Factors influencing the reproductive development and early life history of blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone

by

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Abstract

A study was initiated to determine the effect of selected factors on the reproductive development and early life history of blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone relevant to their wild fisheries or aquaculture. In both species, the rate of gonadal and larval development was proportional to water temperature, but the relationship was not simply multiplicative, rather there was a critical minimum water temperature below which development was arrested, known as the Biological Zero Point (BZP). The BZP for gonadal development was 7.8ºC for *H. rubra* and 6.9ºC for *H. laevigata*. Corresponding BZP values for larval development were 7.8ºC and 7.2ºC, respectively. Observations of larval development relative to temperature enabled a description of the Effective Accumulative Temperature (EAT; the cumulative difference between the culture temperature and the BZP, calculated hourly) for prominent developmental stages. The difference between the EAT for metamorphic competence and that for hatchout (i.e. the interval during which the larvae remain in the water column) was 1120 and 1160 EATºC-h for blacklip and greenlip abalone, respectively. These values, in combination with water temperature data, enable the prediction of the dispersal window for each species *in situ*. Spawning performance of blacklip and greenlip abalone was also affected by temperature, with both sexes of each species producing significantly more gametes when conditioned at 16ºC than 18ºC. Sperm production of *H. rubra* was an order of magnitude greater than that of equivalent sized *H. laevigata*. There was no apparent difference in the lipid or fatty acid composition of the ovary or testis between pre- and post-spawning animals of either species, presumably because of partial spawning and/or incomplete resorption of the gonad. Likewise, a 4ºC difference in conditioning temperature (i.e. 14ºC vs 18ºC) was insufficient to elicit changes in tissue biochemistry. There was a significant interaction between sperm density and contact time on the fertilisation success of eggs from both blacklip and greenlip abalone. Prolonged exposure (> 1200 s for *H. rubra* and > 480 s for *H. laevigata*) to concentrated sperm (i.e. 10^7 ml^{-1}) resulted in egg destruction. Analysis of CoVariance of F_{50} values (i.e. the sperm concentration required for 50%

fertilisation, derived from the linear regression of logit (proportion of eggs fertilised) versus sperm density) between species across a range of contact times demonstrated that contact time had a significant effect ($p < 0.001$) whereas species did not ($p = 0.22$). The lack of a species effect suggests that the fertilisation potential of blacklip and greenlip abalone eggs are similar, at least across the range of sperm densities and contact times used.

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Table of contents

List of tables

List of figures

Chapter 1 General Introduction

1.1 General Background

Abalone are commercially important marine gastropods (Archeogastropoda: Haliotidae) of which there are 55 extant and 40 extinct species (Geiger, 1998; Geiger and Groves, 1999) classified in a single genus *Haliotis*. They inhabit rocky reefs and boulder fields from the intertidal to a depth of 100 m (Sloan and Breen, 1988) and are native to most waters except those around South America and the Atlantic coast of North America (Hahn, 1989a). The Californian red abalone (*H. rufescens*) and the Japanese "Ezo" abalone (*H. discus hannai*) have also been introduced to Chilean waters (Flores-Aguilar, 2003).

Abalone are dioecious broadcast spawners and their life history can be broadly categorized into five stages: embryo, larvae, postlarvae, juvenile and adult. The embryonic stage typically lasts a number of hours and concludes with the hatching of the ciliated trochophore. The larvae are pelagic and lecithotrophic (i.e. non-feeding) and remain in the water column for 2-15 days depending on species and water temperature (Leighton, 1972; Sawatpeera et al., 2001). In the presence of certain chemical cues (e.g. Gamma aminobutyric acid, Morse et al., 1979) the larvae return to the benthos, metamorphose and begin to feed on benthic microflora (predominantly diatoms). From this point they are known as postlarvae. This stage lasts for about two months, during which the animal grows to 1.5-2.5 mm (Leighton, 2000). The end of the postlarval stage is signified by the appearance of the first respiratory pore. As juveniles mature, there is a gradual transition in diet from micro- to macroalgae. The size and age at which the gonad develops varies both within and between species and is dependant on factors such as temperature and food availability. Tropical species grow faster and mature earlier, but do not grow as large as temperate species. Growth of abalone during the postlarval and juvenile stages is exponential, but slows once they reach sexual maturity. The largest species, *H. rufescens* (red abalone) can reach 31 cm and almost 5 kg (Leighton, 2000).

1.2 Abalone fisheries

Abalone have been fished since early history, with the first reference to abalone divers in Japan dating from 30 A.D (Hahn, 1989b). However, it is not known whether the practice started in Japan or China. The popularity of abalone in southeast Asia spread with the gradual movement of the Chinese to other regions (e.g. Taiwan and Korea, Cuthbertson, 1978) but continuous unsustainable levels of harvesting in most of these countries has depleted or destroyed their fisheries. Abalone are particularly susceptible to overfishing owing to their ease of capture, slow growth and unpredictable recruitment (Tegner and Bulter, 1989). Other factors which impact abalone populations include El Niño events, disease and pollution. Most countries with viable abalone fisheries are attempting to reduce human impacts on abalone stocks by minimizing industrial discharge and placing restrictions (e.g. quotas, gear controls and seasonal closures) on recreational and/or commercial fisheries.

Today, the major abalone fishing nations are Australia, Japan, Mexico, New Zealand, South Africa and the United States. Recent (2002) annual catch statistics (from commercial and/or recreational fisheries) for these countries are 6062 t, 2682 t, 1616 t, 1553 t, 1281 t and 243 t, respectively (Gordon and Cook, 2004). The world market for abalone relies on about 15 species, with those sold in greatest quantities being *H. diversicolor* and *H. discus hannai*. The largest consumer nations are Japan, Hong Kong and China (through Hong Kong).

California has the oldest fishery outside of Asia, with records dating back to the mid 1800's (Cox, 1962). Intertidal and shallow water species (e.g. *H. rufescens*, *H. fulgens* and *H. cracherodii*) were collected and the meat dried for Asian markets (Leighton, 2000). By the mid 1900's, improvements in dive technology led to fishing in deeper waters, with the annual catch exceeding 2000 t until the early 1970's. However, the harvest gradually declined so that by 1996 it was less than 10% of earlier levels (Leighton, 2000). Both commercial and recreational fisheries for *H. cracherodii*, *H. sorenseni*, *H. fulgens* and *H. corrugata* were closed in 1996, and in May 1997 both sectors were excluded from fishing *H. rufescens* south of San Francisco (Leighton, 2000).

The Mexican abalone fishery relies on *H. fulgens*, *H. corrugata* and *H. cracherodii*. In the early 1920's (when records began) catches were moderate (1721 t in 1923) but by 1950 they had become unsustainably high (6000 t, Guzman del Proo, 1992). There was an immediate crash in 1951 to < 1500 t, followed by a fluctuation in catch of between 1500 t and 3500 t that continued until 1971. Seasonal closures, quotas and alterations to size limits were progressively implemented but the catch continued to decline. By 1983 it fell to < 500 t (Guzman del Proo, 1992). The catch increased again (to 2500 t) in the late 1980's and early 1990's but has since returned to around 500 t (M. Del-Rio Portillo, pers. comm.).

The modern commercial fishery for paua, *Haliotis iris*, in New Zealand dates from the mid 1940's (Schiel, 1992). Initially, only the shell was marketed (owing to its vivid colouration) but by the late 1950's both shell and meat were sold. Over-exploitation in subsequent years led to a 4-month nation-wide closure of the fishery in 1972 and a restriction on meat export in 1973 (Cooper, 1976). The ban was lifted in late 1990 (Schiel, 1992). The fishery is now managed by an individually transferable quota (ITQ) system across 10 fishing zones, each with a set total allowable catch (TAC, Schiel, 1992). Effort is restricted through the regulation of a snorkel-only fishery.

The South African abalone fishery began in 1949 and is reliant on one species, *Haliotis midae*. The annual harvest fluctuated between 500 t and 1500 t for many years, but in 1965 reached 2800 t (Tarr, 1992). From this point on, the catch (and catch per unit effort) rapidly declined, due to overfishing. In 1968, a production quota of 2316 t was imposed but had no effect (i.e. it was not reached). The quota was decreased annually and first limited the catch (at 1362 t) in 1970 (Tarr, 1992). The quota has remained around 600 t since the early 1970's. Since 1994, intense poaching activities, run by organised crime syndicates, have had devastating effects on the fishery (Tarr, 2000). In early 2004, the South African Government was moving towards a moratorium on recreational harvest of abalone and listing it as a vulnerable species.

The two main Haliotid species fished in southern Australian waters are the blacklip (*H. rubra*) and greenlip (*H. laevigata*) abalone. Blacklips are distributed from Rottnest Island, Western Australia (WA) to Coff's Harbour, New South Wales (NSW) as well as the Bass Strait islands and around Tasmania (Shepherd, 1973). Greenlip abalone have a similar western limit (Cape Naturaliste, WA) but their range only extends to Corner Inlet in Victoria, the Bass Strait islands and the north coast of Tasmania (Shepherd, 1973). Greenlip abalone form the majority of the catch in WA, whereas in NSW, Victoria (Vic), Tasmania (Tas) and South Australia (SA), most (or all) of the catch is blacklip. Recent (yr 2003 or 2003-2004 season depending on state) TAC limits for blacklips were 281 t, 1396 t, 2467.5 t, 482 t and 37.4 t for NSW, Vic, Tas, SA and WA, respectively (D. Worthington, pers. comm., www.dpi.vic.gov.au, Anon, 2002, S. Mayfield, pers. comm. A. Hart, pers. comm.). Corresponding figures for greenlips were 0 t, 0 t, 140 t, 353 t and 202.5 t, respectively.

The Tasmanian abalone fishery began in 1963 (Cuthbertson, 1978), after a minimum size limit (127 mm SL) was set the previous year (Prince and Shepherd, 1992). Commercial abalone licenses were introduced in 1965 and the number of divers capped at 120 in 1969 (Anon, 2000). A further 5 licenses, to fish the Furneaux Group only, were granted in 1972. Licenses became transferable in 1974 and annual catches gradually rose to a peak of 4500 t in 1984. The following year an ITQ system was introduced (Prince and Shepherd, 1992), with each quota unit equivalent to 1.1 tonnes of live abalone caught. This equated to TAC of 3806 t. Since then, minimum size limits and the value of each quota has been varied several times. In 2000, the fishery was divided into three zones, then (in 2003) five zones; four for blacklips and one for greenlips (www.dpiwe.tas.gov.au). The value of the catch in 2002 was $115 million (www.dpiwe.tas.gov.au). The corresponding figure for 2003, although not yet released, was significantly depressed by a reduction in demand, especially in east Asia, caused by Severe Acute Respiratory Syndrome (SARS).

1.3 Abalone culture

Research on the culture of abalone began in Japan, with early works describing the larval development and small scale propagation of *H. gigantea* (Murayama, 1935) and *H. discus* (Ino, 1952). During the 1960's, over a dozen government laboratories began programs to develop hatchery systems (Leighton, 2000). The most significant developments arose in the early 1970's, primarily due to the work of Nagahisa Uki and Shōgo Kikuchi. These researchers published a series of works (in Japanese) on the artificial spawning of abalone (*H. discus*, *H. discus hannai* and *H. gigantea*) covering topics such as the effect of temperature and nutrition on broodstock conditioning, ultraviolet (UV) induction of spawning and optimal sperm density for fertilization (summarized in English in Uki and Kikuchi, 1984). A greater understanding of the process of gonad maturation and the ability to spawn broodstock on demand (using UV induction) greatly improved hatchery and nursery production.

The focus of abalone propagation in Japan is on fisheries enhancement rather than captive growout. At present, 34 prefectural research stations produce seed of one or more of four species (two of which have two subspecies; N. Takiguchi, pers. comm.) that are distributed for release by fishing cooperatives. Currently, nearly 30 million seed are released annually (Kawamura, 2003) and the annual harvest (also managed by fishing cooperatives) stands at 2682 t (2002 figure, Gordon and Cook, 2004). A further 200 t is cultured entirely in captivity (Gordon and Cook, 2004).

Attempts to culture abalone in California began in the mid 1960's (Leighton, 2000). While there was some technology transfer from Japan, it was soon apparent that hatchery techniques would have to be tailored to the needs of local species, the red abalone *H. rufescens* being the preferred candidate. This research was conducted by a small number of private companies and government agencies and by the early 1970's one group (California Marine Associates) had succeeded in growing product to market size (Leighton, 2000). Their success was the catalyst

for further research and investment in the industry. At present, there are 11 groups along the Californian coast that are producing or intend to produce commercial quantities of abalone (Leighton, 2000). The only other abalone farm in the USA cultures the introduced Japanese abalone *H. discus hannai* on the Kona coast of Hawaii. As of 2002, production of American abalone farms was 169 t (Gordon and Cook, 2004) with the total value of abalone products at $US 5.7 million (Seavey, 2003).

The heightened interest in abalone research in the USA during the 1970's led to the important discovery that low (5mM) concentrations of hydrogen peroxide (H_2O_2) also induced abalone to spawn (Morse et al., 1977). These authors proposed that one or more products of the decomposition of H_2O_2 (e.g. the hydroperoxy free radical, HOO·, or the peroxy diradical, ·OO·) act on the enzyme system that produces prostaglandin, which in turn initiates spawning. It is thought that UV irradiation of seawater produces similar free radicals, but the donor molecule is ozone (O_3) rather than H_2O_2 (ozone being produced by the photolysis of dissolved oxygen in seawater). While the peroxide method was developed in the USA, not all hatcheries there use it, with several still preferring to use the UV method.

Propagation of abalone in South Korea, China and Taiwan began in the early 1970's. The main species produced in South Korea and northern China is *H. discus hannai*, while in southern China and Taiwan it is *H. diversicolor* (Chen, 1989; Yoo, 1989; Nie, 1992). Hatcheries in all three countries use the UV method to induce spawning. In South Korea, abalone seed are produced for fisheries enhancement (Yoo, 1989), whereas in China and Taiwan they are grown out in captivity, either in subtidal cages, land-based systems or intertidal ponds. China and Taiwan are the two largest producers of cultured abalone with 2002 annual figures at 4500 and 3000 t, respectively (Gordon and Cook, 2004). No recent production figures for South Korea are available.

In the 1980's, proponents of abalone aquaculture in the USA sold their technology to countries such as Mexico and Chile (Viana, 2002). The two farms currently operating in Mexico culture *H. rufescens* and *H. fulgens*, with a combined annual 2002 production of 53 t (Viana, 2002, Gordon and Cook, 2004). Chilean abalone aquaculture (which started in the early 1990's) is based on the introduced species *H. rufescens* (from North America) and *H. discus hannai* (from Japan). Both are c cultured in northern Chile, predominantly in large land-based facilities, whereas in the south only *H. rufescens* is grown in subtidal cages (Flores-Aguilar, 2003). There are currently 8 farms in the north and 12 farms in the south, with a combined annual (2002) production of 150 t (Flores-Aguilar, 2003, Gordon and Cook, 2004).

Study on the culture of *H. midae* in South Africa began in 1981 (Genade et al., 1988) but it was not until the early 1990's that a systematic research program was initiated (Sales and Britz, 2001). The industry now consists of 12 farms, the majority being land based, with at least one involved in reseeding (Sales and Britz, 2001). Hatcheries typically use H_2O_2 to induce spawning, with one farm having a strict protocol of spawning broodstock every 3 months (regardless of larval needs) to ensure a predictable spawning response when required (M. Miles, pers. comm.). Total production of South African cultured abalone in 2002 was 450 t (Gordon and Cook, 2004) with projections for 2004 in the vicinity of 800 t (Sales and Britz, 2001).

Spawning trials (using H_2O_2) of blackfoot abalone (*H. iris* or paua) in New Zealand began in 1980 and by the late 1980's two land based paua farms had established (Tong and Moss, 1992). The number of farms has since grown to 22 (Kabir, 2001) with the primary aim of most operations being pearl rather than meat production. Juvenile paua are 'seeded' with nuclei which are gradually covered in nacre produced by the mantle tissue. The resultant 'mabe' or half pearls are removed 2-3 years after nucleation and used to produce jewelry such as earrings and necklaces.

Culture experiments on Australian abalone (*H. rubra* and *H. laevigata*) began in Tasmania and Port Lincoln, South Australia in the early 1980's (Sumner and Grant, 1981; Hone and Fleming, 1998). By 1990, there were approximately 10 small farms operating and in 1993 a national program of industry consultation and strategic research was initiated. This collaboration between industry and research providers has resulted in the development of artificial diets, improvements in larval settlement and juvenile growth and genetic improvement programs. Current (2002) annual production from the nearly 40 abalone farms in Australia is 162 t (Gordon and Cook, 2004). Most of these are land based and either produce their own seed or buy in from elsewhere. Hatchery and nursery systems are similar between farms (i.e. use UV induction and conventional settlement plates) but growout systems vary markedly. Those in use today include: raceways (up to 1 m deep) with concrete blocks or tiles for hides; pipe systems, which use hundreds of lengths of 150 mm diameter PVC pipe; maze tanks, 1.2 x 3.0 m moulded polypropylene tubs with a series of straights and 180º turns; and slab tanks, concrete slabs with a low perimeter wall, shallow water (4-5 cm) and occasional flushing from a "tipper". Species differences in behaviour mean that not all systems are used to culture both species. Blacklip abalone are more cryptic than greenlips and so are not suited to maze or slab tanks, neither of which offer any shelter. Other, less frequently used systems for abalone culture in Australia include subtidal cages and barrels and also large cargo vessels fitted out with maze tanks. The latter method has the advantage of being able to follow a water mass of a desired temperature or move to avoid disease or pollution.

Despite there being many abalone farms in Australia, consistent production of seed is still a problem. Almost all hatcheries collect adults from the wild, but spatial and temporal variations in the availability of gravid broodstock and/or the stresses of capture and transport often compromise induction success. Hence, a reliable means of larval supply is vital for the expansion of the industry.

1.4 Reproductive biology and early life history

Artificial control of gonad maturation (i.e. conditioning) of abalone can be achieved through the provision of a favourable physico-chemical environment. This includes a stable water temperature that optimises gonad growth, high levels of dissolved oxygen, low levels of nitrates and ammonia and a pH of 7.5–8.5. Nutrition is also important during this process and broodstock should be fed a high quality diet in amounts slightly in excess of their needs (Uki and Kikuchi, 1982).

Temperature is the main factor influencing the rate of gonad development in most species of abalone. Its effect is cumulative above a certain threshold temperature that varies between species. Kikuchi and Uki (1974b) were the first to record this phenomenon, and named the threshold temperature the biological zero point (BZP). By subtracting the BZP from the daily water temperature and summing this figure over the culture time (in days) they were able to describe the Effective Accumulative Temperature in degree days (EATºC-days) for gonad conditioning of two Japanese abalone species. At present, there is only one account of the EAT for conditioning of southern hemisphere abalones, that of Kabir (2001) on *H. australis* and *H. iris*. Determination of the BZP for gonad development and the optimal EAT for spawning of blacklip and greenlip abalone would be of considerable benefit to hatcheries as they could implement a conditioning regime that resulted in consistently high spawning performance, both in terms of the spawning rate and number of gametes produced. This in turn would greatly improve hatchery efficiency.

The lecithotrophic larval stage of abalone demands that the egg contains sufficient energy reserves to last for several days. These reserves consist primarily of lipid (Moran and Manahan, 2003) which, combined with high fecundity of abalone, means that oogenesis is an energetically demanding process. Fatty acids (FA) are perhaps the most important lipids as they are the major component of cell membranes and in some cases are hormone precursors. Haliotids cannot

synthesize all the FA required for normal cellular function and growth (Uki et al., 1986), and rely on dietary sources of these essential fatty acids (EFA) to fulfill their requirements. Restricting the intake, either through reduced feed rations or provision of feeds low in EFA, results in suboptimal growth of abalone (Uki et al., 1986; Floreto et al., 1996; Mai et al., 1996; Dunstan et al., 2000).

In several countries where abalone are farmed, economic and/or ecological concerns regarding the collection of macroalgae for abalone culture have led to the development of formulated feeds. These feeds are usually composed of a mixture of animal and plant products, and as such have very different FA profiles to those of macroalgae. As yet, the effect of formulated feeds on the lipid and FA profile of somatic and gonadal tissues from blacklip and greenlip abalone (the main species cultured in Australia) has not been examined. Identifying the FA important to gonad development may aid in formulating more suitable broodstock feeds for these species.

Diet is not the only factor influencing the FA composition of marine invertebrates. Freezing points of FA are relatively high and inversely related to the degree of unsaturation. Hence, low temperatures may lead to saturated FA freezing, thus reducing membrane fluidity and disrupting membrane function. Several aquatic invertebrates are able to compensate for this by increasing the proportion of unsaturated FA in cell membranes at low temperatures (Lehti-Koivunen and Kivivuori, 1998; Hall et al., 2002), a phenomenon known as homeoviscous adaptation (Sinensky, 1974). The capacity of abalone to alter their FA profile in response to different temperatures has not been studied. Separating the potential effect of temperature from that of diet may assist in feed formulation.

Given the importance of the fishery to the Tasmanian economy, significant effort has been directed towards monitoring the status of the stocks during the last two decades. Reports generated from surveys have improved our knowledge of growth rates and fecundity of greenlip, and in particular, blacklip abalone. However, one area that has received little attention is the early life history of these species.

Since abalone are broadcast spawners, a reduction in density of mature abalone through fishing or other events has serious implications on fertilization success. In greenlip abalone, separation distances of just two metres can result in a 45% decrease in fertilization success compared to that of adjacent animals (Babcock and Keesing, 1999). In extreme cases, animal density may be so low that by the time sperm reaches an egg, fertilization is highly unlikely, a phenomenon known as the Allee effect (Allee, 1931). With the exception of the work of Babcock and Keesing (1999) on greenlip abalone, there is scant information on the fertilization biology of the two most commercially important haliotids in Australia. Factors requiring clarification for one or both species include the effects of sperm density, gamete contact time and gamete age on fertilization success. Descriptions of sperm morphology are also lacking for blacklip abalone.

Temperature also has a major influence on the early life history of abalone. It dictates the rate of larval development and in so doing affects the duration of the dispersal window (i.e. the interval between hatchout and metamorphic competence). The rate of larval development in abalone is not simply multiplicative (i.e. does not proceed twice as fast if one doubles the temperature) rather, there is a critical minimum temperature (the BZP) below which larval development is arrested (Seki and Kan-no, 1977). Furthermore, the appearance of each stage corresponds to the cumulative difference between water temperature and the BZP (i.e. the EAT). The EAT for each stage (expressed in EATºC-h) is constant between the BZP and the upper thermal limit of the species and provides a means of predicting its appearance when the timing of fertilization and water temperature are known. The ability to predict the duration of the dispersal window from water temperature is necessary to develop models of larval transport for haliotids. Knowledge of the EAT for hatchout and settlement would also enable abalone hatchery managers to control the onset of these stages by manipulating water temperature.

1.5 Objectives of study

The broad aim of this study was to address deficiencies in our knowledge of the reproductive processes of blacklip (*H. rubra*) and greenlip (*H. laevigata*) abalone in order to improve their hatchery production and aid in the management of their fisheries. The hypotheses to be tested were:

- Do these species conform to the BZP model of reproductive development?
- Does temperature and/or conditioning interval influence spawning success?
- Does temperature and/or spawning status affect the lipid and FA composition of somatic and gonadal tissues?
- Does sperm density and/or contact time influence fertilization success?
- Does temperature affect embryonic and larval development?

Each topic is addressed in a separate chapter (each of which contains several sub-topics) outlined below:

Chapter 2 – describes the effect of water temperature on gonad development of both sexes of blacklip and greenlip abalone. Several indices were used to quantify development: descriptors of gross structure include the Visual Gonad Index (VGI) and Estimate of Gonad Volume (EGV). The latter index was divided by shucked animal weight to provide a size-independent measure of gonad development, the Modified Gonad Bulk Index (MGBI). Oocyte diameter ratio (ODR), standardized oocyte diameter, oocyte area and oocyte volume (based on an ellipsoid) were used as descriptors of ovarian microstructure. For each sex and species, the rate of increase in the VGI, MGBI and oocyte volume (females only) of animals held at different temperatures were used to estimate the BZP.

Chapter 3 – examines the effect of two temperatures and five conditioning intervals on the spawning success (in terms of the percentage of spawners, repeat spawners and gamete production) of both sexes of blacklip and greenlip abalone over two conditioning cycles. This information will benefit Australian abalone hatcheries as it identifies the optimal temperature and EAT interval for repeated spawnings of these species.

Chapter 4 – documents the effect of temperature and spawning status on the lipid and FA composition of blacklip and greenlip abalone fed a formulated feed. Foot, digestive gland and gonad samples were analyzed in order to determine where particular FA are synthesized, stored or metabolized. Tissue FA profiles from abalone fed a formulated feed are compared to those from macroalgal feeding trials to determine if the formulated feed can be further improved.

Chapter 5 – investigates the interaction between sperm density and gamete contact time on the fertilization success of blacklip and greenlip abalone. These data were then logit transformed in order to facilitate inter-specific comparisons in fertilisation success across a range of gamete contact times and sperm densities A description of sperm morphology for both species is also provided.

Chapter 6 – reports on the effect of temperature on the larval development of blacklip and greenlip abalone. Stages examined include the first and second polar body release, first and second cell division, hatchout, completion of the velum, torsion and the formation of the fourth tubule on the cephalic tentacle (i.e. metamorphic competence). The duration of the dispersal window (i.e. between hatchout and metamorphic competence) is important to early life history models for Haliotids.

Chapter 7 – summarizes and integrates the main findings of preceding chapters.

1.6 Notes on this study

Each of the five research chapters included in this thesis contain a manuscript written in the format of the journals Aquaculture, Aquaculture Research, Invertebrate Reproduction and Development or the Journal of Shellfish Research. Using this style has necessitated some overlap in introduction, methods and reference sections of the research chapters. Publications are co-authored by the candidate's supervisor or co-supervisor/s in recognition of intellectual and technical contribution given. The status of each publication derived from the study is given below.

Chapter 2 – Temperature effects on the dynamics of gonad and oocyte development in captive wild-caught blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. Invertebrate Reproduction and Development. 45. 185-196.

Chapter 3 – The effect of temperature and conditioning interval on the spawning success of wild-caught blacklip (*Haliotis rubra*, Leach 1814) and greenlip (*H. laevigata*, Donovan 1808) abalone. Aquaculture Research. 36. 654-665.

Chapter 4 – Lipid and fatty acid composition of pre- and post-spawning blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone conditioned at two temperatures on a formulated feed. Aquaculture, 242. 297-311.

Chapter 5 – The effects of sperm density and gamete contact time on the fertilisation success of blacklip (*Haliotis rubra*; Leach, 1814) and greenlip (*H. laevigata*; Donovan, 1808) Journal of Shellfish Research. In press.

Chapter 6 – Temperature effects on embryonic and larval development of blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. Invertebrate Reproduction and Development. 45. 197-203.

The research described in Chapters 2 and 3 was part of a larger study funded by the Australian Fisheries Research and Development Corporation managed through the Abalone Aquaculture Subprogram (project number 2000/204).

1.7 Glossary

Absolute oocyte area (OA_{abs}): the area inside the perimeter of the oocyte.

Biological Zero Point (BZP): the temperature below which gonadal (or larval) development is arrested.

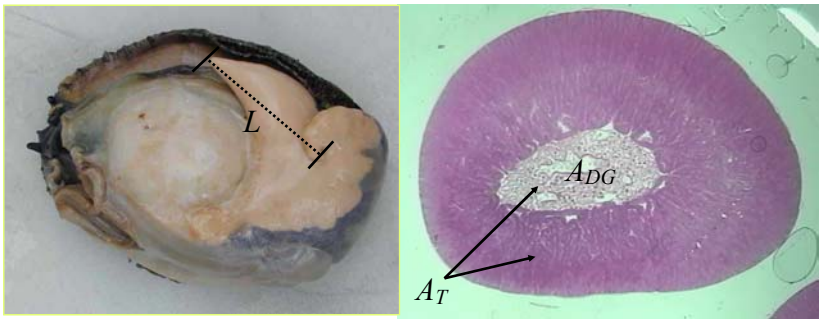
Effective Accumulative Temperature (EAT): the sum of the difference between the culture temperature and the BZP, calculated daily for gonad development and hourly for larval development.

Estimate of Gonad Volume (EGV): calculated from the length of the conical appendage (L) and the cross-sectional areas of the conical appendage (A_T) and the digestive gland (A_{DG}) according to the formula:

$$EGV = \frac{A_T L}{6}\left(8 - \left(\sqrt{\frac{A_{DG}}{A_T}} + 1\right)^3\right)$$

derived by Lleonart (1992).

Figure 1.1 Explanatory diagram showing the length (L) and the cross-sectional areas of the conical appendage (A_T) and the digestive gland (A_{DG}).



Maximum diameter ($max_{Ø}$): the greatest uninterrupted distance inside the perimeter of the oocyte.

Maximum radius (max_r): = $max_{Ø}/2$.

Mean radius ($mean_r$): = min_r + max_r/2.

Minimum diameter ($min_Ø$): the greatest distance perpendicular to the $max_Ø$.

Minimum radius (min_r): = $min_Ø$/2.

Modified Gonad Bulk Index (MGBI): a size independent estimate of gonad bulk, calculated according to the formula: MGBI = EGV/ WW where WW = the shucked wet weight of the animal.

Standardized diameter ($stand_Ø$): = $min_Ø$ + $max_Ø$/2.

1.8 References

Allee, W.C., 1931. Animal aggregations: a study in general sociology. University of Chicago Press, Chicago, 431 pp.

Anon, 2000. The Tasmanian abalone fishery revised policy paper. Department of Primary Industries Water & Environment, 82 pp.

Anon, 2002. Explanation of zones and size limits for the 2003 abalone year. Department of Primary Industries Water & Environment, Tasmania, 25 pp.

Babcock, R. and Keesing, J., 1999. Fertilization biology of the abalone *Haliotis laevigata*: Laboratory and field studies. Can. J. Fish. Aquat. Sci. 56, 1668-1678

Chen, H.-C., 1989. Farming the small abalone, *Haliotis diversicolor supertexta*, in Taiwan. In: Hahn, K.O. (Ed.), The Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, pp 265-283.

Cooper, 1976. Paua fishery - past, present and future. Catch '76. 3, 3-7

Cox, K.W., 1962. California abalones, family Haliotidae, Calif. Fish Game, Fish Bull., 118, 113 pp.

Cuthbertson, A., 1978. The abalone culture handbook. Department of Sea Fisheries, Tasmania, 37 pp.

Dunstan, G.A., Volkman, J.K. and Maguire, G.B., 2000. Optimisation of essential lipids in artificial feeds for Australian abalone. CSIRO Division of Marine Research, Hobart, Fisheries Research and Development Corporation Project 94/85 Final Report, 68 pp.

Flores-Aguilar, R.A., 2003. Ocean-based abalone culture in southern Chile, industry status and perspectives. 5th International abalone symposium, Qingdao, China, 116-117.

Floreto, E.A.T., Teshima, S. and Koshio, S., 1996. The effects of seaweed diets on the lipid and fatty acids of the Japanese disc abalone *Haliotis discus hannai*. Fish. Sci. 62, 582-588

Geiger, D.L., 1998. Recent genera and species of the family Haliotidae Rafinesque, 1815 (Gastropoda: Vetigastropoda). Nautilus 11, 85-116

Geiger, D.L. and Groves, L.T., 1999. Review of fossil abalone (Gastropoda: Vetigastropoda: Haliotidae) with comparison to recent species. J. Paleontol. 73, 872-885

Genade, A.B., Hirst, A.L. and Smit, C.J., 1988. Observations on the spawning, development and rearing of the South African Abalone *Haliotis midae* Linne. S. Afric J. Mar. Sci. 6, 3-12

Gordon, H.R., and Cook, P.A. 2004. World abalone fisheries and aquaculture update: Supply and market dynamics. J. Shellfish Res. 23, 935-939

Grant, A. and Tyler, P.A., 1983a. The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturity indices. Int. J. Invertebr. Reprod. 6, 259-269

Grant, A. and Tyler, P.A., 1983b. The analysis of data in studies of invertebrate reproduction. II. The analysis of oocyte size/frequency data, and comparison of different types of data. Int. J. Invertebr. Reprod. 6, 271-283

Guzman del Proo, S.A., 1992. A review of the biology of abalone and its fishery in Mexico. In: Shepherd, S.A., Tegner, M.J., Guzman del Proo, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp 341-360.

Hahn, K.O., 1989a. Survey of the commercially important abalone species in the world. In: Hahn, K.O. (Ed.), Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, pp 3-11.

Hahn, K.O., 1989b. Abalone aquaculture in Japan. In: Hahn, K.O. (Ed.), The Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, pp 185-194.

Hall, J.M., Parrish, C.C. and Thompson, R.J., 2002. Eicosapentaenoic acid regulates scallop (*Placopecten magellanicus*) membrane fluidity in response to cold. Biol. Bull. 202, 201-203

Harrison, A.J. and Grant, J.F., 1971. Progress in abalone research. Tasmanian Fish. Res. 5, 1-10

Hone, P.W. and Fleming, A.E., 1998. 'Abalone.' In: Hyde, K. (Ed.), The new rural industries - A handbook for farmers and investors. Rural Industries and Development Corporation, Canberra, pp 83-90.

Ino, T., 1952. Biological studies on the propagation of the Japanese abalone (Genus *Haliotis*). Bull. Tokai Reg. Fish. Res. Lab. 5, 1-108

Kabir, N.M.J., 2001. Environmental, chemical and hormonal regulation of reproduction in two commercially important New Zealand abalone, *Haliotis iris* and *H. australis*. PhD thesis, University of Otago, Dunedin, 236 pp.

Kawamura, T., 2003. Abalone fisheries and stock enhancement in Japan. 5th International abalone symposium, Qingdao, China, 119-120.

Kikuchi, S. and Uki, N., 1974a. Technical study of artificial spawning of abalone, genus *Haliotis* I. Relationship between water temperature and advancing sexual maturity of *Haliotis discus hannai* Ino. Bull. Tohoku Reg. Fish. Res. Lab. 33, 69-78

Kikuchi, S. and Uki, N., 1974b. Technical study of artificial spawning of abalone, genus *Haliotis* V. Relationship between water temperature and advancing sexual maturity of *Haliotis discus* Reeve. Bull. Tohoku Reg. Fish. Res. Lab. 34, 77-85 (in Japanese with English abstract)

Lehti-Koivunen, S.M. and Kivivuori, L.A., 1998. Fluidity of neuronal membranes of crayfish (*Astacus astacus* L.) acclimated to 5ºC and 20ºC. Comp. Biochem. Physiol. A 119, 773-779

Leighton, D.L., 1972. Laboratory observations on the early growth of the abalone, *Haliotis sorenseni*, and the effect of temperature on larval development and settling success. Fish. Bull. 70, 373-381

Leighton, D.L., 2000. The Biology and Culture of California Abalones. Dorrance Publishing Co., Inc., Pittsburg, Pennsylvania, 216 pp.

Lleonart, M., 1992. A gonad conditioning study of the greenlip abalone *Haliotis laevigata*. MS thesis, University of Tasmania, Launceston, 162 pp.

Mai, K., Mercer, J.P. and Donlon, J., 1996. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. Aquaculture 139, 77-89

Moran, A.L. and Manahan, D.T., 2003. Energy metabolism during larval development of green and white abalone, *Haliotis fulgens* and *H. sorenseni*. Biol. Bull. 204, 270-277

Morse, D.E., Duncan, H., Hooker, N. and Morse, A., 1977. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. Science 196, 298-300

Morse, D.E., Hooker, N., Duncan, H. and Jensen, L., 1979. Gamma-aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 203, 407-410

Murayama, S., 1935. On the development of the Japanese abalone, *Haliotis gigantea*. J. Coll. Agri. Tokyo Imp. Univ. 13, 227-231

Newman, G.C., 1967. Reproduction of the South African abalone *Haliotis midae*. Investl Rep. Div. Sea Fish. S. Afr. 64, 1-24

Nie, Z.Q., 1992. A review of abalone culture in China. In: Shepherd, S.A., Tegner, M.J., Guzman del Proo, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp 592-602.

Prince, J.D. and Shepherd, S.A., 1992. Australian abalone fisheries and their management. In: Shepherd, S.A., Tegner, M.J., Guzman del Proo, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp 407-426.

Sales, J. and Britz, P.J., 2001. Research on abalone (*Haliotis midae* L.) cultivation in South Africa. Aquaculture Res. 32, 863-874

Sawatpeera, S., Upatham, E.S., Kruatrachue, M., Chitramvong, Y.P., Songchaeng, P., Pumthong, T. and Nugranad, J., 2001. Larval development in *Haliotis asinina* Linnaeus. J. Shellfish Res. 20, 593-601

Schiel, D.R., 1992. The paua (abalone) fishery of New Zealand. In: Shepherd, S.A., Tegner, M.J., Guzman del Proo, S.A. (Eds.), Abalone of the world: biology, fisheries and culture. Blackwell Scientific Publications, Oxford, pp 427-437.

Seavey, A., 2003. The status of abalone culture in the United States. 5th International Abalone Symposium, Qingdao, China, 120.

Seki, T. and Kan-no, H., 1977. Synchronized control of early life in the abalone, *Haliotis discus hannai* Ino, Haliotidae, Gastropoda. Bull. Tohoku Reg. Fish. Res. Lab. 38, 143-153

Shepherd, S.A., 1973. Studies on southern Australian abalone (Genus *Haliotis*). I. Ecology of five sympatric species. Aust. J. Mar. Freshwat. Res. 24, 217-257

Shepherd, S.A. and Laws, H.M., 1974. Studies on Southern Australian abalone (Genus *Haliotis*) II. Reproduction of five species. Aust. J. Mar. Freshwat. Res. 24, 49-62

Sinensky, M., 1974. Homeoviscous adaptation - a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 71, 522-525

Sloan, N.A. and Breen, P.A., 1988. Northern abalone, *Haliotis kamtschatkana* in British Columbia: Fisheries and synopsis of life history information. Department of Fisheries and Oceans, Ottawa, 103, 46 pp.

Sumner, C. and Grant, J., 1981. TFDA experimental abalone hatchery making progress. Aust. Fish. 6, 14-15

Tarr, R.J.Q., 1992. The abalone fishery of South Africa. In: Shepherd, S.A., Tegner, M.J., Guzman del Proo, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp 438-447.

Tarr, R.J.Q., 2000. The South African abalone (*Haliotis midae*) fishery: a decade of challenges and change. In: Campbell, A. (Ed.), Workshop on rebuilding abalone stocks in British Columbia. Can. Spec. Publ. Fish. Aquat. Sci., pp 32-40.

Tegner, M.J. and Bulter, R.A., 1989. Abalone seeding. In: Hahn, K.O. (Ed.), Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, pp 157-182.

Tong, L.J. and Moss, G.A., 1992. The New Zealand culture system for abalone. In: Shepherd, S.A., Tegner, M.J., Guzman del Proo, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp 583-591.

Uki, N. and Kikuchi, S., 1982. Technical study on artificial spawning of abalone, genus *Haliotis* IX. Influence of food levels on maturation and spawning of the abalone, *Haliotis discus hannai* related to effective accumulative temperature. Bull. Tohoku Reg. Fish. Res. Lab. 45, 45-53

Uki, N. and Kikuchi, S., 1984. Regulation of maturation and spawning of an abalone, *Haliotis* (Gastropoda) by external environmental factors. Aquaculture 39, 247-261

Uki, N., Sugiura, M. and Watanabe, T., 1986. Requirements of essential fatty acids in the abalone *Haliotis discus hannai*. Bull. Jap. Soc. Sci. Fish. 52, 1013-1023

Viana, M.T., 2002. Abalone aquaculture, an overview. World Aquaculture Magazine 33, 34-39.

Yoo, S.K., 1989. Abalone farming in Korea. In: Hahn, K.O. (Ed.), The Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, pp 255-263.