oxygen necessary for nitrification reactions and avoids dead spots in the biofilter.

[10] Nitrification is an aerobic process carried out by various genera of bacteria. Primarily *Nitrosomonas* for NH_3 to NO_2^- and *Nitrobacter*, for NO_2^- to NO_3^-.
[11] In abalone culture, nitrate concentration is not recommended to exceed 50 mg/L for long term exposure.

1.5.6 Oxygenation and degassing

The availability of dissolved oxygen is a primary limiting factor for growth (Brett, 1979) in intensive aquaculture systems. Although growth is not directly stimulated by oxygen, dissolved oxygen (DO) concentration limits the scope of metabolism, as oxygen is consumed in cellular respiration as a reactant with food energy (Campbell and Reece, 2002). In aquaculture, oxygenation of the culture water is critical to supply DO to the cultured animals and bacteria in the biofilter. This can be achieved a number of different ways, and varies depending on the culture method (see Fig. 1.12, A & B). Oxygen enters the water by diffusion from the air. When water is in contact with the air, dissolved atmospheric gases (oxygen, nitrogen and carbon dioxide) diffuse in or out of the water to reach equilibrium with the gases in the air. The primary factors that affect the rate of gas transfer, are the area of the gas to liquid interface and the relative partial pressures of the gases in the air phase and the water phase (Timmons et al., 2007b).

Figure 1.12 Tippers (A) promote good water movement to encourage aeration and an even distribution of food pellets in the culture trays. Cascade column aerators (B) are capable of treating large volumes of water and are commonly used in intensive aquaculture operations. Photo B: P. Heath (NIWA).

Unlike fish, that can swim or pump water over the gills by ram ventilation, abalone rely heavily on passive water motion to supply oxygenated water to the gills. Although abalone have a limited ability to regulate and store oxygen (Jan and Chang, 1983; Ragg and Taylor, 2006) (adaptations essential for survival, as abalone lack the mobility to avoid acute changes in oxygen concentration), low dissolved oxygen concentration has been shown to reduce growth rate in abalone (Harris et al., 1999b; Miller et al., 2010). There is an observable difference in growth rates of juvenile greenlip abalone when dissolved oxygen concentrations dip below 80% saturation (Harris et al., 1999b). It is therefore critical to ensure that abalone stock tanks are well aerated to maximise growth. The biofilter is also reliant on dissolved oxygen, as the nitrification process consumes oxygen to convert ammonia to nitrate (Timmons et al., 2007a).

In aquaculture, aerating the culture water achieves a dual purpose of oxygenation and stripping dissolved carbon dioxide from the water. Carbon dioxide is a very soluble gas, and will quickly accumulate in the water if it is not removed through aeration. In abalone culture, a build up of carbon dioxide in the culture water reduces pH and can lead to reduced growth and increased shell erosion (Harris et al., 1999a; Heath and Tait, 2006b; Merino et al., 2010) (note; the nature of carbon dioxide in seawater and problems associated with low pH of the culture water will be discussed in detail in Chapter 2). Low pH can also effect the efficiency of the biofilter, as metabolic rates of *nitrosomas* and *nitrobacter* bacteria have been shown to be sensitive to pH (Grady and Lim, 1980).

Pure oxygen is commonly dosed directly into RAS to increase the carrying capacity (maximum biological load) of the system, and support an increase in stocking densities (Colt and Watten, 1988). Systems operating in this manner are particularly vulnerable to carbon dioxide accumulation as for every 1 mg of oxygen consumed, approximately 1.4 mg of carbon dioxide is produced. This means even in a moderately loaded system (i.e. 30 kg/m^3 biomass/water) carbon dioxide can accumulate very quickly and lower pH.

1.5.7 The rise of RAS

Globally, sea-based aquaculture produces millions of metric tonnes of seafood every year, and is necessary to produce food for the global population and balance the demand made on wild fisheries. Sea-based aquaculture is essential, and will be a part of the aquaculture landscape for generations to come. However, in its current form, sea-based aquaculture (specifically net pen fish farming) is not sustainable in the long term. Fundamental limitations that will limit expansion in the future include, competition for marine space, susceptibility to disease, dependence on an appropriate site, direct environmental impact, and indirect ecosystem effects. Land-based recirculation aquaculture can provide a safe, consistent supply of aquaculture products, that are produced in an environmentally compatible way. Land-based systems are not limited by climate, are flexible and adaptable, and are infinitely expandable. In countries where aquaculture is developed, the amount of new marine space available (through government regulation or appropriate conditions for culture) for new aquaculture operations is in short supply. As water space appropriate for aquaculture becomes more and more scarce, land-based culture operations will become important for the growth of the aquaculture industry in the future.

1.5.8 Advantages and disadvantages of RAS

A key advantage of recycling culture water is the complete control over all the water parameters for production, and the scope to provide conditions that are optimal for growth of the target species. Water temperature is a key parameter and has a heavy influence on growth rate of aquatic animals. Water temperatures in sea-based systems are controlled by season, climate and local weather conditions, and can affect growth rate when water temperatures move outside of an optimal growth range (see section 1.4.2). Optimal growth critically speeds up production time. Additionally, a consistent stable culture environment promotes uniform growth. This enables the operator to accurately predict the size of the cultured animal at a certain stage, which can aid sales and marketing by development of a steady harvesting regime.

The physical isolation of RAS from the environment avoids some major production hurdles experienced by sea-based operations. The impact of large predators on sea-

based fish culture operations is extremely problematic for the fin fish industry. King Salmon (*Oncorhynchus tshawytscha*) operations in New Zealand operate under constant threat from large predatory seals and sharks that attack the net pens to try and access the fish (M Whipp, pers. comm., Nov 2010). There is a direct financial cost associated with maintenance and repair of nets and installation of appropriate deterrent measures. The presence of a large predator can also affect appetite and behaviour of the cultured fish and consequently there is an indirect cost of growth rate through poor feed conversion efficiency (Nash et al., 2000). It is estimated that 10% of the farm gate value of Atlantic salmon (*Salmo salar*) cultured in Maine is lost to seal predation (Morris, 1996). Due to their physical isolation, land-based systems are under no threat from marine predators.

Perhaps the biggest advantage of RAS is that of disease prevention. The problems of disease are particularly acute in sea-based fin fish farms. The impact of parasites and disease are major issues preventing the further expansion of the developed Atlantic salmon industry. The most damaging parasite to the salmon industry in Europe and the Americas is the ecto-parasitic sea louse (Costello, 2006). The impact of sea lice on salmon ranges from mild skin damage to stress induced mortality. Despite a major research effort over the last 30 years, lice control is estimated to cost the global salmon industry $430 M US annually (Costello, 2009).

The risks associated with intensive net pen systems are high because the culture environment is open to the outside environment. Net pens provide some protection for the fish from predators, but allow free transmission of pathogens between farmed and wild fish. Farmed fish are generally held in one area at unnaturally high stocking densities. The close proximity of the fish within the net pen can facilitate the spread of disease (Murray and Peeler, 2005), and if left untreated, a farm can become a pathogen reservoir that can affect the surrounding environment. The risk of disease and pathogen transfer is reduced considerably by using RAS, as incoming water can be filtered and treated to remove larval life stages of parasitic organisms, and culture water is continuously treated (commonly by UV radiation, and/or dosing ozone) to kill any pathogens present in the culture tanks. In addition, if treatment steps need to be taken, it is relatively easy to medicate animals in closed tank systems (common treatments are added to the water as a bath). Because of the physical isolation of land-

based systems, the risk of transmitting diseases to natural populations is low; effluent water can be captured and treated before re-entering the environment.

In New Zealand there are regulations surrounding the impact on the environment when establishing new aquaculture farms. As these regulations will likely become more stringent in the future, managing aquaculture waste will become increasingly important. In RAS, recycling culture water allows the minimal use of source water. Because RAS use extremely low rates of make up water, recycling systems can readily capture from 80 to 100% of solid wastes produced (Timmons et al., 2007c). The facility to concentrate solid wastes in RAS means aquaculture waste can be collected and treated economically and efficiently (Summerfelt et al., 2000a). RAS offers an environmental advantage over more conventional methods of aquaculture, as output of organic pollution of a well managed RAS system is very small.

Recirculating aquaculture systems have many advantages over existing sea-based or flow through culture methods. There are however, drawbacks. Water quality management is paramount to an effective operation. The operator needs an expert understanding of the mechanisms and technology used in each step of the water treatment process. If one part of the treatment process breaks down or reaches its limit, the resulting shift in water quality can potentially be catastrophic for the cultured animals. A robust management regime can minimise risk associated with inevitable system failures, however continuous monitoring systems are critical for success using RAS.

The energy requirements to continuously pump and clean seawater to maintain water quality can be one of a number of significant costs for a large RAS. The relatively high production cost is one of the reasons species selection for land-based aquaculture is generally limited to high value species such as finfish. For RAS to be competitive with sea-based operations they need to be of a significantly large size to achieve economies of scale. There is considerable capital investment required to set up a fully functioning RAS, and as the risks in aquaculture are also considerable, the initial start up costs of a large scale RAS can be intimidating for many investors.

1.6 pH

1.6.1 General

The pH of a solution can be described as a measure of the relative acidity or basicity, and is measured on a scale of 0 to 14 with 7.0 corresponding to the neutral point. An acid or a base added to water releases ions of hydrogen, H^+, or hydroxyl, OH^- respectively. Solutions where H^+ predominates are acidic, and have pH values below 7.0. Solutions where OH^- dominate are basic, and have pH values above 7.0. Changes in pH are logarithmic, whereby the concentration of H^+ or OH^- increases or decreases by a factor of 10 with the change of 1 pH unit. This means at pH 6 there are 100 times more H^+ ions than at pH 8. pH is an important water parameter in an aquatic system because many chemical reactions that affect the living tissue of aquatic animals are pH dependent (Spotte, 1992). The pH of seawater is slightly basic, being relatively stable between 8.0 and 8.5 (Timmons et al., 2007e).

In general terms, the pH of seawater is closely tied to the amount of carbon dioxide (CO_2) dissolved in water, and to the alkalinity[12]. If uncorrected, pH in a closed aquaculture system is depressed through production of CO_2 by cultured organisms (including bacteria) and erosion of alkalinity by nitrifying bacteria in the biofilter. To balance pH there must be systems in place to raise pH back toward a target level. Physical methods of raising pH involve stripping accumulated CO_2 from the water by aeration. As CO_2 moves out of water supersaturated with CO_2, the pH will rise toward an equilibrium. The pH of water at equilibrium with the gases in the air is determined by the alkalinity of the water. Alkalinity resists change in pH by interacting with the H^+ ions that are released by the addition of an acid or hydration of CO_2. The addition of buffering chemicals can restore depleted alkalinity through the addition of anions. This maintains the buffering capacity of the water and fortifies the water to resist sharp drops in pH.

[12] Alkalinity is a net negative charge of all anions in the water that interact with H^+ ions

1.6.2 CO_2 and the carbonate system

pH and the concentration of CO_2 in the water are linked. In aquaculture, CO_2 is produced by respiration of the cultured animals and micro organisms in the system. However, unlike other soluble atmospheric gases, such as nitrogen or oxygen, hydrated CO_2 reacts with water and is immediately assimilated into a chemical equilibrium system. When CO_2 dissolves into water it is converted into a number of inorganic carbon species. The chemical interactions of water, carbon dioxide, and the anions bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), control the pH of seawater through equilibrium reactions (Skirrow, 1975).

Equation 1.1. Carbonate equilibrium system in water

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (1.1)$$

CO_2 dissolves into the water and forms carbonic acid (H_2CO_3) that readily disassociates into bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) ions. The formation of both carbonate and bicarbonate ions releases H^+ ions into the water, resulting in a lowering of the pH (Stumm and Morgan, 1996). Cultured animals in closed aquaculture systems produce CO_2 and trigger the release of H^+ from the formation of new HCO_3^- and CO_3^{2-} that lowers the pH of the system water. In intensive RAS, CO_2 is degassed through heavy aeration of the culture water. CO_2 that exits the water back into the atmosphere allows surplus H^+ to be absorbed back into the carbonate system and will subsequently raise pH.

The relative proportion of the carbonate species varies with pH, and at a given pH only one species predominates. In seawater, with a pH of 8.0 to 8.4, the largest proportion exists as HCO_3^- (see Fig. 1.12).

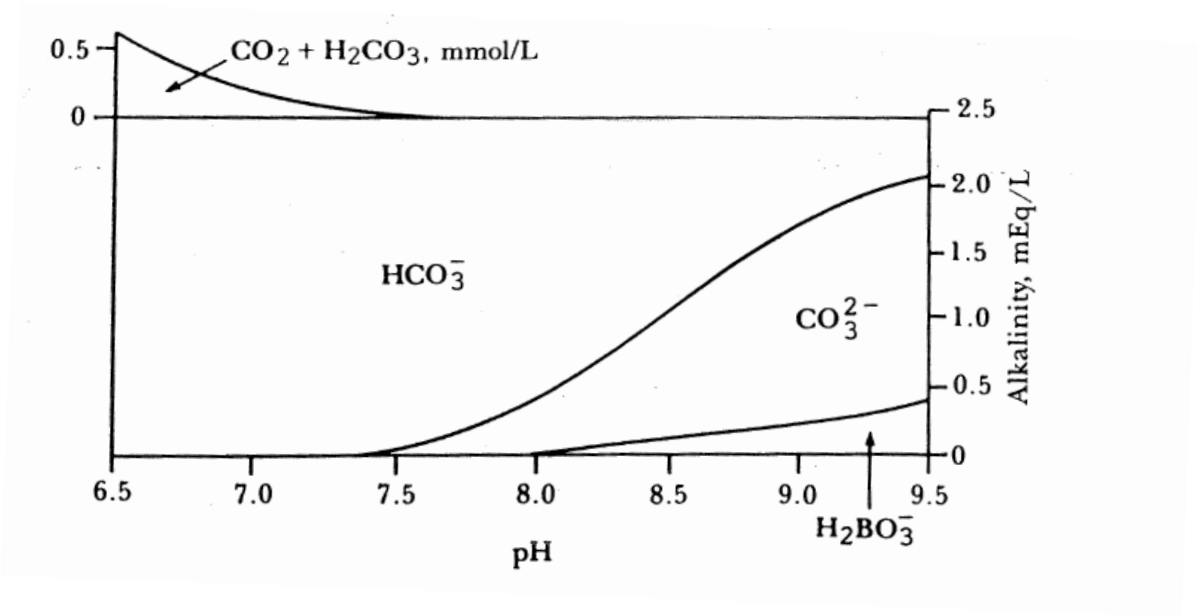


Figure 1.13 Variation in the proportions of carbonate species of seawater with changes in pH. Source: Spotte (1992).

The sum of all the inorganic carbon species in the carbonate system represents total inorganic carbon concentration or C_T (equation 1.2). C_T increases or decreases proportionately with the addition or removal of CO_2 (Moran, 2010a).

$$C_T = [CO_{2(aq)}] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]^* \quad (1.2)$$

*Note, square brackets represent molar concentration

The degree to which CO_2 dissolves into water is determined by the solubility constant K_0 (equation 1.3), that is determined by temperature and ionic composition of the water (Weiss, 1974).

$$K_0 = \frac{[CO_{2(aq)}][H_2CO_3]}{[CO_{2(g)}]} \quad (1.3)$$

Similarly, the relative concentrations of HCO_3^- and CO_3^{2-} at equilibrium can be calculated by the disassociation constants K_1 and K_2 (see equations 1.4, 1.5) that are also dependent on the temperature and ionic composition of the water (Millero et al., 2007).

$$K_1 = \frac{[H^+][HCO_3^-]}{[CO_{2(aq)}][H_2CO_3]} \quad (1.4)$$

$$K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \quad (1.5)$$

1.6.3 CO_2 production

In a coastal marine system, pH is typically not constant and exhibits a natural diurnal cycle (see below Fig. 1.13). This is due to the combined biological processes of photosynthesis and respiration and their influence on the carbonate system. All organisms, including plants respire continuously, which is the process of converting carbohydrate into energy that consumes O_2 and releases CO_2. Addition of CO_2 into the carbonate system depresses pH, however during daylight hours, a rise in pH is commonly observed as CO_2 in consumed by photosynthetic organisms. Photosynthesis is the process of converting CO_2 and water into carbohydrates and oxygen. The amplitude of change can be dramatic. A change of up 1.8 pH units (pH 7.4 to 9.2) driven by algal mats has been observed on shallow tidal flats in peak sunshine by (Oppenheimer and Master, 1965). The amplitude of pH changes is influenced by many factors in a marine system, including nutrient availability, light intensity, temperature and water movement (Oppenheimer and Master, 1965)

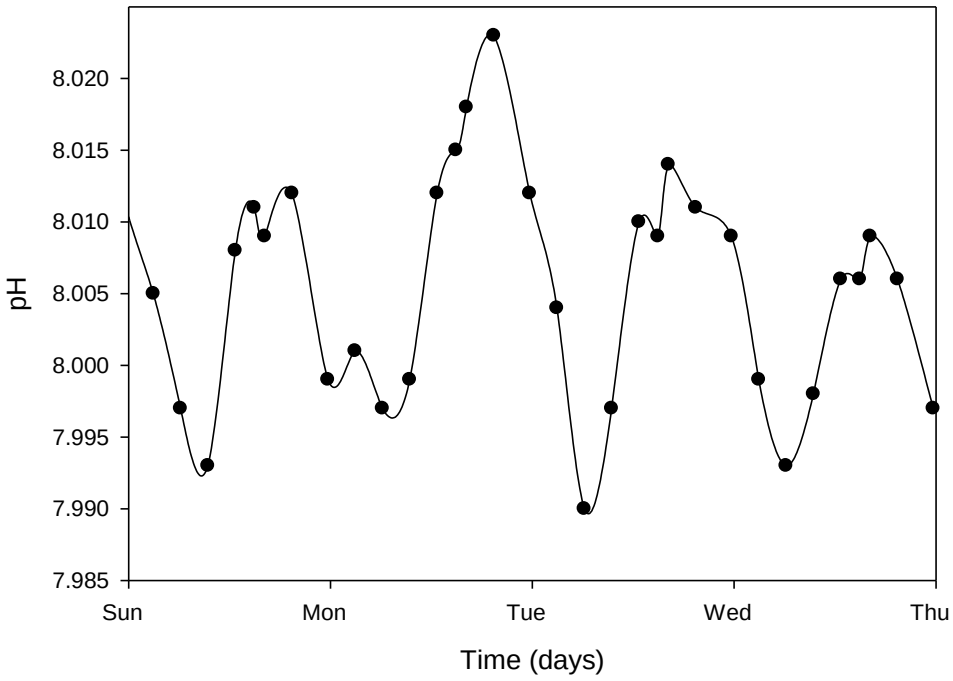


Figure 1.14 pH of natural seawater in Wellington Harbour at the Mahanga Bay research institute. Tick marks represent 00:00 midnight.

In aquaculture the rate of respiration will drive CO_2 production within the system. Respiration rate has important consequences for pH, as each mg of oxygen consumed produces 1.38 mg of CO_2 (Stumm and Morgan, 1996). Periods of high activity influence CO_2 production and pH through increased respiration and can lead to swings in pH in the culture tanks (see below Fig. 1.14). Cultured juvenile pāua exhibit a diurnal activity cycle where after dusk, there is a pulse of foraging activity in the culture tanks (G Moss, pers. comm., Mar 2011). Shifts in water temperature can also influence the respiration rate in abalone. Several studies have demonstrated a linear relationship between increasing oxygen consumption with increased temperature up to 20°C (Hahn, 1989c; Kikuchi and Uki, 1974). This has important implications for abalone culture. CO_2 production is not uniform, and is influenced by a change in water temperature and activity in the tanks. This may lead to periods when pH may drop to dangerous levels (e.g. during feeding periods in the warmer months) if pH treatment systems are not effective enough.

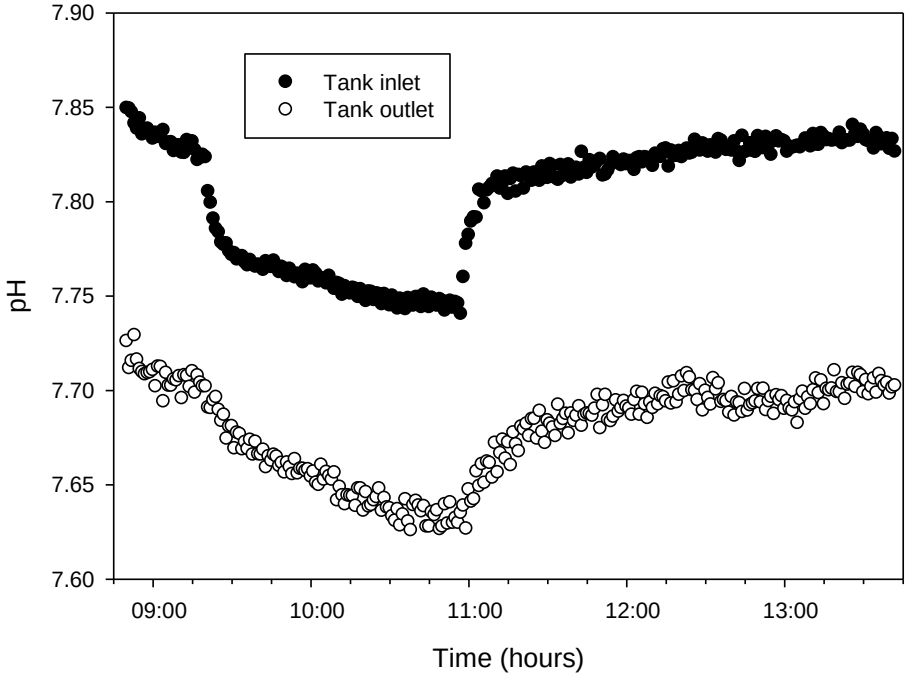


Figure 1.15 Variation in pH in a pilot scale pāua RAS. Trays were fed at 9:00 and held approximately 200 kg of pāua. No buffering chemicals were used in this system (i.e. pH was controlled by degassing only).

CO_2 management and pH control is particularly important in closed systems as CO_2 can accumulate quickly and potentially threaten stock health. An average respiration rate for 35 mm pāua reared at 18°C is 80 mg O_2/kg/hr (Heath and Tait, 2006a), which produces approximately 110 mg CO_2. In a small 5 T production system, this equates to 550 g of CO_2 being dissolved into the culture water every hour. Managing CO_2 concentration through degassing and/or alkalinity supplementation is therefore one of the most critical factors of managing an effective abalone recirculation system.

1.6.4 Alkalinity

The degree of change in pH from CO_2 addition is controlled by alkalinity. In broad terms, alkalinity is the buffering capacity or acid neutralising capacity of the water. The relative stability of pH in seawater compared to freshwater is the result of a relatively high alkalinity. Alkalinity is defined as the net negative charge of all anions that interact with H^+ ions. In seawater, the carbonate system (HCO_3^- and CO_3^{2-}) and borate ($B(OH)_4^-$) contribute the vast majority of anions to alkalinity (Weyl, 1970).

Because of the low concentration of borate in normal pH seawater (see Fig. 1.12), in practical terms, the sum of HCO_3^- and CO_3^{2-} determines alkalinity. The alkalinity[13] of seawater typically ranges from 120 – 150 mg/$CaCO_3$ L.

Any activities that cause HCO_3^- and CO_3^{2-} to diminish, erodes alkalinity and the ability of the seawater to buffer pH change. In recirculation aquaculture, alkalinity is consumed by the biofilter. During nitrification, ionized ammonia (NH_4^+) and nitrite (NO_2^-) is oxidized by nitrifying bacteria to produce energy. This process requires bicarbonate and oxygen. Alkalinity is consumed at the rate of 1.98 moles of HCO_3^- for every mole of NH_4^+ oxidized (J J Bisogni and Timmons, 1994). It is therefore important to supplement alkalinity at a rate equal to its removal from the biofilter. Failure to do so will remove the buffering capacity of the water and lead to a drop in pH in the culture tanks (see below Fig. 1.15).

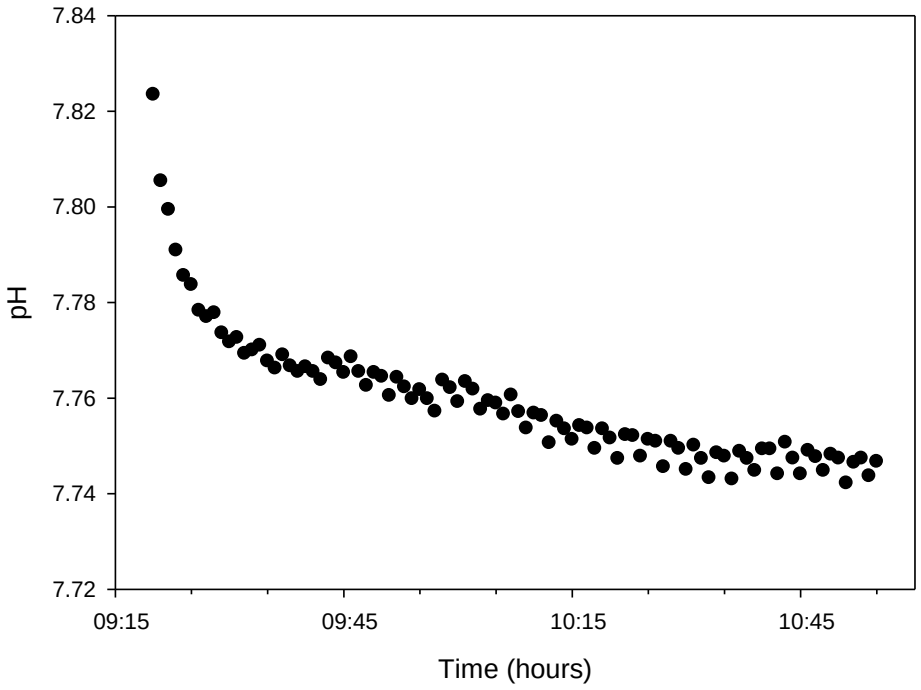


Figure 1.16 pH variation in a pilot scale pāua RAS with no addition of alkalinity chemicals.

[13] Calcium carbonate ($CaCO_3$) is the primary unit for expression of alkalinity in aquaculture, and will be used in this thesis. Sometimes alkalinity is expressed in milliequivalents/Litre (meq/L), or degrees of hardness (dKH).

The flux of CO_2 from the water to the gas phase does not affect the alkalinity of the water. CO_2 is assimilated into the carbonate system and total carbon increases by the amount of that addition, however, because CO_2 does not carry an electrical charge the alkalinity remains unchanged (Spotte, 1992). Alkalinity can be altered to a desired concentration by dosing a strong acid or a base into the system water. Addition of a strong acid e.g. hydrochloric acid (HCl), decreases alkalinity by releasing H^+ ions that consume available anions in the water. Conversely, the addition of a strong base e.g. sodium hydroxide (NaOH) raises alkalinity by the addition of OH^- ions, and can restore or enhance the buffering capacity of the water. Alkalinity supplementation will be discussed in detail in Chapter 3.

1.7 Objectives and aims

Recirculation aquaculture is not a new science and is used in commercial applications all over the world. The majority of species cultured in RAS have been freshwater species, and most studies examining aspects of water quality have focused on freshwater. As the aquaculture industry expands, recirculating aquaculture will become more important in the culture of marine species. There are however key areas of knowledge that have not been investigated within marine RAS, and these could have a significant impact on the development of this technology.

I have selected one of these key areas for my research programme, to address the emerging problems surrounding pH management in land-based seawater aquaculture systems. This thesis will investigate two methods to control pH in seawater aquaculture systems, specifically related to the culture of pāua. I have also included a supplementary chapter to explore the effects of lowered pH on pāua growth and examine the impact on shell mineralogy.

To date there have not been any studies examining CO_2 removal through a cascade column in seawater, and an apparent absence of data surrounding the removal efficiencies of dissolved CO_2 under a concentration of 10 mg/L (equating to approximately $\geq$ pH 7.4).

Industrial grade calcium hydroxide ($Ca(OH)_2$) is used to raise pH and add alkalinity to the culture water of commercial pāua farms around New Zealand, but there have not been any studies examining the direct effect of buffered seawater[14] on pāua growth. There has also been no comparison of buffering chemicals to compare suitability for use in pāua culture.

There is also limited information surrounding the direct effect of lowered pH on biomineralisation and shell dissolution in abalone. These are two areas that have largely been ignored despite the expansion of land-based abalone systems around the world and the apparent sensitivity of abalone to low pH conditions.

1.7.1 Aims

The overall aim of this research programme is to assess two methods of pH control used in closed aquaculture systems designed for the culture of pāua, and examine the effects of lowered pH on pāua. Specific aims are as follows;

1. Evaluate the effectiveness of CO_2 removal through a packed column aerator in seawater at an influent water pH of 7.4, 7.6 and 7.8.

2. Measure the effect of buffered seawater on the growth and development of juvenile pāua.

3. Assess the impact of low pH (7.6) water on juvenile pāua, by examining growth, measuring shell thickness, and examining shell composition to determine sensitivity of juvenile pāua to lowered pH.

[14] In this thesis, reference to 'buffered seawater' refers to seawater in which the pH has been altered by alkalinity chemicals.

Chapter 2

Limitations of Degassing Columns at High pH

2.1. Introduction

2.1.1. Overview

There is growing interest in the use of high intensity recirculated aquaculture systems (RAS) for production of marine species, because they offer greater control over water quality and can produce higher yields per unit of water space than flow through or cage farming systems. Within a RAS it is necessary to continuously treat the system water to prevent a build up of organic and inorganic byproducts that if allowed to accumulate would be toxic to the cultured stock. Ammonia, carbon dioxide, suspended solids and dissolved oxygen are some of the key water quality parameters that require careful management, because changes in the levels of these parameters may have serious consequences for the health of the cultured animals (Timmons et al., 2007e).

The effects of accumulation of carbon dioxide (CO_2) and on the resultant lowering the pH of the culture water have been well documented (Summerfelt et al., 2000b; Wurts and Durborow, 1992). Increased CO_2 levels have been shown to compromise the respiration of fishes by reducing the capacity of the blood to transport oxygen (Colt and Orwicz, 1991). Identifying the biological limitations of pH and CO_2 on target species, and developing appropriate methods to mitigate accumulation of CO_2 in culture systems are therefore critical steps to developing successful high intensity farming of new species.

In New Zealand there is growing industry interest in the cultivation of pāua using RAS. Unlike many fish culture operations, where culture water can be maintained at low pH without any observed adverse effects on stock health (Eshchar et al., 2006; Good et al., 2010), cultured abalone appear to be more susceptible to relatively small changes in pH (Harris et al., 1999a). Studies at the *National Institute of Water and*

Atmosphere (*NIWA*) cool water facility in Wellington, New Zealand have shown low pH to be linked to shell dissolution in pāua. Shell dissolution can make the abalone vulnerable to shell breakage when handing and transporting. Anecdotal evidence suggests the shell will begin to dissolve when the pH of the culture water drops to approximately pH 7.6. Observations on other shelled organisms show calcification rates may exhibit a strong decline as a function of decreasing pH, and increasing partial pressure of CO_2 (Gazeau et al., 2007; Ries et al., 2009). Managing pH to ensure the culture water is maintained above pH 7.6 appears important to running an efficient pāua culture operation.

2.1.2 Carbon dioxide in water

A build up of dissolved CO_2 in aquaculture systems is generally undesirable, and degassing processes have been developed to remove CO_2 from the culture water. Techniques used to remove CO_2 include surface agitators (Boyd, 1998), cascading weirs, bubble columns or airlifts pumps (Moran, 2010b) and aerated packed columns. Aerated packed columns are commonly used in intensive aquaculture and are normally considered to be one of the most effective means of removing CO_2 (Grace and Piedrahita, 1994; Summerfelt et al., 2000b).

Because of the greater emphasis on freshwater species in recirculation aquaculture, the majority of column degassing data are from freshwater experiments and focus on dissolved CO_2 concentrations greater than 10 mg L^{-1} (Moran, 2010a; Summerfelt et al., 2003; Summerfelt et al., 2000b) (equating to pH less than ~7.5). CO_2 degassing data in marine aquaculture systems is limited. Eshchar et al. (2003) compared CO_2 removal rates between a paddlewheel and submerged aerator in a tank of seawater, however studies on CO_2 removal in seawater by column degassing are limited to modelling data derived from comparing degassing efficiency at different salinities (Moran, 2010a). There is also an apparent absence of degassing data in cascade columns at influent CO_2 concentrations <10 mg L^{-1} in either fresh or seawater.

Unlike oxygen (O_2) or nitrogen (N_2) gas, that dissolve but do not react with water, when CO_2 dissolves in water it is assimilated into a chemical equilibrium system (Eqn. 2.1).

Equation 2.1. Carbonate equilibrium system in water

$$CO_{2\,(g)} \underset{K_0}{\leftrightarrow} CO_{2\,(aq)} + H_2O \underset{K_1}{\leftrightarrow} H_2CO_3 \underset{K_2}{\leftrightarrow} HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+ \qquad (2.1)$$

When CO_2 dissolves into water it is converted into a number of inorganic carbon species. Aqueous CO_2 forms carbonic acid (H_2CO_3) that readily disassociates into bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) ions. The formation of both carbonate and bicarbonate releases H^+ ions into the water, resulting in lowered pH. The degree of change in pH from CO_2 addition is controlled by alkalinity (Stumm and Morgan, 1996), and solubility constants (K_0, K_1 and K_2) that are dependent on temperature and salinity (Weiss, 1974).

Removal of aqueous CO_2 by degassing moves the carbonate equilibrium equation to the left, free H^+ ions form new water molecules that result in an increase in pH. The addition of a strong base such as sodium hydroxide (NaOH) or calcium hydroxide ($Ca(OH)_2$) moves the equation to the right. Hydroxyl ions that are released through the disassociation of the dissolved base are assimilated into the carbonate system that increases alkalinity and raises the pH of the water.

Within RAS pH control is normally achieved by removal of CO_2 by degassing. The addition of chemicals to increase alkalinity to control pH has generally been considered as problematic due to the associated cost, handling hazards, and potential for dosing malfunction that may compromise water quality. Increasing alkalinity does not remove excess CO_2 and has been shown to cause health problems such as nephrocalcinosis in fish (Chen et al., 2001).

pH and CO_2 are strongly linked, and given that direct measurement of dissolved CO_2 is difficult in seawater, most commercial systems operators use pH as a proxy for determining CO_2 concentration in the water. In this study pH is used as the primary unit of expression of CO_2 levels in the culture water. pH is more readily measured than dissolved CO_2, and can be used to back calculate CO_2 levels if alkalinity and the solubility constants of temperature and salinity are known.

This study aimed to expand on existing studies related to degassing at low pH by testing the efficiency of a cascade column degassing unit at removing CO_2 (therefore raising pH) in seawater at relatively high pH.

2.2. Materials and Methods

2.2.1. Overview

This study tested the effectiveness of a cascade column to control pH by manipulating water temperature, hydraulic loading, counter current airflow and media height. Trials were performed on a single cascade column system that was constructed at *NIWA*'s Mahanga Bay Aquaculture facility, Wellington, New Zealand.

Food grade CO_2 was dosed via a venturi valve (see below Fig 2.1, A) to lower pH of the water to 7.8, 7.6, or 7.4 that was used as influent water treatments. Four control variables were independently manipulated within each influent water treatment; water flow, water temperature, packing media height and counter current air flow. The test parameters for these variables were as follows;

- Water flow; 70, 85, 100, 115, 130 L min^{-1}
- Water temperature: 12, 15, 18, 21, 24°C
- Packing media height: 55, 110, 150 cm
- Counter-current airflow: (L min^{-1}) (ratio of air to water): 0:1, 5:1, 10:1, 15:1, 20:1

Experimental constants were set at 18°C, 100 L min^{-1}, 150 cm media height, and 5:1 air/water. Data were recorded in real time by a data logging system (dataTaker).

The design of the cascade column was consistent with that of a typical cascade column (Colt and Bouck, 1984; Timmons et al., 2002), being comprised of a vertically positioned cylindrical column enclosing an open structured packing media. Influent water entered the top of the column, and flowed down over the packing media into the main reservoir sump below. Air at ambient temperature entered the

column at the base through a series of vents, and was pulled up through the media in a counter current flow driven by a blower unit.

Figure 2.1 Selected components of the experimental system to test CO_2 removal at high pH. Venturi plumbing arrangement and regulating valve to control input of CO_2 into the system water (A). A bird's eye view of the air tight box, showing the top of the media blocks (B). A variable speed air blower used to generate counter current airflow (C). Degassing column and main reservoir sump (D). The top of the column is fed through the ceiling to a mezzanine floor.

To control the water temperature, system water was pumped through a 21 kW commercial heat chill unit that was installed into a parallel plumbing circuit in the adjacent room. Water was circulated through the heat chill unit by a 1.5 kW centrifugal pump, and returned to the main sump via a CO_2 dosing valve. Water was pumped to the top of the column by a 1.5 kW centrifugal pump. Hydraulic flow was

manipulated by a ball-valve and measured using a f-2000 series paddlewheel flow meter (Blue-White Industries Ltd). The media material BioTube (ACE NZ Ltd) had a total surface area of 250 m^2/m^3. Influent water was sprayed into the top of the media through a *spiraljet* 40 mm PVC distribution nozzle. Adjusting water flow caused subtle changes to the trajectory of water exiting through the *spiraljet* nozzle. Before each test the nozzle was adjusted vertically within the column to compensate for this change, and ensured the spray margin was even with the top of the media. A 9 kW variable speed air blower was installed to generate a counter current air flow and connected to the top of the column via an airtight box and 400 mm ducting. Air flow was measured by an *E&E* electronic air velocity sensor installed into the ducting. Ambient air entered the base of the column through a series of vents located just below the media blocks. To avoid influence from CO_2 laden air seeping up from the main collection sump into the column, a lid was fitted over the main sump and a small extractor fan was installed to remove contaminated air from the space above the sump. The entire system contained approximately 600 L^{-1} of seawater. Ambient air temperature was recorded using a PT100 probe (Servotech NZ), but not controlled. pH was measured by plastic body electrodes from Sensorex (USA) to an accuracy of 0.01 of a pH unit.

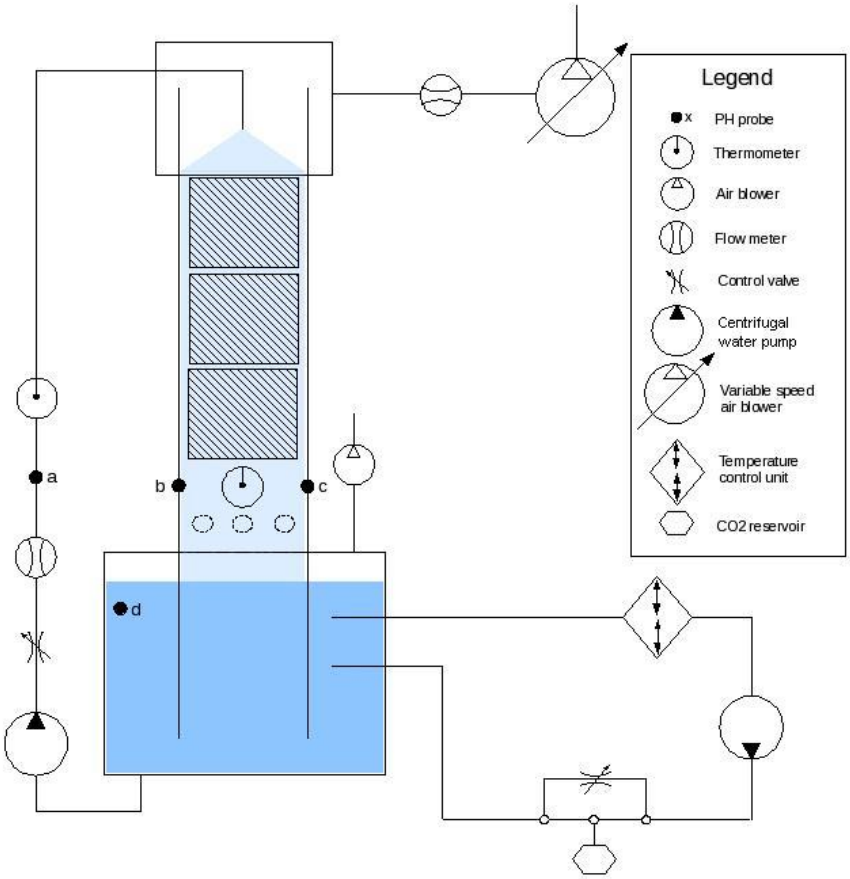


Figure 2.2 Experimental column design to test CO_2 removal at high pH.

Total height of column was 3 m, with an internal diameter of 380 mm. Media blocks were suspended inside the column by 2 x 8 mm PVC rods inserted through the column wall. Each block of media used in this study were made up of 57 x 40 mm diameter tubes of BioTube packing material.

2.2.2. Test procedure

The pH of the water was measured in the supply pipe immediately before entering the column, and measured again immediately after exiting the column. The net difference in pH between these two points was used as a measure of the efficiency of the column.

The pH of the influent water was measured by a single in-line pH probe, effluent water measurements were taken by two pH probes fitted into water collecting pods

located approximately 100 mm below the lowest point of the media. The mean readings of these two probes were used to determine effluent pH. All pH probes were calibrated daily before use, by using buffer solution pH 7 and 10. Probes were stored in buffer solution pH 4 when not in use.

Before each measurement, the experimental parameters were set and the system was allowed time to stabilize. Once all control variables were stable five minutes of data (logged value every 5 seconds) were recorded. Each data point reported on the figures below represents a mean value of the data recorded over the 5 minute test period. Standard deviations of the data points are reported on the associated tables. Water within the system was changed daily before testing and alkalinity tested daily by a carbonate test kit (Aquarium Pharmaceuticals, Inc).

2.3. Results

2.3.1. Impact of water flow on pH

The efficiency of the column was found to decrease with both increased hydraulic load and increased pH such that the highest level of pH change was observed at pH 7.4 and a hydraulic load of 70 L min^{-1} (Fig. 2.3). The difference in observed pH change between the lowest (70 L min^{-1}) and the highest (130 L min^{-1}) flow rate was 0.04, 0.04 and 0.03 of a pH unit within influent water treatments of pH 7.4, 7.6 and 7.8 respectively.

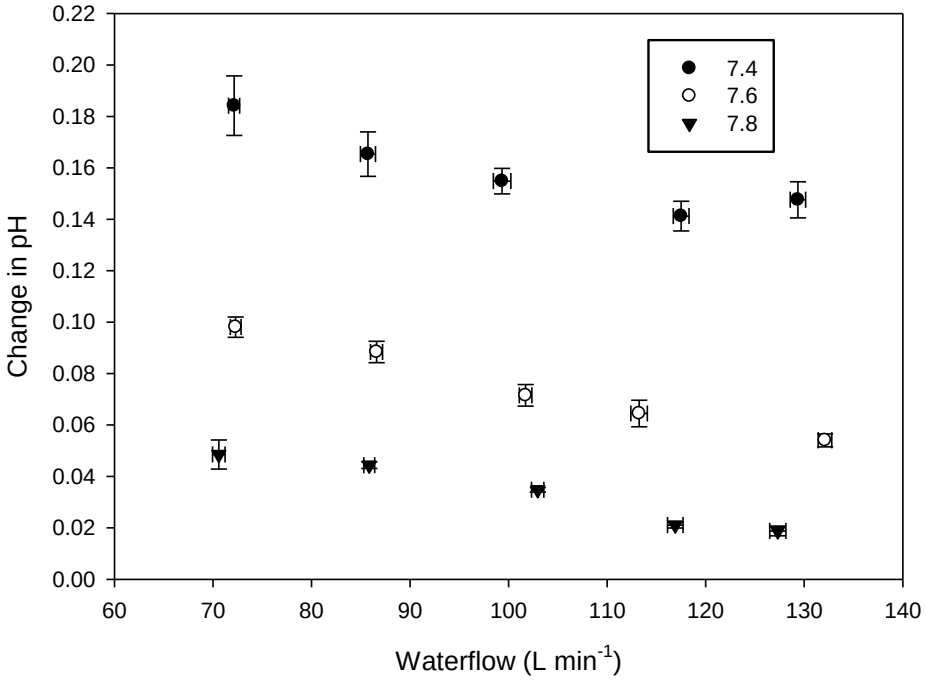


Figure 2.3 The net gain of pH through a cascade column, and the effect of hydraulic loading (L min^{-1}). Influent water pH levels of pH 7.4, 7.6 and 7.8.

2.3.2. Impact of media height on pH

Media height had an observable effect at an influent pH of 7.4, and a very minor influence on an influent pH of 7.6 and 7.8 (Fig. 2.4). The greatest difference in pH between low (55 cm) and high (150 cm) media height was 0.1 of a pH unit observed at influent water pH of 7.4. Difference in pH gain between high and low media height at influent waters of 7.6 and 7.8 were small, 0.02 and 0.01 of a pH unit respectively.

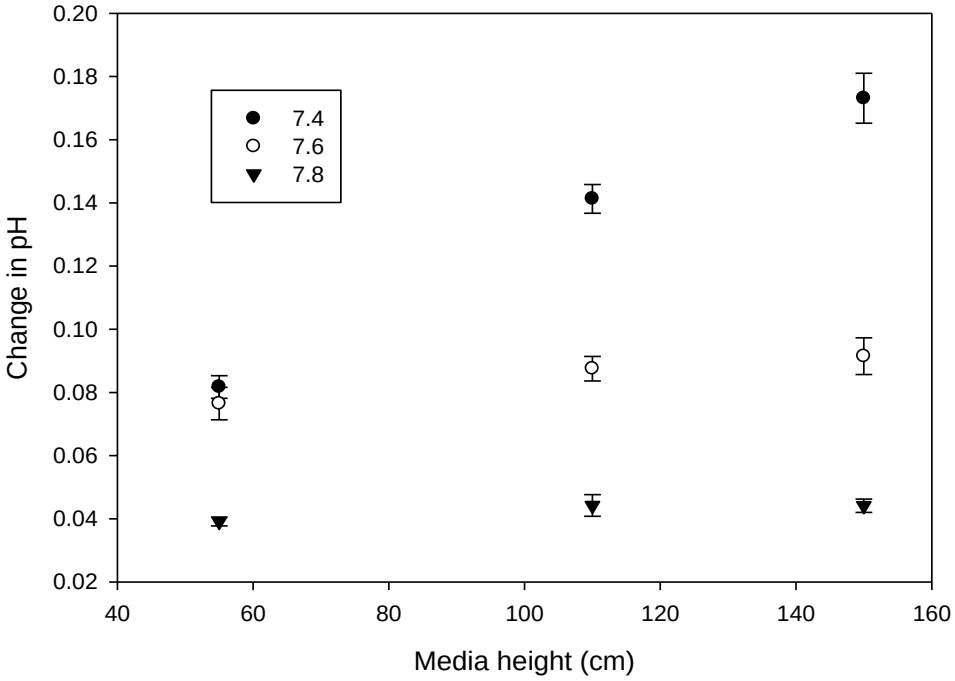


Figure 2.4 The net gain of pH through a cascade column, and the effect of packing media height. Influent water pH levels of 7.4, 7.6 and 7.8.

2.3.3. Impact of counter current airflow on pH

Over the range of influent pH treatments tested, results showed that counter current airflow had a minor effect on degassing efficiency (Fig. 2.5). The greatest difference between concurrent airflow (where the air blower was turned off) and maximum counter current airflow with a ratio of 20:1 (air:water) was 0.04 of a pH unit. Note that the negative airflow measurements shown in Fig. 2.5 were recorded where the air blower was not operational. These values represent concurrent airflow, passive air movement down the column with the falling water.

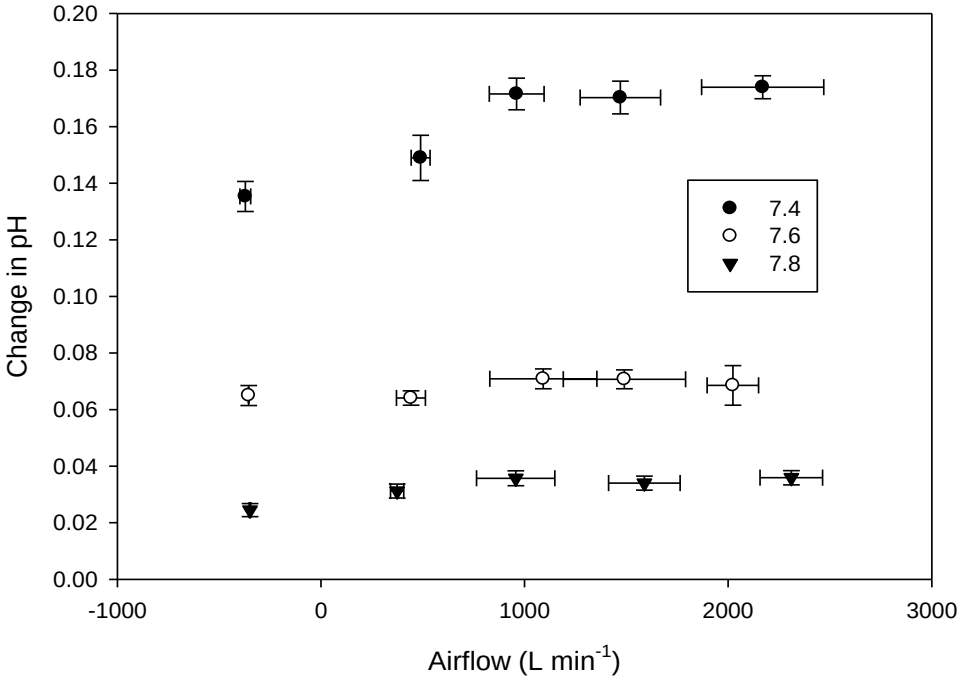


Figure 2.5 The net gain of pH through a cascade column, and the effect of counter current airflow (L min^{-1}). Influent water pH levels of 7.4, 7.6 and 7.8.

2.3.4 Impact of temperature on pH

Compared to the other parameters tested in this study, temperature had more influence on the change of pH through a cascade column than any other treatment variable (Fig. 2.6). Temperature had a similar effect on pH gain across all influent pH levels tested. The difference in pH gain between 12°C and 24°C was 0.12, 0.12 and 0.08 from influent waters of pH 7.4, 7.6 and 7.8 respectively.

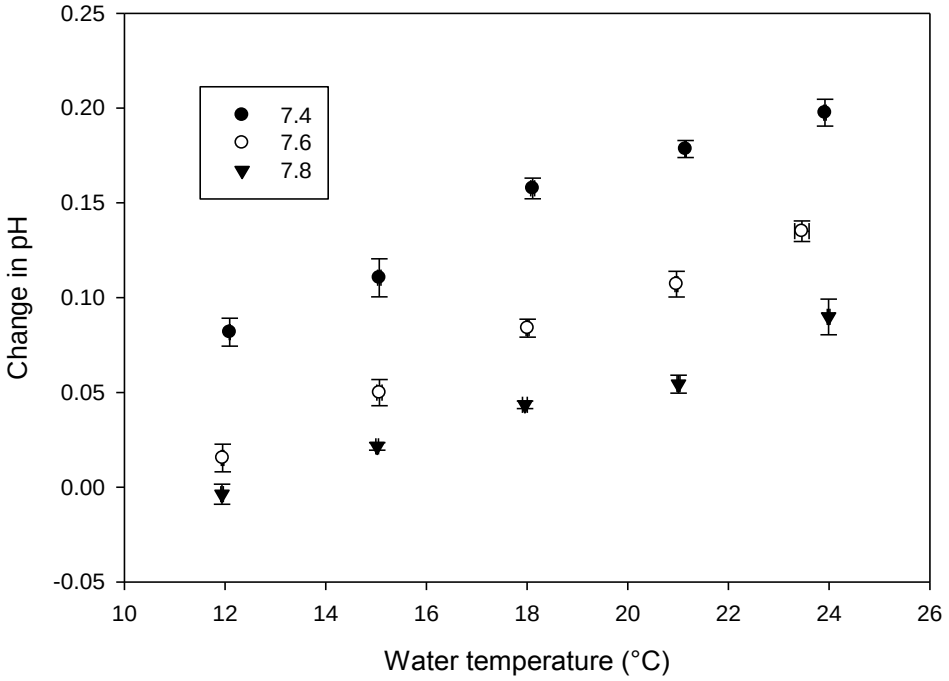


Figure 2.6 The net gain of pH through a cascade column, and the effect of temperature (°C). Influent water treatments of pH 7.4, 7.6 and 7.8.

2.4. Discussion

2.4.1 Column configuration

The movement of water over packing media has a large influence in determining how effective a column design is in removing supersaturated gases from the water (Colt and Bouck, 1984). Evenly distributed flow over the media and low hydraulic loads will promote good air to water contact and efficient degassing. Conversely, uneven flow and flow down the sidewalls of a column will compromise gas removal efficiency by lowered contact of water to air (Colt and Bouck, 1984). pH gain over the influent pH treatments tested were similarly affected by changes in hydraulic loading. The fraction of pH restored between the highest (130 L min^{-1}) and lowest (70 L min^{-1}) water flows was small, however there was a trend of increasing CO_2 removal efficiency observed over all influent pH levels by reducing water flow into the column (Fig. 2.3).

In column degassing packing media is used to break up water flow and provide a large contact area to promote a high rate of gas exchange. A tall stack of media is desirable to provide enough surface area for effective degassing, adjusting media height affects packing contact area and water residence time in the column and has been shown to have a positive effect on carbon dioxide stripping efficiency (Summerfelt et al., 2000b). There are practical difficulties associated with tall columns. Additional pumping cost, difficult access and ceiling height limitations restrict the build height of a degassing column. Many operators use air blowers to manipulate air to water contact and increase removal efficiency of short stacks of packing media, however minimum packing depth remains an important factor for effective degassing in column design. In this study media height only influenced pH gain at an influent pH of 7.4. Influent pH of 7.6 and 7.8 were largely unaffected by packing height (Fig. 2.4), and suggest changes in media volume within the heights tested has little effect on removal of CO_2 at influent waters of pH $\geq$ 7.6. Due to the importance of contact area in gas transfer applications, an acute change in pH was expected by manipulating packing height, however the expected change only manifested itself at the lowest influent pH treatment of pH 7.4.

It is well established that manipulating air to water contact through packing height, water flow, and counter current airflow effect removal efficiency of CO_2 through a packed column aerator (Grace and Piedrahita, 1994; Summerfelt et al., 2003; Summerfelt et al., 2000b). It has been suggested that optimal air to water CO_2 stripping ratios lie between 5 to 10 parts air to 1 part water (Summerfelt et al., 2003). The results in this study suggest that increasing air to water contact by using an air blower unit is of little practical significance when degassing by cascade column in seawater at an influent pH $\geq$ 7.4. Moran (2010b) observed counter current airflow did not improve CO_2 removal efficiency using a packed column design. He suggested that the design of the degassing apparatus used in his study promoted enough passive movement of air through the column that stripping performance was not enhanced by the inclusion of counter current air blower (Moran, 2010a). In this study the effect of increasing airflow on pH was most pronounced at an influent pH of 7.4, however overall, airflow had only minor effect on degassing efficiency (Fig. 2.5). The greatest difference between tests with a non operational air blower (that allowed only passive movement of air in the column) and a counter current airflow of 20:1 air:water was

only 0.039 of a pH unit. In this study, column design allowed for free movement of air through the column when the air blower was not operating. Additionally, the use of open tubular media and low hydraulic loading, it is likely that the column was already operating at near maximum stripping efficiency without the addition of counter current airflow.

2.4.2 Temperature

The solubility of CO_2 changes with temperature and salinity (Weiss, 1974). The direct effect of solubility constants (Eqn. 2.1) on CO_2 removal by degassing was observed by changing water temperature (Fig. 2.6). In this study, manipulating water temperature promoted the greatest observable difference in pH gain across all three influent pH treatments tested. As demonstrated, CO_2 removal efficiency changes with temperature. Removal efficiencies of steady state CO_2 degassing unit will therefore be affected when used in operations where water temperature is not controlled and fluctuate over the day, and from season to season. Given that the difference in pH gain over a 12 °C temperature range is approximately 0.1 of a pH unit, the impact of temperature change in an intensive farming situation may be of little practical significance. However trends observed in this study suggest an added benefit to water temperature control common in RAS, could also be to dampen variability in CO_2 removal efficiency, and facilitate a greater accuracy in management of culture conditions.

2.4.3 Difficulties in carbon dioxide degassing at high pH

The small differences in pH gains between airflow rates, water flow and media height tests, suggest that pH gain was not restricted by column configuration, but possibly by the low concentration of CO_2 in the influent water and chemical reaction dynamics of the carbonate equilibrium system. Removing CO_2 by degassing extracts only a small fraction of total inorganic carbon (C_t) present in the water. Once CO_2 is removed, the carbonate system re-establishes equilibrium by forming new CO_2 from H_2CO_3 and HCO_3^- (Eqn. 2.1). The rate of replenishment of new CO_2 from HCO_3^- is a slow process, and is estimated to take 20 to 30 seconds to re-establish equilibrium (Grace and Piedrahita, 1994; Stumm and Morgan, 1996). This delay generally exceeds the

residence time of the typical cascade column design. The slow reaction time of the carbonate system acts like a bottle neck in a typical cascade column, because during degassing, access to new CO_2 formed from the carbonate pool is limited by the time taken to re establish equilibrium. In this study residence time of the column was estimated to be approximately 10 seconds, therefore it is unlikely given the slow chemical reaction time that there was any replenishment of new CO_2 from the carbonate system. Given there is delay accessing new CO_2 from the carbonate pool, columns that promote a long water residence times are more likely to be effective at raising pH. Multiple passes of culture water through a single column, or water passed through a series of separate degassing units may also help alleviate the problem of CO_2 access, however these methods using existing CO_2 degassing technology are likely to be expensive and cost prohibitive in a commercial operation.

Within the column, the rate of gas transfer is primarily driven by the relative concentrations of CO_2 between the water and air phase, at high influent pH column stripping is less effective because there is a relatively small fraction of CO_2 available to degas. This appears to be one of the fundamental limitations to effective CO_2 degassing in waters of high pH. The impact of low concentrations of CO_2 in high pH water on column degassing is well illustrated in the media height test (Fig. 2.4). Media height only has an effect at a low influent pH, where the concentration of aqueous CO_2 is relatively high. This demonstrates that in column degassing, removal of CO_2 to increase pH is influenced by the sum of aqueous CO_2 in the water. Low total gas transfer at high pH, combined with limited replenishment of new CO_2 from the carbonate system present a significant restriction to effective removal of CO_2 by column degassing at high influent water pH.

2.5 Conclusions

Removing enough CO_2 to lift pH above pH 7.6 is difficult to achieve in a single pass through a typical cascade column degassing unit. Trends observed in this study suggests effectiveness of a cascade column at degassing influent waters pH $\geq$7.4 is largely determined by characteristics of the water chemistry, not by the configuration of the column. The small concentration of aqueous CO_2 in high pH water and the slow reaction time of the carbonate system act like a bottleneck to effective degassing

of high influent pH water. Manipulating air to water contact either through increasing airflow or increasing the height of the packing media had only a minor influence in increasing CO_2 removal efficiency using influent pH >7.4. Pāua farming operations where the pH of culture water needs to be maintained at high levels to avoid compromising animal health must therefore look to other techniques, such as alkalinity dosing, to increase pH.

Chapter 3

The Effect of Alkalinity Chemicals on the Growth of the New Zealand Abalone, *Haliotis iris*.

3.1 Introduction

Commercial pāua farming in New Zealand has struggled to become established in the last 20 years. The failure of the industry to develop is in part due to slow growth rates of pāua caused by fluctuating seawater temperatures and poor system design (Heath and Tait, 2006b). If the pāua industry is to become competitive with international abalone operations, pāua farmers must look to continually improve productivity and reduce production costs. Providing conditions that promote optimal growth is paramount to the effective and economic culture of pāua. Recirculating aquaculture system (RAS) technology is used in the commercial production of abalone in New Zealand. RAS is particularly advantageous in abalone culture, as it provides scope to manipulate water conditions to promote fast growth, in ways that are not possible or are cost prohibitive in open aquaculture systems such as net pens or land-based flow through systems.

The accumulation of respired carbon dioxide (CO_2) in the culture water can be problematic in RAS, because typically there is a high ratio of biomass to culture water. Stocking densities of pāua in shallow grow out trays can reach 150 kg/m^3 biomass/culture water (G Moss, pers. comm., Apr 2011). At these high densities respired CO_2 can accumulate quickly and lower pH. Abalone growth has been shown to be negatively affected by lowered pH in two species of Australian abalone (Harris et al., 1999a). Shell erosion, characterized by a shiny appearance (dissolution of the upper prismatic layer, revealing the inner nacreous layer), has also been observed in abalone cultured in low pH conditions (Heath and Tait, 2006b; Merino et al., 2010). Anecdotal evidence suggests shell erosion begins to occur around pH 7.7 (G Moss, pers. comm., 2008).

Usually, in closed aquaculture systems, pH is controlled through vigorous aeration or degassing systems. However results from Chapter 2 suggest CO_2 removal by aeration is not an efficient method to maintain pH of seawater above 7.6. Although gas exchange is still an important component of an abalone RAS, it is not effective enough to use as a solitary means of pH control. Dissolved CO_2 concentration, and by proxy pH, is determined by equilibrium reactions of the carbonate system (see background section, pH). pH can be manipulated by physical means e.g. CO_2 stripping, or through chemical means, by dosing alkalinity supplements. It is necessary in RAS to dose system water with an alkalinity supplement to replace the alkalinity used up by nitrification reactions within the biofilter (Timmons et al., 2007a). Dosing alkalinity beyond the requirements of the biofilter raises pH and is an important part of maintaining pH above 7.7 in abalone RAS.

There are many chemical substances capable of replacing alkalinity in water (see Table 3.1), however information on the relative suitability of these substances in aquaculture applications is sparse. The aim of this study was to assess the direct effect of buffered seawater on the growth of juvenile (30 mm) pāua, and evaluate the appropriateness of sodium hydroxide (NaOH), food grade calcium hydroxide ($Ca(OH)_2$) and industrial grade calcium hydroxide as buffering chemicals for pāua culture.

3.2 Background

Table 3.1 Substances used to replace alkalinity. Source: Heath and Tait (2006a)

Chemical formula	Name	g/eq	Solubility	Rate of Solubility
NaOH	Sodium hydroxide	40	High	High
Na_2CO_3	Sodium carbonate	53	High	High
$NaHCO_3$	Sodium bicarbonate	83	High	High
$CaCO_3$	Calcium carbonate	50	Moderate	Moderate
CaO	Slaked lime	28	High	Moderate
$Ca(OH)_2$	Calcium hydroxide (Hydrated lime)	37	High	Moderate
$CaMg(CO_3)_2$	Dolomite	46	Moderate	Slow
$MgCO_3$	Magnesium carbonate	42	Moderate	Slow
$Mg(OH_2)$	Magnesium hydroxide	29	Moderate	Slow

Calcium hydroxide is commonly used to buffer seawater in pāua RAS in New Zealand. The use of food grade $Ca(OH)_2$ has been recommended for pāua culture because it is thought the surplus calcium ions added to the system may enhance shell calcification, although this has never been thouroghly tested. Because $Ca(OH)_2$ is only moderately soluble, it is often added to aquaculture systems as a slurry. $Ca(OH)_2$ and NaOH solutions are extremely basic[15] and adding these solutions into the system water can rapidly raise pH at the dosing site and affect the balance of the system. For

[15] Sodium hydroxide or caustic soda is highly corrosive, and is widely available as a drain cleaning product.

this reason, alkalinity supplements are recommended to be added into the system slowly, to avoid sudden increases in pH.

$Ca(OH)_2$ or hydrated lime is derived from solid calcium carbonate ($CaCO_3$) limestone deposits. Limestone is quarried and processed to produce $Ca(OH)_2$, which is used in huge quantities in agricultural and industrial applications both in New Zealand and overseas. Original limestone deposits used to manufacture $Ca(OH)_2$ generally contain a small percentage of impurities such as heavy metals, alkalies, and phosphates that are not removed in the processing steps. The relative concentration of these impurities determine the grade of $Ca(OH)_2$, and if the product can be used in food, analytical or industrial applications. To be classified as a food grade $Ca(OH)_2$, the product must meet specific purity standards that are set by food and nutrition administrating bodies (that may differ from country to country) through central government.

3.2.1 Chemical interaction

When strong bases such as sodium hydroxide or calcium hydroxide are hydrated, ionization occurs and the Ca^{2+} and Na^{+} ions immediately disassociate from the hydroxide (OH^-) anions. OH^- is assimilated into the carbonate equibrium system, and combines with aqueous CO_2 to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) anions (see equations 3.1 & 3.2). The addition of these anions to the water increases the alkalinity. A simplified formula of the reaction is as follows;

$$OH^- + CO_2 \rightarrow HCO_3^- \quad (3.1)$$

$$OH^- + HCO_3^- \rightarrow CO_3^{2-} + H_2O \quad (3.2)$$

The net effect of the addition of a strong base (i.e. NaOH, $Ca(OH)_2$) into seawater is an increase in total carbon (C_t) (through an increase in bicarbonate and carbonate concentration), and an increase in pH.

3.3 Materials and Methods

Hatchery reared juvenille pāua (*H. iris*) that measured approximately 30 mm shell length (SL) were used in this trial. Experimental animals were selected from a pool of approximately 4000 individuals, which had been produced from a single mass spawning of broodstock animals at NIWA's Mahanga Bay hatchery, Wellington, New Zealand. Experimental animals were selected by size (larger, faster growing of the group were approximately 30 mm) and healthy appearance, e.g. shell was intact, no cracking or shiny appearance, no obvious signs of pH stress.

The 960 animals used in this trial were placed into pāua seed trays (see below, Fig. 3.2, B) that contained 6 channels per tray. 20 individuals were assigned to each channel. Each buffered seawater treatment had 6 replicate channels, that were randomly assigned within a two tray block design to account for any disturbance by an adjacent thoroughfare. The culture trays were covered so that experimental animals were kept in continuous darkness (excluding 5 minute service periods every other day). Each channel held approximatly 1.7 L of water, and received new water at a rate of approximatly 800 mL/min. The culture water was not recycled. Incoming water temperature was controlled by a 11 kW heat/chill unit and maintained at 18.07 °C ± 0.33 over the duration of the experiment. Each tray was flushed with clean seawater (same temperature as culture water) every other day to remove uneaten food and faecal material. A commercially available formulated food (6 mm ABGRO, E.N Hutchinson Ltd.) was fed *ad libitum*.

Measurements of dissolved oxygen (DO), ammonia and alkalinity were recorded weekly. DO was measured using a WTE 340i multimeter (WTW Ltd Weiheim Germany) and ammonia and alkalinity were measured using a Palintest photometer test kit (Palintest Ltd UK). DO was not observed to fall below 90% saturation in the culture channels. Ammonia samples were taken from a common effluent water drain and did not exceed 0.04 mg TAN/L. Average total alkalinity was recorded and ranged between 95.6 and 104 mg $CaCO_3$/L across all buffered seawater treatments including the control. No mortalities were recorded throughout the duration of the trial.

Animals were measured and weighed at day 0, 30, 58, and 85 to monitor growth. On these days animals were removed from the experimental system and were weighed (wet weight) in air to the nearest 0.1 g using digital scales (Sartorius), and measured to the nearest 0.01 mm using digital calipers (Sylvac, Switzerland). The animals were out of water for no more than 10 minutes for each census.

Growth increments between measurements were calculated as micrometres per day (μm/day) and used the following equation;

(Length at time 2 – length at time 1) / (Time 2 – time1) x 1000 (3.3)

3.3.1 Experimental system.

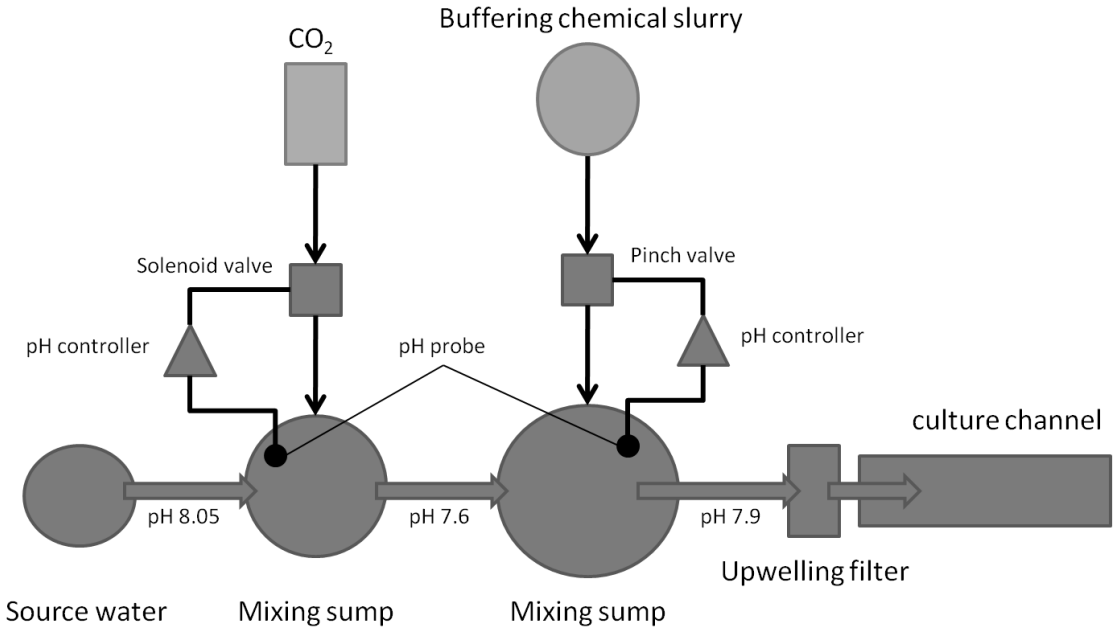


Figure 3.1 Basic flow diagram of experimental system used to test the effect of alkalinity chemicals on the growth of pāua.

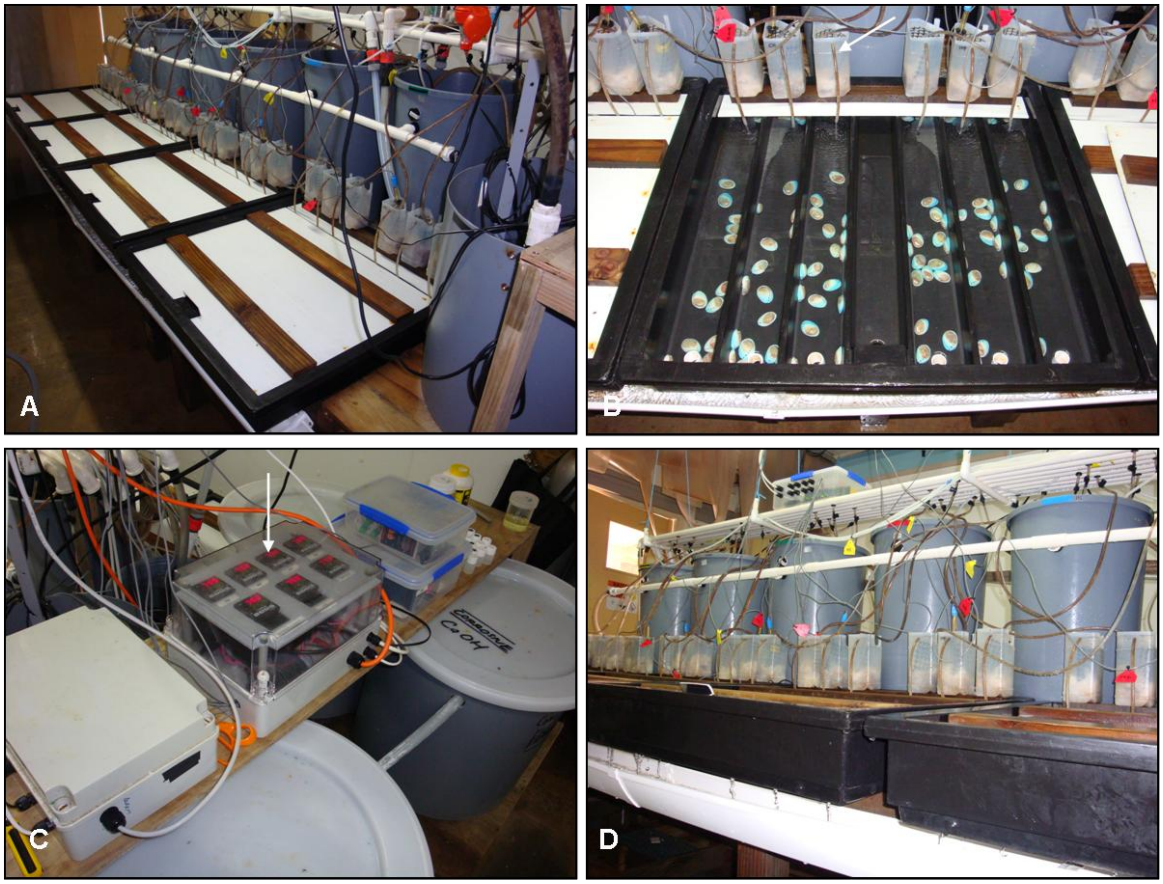


Figure 3.2 Components of the experimental system. (A) Completed system showing 4 of the 8 culture trays, covered. An uncovered tray (B) showing the 6 replicate culture channels. Channels are being fed with buffered seawater via upwelling wool filters (arrow). System electronics (C), pH controllers (arrow) that regulated the flow of buffering chemicals into the mixing sumps. Six gray mixing sumps (one obscured) (D), where buffering chemicals and low pH seawater were mixed before entering the culture channels.

The pH of the pre-buffered experimental water was initially dropped to a target pH of 7.6 by the addition of food grade CO_2 through an air diffuser and thin walled silicon tubing. CO_2 addition was regulated by a PHCN-70 pH controller (Omega, UK). The pH of this experimental water was maintained at a target pH of 7.6. The pH of the control seawater was dropped to pH 7.9 from ambient by the addition of CO_2. No chemicals were used to adjust pH in the control.

The low pH (7.6) water was pumped into 50 L mixing sumps, where buffering chemicals were mixed with the low pH water before being fed into the culture trays. The pH of the low pH water was chemically adjusted up to a target pH of 7.9 (see

Table 3.2) and fed by gravity into the pāua trays. The addition of chemicals was controlled by dosing pumps linked to PHCN-70 pH controllers (Omega, UK).

pH was monitored continuously in all treatments with an independent data logger system (Datataker Ltd). pH was measured by plastic body electrodes (Sensorex, USA). Regulating probes were calibrated weekly using pH 7 and 10 buffer solutions (Scharlau). Monitoring probes were calibrated weekly with seawater AMP and TRIS buffer solutions.

Table 3.2 Average pH of experimental seawater

Treatment	Mean pH ± standard deviation
Control	7.90 ± 0.03
NaOH 1	7.89 ± 0.03
NaOH 2	7.92 ± 0.04
$Ca(OH)_2$ fd 1	7.90 ± 0.04
$Ca(OH)_2$ fd 2	7.89 ± 0.03
$Ca(OH)_2$ ind 1	7.88 ± 0.04
$Ca(OH)_2$ ind 2	7.89 ± 0.04
Pre-buffered source	7.59 ± 0.06

pH data were collected continuously over the duration of the experiment, at a rate of one reading per minute. Daily means were summed and averaged over the experimental period to obtain overall means presented in this table. Of the 85 days the system was running, 10 days of pH data were lost due to file corruption. Systems were running as normal during these periods.

3.3.2 Treatments

Three chemicals were tested in this experiment, sodium hydroxide, and two grades (industrial and food grade) of calcium hydroxide (see appendix 1 for chemical analysis). Because of the low solubility of $Ca(OH)_2$, the two $Ca(OH)_2$ treatments were dosed into the mixing tanks as a slurry. Due to the potential for heavy mortality if for any reason buffering chemicals were over dosed into the system, each of the buffer treatments was trialed in duplicate in case of system failure. Statistical analysis

showed that the duplicate treatments were not significantly different (P>0.251) therefore we have pooled the data for analysis.

3.3.3 Analysis

Comparison of shell length and growth rates between treatments was by analysis of variance (ANOVA). A Tukey test was used for pairwise multiple comparison of values. Comparison of means between duplicate treatments was by t- test. All statistical analyses were performed using Sigma stat Version 11.0.

3.4 Results

Table 3.3 Average shell length and wet weight of experimental animals at day 0 (initial) and day 85 (final).

Treatment	*n*	Initial		Final	
		Mean shell length (mm ± se)	Mean body wet weight (g ± se)	Mean shell length (mm ± se)	Mean body wet weight (g ± se)
Control	6	31.73 ± 0.28	3.7 ± 0.1	38.99 ± 0.42	6.0 ± 0.3
NaOH	12	31.27 ± 0.24	3.7 ± 0.0	38.27 ± 0.29	5.8 ± 0.1
$Ca(OH)_2$ fd*	12	31.60 ± 0.24	3.9 ± 0.1	38.89 ± 0.32	6.2 ± 0.2
$Ca(OH)_2$ ind**	12	31.70 ± 0.26	3.8 ± 0.1	38.85 ± 0.23	6.1 ± 0.1

*,** fd and ind represent food and industrial grades, respectively.

3.4.1 Impact of buffered seawater on shell length

The average length of pāua increased in all treatments over the duration of the experiment (Fig. 3.3). ANOVA showed that there were no significant differences between treatments on any of the four sampling occasions (P always >0.338). A two way ANOVA was used to examine the effect of time and treatment on the mean size of pāua at days 30, 58 and 85. As expected there was a significant difference with time ($P < 0.001$). But there was also a statistically significant difference detected between treatments ($P = 0.039$), and there was no significant time, treatment interaction observed ($P = 1.0$). A pairwise comparison (Tukey) test on the treatments showed NaOH was significantly different ($P < 0.1$) from $Ca(OH)_2$ (fd) and $Ca(OH)_2$

(ind) (P always < 0.096), however NaOH was not significantly different from the control (P = 0.129)

3.4.2 Average growth rate

Analysis of daily incremental shell growth (Fig. 3.4) using ANOVA showed the average growth rate was not significantly different between treatments on any sampling occasion (P always > 0.702). Average growth rates were significantly different (P <0.001) between days 0 to 30, and days 31 to 58 and 59 to 85 over all treatments. In the first 30 days of the experiment, the average growth rate of 113.8 μm/day was recorded across all treatments. This growth rate dropped to 69.7 and 67.0 μm/day between days 31 to 58, and 59 to 85 respectively.

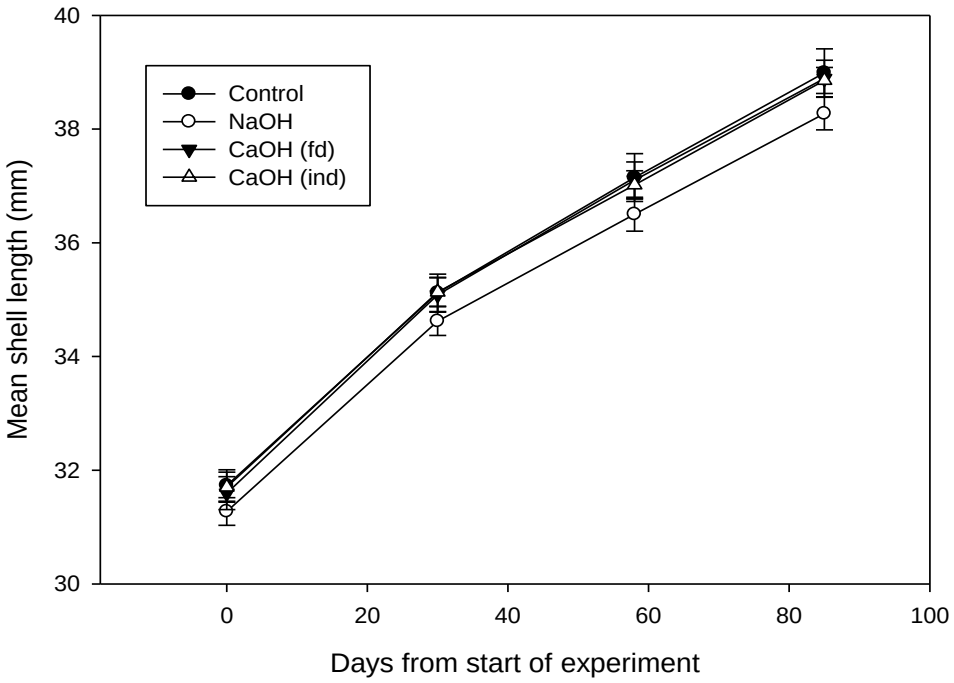


Figure 3.3 Length of pāua in each buffered seawater treatment, at each sampling interval. (average size (mm) ± standard error).

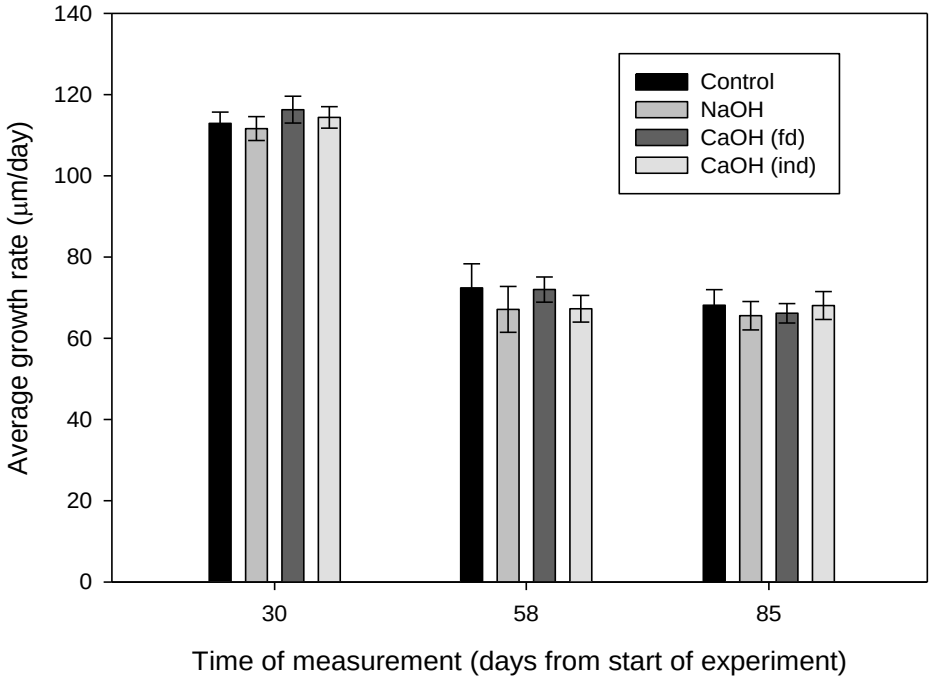


Figure 3.4 Average daily growth rates for pāua between each buffered seawater treatment and each sampling occasion. Average growth rate (µm/day) ± standard error.

3.4.3 Impact of buffered seawater on weight

The average weight of pāua increased in all treatments over the duration of the experiment (Fig. 3.5). ANOVA showed that there were no significant differences between treatments on any of the four sampling occasions (P always > 0.219). A two way ANOVA was used to examine the effect of time and treatment on the mean size of pāua at days 30, 58 and 85. There was an expected significant difference with time ($P < 0.001$), however there was no statistically significant difference ($P = 0.080$) detected between treatments. There was no significant time x treatment interaction observed ($P = 0.993$).

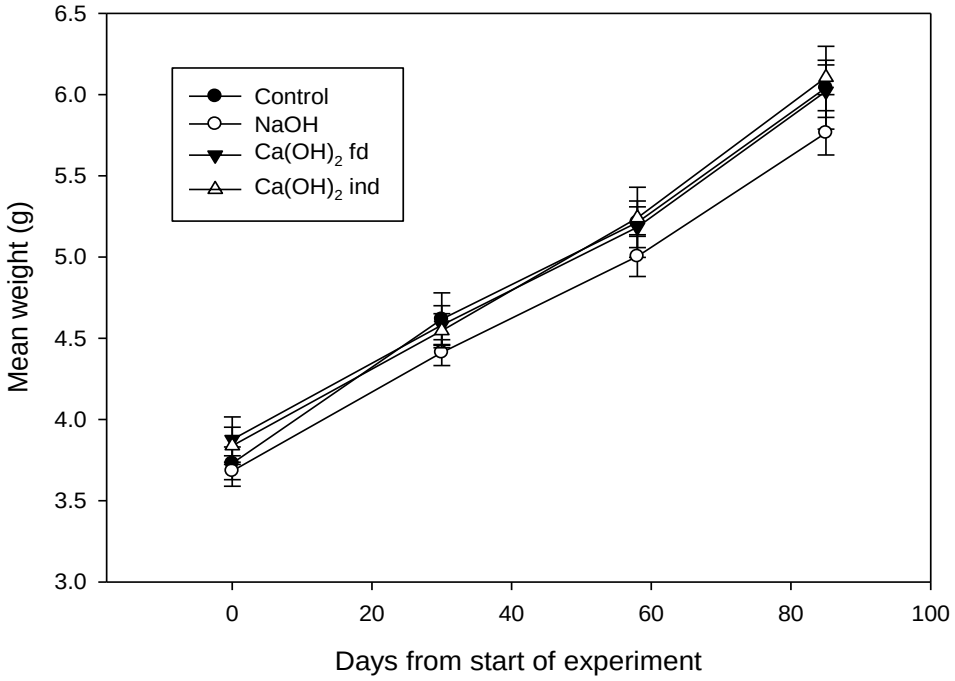


Figure 3.5 Average weight for pāua between each buffered seawater treatment and each sampling occasion. Average wet weight (g) ± standard error.

3.5 Discussion

Buffering water in abalone RAS is necessary to maintain pH above 7.7 to protect the animals from shell erosion. Shell erosion has several detrimental effects on the animal: not only does it make the animal more susceptible to damage but shell dissolution also reduces the overall weight (discussed in detail in Chapter 4). Abalone is commonly traded live in the shell, and value is determined by wet weight. Shell erosion can impact profitability by a reduction of weight, and can be a significant cost to a large abalone farm (Merino et al., 2010).

From the results in this study the use of $Ca(OH)_2$ is recommended for seawater buffering in abalone RAS. Differences in growth were not detected comparing daily incremental growth rate between treatments, however there was a significant difference ($P = 0.039$) in mean shell length between treatments. The greatest difference in average shell length occurred between the NaOH treatment, and

industrial and food grade $Ca(OH)_2$ treatments (see above section 3.4.1). This suggests that a higher concentration of Na^+ (sodium) ions in the culture water has a slightly negative influence on shell growth, and a higher concentration of Ca^{2+} (calcium) ions has a slight benefit. The addition of more Ca^{2+} into the water would appear to be favourable for abalone growth as it is a primary ion used in the production of the calcium carbonate shell. This is however speculative as it is impossible to rule out the negative (or positive) effects of impurities present in the buffering chemicals. Despite this, based on the results from this study NaOH is not recommended for use as a buffering agent for pāua RAS.

A bioenergetic assessment of pāua cultured in buffered seawater would be useful to define the affects of chemical buffers on shell growth. Slower shell growth, observed in the NaOH treatment, could be symptomatic of a reallocation of energy resources away from somatic growth to compensate for less favourable conditions. Further research on the effect of buffered seawater on abalone physiology is required to understand this interaction completely, and clarify the effects of NaOH on pāua growth.

There was an effort to promote high uniform growth rates of pāua in this experiment. Growth rates of juvenile abalone are very variable, even among siblings in the laboratory (Leighton, 1974). Access to food and variation in environmental conditions are factors likely to contribute to variation in growth (Day and Fleming, 1992a), however it is clear that some variation in growth is due to genetic influence, and a single cohort will not exhibit a uniform growth even being cultured under similar conditions (Heath and Moss, 2009). Pāua used in this experiment were selected by shell length from a single cohort of animals to minimize the effect of genetic variability. Additionally, environmental factors known to influence growth such as temperature, stocking density, food availability and photoperiod were optimized to promote fast uniform growth across the trial.

The higher growth rates (average 113.8 μm/day) observed at the start of the trial (Days 0 to 30) are likely due to a period of compensatory growth (Fig. 3.4). Compensatory growth is a phenomenon that is well documented in aquatic animals, and is defined by a temporary increase in growth rate after a period of restricted

development (usually associated with reduced feeding) (Hornick et al., 2000). The original cohort of animals was held in a flow through tipper-tray system under ambient seawater conditions. These animals were held at a high stocking density (>100% cover)[16] and as a result probably had limited opportunity to access food. Moving the experimental animals from ambient water temperature (12.2 °C) into a temperature that was optimal for growth (17.9 °C) (see section 1.4.2, temperature, Fig. 1.6), a reduction in stocking density (Clarke and Creese, 1998; Heath and Moss, 2009), and sudden access to abundant food are factors likely to have contributed to increased growth rates observed in days 0 to 30. A period of initial fast growth was not unexpected, and the trial was run for a targeted 3 months to accommodate this change.

Overall growth rates observed in this experiment are comparable to other data around juvenile pāua growth. In a survey of New Zealand pāua farms, Chen (2001) found that a growth rate of 66 to 83 μm/day was observed in summer conditions, where temperatures ranged from 17 – 19 °C. Other studies at the Mahanga Bay research institute have shown an average growth rate of pāua of this size at 18 °C to be around 80 to 100 μm/day (G Moss, pers. comm., Mar 2011). During this study, growth rates observed after day 30 (67.0 – 69.7 μm/day) were toward the lower end of expected growth at 18 °C. This is possibly due to the low water velocities (0.0055 m/sec) in the experimental channels. Although channel turnover rate was high (approximately 1 exchange every 130 seconds), pāua growth has been shown to respond positively to increased water movement (Miller et al., 2010).

3.5.1 Problems with seawater buffering

A preliminary trial was undertaken using the experimental setup described above. This trial showed that pāua exhibited reduced feeding and increased mortality in the $Ca(OH)_2$ (fd & ind) treatments. There appeared to be precipitation of the buffering agent and sedimentation in the culture channels that negatively affected the animals. The influence of these fine solids was thought to be the cause for the mortality. Fine solids are known to negatively affect gill function (see section 1.5.4, solids removal),

[16] As abalone live on the substrate, percentage cover is a unit often used in abalone culture to express stocking density. % cover can exceed 100%, as animals can live on top on one another if space is limited.

and in this instance may have contributed to the observed mortality. Additionally, the precipitate may have caused very high alkaline conditions on channel surfaces that may have negatively affected the pāua. Ingestion of the precipitate could have been a contributing factor, but as there was no observed feeding (complete uneaten pellets) and animals appeared to remain stationary (no obvious mucous trials) this is unlikely. No gill histology or chemical analysis of the precipitate was performed so the direct cause of mortality is unknown.

A number of items were adjusted to address the precipitate problem for the experiment. (i) The target pH for the preliminary trial was pH 8.0, this was dropped to 7.9 to reduce the amount of chemical added to the system. This also brought the target pH down in line with levels used by pāua farmers, who buffer to pH 7.9 using industrial grade $Ca(OH)_2$ (R Roberts & M Tait, pers. comm., Oct 2010). (ii) The $Ca(OH)_2$ slurries that were mixed with freshwater in the preliminary trial were mixed with seawater in the final trial. (iii) The capacity of fine wool upwelling filters that were installed pre-culture channels was doubled to increase the effectiveness of suspended solids capture exiting the mixing sumps. (iv) Finally, the volume of water in the culture channels was reduced (approximately 2.8 L to 1.7 L) to increase velocity (from 0.33 to 0.55 cm/sec) in the channels in an effort to minimize sedimentation. These changes contributed to the reduction in observed sedimentation.

The problems encountered with the preliminary trial highlight a potential danger when using buffering agents to control pH. It is possible that agitation of the culture water in a farm would reduce the problem of fine solids settling in the culture trays. This experiment minimised agitation of the culture water to avoid excess gas exchange (to maintain a constant pH). The addition of tippers or high water velocities that are common in commercial pāua RAS would likely minimise the problem of sedimentation.

Another potential issue could be that the impurities that are present in chemicals commonly used to raise alkalinity may accumulate in a system over time. $Ca(OH)_2$ is manufactured directly from natural limestone, so impurities present in $Ca(OH)_2$ are a function of the geology of the ore and may vary from quarry location. Variable concentrations of silicon, aluminium, iron, sulphur and heavy metals are commonly detected in limestone (Oates, 1998), and are transferred directly into an aquaculture

system with the addition of the processed $Ca(OH)_2$. Although the majority of these compounds are inert and harmless in an aquatic system, impurities known to be harmful to aquatic organisms (e.g. copper) are often present. Copper can precipitate out of solution in normal ambient seawater pH (8 to 8.2) and accumulate in a closed system over time (Holmes-Farley, 2011). Although impurities may exist in the $Ca(OH)_2$ at very low concentrations (<1 mg/kg), the large volumes of chemical needed to raise the pH in RAS could potentially introduce a risk of contamination. In a 80 T pāua RAS facility (OceanNZ Blue Ltd), 60 kg of $Ca(OH)_2$ is used every day to maintain the pH at 7.9 (R Roberts, pers. comm., Oct 2010). Care must be taken to regularly flush precipitate from the system to avoid contamination.

3.5.2 Mineralisation

There is interest in the scientific community on the impact of ocean acidification and its affect on marine calcifying organisms. Saturation levels of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) anions in the water are thought to be important in biomineralisation. A decrease in pH shifts the carbonate equilibrium system to the left, that results in an increase in dissolved carbon dioxide and carbonic acid, and a decrease in bicarbonate and carbonate anions. Marine calcifiers exhibit variable responses to low pH (Ries et al., 2009). The broad range of responses make it difficult to predict the effects of low pH water on shell mineralisation in abalone.

There is a suggestion that coral growth in natural reefs is limited by the availability of HCO_3^- and CO_3^{2-} anions, and growth can be increased by artificial manipulation of alkalinity. Anecdotal evidence (originating from reef enthusiasts who keep corals for ornamental purposes) suggest biomineralisation rates of hermatypic corals[17] can be enhanced by raising alkalinity and calcium in the culture water. There is at least one study in the literature that demonstrates enhancement of growth by supplementing alkalinity and raising pH in the laboratory (Marubini and Thake, 1999) and there are long term studies that show a linear reduction in calcification in hard corals with an associated reduction in Ca^{2+} and carbonate (CO_3^{2-}) anion concentration (Langdon et al., 2000).

[17] Reef building corals that host zooxanthellae, symbiotic algae.

To my knowledge there is no evidence in the literature that describes the effect of increased alkalinity in calcification in molluscs, however there are studies that observe a linear decline in calcification of molluscs with a reduction in pH and CO_3^{2-} anions (Gazeau et al., 2007; Ries et al., 2009). Results from this study (see section 3.4.1) suggest a slight increase in Ca^{2+} ion concentration and alkalinity through the addition of $Ca(OH)_2$ may be beneficial to pāua shell growth. Because it is necessary to buffer water to avoid pH problems, it is convenient to increase alkalinity beyond natural levels in abalone RAS. RAS are closed systems and alkalinity and calcium concentration levels can be modified relatively easily. Future work should examine calcification of abalone in high alkalinity water, to see if any parallels exist between reef building corals and abalone. The potential to enhance growth through increased mineralisation rate is important to the continued development of abalone culture, and could be another benefit to culturing abalone in RAS.

Chapter 4

The Effect of Lowered pH on Biomineralisation and Shell Dissolution of Pāua.

A preliminary investigation

In New Zealand pāua are farmed intensively using recirculation aquaculture technology (RAS). This method of culture can be susceptible to carbon dioxide (CO_2) accumulation and subsequent pH instability. In this research programme I have explored two primary methods to control pH in seawater aquaculture systems, however it is also pertinent to define the effects of pH on the target species to understand their biological limitations.

4.1 Introduction

The effects of pH on abalone shell are well recognised. The symptoms of shell erosion include fragile shells that are easily damaged, respiratory pores that are not well defined, and 'shiny' areas that appear where the prismatic layer has dissolved away and the iridescent nacre can be seen (see below Fig. 4.1). Compared to wild pāua that are exposed to predators and vigorous wave action, protection from a large shell is perhaps not as important in an aquaculture situation. However, the appearance of the aquaculture abalone is very important for sales and marketing, and a healthy looking shell is generally indicative of a well managed abalone farm. Abalone that are traded live are sold by the kilogram with the shell on. Poor pH management may influence return on sales, as shell erosion caused by exposure to low pH water, effectively reduces the weight of animals destined for market. Live abalone generally attract the best price (compared to frozen or canned products); it is therefore important to promote healthy shell growth and minimise shell erosion particularly when trading live abalone.

Fig 4.1 Effects of low pH water on pāua shell. Chronic exposure to low pH conditions can cause fragile shells, that may lead to breaks along the margin of respiratory pores (A, left). The shiny appearance of pāua is a symptom of shell dissolution (B, left), where the prismatic layer has completely dissolved away revealing the nacre. Photos: G. Moss (NIWA).

The mineralogy and microstructure of the molluscan shell have been widely examined. The interest in mollusc shells is due primarily to the great mechanical strength of the nacreous layer, created by the unique structural arrangement of aragonite crystals in association with an organic laminate. The molluscan shell is composed primarily of two crystal polymorphs of calcium carbonate ($CaCO_3$); aragonite and calcite. The majority of gastropod shells consist entirely of aragonite, only a few taxa incorporate calcite into their shell mineralogy (Boggild, 1930), and Haliotidae is one.

The mineralogy of abalone shell is of particular interest as the relative proportions of calcite versus aragonite present in the upper prismatic layer may influence rates of shell dissolution in low pH water. Aragonite is more soluble than calcite (Morse et al., 2007) and therefore abalone with a higher proportion of aragonite in their prismatic layer may be more likely to suffer from shell dissolution. Variation of shell mineralogy between species is well known within the Haliotids (Dauphin et al., 1989), and there has been wide variation detected within mature pāua shells collected from wild populations (Gray and Smith, 2004). Additionally, different species of abalone at different stages of ontogeny have different proportions of aragonite and calcite in their shells. He and Mai (2001) observed a steady increase in the proportion of calcite relative to shell length in the shells of *Haliotis discus hannai*. Given the difference in solubility of $CaCO_3$ polymorphs in abalone shell, and the variable

proportion of these present in the prismatic layers of the shells, some species and life stages will be more vulnerable to shell dissolution than others.

4.2 Background

4.2.1 The shell

The abalone shell is a complex structure. The shell is predominantly constructed of $CaCO_3$ that accounts for 95 to 99% by weight: the remaining fraction is composed of an organic matrix component (Marin and Luquet, 2004). During biomineralisation, calcium and carbonate ions are pumped through the epithelial cells of the mantle into a secreted extracellular protein matrix called the periostracum (Marin and Luquet, 2004). The periostracum matrix is a necessary precursor to biomineralisation, as it provides a surface for $CaCO_3$ crystal development, and a controlled environment for mineralisation to occur (Harper, 1997). The periostracum is retained in the developing shell, and covers the deposited $CaCO_3$ shell as a laminate (see Fig. 4.2). This provides some protection from erosion by fouling organisms and corrosion by seawater, however the effectiveness of this primary barrier appears to vary widely among the molluscs. In abalone, the periostracum is very thin (100 – 200 nm), and offers very little in the way of physical protection (Shepard et al., 1995).

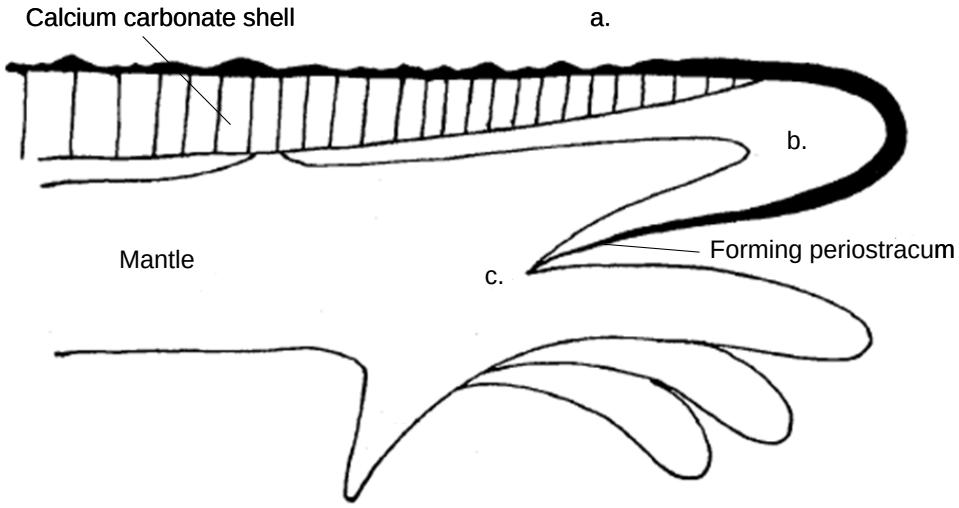


Figure 4.2 Simplified structure of molluscan shell illustrating shell formation. (a.) Outer periostracum (b.) extrapallial space (c.) periostracal groove. Source: Adapted from Harper (1997)

Excluding the periostracum, the $CaCO_3$ shell of a mature animal is divided into two primary layers. The outer prismatic layer may contain aragonite and calcite crystals, the relative proportions of which are dependent on species and developmental stage (Dauphin et al., 1989; He and Mai, 2001). The innermost layer is the nacreous pearl layer, composed exclusively of aragonite and differs from the upper prismatic layer in the arrangement of the crystals. Nacre is relatively strong, and is made from disc shape tablets that form columns perpendicular to the shell surface (Ruppert et al., 2004). The physical arrangement of the crystals, the protein matrix and the chitin that binds the columns together are credited for its strength. The lustrous nacre is smooth and deposited continuously by the mantle. This adaptation protects the soft tissue from irritating debris by entombing them in nacre, often producing 'pearls' that can be cut from the shell and manufactured into products for the jewellery trade.

4.2.2 The energetic cost of biomineralisation

The basic mechanisms of biomineralisation are described above. However, given that this research is focused on the culture of abalone for commercial purposes, outlining the energetic cost of shell formation is useful.

Calcification itself takes place within the secreted periostracum, in a small compartment termed the extrapallial space (EPS). The EPS creates a suitable environment in which calcification can occur, and is a necessary precursor to mineralisation. The calcium and carbonate ions, or the building blocks of the calcium carbonate shell, are acquired from the surrounding seawater or from food (Marin et al., 2007). However, given the relative abundance of these ions in seawater (at low and middle latitudes), it seems likely that a large proportion of these ions are sourced from the seawater. In molluscs, the calcifying ions enter the animal over the gills, gut or over the body surface, and are transported to the mantle epithelium via the hemolymph. They are then actively pumped into the EPS by calcium and bicarbonate channels (Marin and Luquet, 2004).

There is an obvious metabolic cost associated with forming a large protective shell, however little is known about the exact energetic requirement of shell production. It appears that the greatest proportion of the energy required for mineralisation is synthesis of the organic component of the shell. Palmer (1992) estimated the energetic cost of calcification in two rocky shore whelks, and showed the total cost of the organic components of the periostracum was considerably higher than the production of $CaCO_3$. He also noted that groups of snails that produced extra shell material consumed relatively more food (Palmer, 1992), indicating that the energetic requirement for animals that secrete a relatively large thick shell may be higher.

Aquaculture pāua are vulnerable to shell damage, as shells in cultured pāua are typically much thinner due to relatively fast growth. As such, shells can easily crack during handling and transfer, particularly in the margin of new growth that is relatively thin compared to older shell. The shell repair process in molluscs has been shown to be energetically costly (Palmer, 1983). A damage event has been shown to trigger behavioural and physiological responses in the animal that incur an energetic cost (Fleury et al., 2008). Although there is limited information on the effect of shell damage on growth rates in abalone, anecdotal evidence suggests that when a pāua shell is cracked or damaged, growth rates post damage are compromised. This would indicate that there is a significant portion of energy being channelled into shell repair and recovery and away from somatic growth, and highlights the importance of protecting shells from damage in an aquaculture situation.

The direct energetic cost of shell dissolution is an area that is largely unexplored. However one study has shown that shell erosion does not incur a short term energetic cost as with an acute shell damage event (Day et al., 2000). Shell erosion may not directly influence the energetic cost of mineralisation, however it may have an indirect cost, as thin shells are more fragile and prone to handling damage.

The objectives of this chapter are to; (i) define the $CaCO_3$ composition of pāua shell by assessing calcium carbonate proportions of aragonite and calcite to determine sensitivity to pH, and (ii) to assess shell deposition and dissolution dynamics in 30 – 40 mm juvenile pāua cultured in water of different pH (pH 7.6 and 7.9) by monitoring shell growth and determining shell thickness.

4.3 Materials and Methods

Animals used in this experiment were part of the buffered seawater trial presented in Chapter 3. Please refer to *3.3 Materials and Methods* and *3.3.1 Experimental System*, for a description of experimental design, culture conditions and experimental system function. pH of the seawater used in this experiment was manipulated solely by the addition of food grade CO_2, no chemical buffers were used to alter pH. In the two treatments, CO_2 was used to lower the pH of the water down to pH 7.90 (± 0.03) and 7.59 (± 0.06) (daily average ± standard deviation).

4.3.1 Shell dissolution

Shell thickness was assessed to examine the effect of shell dissolution. Three to five shells were randomly selected from each replicate channel, and used in the final assessment. At the time of harvest, animals were shucked from the shells and the shells were cleaned to remove any excess organic material. An initial wet weight was recorded, and then shells were dried in a oven at 50 °C for 24 hours to obtain a dried weight. The shells were then weighed (to the nearest 0.01 g) using digital scales (Denver Instruments, USA), and the shell area was calculated using image processing software (ImageJ, version 1.45b). Shell thickness was estimated using the weight per unit area and calculated with the following equation;

Shell thickness = dry weight (mg) / shell area (mm^2) (4.1)

4.3.2 Calcification rate and growth

Animals were measured and weighed at day 0, 30, 58, and 85 to monitor growth. On these days animals were removed from the experimental system and were weighed (wet weight) in air to the nearest 0.1 g using digital scales (Sartorius), and measured to the nearest 0.01 mm using digital calipers (Sylvac, Switzerland). The animals were out of water for no more than 10 minutes for each census.

Growth increment between measurements in micrometers per day (μm/day) was calculated as follows (eqn 4.2);

(Increase in shell length between sampling periods (mm) / number of days) x 1000

4.3.3 Shell composition

4.3.3.1 Raman spectroscopy

Two methods were used to determine the mineral composition of the pāua shells used in this trial. Raman spectroscopy (Horiba Jobin Yvon, LabRam HR) allowed determination of the transition in crystal polymorphs through the depth of the shell. Shells were prepared for analysis by cutting a cross section across the margin of new growth (see Fig. 4.2, A) using a circular diamond saw (South Bay Technology, USA). The sectioned fragments were set in resin (two part araldite glue) and flattened using sandpaper (wet and dry, P1200) before sampling. Using a green laser, with a wavelength of 532 nm and resolution of approximately 2 μm, samples were taken at approximately 100 μm increments from the dorsal surface down through the shell to the inside margin. This was repeated three times at regular intervals across the shell edge for accuracy (see Fig. 4.2, C.).

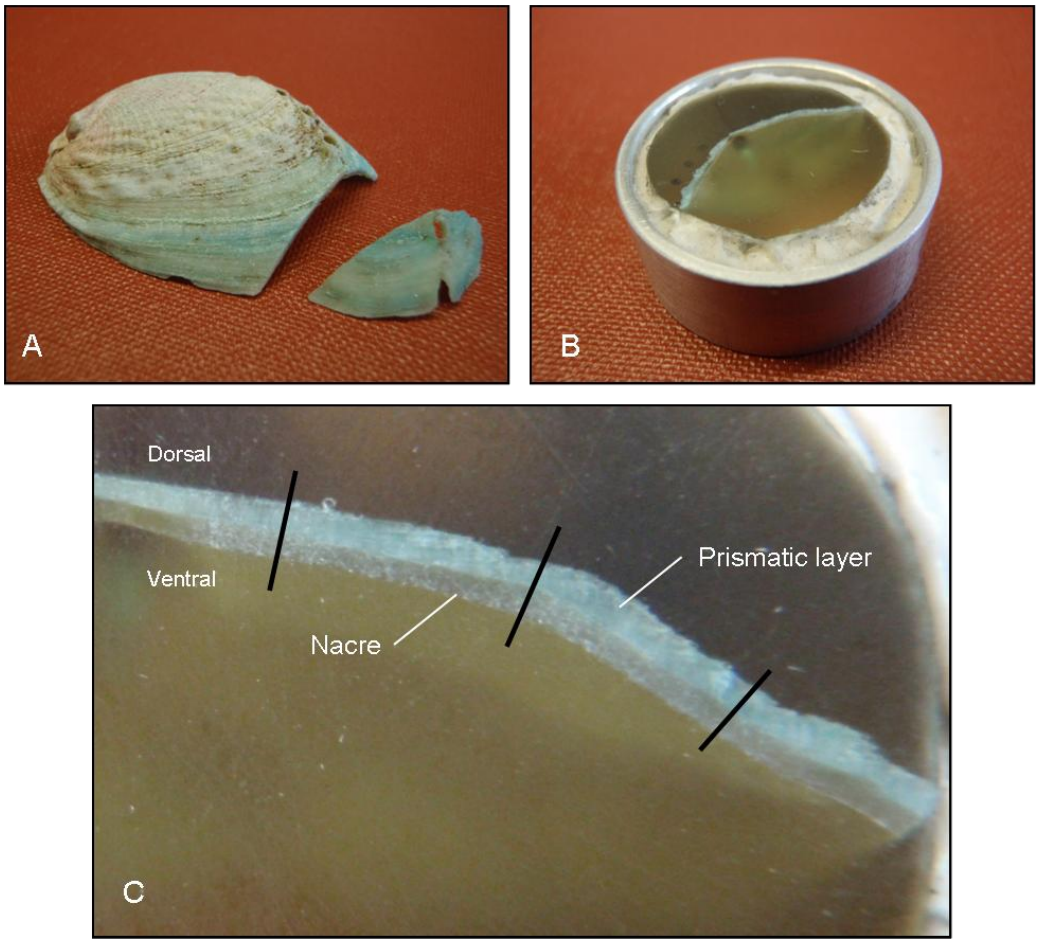


Figure 4.3 A section of shell cut from the margin of new growth that was used for Raman analysis (A). A potting mould showing the prepared section (B). A cross section view of shell used for analysis (C). The black lines represent the three sampling profiles, where Raman samples were taken at approximately 100 μm increments from the dorsal to the ventral surface.

4.3.3.2 X-ray diffraction

To verify the results from the Raman investigation, X-ray diffraction (XRD) was used to assess and confirm proportions of aragonite and calcite in the total shell. For each replicate (n = 6) three shells were selected at random and ground into a fine powder using a geological grinding mill (Tema). Diffraction data were obtained from the ground samples, and were assessed using the X'pert PRO MPD X (software by PANalytical). The relative proportion of calcite to aragonite in the samples was estimated by using formulae and calibration data presented in Dickinson & McGrath (2001). Analyses were performed using the reflection peaks at 29.5° 2θ for calcite and 45.9° 2θ for aragonite.

Proportions of calcite to aragonite were calculated using the following equation;

$$X_c = X_a \frac{A_c}{A_a \times 3.5} \tag{4.3}$$

Where A_i is the peak height, and X_i is the fraction of calcite or aragonite in the total sample (subscripts a and c correspond to aragonite and calcite, respectively). We assume that $X_a + X_c = 1$, since there was no evidence of any diffraction peaks that could not be attributed to either calcite or aragonite[18].

4.3.4 Statistical analysis

Comparison of shell length and growth rates between treatments was by analysis of variance (ANOVA). A Tukey test was used for pairwise multiple comparison of values. All statistical analyses were performed using Sigma stat Version 11.0.

4.4 Results

4.4.1 Pāua growth at pH 7.6 and 7.9

Absolute calcification rate was measured as a daily incremental growth rate and is summarised in Table 4.1. Although pāua weight and total shell length were not directly used in shell calcification calculations, they are still useful measurements in aquaculture and provide additional information on the effects of low pH conditions in pāua culture. For these reasons, and for completeness, average shell length and average wet weight are included in the table. Over the 85 day culture period there was a statistically significant difference between mean daily growth rates of pāua cultured at pH 7.6 and 7.9 ($P = 0.049$).

[18] The $CaCO_3$ polymorphs vaterite and dolomite have also been detected in molluscan shells, at relatively low concentrations.

Table 4.1 Summary of initial and final lengths and wet weights, and daily incremental growth rate of pāua cultured at pH 7.6 and 7.9.

Treatment	n	Initial (day 0)		Final (day 85)		
		Average shell length (mm ± se)	Average wet weight (g ± se)	Average shell length (mm ± se)	Average wet weight (g ± se)	Mean daily growth rate (μm/day ± se)
pH 7.9	**6**	**31.73 ± 0.28**	**3.73 ± 0.1**	**38.99 ± 0.42**	**6.04 ± 0.3**	**85.35 ± 2.13**
pH 7.6	**6**	**31.37 ± 0.65**	**3.61 ± 0.2**	**37.78 ± 0.77**	**5.62 ± 0.3**	**75.45 ± 3.87**

4.4.1.1 Impact of low pH on shell length

The average length of pāua increased in both treatments over the duration of the experiment (Fig. 4.4). A two way ANOVA was used to examine the effect of time and treatment on the mean size of pāua at days 30, 58 and 85. ANOVA detected a significant difference between treatments after allowing for the effects of time, and a pairwise comparison (Tukey) of the treatments showed a significant difference between the shell length of pāua reared in pH 7.9 and 7.6 ($P = 0.016$). There was no significant difference between treatments at day 0 ($P = 0.617$).

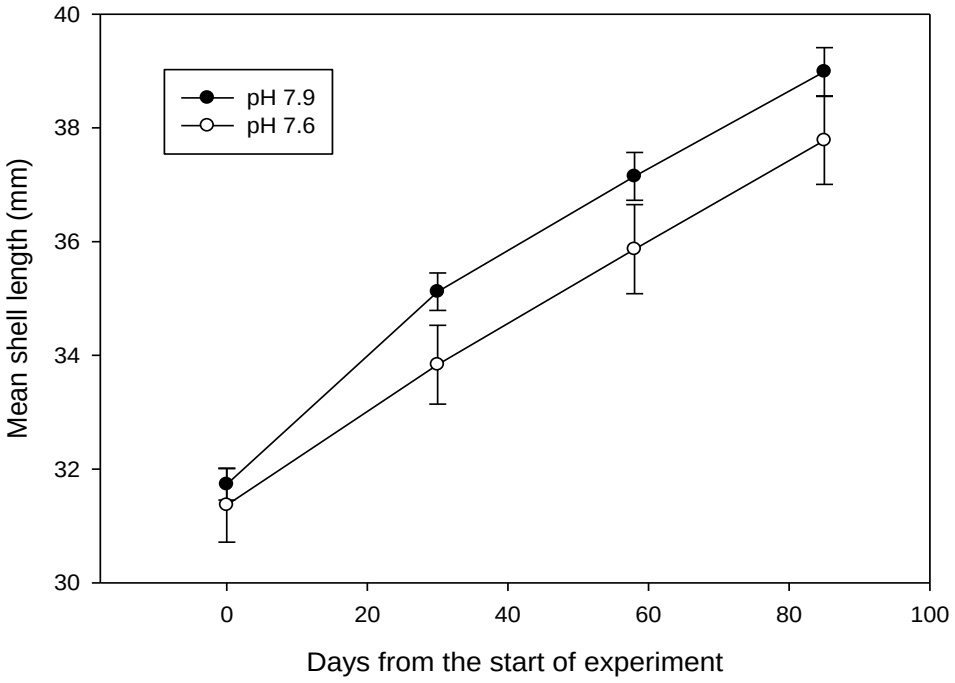


Figure 4.4 Average shell length of pāua cultured at pH 7.6 and 7.9 at each sampling occasion. Average size (mm) ± standard error.

4.4.1.2 Average incremental growth rate

Analysis of daily incremental shell growth (Fig. 4.5) using a t-test showed the average growth rate was significantly different between treatments between days 1 to 30 ($P < 0.001$) but not between days 31 to 58 and 59 to 85 (P always > 0.598). A two way ANOVA and Tukey pairwise comparison was used to examine the effect of time on the average growth rate of pāua at days 30, 58 and 85. Average growth rates were significantly different ($P < 0.001$) between days 0 to 30, and days 31 to 58 and 59 to 85 in pH 7.9, but no significant differences are detected in growth rates of pāua cultured in pH 7.6 at any of the sampling occasions (P always > 0.181).

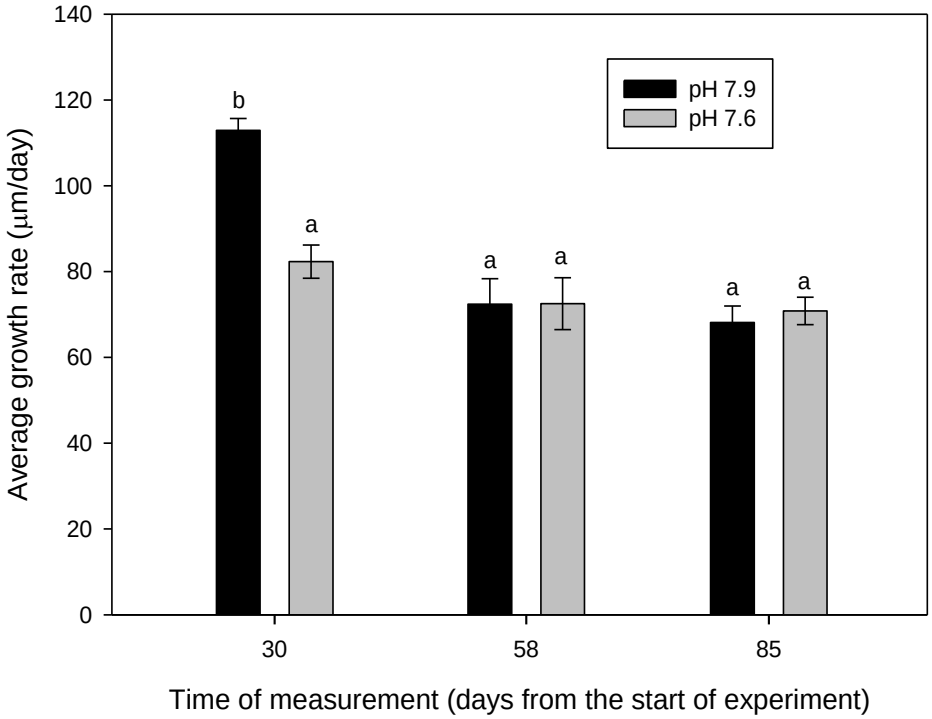


Figure 4.5 Average daily incremental growth rate for pāua cultured at pH 7.6 and 7.9 between each sampling occasion. Average growth rate (µm/day) ± standard error. A & B show differences at the 99% significance level.

4.4.1.3 Impact of pH on weight

The average weight of pāua increased in all treatments over the duration of the experiment (Fig. 4.6). A two way ANOVA was used to examine the effect of time and treatment on the mean size of pāua at days 30, 58 and 85. There was no statistically significant difference (P = 0.052) detected between treatments after allowing for the effects of time.

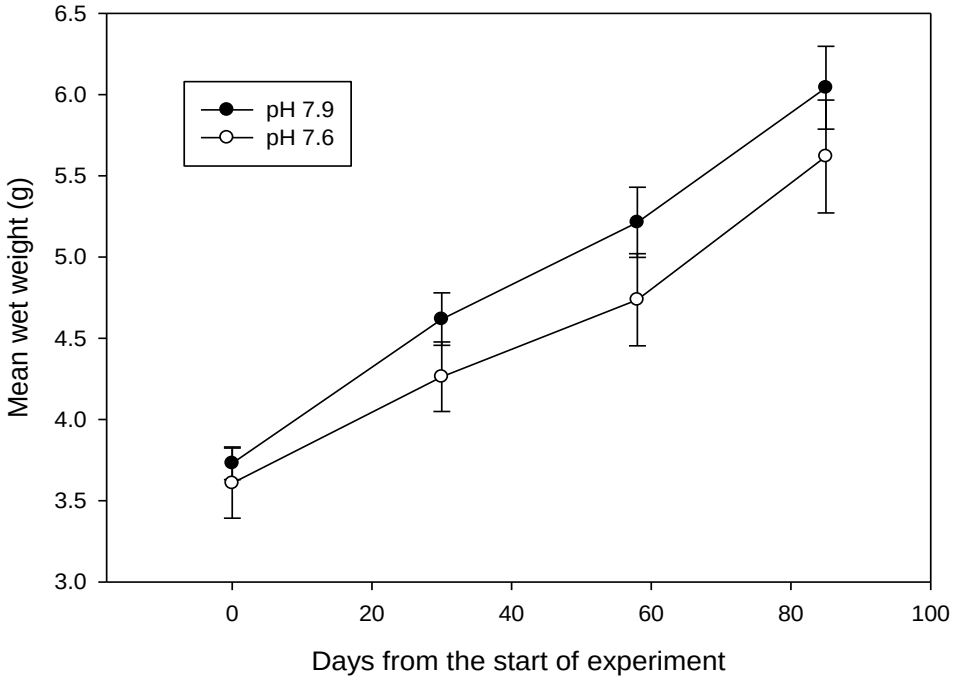


Figure 4.6 Average wet weight of pāua cultured at pH 7.6 and 7.9 between each sampling occasion. Average weight (g) ± standard error.

4.4.2 Shell thickness

Shell thickness, calculated from the dry weight of the shell and shell area, was used to assess the relative effect of shell dissolution in pāua cultured at pH 7.6 and 7.9. Analysis of the data summarised in Table 4.2 suggest dissolution does occur in pāua cultured in low pH conditions. Unfortunately, this analysis lacks a true control, as there was no scope to include an additional ambient pH treatment, to evaluate dissolution at pH 7.9. However, even though source water was lowered from ambient pH (approximately 8.05) to 7.9, no physical signs of dissolution were observed in pāua cultured at pH 7.9.

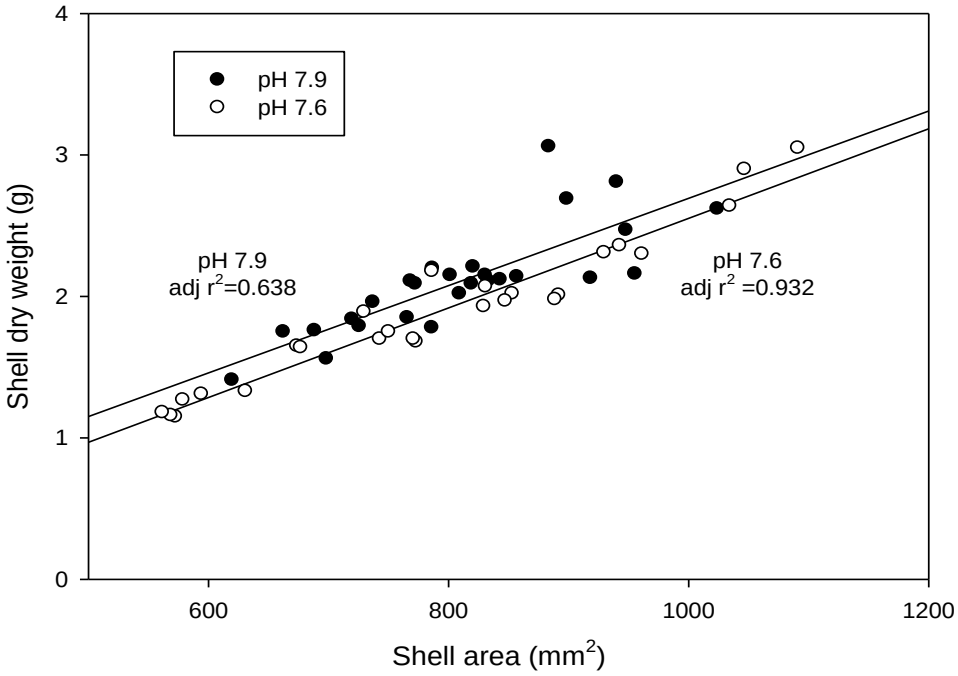


Figure 4.7 Shell area versus shell weight of individual pāua shells cultured at pH 7.6 and 7.9.

Table 4.2 Average shell thickness (per unit area) between the shells of pāua cultured at pH 7.9 to 7.6.

Treatment	Individual shells assessed	Weight (mg) per unit area (mm^2) ± se
7.6	**26**	**2.38 ± 0.06**
7.9	**27**	**2.60 ± 0.06**

Figure 4.6 plots the relationship between shell area versus shell dry weight of the shells of pāua cultured at pH 7.6 and 7.9. Each data point represents a single shell. A t-test comparison of average weight per unit area (Table 4.2) showed there was a significant difference in shell thickness between the shells of pāua cultured at pH 7.9 to 7.6 (P = 0.020).

4.4.3 Shell composition

Raman spectroscopy was used to determine what polymorphs of $CaCO_3$ were present in the upper prismatic layer and inner nacreous layer by sampling at multiple points through a cross section of the shell, from dorsal to the ventral side. From these data the relative proportions of the two shell layers could be determined and estimates of

the likely total proportion of aragonite and calcite in the entire shell made. X-ray diffraction was used to support the observations from the Raman analysis and confirm the absolute proportion of aragonite and calcite in the total shell.

4.4.3.1 Raman spectroscopy

A single shell from each treatment was analysed using Raman spectroscopy to assess the ratio of calcite to aragonite in the new margin of growth. Lattice mode peaks (i.e. peaks at low wavenumbers <300 cm^{-1}) were used to differentiate between calcite and aragonite (see below Fig. 4.7). Each polymorph shares a peak at 155 cm^{-1}, however pure aragonite has a peak at 206 cm^{-1}, and calcite a peak at 281 cm^{-1} (Wehrmeister et al., 2011). In this study these two peaks were used for diagnostics, where the presence or absence of peaks at 206 and 281 cm^{-1} determined the mineralogy at the sampling site.

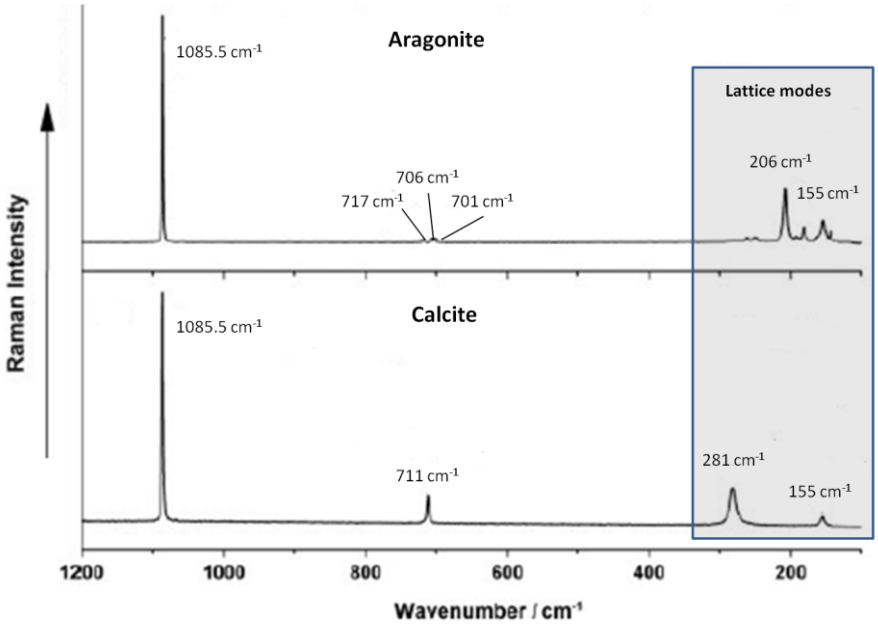


Figure 4.8 Raman spectra of aragonite and calcite. Note; the lattice mode peaks are easily differentiated between the two polymorphs. Source: Obtained from the Raman database of the Department of Geosciences, Johannes Gutenberg-University, Mainz *in Wehrmeister et al.* (2011)

In Figs. 4.9 and 4.10 are shown representative Raman spectra as a function of distance from the dorsal surface and position on the shell, for pāua grown at pH 7.6 and 7.9 respectively.

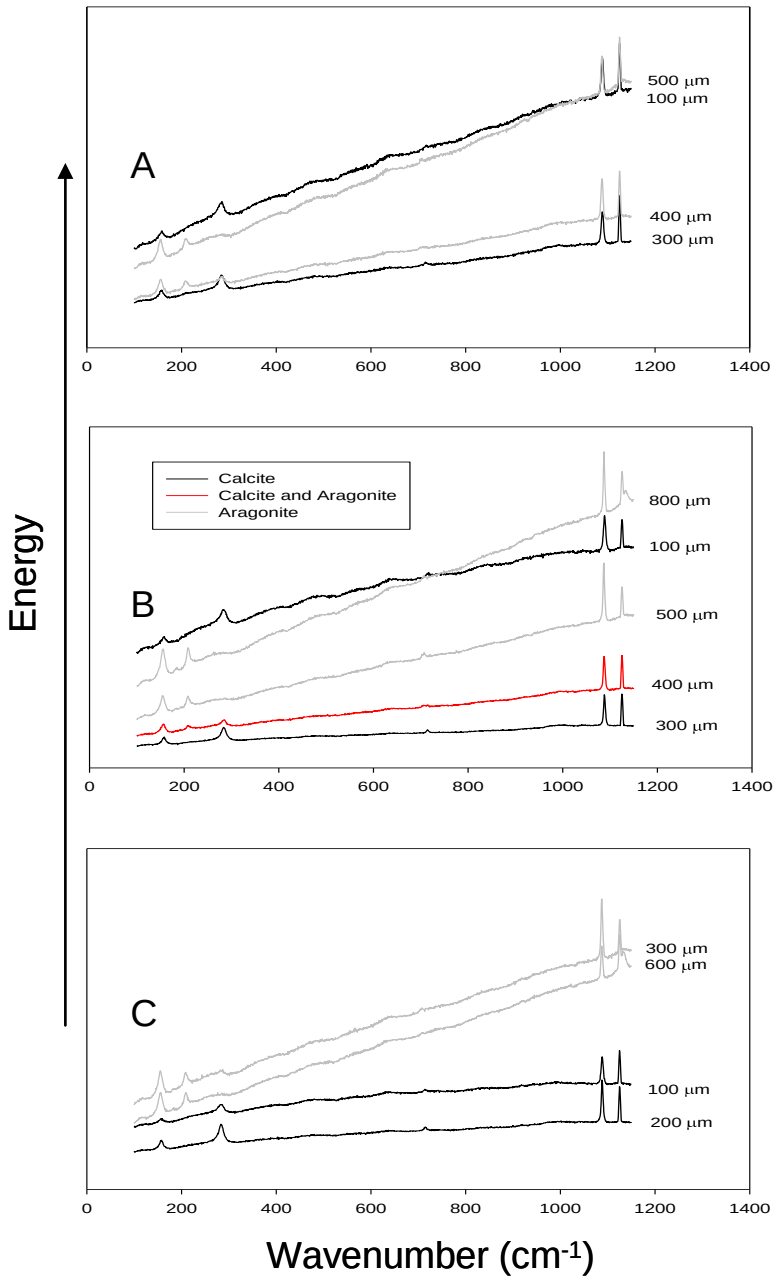


Figure 4.9 Representative Raman spectra taken at 100 µm increments through a shell deposited at pH 7.6. A, B and C correspond to the three individual sampling profiles (see Fig 4.3, C), with A being the closest to the distal margin of the shell. The spectra are labelled in 100 µm increments, which correspond to the depth into the shell. Not all spectra are shown for clarity.

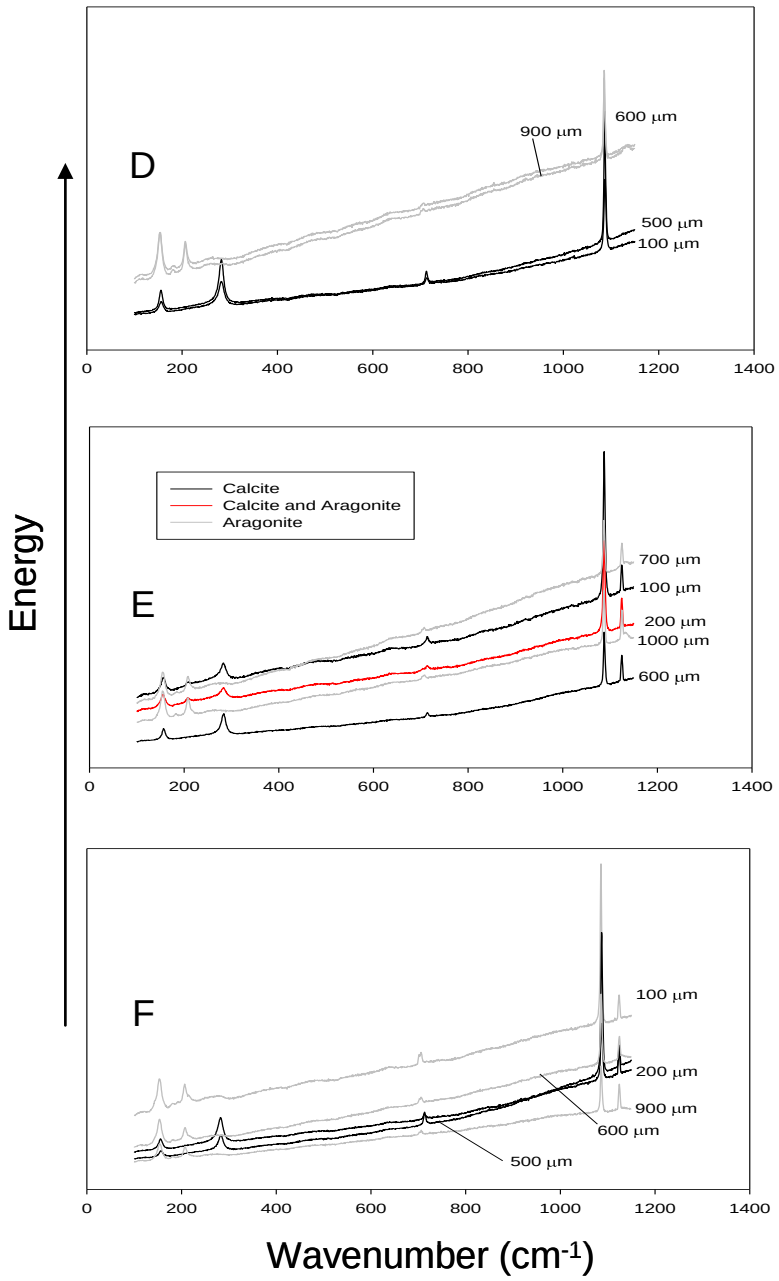


Figure 4.10 Representative Raman spectra taken at 100 µm increments through the shell deposited at pH 7.9. D, E and F, correspond to the three individual sampling profiles (see Fig. 4.3, C). Not all spectra are shown for clarity.

All Raman spectra generated (Figs. 4.9 & 4.10) showed a strong peak at approximately 1087 cm^{-1}, indicative of a calcium carbonate sample. All spectra could be identified as either aragonite or calcite (or containing both) by clear peaks in the lattice modes region of the spectra.

Table 4.3 displays the polymorphs detected at each sampling site, and their corresponding depth into the shell. This table also provides approximate thickness of shell at the new margin of growth. Table 4.4 is a summary of the lattice mode peaks for each of the spectra shown in Figs. 4.9 and 4.10 corresponding to the transition from calcite to aragonite. Table 4.5 summarises the proportions of the upper prismatic layer to the lower nacreous layer compared to total shell thickness.

Table 4.3 Polymorphs of calcium carbonate present in the margin of new growth of pāua cultured at pH 7.6 and 7.9.

	pH 7.6 (Fig. 4.8)			pH 7.9 (Fig. 4.9)		
(μm)	(A)	(B)	(C)	(A)	(B)	(C)
100	Calcite	Calcite	Calcite	Calcite	Calcite	Aragonite
200	Calcite	Calcite	Calcite	Calcite	Cal/Arag	Calcite
300	Calcite	Calcite	Aragonite	Calcite	Calcite	Calcite
400	Aragonite	Cal/Arag	Aragonite	Calcite	Calcite	Calcite
500	Aragonite	Aragonite	Aragonite	Calcite	Calcite	Calcite
600		Aragonite	Aragonite	Aragonite	Calcite	Aragonite
700		Aragonite		Aragonite	Aragonite	Aragonite
800				Aragonite	Aragonite	Aragonite
900				Aragonite	Aragonite	Aragonite
1000					Aragonite	

Table 4.4 Wavenumbers of lattice mode peaks of spectra at the transition from calcite to aragonite (upper prismatic to inner nacreous layer).

Figure	Shell depth	Wavenumber (cm^{-1})		Polymorph
4.5 A	**300**	**157.6**	**283.9**	**C**
	400	**155.5**	**208.3**	**A**
4.5 B	**400**	**156.5**	**284.4**	**C**
	500	**154.4**	**208.3**	**A**
4.5 C	**200**	**158.1**	**283.4**	**C**
	300	**155.5**	**208.9**	**A**
4.6 D	**500**	**155.5**	**282.3**	**C**
	600	**154.4**	**206.7**	**A**
4.6 E	**600**	**156.5**	**283.9**	**C**
	700	**155.0**	**208.3**	**A**
4.6 F	**400**	**156.0**	**283.9**	**C**
	500	**153.9**	**207.3**	**A**

Note: Letters A and C in the polymorph column correspond to aragonite and calcite respectively.

In most of the Raman spectra presented above there was significant florescence observed (i.e. energy gradually increasing with wavenumber). This phenomenon has been observed in Raman analysis of other molluscan shells (Wehrmeister et al., 2011), and is due to the interference of organic molecules in the associated mineralogy influencing energy output. In Raman spectroscopy the visible light of the laser interacts with the molecules in the sample causing electronic excitation as well as vibrational excitation. Electronic excitation is significantly more dominant, and as a result, emission as florescence is seen in the background. The amount of background excitation can also be influenced by the wavelength of the laser. Soldati et al.(2008) used Raman spectroscopy to examine the $CaCO_3$ nacre from freshwater cultured pearls and observed an increase in florescence as a function of wavelength. They found excitation with shorter wavelengths produced higher peak intensities and relatively higher florescence. Although there was significant background florescence observed in many of the spectra in this study, the diagnostic peaks in the lattice mode

were clearly distinguishable, and therefore did not interfere with identification of the crystal polymorphs.

Table 4.5 Percentage of prismatic and nacreous layers of a pāua shell cultured at pH 7.6 and pH 7.9 in the margin of new growth that occurred over the duration of the trial.

	7.6			7.9		
	A	**B**	**C**	**D**	**E**	**F**
Prismatic layer (%)	**60**	**56**	**33**	**56**	**60**	**56**
Nacreous layer (%)	**40**	**44**	**67**	**44**	**40**	**44**
Profile depth (μm)	**500**	**700**	**600**	**900**	**1000**	**900**

In both shells there was a clear transition from calcite to aragonite approximately half way through the shell. This is the transition from the upper prismatic layer (primarily calcite) into the lower nacreous layer (exclusively aragonite). In three of the Raman spectra presented above (Fig. 4.9 B, & Fig. 4.10 E, F) there was also aragonite detected in the upper prismatic layer. Additional sampling around the aragonitic sites confirmed that the pockets of aragonite were small and surrounded by calcite. By averaging the percentages of each profile I can calculate a crude estimate of the ratio of the two primary shell layers. In the shell of pāua cultured at 7.6, 49.7% of the sampling sites were identified as prismatic, and 50.3 % nacreous. At pH 7.9, 57.3% were prismatic calcite, and 42.7% aragonitic nacre. The prismatic layer is generally several hundred micrometres thick in the margin of new growth in pāua shells 35 to 40 mm SL.

It is also noted that the shell cultured at pH 7.9 was considerably thicker than the shell cultured at pH 7.6. However, given that only one individual shell from each pH treatment was assessed, I can only speculate at this point as to the influence of low pH in the new margin of growth.

4.4.3.2 X-ray diffraction

X-ray diffraction was used to assess the absolute proportions of calcite versus aragonite in the entire shell of pāua used in this experiment. Fig. 4.11 shows a representative diffractogram indicating the presence of both aragonite and calcite. The XRD data obtained are consistent with the observations made in section 4.3.1 (Raman spectroscopy), and indicate that the proportion of calcite and aragonite in the shell is approximately 50% (see below Fig. 4.12). Results from the XRD analysis suggests that the ratio of aragonite to calcite observed in the new margin of growth during Raman sampling is common throughout the rest of the shell.

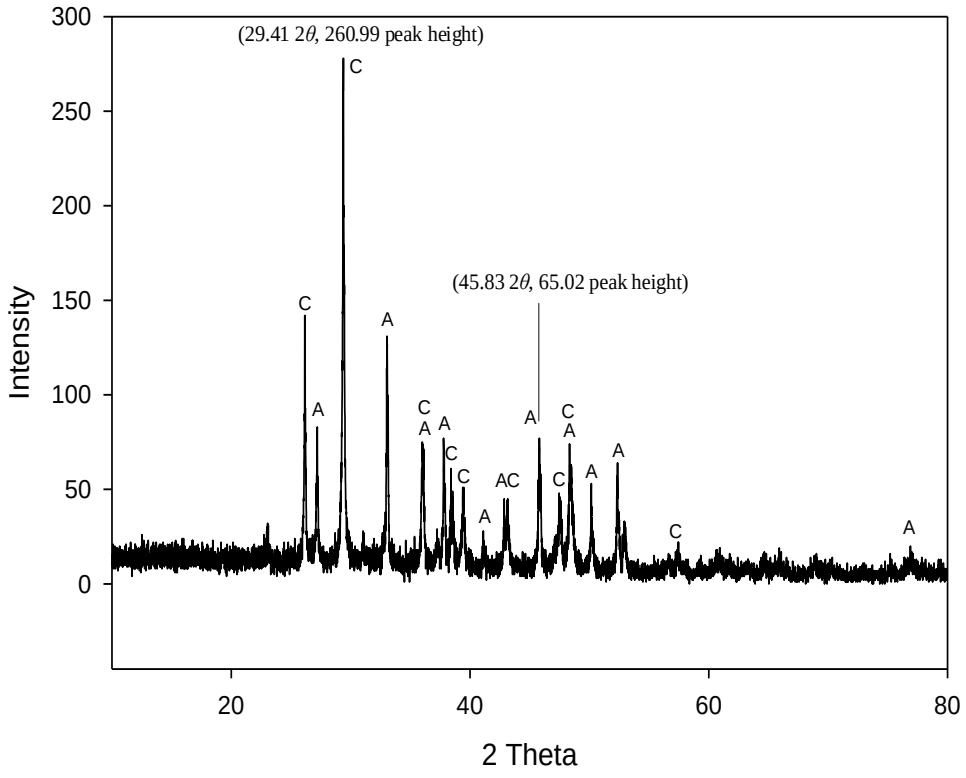


Figure 4.11 X-ray diffractogram of juvenile pāua shells cultured at pH 7.9 (sample 7.9 f, see appendix 1).

Note: In Fig. 4.11, A & C correspond to aragonite and calcite respectively, and indicate which peaks are present in a pure sample. For complete description of peaks and positions for each polymorph refer to appendix 1.

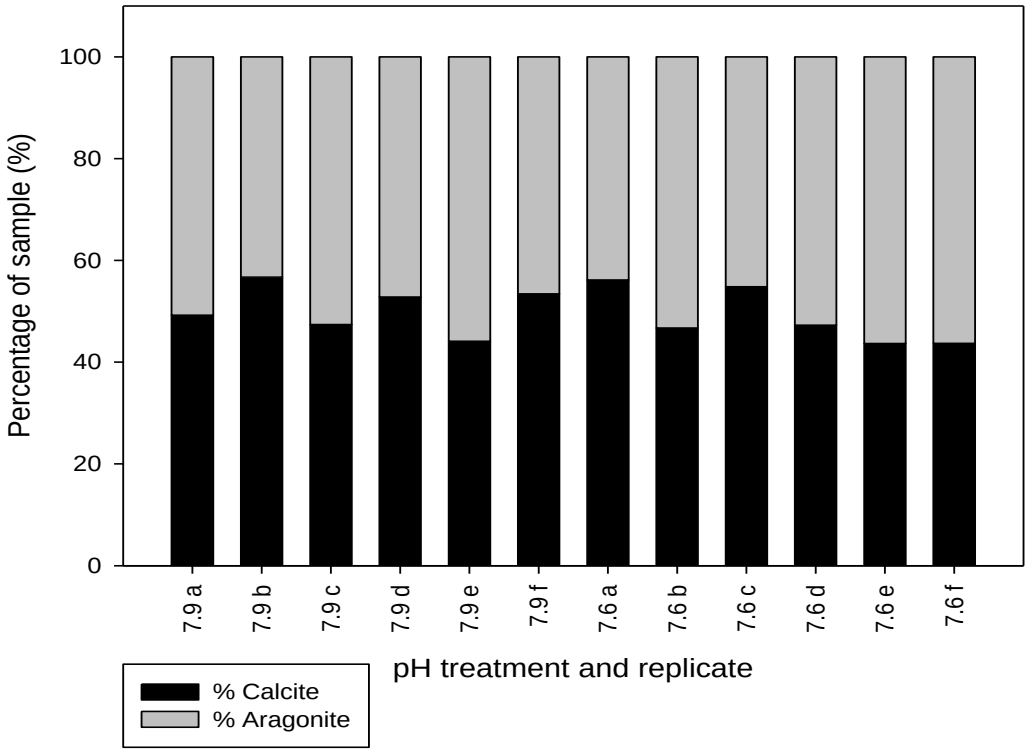


Figure 4.12 The relative proportions of calcite and aragonite in juvenile pāua shell.

A t-test showed that there was no significant difference in the percentage of calcite and aragonite in pāua shells cultured at pH 7.6 and 7.9 (P = 0.532). Pāua shell cultured at pH 7.6 had 49.4% ± 4.6 (± std dev) aragonite, and shells at 7.9 had 51.3% ± 5.5 aragonite present in the sample, taken as an average of the six samples. There appears to be considerable variation between the replicate samples (a to f, Fig. 4.12). Some of this variation could possibly be attributed to developmental stage i.e. a change in mineralogy with size, however no measurements of shell area or length were recorded before the shells were ground up for analysis, and therefore no comparisons can be made.

4.5 Discussion

4.5.1 Shell composition

The mineralogy of abalone shell is important in abalone culture, as shells with proportionately more aragonite in their prismatic layer have been shown to donate

more $CaCO_3$ (i.e. dissolved shell) to the surrounding water in low pH conditions (Merino et al., 2010). It is therefore important to define the mineralogy of the shells of abalone cultured in recirculating aquaculture systems (RAS) to determine their sensitivity to low pH conditions.

Shell composition analysis from this study suggests that the upper prismatic layer of pāua is predominantly calcite, with small pockets of aragonite embedded between the calcitic prisms. This layer is generally several hundred micrometers thick. These observations are consistent with other studies examining the mineralogy of wild pāua shells (Dauphin et al., 1989; Gray and Smith, 2004). An examination of the shell layers using Raman spectroscopy showed the shell was divided into approximately 50% nacre and 50% prismatic layer (see Table 4.5). X-ray diffraction data showed that the relative proportions of calcite to aragonite are approximately 50/50. Thus if we assume that nacre is 100% aragonite, the prismatic layer is therefore almost exclusively composed of calcite.

In cultured pāua, a discrete 'shiny' section at the umbo is commonly observed in animals exposed to low pH water, abalone farmers refer to this as the 'shiny butt syndrome'. In this study, I also observed obvious dissolution occurring at the umbo over the short duration of the trial in the low pH treatment. This area of localized dissolution suggests particular areas of the shell or different life stages of the animal may be more vulnerable to pH damage. Among the Haliotids, change in mineral composition has been associated with developmental stage in two species of commercial abalone including pāua (Gray and Smith, 2004; He and Mai, 2001). In addition, the composition of the shell of post larval and juvenile abalone has been assessed in at least one species, the data show that the shell at these early stages is essentially made from aragonite (Auzoux-Bordenave et al., 2010). It is clear that the mineral composition of abalone changes with ontogeny, and it is likely that early in development, pāua shells are composed primarily of the more soluble aragonite. The appearance of this shiny section and its relatively consistent size (irrespective of overall size of the animal) suggests post larvae and juvenile pāua have a shell composition that is predominantly aragonite to at least 10 mm in length (see Fig. 4.13 A & B) and therefore are more vulnerable to shell dissolution in this area of the shell.

Figure 4.13 Mature and juvenile pāua (approx 100 mm and 45 mm SL respectively), with eroded spire (circled). A shiny area of approximately 10 to 15 mm in length is commonly observed in hatchery reared pāua.

4.5.2 Shell deposition

Molluscan calcification rates show a broad range of responses to lowered pH water (Beniash et al., 2010; Gazeau et al., 2007; Nienhuis et al., 2010; Ries et al., 2009). Differences in calcification rates have even been detected between species of abalone (Harris et al., 1999a). The range in responses highlights the importance of assessing each species individually in its responses to a low pH environment, and is particularly important if that species is cultured commercially. The primary reason surrounding this range of responses is thought to be due to each individual species ability to regulate pH at the site of calcification (Ries et al., 2009). The capability to raise pH at the site of calcification can facilitate $CaCO_3$ precipitation by the conversion of bicarbonate (HCO_3^-) into carbonate (CO_3^{2-}) (see section 1.5, pH) and increasing concentration of CO_3^{2-} available to use in the mineralisation process.

In this study, calcification was assessed by measuring the change in shell length and calculating daily incremental growth rate**.** Over the 85 day culture period there is a significant difference between the treatments (P = 0.049), however once the animals had become acclimatized to their new environment (i.e. from days 31 to 85), the rate of mineralisation (incremental shell growth) does not appear to be influenced by pH of the culture water (see Fig. 4.5).

The ability of pāua to deposit calcium carbonate shell in low pH water is perhaps unsurprising, given that many marine invertebrates can exert tight control over the mineralisation process regardless of ambient seawater pH. The reasons for initial low calcification rates of pāua cultured at pH 7.6 are unknown at this time, however this is possibly due to a period of physiological adaption to the low pH environment.

Pāua have demonstrated they can adapt and continue to secrete shell at a 'normal' rate in a chronic low pH environment, however it is not known if they use more energy to do so. Oxygen (O_2) consumption is a good proxy for energy expenditure, and an increase in respiration rate typically represents a relative increase in energy expenditure. Although O_2 consumption was not measured in this trial, Harris et al. (1999a) observed an increase in O_2 consumption in *H. laevigata* at pH levels approximately 0.5 of a pH unit below and above pH 8. Calcification rates (after day 30) were not affected by low pH water, however it remains to be seen how pH affected resting metabolic rate and influenced food consumption. If there was proportionately more energy being channelled into maintenance pathways to sustain high growth, food conversion ratios could be impacted (see section 1.4.2, temperature) and affect the production efficiency of an abalone farming operation. To my knowledge there are no studies describing the influence of pH on an abalone energy budget. Future work should aim to quantify the energetic cost of acute and chronic exposure to low pH water, to determine the overall cost to an abalone farming operation.

4.5.3 Shell dissolution

Abalone, and all organisms that secrete their $CaCO_3$ shells into direct contact with the surrounding seawater, are vulnerable to shell dissolution. The periostracum that serves as a barrier between the prismatic layer of the shell and the seawater provides some protection, but it can quickly be eroded away and is often absent altogether in older areas of the shell. The morphology of the abalone shell is therefore particularly sensitive to pH damage, and offers little in the way of protection from shell dissolution due to falling seawater pH.

Shell thickness was examined by comparing the shell weight versus shell area between treatments, to see if shell dissolution was occurring at pH 7.6. There was a difference in shell thickness between treatments (see Table 4.2) that suggests that pāua shells are susceptible to shell dissolution at pH 7.6. Analysis of shell thickness is useful, as it provides an estimate of overall weight loss of shell, however it does not indicate where on the shell dissolution occurs. As discussed above, some obvious dissolution that appears localized around the spire of the shell is commonly seen in pāua, and it is likely that a disproportionate amount of shell dissolution occurs in this area. In many of the animals cultured at pH 7.6, dissolution was dramatic, where the prismatic layer was completely eroded away and the inside nacreous layer could be seen. Reasons for this include sensitive mineralogy at early developmental stages, and possibly an absence of physical protection due to erosion of the periostracum at the oldest part of the shell. Dissolution could also have been occurring around the margin of new growth, as observations from section 4.3.1 (Raman spectroscopy) suggest the shell was much thicker in the newly formed shell in pāua cultured in pH 7.9 (see Table 4.3). However, it is unclear whether or not low pH affects newly formed shell in pāua. Further sampling is needed to define shell thickness in low pH environments.

Chapter 5

General Discussion

5.1 Summary and general recommendations

In New Zealand, pāua are farmed commercially in land-based high intensity recirculating aquaculture systems (RAS). RAS provide scope to manipulate environmental conditions and promote fast growth necessary to operate a profitable aquaculture business. Careful management of RAS is important as water quality parameters such as ammonia, oxygen and suspended solids loading can fluctuate quickly and threaten the health of the stock. Among these parameters is dissolved carbon dioxide (CO_2), that can rapidly accumulate in the water and have a significant impact on the pH of the culture water. A reduction in growth rate (Harris et al., 1999a) and shell dissolution and deformation (Heath and Tait, 2006b) has been observed in abalone grown in lowered pH conditions. Developing appropriate methods to mitigate the accumulation of CO_2 in RAS is a critical step toward developing a successful high intensity pāua farming industry in New Zealand. The research presented in this thesis has been focused primarily on two methods commonly used in aquaculture to raise pH of seawater, namely CO_2 degassing and alkalinity dosing. I have shown here that CO_2 degassing through a cascade column is not efficient enough at removing CO_2 from seawater and maintaining pH at levels suitable for pāua culture (i.e. pH 7.9 to 8.2). Although degassing remains an important and necessary component of a RAS (for oxygenation and degassing of other atmospheric gases such as nitrogen), degassing is not recommended as a primary method of pH control in pāua culture. The primary technique recommended for pH control in pāua culture is alkalinity dosing. Alkalinity dosing is incorporated into RAS to replace alkalinity consumed by the biofilter (Timmons et al., 2007a) and raise pH. I observed no significant negative effects on the growth of pāua using seawater buffered with either sodium hydroxide or food and industrial grades of calcium hydroxide. The continued use of industrial grade calcium hydroxide in pāua RAS is therefore recommended as a means to raise pH.

5.2 Summary of results

In Chapter 2 the efficiency of CO_2 removal through a packed column aerator was examined. Anecdotal evidence suggests the effects of pH begin to appear on the shells of pāua at approximately pH 7.7 (G Moss, pers. comm., 2008). The apparent inefficiency observed in degassing applications in seawater, and the lack of information surrounding CO_2 removal in seawater at greater than pH 7.4, justified an investigation into CO_2 degassing efficiencies in seawater systems. Previous studies, examining CO_2 removal in a cascade column design, suggest air to water ratio is the primary factor influencing degassing efficiency (Summerfelt et al., 2003; Summerfelt et al., 2000b). The data presented in Chapter 2 showed that manipulating countercurrent airflow, media height and hydraulic loading, have little affect on the degassing efficiency in seawater at the experimental influent pH of 7.4, 7.6 and 7.8. The difficulties observed in degassing at these pH levels appear to be coupled to the limitations of the water chemistry. At a pH that is appropriate for abalone culture only a small fraction of aqueous CO_2 is available to degas. The slow reaction time of the carbonate system re-establishing equilibrium during degassing (Grace and Piedrahita, 1994; Stumm and Morgan, 1996) effectively acts as a bottleneck, limiting the amount of CO_2 that can be removed from the water over the short throughput time of a cascade column. It is therefore difficult to remove enough CO_2 to lift pH to acceptable levels in a single pass through a cascade column. Only degassing apparatus that promotes a long residence time may be effective in raising pH toward a target pH of 7.9.

In Chapter 3 the effect of buffered seawater (i.e. low pH water that had been corrected using alkalinity chemicals) on the growth of pāua was examined. The observed inefficiencies of degassing methods highlighted the importance of using buffering chemicals to control pH. However little was known about the direct effect of using buffering chemicals on the growth of abalone. Industrial grade calcium hydroxide is currently being used for pH and alkalinity control in commercial pāua farming operations around New Zealand. Incremental growth rate was used as a primary factor to evaluate the appropriateness of three commercially available alkalinity supplements for use in pāua RAS. There were no significant differences detected in growth rate between the treatments, however there was a suggestion by comparing

differences in shell length, that sodium hydroxide had a slightly detrimental effect, and calcium hydroxide a slightly positive effect on shell deposition rates. From the results of this trial I concluded that the effect of buffered seawater on growth was minor.

As a supplement to the investigation of pH control methods described in Chapters 2 and 3, a preliminary investigation into the vulnerability of pāua to low pH conditions was described in Chapter 4 by examining the effect of pH on shell calcification rates and shell dissolution in pāua. Exposure to low pH water has been linked to shell dissolution in abalone, however the effect of shell dissolution has been shown to be more pronounced in some species more than others (Merino et al., 2010). The relative proportions of the two primary calcium carbonate polymorphs, aragonite and calcite, vary in the upper prismatic layer of abalone (Dauphin et al., 1989). The upper prismatic layer is largely in direct contact with the seawater, and shells with a higher proportion of the more soluble aragonite (Morse et al., 2007) appear relatively vulnerable to shell dissolution. X-ray diffraction and Raman spectroscopy analysis revealed that the composition of the prismatic layer of pāua used in this trial (~35 – 40 mm SL) was predominantly less soluble calcite. The composition of the shell suggests pāua are relatively tolerant of shell dissolution compared to other abalone species with a higher proportion of aragonite in their prismatic layer. However, shell thickness analysis detected a significant amount of dissolution occurring in pāua cultured at 7.6 compared to 7.9, indicating that dissolution does occur, and is indeed a problem in pāua aquaculture when pH falls below 7.7. Results presented in Chapter 4 suggest pāua do have the physiological capability to regulate biomineralisation processes in low pH conditions. Shell deposition rates were initially significantly lower in pāua cultured at pH 7.6, a trend that was consistent with observations made in similar studies of abalone (Harris et al., 1999a). However, after a period of acclimatization, deposition rates were comparable to those cultured at pH 7.9.

5.3 Final remarks

Pāua farming is not new to New Zealand, however the industry has remained small over the past few decades and failed to establish momentum in the aquaculture sector. One of the primary reasons for its lack of progress has been linked to gaps in

knowledge surrounding optimal culture conditions. The use of RAS in abalone farming brings with it many advantages, above all the ability to manipulate water temperature to provide optimal conditions for fast growth. With RAS, managing water quality becomes paramount, as small changes in water quality parameters like pH can restrict growth and compromise productivity. pH must remain above pH 7.7 in the culture tanks to promote fast growth and avoid the negative effects of shell dissolution. The research presented in this thesis surrounding degassing efficiency and alkalinity dosing provides a reference for operators present and future to develop and refine systems to control pH in RAS. It is important that the limitations of degassing as a means to control pH be recognized in the natural pH range of seawater. Globally, as the application of RAS continues to expand into marine species, information surrounding pH management in closed systems is important for development of this sector. This is particularly relevant in the culture of pH sensitive species, such as abalone.

5.4 Future directions

The nature of pH in RAS is dynamic and may fluctuate within a system depending on factors such as water temperature, feeding activity, and alkalinity. Data presented in this thesis have shown that pāua can adapt and grow remarkably well in chronic low pH conditions, however it remains to be seen if similar growth rates can be achieved when pH is fluctuating as is common in high intensity aquaculture situations. There is an energetic cost associated with living outside a normal range of pH. As bio-energetic analysis is particularly important in an aquaculture situation, this cost should be examined and the effect of variable pH on growth rate and food conversion efficiencies investigated.

The rate of shell growth may ultimately limit the rate of body growth in abalone, so providing an environment optimal for shell growth is important in a commercial aquaculture situation. There is a suggestion from the results presented in Chapter 3 and anecdotal evidence from ornamental coral enthusiasts, that biominerisation rates can be increased by raising calcium and alkalinity concentrations in the water. I believe this avenue of thought requires further investigation, as manipulating calcium and alkalinity concentrations would be relatively straight forward in RAS. The

potential to increase biominerisation rates and avoid shell dissolution may have a significant impact on the profitability of an abalone farm.

Appendix 1

1.1 Description of diagnostic peaks used in the XRD analysis of pāua shells presented in Fig. 4.12.

Sample	Diffraction angle (2θ)	Peak height (counts per second)
7.9 a	29.4105	195.3
	45.8401	57.54
7.9 b	29.4487	202.15
	45.8275	44.1
7.9 c	29.3938	184.64
	45.8316	58.59
7.9 d	29.4023	271.35
	45.8253	69.36
7.9 e	29.3662	187.54
	45.7824	67.91
7.9 f	29.4065	260.99
	45.8285	65.02
7.6 a	29.4083	246.97
	45.8273	55.11
7.6 b	29.4499	214.93
	45.8796	70.03
7.6 c	29.4083	239.51
	45.827	56.43
7.6 d	29.388	212.11
	45.8145	67.58
7.6 e	29.3677	173.09
	45.8085	63.76
7.6 f	29.3956	145.14
	45.772	53.4

1.2 Peak descriptions from XRD analysis of pure calcite and aragonite samples.

	Calcite		Aragonite	
No.	2 Theta (°)	Intensity (%)	2 Theta (°)	Intensity (%)
1	23.0	12.0	21.0	2.0
2	29.4	100.0	26.2	100.0
3	31.4	3.0	27.2	52.0
4	35.9	14.0	31.1	4.0
5	39.4	18.0	32.7	9.0
6	43.1	18.0	33.1	46.0

7	47.1	5.0	36.1	33.0
8	47.4	17.0	37.2	14.0
9	48.5	17.0	37.9	38.0
10	56.5	4.0	38.4	31.0
11	57.4	8.0	38.6	6.0
12	58.0	2.0	41.2	11.0
13	60.6	5.0	42.9	23.0
14	60.9	4.0	45.8	65.0
15	61.3	3.0	48.3	32.0
16	63.0	2.0	48.4	25.0
17	64.6	5.0	50.2	23.0
18	65.5	3.0	51.9	4.0
19	69.2	1.0	52.4	25.0
20	70.2	2.0	52.9	15.0
21	72.8	2.0	53.9	3.0
22	73.7	1.0	59.3	4.0
23	76.3	1.0	60.2	2.0
24	77.1	2.0	61.8	4.0
25	80.9	1.0	62.9	3.0
26	81.5	3.0	63.3	5.0
27	82.1	1.0	66.1	5.0
28	83.7	3.0	66.5	3.0
29	84.7	1.0	68.7	3.0
30	86.4	1.0	69.1	3.0
31	93.0	1.0	70.9	2.0
32	94.7	3.0	75.3	6.0
33	95.0	4.0	76.8	7.0
34	96.1	2.0	78.0	5.0
35	97.6	1.0	79.4	6.0
36	99.1	2.0	80.7	5.0
37	102.2	1.0	82.2	6.0
38	102.9	1.0	83.2	3.0
39	103.8	1.0		
40	104.1	3.0		
41	105.8	2.0		
42	106.1	4.0		
43	107.3	1.0		
44	109.5	2.0		
45	110.4	2.0		

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