Reseeding of *Haliotis iris* in a customary fisheries context

by

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Abstract

Globally, there is an increasing demand for fish which is accompanied by global trends of declining wild fish stocks. Fisheries managers are looking for alternative techniques to increase production and to more effectively manage stocks. Reseeding is a technique aimed at increasing wild abalone populations via the addition of juveniles that have been raised in aquaculture, often on a large scale. Large scale releases of hatchery reared animals into the wild have the potential to cause adverse genetic effects on the recipient wild population. These adverse effects can often result from events that occur at the hatchery where the animals were bred and reared. If appropriate numbers of brood stock and gamete mixing protocols are not employed at the hatchery, then the hatchery reared animals may exhibit inbreeding depression. In addition to this, a mass release of such individuals can result in outbreeding depression in the wild recipient population. In order to minimise the genetic risk and identify hatchery reared animals post-release procedures for genetic monitoring and genetic identification should be in place.

There is interest from commercial, recreational and iwi groups in New Zealand in the application of abalone reseeding. The interest comes is due to the potential for use in replenishing and sustaining stocks of the blackfoot abalone, Haliotis iris. In April 2013, reseeding of juvenile H. iris was undertaken in the East Otago Taiāpure and the Punawai O Tōriki Mātaitai, two customary management areas in Otago, New Zealand. This reseeding event has been used as a case study to assess the use of reseeding into customary areas. The survival of the reseeded animals was tracked. The released animals were compared in length and number to the resident wild juvenile H. iris. The DNA microsatellite markers were used to determine the suitability of individuals for reseeding by analysing the genetic diversity and genetic population structure of the hatchery reared and wild juvenile H. iris. The use of DNA microsatellite markers was also examined for use in distinguishing between wild and hatchery animals post-reseeding.

An estimated 125543 ± 17405 (1 SE) juvenile H. iris were released in the East Otago Taiāpure and the Punawai O Tōriki Mātaitai. The survival of the released animals was very low (<0.05%). There was no difference between the number of wild and reseeded
juvenile *H. iris* seen at the reseeding sights after the release. The pre-reseeding and post-reseeding animals were not significantly different in length, but both differed significantly from the wild juveniles present. The genetic monitoring concluded that there was reduced genetic diversity in the hatchery populations compared to the wild *H. iris*. There was genetic population differentiation between two of the hatchery populations and the wild populations. The use of microsatellite markers to identify the hatchery populations worked best when the hatchery populations were compared to a combined wild population. However, there was still variable success with correctly assigned individuals ranging from 67.1 – 98.6%. The results from the use of the microsatellite markers to identify the origin of individuals indicate that it is not 100% reliable and this should be considered before applying its use in future reseeding efforts.

The results suggest that the use of reseeding in customary management areas requires further investigation. This will help to improve the survival of the released individuals. The implementation of genetic monitoring at the hatchery level is key to minimising genetic risks. The use of microsatellite markers, as used in this study, to identify released animals will produce unreliable results when attempting to identify animals in the future. Overall, the use of reseeding in the East Otago Taiāpure and the Punawai O Tōriki Mātaitai indicated that reseeding methods need to be refined and reviewed to assess the success of reseeding in customary areas. It was seen that genetic monitoring is a useful tool to identify and minimise genetic risks when reseeding. It is recommended that it should be used in all stock enhancement programs in general.
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1. General Introduction

1.1 Stock enhancement of fisheries

In an effort to eliminate ‘the race for fish’ countries are looking towards alternative management practices (Hilborn et al., 2003). The current levels of catch may not be sustainable and so many capture fisheries are looking to intervene and improve production and management (Bartley & Bell, 2008). Enhancement of a fishery is one of these practices. Habitat restoration or construction and enhancement by stocking of juveniles have been the two traditional ways to enhance a fishery (Hilborn, 1998; Le Vay et al., 2008). ‘Restocking’ and ‘stock enhancement’ are two management tools that have the potential to help rebuild severely depleted fisheries (Bell et al., 2006). These two tools intervene by releasing cultured juveniles. This is done to either restore the spawning biomass (restocking) or overcome recruitment limitation (stock enhancement) (Bell et al., 2006). In a more general sense, stock enhancement is the hatchery-production of fish to a certain size for the purpose of releasing them into a wild fishery (Molony et al., 2003). There is a third tool, sea ranching, where cultured juveniles are released for harvest at a larger size (Bell et al., 2008). The duration of the stock enhancement will vary depending on the intended purpose. Long term programmes will likely help supplement natural recruitment while temporary programmes will be to rebuild depleted populations (Lorenzen, 2005).

To date there have been numerous examples of stock enhancement programmes worldwide. These have involved both marine vertebrates and invertebrates (Bell et al., 2006). In 2006, the count stood at 33 countries having undertaken stock enhancement with 59 different species (Kitada & Kishino, 2006). The potential for the release of cultured juveniles to augment coastal fisheries has drawn interest from a growing number of countries. This has led to investigation and research of stock enhancement (Bell et al., 2008). This growing interest sparked the development of a ‘responsible approach’ to hatchery-based marine enhancement. This is thought to be essential to the control and optimisation of the process (Blankenship & Leber, 1995). The 10 components outlined, in brief, were; select a target species; develop a species management plan; define quantitative measures of success; use genetic resource management to avoid deleterious genetic effects; use of disease and health management; consider ecological, biological, and life history patterns when forming objectives and
tactics; identify released hatchery fish and assess stocking effects; use an empirical
process for defining optimum release strategies; identify economic and policy
guidelines; and use adaptive management.

The potential benefits of stock enhancement are emphasised by the Japanese scallop,
_Patinopecten yessoensis_, fishery in Hokkaido, Japan (Uki, 2006; Bell et al., 2006). The
fishery collapsed in 1945 and consistently landed <100 tonnes p.a. prior to the
development and implementation of methods for stock enhancement (Uki, 2006). The
result of this breakthrough has seen the fishery restored and enhanced from <100 tonnes
p.a. to 40,000 tonnes p.a., consistently (Uki, 2006). With other fisheries adopting the
techniques developed the total Hokkaido scallop fishery lands approximately 300,000
tonnes p.a. (Uki, 2006). This is one of the best examples of a successful stock
enhancement initiative (Bell et al., 2006). The reality for most stock enhancement
initiatives, is that practices are far from perfect, in fact, many stock enhancement
programmes have failed to meet expectations (Bell et al., 2006).

1.1.1 Reseeding of abalone

The stock enhancement of abalone is commonly referred to as ‘reseeding’ or ‘seeding’.
The motives behind reseeding abalone are the usually the same as those that motivate
the stock enhancement of other species. The potential benefits of overcoming
recruitment failure (McShane, 1995b) to enhance wild populations with hatchery-
produced abalone, to maintain viable fisheries, has attracted the interest of countries
across the globe (Dixon et al., 2006; Roberts et al., 2007; Heasman et al., 2004; Kojima,
1995; Schiel, 1993). A considerable amount of research and resources have been
dedicated to investigating reseeding in Australia (Goodsell et al., 2006; Heasman, 2006;
Dixon et al., 2006), New Zealand (Schiel, 1993; Roberts et al., 2007), South Africa (de
Waal, 2010; de Waal et al., 2003; de Waal & Cook, 2001), and Japan (Kojima, 1995;
Hamasaki & Kitada, 2008). These countries have considerable wild abalone fisheries so
it has been in their interest to research reseeding (Prince, 2003).

It is thought that abalone are a suitable species for the application of reseeding
(Shepherd et al., 2000). In practice, the success of reseeding abalone has been highly
variable. This is thought to be due to multiple factors often acting at the same time.
Before leaving the hatchery, behavioural deficits may already be present in juveniles,
causing them to be vulnerable to predation upon release (Schiel & Welden, 1987;
Brown & Day, 2002). Handling stress, during transportation and reseeding, is likely to
cause mortality (Heasman et al., 2004). This may be able to be reduced by using adult shells as a reseeding device (James et al., 2007). The survival of the juveniles following reseeding increases with increasing size, but it is more expensive to culture juveniles to larger sizes (Roberts et al., 2007). The characteristics of the reseeding site is highly important with factors such as; sediment movement; wave exposure; substrate type; substrate complexity; and size all contributing to the survival of reseeded juveniles (de Waal & Cook, 2001; de Waal et al., 2003; Dixon et al., 2006). The density at which the seed is released can negatively affect the survival and may lead to mortality as they grow. This is because of decreased access to food and space (Goodsell et al., 2006; Roberts et al., 2007; Heasman, 2006). The reality of abalone reseeding, is that it is not as simple as it initially perceived. Like other forms of stock enhancement, the lessons learnt from past attempts must be taken on board, if it is to be a viable fisheries management tool (Bell et al., 2006).

There are problems surrounding the reseeding of abalone, which are highlighted by the variability of the success that reseeding efforts have had in the past (Roberts et al., 2007; Schiel, 1993; Dixon et al., 2006; Hamasaki & Kitada, 2008; de Waal et al., 2013; Searcy-Bernal et al., 2013). The main concerns are centred around the survival of the reseeded abalone. The economic viability of reseeding often depends on the ability to recapture individuals for commercial sale (Hamasaki & Kitada, 2008; Roberts et al., 2007). Research has focused on ways to increase survival rates, for example, site selection (de Waal et al., 2013; Roberts et al., 2007; Schiel, 1993), release techniques (Bird et al., 2009), substrate construction (Dixon et al., 2006), predation (Werner et al., 1995), and release density (Roberts et al., 2007; Goodsell et al., 2006) among other topics have been investigated. The tracking of reseeded individuals to determine survival rates has been particularly problematic. This has led to the development of a variety of techniques to identify reseeded individuals (Pirker & Schiel, 1993; Prince, 1991; McCowan, 2012; Poore, 1972a; Gallardo et al., 2003).

There are genetic concerns to do with the reseeding of abalone. These concerns mainly involve the potential for detrimental genetic impacts on the wild populations caused by genetic mixing with the reseeded populations (Roodt-Wilding, 2007; Le Vay et al., 2007; Bert et al., 2007; Laikre et al., 2010). The use of hatchery stock bred with small numbers of brood stock is a common cause for concern (Evans et al., 2004; Roodt-Wilding, 2007; Smith & Conroy, 1992). The potential implications for the genetic concerns are discussed further in Chapter 3.
1.1.2 Reseeding of *Haliotis iris*

There are three discrete species of abalone in New Zealand. These species are collectively known as pāua. The species are commonly referred to as the blackfoot abalone (*Haliotis iris*), the White foot abalone (*Haliotis virginea*) and the Yellow foot abalone (*Haliotis australis*) (Poore, 1972a). *H. iris* is the largest of the three species (Sainsbury, 1982) and is the focus of the present study.

The technology and methods for culturing *H. iris* have been developed relatively recently in comparison to the rest of the world. The 1980’s saw the undertaking of a project focusing on determining whether *H. iris* could be produced in a hatchery for use in reseeding and enhancement trials. This project involved the research of the majority of the *H. iris* life history. This included breeding, larval rearing, settlement and juvenile production. The work was carried out by the New Zealand Fisheries Research Division (Tong & Moss, 1992). Today there are approximately 13 farms producing *H. iris* in New Zealand (www.nzafa.org.nz).

The initial investigations into *H. iris* stock enhancement were by release of cultured larvae (Tong et al., 1987; Schiel, 1992). This method soon gave way to research into juvenile reseeding. The first investigations into the viability of *H. iris* reseeding were carried out at an experimental scale with juvenile *H. iris* produced using the methods described by Tong & Moss (1992). The focus was on investigating growth and survival (Schiel, 1992). These early experiments found that predators had a minimal detectable effect on survival. It was found that substratum, which was likely to move during storms, had major effects on survival (Schiel, 1992). After 3 years, survival of juveniles was as high as 24% (Schiel, 1992). Following these investigations, a large-scale investigation took place on the Chatham Islands. A total of Eighty thousand juvenile *H. iris*, of varying sizes (3-30mm), were reseeded into 8 sites and exhibited a high variation in survival (7.5 – 53.8%) (Schiel, 1993). Again, the importance of site selection was emphasised. Sites comprised primarily of extensive boulders on rocky reefs were chosen (Schiel, 1993).

Following a reduction in the TACC between 1999 and 2004, research was initiated by quota holders into stock enhancement in Marlborough, New Zealand (Roberts et al., 2007). The focus of this study included; economically optimum seed size; effects of seeding density; growth and survival of seeded *H. iris* through to 125mm; and economic viability of *H. iris* reseeding in this area (Roberts et al., 2007). The findings of the this
study reiterated that site selection is highly important, as it was shown to effect the survival of the reseeds (Roberts et al., 2007). The sites with the lowest survival in the study were affected by the movement of substrate due to storms (Roberts et al., 2007). The main conclusions drawn from the study were that reseeding is economically viable, but care must be used when choosing sites and habitat to reseed. In addition, large scale reseeding should be monitored for the survival of animals to harvestable size, in order to determine success (Roberts et al., 2007).

1.2 Abalone fisheries decline and mismanagement

From the 1970’s, a global trend of declining landings has been seen across the board in abalone fisheries. In 1968, the peak of global abalone, wild caught, production occurred. It peaked at 27,600 t/annum, and then decreased to approximately 60% to ~10,000 t/annum in the early millennium (Prince, 2005). There is commercial interest in approximately, 14 species worldwide (Roodt-Wilding, 2007). The estimated worth of the global abalone fishery is $100 million. This is spread across seven countries (Miller et al., 2009). On a global scale, it appears that abalone fisheries are being mismanaged, with few exceptions. The Californian abalone fishery highlights the potential repercussions for fishery mismanagement (Karpov et al., 2000). Other examples of countries with closed and/or collapsed abalone stock include the fisheries in Alaska and Canada (British Columbia) (Hilborn et al., 2003), South Africa (Raemaekers & Britz, 2009), and Waterlo Bay, Australia (Dowling et al., 2004). The inappropriate management of the remaining abalone fisheries is on-going. This is indicated by fisheries that are exhibiting a decline in the Total Allowable Catch (TAC). These fisheries include those in Australia (New South Wales) (Heasman et al., 2004), Europe (Huchette & Clavier, 2004), New Zealand (Roberts et al., 2007), Mexico (Searcy-Bernal et al., 2013) and Japan (Uchino et al., 2004).

The most famous and well documented case of a collapsed abalone fishery, is the case of the Californian abalone fishery. At its peak, approximately, 2000 t/annum of abalone were landed. However, due management failure, the fishery collapsed and was subsequently completely closed to commercial fishers (Karpov et al., 2000; Leiva & Castilla, 2002). This fishery collapse occurred after 150 years of commercial fishing. This included the five species present on the Californian coast; *Haliotis corrugate* (pink abalone), *Haliotis fulgens* (green abalone), *Haliotis rufescens* (red abalone), *Haliotis sorensei* (white abalone) and *Haliotis cracherodii* (black abalone) (Cox, 1960). Initially, red and pink abalone were the species focused on. These two species were
extracted during the 1940 – 50’s, with landings increasing during this time period. This was followed by a stabilisation in landings during the 1960’s. This occurred before decreases in landings that are typical of an overexploitation phase. However, the overexploitation of the red and pink abalone was overlooked. The overexploitation was overlooked because the fishery management was being based on the landings of multiple species. The decline of the red and pink abalone landings was obscured by the increased landings of the black, green and white abalone. This ultimately led to the mismanagement, and overexploitation of the Californian abalone fishery (Tegner, 1989; Leiva & Castilla, 2002; Karpov et al., 2000).

The closure of the abalone fishery on the Eastern Cape of South Africa, has been mainly attributed to the emergence of illicit abalone trade in 1997. By 2005, a highly specialised fleet piloted by illegal fishers was harvesting 1000 – 2000 tons per year (Raemaekers & Britz, 2009). The failure of the South African government to enforce sea-based compliance, and issue fishing rights, has allowed the abalone density and size to decrease significantly (Raemaekers & Britz, 2009).

The collapse and decline of many of the world’s abalone fisheries has been attributed to overexploitation, illegal harvesting, disease, and habitat degradation (Cook & Gordon, 2010). However, it should also be noted that the Japanese abalone fishery has seen a decrease in the landings. The decrease was from 600 – 800t in 1979 to less than 100t in 1997 (Uchino et al., 2004). Investigations into possible causes indicated the decline seemed to be related to overfishing.

1.2.1 New Zealand Haliotis iris fishery

Haliotis iris is highly sought after by customary, recreational and commercial fishers. A commercial fishery has existed since the mid 1960’s (Sainsbury, 1982). This fishery now lands approximately 1500 tons of catch a year, making it one of the largest abalone fisheries in the world (Cook & Gordon, 2010). A minimum legal size (MLS) of 125mm, no use of underwater breathing apparatus (UBA), landing in a measurable state regulations, and a limit of 10 per person per day are enforced for both recreational and commercial divers (Ministry for Primary Industries, 2013). Unlike recreational divers, commercial divers are not limited to 10 H. iris per person per day. The landing from recreational and illegal fisheries is also significant. There are estimates of the take being 1000 tons for the illegal fishery (Cook & Gordon, 2010). The recreational catch is unknown. The introduction of the Fisheries Amendment Act 1986, brought the H. iris
fishery into the Quota Management System (McShane et al., 1994). The two key
components of the Quota Management framework are total allowable catch (TAC) and
individual transferable quota (ITQ) rights. The total allowable commercial catch
(TACC) is set based on the TAC and is then distributed between the 11 quota
management areas (QMA’s)(Batstone & Sharp, 1999). Nationwide, TACC has been
reduced 18% between 1999 – 2004, in the Marlborough (PAU7) and Stewart Island
(PAU5B) QMA reductions of 40% have occurred (Roberts et al., 2007). The scale of
the QMA’s is thought to be inappropriate, with areas being in the region of 100s to
1000s of km of coastline (McShane et al., 1994). This scale is much larger than the
scale of what should be considered an abalone stock (Shepherd & Brown, 1993).

1.2.2 Customary fisheries
Traditionally Māori have an intimate relationship with the moana (sea) and kaimoana
(seafood). Māori consider certain species to be taonga (treasures) (Bess, 2011;
McCarthy et al., 2013). Haliotis iris is considered as one of the taonga species.
Following the introduction of the QMS, by the Fisheries Amendment Act 1986, many
part-time Māori fishers were left without any allocation of ITQ. These grievances
stemmed from Māori fishing rights not being upheld. The rights were not upheld despite
a guarantee under the Treaty of Waitangi (Bess, 2011). The follow-up piece of
legislation was the Treaty of Waitangi (Fisheries Claims) Settlement Act 1992. This Act
gave the entitlement of various customary and commercial fishing rights to Māori. This
enabled Māori to practice rangatiratanga (sovereignty or chieftainship) over customary
management areas (CMA’s), mātaitai and taiāpure.

Traditionally, Māori hapu (groups of families living close together) practiced fisheries
management in a sustainable manner. Management would be conducted in the fisheries
that usually resided adjacent to the land they lived on (Bess, 2011; Hepburn et al.,
2010). Through the application of tikanga (traditional practices and customs), and
kaitiakitanga (guardianship), fisheries were managed using various tools such as rahui
(closures), stock rotation and even stock enhancement (Bird et al., 2009; Bess &
Rallapudi, 2007; Bess, 2011). There is also evidence of local over-exploitation of
marine resources by Māori (Smith,2011a; Smith 2011b). However, despite having the
rights to now manage and co-manage traditional fisheries there is still on-going concern
for the state of the fisheries, and the continuing depletion of inshore fisheries stocks.
The depletion of H. iris stocks are of particular concern to Māori (McCarthy et al.,
2013). The unique situation that CMA’s provide, in terms of spatial scale and local
fishery bylaws and regulations, offers a potentially useful tool for the management of *H. iris* stocks (Hepburn et al., 2010). This is of interest as *H. iris* has been identified as the top species of concern for fishers, both Māori and non-Māori, in New Zealand (McCarthy et al., 2013).

### 1.2.2.1 Mātaitai reserves

Mātaitai reserves are established on traditional fishing grounds for the purpose of recognising, and providing for, customary management practices and food gathering. The Minister of Fisheries may establish a mātaitai reserve following application by tangata whenua. In general, mātaitai reserves; exclude commercial fishing; allow for bylaws for fishing to be made; do not prevent access to beaches or rivers not on private land; and do not require recreational fishers to obtain permits or prevent non-Māori from fishing ([www.fish.govt.nz](http://www.fish.govt.nz)). Mātaitai allow for a greater level of management control than taitāpure. As the tangata tiaki/kaitiaki, Māori are empowered to make bylaws within the mātaitai area for the purposes of sustainable harvesting of fish (Memon et al., 2003).

### 1.2.2.2 Taiāpure reserves

Taiāpure (local fisheries), are a tool for tangata whenua to make use of in an effort to become involved in the management of both the commercial and non-commercial fishing in the area. All fishing, recreational, customary, and commercial, can continue within a taiāpure. A management committee is appointed to a taiāpure. The committee is often comprised of local fisheries stakeholders and local iwi members. Recommendations for regulations are made by the management committee to the Minister of Fisheries. The regulations may relate to; species that may be taken; quantity that may be taken; dates and seasons when fishing is allowed; size limits of fish to be taken; methods to be used; and areas that are allowed to be fished. The key advantage of a taiāpure over a mātaitai, is that bylaws may be implemented in a short timeframe (40 days) and so reactive measures to aid the sustainable management of the fishery can be put in place faster, if needed.

### 1.2.2.3 The East Otago taiāpure

Following a decade of deliberation, after the initial proposal in 1989, the East Otago taiāpure (EOT) was established in 1999 (Hepburn et al., 2010). In 2001, the East Otago taiāpure Management Committee (EOTMC) was established. The EOTMC works to maintain and enhance the fisheries and habitats in the EOT for future generations.
(Hepburn et al., 2010). The philosophy that the EOTMC operates under is “Ki Uta ki Tai: From the Mountains to the Sea”. This represents a holistic approach to management that incorporates different world views, perspectives and backgrounds (Hepburn et al., 2010). The members of the EOTMC reflect this approach with 50% being from the local iwi, Kati Huirapa, and the other 50% being made up of community stakeholder groups. These groups include River-Estuary Care Waikouaiti, the University of Otago, commercial fishers, and a recreational group, Eastern Boating and Fishing Club (Hepburn et al., 2010).

The area of the EOT is defined by a line commencing at Cornish Head. The line travels towards Brinns Point then to Warrington Spit in a South-Westerly direction. It then turns and heads to Potato Point in an Easterly direction (East Otago taiāpure Management Commitee, 2008), see Figure 1.1. The EOT includes all of the marine and estuarine waters to the mean high tide water mark, this includes a variety of marine and estuarine habitats which are home to many species, including H. iris. These species are of interest customarily, recreationally and commercially (Hepburn et al., 2010).

The management plan of the EOTMC outlines the guiding principles, and objectives, that govern the direction of the EOTMC. The issues that are relevant to the present study, are that, within the EOT, overfishing has led to depletion of fish stocks, and decreased access to supplies of healthy fisheries resources (East Otago Taiāpure Management Commitee, 2008).

1.2.2.4 Punawai o Toriki mātaitai

The Punawai o Tōriki Mātaitai (PWTM) was initially proposed in 2005 by Waikōau Ngāi Tahu Rūnanga (S.O.) Incorporated. Local concerns that the existing quota management regime was an inappropriate means of managing the area, which is of special customary significance, prompted the proposition. The area consists of large areas of intertidal zone, which is important habitat for a wide range of customarily important shellfish, including H. iris. The area is of customary significance to the Ngāi Tahu Whānau and the local community, so the management body aims to ensure that the Ngāi Tahu Whānau have access to and use of abundant mahinga kai (food gathering); to ensure that current and future generations of Ngāi Tahu Whānau are able to exercise their customary rights of rangatiratanga (chieftainship), and kaitiakitanga (guardianship) responsibilities, in the management of the PWTM. This is in order to
prevent further degradation of the mauri (life force), and wairua (spirit) of PWTM. This is seen as exercising their customary rights

The PWTM encompasses 2.34km² of coastal waters from the mean high water mark outwards. The seaward boundaries are defined by a line starting at O Raki Waea, heading east approximately 1 km, then south approximately 2 km before heading south, then south-west to approximately the breakwater at Tirohanga.
Figure 1.1 – Map of the East Otago taiāpure
(adapted from EOTMC, 2008)
1.3 General information on *Haliotis iris*

1.3.1 Distribution and habitat

*Haliotis iris* occurs sporadically along the rocky shores hard substrates of New Zealand, including Stewart Island, the Snares Islands and the Chatham Islands (Poore, 1972a; Sainsbury, 1982). *H. iris* can be found in the sublittoral fringe and subtidally on these hard substrata (Preece & Mladenov, 1999). *H. iris* are present predominantly in < 6m depth (McShane & Naylor, 1995a), but can also occur down to 10m depth, little (<2m) individual movement, by adults, appears to occur (Poore, 1972b).

1.3.2 Growth and diet

Ageing of individual *Haliotis iris* is still difficult to determine, this is due to the lack of an accurate method. Methods looking at shell growth rings (Schiel & Breen, 1991) and variations in ratios of carbon isotopes (Naylor et al., 2007) showed no consistent patterns that can be used to identify age. Tag-recapture studies have been extensively used to determine estimates of the age and growth. *H. iris* exhibits demographic variation across New Zealand in respect to its growth (Poore, 1972c; Sainsbury, 1982; McShane & Naylor, 1995b). Individuals have been reported to reach sizes of 188 - 195mm in length (Preece & Mladenov, 1999; Schiel & Breen, 1991). Environmental conditions play a role in growth rates. Individuals found off headlands, have been found to grow at faster rates than those in bays. These individuals can be separated by as little as 200m. The difference is potentially due to differing levels of exposure and/or food delivery (McShane & Naylor, 1995b). Food availability has been suggested multiple times as a dominant driver for growth (Poore, 1972c; Poore, 1972b; Sainsbury, 1982).

Differences in growth rates are thought to account for the variation in size at sexual maturity. Faster growing populations, found off headlands, have been seen to reach sexual maturity at a smaller size than those in sheltered bays (McShane & Naylor, 1995b). The size at sexual maturity decreases with increasing sea surface temperature (Naylor et al., 2006). Animals found in waters with high mean monthly sea surface temperatures, grow at a slower rate than those animals found in areas with lower mean monthly sea surface temperatures (Naylor et al., 2006).

*H. iris* feeds mainly on drift algae but can change its feeding strategy to incorporate attached seaweed. This strategy change depends on the availability and source of food (Poore, 1972a; Cornwall et al., 2009). A preference for the brown algal species over red
13 and green algae exists (Cornwall et al., 2009). However, the greatest growth rates are
witnessed when a mixture of the species are fed to *H. iris* (Stuart & Brown, 1994).

### 1.3.3 Reproduction and development

*Haliotis iris* are dioecious broadcast spawners. They have very little sexual dimorphism,
except for the colouration of the gonad (Wilson & Schiel, 1995). The female gonad is
green in colouration while the male is a creamy colour (Poore, 1972d; Wilson & Schiel,
1995). It is thought that the *H. iris* aggregate in close proximity to one another in order
to increase the chance of fertilisation success (McShane, 1995b). This is a common
behaviour of abalone elsewhere in the world (Shepherd & Partington, 1995). Marine
invertebrates will often use this aggregation strategy, along with synchrony of spawning
and large sperm volumes, to increase fertilisation success (Giese & Pearse, 1974).

The cues and timing for spawning in *H. iris* remain relatively unknown. The timing of
spawning and gonad development have been studied multiple times revealing that there
is demographic variation (Sainsbury, 1982; Wilson & Schiel, 1995; McShane & Naylor,
1996). The appearance of recruits and fluctuations in the seasonal macroscopic gonad
index indicate that spawning is annual. However, inter-annual variation occurs in the
scale and frequency of spawning events as well as the timing of recruitment (McShane
& Naylor, 1996). The spawning will also vary depending on the location of the
population. *H. iris* spawning in southern localities of New Zealand have been found to
have a different spawning cycle than those in localities that are further north (Poore,
1972d; Wilson & Schiel, 1995). A 4 year observational study of the breeding season
showed no spawning during 2 of the 4 years, and offered no explanation as to why
spawning failure occurred (Sainsbury, 1982).

Following spawning, and successful fertilisation, the fertilised egg will be negatively
buoyant for the first 12 hrs before first becoming a trocophore larvae, followed by a
pelagic veliger stage prior to settlement (Tong et al., 1992). The larvae may stay in the
pelagic stage for up to several weeks before post-larval performance deteriorates
(Roberts & Lapworth, 2001). Dispersal of the larvae primarily occurs during the pelagic
stage. Dispersal is effected by point of release, shape of coastal features and the
hydrodynamic conditions present (Stephens et al., 2006). Following the pelagic stage,
laboratory studies have shown that new *H. iris* recruits are mainly found on crustose
coralline algae (Moss & Tong, 1992; Roberts et al., 2004). The stage of the larval
development effects the settlement of the larval *H. iris*. Larvae with 3 or more rows of
teeth (7-8 days old) will settle. However, larvae with 8 rows of teeth (9 or more days old) or more will settle, stay and survive longer on crustose coralline algae covered surfaces (Moss & Tong, 1992). Shallow habitat (1-2m) offers poor survival in the weeks following settlement, this is due to an association with fine sediment accumulation and greater water movement. Larvae settling at deeper depths (6 – 8m) have greater survival in comparison to those at shallower depths (McShane & Naylor, 1995a).

1.3.4 Juvenile characteristics

*Haliotis iris* that are considered juvenile are reproductively immature and generally <70mm in length (McShane & Naylor, 1996; Roberts et al., 2007). They are most active at night while during the day they are cryptic and are found beneath boulders. This behaviour lasts for approximately 3 years or while they are 5 – 70mm in length (Roberts et al., 2007). There appears to be a preference by juveniles for shallow depths (McShane & Naylor, 1996). The relative abundance of juveniles compared to adults suggests that the rate of recruitment for *H. iris* is low (McShane et al., 1994). The abundance of juveniles is weakly correlated with large patches of adults, but it is speculated that the availability of juvenile habitat, wave exposure and water movement, act as factors influencing the presence of juveniles (McShane, 1995a). Unlike adult *H. iris*, juveniles feed on attached microalgae and seaweed. The size when the switch from attached seaweed to drift seaweed occurs is unknown, it is speculated to be as small as 5mm (Roberts, 2003).

1.4 The use of genetic tools in fisheries management

Genetic tools are now considered to be essential to the effective management of hatchery reared fish, especially when release of cultured fish into wild populations is planned for restocking and stock enhancement (Roodt-Wilding, 2007). Introductions of genotypes that are not representative of the wild population can result in deleterious effects (Ward, 2006). Hatchery breeding procedures can, potentially, have a bottleneck effect on the resulting offspring. If the contributing parents are not representative of the wild recipient population the bottleneck effect can occur (Hedgecock & Sly, 1990). Cases of this have already occurred in abalone reseeding programmes (Roodt-Wilding, 2007).

The main genetic concerns surrounding restocking and stock enhancement are based on inbreeding depression and outbreeding depression (Roodt-Wilding, 2007; Ward, 2006). Outbreeding depression can occur when hybrid offspring with reduced fitness are
produced. This population is then crossed with a resident wild population (Lynch, 1991; Ward, 2006). Inbreeding depression occurs when large numbers of closely related genotypes are released and swamp the wild population, potentially resulting in a loss of fitness (Ward, 2006; Ryman & Laikre, 1991).

It is has been suggested by Roodt-Wilding (2007) that the negative effects of restocking, stock enhancement and ranching, can be minimised by; avoiding swamping the population with closely related genotypes by utilising large number of brood stock; ensuring the genetic diversity of the natural population is represented in the brood stock; and the monitoring of hatchery practices to avoid adaptation to the cultured environment. One of the most reliable and commonly used ways to assess the population structure of hatchery and wild stocks, is to use microsatellite markers (Roodt-Wilding, 2007; Ward, 2006). This technique has already been developed for use on *Haliotis iris* and will be will be elaborated on in Chapter 3 and 4.

### 1.4.1 Genetic work carried out on *Haliotis iris*

There is relatively little genetic work that has been conducted on *Haliotis iris* in comparison to other *Haliotis* species. The genetic population structure of *H. iris* around New Zealand has been studied. This was carried out to identify gene flow between geographic locations (Will et al., 2011). The study found that there was four phylogeographic breaks were present. The breaks occurred across the Chatham rise, in the western Cook Strait region, along the southeast coast of the South Island, and at East Cape in the North Island. This highlights how genetic markers can also be used to identify population structures that already exist in fisheries.

A study between a population of hatchery reared and wild *H. iris* found that significant genetic differences existed. It was observed that a change in allele frequency, loss of rare alleles, and a reduction in heterozygosity existed (Smith & Conroy, 1992). The study recommended that, when spawning, a minimum of 5 male and 5 female parents were needed. This is to maintain genetic variation which, in practice, translated to 10-3 males and 25 – 50 females due to the difficulty of inducing spawning (Smith & Conroy, 1992). The genetic considerations and the associated risks of reseeding abalone will be elaborated on further in Chapters 3 and 4.

### 1.4.2 Research context and customary reseeding case study

In April 2013, an opportunity to positively influence the declining trend of *Haliotis iris* stocks within the East Otago Taiāpūre (EOT) and the Punawai O Tōrīki Mātaitai
(PWTH) arose. Reseeding was used in an attempt to contribute to the on-going sustainability of the fishery. It was hoped that the addition of juvenile *H. iris* would bolster the number of reproductively mature animals in the following years. Additional reproductively mature animals would then improve the resilience of the population to recruitment variation, and thus make the fishery more sustainable. This reseeding was undertaken with the local community in mind and was hoped to replenish the local *H. iris* fishery. The EOTMC, in conjunction with the University of Otago and Te Rūnanga o Ngai Tahu, received juvenile *H. iris* from Ocean Beach Properties. Ocean Beach Properties is located at the southern end of New Zealand in Bluff. Bluff is ~ 220km South-West of the University of Otago, and ~ 260km South West of the EOT. The organisation of packaging, transport and out planting of the juvenile *H. iris* occurred within the timeframe of one week. One week is an extremely timeframe for a reseeding event to be planned. The reseeding is explained in further detail below.

The reseeding opportunity arose when Dr. Chris Hepburn (University of Otago, Marine Science Department) was contacted in regards to the availability of juvenile *H. iris* stock that were ready for reseeding. The stock was offered at no monetary cost, however, the labour and other associated costs to reseed were to be at the expense of Dr. Chris Hepburn. The stock had to be moved within a week because of changes at the Ocean Beach Properties facility. These changes meant there would be no available space to house the animals. The University of Otago did not have the capacity to house the animals. The entire operation had to be carried out with limited time of one week. This meant that issues that were encountered were dealt with in an impromptu manner. This often left the science accompanying the operation by the way side. Unfortunately this was because time and resources were limited or simply not available.

The scientific work that this thesis has conducted was undertaken ad hoc due to the reseeding event being unexpected. It is wished to be pointed out that the usual process of reseeding would take on careful planning and preparation which would usually take place over years rather than one week. The research work carried out should be considered in this context.

The legality of the reseeding required permission granted by the Ministry of Primary Industries. The permit hinged on the juveniles passing a test showing that they were clear of disease. This clearance was only granted hours before the reseeding began, highlighted the small timeframe in which this task was being conducted. The reseeding
was accomplished through a ground swell of community support of approximately 200 persons. Local residents, iwi members Kati Huirapa ki Puketeraki, community groups, University of Otago staff and students participated in all facets of the reseeding process.

1.4.3 Summary and overview of chapters

There is a need for alternative fisheries management strategies for abalone. Current management practices are unable to halt the decline in the landings of major world fisheries (Prince, 2005). The unique position of CMA’s in New Zealand fisheries provides an opportunity for a local community based approach to fisheries management (Bess, 2011; McCarthy et al., 2013). *Haliotis iris* is of particular interest to Māori due to its status as a taonga species (Bess, 2011; McCarthy et al., 2013). There is on-going concern that it is declining within traditional fishing areas, where taiāpure and mātaitai are often situated (McCarthy et al., 2013). One of the suggested solutions to declining abalone stocks, has been the use of reseeding to replenish and ensure the sustainability of the fishery (Dixon et al., 2006; Heasman et al., 2004; Roberts et al., 2007). However, the theoretical simplicity of reseeding masks the underlying complexity of the issues surrounding it as a management option (Molony et al., 2003).

The aim of the present study was to help assess the viability of reseeding into customary management areas. This was done with genetic tools in order to investigate the use of genetic tools. These tools were used in distinguishing between the wild resident population and the hatchery-reared population. The results will be used to make recommendations for future reseeding efforts undertaken in a customary fisheries context.

Chapter 2 looks at the initial survival of reseeded *H. iris* into the customary management areas of the East Otago taiāpure and the Punawai O Tōriki mātaitai. The survival of the hatchery individuals gives an estimate of the success of the reseeding.

Chapter 3 investigated the use of microsatellite markers to determine the genetic diversity. This was used to compare hatchery-reared stock and wild populations in the East Otago taiāpure and the Punawai O Tōriki mātaitai. The genetic population structure of the hatchery and wild populations was also investigated.

Chapter 4 investigates the potential for the use microsatellite markers to genetically distinguish between the reseeded populations from the wild resident populations. This
was carried out to establish if genetic identification could play a future role in genetic
monitoring and survival rate estimation.

Chapter 5 will discuss the results from the preceding chapters in a fisheries management
context, specifically relating to the East Otago taiāpure and the Punawai O Tōrīki
mātaitai and make recommendations for future reseeding programmes.
2. Reseeding in customary management areas:
Quantification and survival estimation of reseeded stock

2.1 Introduction

2.1.1 Decline of Haliotis iris stocks in NZ

There is concern over declining Haliotis iris stocks in New Zealand as national abalone fisheries follow the worldwide trend of decreasing stocks (Prince, 2003). A decrease in Total Allowable Commercial Catch (TACC) of 1267 to 1056 tonnes occurred between 1986/87 – 2004/2005 (Kahui & Alexander, 2007). The current TACC stands at approximately 1058 tonnes, however the recorded commercial catch has been below the TACC since at least 2007 (Ministry of Primary Industries, 2014). Reduction in TACC has historically occurred over concern about the sustainability of the resource and aims to aid in the rebuilding of stocks (Paua Industry Council Ltd, 2013a). One of the major challenges for abalone fisheries is to address ‘the tyranny of scale’. This challenge is the adaptation of monitoring, assessment, and management to abalone micro-stocks, which span areas as small as 100s – 1000s of meters (Prince, 2005). This issue is relevant in New Zealand where managers have historically grouped H. iris stocks into areas that cover of hundreds of kilometers (McShane et al., 1994; McShane et al., 1994; McShane & Naylor, 1996). There are new management strategies being implemented by the Paua Industry Council Ltd (PICL), which propose to create 100 micromanagement zones from the current management areas (Paua Industry Council Ltd, 2013b). PICL provides the commercial H. iris quota owners with a range of technical, administrative, research, and management assistance (Paua Industry Council Ltd, 2013c). Managing abalone at spatial scales that are too large is problematic. This is because of certain characteristics including restricted movement and dispersal, highly variable patterns of growth, variable fecundity, and highly aggregated populations (Prince, 2005; McShane, 1995b; Hobday et al., 2001). H. iris exhibits these same life history traits with aggregations (McShane, 1995a; McShane, 1996), large variations in growth and fecundity (Sainsbury, 1982; McShane & Naylor, 1995b; Schiel & Breen, 1991; Hooker & Creese, 1995), limited movement (Poore, 1972b), and limited dispersal of larvae (McShane & Naylor, 1996; Roberts & Lapworth, 2001; Roberts et al., 2007; Stephens et al., 2006). These characteristics leave them vulnerable to serial depletion and overfishing, which
have shown to lead to collapsed abalone fisheries (McShane et al., 1994; Karpov et al., 2000).

In particular, the recruitment of abalone and H. iris is highly variable making it difficult for fisheries managers to make effective management decisions (McShane, 1995b).

There is evidence showing that abalone species, including H. iris, locally recruit (McShane et al., 1988; McShane, 1995b; Stephens et al., 2006). This is a primary argument for the implementation of fine-scale management (Prince, 2005; McShane, 1995b). Fine-scale management at a reef-by-reef scale can allow for management decisions that account for the demographic variability that Haliotis species exhibit (Dowling et al., 2004; McShane, 1995b; Prince, 2005).

2.1.2 Alternative management strategies

One fisheries management tool allowing for the fine-scale management of Haliotis iris that already exists in New Zealand legislation is customary management areas (CMAs), specifically mātaitai and taitāpure (Bess, 2011; Bess, 2001; Hepburn et al., 2010). CMAs are fisheries tools developed to give effect to the obligations of the Treaty of Waitangi Fisheries Claims Settlement Act 1992 which helps to recognise the use and management practices of Māori in exercising non-commercial fishing rights (Ministry for Primary Industries, 2013a). In fact, mātaitai and taitāpure have been applied for in response to concern over declining H. iris stocks and increased difficulty of access (McCarthy et al., 2013; Bird et al., 2009). The East Otago taitāpure (EOT) was applied for in response to declining H. iris stocks. The elders of Kati Huirapa Runaka ki Puketeraki were concerned that H. iris stocks within the EOT were declining (Hepburn et al., 2010). Following this, new regulations were put in place to help protect H. iris stocks in the EOT. These regulations included reduced daily bag limit of 5 H. iris per person per day (down from 10) and closure of a significant H. iris fishing area around the Huriawa peninsula (Hepburn et al., 2010). One of the actions outlined in the East Otago taitāpure Management plan is the undertaking of shellfish reseeding initiatives, specifically H. iris (East Otago Taiāpure Management Committee, 2008).

2.1.3 Reseeding to replenish stocks

A potentially powerful combination of management strategies is to use fine-scale management and reseeding to overcome recruitment failure. Japan has made the most progress in implementing commercial scale reseeding, and is currently the only nation to do so (Hamasaki & Kitada, 2008). The large-scale reseeding has been on-going since
the 1960’s with large variations in recapture rates (1.4 – 23.8%), yield per release (YPR) (3.1-60.3g/individual), and contribution to total catch (6.9 – 83.5%) (Hamasaki & Kitada, 2008). Large numbers of juveniles have been released, 10.5 million in 1980, which then increased steadily to 30.3 million in 2000, before declining to 24.3 million in 2004 (Hamasaki & Kitada, 2008). Despite this, there has been an overall decline in total landings of abalone in Japan (~6500 t in 1970 to ~2000 t in the mid-1990s)(Hamasaki & Kitada, 2008). The case of the Japanese reseeding highlights the considerations that need to be made when undertaking stock enhancement.

2.1.3.1 Reseeding examples and results

Earlier studies on reseeding H. iris have shown promising survival rates, but have not been deemed economically viable (Booth & Cox, 2003). A more recent study modelled the economic viability of reseeding. A return on investment of 20%yr⁻¹ at 10% survival to harvest, with a cost of NZ$0.32 per 10mm shell length of seed was forecast (Roberts et al., 2007). With no long term studies providing evidence that reseeding is viable, the commercial industry has not yet invested in large scale reseeding programmes. However, there is continued interest from iwi and community groups that wish to restore their local fisheries for recreational or customary purposes (Turner et al., 2013; McCarth et al., 2013). This was the basis for the reseeding that took place in the East Otago Taiāpure and Punawai O Tōriki Mātaitai (PWTM). Other iwi have attempted reseeding initiatives using juvenile H. iris. This was undertaken by Ngāti Kahungunu Iwi Incorporated (Whyte & Craig, 2012). The reseeding was undertaken due to concerns over diminishing stocks. It is unclear, however, if monitoring of the released animals is going to be undertaken.

2.1.4 Common factors relating to survival during reseeding

Previous work on abalone species has indicated that reseeding is quite complex and not as simple as growing and releasing the animals for later harvest. During reseeding, the survival of the animals can be influenced by numerous factors. These factors are behavioural deficits (Schiel & Welden, 1987; Brown & Day, 2002), transport stress (Heasman et al., 2004), seeding size (Schiel, 1992; de Waal & Cook, 2001; Dixon et al., 2006; Roberts et al., 2007), site selection (Schiel, 1992; de Waal & Cook, 2001; de Waal, 2010; Roberts et al., 2007; de Waal et al., 2013), release density (Goodsell et al., 2006; Roberts et al., 2007), and release technique (Guzman del Proo et al., 2004). All of these factors can work to reduce or improve the survival rates depending on how they are considered and utilised.
Behavioural deficits can occur in animals that have undergone long-term captivity since birth, this may lead to differences in responses to environmental cues and lower the chances of survival (see review by Brown & Day 2002). Laboratory predation experiments have shown that wild *Haliotis rufescens* experienced significantly lower mortality than cultured animals which was linked to sluggish responses (Schiel & Welden, 1987). However, field experiments of translocated wild, and reseeded hatchery, juvenile *Haliotis fulgens* showed no significant difference in natural mortality rates (Guzman del Proo et al., 2004).

Transport stress has been researched using *Haliotis rubra*. This was done to as a need arose to deploy batches of several hundred thousand animals for reseeding (Heasman et al., 2004). *H. rubra* animals showed that being transported in damp conditions, as opposed to wet, at 14°C allowed for up to 95% survival after 48hrs (Heasman et al., 2004). Metabolic stress, as indicated by increased lactate and tauropine with an associated decrease in ATP and energy charge, has been seen in *Haliotis iris* during air exposure. This suggests that this will affect survival rates during transport (Wells & Baldwin, 1995).

There is increased survival with increasing seed size of released *H. iris* (Roberts et al., 2007), *Haliotis midae* (de Waal & Cook, 2001), *Haliotis mariae* (de Waal et al., 2013), *Haliotis kamtschatkana* (Emmett & Jamieson, 1989). This is thought to be because of predator-prey relationships. It is generally accepted that survival increases with seed size up until 30mm, so it is recommended that released animals should be >30mm for best survival rates (de Waal & Cook, 2001; de Waal et al., 2003; Roberts et al., 2007; Hamasaki & Kitada, 2008).

Site selection is considered to be one of the major factors that influences survival during reseeding abalone. There are multiple site characteristics that should be considered. This is mentioned throughout the literature (Hamasaki & Kitada, 2008; Dixon et al., 2006; Rogers-Bennett & Pearse, 2001; Searcy-Bernal et al., 2013), highlighting the importance of taking care to identify suitable areas of habitat prior to reseeding. The characteristics that have been considered in previous studies emphasise the range of influences that can be present at a site. The range that has been considered includes characteristics such as the presence of urchins (Andrew & Underwood, 1992; de Waal, 2010), presence of adult abalone (Naylor & McShane, 2001), wave exposure (Schiel,
Release density is thought to influence survival indirectly due to the competition for resources. This occurs between the reseeded juvenile abalone and the wild juveniles that may already be present at the reseeding site (Hamasaki & Kitada, 2008; Baskett & Waples, 2013). Release densities of up to 300 m$^{-2}$ have been seen to still allow good growth and survival or *H. iris* (Roberts et al., 2007). However, there is also an argument that the release density should be at a density that matches the natural density of the wild population (Goodsell et al., 2006). The use of different release devices is thought to aid in the survival of reseeded juveniles. Handling devices help to decrease handling stress and can also provide predator refuge upon the immediate release (Guzman del Proo et al., 2004; Dixon et al., 2006).

### 2.1.5 Aims

The overall goal of the present study was to generate an idea of the success of the reseeding that occurred in April 2013. Specifically, the intention was to provide estimates of the number of juvenile *Haliotis iris* reseeded into the EOT and the PWTM. And to provide an estimate of the proportion of juvenile *H. iris* that survived reseeding at sites in the EOT and PWTM. In addition to this, a comparison of the proportion of surviving reseeded individuals to the wild resident juvenile *H. iris* was conducted. This was carried out to try understand if the reseeding had enhanced the juvenile abundance at the reseeding sites. A comparison of the lengths frequency distribution of the pre-reseeding hatchery *H. iris* against the surveyed wild and post-reseeding juveniles was carried out. This was to get an indication of survival, as the size at reseeding is seen as a good indicator of survival (Roberts et al., 2007). It is hypothesised that the survival rates found in the present study will be low in comparison to those found in other studies. This is hypothesised for multiple reasons which include the animals appearing lethargic and stressed during reseeding. They were not cooled during transport, which took 24-48hrs depending on the site, and human error during reseeding process potentially causing mortality. It is also hypothesised that the total number of wild juvenile *H. iris* will be less than the number of reseeded individuals. This is predicted because of the large quantity individuals reseeded.
2.2 Methods

2.2.1 Permit and Histopathology report

Before the release of Haliotis iris for reseeding, or any stock enhancement for that matter, a permit was obtained from the Ministry of Primary Industries. The permit included general information on the reseeding. This information included the location, GPS coordinates, number of reseeds, time and date of release, brood stock source, hatchery location and protocols. In addition to this information, the permit required a histopathology report to ensure no harmful diseases or parasites were likely to be introduced into the recipient site via the reseeds. The permit was granted, indicating the animals were clear of disease prior to release. A list of what was required for the permit can be found in the Section 7.1 of the Appendix. The histopathology report provided by the Cawthron Institute can be found in Section 7.2.

2.2.2 Housing, packaging and transportation of hatchery juveniles prior to release

The juvenile Haliotis iris were housed at Ocean Beach Properties prior to release. Ocean Beach Properties is located in Bluff, Otago, New Zealand (Address: 250 Ocean Beach Rd, Ocean Beach 9814, New Zealand. Phone: + 64-3-2128659). The tanks were supplied with filtered sea water via a flow-through system. The animals were bred and raised at this facility. The animals were bred from adult brood stock from two locations, Bluff and Kaikoura. The animals were fed with naturally occurring seaweeds that were sourced from local beaches. Prior to packaging for transport, the tanks housing the animals were drained of all seawater. The animals were then induced to release from the sides of the tank by application of a solution. The solution was comprised of 5% white vinegar 95% seawater. Once the animals had released from the tank, they were then placed on mesh fabric in buckets. The animals were placed on the mesh to avoid them attaching to the buckets. The buckets were supplied with flow-through seawater. This was done in order to wash off the vinegar solution (Figure 2.1). The animals were washed for approximately 5min. Following washing, the animals were placed into mesh bags (Figure 2.2). The mesh bags were then suspended in v-tanks supplied with flow-through seawater (Figure 2.3). The animals were kept in these tanks until the logistics of the operation dictated that it was time to transport them. During transit the animals were placed into polystyrene containers. The containers were lined with newspaper with a layer of bubble wrap on top. The purpose of the newspaper was to absorb excreted wastes from the juveniles. The bubble wrap raised the animals above any excreted
The animals were transported by car from Ocean Beach Properties in Bluff to the PWTM, approximately 2hrs drive, and the EOT, approximately 3 hrs drive. This took place on the 2 – 5th of April 2013. This mode of transport was chosen as it was the fastest and most affordable option available. The polystyrene boxes were transported in a trailer and on the bed of a truck. Once the animals had left the hatchery they were exposed to the ambient temperature within the polystyrene bins. The animals were not submerged in seawater again until they were released. No temperature control or recording was put in place. Attempts were made to keep the animals as cool as possible by avoiding direct sunlight and ensuring adequate shade.

Figure 2.1 - Collected juvenile *H. iris* on mesh in buckets. The animals are being washed with seawater to remove vinegar solution.
2.2.3 Site Selection

The selection of the sites to be reseeded was based on previous survey work carried out by (Hepburn et al., 2010; Richards, 2010; McCowan, 2012). This work identified suitable areas that contained juvenile *Haliotis iris* habitat. In general, these sites were below the low-tide mark, and had crustose coralline algae present. The sites also
contained complex substrate habitat. This consisted of boulders on boulders; boulders on reef; or cobble on reef. These areas provide cryptic habitat that juvenile *H. iris* occupy (Schiel, 1993; Roberts et al., 2007). In addition to this, sites were preferred if they were less exposed to wave action, either by protection via fringing reefs or being located on the inside of bays.

### 2.2.4 Release of juveniles

All animals released into the PWTM were released within 24hrs of leaving hatchery. The animals released into the EOT were all released within 48hrs of leaving the hatchery. The majority of animals released on the second day. This was due to time and safety concerns to do with divers being in the water. No reseeding was undertaken when there was not sufficient daylight. The mesh bags containing the animals were taken by divers and placed into the selected sites by hand. If possible, the animals were placed into cracks and crevices that provided cryptic habitat and protection from predators. Divers were directed to release the animals at a density no greater than 300 m$^{-2}$ (Roberts et al., 2007). However, this was not specifically measured. Observations suggest that densities were below this (pers.obs). The animals were released at a depth of 0.5 – 1.5m. The size of the area reseeded was about 100m stretch of coast from the low tide mark to 1.5m depth. This was the area at each location. There were mortalities that occurred but were not recorded. It was apparent that the mortalities had occurred during both the transport and release of the animals (personal observation).

### 2.2.5 Estimation of quantity and length of juveniles released

The initial estimation of the quantity and length of the *Haliotis iris* being reseeded was carried out hastily at Ocean Beach Properties. In an effort to obtain better estimates the quantity and length of the juveniles were estimated a second time. This was done immediately prior to release. A sub-sample of the juveniles to be reseeded was taken from the population. *H. iris* were selected by haphazardly choosing one of the mesh bags containing the animals from each of the polystyrene boxes used for transport. The number of individuals in each bag was recorded. The mean (±1 standard error) number of individuals per bag was calculated. The number of bags reseeded into each site was recorded and multiplied by the mean (±1 standard error) number of individuals per bag to estimate the number of individuals released at each site (N = 9).

The length, in millimetres, of the first 50 individuals from 15 bags was recorded. The bags were sampled haphazardly from the polystyrene boxes the animals were
transported in, as these boxes contained animals from the same tanks. Only one bag was taken from each of the boxes. The lengths were then averaged to estimate the average size of the animals released.

### 2.2.6 Post-reseeding surveys

The reseeded sites were surveyed at 7 months post-reseeding. Each site was marked by using Expocrete, a type of cement usable in marine environments, and a plastic tag to define the length of the area. Post-reseeding surveys were conducted in a similar manner to the complete surveys conducted by Roberts et al (2007). The reseeded areas were actively surveyed by searching crevices and overturning all possible boulders. Any boulders that could not be overturned were searched visibly or by hand as thoroughly as possible. The number and length of the *Haliotis iris* that were found at each site were recorded. This included both wild and hatchery animals. The hatchery animals could be identified easily due to the distinct blue-green colouration of their shells. Only animals below 70mm were measured, as these are considered juveniles. These surveys were extremely labour intensive and often had to be conducted by snorkelling. This type of survey only provides a minimum estimate of survival. This is because some animals were missed due to juveniles being present beneath immobile boulders and difficult to search crevices. The sites surveyed were chosen due to their accessibility and time constraints. The post-reseeding surveys were carried out at Warrington, Brinns Point, and PWTM (N = 3). The Warrington and Brinns Point sites were located within the EOT. The third site at PWTM consisted of three sub-sites Te Tau o Waea, O Raki Waea, and Taumata Kotare. The data from these three sub sites was pooled as only the total number reseeded into the PWTM was known. The number reseeded into each individual site was not known.

### 2.2.7 Time-series of wild and reseeded juvenile numbers

A sub-site at Warrington was surveyed 3 times at 1, 2 and 28 weeks post reseeding. This survey used 3 permanent transects to monitor the change in total number of wild and reseeded *Haliotis iris* across time. Each transect was 30m x 2m. The transect area was searched using the same methods as described in the post-reseeding surveys. The search area was limited to the transects.

### 2.2.8 Estimation of survival of reseeded individuals

The minimum estimate of survival for reseeded individuals was calculated as a percentage. The number of *Haliotis iris* released at the site divided by the number found
in the 7 month post reseeding survey, multiplied by one hundred. The exception to this was the Brinns Point survey which was surveyed 2 weeks post-reseeding. The reseeding at Brinns Point saw many lifeless animals at the time of release, indicating mortality. This survey found no surviving individuals, only large amounts of empty shells, so this site was not surveyed again under the assumption that no individuals survived.

2.2.9 Data Analysis

An analysis of the lengths of juvenile *Haliotis iris* pre-reseeding, post-reseeding and wild was carried out using pooled length data from the post-reseeding surveys and the initial length estimation of the hatchery juveniles. This analysis was under the null hypothesis that the groups did not differ significantly in length. Length frequency diagrams were constructed to visually compare the lengths of the hatchery *H. iris* pre and post reseeding along with the wild length data. The data was examined for normality using a Shapiro-Wilks test. The data failed the Shapiro-Wilks test so a Kruskal-Wallis nonparametric test was employed. A Kruskal-Wallis nonparametric test was used to check for significant differences between the groups, followed by a post-hoc Tukey-Kramer HSD to identify the group pairs that differed significantly.

The time-series of estimated wild and reseeded juvenile *H. iris* abundance at 1, 2 and 28 weeks in permanent transects was tested for effects of origin of juveniles (wild or reseeded) and time using a regression analysis of variance.

The total number of reseeded and wild juvenile *H. iris* across of the 5 reseeded sites tested for normal distribution by a Shapiro-Wilks test, while Levene and Bartletts tests were used to determine equal variance. This was followed by a one-way ANOVA to test for significant differences between the number of wild and reseeded juveniles present at 3 reseeded sites (Warrington, Brinns Point, and PWTM) post-reseeding.

All analysis were carried out using JMP v.11 while the graphs were constructed using Microsoft Excel 2010.
2.3 Results

The estimated total number of juvenile *Haliotis iris* reseeded, into both the EOT and PWTM, showed large variation with an estimated mean total of $125543 \pm 17405$ (Table 2.1). The minimum survival rate of the reseeded juvenile *H. iris* was extremely low. The minimum survival rates ranged from $0.014 – 0.434 \%$ of the estimated mean number reseeded into the respective site (Table 2.2).

The length-frequency data was not normally distributed. This was identified using a Shapiro-Wilks Goodness-of-Fit test. Following this a nonparametric Kruskal-Wallis with a 1-way test, Chi Square Approximation, was used to test for the differences between the groups. The results showed that there was a significant difference between the groups ($\chi^2_{(2,1070)}=231.55, p = 0.0001$). A post-hoc Tukey-Kramer HSD revealed a significant difference between the wild and other groups, both pre-reseeding and post-reseeding. There was no significant difference found between the pre-reseeding and post-reseeding group (Figure 2.4).

The time-series of the mean number of wild and reseeded juvenile *H. iris* at Warrington, post-reseeding, showed no significant difference. This was following a regression analysis of variance between the wild and reseeded juveniles ($R^2=0.169, p=0.4436$) (Figure 2.5). No difference was found over the time post-reseeding. No correlation between the origin of the animals, the time post-reseeding and the number found was observed (Table 2.3).

The mean number of juvenile wild and reseeded *H. iris* found at Warrington, Brinns Point, and PWTM was shown to be normally distributed (Shapiro-Wilks test) and had equal variance (Levene and Bartlett’s test). There was no difference between the mean number of wild and reseeded juveniles across the 3 sites (One-way ANOVA $F_{(1,5)} = 1.0256, p = 0.184$), see Figure 2.6.
Table 2.1 - The estimated number of hatchery reared *H. iris* released into sites in the East Otago taiāpure and the Punawai O Tōriki mātaitai.

<table>
<thead>
<tr>
<th>Site</th>
<th>Estimated number released ± 1 S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warrington</td>
<td>17038 ± 2362</td>
</tr>
<tr>
<td>Puketeraki</td>
<td>14796 ± 2051</td>
</tr>
<tr>
<td>Matainaka</td>
<td>19280 ± 2673</td>
</tr>
<tr>
<td>Omimi</td>
<td>13003 ± 1803</td>
</tr>
<tr>
<td>Big Rock sheltered</td>
<td>9416 ± 1305</td>
</tr>
<tr>
<td>Big Rock exposed</td>
<td>8519 ± 1181</td>
</tr>
<tr>
<td>Mapoutahi</td>
<td>12106 ± 1678</td>
</tr>
<tr>
<td>Brinns Point</td>
<td>13899 ± 1927</td>
</tr>
<tr>
<td>PWTM</td>
<td>17486 ± 2424</td>
</tr>
<tr>
<td>TOTAL</td>
<td>125543 ± 17405</td>
</tr>
</tbody>
</table>

Table 2.2 – Estimated minimum proportion of surviving reseeded juvenile *H. iris* post-reseeding. Survival estimates are based on post-reseeding surveys 2 weeks (Brinns Point) and 28 weeks after (Warrington and PWTM) reseeding.

<table>
<thead>
<tr>
<th>Estimated number of <em>H. iris</em> released ± 1 S.E.</th>
<th>Minimum number of survivors</th>
<th>% survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warrington</td>
<td>17038 ± 2362</td>
<td>74</td>
</tr>
<tr>
<td>PWTM</td>
<td>17486 ± 2424</td>
<td>16</td>
</tr>
<tr>
<td>Brinns Point</td>
<td>13899 ± 1927</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 2.4 – Length-frequency diagrams of the juvenile *H. iris* in the hatchery stock pre-reseeding (A), post-reseeding (B), and the surveyed wild stock (C). n = 750 (A), n = 90 (B), n = 230 (C).
Figure 2.5 – Time series of the mean number of wild and reseeded juvenile *H. iris* at Warrington post-reseeding across permanent transects.

Table 2.3 – Results of regression analysis of variance on the time-series of reseeded and wild juvenile *H. iris* at Warrington.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>1</td>
<td>40.5</td>
<td>0.1521</td>
<td>0.7024</td>
</tr>
<tr>
<td>Week</td>
<td>1</td>
<td>237.565</td>
<td>0.8921</td>
<td>0.3609</td>
</tr>
<tr>
<td>Origin-Week</td>
<td>1</td>
<td>480.172</td>
<td>1.8032</td>
<td>0.2007</td>
</tr>
</tbody>
</table>
Figure 2.6 - Total number of wild and reseeded juvenile *H. iris* found during post-reseeding surveys. The East Otago taiāpūre sites are Warrington and Brinns. The Punawai O Tōriki Mātaitai.
2.4 Discussion

2.4.1 Factors influencing the survival of reseeded juvenile *Haliotis iris*

The survival rates found in the present study were very low (<0.05%). The discussion below considers factors that, individually, may have contributed to the low survival. However, the resulting low survival rates are most likely due to a combination of factors acting together.

2.4.1.1 Survey technique

One of the underlying problems with reseeding studies, or studies of juvenile abalone populations in general, is the ability to accurately estimate the abundance of the animals in the wild. This is due to the cryptic habitat the juveniles occupy which makes it difficult to sufficiently sample them (McShane & Smith, 1988). The juvenile *Haliotis iris* occupy cryptic habitat on shallow rocky reefs (<5m depth) with microhabitats beneath boulders and within crevices (Roberts et al., 2007; Schiel, 1993). These microhabitats have little to no sediment (Chew et al., 2013). The problem with sampling this type of substrate is that it is physically difficult to access the spaces beneath boulders. This is especially the case if there are multiple layers of boulders that are too large to move. However, complete survey methods have been employed to estimate the survival rates of reseeded *H. iris* in the past (Roberts et al., 2007). This technique is extremely labour intensive as all crevices and boulders are searched and turned over, if possible. This technique only provides a minimum estimate of survival because individuals are missed if they are underneath boulders or within crevices that cannot be searched (Guzman del Proo et al., 2004; Roberts et al., 2007). Roberts et al (2007) used a combination of complete surveys and randomly placed quadrats to estimate survival. This combination showed survival averaged $21 \pm 6\%$ (95% CI) for 5mm seed, $49 \pm 13\%$ (95% CI) for 20mm seed at 3 – 4.5 months post-release, with an average survival of 13.8% at 20 months. The post-reseeding surveys undertaken in the present study are similar to the complete surveys used by Roberts et al (2007) and so only a minimum estimate of survival is provided. This is because reseeded animals beneath boulders and crevices that were difficult to search would have been missed. The true survival rates may be higher. However, the survival rates in the present study were still unusually low (<0.05%), especially when considering the survival rates found at 20 months by Roberts et al (2007).
2.4.1.2 Release technique

The animals in the present study were released in an inconsistent manner. Attempts were made to place animals into or beneath crevices and boulders. Due to the difference in the divers’ free-diving ability and exposure to waves it was not always possible to wait for animals to attach. It is thought that the use of seeding release devices during the release of the animals can improve survival (McCormick & Herbinson, 1994; Goodsell et al., 2006). An annual survival rate of 30% over 3 years was found for Haliotis fulgens in a study that released 529 cultivated juveniles (Guzman del Proo et al., 2004). The release method used by Guzman Del Proo et al. (2004) took more care to place animals than the present study which may have increased the survival rate. The juveniles were either placed out in batches of 25 – 30 inside of PVC tubes or attached by hand to the underside of boulders. This was at a density of 4 – 7 per boulder (Guzman del Proo et al., 2004). The use of PVC piping is thought to reduce the stress of handling and initially protect from predation (McCormick & Herbinson, 1994; Dixon et al., 2006). Attachment of the individuals, onto boulders, would have allowed the animals to move away without having to right themselves. During righting a juvenile is exposed to higher risk of predation (Chew et al., 2013; Donovan et al., 1999). The use of seeding devices is not always linked with high survival rates. A seeding device used by Goodsell et al. (2006) showed recovery rates, equivalent to survival rates of reseeded individuals at 0.05 – 2% recovery after 6 months. The use of a seeding device in the present study may have helped to increase the survival of the reseeded animals. If animals were already in seeding devices then the divers would have been able to place the devices into cracks quickly and efficiently without handling the juvenile Haliotis iris. In addition, predation by finfish and starfish present (pers.obs) may have been reduced while the juveniles were remained within the device.

2.4.1.3 Release density

The release density of the juvenile Haliotis iris in the present study was higher than that used by Guzman Del Proo et al (2004). However, this is not believed to have contributed to the lower survival rates as juvenile H. iris have been released at densities of up to 300 per m$^{-2}$. Release at this density produced survival rates of up to >40%, over 3 months (Roberts et al., 2007), which was the maximum release density aimed at in the present study. The seeding of small numbers of Haliotis rubra (1 – 3 abalone per m$^{-2}$) to match the density of the natural populations is potentially more productive and ecologically conservative (Goodsell et al., 2006). This is because the release of fewer
and larger *H. rubra* can sustain populations close to the average natural densities of adults (Goodsell et al., 2006). This could be a more productive option for community management areas as it would allow for more care to be taken when releasing animals. The larger size would also mean the animals are more likely to survive (de Waal & Cook, 2001; de Waal et al., 2003; Roberts et al., 2007).

### 2.4.1.4 Conditions during transit

The temperature and air exposure which the animals were exposed to during the transit between hatchery and release sites is likely have caused high mortality prior to reseeding. The temperature that the animals were exposed to between the hatchery and reseeding was the ambient temperature. It was, unfortunately, not measured. Juvenile *Haliotis iris*, 10 – 60mm in length, have a critical thermal maximum, when in seawater, of approximately 28 – 29 °C. A critical thermal maximum is the point when 50% mortality occurs (Searle et al., 2006). It is also known that exposure to air causes a stress response in the animals. Larger animals are survive for longer when exposed to air (Wells & Baldwin, 1995). Experiments to improve transport of *H. rubra* for reseeding showed that being transported in damp conditions, as opposed to wet, at 14°C allowed for 95% survival after 48hrs (Heasman et al., 2004). The lack of temperature control and exposure to air during the transport of these animals is likely to have caused major mortality during this reseeding. This is thought to have contributed to the low survival that was seen at Brinns Point. The animals reseeded at Brinns Point were released on the 2nd day. Those at Warrington and PWTM were released on the 1st day. Release on the 2nd day would of exposed the animals to the air and temperature for a greater length of time. The use of refrigerated transport, is recommended for any future reseeding efforts.

### 2.4.1.5 Site selection

Site selection is considered to be one of the most important factors influencing the survival of juvenile *Haliotis iris* when reseeding (Schiel, 1993; Roberts et al., 2007). For example, the presence of substrate such as boulders, is thought to cause mortality because of movement during extreme weather events so selection of sites with large boulders or protection from extreme weather can help survival (Schiel, 1993; Roberts et al., 2007). The presence of cryptic habitat for the juvenile *H. iris* to take refuge in is another example, and should be considered when selecting sites (Schiel, 1993; Roberts...
et al., 2007). This is not exclusive to *H. iris*. It has been recommended that site selection for reseeding *Haliotis midae* should take into account the physical substratum’s suitability to provide shelter (de Waal, 2010). There is a positive correlation between *H. midae* survival and stacked boulders with diameter less than 30cm. The stacked boulders provide shelter and habitat (de Waal & Cook, 2001). Site selection was vital to the increase in recovery-rates of *Haliotis mariae* during an experimental reseeding. Sites were selected based on criteria (de Waal et al., 2013). The criteria for selection was; the presence of cracks, crevices and boulders for the juveniles to seek refuge in, the presence of sea urchins and sea cucumbers, the presence of adult and juvenile abalone, abundant crustose coralline algae, and depths <8m (de Waal et al., 2013). The importance of site selection for the reseeding of different species of *Haliotis* is mentioned throughout the literature (Hamasaki & Kitada, 2008; Dixon et al., 2006; Rogers-Bennett & Pearse, 2001; Searcy-Bernal et al., 2013). This highlights the importance of taking care to identify suitable areas of habitat prior to reseeding. The results from the present study also indicate that site selection plays an important role. The difference in the results from the post-reseeding surveys, where Warrington displays a much greater total for wild and reseeded than the other sites, helps to show this. The substrate at Warrington was much more complex. Layers of boulders on boulders which appeared to provide much greater interstitial space, the space beneath boulders, were found at Warrington. Interstitial space is positively associated with *H. iris* abundance (Aguirre & McNaught, 2012).

The time between the reseeding and post reseeding survey of PWTM included an unusual hydrological event. Large flooding in the catchment that surrounds the PWTM area caused an excess of freshwater to exit the river mouth that is located close to the PWTM (<1km). The flooding event was followed by major a die-off of *H. iris* within the PWTM. Piles of hundreds of dead *H. iris* were washed up on the beach following the event. It is thought that the excess freshwater exited the river mouth and washed along the coast and over the reefs where the *H. iris* were. The freshwater then caused the large die-off of the *H.iris*. It is possible that this event also negatively influence the survival of the reseeded juveniles at PWTM.

### 2.4.1.6 Emigration from release site

The present study did not take into account the movement of hatchery juveniles out of the reseeded areas. If the reseeded *Haliotis iris* had dispersed outside of the survey areas then a bias in the estimate of survival rate would exist. Emigration of reseeded juveniles
has been considered in studies of *Haliotis midae* and has shown juveniles will move less than 5m (de Waal et al., 2003). It was argued that the juveniles will actively disperse when exposed to adverse weather conditions such as wave and surf action (de Waal et al., 2003). This may have been the case for the animals released in the PWTM where it is often exposed to adverse weather, especially during the winter months.

### 2.4.1.7 Size

The size of the released individuals is positively related to the survival rates of reseeded abalone. Larger individuals are more likely to survive. *Haliotis iris* show an increase in survival rate with increased seeding size, however when this is weighed against the cost of the larger seed size then the economic benefit is lost (Roberts et al., 2007). A direct link between short-term survival, 3 months, and release size of *Haliotis midae* has been established (de Waal & Cook, 2001). Survival was seen to increase from a minimum average of 24% for seed 13.87 mm (SD ± 1.73mm) in length to 59% for seed 26.61 mm (SD ± 1.33 mm) (de Waal & Cook, 2001). *Haliotis mariae* also exhibited a positive relationship between average seed size and increased recovery rates (de Waal et al., 2013). It is generally accepted that survival increases with seed size up until 30mm (de Waal & Cook, 2001; de Waal et al., 2003; Roberts et al., 2007). The size of the animals reseeded in the present study was approximately 30mm. This suggests that the proportion of animals to survive should have been higher. It is then indicated that other factors have influenced the survival. The finding of no difference between the pre-reseed and post – reseed lengths also supports this theory. If there was size related survival at work, then you would expect to see a selection for the larger sizes, especially those >30mm (de Waal & Cook, 2001; de Waal et al., 2003; Roberts et al., 2007). The significantly greater mean length of the wild animals is not surprising as the hatchery bred animals were spawned in a few one-off spawnings. The length-frequency distribution of the juvenile wild animals likely shows the lower end of the length frequency distribution of the wild population. Larger *H. iris* are also easier to find as they emerge from cryptic habitat. This may account for the greater number of wild *H. iris* found close to the 70mm limit (Naylor et al., 2006; Aguirre & McNaught, 2012)

### 2.4.2 Proportion of wild and reseeded juvenile *Haliotis iris* post-reseeding

There was no difference in the total number of wild and reseeded juvenile *Haliotis iris* found in the present study. However, all sites except for Brinns Point showed a greater number of wild individuals. The lack of wild individuals and low number of reseeded individuals at Brinns Point is potentially because the area reseeded was not suitable.
This contrasts with the Warrington reseeding site, which shows the greatest proportion of surviving individuals. This could be explained by a number of factors. These factors include the presence of a limiting factor, in terms of the carrying capacity, for juvenile *H. iris* at the sites, the presence of suitable refuge habitat, and interactions with predators. Predators have been seen to limit the number of prey in marine reef environments (Menge & Lubchenco, 1981; Brown & Day, 2002). During surveys, the wild and reseeded *H. iris* were found in cryptic habitat beneath rocks (personal observation). Predatory fish were seen to feed on exposed juveniles when rocks were flipped during surveys (personal observation). Assuming that the cryptic habitat was limiting, and was close to carrying capacity before the reseeding, then the low survival of hatchery-reared animals could be due to exposure to predators and a lack of cryptic habitat.

The lack of baseline wild juvenile abundance data means that it cannot be tested to see if the release of hatchery reared juveniles has increased the overall abundance at the reseeding sites. The proportion of wild to reseeded individuals at Warrington indicates that reseeding has the potential to increase the juvenile abundance by ~ 43%. However, it is conversely possible that the presence of reseeded individuals has caused the displacement of wild juvenile *H. iris* that were already present (Leber et al., 1995; Molony et al., 2003).

The time-series data showed no difference in total wild and reseeded juvenile *H. iris* at 1, 2 and 28 weeks post reseeding. The data suggests that there may have been an increase in the mean number of wild individuals, but this was not detected. If it was present it is possible that the ability of the divers to accurately identify and find the cryptic wild animals improved over time. The shells of the hatchery reared individuals are easily distinguishable in the cryptic habitat where as those of the wild individuals tend to blend in with the surrounding environment (pers. obs).

This study could be improved using some of the following suggestions. Prior to the reseeding, baseline surveys should be undertaken. The same survey technique should have then been used to carry out the post-reseeding surveys. The areas to be reseeded should have been clearly defined. In addition, all of those volunteers involved should have been briefed prior to the reseeding so that they understood the scientific significance of the work and importance of the techniques used.
During transport temperature recordings should be taken. This is to record the ambient
temperature and the temperature inside the polystyrene boxes. These recordings would
be carried out at regular intervals, for example every 30min, to monitor the temperature
that the animals were exposed to. Before releasing the animals into the sites, all
mortalities should be removed. The number of mortalities would then be recorded. This
would allow for estimation of mortality caused during transport and would ensure only
healthy alive individuals were reseeded.

In addition, sub-sites should be established within each reseeding area. Into each sub-
site, known quantities of individuals should be reseeded. This would give a more
comprehensive and robust estimate of survival following post-reseeding surveys. When
used in conjunction with the baseline surveys, it would be possible to see how the
reseeding contributed to the abundance of *H. iris* in the areas. It would also allow for
comparison of wild and reseeded *H. iris* abundance post-reseeding.
3. Genetic considerations on the introduction of hatchery-reared *Haliotis iris* into the East Otago Taiāpūre

3.1 Introduction

3.1.1 Genetic risks associated with stock enhancement

Large scale releases of animals reared in captivity for the purpose of stock enhancement have the potential for downstream adverse genetic changes (Laikre et al., 2010; Bell et al., 2006). The interbreeding between the recipient wild population and the offspring raised for stock enhancement is the mechanism through which the adverse genetic effects occur (Utter, 1998). These changes have been grouped into four types: loss of genetic variation; loss of adaptations; change in population composition; and change in population structure (Laikre et al., 2010). Despite the gene-level associated risks that are connected with such releases, it appears that releases are largely neglected when it comes to genetic monitoring (Laikre et al., 2010).

Many of the important decisions that can lead to deleterious genetic effects downstream occur at the hatchery level (Le Vay et al., 2007; Bert et al., 2007; Evans et al., 2004; Laikre et al., 2010; Roodt-Wilding, 2007). The use of brood stock that are representative of the wild population is crucial to avoid reduction in the effective population size and helps to avoid a genetic bottleneck (Le Vay et al., 2007; Laikre et al., 2010). The bottleneck effect occurs when a new population is establish with a small number of individuals from a larger population, or alternatively, when there is a sharp reduction in population size. There is an associated reduction in the gene pool due to the small number individuals contributing reproductively to the population, which in turn causes genetic drift (Nossal et al., 2004). The loss of genetic make-up is difficult, if not impossible, to recover once it occurs. Care must be taken to preserve the genetic variation found in the recipient population (Bert et al., 2007). The presence of negative fitness effects and reduced genetic variation on hatchery-reared fish are shown to be present throughout the stock enhancement literature. This suggests hatchery practices need to be improved (Araki & Schmid, 2010). Effective genetic monitoring strategies for stock enhancement should be able to detect genetic diversity, fitness and effective population size differences between the wild and hatchery populations (Bert et al.,
The concern over the genetic risks associated with releasing hatchery-reared progeny are not new. The concern over finfish releases have existed for over 35 years (Utter, 1998). There are lessons to be learnt from this history about the management and conservation of genetic variation (Utter, 1998; Nagata et al., 2011). Due to unregulated activity that spans centuries, finfish releases have encountered problems with hybrid swarms, losses in genetic variation in cultured and wild populations, and adaptive divergences between cultured and wild populations (Utter, 1998; Hindar et al., 1991). Large scale releases of finfish can change the genetic characteristics of a population via gene flow. This potentially results in changes in population genetic structure, change of genetic composition, breakdown of intrinsic/extrinsic genetic adaptations, and loss of genetic diversity (Laikre et al., 2010).

3.1.2 Genetic risks associate with reseeding abalone and need for monitoring

The husbandry practices at the hatchery level can lead to deleterious genetic effects. These effects include a reduction of the effective population size, genetic drift, artificial selection, inbreeding and outbreeding depression (Hindar et al., 1991; Roodt-Wilding, 2007). Studies on different species of *Haliotis* that report negative genetic effects, or the potential for them to occur, have been seen in studies of hatchery reared abalone (Smith & Conroy, 1992; Evans et al., 2004; Li et al., 2004). Comparisons made between hatchery populations of *Haliotis midae* (South Africa) and *Haliotis rubra* and their respective wild populations showed a decline in genetic diversity (number of alleles per locus) of 35 – 62% (Evans et al., 2004). The present study demonstrated that changes in allele frequencies between hatchery and wild stocks can easily occur and may result in the loss of common alleles and the promotion of rare alleles (Evans et al., 2004). Another comparison of three strains of hatchery stock and two wild populations showed the presence of a bottleneck effect in the hatchery environment (Li et al., 2004). The hatchery strains showed a significant reduction in the number of alleles per locus. An average of only 24% of the microsatellite alleles present in the wild populations were expressed in the hatchery populations (Li et al., 2004). Release of hatchery reared individuals with decreased genetic diversity can then lead to a decreased effective population size. This can lead to a reduction in genetic fitness of the wild population (Roodt-Wilding, 2007).
Local adaptation of marine invertebrates has been previously thought to only occur in rare circumstances, especially in animals with planktonic dispersal. Recently, increasing evidence has shown that it is not as rare as previously thought (Sanford & Kelly, 2011). Local adaptation has been observed in *Haliotis rufescens* (De Wit & Palumbi, 2013).

Reseeding abalone, and stock enhancement in general, can promote or suppress the prevalence specific alleles in hatchery populations. Suppression of these alleles can potentially remove local adaptation (Tringali & Bert, 1998; Evans et al., 2004; Smith & Conroy, 1992). Loss of local adaptation can leave the released hatchery individuals with decreased fitness in comparison to the wild resident animals. This may lead to outbreeding depression and a loss of fitness in the wild population (Roodt-Wilding, 2007; Sanford & Kelly, 2011).

The associated genetic risks with reseeding underline the need for genetic monitoring in *Haliotis iris* reseeding programs. Genetic monitoring should involve a baseline study of the wild populations genetic profile and continued monitoring post-reseeding. In addition, identifying genetically representative brood stock and genetic analysis of hatchery populations should be carried out prior to reseeding (Roodt-Wilding, 2007; Ward, 2006). In order to understand the impact the release of hatchery abalone has on the genetic structure of the wild recipient population genetic characterisation of the hatchery strain is necessary (Li et al., 2004). The use of genetic tags for identifying released animals is also possible (Roodt-Wilding, 2007) and is discussed further in Chapter 4.

### 3.1.3 Aims

The first aim of the present study was to assess three different hatchery populations of *Haliotis iris* for their potential to have an adverse genetic impact on the recipient wild populations in the East Otago Taiāpure (EOT) and the Puna-wai o Toriki Mataitai (PWTM). The wild populations were previously studied for their fine-scale genetic population structure within the respective marine reserves. This offered an opportunity to compare the hatchery-reared populations against the baseline genetic structure present in the reserves (McCowan, 2012). Future opportunities to study the genetic structure of the resultant admixture population also exist. Microsatellite markers were used to assess the genetic suitability of the hatchery populations reseeded. Specifically, indices of genetic diversity (heterozygosity, genetic relatedness, effective population size, and number of alleles) and population differentiation (Fst, genetic distance and STRUCTURE analysis) will be assessed. It is hypothesised that the hatchery
populations will exhibit less genetic diversity than the wild populations. It is also 
hypothesised that the hatchery populations will show differentiation from one another 
and the wild populations.

3.2 Methods

3.2.1 Origin of hatchery populations
The Bluff and Kaikoura ‘populations’ were spawned, reared and housed at Ocean Beach 
Properties prior to reseeding. This included being spawned from brood stock from 
different localities around New Zealand. The designation of the populations as ‘Bluff’ 
and ‘Kaikoura’ refer to the locations where the brood stocks were sourced from.

‘Population X’ is a population of unknown origin. This is due to incomplete records of 
both hatchery procedures and brood stock from the hatchery that Population X was 
sourced. The animals that were used as brood stock were reportedly from the Shag Point 
area north of the EOT. The juvenile animals were moved to PML in July 2012.

Unfortunately, due to circumstances beyond the author’s control the location and source 
of the stock cannot be disclosed. This is in order to avoid conflict with the hatchery 
owner.

3.2.2 EOT and PWTM population sample collection
Prior to reseeding, baseline genetic surveys were conducted by Tom McCowan as part 
of his PhD thesis (McCowan, 2012). The methods used to sample the wild populations 
at EOT and PWTM are described briefly. Samples were collected at various locations 
within the EOT (6 sites) and PWTM (5 sites). The present study investigated fine-scale 
management and so the locations were chosen at relevant spatial scales for Haliotis iris 
fisheries management. This is also known as ‘reef by reef’ scales. The H. iris were 
selected at random within the defined areas to be studied. All H. iris were collected by 
snorkel (for adults) or by overturning boulders in the intertidal zone (for juveniles).

Following tissue sampling, all H. iris were returned as close as possible to the location 
that they were collected.

3.2.3 Hatchery population sample collection
During the entire process all populations were kept separate. It is assumed no genetic 
mixing or misidentification of individuals occurred. 50 animals from each population, 
for a total of 150 animals, were selected for tissue sampling. The animals were frozen
and stored in -20°C freezer at the University of Otago, Portobello Marine Laboratory (PML) before being sampled for tissue.

### 3.2.4 Tissue sampling

Tissue sampling was carried in the field for EOT and PWTM populations. The sampling for Bluff, Kaikoura and Population X hatchery populations was carried out at PML. Despite the difference in sampling in the field and the laboratory the procedures remained the same. All individuals were measured using callipers to the nearest mm. A scalpel blade was used to remove approximately 5mm$^2$ of epipodal tissue or tentacle from the foot of the *Haliotis iris*. The tissue was then placed into 1.5mL Eppendorf tubes. The tubes were then filled with 100% isopropanol. Between sampling all equipment was washed down to avoid contamination between samples. All samples were then stored in a -20°C freezer, the EOT and PWTM samples were stored at the Department of Anatomy, University of Otago, while the Bluff, Kaikoura and Population X hatchery samples were stored at PML.

### 3.2.5 DNA extraction and genotyping

Genomnz™ ([www.genomnz.co.nz](http://www.genomnz.co.nz)) conducted the sample extraction and genotyping. The following methods are the same as those used by Tom McCowan to process the EOT and PWTM samples. Extraction of DNA from each sample involved modified chelex-based extraction (Walsh et al., 1991). The epipodal or tentacle tissue was incubated in 200μl of chelex solution (5% Chelex 100, 0.1% Tween 20, Proteinase K (20mg/mL)) at 60°C overnight. Deactivation of the proteinase K was achieved by vortexing and boiling for 10mins following incubation. Each samples was vortexed and centrifuged (13,000rpm for 5mins). Supernatant was then transferred to a fresh tube. The samples were stored at -20°C before PCR was carried out. Table 3.1 shows the 10 microsatellite markers multiplex used in the PCR. The analysis of the microsatellite loci was carried out on an Applied Biosystems 3730. DNA Analyser. The analyser was set to default options and genotypes were scored using GeneMapper. The quality control was the same as that used by Tom McCowan (2012).

### 3.2.6 Analyses of genetic diversity

GenAlex 6.5 (Peakall & Smouse, 2012) software was used to determine the difference in pairwise relatedness (Queller & Goodnight, 1989) within and among the wild and hatchery populations. Prior to analysis samples that had incomplete microsatellite data were excluded from the analysis to allow it to run. Following the calculations of
individual pairwise relatedness, bootstrapping resampling was carried out. This was done to estimate the mean and confidence intervals of the null hypothesis of ‘no difference’ in relatedness. 1000 permutations and 1000 bootstraps were used in for the estimation. In addition to this, GenAlex was also used to calculate indicators of genetic diversity; observed heterozygosity (H₀); unbiased expected heterozygosity (Hₑ); number of alleles (Nₐ); and number of effective alleles (Nₑ). All indices values were taken from the average found across the 10 microsatellite loci for each population. The effective population size and associated confidence intervals were estimated using NeEstimator 2.0 (Do et al., 2013). The Molecular Coancestry method was used and all allele frequencies were taken into account when calculating the estimates.

3.2.7 Analysis of genetic population structure

Pairwise Fst values between each population were calculated using Fstat 2.9.3 (Goudet, 2001) to determine if significant population differentiation occurred between the hatchery populations and the wild populations. The p-values for the pairwise Fst estimates were obtained after standard Bonferroni corrections. GenAlex 6.5 (Peakall & Smouse, 2012) was also used to calculate unbiased Nei’s genetic distance between the populations to give an indication of the genetic divergence between the hatchery and wild populations.

The most likely number of groups (k) was determined using the software STRUCTURE 2.3.4 (Pritchard, J. K., Stephens, and Donnelly, 2000). STRUCTURE 2.3.4 uses Monte Carlo Markov Chain Bayesian clustering to maximise within cluster Hardy-Weinburg and linkage equilibrium. The highest probability of membership is used to assign individuals to clusters. Five independent runs were made for each k value which was set at twice the number of input populations. The program was run over a burnin period of 10,000 followed by 100,000 iterations. The most likely number of clusters was identified by calculating ΔK as described in (Evanno et al., 2005).
Table 3.1 - Properties of the 10 microsatellite markers used for genetic analysis.
Adapted from McCowan (2012)

<table>
<thead>
<tr>
<th>Micro ID</th>
<th>SSR Motif</th>
<th>Repeats</th>
<th>PCR Temp</th>
<th>Primer forward then reverse; 5’-3’</th>
<th>Approx. size (BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB3</td>
<td>GATA</td>
<td>13</td>
<td>58</td>
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</tr>
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<td>80</td>
</tr>
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<td>56</td>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
<td>EDN91</td>
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<td>10</td>
<td>56</td>
<td>CAT TAC GAA AAG CTG CAA ACG AAC GGT GAA TAG CAT TCT GGA</td>
<td></td>
</tr>
</tbody>
</table>
3.3 Results

3.3.1 Analysis of genetic diversity

The results primarily consider the differences between the populations that have been spawned and raised under hatchery conditions (Bluff, Kaikoura and Population X) and the populations that have been sampled in the wild (PWTM and EOT). These two groups are collectively referred to as the ‘hatchery’ or ‘wild’ populations.

The diversity indices for the hatchery populations and the wild populations are shown in Table 3.2. The hatchery populations Bluff (11.5), Kaikoura (10.1), and Population X (6.9) had lower number of alleles than the wild populations PWTM (13.2) and EOT (14.1). The same trend was seen for the number of effective alleles; Bluff (5.971); Kaikoura (5.651); Population X (4.436); PWTM (6.231); and EOT (6.271). The observed heterozygosity was higher than the expected heterozygosity in the Kaikoura and Population X populations. The other three populations showed lower observed heterozygosity than expected heterozygosity. However, none of the results showed a significant difference between the observed and expected heterozygosity.

The effective population size was calculated for each of the hatchery and wild populations. Both of the wild populations showed considerably larger effective population sizes. The EOT and PWTM were calculated as 338.4 (95% CI = 0.3 – 1699.6) and 71.4 (95% CI = 5.2 – 222.5) respectively. In comparison the mean effective population sizes calculated for the hatchery populations were low; Bluff = 31.2 (95% CI = 3.7 – 87); Kaikoura = 13.2 (95% CI = 6.6 – 22.1); and Population X = 9.7 (95% CI = 6.6 – 13.5). The overlapping confidence intervals of the effective population sizes indicate that there is no significant difference between the populations.

3.3.2 Genetic analysis of relatedness within populations

Figure 3.1 shows the Queller-Goodnight relatedness (r) within each population being compared to the null hypothesis of ‘no difference’ (r = 0) across the populations. Figure 3.1 compares the hatchery populations to the wild populations. There was a significantly greater relatedness than 0 for the EOT (p<0.01), PWTM (p<0.05), Kaikoura (p<0.001), and Population X (p<0.001) while the Bluff population showed no difference (p=0.392). The greatest departure from 0 was seen for Population X (r = 0.134 [95% CI = 0.119, 0.146]), followed by Kaikoura (r = 0.039 [95% CI = 0.026,0.051]). The significantly greater difference found for the PWTM population (r = 0.005 [95% CI = 0.003, 0.008]) had an overlap of the corresponding 95% CI with the
null hypothesis 95% CI [-0.009, 0.005]. The significantly greater difference above 0 found for the EOT population was \( r = 0.009 \) [ 95% CI = 0.007, 0.0011].

### 3.3.3 Genetic analysis of population structure and divergence

Table 3.4 shows the comparison of the pairwise Nei’s genetic distance between the wild population and the hatchery populations showed a variation in genetic distances. The greatest genetic distance was seen between Population X and all of the other populations; PWTM (0.216); EOT (0.207); Bluff (0.200); and Kaikoura (0.178).

The pairwise Fst values showed significant differentiation between Population X and all of the other populations which ranged from Fst = 0.043 – 0.049. Significant differentiation was also seen between the Kaikoura population and the PWTM (Fst = 0.012) and EOT (Fst = 0.011) populations. There was nearly no significant differentiation found between the Bluff, PWTM and EOT populations which ranged close to zero, Fst = -0.0007 – 0.0004. The Fst value between Bluff and Kaikoura also showed extremely low differentiation, Fst = 0.003.

The STRUCTURE 2.3.4 (Pritchard, J. K., Stephens, and Donnelly, 2000) analyses are shown as bar plots in Figures 3.2, 3.4, 3.6, and 3.8. These bar plots give a visual representation of the population structure between the samples populations. Each vertical line indicates an individual. The contribution of each colour to the vertical line gives the probability of the individual belonging to a certain population. The sampling populations are labelled below the figures and are separated by vertical black lines within the bar plots.

Figure 3.2 shows the bar plot for k = 3 for the hatchery and EOT populations. Visual analysis suggests a distinct population differentiation between Population X and the other three populations. There is also less distinguishable population differentiation between the Kaikoura population and the Bluff and EOT population. The ΔK plot based on the methodology by Evanno et al (2005) indicate that the likely number of populations is 3 (Figure 3.3).

The STRUCTURE analysis was repeated without the Population X data as a distinct differentiation can cause masking of potential subpopulations within other samples (Pritchard, J. K., Stephens, and Donnelly, 2000). Figure 3.4 shows the bar plots for the Bluff, Kaikoura and EOT populations for k = 2, k = 4, and k = 5. While the ΔK plot (Figure 3.5) suggests the most likely number of populations as 4 with a slight peak at 2
and 5 the bar plots are less distinct with the most notable difference being the Kaikoura population compared to the Bluff and EOT population for all three k values.

Figure 3.6 looks at the STRUCTURE bar plots for k = 2, k = 3, and k = 6 between the hatchery populations and the EOT population. The ΔK plot (Figure 3.7) shows a peak at k = 2 with another two smaller peaks at k = 3 and k = 6. This suggests that there are two distinct populations when compared with the k = 2 bar plot, with Population X showing distinct differentiation to the other three populations, this differentiation is also present in the k = 3 and k = 6 bar plots.

Figure 3.8 shows the STRUCTURE bar plot, k = 3, for the Bluff, Kaikoura and PWTM populations. There is a slight difference seen between the Kaikoura population the other two populations. The difference between the Bluff and PWTM population is less pronounced. The ΔK plot (Figure 3.9) indicates that the most likely number of populations is 3.
Table 3.2 - Average diversity indices across all loci for the hatchery and wild population showing the number of samples (n), the number of alleles (NA), the effective number of alleles (Ne), the observed heterozygosity (Ho), and the expected heterozygosity (He).

<table>
<thead>
<tr>
<th></th>
<th>Bluff</th>
<th>Kaikoura</th>
<th>Population X</th>
<th>PWTM</th>
<th>EOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>46</td>
<td>48</td>
<td>46</td>
<td>147</td>
<td>229</td>
</tr>
<tr>
<td>NA</td>
<td>11.5</td>
<td>10.1</td>
<td>6.9</td>
<td>13.2</td>
<td>14.01</td>
</tr>
<tr>
<td>Ne</td>
<td>5.971</td>
<td>5.651</td>
<td>4.436</td>
<td>6.231</td>
<td>6.271</td>
</tr>
<tr>
<td>Ho</td>
<td>0.798</td>
<td>0.813</td>
<td>0.785</td>
<td>0.803</td>
<td>0.806</td>
</tr>
<tr>
<td>He</td>
<td>0.813</td>
<td>0.796</td>
<td>0.76</td>
<td>0.816</td>
<td>0.814</td>
</tr>
</tbody>
</table>
Figure 3.1 - Mean within population pairwise values, and 95% CI, for relatedness (r) for the Bluff, Kaikoura, Population X, and Puna-wai o Toriki Mātaitai and East Otago Taiāpure populations. A comparison is made against the null hypothesis of 'No difference' (r = 0) across the populations as determined by permutation.

Table 3.3 - Estimated effective population size (Ne) for Hatchery and Wild populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Hatchery Population</th>
<th>Wild Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bluff</td>
<td>Kaikoura</td>
</tr>
<tr>
<td>Mean sample size</td>
<td>46.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Estimated Ne</td>
<td>31.2</td>
<td>13.2</td>
</tr>
<tr>
<td>95 % CI for Ne</td>
<td>3.7 – 87</td>
<td>6.6 – 22.1</td>
</tr>
</tbody>
</table>
Table 3.4 - Pairwise Population Matrix of Unbiased Nei's Genetic Distance (above the diagonal) and pairwise Fst Values (below the diagonal) Comparing the Hatchery Population to the wild populations. * significant differences in pairwise Fst values at P<0.01.

<table>
<thead>
<tr>
<th></th>
<th>Bluff</th>
<th>Kaikoura</th>
<th>Population X</th>
<th>PWTM</th>
<th>EOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluff</td>
<td>0</td>
<td>0.014</td>
<td>0.200</td>
<td>0</td>
<td>0.032</td>
</tr>
<tr>
<td>Kaikoura</td>
<td>0.0033</td>
<td>0</td>
<td>0.17816</td>
<td>0.052</td>
<td>0.081</td>
</tr>
<tr>
<td>Population X</td>
<td>0.0464*</td>
<td>0.0434*</td>
<td>0</td>
<td>0.216</td>
<td>0.207</td>
</tr>
<tr>
<td>PWTM</td>
<td>-0.0005</td>
<td>0.0117*</td>
<td>0.0486*</td>
<td>0</td>
<td>0.023</td>
</tr>
<tr>
<td>EOT</td>
<td>0.0004</td>
<td>0.0106*</td>
<td>0.0441*</td>
<td>-0.0007</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3.2 - - STRUCTURE 2.3.4 (Pritchard et al, 2000a) bar plots for Bluff, Kaikoura, Population X and the East Otago Taiāpure population at k = 3.
Figure 3.3 - ΔK vs K for STRUCTURE 2.3.4 (Pritchard et al, 2000a) analyses, based on methodology described in Evanno et al (2005) for Bluff, Kaikoura, Population X and the East Otago Taiāpure population.
Figure 3.4 - STRUCTURE 2.3.4 (Pritchard et al, 2000a) bar plots for Bluff, Kaikoura, and the East Otago Taiāpure population at $k = 2$, $k = 4$, $k = 5$.

Figure 3.5 - $\Delta K$ vs $K$ for STRUCTURE 2.3.4 (Pritchard et al, 2000a) analyses, based on methodology described in Evanno et al (2005) for Bluff, Kaikoura, and the East Otago Taiāpure population.
Figure 3.6 - STRUCTURE 2.3.4 (Pritchard et al, 2000a) bar plots for Bluff, Kaikoura, Population X, and the Puna-wai o Toriki Mataitai population at $k = 2$, $k = 3$ and $k = 6$.

Figure 3.7 - $\Delta K$ vs $K$ for STRUCTURE 2.3.4 (Pritchard et al, 2000a) analyses, based on methodology described in Evanno et al (2005) for Bluff, Kaikoura, Population X, and the Puna-wai o Toriki Mataitai population.
Figure 3.8 - STRUCTURE 2.3.4 (Pritchard et al, 2000a) bar plots for Bluff, Kaikoura, and the Puna-wai o Toriki Mataitai population at k = 3.

Figure 3.9 - ΔK vs K for STRUCTURE 2.3.4 (Pritchard et al, 2000a) analyses, based on methodology described in Evanno et al (2005) for Bluff, Kaikoura, Population X, and the Puna-wai o Toriki Mataitai populations.
3.4 Discussion

3.4.1 Comparison of genetic diversity between hatchery and wild populations

For this discussion it has been assumed that the levels of genetic diversity in the wild populations are similar to the source populations of the brood stock that were used to spawn the hatchery populations. Any differences seen between the hatchery and wild populations have been discussed under this assumption.

The results from the genetic analysis of diversity indices, relatedness and effective population size suggest that there is less genetic diversity in the hatchery populations compared to the wild populations. This is a common occurrence in Haliotis hatchery populations. The bottleneck effect will occur in hatcheries due to a small number of brood stock (Roodt-Wilding, 2007). This occurrence is not limited to abalone and is seen in the stock enhancement of other species both terrestrial, freshwater and marine (Schwartz et al., 2007; Hedgecock & Sly, 1990; Laikre et al., 2010; González-Wangüemert et al., 2012). The number of alleles and effective number of alleles for the hatchery populations were lower when compared to the EOT and PWTM populations. However, the expected heterozygosity and observed heterozygosity remained similar between the hatchery and wild populations for both analyses. A decrease in genetic diversity, indicated by loss in number of alleles, with no associated loss in heterozygosity is not an unexpected result. This has been observed in past studies comparing hatchery and wild populations of Haliotis rubra and Haliotis midae (Evans et al., 2004). This can be explained by a short-term population bottleneck where rare alleles are lost but no loss of heterozygosity is seen (Nei et al., 1975; Evans et al., 2004). As heterozygosity is insensitive to genetic changes in first generations of cultivated aquaculture populations, then the loss of rare alleles from hatchery stocks can be more meaningful than a change in heterozygosity (Hedgecock & Sly, 1990; Evans et al., 2004). This has been documented in scallops as well (Hold et al., 2013). However, other studies of Haliotis iris (Smith & Conroy, 1992; McCowan, 2012) and Haliotis discus hannai (Li et al., 2004) report reduced heterozygosity in hatchery populations being associated with a reduction in genetic diversity.

The smaller estimated effective population sizes of the hatchery populations indicate that a reduction in genetic diversity has occurred (Evans et al., 2004; Ryman & Laikre, 1991). Because of this there is an associated increased chance of loss of rare alleles and
other deleterious allele frequency effects (Tringali & Bert, 1998; Ryman & Laikre, 1991). Simulations of stock enhancement using hatchery reared scallops have shown that release of hatchery seed can lead to a decrease in the wild populations effective size (Hold et al., 2013). The variation in the effective population size between the hatchery populations and the wild populations should be monitored in the future.

The loss of genetic diversity could be due to an isolated spawning event with only a few contributing parents (Rhode et al., 2012). The maintenance of the genetic diversity of hatchery populations should occur throughout the stock enhancement process. In particular, selection of brood stock representative of the wild population, and ensuring sufficient individual parental contribution during spawning should be focused. This should be done to ensure that genetic diversity is maintained between the wild and hatchery populations (Bert et al., 2007).

The difference in genetic diversity between the hatchery and wild populations indicates that a change in the genetic diversity occurred at the hatchery. It is important to note that between the hatchery populations there are differences in the genetic diversity. This indicates that the efforts to maintain genetic diversity at the hatchery have differed. The Kaikoura population shows lower genetic diversity than the Bluff population. However, in comparison to the wild populations these two hatchery populations are relatively similar. The low Fst and genetic distance between these two populations gives further evidence. This may reflect the husbandry practices of the hatcheries that reared each population. The Bluff and Kaikoura populations originate from the Ocean Beach Properties hatchery and so the same husbandry practices are likely to have been followed. Whereas, Population X, which originates from another hatchery, shows less genetic diversity than the other two hatchery populations. Previous studies have noted that inappropriate husbandry practices may produce a high proportion of related individuals if there is discrepancy between the number of brood stock used to spawn and the number of brood stock that contribute to the resulting brood (Evans et al., 2004).

The relatedness of the hatchery populations compared to the wild populations show interesting variation. The Bluff population shows very similar levels of within population relatedness to both the EOT and PWTM populations. The Bluff population is the only population that was found to not be significantly higher than zero showing no relatedness. While both the wild populations were significantly higher than zero in their
respective analysis the level of relatedness is still very low. To put it in perspective both
wild populations showed mean relatedness to be 0.004 which is close to the level
expected between fourth cousins or unrelated individuals (Queller & Goodnight, 1989).
Kaikoura shows 0.03 which is the level between second cousins (Queller & Goodnight,
1989). Population X shows unusually high relatedness showing (0.128) that this
population consists predominantly of first cousins or half-siblings (Queller &
Goodnight, 1989). In a breeding context, it has been suggested that at least 10-13 males
and 25-50 females are used to maintain the genetic diversity in H. iris (Smith & Conroy,
1992). The level of relatedness shown by Population X suggests only 4 Adults have
been used to spawn. The high level of relatedness could also be due to variance in the
reproductive success of the brood stock caused by pre-zygotic and post-zygotic factors.
For example, the presence of sperm competition in the Pacific oyster (Crassostrea
gigas) caused a 20% decrease in effective population size which again highlights the
importance of using a limited number of brood stock (Boudry et al., 2002).

3.4.2 Population structure of hatchery and wild populations
Population X is differentiated from both the wild and hatchery populations. This is
suggested by the results from the STRUCTURE plots, Nei’s genetic distance values and
significant pairwise Fst values. The Kaikoura population appears to be differentiated
from the wild populations but to a lesser degree than Population X. The Bluff
population did not appear to differentiate from either of the wild populations. The
hatchery results portray a spectrum of differentiation from the wild population which
emphasises that the proper maintenance of genetic diversity and structure during the
rearing process can produce hatchery stock similar to the wild resident population.

The use of microsatellite analysis for determining population differentiation between
hatchery and wild strains has been used in abalone previous with success (Li et al.,
2004). Significant estimates of Fst ranging between 0.059 – 0.427 (P<0.01) were
detected between the hatchery and wild populations in Haliotis discus hannai (Li et al.,
2004). The study also analysed the genetic distances between individuals which showed
clear clustering of the different hatchery populations (Li et al., 2004). The pairwise Fst
values reported in the present study were highly significant but were very low indicating
that the allele frequencies between the populations are similar (Holsinger & Weir,
2009). The possible exception was Population X, and the Kaikoura population to a
lesser extent.
It is possible for abalone larvae to disperse over long distances and therefore have potential for gene flow over these distances (Hamm & Burton, 2000). Unbiased Nei’s genetic distance has been used to detect very small genetic distances (0.001) between populations of *Haliotis cracherodii* separated by ~10km. No relationship between genetic distance and geographic distances (up to ~300km) was observed (Hamm & Burton, 2000). *Haliotis rubra* has shown that genetic isolation by distance can occur over distances of 500km. Individuals outside of this ‘genetic neighbourhood’ area will have a greater genetic distance than animals sampled within the same locality (Brown, 1991). *Haliotis laevigata* differs in this regard across South Australia, where genetic distances are large between populations that are geographically close. This showed a genetic neighbourhood of 0km (Brown & Murray, 1992). However, the small-scale genetic heterogeneity of *H.rubra* indicated localised recruitment (Brown & Murray, 1992). The absence of genetic differentiation between EOT and PWTM populations is in agreement with the findings from the previous work done on *Haliotid* species. The use of microsatellites for precise estimation of genetic distances has been found to need sample sizes of $50 \leq N \leq 100$ (Ruzzante, 1998). The estimates of genetic distance for the hatchery populations may be less precise than the wild populations, however, the use of 10 microsatellites may have compensated for this (Ruzzante, 1998).

Although not the intention of the present study, the results also support the findings of low to moderate genetic differentiation across the distribution *Haliotis iris* in New Zealand (Will et al., 2011). The study by Will et al. (2011) did find a phylogeographic break on the southeast coast of the South Island where the present study was conducted but there was no sampling conducted near the EOT in the study. The phylogeographic break may then be present due to environmental factors north of the EOT. This may also explain the population differentiation between the wild populations and the Kaikoura population as the brood stock of this population was sourced north of the break. Whereas, there is no phylogeographic break between the Bluff brood stock and either of the wild populations. Reseeding programmes in the future may find it wise to select brood stock from between the phylogeographic breaks. However, without a genetic baseline profile of the brood stock and the source population the discussion is purely speculative.

In summary, the genetic analysis of the hatchery population, in particular Population X, has highlighted the need for maintenance of genetic diversity between the wild populations and the hatchery (Roodt-Wilding, 2007). It also highlighted that the
development of abalone conservation and management plans requires an understanding of dispersal and genetic population structure (Hamm & Burton, 2000). Future monitoring of the released hatchery populations should be undertaken to identify potential genetic impacts on the wild populations in the EOT and PWTM. The magnitude of these effects will likely be very small. This is due to the low difference in genetic diversity and the low population differentiation that the Bluff and Kaikoura stocks displayed compared to the wild populations.
4. Chapter 4 – Genetic identification of the reseeded populations in the East Otago taiāpure and Punawai O Tōrika mātaitai

4.1 Introduction

4.1.1 Use of genetic tools in fisheries management

The development of molecular genetic markers has given fisheries managers access to genetic population data. Genetic population data can provide valuable information on the management, conservation and ecology of populations (Lorenzen, 2005; Schwartz et al., 2007; Roodt-Wilding, 2007; Bartley & Bell, 2008). The use of genetic markers can allow managers to chose brood stock genetically representative of the population (Le Vay et al., 2007; Seamons et al., 2012; Gruenthal & Drawbridge, 2012), avoid the potential genetic risks of large scale releases (Laikre et al., 2010), and monitor the resulting genetic impacts of these releases (Lorenzen, 2005; Schwartz et al., 2007).

This genetic information has been used to define and differentiate within stocks of *H. laevigata* and *H. rubra* in South Australia (Shepherd & Brown, 1993). The genetic information allowed the researchers to define minimum genetically viable populations and to determine genetic neighbourhoods (Shepherd & Brown, 1993). The study identified, using a case study of collapsed abalone stocks, that a contributing factor in the collapse was that a marine protected area had only partly protected the metapopulation. If the marine protected area had been large enough then it would of lessened the risk of the number of adults declining (Shepherd & Brown, 1993). In addition, species specific recommendations, for the roles of marine protected areas, were made. The use of many small 'stepping-stone' areas was recommended for *H. rubra*, while a few large areas were recommended to protect *H. laevigata* populations. It was also warned that overenthusiastic use of marine protected areas may actually be detrimental to the population structure due to overfishing of other areas (Shepherd & Brown, 1993). This example highlights how the genetic information can be used in a multitude of ways by fisheries manages to more effectively manage stocks.
4.1.2 The use of genetic markers in stock enhancement

In the context of stock enhancement, genetic information is valuable. This is because differentiating between stocked and wild individuals is a key issue in the monitoring and assessing stock enhancement work (Bartley, 1999). Of particular interest is the ability to distinguish between hatchery bred and wild stocks once the hatchery population has been released (Roodt-Wilding, 2007; Bartley, 1999). This is of interest because it allows for estimation of the survival rate of the released stock (Sekino, Saitoh, et al., 2005). In addition, it can be beneficial for use in genetic monitoring of the resultant admixture population. This allows for assessments to be made of the genetic impact of a release (Gruenthal et al., 2014). The use of genetic tagging has already been used successfully in multiple fisheries. These fisheries include the fishery for Japanese flounder (Paralichthys olivaceus) (Sekino, Saitoh, et al., 2005), salmonids (Steele et al., 2013; Hindar et al., 1991; Utter, 1998), and mud crabs (Scylla paramamosain) (Obata et al., 2006) among others. The use of a genetic marker for mitochondrial DNA has allowed the contribution of released juvenile mud crab Scylla paramamosain to the total catch to be calculated (Obata et al., 2006). The genetic marker enabled discrimination between the released and wild stocks (Obata et al., 2006). However, in this case, it was recommended that other means of tagging should be used because the genetic marker required a large releases of juveniles to achieve sufficient statistical power, which in turn, increased the risk of long-term deleterious effects on the wild population (Obata et al., 2006).

4.1.3 Use of genetic markers in abalone fisheries management

The use of genetic markers to gain genetic information for fisheries management has already been utilised in multiple species of abalone (Roodt-Wilding, 2007). The relative ease of non-destructive sampling of abalone, the relatively affordable cost, and the ability to undertake parentage assignment makes genetic tagging via genetic markers very attractive to abalone fisheries managers (Roodt-Wilding, 2007; Slabbert & Roodt-Wilding, 2006). The main use of genetic markers in abalone stock enhancement has been to determine aspects of population structure and biology. Allozyme, protein electrophoresis, and mitochondrial DNA analysis have been used to track the success of a large scale release of H. rufescens. These techniques were effective for tracking population bottlenecks in the hatchery populations (Gaffney et al., 1996; Burton & Tegner, 2000). A panel of microsatellite markers was used to detect evidence of non-random mating in two admixture populations of H. discus hannai. The study showed the
potential of microsatellites as a means to examine released abalone and their reproductive impact (Sekino, Saido, et al., 2005). Genotyping of H. asinina larvae using microsatellites was carried out to determine the parentage of individual larvae in the hatchery (Selvamani et al., 2001). More recently use of restriction site associated DNA sequencing analysis (RADSeq) identified H. fulgens as being panmictic. The findings have interesting implications for brood stock sourcing and translocation limitations (Gruenthal et al., 2014).

4.1.4 Genetic tagging of Haliotis iris

Like other species of Haliotis with commercial importance, interest in reseeding of H. iris has prompted the need for a genetic tag. Previous work has shown that a microsatellite tag has been developed for use on H. iris (McCowan, 2012). This work used a microsatellite based population assignment to identify recaptured individuals as originating from either a hatchery or a wild population (McCowan, 2012). The results from the work by McCowan (2012) found that when using the hatchery population as a reference 99.4% of individuals could be assigned to their origin population. However, when the wild population was used as a reference assignment dropped to 70.6% of individuals.

The benefit of being able to genetically profile a hatchery population, by sampling from the brood, is that there is no need for analysis of the parents. So in an event where parentage is unknown, the offspring may still be of used for reseeding, assuming that they are appropriate stock to reseed.

4.1.5 Alternative methods of tagging

Alternative ways to identify reseeded abalone individuals have been used which have been broadly described as physical, chemical, and dietary (McCowan, 2012). The following examples are of different tag types and how they have been used. The use of numbered discs as a physical tag has been demonstrated in H. rubra (Prince, 1991). Retention and identification of physical tags, such as the numbered discs, can be problematic. Over time, the number on the tag can become ineligible from shell encrusting epibiota (Prince, 1991). Tetracycline has been used to identify H. iris by either immersing or injecting the animal. Tetracycline acts as a fluorescent tag (Pirker & Schiel, 1993). Dietary markers have proven useful as they are easily applied to juvenile abalone in the hatchery via artificial feed. H. iris retain a blue-green colour from the artificial hatchery feed (Gallardo et al., 2003; Roberts et al., 2007). This is a reliable and
relatively easy form of tag to be employed. However, it encounters the same problem as physical tags when shell encrusting epibiota prevent the colouration from being visible. (Roberts et al., 2007; Roodt-Wilding, 2007; McCowan, 2012). The genetic tags have the additional advantages of being usable for all life stages, not being size dependent, and the usual adverse effects of attaching a physical tag are not present (Obata et al., 2006).

4.1.6 Aims
The aim of the present study was to determine if the hatchery bred stocks could be successfully identified. This was done to see if it is viable to identify the hatchery population without the genetic information of the parents. The parental genetic information is not always available, as is the case in the present study. It is still important to be able to identify the released animals in the absence of this information. This information is used when the animals are released into the wild and need to be distinguished from the wild stock. This is done in order to assess the survival and abundance of the reseeded animals at a later date. Using each of the sampled populations individually, both wild and hatchery, as a reference is thought to be best option for correctly identifying individuals via genetic markers. Using a panel of 10 microsatellite markers, a random selection of individuals was tested for their probability of being assigned to a population. It was hypothesised that sufficient genetic differentiation would have occurred during the breeding of the hatchery populations to allow them to be successfully assigned to their respective original population.

4.2 Methods
4.2.1 Sample collection
The methods for the sample collection for each population were the same as those explained in Chapter 3.

4.2.2 DNA extraction and genotyping
The methods for the DNA extraction and genotyping of the populations are the same as those explained in Chapter 3.

4.2.3 Population assignment of hatchery individuals
The software GeneClass 2.0 (Piry et al., 2004) was used for the population assignment. All hatchery (Bluff, Kaikoura, and Population X) and wild (EOT and PWTM) populations were used in one assignment procedure. Further assignment procedures were undertaken in an attempt to increase the accuracy of the procedure by combining
the two wild populations. Each of the hatchery populations were run against the
combined wild population in individual assignment procedures. Prior to analysis all
individuals with missing loci data were excluded from the population. This was carried
out to ensure all individuals had the same number of loci for the assignment. Having a
different number of loci results in the criterion values not being able to be compared
(Piry et al., 2004). ‘Self-assignment’ was used to assign each individual. This was
because the individuals originated from the reference populations. ‘Self-assignment’
involves the exclusion of the individual from the reference population during
computation (Piry et al., 2004). Bayesian methods based on Rannala & Mountain
(1997) were used to calculate the probability of each individual belonging to each of the
populations at a threshold of 0.05. A simulation of 10,000 individuals, with a Type 1
error of 0.01, were scored over all loci using Monte-Carlo resampling based on the
simulation algorithm by Paetkau et al. (2004). The highest probability calculated for an
individual genotype occurring in a given population was used to determine which
population an individual was assigned to. This allowed for calculation of the proportion
of correctly assigned individuals and incorrectly assigned individuals. An analysis of
the probability of the assignments was undertaken as well. A quality index was also
calculated as the mean value of the scores of each individual in the population it belongs
to (Piry et al., 2004). This was carried out to determine the confidence in the
assignments. The proportion of a population that had a probability of ≥0.95, ≥0.90,
≥0.75, ≥0.50, and ≤0.05 of being assigned to any of the populations was also calculated.

4.3 Results
The proportion of correctly assigned individual genotypes ranged from 25% - 82.6%
across all of the five populations (Table 4.1). The Kaikoura population showed the
lowest proportion of correctly assigned individuals at 25%, followed by Bluff (28.3%),
EOT (29.7%), PWTM (38.1%), and Population X (82.6%). Population X was distinct in
its assignment compared to the other 4 populations. PWTM and Population X were the
only two populations that had the majority of the individual genotypes correctly
assigned to the origin population. The other populations showed greater assignment of
individuals to alternate populations; Bluff showed 30.4% assigned to PWTM; Kaikoura
had 41.7% assigned to Bluff; and 48.5% of the EOT population was assigned to
PWTM. There was consistently low assignment of Population X genotypes to the other
4 populations with 0% being assigned to Kaikoura, 4.3% to both wild populations and
8.7% assigned to the Bluff population.
Population X appears to be genetically distinct with 0% of individuals from other populations being assigned to Population X (Table 4.1). Kaikoura also showed low levels of individuals from other populations being assigned with 0%, 1.4%, 1.7%, and 13% for Population X, PWTM, EOT and Bluff respectively. The populations of Bluff, PWTM and EOT followed a similar assignment pattern indicating that they are genetically similar.

The results show that a higher quality index and a higher proportion of correctly assigned individuals were attained when pooling the two wild populations into one ‘combined wild’ population (Table 4.2). This is shown as the quality index and proportion of correctly assigned individuals are higher in all of the analyses when compared to the ‘All populations’ analysis. This analysis was a self-assignment analysis which assigned individuals to each of the 5 populations. Out of the populations using the combined wild population as a reference, Population X showed the highest quality index (97.79%) and correct assignment of individuals (98.6%). Referencing each of the populations against all of the other populations gave the lowest proportion of correctly assigned individuals and quality index were produced (36.1% and 31.21% respectively).

The proportion of individuals from the bluff population that were assigned to the Bluff population at a probability of ≥0.95 was 0% (Table 4.3). Again, Population X appeared to be distinct showing of 93.8 – 100% individuals from the other populations being excluded from Population X at a probability of <0.05. There was consistently low (0 – 8.7%) assignment of individuals with a probability of ≥0.95 indicating that assignments in this self-analysis were not reliable.
Table 4.1 - The proportion of individuals from each origin population assigned to the Bluff, Kaikoura, Population X, PWTM or EOT population. The proportion of correctly assigned individuals is shown in the grey boxes of the respective population. Bluff n = 46, Kaikoura n = 48, Population X n = 46, PWTM n = 147, EOT n = 229.

<table>
<thead>
<tr>
<th>Origin population</th>
<th>Proportion of origin population (%) assigned to population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bluff</td>
</tr>
<tr>
<td>Bluff</td>
<td>28.3</td>
</tr>
<tr>
<td>Kaikoura</td>
<td>41.7</td>
</tr>
<tr>
<td>Population X</td>
<td>8.7</td>
</tr>
<tr>
<td>PWTM</td>
<td>25.2</td>
</tr>
<tr>
<td>EOT</td>
<td>20.1</td>
</tr>
</tbody>
</table>

Table 4.2 - Summary statistics of self-assignment analysis between the wild and hatchery populations. The proportion of correctly assigned individuals and the quality index is shown. The quality index is computed as the mean value of the scores of each individual in the population it belongs (Piry et al., 2004). The combined wild population is a pooled populations of the PWTM and EOT samples.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Correctly assigned individuals (%)</th>
<th>Quality index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluff - Combined wild</td>
<td>67.1</td>
<td>54.49</td>
</tr>
<tr>
<td>Kaikoura - Combined wild</td>
<td>85.4</td>
<td>66.83</td>
</tr>
<tr>
<td>Population X - Combined wild</td>
<td>98.6</td>
<td>97.79</td>
</tr>
<tr>
<td>Bluff + Kaikoura – Combined wild</td>
<td>76.4</td>
<td>58.88</td>
</tr>
<tr>
<td>Combined hatchery - Combined wild</td>
<td>78.5</td>
<td>63.71</td>
</tr>
<tr>
<td>All populations</td>
<td>36.1</td>
<td>31.21</td>
</tr>
</tbody>
</table>
Table 4.3 – Proportion of individuals from each origin population at different levels of probability of occurring in the populations. Bluff n = 46, Kaikoura n = 48, Population X n = 46, PWTM n = 147, EOT n = 229.

<table>
<thead>
<tr>
<th>Origin Population</th>
<th>Probability belonging to population</th>
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<th>Kaikoura</th>
<th>Population X</th>
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4.4 Discussion

The results from the self-assignment analysis revealed that the hatchery populations have varying levels of assignment probability. This indicates that the ability to detect the released hatchery individuals in the future differs between the populations.

Population X showed a very high probability of being able to correctly identify individuals with high confidence. This is due to its distinct genetic profile (see Chapter 3). The distinct genetic profile of Population X is also highlighted in the results from the present study. While Population X has not been reseeded, the inclusion of it in the assignment analysis highlights how a genetically distinct population could potentially be easily identified in a reseeding situation. However, the use of such a population is likely to encounter problems and is not recommended. A genetically distinct population of abalone from a hatchery may have lower genetic diversity (Evans et al., 2004; Li et al., 2004) and a limited effective population size (Evans et al., 2004; González-Wangüemert et al., 2012). It can also exhibit adaptation to the hatchery environment which can promote artificially high survival of animals with reduced fitness (Roodt-Wilding, 2007). Such a population can then pose a genetic risk to the wild population through outbreeding depression (Lynch, 1991).

The results of the self-assignment analysis of the Bluff and Kaikoura populations are of more importance. These populations were reseeded into the PWTM and EOT. The results show that the reference populations will unreliably identify reseeded individuals from wild individuals. This is important to consider if genetic surveys are carried out in the future. The use of the same microsatellite panel and similar genetic assignment by McCowan (2012) produced highly reliably identification when the hatchery population was used as a reference. Of the recaptured individuals 99.4% could be correctly assigned to the population that they originated from (McCowan, 2012). The sample size used was much larger (n = 240) than the hatchery sample sizes in the present study (Bluff n = 46, Kaikoura n = 48, Population X n = 46). This could explain the lack of correctly assigned individuals. There is potential to further sample and analyse tissue samples from the Bluff and Kaikoura populations. This is due to the collection of excess tissue samples prior to reseeding. The inclusion of this genotyping data could bring the sampled number to approximately n = 100 for each population. This may be sufficient to accurately identify and assign individuals. It is also important to point out that the work carried out by McCowan (2012) encountered problems at the hatchery. These problems resulted in the reseeded individuals only having parental contribution from 1
male and 1 female. In comparison to Population X, which is thought to have 4 contributing parents (see Chapter 3), the proportion of correctly assigned individuals is relatively similar. The proportion of correctly assigned individuals for the Bluff and Kaikoura analysis may then reflect a larger number of brood stock having been used. The recommended number of brood stock used to spawn individuals is generally 10 males and 10 females (Tong & Moss, 1992; Smith & Conroy, 1992). If it is assumed that this recommendation has been followed, then it would be expected that the individuals from the Ocean Beach Properties hatchery would be less genetically distinct from the wild populations. If the population and were less distinct then it is less likely to be correctly assigned.

Another potential way to improve the identification of the reseeded individuals in the present study is to sample the brood stock used to spawn the reseeded populations. This is a viable option as the brood stock animals are still being housed at Ocean Beach Properties in Bluff. Parentage assignment involves the retrospective assignment of individuals to a ‘family group’ or parents. It has been previously used in H. asinina to identify differences in parental contribution at the hatchery (Selvamani et al., 2001). It has also been previously used in H. iris to estimate parental contribution and distinguish between reseeded and wild individuals (McCowan, 2012). The use of microsatellites is highly recommended for the use in parentage analysis (Jones et al., 2010; McCowan, 2012). This could be achieved by genotyping the brood stock, in the same manner used by the present study and by McCowan (2012). It is likely that this would provide more accurate data as all possible genotype combinations could theoretically be simulated (Jones et al., 2010).

There are alternative ways to identify reseeded abalone individuals that have been used with success in the past. Besides the use of genetic tagging, the other methods are broadly described as physical, chemical, and dietary (McCowan, 2012). Immersion or injection of H. iris with tetracycline has been studied and identified as a means to fluorescently tag individuals (Pirker & Schiel, 1993). However, adult H. iris were either sluggish or incapable of righting themselves following injection, indicating that immersion was the better option (Pirker & Schiel, 1993). The use of numbered discs as a physical tag has been demonstrated in H. rubra but incurred an initial tag loss of 4-16% with an additional 4-35% of tags shed annually (Prince, 1991). This technique may not be appropriate for juvenile H. iris due to the size of the tag (14mm diameter). Similar physical tags have also been used on H. iris (Poore, 1972a). Retention and
identification of tags can be problematic as shell encrusting epibiota can remove the tag or make the identifying number ineligible (Prince, 1991). Dietary markers have proven useful as they are easily applied to juvenile abalone in the hatchery via artificial feed (Gallardo et al., 2003; Roberts et al., 2007). The use of artificial feed in *H. iris* hatcheries causes the shell to produce a blue/green colour as it grows, clearly distinguishable from the naturally occurring shell colouration in the wild (Schiel, 1993; Roberts et al., 2007). This is a reliable and relatively easy form of tag to be employed. However, following encrustation of the shell by epibiota, the tag will be no longer visible (Roberts et al., 2007; Roodt-Wilding, 2007; McCowan, 2012). The shortfalls of physical, chemical, and dietary tags in *H. iris* studies can be overcome by use of genetic tags as they have no retention issues, are identifiable during all life stages, can be applied to all size classes, have no effect on the individual, and can be achieved via non-destructive sampling (McCowan, 2012). In addition, genetic markers can be applied to wild animals to assess the genetic impacts of the release of hatchery reared animals (Gruenthal & Drawbridge, 2012). It is then recommended that because of the advantages of genetic tags, the method used in the present study should be continued to be used. However, efforts must be taken to improve the accuracy via parentage analysis and/or increasing sample size for the results to be reliable. This recommendation is for future work on the present study and any *H. iris* reseeding efforts in the future.
5. Conclusions and recommendations for reseeding customary management areas

5.1 Overview

The need for effective and alternative management techniques in fisheries has never been greater due to the increasing demand for fish (Watson & Pauly, 2001; Hilborn et al., 2003). The global abalone fisheries are no exception. The global abundance of abalone has been declining over the past 50 years highlighting the need for change (Shepherd & Brown, 1993). The fisheries in New Zealand show similar trends to the global fisheries, with many important species exhibiting a reduction to a fraction of their virgin biomass (Gibbs, 2008). There is a need for fisheries managers to intervene by using stock enhancement. This will help to improve the production of the fisheries which is important as current catch levels are considered as unsustainable (Bartley & Bell, 2008). The release of hatchery reared *H. iris* juveniles into the Marlborough Sounds, New Zealand, was initiated by the Quota holders that were interested in the viability of stock enhancement of the *H. iris* fishery (Roberts et al., 2007). This showed the commercial interest in New Zealand. There is also interest in the use of *H. iris* stock enhancement to aid in the replenishment of customary management areas, such as the East Otago taiāpure and the Punawai O Tōrīki mātaitai. (EOTMC 2008). The concern of Māori and non-Māori over declining fisheries stocks ranked *H. iris* as the number one species of significance (McCarthy et al., 2013). This partially explains the interest of recreational and customary stakeholders in reseeding. Customary management areas provide an opportunity to manage stocks of *H. iris* at a fine scale. This is seen as essential for effective management as it makes allowances for the demographic variability which is present (McShane & Naylor, 1995b; Dowling et al., 2004; Prince, 2005; Hepburn et al., 2010). There is potential to combine both fine-scale management and reseeding to effectively manage and sustain the *H. iris* stocks present within customary fisheries areas. However, reseeding comes with its own suite of associated problems and the relatively simple overlying concept of releasing hatchery-reared stock to supplement the wild fisheries is in actuality very complex (Molony et al., 2003).

The potential success of reseeding efforts into customary fisheries areas should be established to determine if it is an effective management technique. Unlike commercial reseeding, the viability of reseeding into customary management areas would place less
weight on the economic returns and more weight on the reseed survival and overall improvement of the abundance of *H. iris*. To understand this better it is important to consider this in regards to Te Ao Māori (the Māori world view). All aspects of the natural world are considered to be alive, related, and to possess mauri (life force). Mauri should not be greatly altered and must be maintained at equilibrium. Mauri-ora, a higher form of mauri, is possessed by humans which requires them to exercise kaitiakitanga, a responsibility towards other living things. This is inclusive of maintaining the mauri of the marine environment and its inhabitants (Booth & Cox, 2003). In other words, reseeding to replenish depleted wild stocks would be considered practicing kaitiakitanga to replenish the mauri of the marine environment. With this in mind it is easy to see that successful reseeding in customary management areas would more appropriately be measured as the improved abundance and reseeded survival of *H. iris*.

Careful consideration of the potential impacts and success of reseeding efforts into customary management areas should be undertaken, as would be expected for stock enhancement in general (Molony et al., 2003; Bell et al., 2006; Roodt-Wilding, 2007; Hamasaki & Kitada, 2008; Gruenthal & Drawbridge, 2012). One of the major concerns that surrounds the release of large numbers of hatchery reared individuals is the genetic impacts that may result from the hatchery population breeding with the wild population, in turn, potentially compromising the genetic diversity of the wild population (Laikre et al., 2010). The use of genetic considerations and tools for abalone has been reviewed by Roodt-Wilding (2007) who pointed out the benefits of genetic tools. These benefits included being able to monitor for adverse genetic impacts pre and post-reseeding as well as being able to identify reseeded individuals. Identification is a key factor in being able to assess the success of a reseeding.

The overall aim of the present study was to assess the reseeding event that occurred at the East Otago tainui and the Punawai O Tōriki mātaitai in April 2013. The study considered the success and use of genetic tools in the context that has been summarised above.

5.1.1 Summary of main findings

The present study found that the survival of the juvenile *H. iris* reseeded into the East Otago tainui and Punawai O Tōriki was very poor, with minimum estimations of survival showing that less than 0.05% of the reseeded individuals survived.
The analysis of the genetic diversity of the hatchery and wild populations showed that the animals reared in the hatchery showed less genetic diversity than the wild populations in both the EOT and PWTM. In particular, Population X, which was not reseeded, showed a large reduction in genetic diversity. The Kaikoura and Bluff hatchery populations showed a reduction in genetic diversity but to a lesser degree. There was a distinct population differentiation between Population X and all of the other wild and hatchery populations. The Kaikoura hatchery population also showed differentiation from the wild populations, while the Bluff hatchery population did not.

The use of genetic markers to identify the reseeded stock was not successful. The quality index and proportion of correctly identified individuals were too low for reliable identification. This contrasted Population X which showed that greater than 95% of individuals could be correctly identified with a quality index also greater than 95%.

5.2 General Discussion

5.2.1 Genetic considerations – Hatchery management and procedures

The loss of genetic diversity between hatchery reared stock and the associated wild populations has been well documented (Hindar et al., 1991; Roodt-Wilding, 2007; Laikre et al., 2010). The means to minimise this loss have been identified and include choosing wild brood stock that is representative of the population, using adequate numbers of brood stock and ensuring that there is sufficient parental contribution (Smith & Conroy, 1992; Evans et al., 2004; Li et al., 2004; Roodt-Wilding, 2007). This is taken into consideration in an effort to minimise the negative genetic impacts on the wild population that will receive the hatchery reared stock. The results from the genetic analysis showed that Population X had experienced a reduction in genetic diversity compared to the natural populations. The Bluff and Kaikoura populations also showed a reduction in genetic diversity to a lesser degree than Population X. However, the genetic analysis indicated that a large scale release of Population X individuals would have had a high risk of negative genetic impacts on the wild population present (González-Wangüemert et al., 2012). This reduction in genetic diversity highlights the need for genetic monitoring of hatchery populations prior to release and the improvement of hatchery practices. The present study and the work carried out by McCowan (2012) shows that the genetic tools are available to assess the suitability of hatchery reared *H. iris* for release. In addition, the procedures and estimated number of parents required to maintain sufficient genetic diversity in cultured *H. iris* populations has been established.
The use of this information in future reseeding programmes of *H. iris* should be implemented as it would stop the release of genetically unsuitable stock, and consequently, prevent any adverse genetic impacts on the wild population.

### 5.2.2 Genetic considerations – Impacts on the wild population

The use of genetic tools to assess the genetic diversity and genetic population structure of the released hatchery individuals proved to be valuable in providing information on the genetic differentiation of the reseeded Kaikoura stock. There are potential implications for large scale releases of individuals that are genetically similar (Laikre et al., 2010; Roodt-Wilding, 2007). The lack of differentiation seen between the Bluff and wild populations in the EOT and PWTM indicates that there is no immediate concern surrounding their release, especially considering the low survival. The release of stock, such as the Kaikoura stock, into the EOT and PWTM could impact the wild population via a reduction in the genetic effective population size, loss of fitness, outbreeding depression, and inbreeding depression (see review by Roodt-Wilding 2007). The impact of large scale release of abalone appears to have not been studied so other species are used as examples to highlight potential consequences. Lindley et al. (2009) investigated the large scale recruitment failure of the fall-run Chinook salmon in the Central Valley of California. A significant contributing factor was the widespread large scale releases of salmon had caused the genetic homogenisation of the metapopulation. This resulted in a loss of environmental buffering that would have been provided by diverse natural populations. In the context of the EOT and PWTM reseeding, there is unlikely to be adverse genetic consequences because the release was a one off event and the low survival of hatchery stock indicates that very few will reproductively contribute to the population in the future. As it appears unlikely that many animals will reproduce there is little or no genetic risk. Consistent large scale releases of genetically similar *H. iris* could cause homogenisation of the population within the EOT. However, the local recruitment of *H. iris* would likely limit this geographically (Wilson & Schiel, 1995; Stephens et al., 2006). Regardless, this should be considered if future reseeding is to be undertaken. Despite a low risk of adverse genetic impacts, genetic monitoring of the mixed hatchery and wild population in the EOT and PWTM should be undertaken. This would allow for detection of alterations to the genetic characteristics of the natural population and identification of potential biological consequences which will allow for effective fisheries management (Schwartz et al., 2007; Laikre et al., 2010).
Genetic identification of the reseeded animals does not appear to be reliable with the technique used in the present study. The animals will be temporarily identifiable for approximately 2-3 years because of the distinctive colouration of the shells of the animals (Gallardo et al., 2003). As the shells of the animals become encrusted with epibiota they will be increasingly difficult to identify (Prince, 1991). In future reseeding work, it would be wise to make use of parentage assignment to aid in the identification of animals. Parentage assignment has been used in previous work carried out on H. asinina larvae (Selvamani et al., 2001). The parents of the larvae were genotyped using microsatellite markers which allowed for all of the genotyped larvae to be assigned to their respective parents (Selvamani et al., 2001). There has also been successful use of parentage analysis to distinguish individual hatchery and wild H. iris with 91.6% of individuals being correctly assigned (McCowan, 2012). In addition to this, parentage analysis can be used to determine the contribution of individual parents to the offspring population (Selvamani et al., 2001; McCowan, 2012).

5.2.3 Reseeding as a fisheries management technique in customary management areas

The results from the present study indicate that reseeding juvenile H. iris into customary management areas may not be an effective means to sustain wild populations of H. iris. The fine-scale management of H. iris stocks that can be achieved by using customary management areas (Hepburn et al., 2010) is likely to be more effective if it focuses on other means of sustaining the H. iris stocks. Stock enhancement is thought to be an ineffective means of managing the sustainability of a fisheries stock if it is the only management intervention used. If the fishing effort is limited and / or habitat restoration is undertaken then stock enhancement is more effective (Penman & McAndrew, 1998).

The reduced daily bag limits for recreational fishers and exclusion of commercial fishing of H. iris in the EOT and PWTM may then be a more effective means of fisheries management. However, because of the rushed nature of this reseeding effort and the inaccuracy of the survival estimates the use of reseeding as a fisheries management technique in customary management areas is still up for debate.

The calculations of the survival of juvenile H. iris suffered from poor scientific methods during the course of this study. In terms of the success of reseeding, as judged by the survival of the reseeded animals, it would be unwise to draw conclusions from the results presented in this study. The author believes that reseeding may still be a viable option for customary fisheries management. However, further study should be
undertaken. The results from this study show that genetic tagging is useful when the population is sufficiently genetically distinct. Unfortunately, for use in reseeding, this is not ideal as the hatchery population should be representative of the wild population. Improvements in successful population assignment are needed before genetic tagging can be considered as a reliable identification method. This study showed that genetic monitoring of the hatchery and wild populations is easily achievable. It is recommended that all future reseeding operations undertake genetic monitoring to help ensure maintenance of genetic diversity between the wild and hatchery populations.
6. References


Goudet, J. (2001) ‘FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)’.


7. Appendix

7.1 List of information required for reseeding permit.

- Details of the *H. iris* reseeding proposal -
  - Contact details of the applicant(s).
  - Reseeding area, with maps showing area.
  - Quantity of animals to be released.
  - Name of the hatchery the animals has been sourced from.
  - Water flow on the hatchery (i.e. Flow through or recirculating).
  - Size range of the animals.
  - Proposed date the animals will be released.
  - Treatment of animals prior to reseeding to remove fouling organisms.
• Confirmation that the *H. iris* seed will not be fed 24 hours before packing out from the hatchery.

• Details of rearing of the *H. iris*—
  • Where the brood stock came from.
  • Confirmation that the animals being released are the first generation from the brood stock.
  • Details of the spawning strategy to ensure the animals are the progeny of several parents.

• Details of the holding site—
  • Location of the holding site.
  • Details of the holding site.
  • Expected time animals will be contained in the holding site.

• Risk of transferring pest organisms with the animals being released—
  • Is *Undaria pinnatifida* present in the location of the hatchery that is providing the animals?

• Histopathology report—
  • Conducted by a qualified fish pathologist.
  • Must be representative sample of animals being used.
  • Sufficient number tested to provide statistically valid results.
  • Report must show *H. iris* shell and body are in good condition and do not harbour pathogens of concern.
  • *H. iris* must be examined for pathogens of concern (fish pathologists should be aware of these).
7.2 Histopathology report

Health assessment of paua (*Haliotis iris*)
for reseeding

Prepared by
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Cawthron Institute

For
Storm Stanley
Pauamac5

10 April 2013

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Summary

No significant pathologies or pathogens were seen in a sample of 68 *Haliotis iris* of size range 12-51 mm with a mean size of 22.3 mm. Despite particular vigilance for important conditions, such as ganglioneuritis and infection with the paua *haplosporidian*, the paua appeared to be free of significant pathogens.

All paua were examined individually upon arrival to assess appearance and condition including coloration and surface texture of soft tissues. All except two paua appeared alive but the live paua all exhibited below normal activity. Nevertheless, there was no discoloration, swelling or eversion of the mouth parts – the latter two features being associated with ganglioneuritis infections.

Histopathological preparation allowed examination of the soft tissues for a range of pathogens/conditions including haplosporidia, epithelial erosion, ciliates associated with the mantle/foot epithelium, bacterial infection, granuloma-like lesions, haemocytic neoplasia-like inflammation and *Perkinsus olsenii*. During examinations, attention was also paid to the possibility of exotic and so far unrecorded pathogens/pathologies such as amyotrophia, withering syndrome, *Labyrinthuloides haliotidis*, *Margolisiella (=Pseudoklossia) haliotis*, *Echinocephalus pseudouncinatus* and viral ganglioneuritis. Only trivial conditions were noted, including a low prevalence (4.4%) of *Scyphidia*-like ciliates associated with the foot epithelium, also 1.5% prevalences in gills and the buccal pouch. Ectocommensal ciliates such as these are common, and are not associated with pathology. Gill filament erosion was noted in 1.5% of paua. These occurrences were free from other signs of pathology. There was widespread (94%) presence of brown ceroid material, particularly in the digestive gland, and lesser prevalences in the right kidney, musculature and left kidney. Such material is suggestive of suboptimal environmental conditions that might explain the depressed activity noted in the paua.

Shell examination completed the assessment. Shell mycosis, the boring mud-worms *Polydora*, *Boccardia* and the, so far unrecorded in NZ, exotic shell-dwelling sabellid *Terebrasabella heterouncinata* were sought. Of the 72 shells examined 69% had evidence of a minor growth-check line, 8.3% showed elongation in a shell hole (trema singular – tremata plural); and in one shell, two tremata had fused. Elongated or fused tremata are suggestive of some stress during shell deposition at that time. Ten (14%) had small ~1mm blister pearls on the inner shell surface and one shell had a small pit on the shell outer surface with another having a fracture hole near the umbo. None of the shell features noted suggested any significant infectious pathological threat.

Introduction

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This is a report on the health status of paua to be used in a reseeding operation. Since translocation of animals carries with it a risk of transfer of potentially damaging pathogens, it is important to survey such animals for significant pathologies and pathogens.

**New Zealand pathogens**

Paua (*Haliotis iris*) have been reported with a range of associated organisms and pathologies. Diggles and Oliver (2005) report haplosporidia, epithelial erosion, rickettsial inclusions in the gut epithelium, protozoa in foot epithelium, bacterial infection (see also Bower 2006a), non-specific necrosis, granuloma-like lesions, haemocytic neoplasia-like inflammation and gregarines (apicomplexans). Diggles et al. (2002) reported a pustule disease caused by *Vibrio* bacteria. Paua also exhibit a fungal shell mycosis (Grindley et al. 1998) as well as the shell boring spionid mud worms *Polydora* and *Boccardia* (Diggles et al. 2002, Bower 2006e) that can be a problem in culture. Severe cases of mudworm can cause significant shell embrittlement (Webb pers. obs.). In addition to shell damage, there can be loss of condition: *H. iris* infected with *Polydora hoplura* can be underweight and produce abnormal deposits of conchiolin (Diggles, Oliver 2005).

Despite their nuisance value, none of these pathologies currently presents an insurmountable obstacle to the NZ abalone industry. Hine (1997) in his review of the health of commercially important New Zealand molluscs mentions only fungal shell disease and even that, he says, is not a cause of significant mortality. In contrast, Diggles and Oliver (2005) add that a haplosporidian has been associated with mortalities – some of them significant. In addition, potential problems could arise from rickettsia, granuloma-like lesions, inflammatory lesions suggestive of hemocytic neoplasia, mudworm and fungal infections as discussed by Diggles and Oliver (2005). Clearly, continued vigilance is required.

A further indigenous threat may come from *Perkinsus olsenii*. Paua have not been reported with this pathogen, but it is noted in the Northland bivalves *Austrovenus stutchburyi*, *Macomona liliana*, *Barbatia novaezelandia* and *Paphies australis* (Diggles et al. 2002). In Australia the same *Perkinsus* species can infect the abalones *H. rubra*, *H. laevigata*, *H. cyclobates* and *H. scalaris* (Bower 2007). The lack of host specificity shown by this parasite – infecting both bivalves and gastropods – suggests that paua would not be invariably immune.

In previous surveys Webb (pers. Obs.) has noted a *Nematopsis*-like protozoan encysted in small numbers in the gills and foot musculature, and also a gregarine infection in the gut lumen. Small numbers of rickettsia-like organisms have been noted in the gill of one paua. In addition, a gregarine (protozoan) has been implicated in the production of shell scars running parallel with the stomata. These appear to be of trivial pathological impact.

**Exotic pathogens**

Although not reported in New Zealand, vigilance is required for the following exotic abalone pathogens: amyotrophy (probably viral), the rickettsial withering syndrome from the West Coast of USA (Bower 2006d, Diggles et al. 2002); the shell-dwelling sabellid *Terebrasabella heteroucincnata* (Bower 2006b); *Labyrinthuloides haliotidis* a protist in *H. kamtschatkana* and *H. rufescens* (Bower, Meyer 2005) and the kidney coccidian *Margolisiella (=Pseudoklossia) haliotis* from the West Coast of USA (Bower 2006c). A more distasteful but possibly less likely threat comes from the nematode *Echinocephalus pseudouncinatus* (Bower 2001). It weakens the foot muscle and allows easier detachment from the substratum. The usual final hosts are certain sharks and rays, but human consumption of the live worms in undercooked abalone may allow migration of the larvae through human tissues.
The most immediate exotic threat to New Zealand abalone is from viral ganglioneuritis which now has been reported from farmed *H. laevigata* and *H. rubra* in Australia (Hooper et al. 2007) where it is associated with mortalities. It is thought to have come from the Far East where farmed abalone have been reported with similar herpes-like viruses (Chang et al. 2005, Wang et al. 2004). There is evidence to suggest that the virus has spread to wild populations causing significant mortalities among abalone, and possibly other gastropods (Hine 2006). As to the hazard facing New Zealand, Hine (2006) concluded that the taxonomy and geographic isolation of *H. iris* in New Zealand is such that it is currently likely to be free of the virus.
Materials and methods

Test samples

<table>
<thead>
<tr>
<th>Species</th>
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<tr>
<td>Date of arrival at Cawthron</td>
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</tr>
<tr>
<td>Client</td>
<td>Pauamac5</td>
</tr>
<tr>
<td>Contact</td>
<td>Storm Stanley 027 653 1073</td>
</tr>
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<tr>
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Sample processing

External examination: All paua were examined individually upon arrival to assess appearance, activity and condition. They were then processed for histology immediately.

Histology: Sixty eight paua were processed. This sample size approximates to that used by Diggles (2009) for similar paua health testing. Total shell length (the largest dimension) of each was measured to the nearest millimeter.

Larger paua that would not fit whole in a cassette were shucked and three sections cut before fixation (Figure 1). This also allowed easier fixative penetration to minimize post-mortem tissue autolysis (breakdown).
Figure 1. Three sections (~4mm thick) from each larger paua were taken.

Smaller paua (those that would fit whole in a histology cassette) were fixed in the shell but the membrane between the body and shell over the digestive gland area was detached to allow free access to the fixative. All preparations were fixed in 1:9 v/v concentrated formalin/filtered seawater for 48 hours after which they were placed in 70% ethanol. After fixation, smaller paua were then shucked and placed whole, foot down, with up to 3 other paua of the same size in the same cassette (Figure 2). The histology paraffin blocks containing multiple paua were sectioned on two levels about 1mm apart on the plane horizontal to the sole of the foot so as to give sections of musculature and visceral organs for each individual. The tissue sections were cut to 5µm and stained in haematoxylin and eosin. During microscopic examination of the sections, tissues sought included left and right kidney, hypobranchial gland, digestive gland, heart, foot musculature, gill, gastrointestinal epithelium, cerebral ganglia and nerve cords, mantle tissues and buccal pouch.

Figure 2. Smaller paua were sectioned whole – three are on this slide
Shells were examined inside and out (with magnification) for deformities and signs of diseases/pathogens such as fungal shell mycosis, Polydora, Boccardia (Diggins et al. 2002, Bower 2006e), Terebrasabella heterouncinata and signs of significant shell corrosion suggestive of embrittlement caused by shell borers such as the cyanophytes Plectonema, Mastigocoleus, Cyanosaccus, Pleurocapsa, and Hyella (Lukas, Golubic 1981, 1983; Nielsen 1987; Le Campion Alsumard et al. 1995; Mao Che et al. 1996; Kaehler 1999; Kaehler, McQuaid 1999), the barnacle Cryptophialus (Australophialus) melampygos (Batham, Tomlinson 1965) and the Cliona sponges such as C. vastifica and C. eurphylla; the latter two are reported in Haliotis spp. from Southern Australia (VPS 2000).

Results and Discussion

Despite particular vigilance for important conditions such as ganglioneuritis and infection with the haplosporidian, the paua appeared to be free of significant pathogens.

External examination

All except two appeared alive but the live paua all showed below normal activity. Nevertheless, there was no discoloration, swelling or eversion of the mouth parts – the latter two features being associated with ganglioneuritis infections (Hooper et al. 2007). Coloration and surface texture of the flesh was also normal. The two dead paua were also prepared for histological examination.

Shell examination

Of the 72 shells examined 69% had evidence of a minor growth-check line, 8.3% showed elongation in a shell hole (trema singular – tremata plural); and in one shell, two tremata had fused. Elongated or fused tremata are suggestive of some stress during shell deposition at that time. Ten (14%) had small ~1mm blister pearls on the inner shell surface and one shell had a small pit on the shell outer surface with another having a fracture hole near the umbo. None of the shell features noted suggested any significant infectious pathological threat.

Histological examination

Only trivial conditions were noted, including a low prevalence of Scyphidia-like ciliates (Figure 3): these being associated with the foot epithelium 3/68 (4.4%), gills 1/68 (1.5%), and
buccal pouch 1/68 (1.5%). Ectocommensal ciliates such as these are common, and are not associated with pathology. Gill filament erosion was noted in one paua (not infected with ciliates). These occurrences were free from other signs of pathology. There was widespread 64/68 (94%) presence of brown ceroid material, particularly in the digestive gland with lesser prevalences in the right kidney, musculature and left kidney. Such material is suggestive of suboptimal environmental conditions – which might explain the depressed activity noted in the paua.

Figure 3. Ciliates (C) associated with mantle epithelium (M). Scale bar 25μm.
References


