# Spat survival of *Paphies ventricosa* (Toheroa)
# in an Aquaculture setting

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THE UNIVERSITY OF
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*Te Whare Wānanga o Waikato*

## Contents

# Acknowledgments

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# Abstract:

The toheroa (*Papris ventracosta*) is a large intertidal surf cam endemic to New Zealand. This species has a long history with the people of New Zealand in both cultural and economic sense. Exploitation and environmental change have greatly diminished the wild stocks of these animals making a sustainable fishery no longer feasible. Aquaculture has been widely discussed as a potentially profitable option for this species due to its large size, esteemed taste and its social and cultural significance. Aquaculture of toheroa could generate many benefits for the people of the toheroa beaches, as aquaculture has done in other regions of New Zealand. However, the suitability of toheroa for aquaculture is largely unknown, with many questions needing to be answered before that can become a reality. The purpose of this research is to gain a better understanding of the environmental conditions which will support the successful cultivation of wild caught toheroa spat in recirculating aquaculture systems (RAS). This is done by answering questions around knowledge gaps in the biology of the animal. Three experiments were conducted on aspects of the animal's environment and biology. The first experiment sought to identify the presence of microphytobenthos's within the wild population beds of the toheroa. As evidence suggests from research conducted on other species, microphytobenthos is an essential part of the animal's ecosystem which might be a necessary component of a successful spat rearing operation. The experiment showed elevated chlorophyll levels (a proxy for microphytobenthos) within toheroa beds relative to other areas of the beach. The second experiment compared survival and growth rates in sediment vs. non-sediment holding systems. The results of this study indicated the benefits of housing spat in sediments, as demonstrated by differences in measured growth and mortality. A third experiment sought to test for size dependent mortality rates within the RAS. Somewhat

surprisingly, it was found that smaller toheroa (0-10mm) performed better within a system compared to larger ones (20-30). Larger toheroa tended to eject themselves from the sediment constantly, an act which may have compromised their growth and survivorship. The result of these experiments answered questions and raise many more. Potentially through understanding these questions we can create tools which industry and conservation can use. This research show that successful toheroa spat can be successfully integrated into an aquaculture system. With mortality settling the animals feeding and showing healthy condition. However, the next stage would be to optimize this. Toheroa aquaculture being in its infancy requires a wide variety of research before considered feasible. However, results and observations made from this research can help in identifying and specializing future research objectives.

# List of figures

## List of tables:

## Preface:

With understanding the suitability of a species fitness for aquaculture, there is a variety of parameters which need to be understood. Before getting into the nitty and gritty parts of growing toheroa for commercial purposes, there first needs to be a conversation about the potential future of an industry. This conversation must be with the stakeholders, which can be negatively and positively affected by the progression of industry. In the case of this species these stakeholders are primarily the iwi and hapu which call the eastern coast of Northland their home. The have a deep historical relationship with toheroa, any attempt to create an industry from the natural resources provided by the land must include provisions that benefit the lives of the people who live there. If not entirely led by the communities which protect them. Toheroa have had a long history with humans which has not benefited their ecology. Though with aquaculture we could reduce pressure on wild stock, create a better understanding of its biology to promote conservation and create a sustainable industry with permanent jobs within the regions. That would be the aim. However, there are many questions which need to be answered before that can become reality. This thesis aims to try and shed light on one of the simplest and yet arguably most important aspects: spat survivability. What is the ideal condition for spat to survive, thrive and grow out into adulthood?

# Chapter 1: Introductory chapter

## 1.1: Early history of toheroa industry

Throughout the history of toheroa, its high value of a food source has also played into its ecological decline (Ross et al., 2008; Hoby, 1933). Iwi and Hapu of Northland have always had toheroa as part of their culture and history (Ross et al., 2018). This species was once highly abundant on the specific west coast beaches around New Zealand (Ross et al., 2008; Hoby, 1933; Cassie, 1955; Street, 1971; Redfearn, 1974). Some of these main locations include Te Oneroa-a-Tōhē (Ninety Mile Beach), Mitimiti, and Te Oneone Rangatira (Muriwai) beaches in Tai Tokerau, on the Kāpiti-Horowhenua coast from the Rangitikei River to Waikanae Beach, and in Murihiku at Oreti, Bluecliffs and Orepuki Beaches (Ross et al., 2018; Hoby 1933; Cassie 1955; Street 1971; Redfearn 1974). It was only at the turn of the 20th century that there had been any need for restrictions imposed on the wild collection of toheroa (Muron, 2006). Māori had traditionally managed the stock for generations (Ross, et al., 2018). Only due to the modern industrial world catching up, did it even begin to be understood that humans could affect the availability of this naturally abundant species (Redfearn 1974; Murton 2006). Toheroa populations began to decline with the intensification of harvesting by Europeans from the early 1900s (Redfearn, 1974; Murton, 2006).

Toheroa are described by numerous sources as being delightful to eat (Ross, et al., 2018), with Māori coveting them above all other shellfish (Murton, 2006) most likely attributed to the size and ease of capture. However, Māori had a deep connection to their environment linking important aspects of it through genealogy or “whakapapa” (Ross et al., 2018). Māori culture and beliefs are intrinsic with showing respect to your environment as stewards of the land “kaitiakitanga” (Smith, 2013). This respect is reported to be the same respect that you would show your family or your tribe (Smith, 2013). Early harvesting of toheroa came from Māori who traveled far and wide to the coasts (Ross et al., 2018). The gatherers would walk down to the beach and collect toheroa by removing them from the beach and then bringing them behind the sandstone cliffs adjacent to shelter from the strong winds (Taikato, 2022). Everything that they collected from the beach would have to be carried by hand (Taikato, 2022). The excess shell was shucked, and the meat collected in bags for transport (Taikato, 2022). Within the dunes they shucked thousands of shells, which are still there today, as the shell added no value only weight (Ross et al., 2018). The Māori then smoked the animals, stored the meat in bags made of flax and carried them back to their families for food and for trade (Ross et al., 2018). Māori of this time had been known to transport live toheroa around the country (Taikato, 2022). It was thought that the purpose of this was to ether trade or translocate populations so that the resource would be available to them in new areas (Taikato, 2022). In a sense, this was the first commercial industry of toheroa (Taikato, 2022). Wild harvest, which was done at large scales with processing methods, provided food and trading materials for the Hapu (McCarthy et al., 2014). This resource was well protected, and many battles were fought by Māori to protect the area or beach of which they could harvest (Murton, 2006). For people who depend on the natural resources of the land, an abundant food source like toheroa can be the difference between comfortable living and poverty (Liebenberg, 2006).

When expansion of European settlers in the regions of which possessed toheroa, Europeans became more interested in the sought-after shellfish (Ross et al., 2018; Samuel, 1936). The demand was extended further by one event, involving the royal family (Ross et al., 2018). During a royal visit in 1921 Prince Edward attended a royal banquet and by breaking all royal protocol, asked for a second serving of toheroa soup (Ross et al; Stace, 1991). The soup being served to him was done to showcase specific products native to New Zealand (Ross et al., 2018; Stace, 1991). This second serving of soup was reported throughout the world and toheroa became the must-have delicacy for the upper classes of European society (Murton, 2006). Advancement and availability of automobiles made it much easier for 'out of towners' to travel to these areas to participate in the growing trend (Ross et al, 2018). The ease of capture was the toheroas downfall, as one could simply drive along the beach, dig a couple, or bucketful, out of the beach for dinner and be on their way (Ross et al, 2018). The popularity of toheroa began to grow further around the late nineteenth century as well as word of mouth that the species was an excellent bait for fishing (Murton, 2006). With this increasing demand came the first large scale industrial canning operation within the area (Stace, 1991). The canning facility was built near Ripiro beach in the 1890s at Mahuata gap, which grew to be rather successful (Ross et al 2018; Stace, 1991). Its success led to the construction of four other factories along the coastline producing estimated of 20 tons of canned toheroa per annum (Redfern, 1974). The annual tonnage fluctuated throughout the years with the highest record recorded in 1940 with 77 tons (Redfern, 1975). Exporting toheroa became the most profitable revenue stream for the canning industry and tourism to the regions became intensified (Williams, Sim-Smith, et al., 2013). This began to cause tension within the region (Ross et al., 2018) with some being against the exploitation of the land, and some enjoying the economic benefits of which tourism money and industry could bring (Murton, 2006). However, because of this increased demand, toheroa populations started to decline rapidly (Murton 2006; Williams, Ferguson, et al., 2013). The once “free for all” industry became subject to regulation such as camper reserves, season closure with bag and size limits for European recreation harvesting (Ross et al., 2018; Redfearn, 1974). With the population still not recovering, restrictions were introduced for Māori in 1941 (Redfern, 1974). After the fact there has been numerous attempts with different legislative methods trying to promote population recovery but to no avail (Ross et al., 2018). The collapse was caused by both unsustainable industrial methods and poor regulations of recreation catch (Murton, 2006). However, many industries which depend on wild harvest tended to meet the same fate throughout history, if done in an unsustainable manner and without insight into the ecology of the animal (Davis, Gallman & Hutchins, 1988).

Whaling, sealing and wild harvest of kauri timber are historical industries of New Zealand were extremely profitable and helped build the country (Prickett, 2002). However, without the ecological insight needed, they were destined to fail eventually (Prickett, 2002). That this was also the case with toheroa, however it does not have to be, Aquaculture could be an industry where it could be sustainable. Throughout the world bivalve aquaculture is a large part of the Aquacultural sector (van der Schatte et al., 2020). By Understanding these species farmers could cultivate the animals on land sustainable (Shpigel, Neori, Popper & Gordin, 1993). Without putting pressure on wild stocks (Shpigel, Neori, Popper & Gordin, 1993). There is even the possibility aquaculture could one day provide replenishment of wild stocks (Garrett, Dos Santos,

& Jahncke, 1997) which has been done within New Zealand with the species of giant Kokopu, where successful breeds of the fish were released into the wild (Wansbrough, 2022). As a country it is our responsibility to analyze our history, recognize mistakes made and learn from them, then go into the future with new insight and technology to better utilize natural resources to benefit communities and the economy sustainably.

## 1.2 Threats to ecological recovery

There is a wide variety of possible reasons which have been proposed why juvenile toheroa have not recruited to their former populations (William et al., 2013). With total bans on the capture (except for customary rights) it would be expected that the populations have been given the best chance to recover (Williams, et al. 2013). However, this is not the case and even with forty years of protection the populations have failed to recover (Williams, Ferguson, et al. 2013; Berkenbusch et al. 2015). =. Toheroas are annual broadcast spawners and have approximately two spawning events around late spring and summer (Redfern, 1982; Beentjes, Carbines & Willsman,2006; Rapson, 1952). Through analyzing the beach during the collection of the spat we noticed that there was a huge bout of healthy juvenile toheroa littered uniformly across the beach. This goes to show that what limits toheroa recovery within the region was not that the adult's ability to spawn. There must be a bottle neck which limits the ability for these animals to occupy the distribution of which their ancestors did (Ross et al., 2018). This bottleneck is still not understood properly (Ross et al., 2018). However, it is optimistic to note that if their bottle neck is identified, it can then be properly mitigated through management actions (Ros, 2011). This means that the population could have the ability rapidly recover when given the optimal conditions (Ros, 2011). Within laboratory settings is where the parameters which limited spat survival can be identified through carful control and testing (Crisp et al. 2011)

The environment and landscape of the toheroa ecosystems has also been reported to have changed considerably (Bennion et al., 2022). A variety of abiotic and biotic variables have been altered since prehuman colonization. The land use change of the surrounding land has drastically been developed for dairy farming and forestry (Bennion et al., 2022). These industries can inadvertently affect toheroa beds as these practices have major effects of the surface and ground water hydrology (Tong, & Chen, 2002). Stream input has been a major determining variable of the distribution of northern populations of toheroa (Cope, 2018). The reason for this is not entirely understood. Cope (2018) discovered that a water table interaction caused by the stream input likely aided in maintaining saturation conditions. These conditions prevented desiccation of the animals at low tide when they were exposed (Cope, 2018). A reduction of stream input would likely further exacerbate the decline of toheroa beds within the region (Cope, 2018). Climate change is another proposed potential stressor for toheroa recovery (Futter, 2011). The primary food source of toheroa is phytoplankton (Rapson, 1952). The ocean compassing the west coast of New Zealand is subject to seasonal blooms of phytoplankton which are then circulated throughout the ocean currents to become available to toheroa on the coastline (Murphy et al., 2001). Climate change and increasing sea temperature has been known to have major effects on the frequency of the algae blooms and hydrology of ocean currents (Hallegraeff, 2010). Many species of bivalves time their broadcast spawning events around seasonal food availability, i.e. algae blooms (Gadomski, & Lamare,2015) to maximize the survival of their larvae. This becomes increasingly problematic with rising sea temperatures as many bivalve species spawning events are timed and triggered through temperature cues (Redfern, 1972; Gadomski, & Lamare,2015). Changes in the external environment can cause miss timing of spawning events, which do not align with optimal conditions for success for the offspring. Toheroas have distinct differences of spawning patterns between the southern and northern populations (Redfern, 1972; Gadomski, & Lamare, 2015) with northern populations having more events with more variance in timing when compared to southern population (Redfern, 1972; Gadomski, & Lamare,2015). It is suggested (Gadomski & Lamare 2015) that this is likely due to the increased water temperature of the region triggers them to spawn. It is yet unclear if climate change has an effect on the success of toheroa spat survival. However, research into other species of bivalves does suggest that it is a variable to consider.

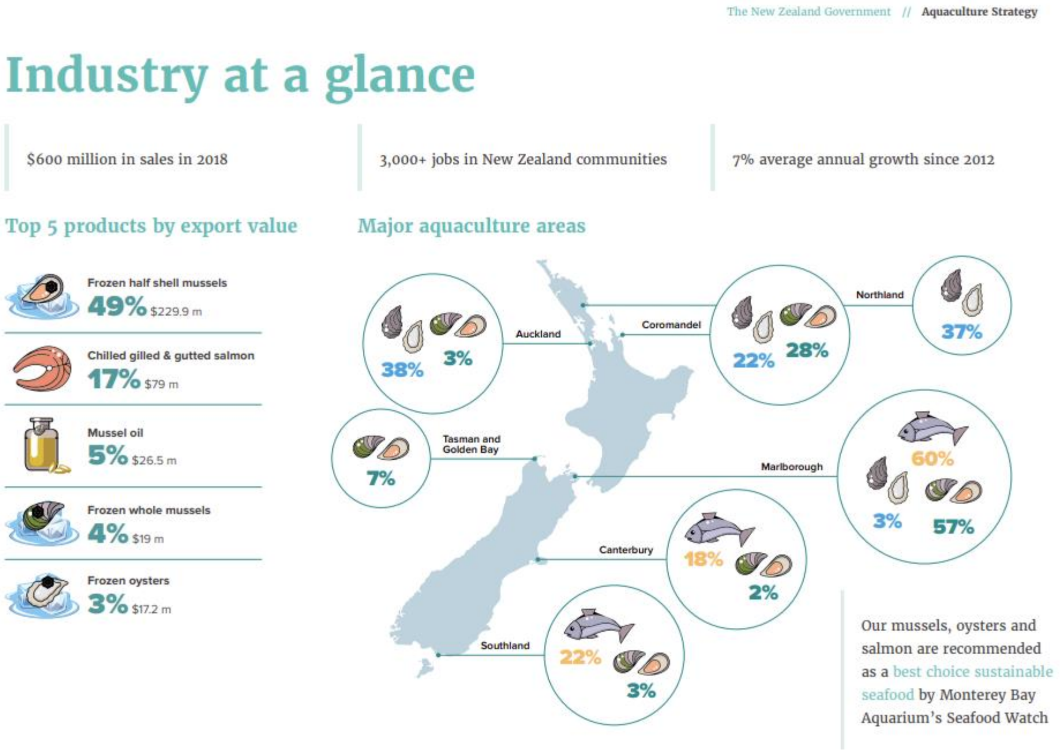
There are also new challenges for the modern toheroa which its ancestors did not have to face. Many of the beaches around New Zealand including the beaches of Northland are regarded as official national highways (o Te Ao Tūroa, 2009). Many people use these west coast beaches as their daily commute and there are even some settlements that are only accessible through driving on the beach (o Te Ao Tūroa, 2009). This is problematic when considering that the life cycle of toheroa which has never had to deal with this stressor before in its evolution. During my own surveys conducted after a spawning even at Ripiro beach we observed that the majority of spat occupied the top five centimeters of the substrate within the bed. Through examining tire marks left behind found thousands of crushed toheroas spat. Due to the feeding strategy of toheroa, they can only go as far into the substate as their siphon allows it, as it needs to reach the surface to feed (Rapson, 1952). Therefore, explaining the shallow depth of juveniles and why adults appear to be less effected by automobiles as they can get deeper (Rapson, 1952). This was confirmed by a study which took place on after the Burt Munro Challenge (O e Ao Tūroa, 2009). The report states that are estimated that 53,000 juvenile toheroas were killed in an 850-metre-long racetrack with an estimated mortality rate of 72 percent (O Te Ao Tūroa, 2009). This is further exacerbated by the location of which many holiday makers and locals decide to do "doughnuts". Because the beach is heavily tidal dominated the beach become narrower as the eb tides come in (Cope, 2018). The widest section of the beach is where the inlet streams enter the beach (Cope, 2018). Theses sections are most popular with donut enthusiasts, as its wide enough to do large turns and safer from the sea. This area however is usually located to an adjacent toheroa bed as the beds in Northland are located around freshwater input. This is the environment where the spat has the optimal conditions for survival (Cope, 2018). However, from my own observation I discovered it could take only a few cars  wipe out a whole generation of recruitment to the bed. With natural toheroa beds in such dwindling supply, it is crucial that education of people using the beach is done. Signs and closers could be implemented and only for a short time after the spawning season. This is because the animals could have the opportunity to grow and bury deeper to be safe from human traffic. Aquaculture could also be an opportunity to mitigate this issue (Diana, 2009) with cultured spat given the time to grow out in captivity and given the size to reach the depths where they would be safe. This could be down with translocation of wild spat after a spawn or through laboratory induced spawning (Diana, 2009)

## 1.3: Aquaculture within New Zealand

The aquaculture sector within New Zealand is dominated by only a few species. These species are green lipped mussel (Perna canaliculus) , salmon (Salmo salar) and oysters (Crassostrea gigas) (MPI, 2022; McGinnis & Collins, 2013). The region of Northland has been heavily involved with the green lipped mussel industry indirectly (Shumway et al., 2003). Most of the spat that has been used to maintain the cultured stock around the country has been collected at 90-mile Beach (Shumway et al., 2003). Without the availability of this spat on this beach the entire industry would not exist (Alfaro & Jeffs, 2003). The Green lipped mussel industry has created thousands of jobs around the country and created lasting infrastructure that benefits local economies (Scott, 2015). However, the benefits of this industry have not been felt as much in the areas of Northland where the spat has been collected. This is a travesty as a natural resource which is only present on the west coast has been used to enrich the people of other regions. With these regions of the east coast of Northland being isolated in comparison, with the social economic state being relatively low, an opportunity to benefit the area was missed. With the closing on the reproductive cycle of green lipped mussels it is expected that the industries dependent on the region with diminish in the future (Alfaro et al., McArdle & Jeffs, 2010). However it should be said that there is still along way to go before this method is scalable to meet the need of the industry. With the potential of toheroa aquaculture there is the opportunity for a local driven industry which would create jobs and benefit local stocks of this special species.

The New Zealand Aquacultural Council commissioned the completion of an aquaculture strategy in 2006 (Council, 2006). This strategy aimed to grow the aquaculture sectors by 1-billion-dollars in 2025 (Council, 2006). However, this strategy was revised by the Ministry of Primary Industries in 2022 (MPI, 2019). This strategy's main aim is to create a 3-billion-dollar a year industry by 2035 (MPI, 2019). Within the new governmental framework, it describes the methods of which the government sets out to achieve its goals through three steps (MPI, 2019). The first maximizing the value of existing farms through innovation (MPI, 2019). The second extending into high value land-based aquaculture and the third is extending aquaculture into the open ocean (MPI, 2019). With these steps the government hopes to achieve the four specific outcome for the industry (MPI, 2019) Outcome 1: "sustainable" A primary industry leading in environmentally sustainable practices across the value chain (MPI, 2019). Outcome 2: "Productive" Aquaculture growth supports regional prosperity (MPI, 2019). Outcome 3: "Resilient" Aquaculture is protected from biological harm and supported in adapting to climate change (MPI, 2019). Outcome 4: "Inclusive" Partnering with Māori and communities on opportunities to realize meaningful jobs, wellbeing, and prosperity (MPI, 2019). Through looking at this strategy, it does appear to have the making of being able to develop the industry fast by maximizing revenue. The reason salmon is so rapidly invested in is because it generates most of the sector's revenue (MPI, 2019) with expansion into offshore fish farms has been proven around the world to rapidly grow the profits while moving away for the controversial methods used on inshore cage farming. However, heavily concentrating on a few species does pose a variety of problems for an industry. Diversification of target species gives an industry flexibility to deal with potential issues. Offshore salmon farms are prone to diseases and environmental concerns, which with modern technology is becoming easier to mitigate (Olesez Myhr, & Rosendal, 2011). But one disease could collapse an industry. The current aquaculture strategy only address's algae as a potential species for diversification (MPI, 2019). New Zealand is home to a range of aquatic species which would be ideal aquaculture targets (McGinnis & Collins, 2013). Diversification of species would potentially allow a greater spectrum of rural regions to benefit from the advancement of aquaculture. The New Zealand government does recognize the economic and social benefits of which aquaculture can make on communities (Carswell, 2015). They have made a large investment in the region of Opotiki to promote large scale commercial open ocean mussel farms (Carswell, 2015). This involves dredging the harbor to accommodate larger mussel barges, funding the construction of a factory, and developing the mussel farms themselves. Opotiki and most of the Eastern Bay of Plenty has had generally lower socio-economic issues when compared to the rest of the country (Carswell, 2015) mainly due to the lack of industry within the area. However, the oceans of the east cape are some of the most productive in New Zealand due to the strong ocean upwelling (Bradford & Roberts,1978). Theses upwellings provide nutrient needed for phytoplankton blooms, which is needed for to sustain large amounts of green lipped mussel (Bradford & Roberts, 1978). Although offshore aquaculture is very well suited to Opotiki, the suitability or offshore ocean farming varies a lot. The farm must be accessible for large vessels, have the correct amount of depth to eliminate benthos contamination, must be productive enough to support growth, be able to have the correct average current speeds and the list goes on (Bradford & Roberts, 1978). There are only a small portion of areas of New Zealand which meet the requirements for offshore aquaculture of our current species (Stenton-Dozy et al., 2021). Land based aquaculture investment would be the best way for all regions to benefit from the growing technological advancement of aquaculture (Tal et al., 2009). Land based aquaculture can be constructed within a range of environments. This is because the systems are self-containing allowing the internal environment to be modified to accommodate the target species (Johnson & Rickard, 2022). Land-based aquaculture of new species has been shown to be successful with new species within New Zealand (Stenton-Dozy et al., 2021). For instance, there has been heavy research into the aquaculture of yellow-tailed kingfish (Symonds et al., 2014) conducted by National Institute of Water and Atmospheric Research (NIWA) within Northland Aquaculture Centre (Fielder, 2013). The research includes an entirely functioning breeding and rearing system on land (Fielder, 2013) which developed methods of growing kingfish from fry to marketable size within a year (Symonds et al., 2014). Culturing this species has the potential to be highly profitable due to its natural traits (Symonds et al., 2014). Extremely fast growth rates (ref), ability to thrive in high stocking density (Moran et al., 2011) and it possess highly marketable meat both domestic and internationally (Poortenaar et al., 2001). However, replicating the success of this facility poses issues which would limit the growth of kingfish farming around other regions of New Zealand (Fielder, 2013). Firstly, the initial costs of building a state-of-the-art facility like this would be extremely high (Engle, 2007) making it more difficult for potential investors to secure funding especially with the inherent risk associated with aquaculture (Engle, 2007). This facility has also repurposed infrastructure unique to the facility. With the facility has access to a large pipeline which brings in a supply of offshore water. This is needed for large amounts of water exchanges as kingfish are fast moving pelagic predators which require lots of freshly oxygenated clean sea water to survive (Fielder, 2013). In aquaculture some species require a large amount of effort and expense to build the environment of which they can thrive (Neori & Nobre, 2012).

However, some species require far less effort and expense (Neori & Nobre, 2012). Diversification of species could allow for smaller scale operations to be easier to establish. Lower tropic-level species such as invertebrates have been utilized around the world (Phillips & Pérez-Ramírez, 2017). Some examples include using corals, crustaceans, bivalves, sea cucumbers and cephalopods (Parks, Pomeroy, & Balboa, 2003). These animals are successful in both large-scale facility and small-scale community operations (Shumway et al., 2003; Pant et al., 2014). Due to the low requirements needed to grow out the species. Within New Zealand there is a large range of native invertebrate species which would be ideal for future aquaculture (Stenton-Dozy et al., 2021), as well at the potential to in integrate them with other species existing species to maximize profits (Stenton-Dozy et al., 2021). Multi-species integration in this manner is referred to as "integrated multi-trophic level aquaculture" (IMTA) (Stenton-Dozy et al., 2021). This field is widely researched within New Zealand for future development of the aquaculture sector (Stenton-Dozy et al., 2021).


The New Zealand Government // Aquaculture Strategy

### Industry at a glance

$600 million in sales in 2018

3,000+ jobs in New Zealand communities

7% average annual growth since 2012

**Top 5 products by export value**

| Product | Share | Value |
|---|---|---|
| Frozen half shell mussels | 49% | $229.9 m |
| Chilled gilled & gutted salmon | 17% | $79 m |
| Mussel oil | 5% | $26.5 m |
| Frozen whole mussels | 4% | $19 m |
| Frozen oysters | 3% | $17.2 m |

**Major aquaculture areas**

Our mussels, oysters and salmon are recommended as a best choice sustainable seafood by Monterey Bay Aquarium's Seafood Watch

Figure 1: Diagram detailing the species and regions involved within the aquacultural sector of New Zealand in 2019 (MPI, 2019).


### The sustainable growth pathway

Towards our goal of $3 billion in annual sales by 2035

**Current state** → **Towards $1 billion** → **Beyond $1 billion** → **Potential $3 billion industry** → **Future state 2035**

Existing industry
- $600 m+ in annual sales
- 3000+ regional jobs
- Sustainable production
- Efficient farming
- $100 m in innovation
- 7% average annual growth

Maximise the value of existing farm space through a strong research, innovation and commercialisation system

Lift industry confidence to invest in growth across the work programme

Wharf and hatchery infrastructure to support growth

World-leading frameworks to enable sustainable open ocean and land-based farming – transformational growth away from many competing uses and values

Extend farming into open ocean →

World-leading trials of open ocean shellfish and seaweed farming

Enable transitional fish farms to build skills and expertise *(6m waves)*

Open ocean farms *(11m waves)*

Extend farming onto land-based systems →

Hatchery-raised juveniles for greater resilience, value and efficiency

Growing juveniles for longer to make best use of space and avoid unfavourable conditions

Farms producing high value seafood and extracts for market

Future industry
- $3 b+ in annual sales
- 6000+ regional jobs
- Sustainable lifecycle
- Highly efficient farming

Image credit: AKVA group

**Our four outcomes**

**Sustainable:** A primary industry leading in environmentally sustainable practices across the value chain

**Productive:** Aquaculture growth supports regional prosperity

**Resilient:** Aquaculture is protected from biological harm and supported in adapting to climate change

**Inclusive:** Partnering with Māori and communities on opportunities to realise meaningful jobs, wellbeing and prosperity

Figure 2: Diagram detailing the sustainable growth pathway proposed by the ministry for primary industry for the aquaculture sector of New Zealand in 2019 (MPI, 2019).

## 1.4: The benefits of aquaculture

Aquaculture is an ever-increasing industry which around the world is becoming the fastest growing sector (Lenzi, 2013). With this growing interest from the private and public sector is becoming importance to recognize and highlight the various benefits of aquaculture (Borja, et al., 2009). Being a coastal island nation, New Zealand has a wide variety of potential target species for culturing. Currently New Zealand only cultures three species to a large scale which are green lipped mussels, salmon, and scallops with a variety of smaller scale species in development (McGinnis & Collins, 2013). The advancements of this industry have the power to bring industry and jobs into once isolated and impoverished areas (Tlusty, 2002). This is clear to see in the examples of the Coromandel and East Cape of the Bay of Plenty of New Zealand (Pambudi & Clough, 2017). The introduction of mussel farms has reignited the local economies of these regions (Scott, 2015). For example, the Coromandel has traditionally been a holiday destination for New Zealanders (Dudding & Ryan, 2000). With the town having far less employment opportunities for residents when not in summer, without tourists to accommodate (Dudding & Ryan, 2000). With governmental assistance, offshore aquaculture for green lipped mussels and oysters was heavily invested within the region (Pambudi & Clough 2017). Today the Coromandel aquaculture industry contributes $77.4 million in GDP to the national economy (Wyatt, 2011). This industry has generated a total of 1,193 full-time jobs, which did previous not exist (Wyatt, 2011). This was possible in the case of the Coromandel as the region's coastal environment made it possible to develop offshore farms (Pambudi & Clough 2017).

However, monetary benefits are not the only positive outcome aquaculture industry can bring to communities (Shang, 1985). Food security for protection against poverty, protection of natural resources for both cultural and conservation purposes are all possible with aquaculture (Shang, 1985). There was a project which was conducted to document the benefits of aquaculture on the livelihoods of Adivasi (ethnic) communities in the North and Northwest of Bangladesh (Pant et al., 2014). Small-scale aquaculture materials were given to 3594 resource-poor Adivasi households with the training on how to operate the systems (Pant et al., 2014). The average income of households rose by 30% annually during the period of the study 2007-2009 (Pant et al., 2014). The case study also documented that the diet of the households improved with excess fish to sell provided households with the ability to have more varied diets, promoting health and wellbeing (Pant et al., 2014). The monthly frequency of fish, meat and egg consumption increased between 2007 and 2009, confirming improved food and nutrition security among project participants (Pant et al., 2014). Although non-project participants also slightly increased their fish consumption, it remained significantly lower than that of the project participants. The results from this research contradict the prevailing view that aquaculture is inappropriate for landless, socially marginalized, and extremely poor communities (Pant et al., 2014). This study shows an alternative use of aquaculture when compared to the tradition form of industrial aquaculture. This is entirely based on profits and export, which has been known to negatively affect marginalized communities (van Mulekom et al., 2006; Golden et al., 2016). A type of community-lead aquaculture could benefit communities living in rural New Zealand focusing on protection of one of their taonga species as well as providing sustainable food and jobs for the local communities. This type of aquaculture isn’t as perfect as it seems and has been criticized in other countries (Bailey, 1988). For instance, shrimp farming in Thailand, which is locally owned, is not well-regulated causes a variety of environmental and social issues (Bailey, 1988). These impacts can be mitigated with RAS online farms with no connection to the sea (Valenti, Kimpara, & Moraes-Valenti, 2018). With RAS systems standardized practices are easier to develop (Valenti, Kimpara, & Moraes-Valenti, 2018). Making the regulation and inspection process more efficient (Valenti, Kimpara, & Moraes-Valenti, 2018). Having systems on land also gives aquaculture ventures the ability to safeguard their stock from any factors relative to climate change (Allison, Andrew & Oliver 2007).

# Chapter 2: Biological factors important in relation to aquaculture

## 2.1: Overview

When attempting to make an advancement in the aquaculture of species there must be first understanding the research which has already been done on it (Hiney et al., 2002). Firstly, as not all species are fit for aquaculture (Hiney et al., 2002) not only in aspects of survivability but also profitability and sustainability. A researcher needs to understand the aspects of the species' life cycle which needs to be explored further to make conclusions. There is not great deal of research out there specific to the application of aquaculture regarding toheroa. However, the perfect aquaculture environment attempts to mimic the optimal ecological environment of which the animal thrives (Badiola, Mendiola & Bostock 2012). In recent years there has been a wide variety of research conducted that examines the ecology of toheroa (Ross et al., 2019), partially driven by decline in distribution and abundance over modern history (Ross et al., 2019; Beentjes & Gilbert, 2006). The main ecological literature was produced between the 1950s to 1980s where detailed articles are the base of our understating of toheroa ecology today; (Redfearn 1974, 1982; Rapson, 1952, 1954; Cassie, 1951, 1955). There have also been extensive toheroa surveys over the period of decline by Beentjes & Ggilbert (2006b). This chapter attempts to showcase the ecological research that has been conducted on the animal and try to use the conclusions found to suggest aquacultural techniques and systems design relevant to the literature.

## 2.2: Life cycle

Toheroas are a large intertidal beach clam which is found primarily on the high-wave action beach of Northland, New Zealand (Ross et al., 2019). Toheroa possess a wedge-shaped shell, with similarities to the Donax genus, with the posterior edge flattened (Hoby, 1933; Stanley, 1970; Redfearn, 1974). Rapson (1952) confirmed Hoby (1933) that between the ages of two to three years toheroa became sexually mature and can participate in broadcast spawning (Redfearn, 1974; Mandeno, 1999). The number of spawning events is typically around two to three times each year for the northern populations (Redfearn, 1974; Mandeno, 1999). The warmer water has also been shown to aid in the recovery of the gonads between these periods (Redfearn, 1974). Redfern (1986) managed to manipulate temperature within a laboratory environment to trigger individual toheroa to spawn and produce fertilized eggs. Redfern (1986) documented the various stage of blastoma division after fertilization and after 22 days from spawn he had successful settled toheroa spat; without hormones injections and solely with temperature shocking (Redfern, 1986). This paper is an important tool for any future aquaculture venture as spawning can be conducted more frequently and to greater success simply by temperature cues (Redfern, 1986). This would eliminate any need for wild harvest of spat like in the green lipped mussel industry and protects wild stocks. However, Redfern (1986) does not go onto rear the spat after settlement (Redfern, 1986). The next stage of research would have to be conducted to close the link between spawning event and large cultured adult. Within this paper lies the framework for that type of research. Focusing on the specific condition of which spat requires to be healthy and grow.

## 2.3: Distribution

The distribution of toheroa is interesting as it has been likely influenced by human intervention (Taikato, 2022). Even with larval dispersal, is unlikely that that larval dispersion would have exceeded 800 kilometres to the South Island (Ross et al., 2018). Māori, who are native to Northland have been reported to translocate these animals across the country in historical texts (Taikato, 2022; Ross et al., 2018). This was either done to trade with other iwi or to take their natural resources with them to seed to areas (Taikato, 2022; Ross et al., 2018). They did this by constructing damp bags made out of kelp (Taikato, 2022). This goes to show the importance that the shellfish have for these people. Due to this translocation, there has been numerous accounts of sub-populations around the country (Ross et al., 2018; Hoby, 1933; Stanley, 1970; Redfearn, 1974). Regarding aquaculture, this historical record answers a crucial scientific step needed to culture the species. That question is, can these animals be transported and safely settled into a new environment. This is crucial as transportation stress can have extremely detrimental effects on the animals (King, 2009). Large amounts of studies have been conducted on the translocation of fish and very little on shellfish species (Shummway, 2011). Stress can have major negative impacts of the health of individual organisms (Iwama, Afonso, & Vijayan, 1998). Some of these include affecting the mucous layers which protect against infections such as diseases and parasite, reducing their ability to feed causing them to starve and sudden death (Iwama, Afonso, & Vijayan, 1998). Aqua culturalist's who would have to translocate toheroa as spat to a rearing tank, would have to aim to mitigate stress as much as they can. Some of the parameters which can be manipulated are stocking density, oxygen injection into the water, chemical sedation, cooling systems, turbulence mitigate and many more (Iwama, Afonso, & Vijayan, 1998). However, as with everything within aquaculture, those can become extremely expensive (Llorente & Luna, 2016). While essential for some aquatic species this might not be the case for toheroa. Which is shown by the historical record of crude traditional translocation methods producing with successful results (Taikato, 2022). This topic would have to further researched as if an industry can simplify the translocation method without increasing mortality, this would greatly reduce the initial experience cost (Llorente & Luna, 2016). With a lower overhead companies would more likely be successful.

The distribution of toheroa is very different from other bivalve species of the same family such as pipis and tuatua (Johyphen et al., 2002). They are found within beach the sand is within the intertidal zonation of heavily exposed sandy beach beaches (Cope 2018; Ross., et al 2018; Johyphen et al., 2002) exposed at low tide and emerge at high tide. Tuatua are described to dominate the sub tidal zone due to their shells being thicker which provides protection from predation and the smashing surf (Duncan Waugh & Greenway 1967). Toheroa require sand which contains sufficient moisture to eliminate desiccation within the warmer months (Cope, 2018; Cassie, 1952). The fine sediment is thought to hold the moisture far better coarse sediment and allows ease of vertical movement in the substrate (Cassie, 1952). Cope (2018) detailed the relationship between surface water hydrology and toheroa bed distribution. The research observed a relationship between adult toheroa distribution and the water table associated around the freshwater stream input (Cope, 2018). Conversely then, why wouldn’t the species thrive in the totally submerged subtidal environment where desiccation and food availability isn't an issue? A study by G. Duncan Waugh & J. P. Greenway (1967) made a discovery that could shed some light on this. The researchers discovered there were many shells of toheroa located within the sediment within the littoral zone which appear to have been predated on by gastropods (Duncan Waugh & Greenway 1967). While proving inconsequential, within the report they cited Rapson (1954) who from between the year of 1961 to 1967 never encountered a gastropod on his transects of the toheroa beds around Dargaville. The researcher presented this evidence of sublittoral populations of toheroa, as the only way that they could have obtained theses hole would have been to inhabit further out to sea (Duncan Waugh & Greenway 1967). However, this report does state that no adult toheroa has been discovered within the subtidal zone by researchers due to the rough surf conditions and low visibility (Duncan Waugh & Greenway 1967; Rapson, 1952). They suggest this evidence as a proxy. However, in an aquacultural sense this can be useful information regarding system design. If the main factor stopping them from being fully submerged is predation and competition, that can easily control within a land-based system. Having animals fully submerged always reduces cost and risk to the animals and ensures that water parameters are always optimal.

1967] WAUGH & GREENWAY—SUB-LITTORAL TOHEROA 409

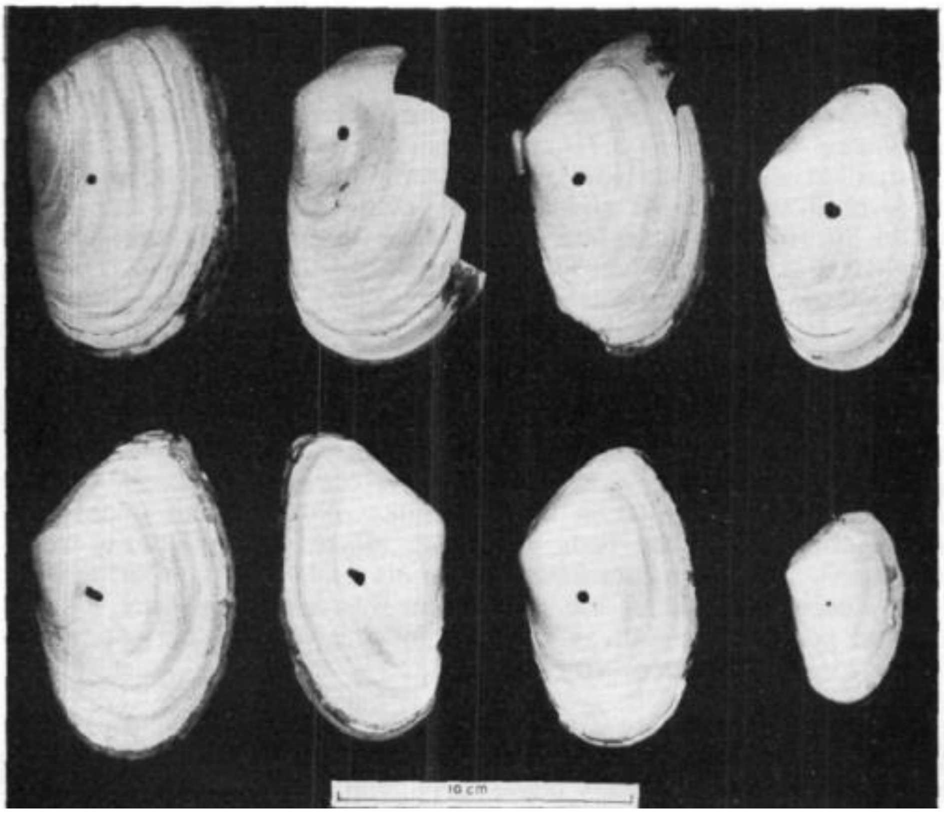


Figure 3: Evidence shown of predation on toheroa from sub-littoral populations of gastropods (Duncan Waugh & Greenway 1967).

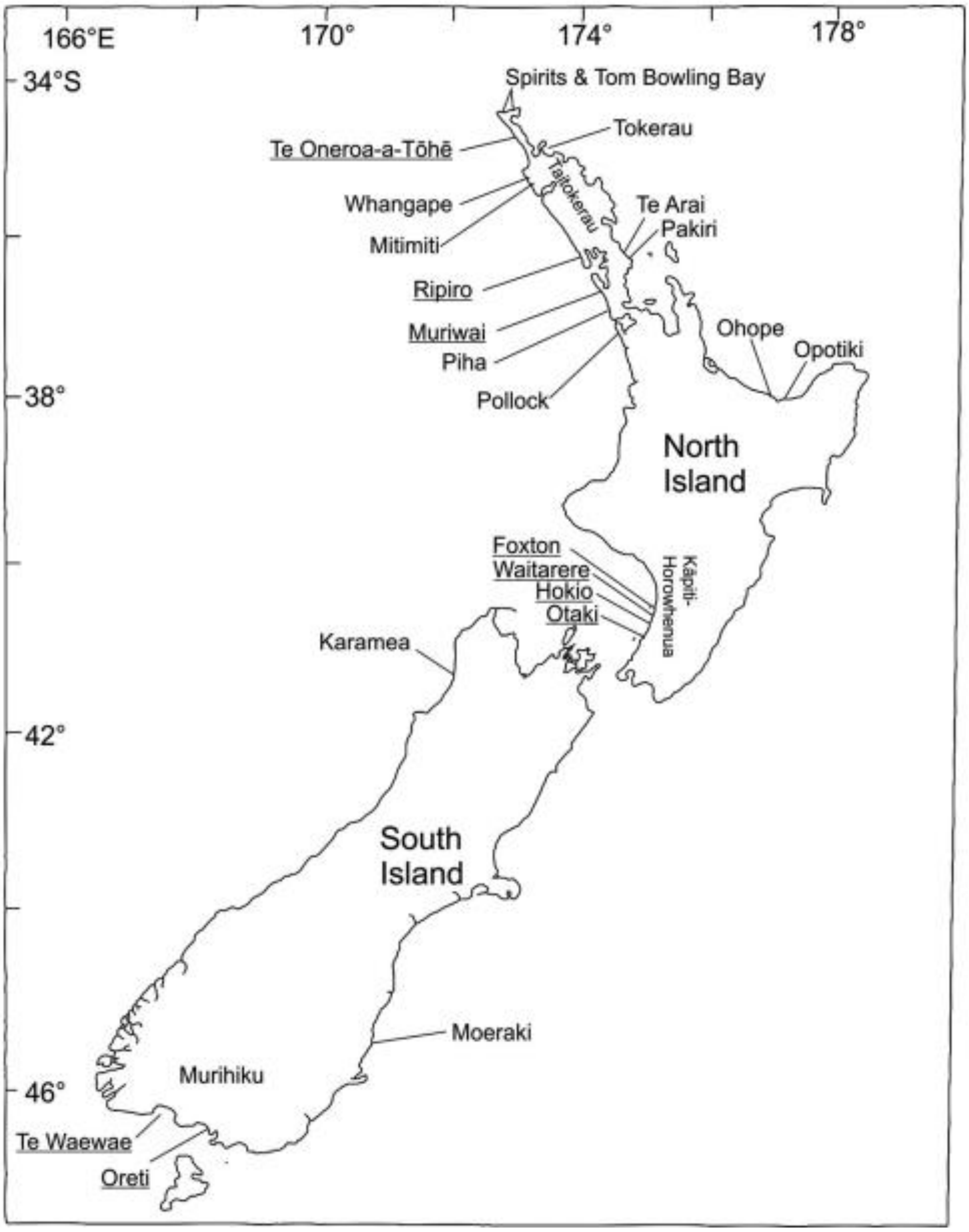


Figure 4: Distribution of know populations toheroa (*Paphies Ventricosa*) within New Zealand (Ross et al., 2018; Redfern, 1974)

## 2.4: Orientation

The animals themselves are a suspension feeding clam whose shell morphology evolved to position themselves upright to feed from the water column (Kondo & Stace). Shell orientation and burrowing ability has been described in a report Kondo & Stace (1995) as a crucial behavior adaptation. Firstly, with the orientation of the shell being uniform in relation to the beach and sea. The report confirms Hoby (1933) observations that the Azimthal Orientation was roughly parallel to the northeastern and southeastern trending shoreline (Kondo & Stace 1995). This is suggested to be an adaptation which aids in keeping the organism's firm without the sand by increasing surface area to combat the strong tidal currents (Kondo & Stace 1995). If the animal naturally orientates due to tidal movement this could cause some issues for aquacultural design. Mimicking tidal moving artificially in a system would be difficult as well as expensive (Marzano & Brizzzi, 2009). Further research would have to be conducted to see if this behavior is necessary for the health of the animal in captivity.

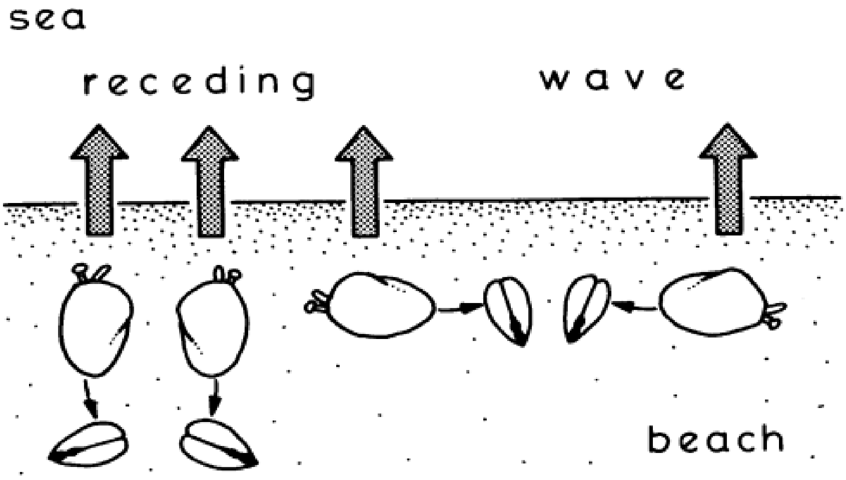


Figure 5: Schematic drawing explaining azimuthal orientation of toheroa (Kondo & Stace, 1995).

## 2.5: Feeding methods

Toheroa are suspension feeding bivalves (Rapson, 1954). They exhibit two siphons which both protrude from the top of the animal (Rosset et al., 2018; Beentjes, et al., 2018). One of these siphons is for feeding (P. M. Ross, M. P. Beentjes, et al., 2018). They protrude beyond the sediment to suck in water from the water column (Rapson, 1954). This water is then pumped down onto the gills of the animal (Rapson, 1954). Bivalves such as these then have very specific methods of sorting what is food and what isn't (Rapson, 1954). Toheroa feed on microalgae and the waters along their habitat are extremely turbid and nutrient poor (Rapson, 1954). For this reason, the animal must not miss opportunities for nutrient uptake through miss identification (Rapson, 1954) as this can waste metabolic energy trying to digest inorganic materials. The second siphon is the exhalent syphon (Rapson, 1954). This too protrudes out of the sediment to expel waste matter which would be then washed away by the surf (Rapson, 1954). These methods make it easier to identity toheroa beds from the surface at low tide (Murton, 2006). The two siphons can leave marks in the sand which identify an active animal (Murton, 2006). This also give a clear perception of size and orientation of the specific animal (Murton, 2006).

Aquaculture of toheroa has the ability to be sustainable in feeding methods which the majority of efforts have not been thus far (Naylor et al., 2009). Salmon farming for instance has some of the most unsustainable feed in the aquaculture sector (Sahlmann et al., 2015). The feed pellets in the early history had been constructed entirely from schooling fish (Sahlmann et al., 2015). This can be rapidly overfished to provide perfect nutrition for higher trophic level fish such as salmon (Sahlmann et al., 2015). However, this is unsustainable, as the feed conversion ratios of salmon require 10 kilograms of school fish to make 1 kilogram of salmon (Pelletier & Tyedmers, 2007), promoting overfishing. When this revelation was made to the public the industry was forced to look for alternatives which would satisfy consumer demand for sustainable practices (Gillund & Myhr, 2010). The industry conducted research in plant-based alternatives such as soybean replacement (Gillund & Myhr, 2010). However the new formulated pellets could only substitute fish meal to a degree. (Gillund & Myhr, 2010). Only 40 percent of the fish meal pellets could be substituted before growth rates of the fish were effected (Gillund & Myhr, 2010). Furthermore, there were further complications with the hybrid feed resulting in fish health problems and flesh abnormalities (Easton et al., 2002). Also reports detail concerns that formulated feeds can have some toxicology concerns within humans especially for pregnant women. (Easton et al., 2002). The solution to this problem is to farm organisms which have a lower metabolic demand and shellfish are the perfect candidate (Willer & Aldridge, 2017). Toheroa exclusively feed on small microalgae within the water which can be rapidly cultivated on mass  (Marshall et al., 2010), making the meat it produces more sustainable than others.

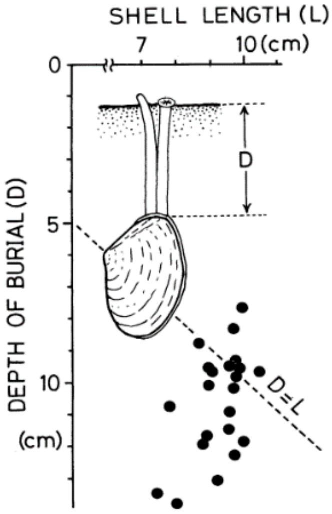


Figure 6: Diagram detailing the feeding method of toheroa in relation to shell length vs burial depth (Kondo & Stace, 1995).

## 2.6 The environment of Ripiro Beach

The environment of which toheroa find themselves in is unique when comparing to the rest of the country, with strong wave exposed beaches and large tidal movements (Ross et al.,2018). The beaches can be described as long stretches of highly exposed beach (Cope, 2018). The beach itself is fringed by large cliffs before returning to pastural farmland forest (Cope, 2018). This area is has small human populations, limiting impact on the environment, however within modern history, this has had extensive changed to the land use which surround the beach (Bennion et al.,2022). A study conducted raises concern around the use of pine-forest and pastural farming and freshwater hydrology mainly because of the effect the tap root has on the natural hydrological process within the region (Froude, 2011). It is reported that this has caused a large majority of the freshwater streams which feed onto the beach to dry up (Froude, 2011). This is further exhibited by the continued frequencies of summer droughts in Northland (Froude, 2011). As previously stated, the beds within the region are dictated by the input of theses streams (Cope, 2018). Pressure on this process from land use change would further reduce the available habitat for spat to inhabit. There has also been reports of which water was being pumped from coastal bore sites into farm across the region adding to the water loss of the streams (Ross et al., 2018).

There are some suggestions that climate change might have a negative impact on toheroa populations, as the primary production of the world's oceans are changing (Doney, et al., 2012). All marine ecosystems are experiencing rising $CO_2$ levels with shifts in temperature, nutrient availability stratification and oxygen concentration (Redi, 2009). Bivalves such as toheroa have the potential to be negatively affected by these changes directly (Shi, et al., 2016). Ocean acidification can chemically limit their ability to calcify their shells (Shi, et al., 2016). Furthermore, a less direct, however, more critical component, is that climate change has a direct impact of the development of phytoplankton and its distribution (Doney, et al., 2012); phytoplankton being the main food source for the toheroa as well as the base for the entire marine ecosystems (Winder & Sommer, 2012). Due to climate change, it has been reported that many of the annual blooms of phytoplankton have changed timing and intensity because of the changing environment (Winder & Sommer, 2012). Theses blooms also have changed the distribution with shifts in the changing water column stratification caused by climate change (Winder & Sommer, 2012). Increased $CO_2$ within the oceans was thought to benefit the production of phytoplankton, however studies now suggest that that increased temperature caused by $CO_2$ within the atmosphere affects their ability to grow and replicate (Toseland, et al., 2013). With temperature appearing to lower the density of ribosome which is needed produce the required amounts of cellular protein (Toseland, et al., 2013). Understanding the natural state of the environment for toheroa is needed to fabricate within an on land system. However, with the change of its natural environment it is difficult to properly assess this, as the baseline environments of which toheroa thrived in abundance have shifted. This could be limiting its recovery. The best avenue to assess the optimal state is to thoroughly assess remaining population environments.

# Chapter 3: System design

The experimental aquaculture system for the trials within this thesis was built in Tauranga, New Zealand to replicate a scalable model which can be used in a larger-scale aquaculture set up. A RAS (recirculating aquaculture system) was thought to be the best method for shellfish culture. By using this system, it gives the culture more control over the water parameters of the system and better limitation of contamination. The water which was used from the system was sourced from the sea which was delivered by truck into a five thousand liter holding tank. This tank was on a separate filtration loop. With having this water on a separate loop, it gave time for the water to be sufficiently cleaned of solids before entering the main system containing the shellfish. Within the holding tank was a pump, ten-micron sock filter, chiller and a bypass line to IBC's which stored the water for the main system. The water for the main system began at the connected IBC, which was then gravity-fed into the first pump. This pump then feeds into another canister filter, then UV filter before going into the main line to feed the internal tanks within the container. The 30L Conical tanks which house the toheroa were located inside, with the water entering from above and draining from the bottom. This design was chosen as a fast way of disposing waste and controlling water quality which is similar to the natural conditions. From the bottom of the tanks the water was fed by gravity into a biofilter within the lab. The biofilter contained an initial sock filter, bio-media, protein skimmer, aeration lines and drain to an external line to a secondary IBC. This secondary IBC is connected to the first which is also gravity fed to control the levels and thus closing the system.

Due to the nature of the tank design flowing from the bottom, special consideration was made to how the shellfish would sit. Aspects of how adults and spat would be housed was considered, so that both could still fit into a modular system. Everything involved with the racking system was made using a 3D printer. Inside the tanks sat a frame that could be submerged into the tank similarly to how a deep fryer works. Within this frame there were grooves within which allowed for different types of racks to be slotted within. Specific racks were fabricated to accommodate different sizes of toheroa, from spat to large adult. With the adult racks, the initial design was plastic grooves where the toheroa would sit upright. However, this proved to be not fit for use as the force of the toheroa expanding their shells caused their shells to break against the plastic. In this case a redesign was done. The racks were changed to be able to accommodate flexible plastic bands, which held the toheroa firmly in place and gave them the ability to contract freely. This method proved more successful. With the spat, this method would not be feasible due to the small size of the individuals. In this case, trays were made which could accommodate replicate jars to house the juvenile toheroa.

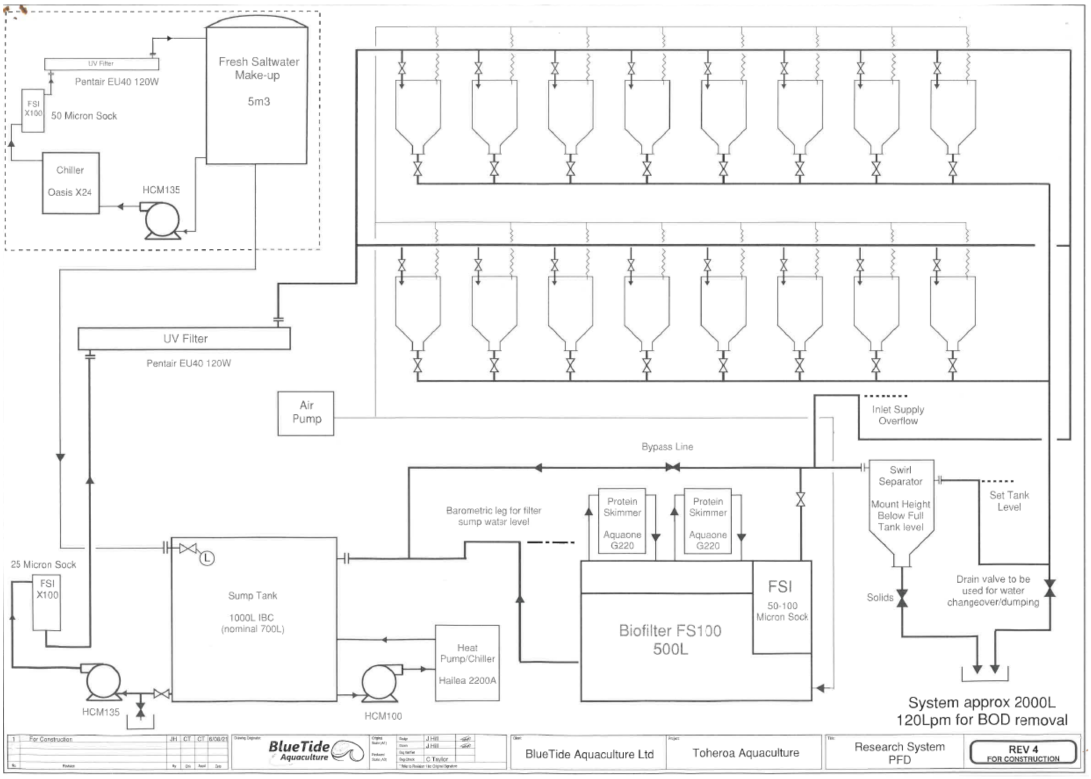


Figure 7: Original design drawing of internal and external pumping configuration for a self-contained toheroa aquaculture facility.

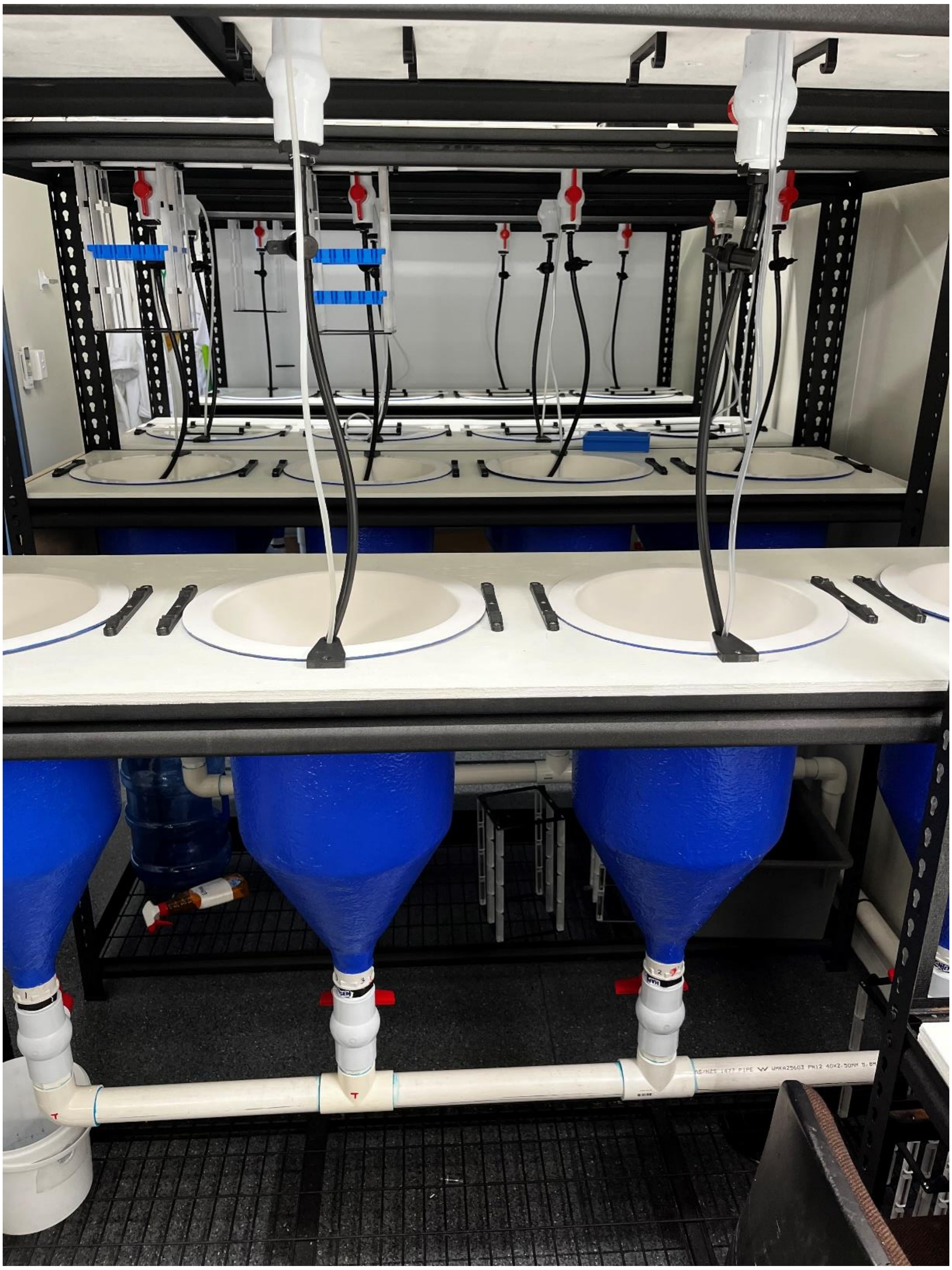


Figure 8: Conical replicate toheroa holding tank layout with racks and associated piping within the laboratory.

Figure 9: Photo showing external system components. Including 10,000 liter initial holding tank (Top right), insulated cover IBC’s (Bottom) and filtration canisters within water chiller (Top left)

Figure 9b: Photo showing the UV sterilization and pump set up inside an enclosed shed to outside of the laboratory.

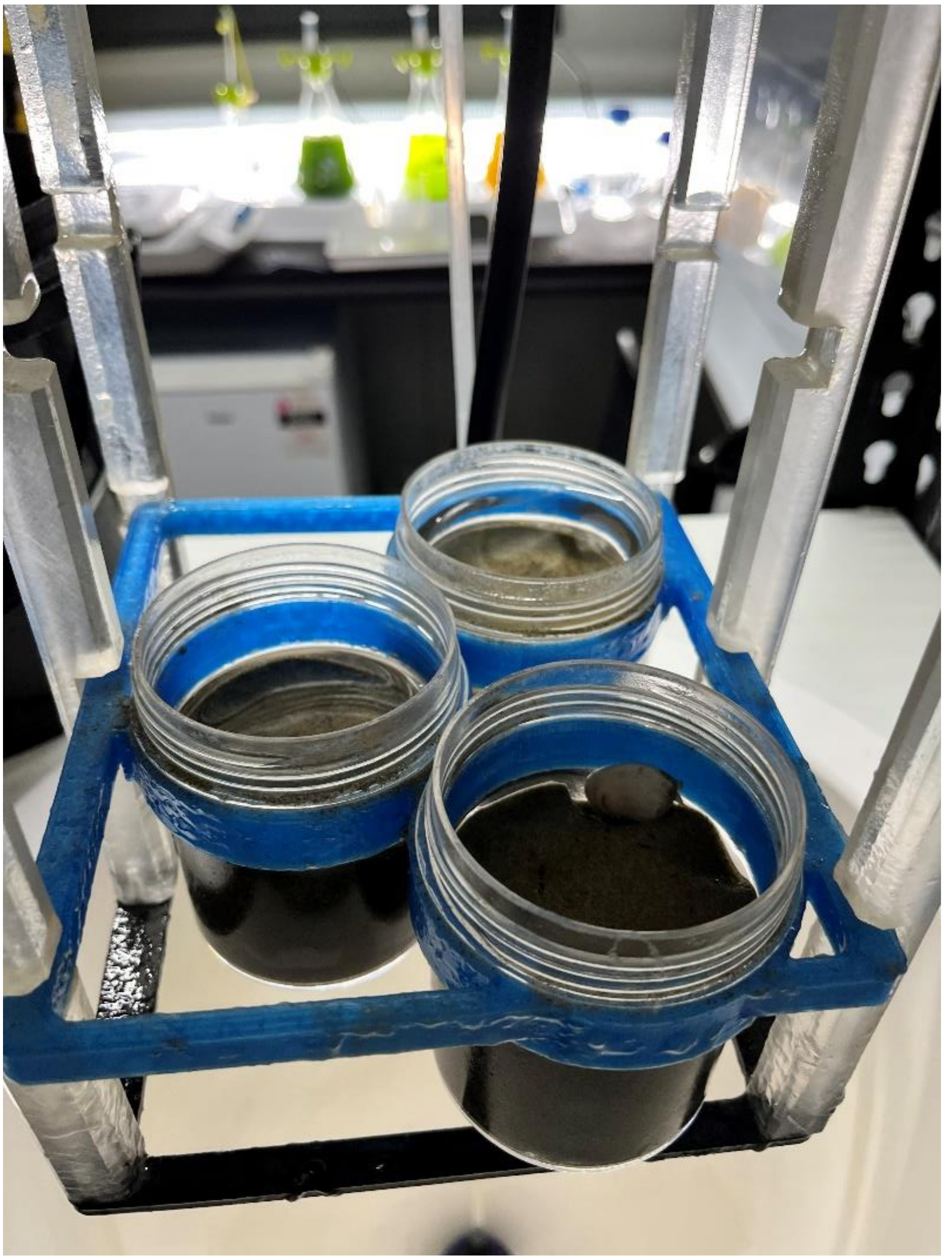


Figure 10: Photo showing the 3D printed rack system designed to hold the replicate jars. Within the jars contain toheroa spat.

# Chapter 4: Experiment 1: Comparing chlorophyll concentration of toheroa beds when compared to none-bed locations of the beach.

## 4.1 Introduction

To understand how a species is best cultivated it is crucial to understand condition in the wild in which the animal thrives (Marzano & Brizzi,2009). With this species there has been many fluctuations to its natural distribution (Cassie, 1955). Theses fluctuations are not entirely understood. Reports from locals have described the toheroa beds to be extremely abundant in its precolonial times before the influx of population and predation pressure from Auckland (Ross et al., 2019). This has also been confirmed by the various middens which are located around the dunes adjacent to the beach (Ross et al 2019). Theses middens were sites where Māori traveled to the coast, shucked the shellfish and discarded the shells in piles so that the meat could be better transported (Taikato, 2022). The middens are good ecological indicators of the condition of toheroa ecology (Taikato, 2022). The overall size was much larger than their modern counterpart, as well as being in far higher abundance (Ross et al., 2018; Hoby 1933; Cassie 1955; Street 1971; Redfearn 1974).

The main goal for any conservationist is to return these stocks to their former state and this poses questions on how to achieve this (Doak et al., 2015). The standard model is to ban/restrict taking of theses animal, which has in the past relaxed pressure on the populations, and they begin to return (Munro & Scott, 1985). This has not been the case with toheroa, even with heavy restrictions the populations still appear to be on the decline (Ross et al., 2018). Through the process of elimination, it is clear to see that there are other variables responsible for the decline other than just harvesting. Through observations done by other researchers, it could be pointed that there is an environmental issue with the recruitment of juveniles (Ross et al., 2018; Bennion et al 2022). Approximately twice a year around the summer months, toheroa have synchronized breeding events (Gadomski & Lamare, 2015). These events are timed through temperature changes in the ocean (Gadomski & Lamare, 2015). Once the gametes have been fertilized, they spend a period drifting with the ocean before settling on the beach (Redfern, 1982). At this stage they bury within the beach and begin to feed and grow into adulthood (Redfern, 1982). However, throughout recent history these juvenile toheroas have not survived long enough to begin to replenish the former stocks (Redfern, 1982). This type of breeding strategy expects large amounts of mortality (Crean & Marshall, 2008). Broadcast spawning is a numbers game with most juveniles perishing naturally (Crean & Marshall, 2008), with most not finding suitable bed habitat to settle (Crean & Marshall, 2008. Within this strategy, however, gives the opportunity for the juveniles to colonize the largest amount of suitable habitat available (Crean & Marshall, 2008). This is the strategy for all toheroa's close relatives such as pipis and tuatua and they can be found in high abundance right across New Zealand (Hooker, 1997). However, toheroa do not appear to be that successful (Cope, 2018), even with the large spawning events being successful in allowing the spat to settle on the beach (Ross et al 2018). There appears to be an environmental bottleneck restricting the chance of the success within the habitat (Ross et al., 2018).

There has been a variety of literature on the distribution of toheroa, when it was described that the adult beds of these species were found around the input of freshwater streams (Redfern, 1972). This has been confirmed through a multitude of other observational research since then. With this clearly being the case with the Northland populations of toheroa, it is not clear to see why the distribution is so different from the southern populations, which are more uniformly distributed across the beach (Gadomski & Lamare, 2015). Although the distribution has been described, there are still a few questions around why the distribution is like this. The most agreed upon explanation is that the toheroa require constant saturation within the sediment (Cope, 2018). This keeps them from desiccating during long periods of tidal emersion (Cope, 2018). This is plausible as Northland as a region of New Zealand is considerably warmer than the rest of the country during the summer months with a higher frequency of drought (Huber, Iroumé & Bathurst 2008). The change this has had on the surface water hydrology has been well documented (Huber, Iroumé & Bathurst 2008). Another contributing factor is that the beach itself, if exposed to high winds, which will dry out the sand rapidly at low tide (Huber, Iroumé & Bathurst 2008). All toheroa are primarily suspension feeders and need their siphon to extend above the sediment to feed (Redfern, 1972; Kondo & Stace, 1995). Because of this they are usually found very shallow in contrast to the adults (Kondo & Stace, 1995). This makes them far more likely to succumb to desiccation through the drying out of the sand (Cope, 2018). Conversely however, there can be some flaws with the saturation explanation. Through my own observations, the streams water distribution is highly varied. Many of the streams are small in comparison to the reported size of the beds they support. Furthermore, the streams are highly susceptible to the strong winds across the beach, causing the flow to bypass many beds all together. This saturation explanation is in contradiction to the intertidal distribution. This is a highly variable environmental parameter; any changes would significantly affect the population's recruitment. If saturation was a critical component, toheroa bed distribution should be further down past the low tide mark, under constant submergence, thus, ensuring that they were constantly saturated and could spend more time feeding. This explanation does not totally explain the reasoning for distribution within the intertidal zone, which is far more hazardous. There have been rudimentary attempts to discover toheroa past the low tide mark which have been unsuccessful (Redfern 1972). This was partly due to the rough nature of the surf at the beaches with low visibility, making manual surveys extremely difficult; as well as an overwhelming population of tuatua which could have outcompeted toheroa for the available habitat. Also, it is interesting to note that the settling periods for theses juvenile shellfish is within the summer months, when freshwater flows are at their lowest and the sun is at the strongest (Crean & Marshall, 2008). If saturation is the crucial component for spat survivability this is by far the worst time of the year for these animals to be settling (Huber, Iroumé & Bathurst 2008). There appears to be many questions which this explanation poses and requires further research. Understanding why the environment of the beds is favorable is the key to understanding the recruitment.

Through my own visits to toheroa beds, there appeared to be an obvious difference between the sediment of the toheroa beds and areas adjacent to it. The coloration of the sediment was extremely green which indicated to me the presence of microphytobenthos within (Waska & Kim, 2010). Production of this kind would likely be caused by the stream input depositing nutrient from the land run off (Waska & Kim, 2010). These nutrients would have been trapped by the sediment, making them available to be used for primary production (Waska & Kim, 2010). Initially, I disregarded this as a potentially food source for the toheroa as the literature stated that settled spat are suspension feeding bivalves (Ross et al, 2019). However, within this sediment we found a larger density of healthy toheroa spat and were found at deeper depths. The question is if this primary production is just a secondary biproduct of the freshwater stream input and if the toheroa only thrive in that location for saturation. Or does the presence of microphytobenthos within the sediment benefit them in some way, at least in the early stages of their lives? It has been shown in literature that benthic productivity is the base for supporting large amounts of diversity and biomass within coastal ecosystems (Haese and Pronk 2011; Jones et al. under review). With most of the primary production being within the sediment in shallow waters created by it (Gargas 1972; Nowicki and Nixon 1985; Varela and Penas 1985; Plantecuny and Bodoy 1987; Jassby et al. 1993; Macintyre and Cullen 1995). There has been a wide range of research conducted into relationships like this primarily within the estuarine environment (Kelly et al. 1985; Nixon et al. 1996). Although their habitats differ greatly, they are still exposed to tidal fluctuations which have huge impacts of the survivorship of the animals that live there. Within shallow estuarine environments, it shows that they are dependent on the exchange of solutes and particle between the pelagic and benthic waters (benthic-pelagic coupling) (Kelly et al. 1985; Nixon et al. 1996). This provides food for the organisms who reside within the sediment as well as providing oxygenation.

The purpose of this study it to confirm if there is a presence of primary producers within the sediment of the toheroa beds when comparing to no bed sediments on the same tidal zone. This experimental chapter aims to simply confirm if there is a parameter within the environment of the beds that differs from areas of beach that cannot support beds. To identify the presence of microphytobenthos, chlorophyll measurements would be taken as a proxy. With this understanding, future researchers could potentially build upon this to understand more deeply a potential biological/ecological relationship between microphytobenthos presence and toheroa spat survivals. Regarding aquaculture, understanding the relationship between spat and its habitat is crucial for successful ventures. Finding crucial parameters to focus on gives aqua-culturalist’s the information to create ideal environments for which there is a maximum chance of that animal reaching adulthood. The best source of that information is the optimal conditions is in the environment which these animals evolved to exploit.

## 4.2: Methods

The sediment samples were taken from beds surrounding Ripiro Beach at Kennedy Bay in Northland, New Zealand. There was a total of six sites which were tested. At three of these sites were toheroa beds and the other three sites were areas approximately two hundred meters away from known bed sites for comparisons. The control site were checked to confirm that there was no animals present. A total of ten samples were taken from each site (sixty in total). They were located along the stream input around Ripiro beach. (Site 1 = Chases gorge, Site 2 = Mahuta Gap, Site 3 = Kopowai). The samples were extracted using 10-centimeter core samples. Then the contents were emptied into small pottles for transportation. The sediment was then cleaned with Cleanaid and rinsed five times with Ultrapure water. The sediment was then frozen in the 70-millimeter plastic pottles at -20 degrees. Once freeze-dried for 24 hours, each of the samples had 0.15 grams of freeze-dried sediment into clean 15 milliliter centrifuge tubes, recording the exact weight to 3 d.p. Once in the 15 milliliter tubes, 10 milliliter of 90 percent buffered acetone was added to the sediment. The next step was to shake the samples vigorously. The processed samples were covered in foil to block out light and then placed in the dark for 24 hours at a constant temperature of 4 degrees. During this time frame the samples were shake once more (when). After this period had concluded, the samples were shaken again before being centrifuged for 10 minutes at 3300 rpm with high brake. The final stage of the preparation before analyses was to allow the samples to stand for 30 minutes in the dark to allow the samples to return to room temperature. The chlorophyll analyses were conducted using a fluorometer, which was turned on and warmed up prior to analysis. The fluorometer was then calibrated to the correct setting for this analysis. The analysis used was a multifactorial ANOVA with replication to compare the concentrations recorded and compare the variance amongst the treatments across the three replicate sites.

Figure 11: Image of stream input onto the beach before the sandstone cliff behind toheroa beds on Ripiro beach.

## 4.3: Results

All three replicate sites showed higher chlorophyll a concentration at all three replicate sites. Site 1 and site 2 described similar results with site 2 showing the lease Recorded. A multi factorial ANOVA was conducted on the total Chlorophyll concentration of the sediment samples which were collected at the sites. All the replication sites showed much higher mean Chlorophyll a concentration within toheroa beds compared to None bed samples. The testing of the ANOVA showed a high statistical difference between the two variables. This is shown through the p value of the test >0.001 (Table 1). As only comparing two variables a post hoc test was not necessary to understanding where the variance lies.

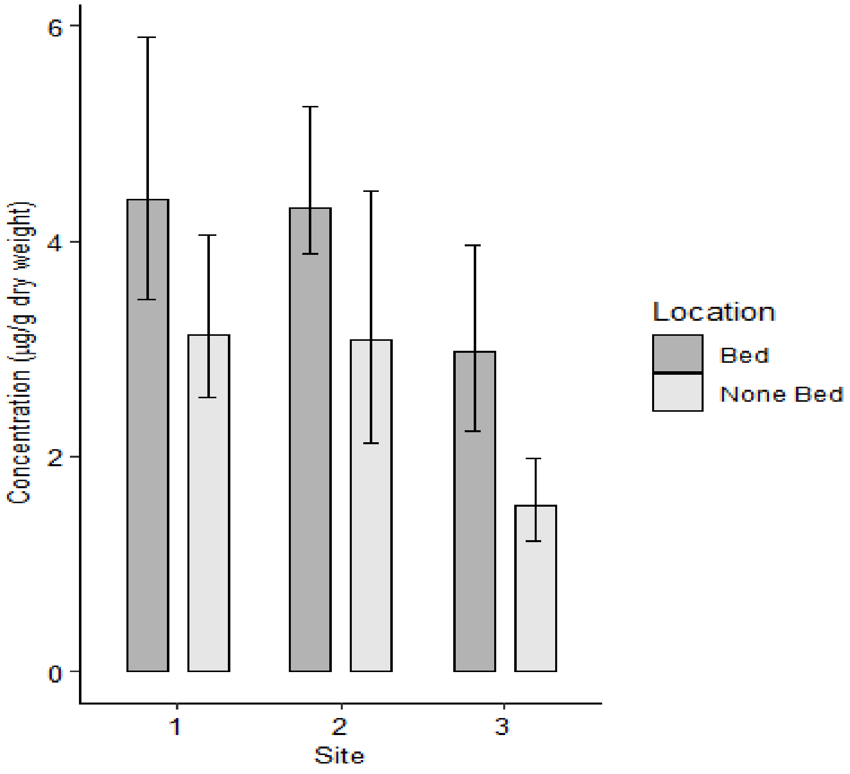


Figure 12: Plot describing the mean total concentration of chlorophyll recorded at each of the replicate sites for samples taken within the bed and outside. (Site 1 = Chases gorge, Site 2 = Mahuta Gap, Site 3 = Kopowai)( Error bar =SE)

*Table 1: Descriptive statistics for comparing total Chlorophyl concentrations within the sediments of Bed and non-bed locations across the study sites.*

ANOVA

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Location vs Concentration | 1 | 25.40 | 25.40 | 31.22 | 0.001 |
| Residuals | 58 | 47.19 | 0.81 | | |

## 4.5 Discussion

The results clearly show that there is a relationship between bed location and chlorophyll concentration. At all three sites the conc of Chl was statistically significantly higher than at non-bed sites. This was to be expected due to the initial observations of coloration differences. However, it is important to note that these results cannot confirm a connection that presence of microphytobenthos promotes juvenile toheroa survival rates. These results only confirm the presence of a biotic variable within the environment deemed naturally optimal for survival. There are a multitude of important reasons why this could be the cause. Most likely it is that saturation levels are described a crucial reason why this bed are distributed around where the input of the freshwater streams are (Cope, 2018). The excess chlorophyll could be a coincidental biproduct of this freshwater input and has no effect of the juvenile toheroa. To gain a full understanding of why the sediment within the toheroa beds contain higher concentration of chlorophyll, further studies would have to be conducted. Stable isotope work would be the best methods to reach conclusions on this topic. With stable isotopes researchers can look at the sources of which the chlorophyll came from (Bidigar et al., 1991). This type of research would indicate whether the microphytobenthos's produce food which the toheroa spat ingest (Fellows et al., 2006) as the isotopes would give an indication that the food of which the animal ate came from a terrestrial or marine nature (Fellows et al., 2006). There are also other reasons why the toheroa might benefit from operating in productive sediments. It is widely shown the benefit microphytobenthos has on sedimentary ecosystems (Hope & Thrush, 2020). In many cases they provide food for the animals who live there, however, they also play a large role in oxygenation of the sediment, both directly and indirectly (Hope & Thrush 2020). In the case of mudflats with high degrees of sediment loading, anoxic layers can form (Needman et al., 2011). With the presence of microphytobenthos, they can support populations of micro and macro fauna which move through the sediment grazing on it (Needman et al., 2011). This movement causes bioturbation within the sediment allowing further penetration of fresh oxygenated water (Hope & Thrush 2020; Needman et al., 2011). This then in turn extends the depth of the anoxic zone (Hope & Thrush 2020; Needman et al., 2011). Increased biodiversity also has several benefits and ecological impacts, such as symbiotic relationships, nutrient recycling disease prevention, food availability etc. (Hope & Thrush 2020; Needman et al., 2011). It is yet unclear if being within this environment is beneficial to toheroa spat survival. However, similar species have been known to benefit in another research (Kiørboe & Mohlenberg 1981, Prins et al. 1991). It has been discovered that suspension feeding bivalves can feed on microphytobenthos in other species (Kiørboe & Mohlenberg 1981, Prins et al. 1991). For example, a study conducted on seasonal patterns in growth and reproductive activity of a suspension-feeder *Laternula Marilina* discovered that the animal was willing to switch feeding methods (Kang et al., 2006). The results show that seasonal development of microphytobenthos was a critical food source around the period of gamete production, which was always around summer (Kang et al., 2006). Other studies show that food dependence on benthic vs pelagic sources for suspension feeding bivalves can be determined by temporal changes of its availability (Kang et al., 1999). Kamerman (1994) discovered that within the Wadden sea many of the deposit and suspension feeding bivalves ate similar food sources (Kamerman, 1994). They did this by comparing the algal composition within the stomachs and found that the species consumed was of similar nature (Kamerman, 1994). Another study conducted by lucas et al. (2000) demonstrated that intertidal sediment rich with microphytobenthos can be resuspended by wave action making them more readily available to suspension feeding bivalves again (Lucas et al., 2000). Implications of this could mean a lot for toheroa spat survival. Even if they are not directly feeding on it within the sediment. The high energy wave action of the beach has the potential to locally resuspend terrestrial microalgae into the pelagic space of which they can then feed on (Lucas et al., 2000). The input of nutrient from the freshwater streams would also help promote coastal micro algae development (Cassie, 1995). There has also been researching into the bacteria composition of toheroa and how it has changed over the years (Bennion et al, 2022). The research conducted indicated a change in the bacteria composition due to the land use change effecting the stream input (Bennion, Ross, Lane & McDonald, 2022). Changes in the bacteria composition of toheroa could be directly affecting the health of the animal and preventing their recovery (Bennion, Ross, Lane & McDonald, 2022). The composition change could have been caused by a changing benthic health of the sediment caused by the streams. Whether this has a direct link to microphytobenthos is currently unknown.

In relation to aquaculture, what does this research and our results mean? This research does not prove a direct link to microphytobenthic presence and toheroa spat survival. However, it does show a link that it has a large presence within its ideal habitat within the beach. This could mean that understanding the microbiological aspects on the sediment would aid in the culturing of the species. Better understanding of the ecosystem function could prove to become a tool for culturalists. Further research could test to see if the animals responded differently to different compositions of sedimentary microalgae. If it benefited their growth rates and through analyzing the gut content to see if they were consuming them. There are many aquacultural instances of using microphytobenthos to prime environments for spat rearing, for example, in the case of New Zealand black foot paua (*Halotis Iris*). After settlement the spat are moved into rearing tanks where microphytobenthos film has been cultured along plates within the tanks (Moss, 1998). The purpose of this it to provide food for the paua as well as ensuring health and immune protection to promote their survival (Moss, 1998). With the potential for toheroa aquaculture still at this stage in its infancy, it is important to use our understanding of its ecology to aid in developing the culture method. There are many questions still to be answered surrounding toheroa ecology which would be crucial in this regard.

# Chapter 5: Experiment Two: The essential need for sediment in relation to survival of toheroa spat

## 5.1: Introduction

The mechanism of which toheroa spat survive needs to be understood for both an aquacultural sense and ecological sense. The optimal conditions for spat survive are not well understood. This is the case for this species however there has been much research done on other species with similar ecology (Utting & Spencer, 1991; Gerasimova & Maximovich, 2013; Bonsdorff Norkko & Bostrom, 1997); Donadi et al., 2013). With all this research the main tread appears to the presence of viable substrate for the animal to settle in, without it the animals simply cannot feed themselves and begin to decimate and die, unless predated on. It is clear to see that in their environment toheroa requires appropriate sediment to be healthy. The animals use the substrate to protect themselves from predation, desiccation, as well as giving them the ability to feed (Joyce, A., & Vogeler, 2018). The toheroa has evolved entirely around operating within sand (Ross et al., 2019). The shell morphology is designed to move up and down within the substate with ease while also using the ability to use the sand to anchor itself firmly to resist being expelled from it (Kondo & Stace, 1995). Toheroas possess a large foot that can be extended from the base of its shell (Redfern 1982). This can be used to move up and down the substate however it can also be used to rapidly bury itself after being expelled from sand. The foot consists of muscle tissue which relative to its size, are very powerful. This is needed to lift the entire weight of the animal from horizontal to vertical in order to descend to safety rapidly (Kondo & Stace, 1995). In an aquaculture setting, substrate such as sand can be problematic. This is because it can rapidly clog filters and disrupt various sensitive equipment which is designed to monitor and clean the water (Bratvold, & Browdy, 2001). This can create a variety of extra expenses to any potential aquaculture venture (Bratvold & Browdy, 2001). The purpose of this experiment is to understand if sand within the system is crucial to the optimal survival of the spat. If it is possible to exclude the need for sand within a system without jeopardizing health of the animal, it would be a huge benefit to a potential industry (Bratvold, & Browdy, 2001). If proven that sand is essential, it would require the technical innovation in system design. This would have to require research into optimal alternative tank designs to cope with sediment loads, biological sediment dynamics, optimal grain size, stocking densities etc. (Van der Schatte Olivier, 2020). Research such as this requires considerable investment and time which would further slowdown the growth of any potential industry (Badiola et al, 2012). However, on a positive note, information obtained from research could be used to benefit management of the wild stocks, as there are some information gaps on the biology of this animal which might be useful in the recovery of the wild stocks (Ross et al., 2019). The purpose of this experiment is a crucial component to keep toheroa spat alive in an aquacultural setting.

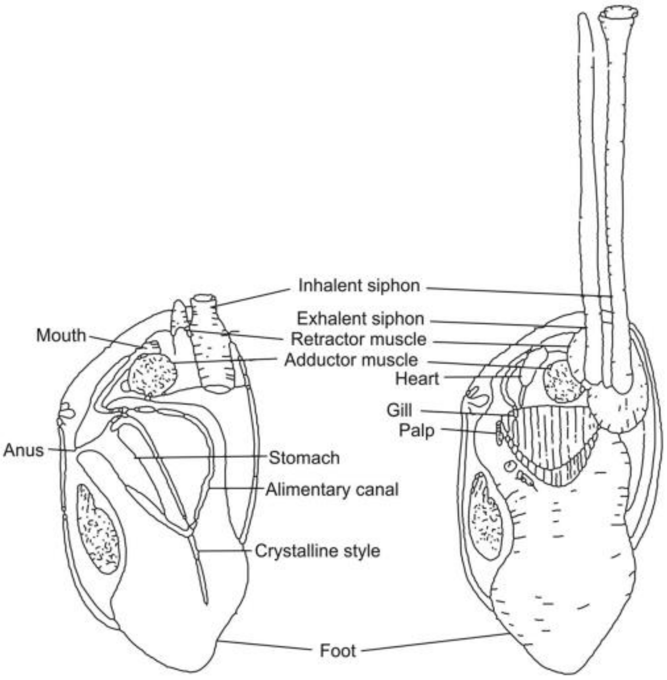


Figure 13: Internal anatomy of *Paphies Ventracosta* with left valve and mantle removed. Figure reproduced from Rapson, (1952)

## 5.1 Methods

The individuals for the experiment were taken from Ripiro Beach . The toheroa spat were harvested in the intertidal beach within the top to 10 centimeters of sediment. The digging was done using a plastic shovel going straight down prying the sediment up in big clumps. It was thought to be the best method to minimize the potential of crushing the delicate toheroa shell. The sediment was then placed into a sieve and mixed with sea water to remove the sediment and reveal the organisms within. Once recovered, the animals were placed into a bucket filled with wet sand and allowed to bury. The reason for using the bucket was to rapidly collect the numbers needed and transport them off the beach before sorting them. This was because the collection was on a time limit. The beds were only uncovered at low tide meaning that there were only a few hours to collect the required animals before the tide came back in.

It was also critical to collect all the animals required within the trial at one time, to minimize stressor bias. After the collection event the animals were left overnight with filtered sea water and aeration. The reasoning of this was to allow the spat to recover from the initial removal. Reburying requires a large amount of energy for the toheroa due to the nature of the muscle type of the foot as well as stress (Kondo & Stace, 1998; Redfern, 1982). The next stage was to assess the individual condition of each animal for the trial as well sort them into size classes. The individuals once removed were graded first by fitness and then by size. Fitness was determined by an overall assessment of the shell condition which involved checking for cracks etc. A secondary test was also performed by testing shell abductor muscle response which was performed by lightly touching the exposed part of their tongue between the shells. If healthy the spat should close their shells tight as a method of protecting themselves from predation. Once an individual spat had been assessed that it has the adequate fitness to be involved within a trial, the next parameter determined was size. To minimize any bias amongst the replicate the animals used for each experiment all belonged to a similar size class. This size class which was selected was between 10-20 millimeters long. This was approximately the middle range of the toheroa spat recovered from the beach. The animals required for this trial where then placed in a large refrigerated chilly bin containing sand and aeration for transport. The sediment was drained slightly to minimize sloshing around during the journey and disrupting the animals. The animals were transported to the University of Waikato's Coastal Marine Field Station to be placed within specially designed tanks for the experiment. After the transport, the animals were assessed again for fitness using the same method to ensure that the transportation had not compromised them. For this experiment there was allocated room for 24 replicates with 12 replicates contained sand and 12 contained in mesh (figure). The replicates consisted of three individual toheroas inside a pottle jar. Before being entered into the pottle, all three toheroas were patted dry with paper towels and weighed together. The weighing was done on scientific balance which was calibrated before the experiment began to ensure accurate results. The individuals were there placed on a white board recording the date with a ruler and a photograph was taken (figure). This was done so that the induvial spat could be accurately measured using a software called Image J. The software calculates the length against a known distance within the image (the ruler) which then can be applied to measuring the toheroa at the widest point on the shell laterally. Using this method can standardize the measuring of every toheroa ensuring accurate reading throughout the trial. The animals were then placed within the 3D printed racking systems designed to house the pottles and then placed within the tanks (figures). The tanks were exposed at the top where the water came in and drained from the bottom. Having the exposed top gave the study the ability to observe the toheroa spat from above without having to disturb them. The daily requirements required the observation to check for mortalities and remove them from the system to avoid causing water quality issues. Mortalities where measured and recorded with the date and time of their discovery. Other daily requirements involved testing water parameters, temperature, and cleaning filtration socks to safeguard against mortality events. This was done to ensure bias potential control. During the first week of the trial, it was allowed that any individual that had died could be replaced within the replicate, as the deaths would likely be due to the stressor of removal and transportation. This also allowed time for the replicates to acclimatize properly to the system and begin feeding. After this time the trial could officially start with full healthy replicates. The trial consisted over eight weeks with two feeds every day. The feeds consisted of shutting off the water circulation of water to the tanks then adding a formulated shellfish diet. Shellfish Diet 1800® is a unique mix of five marine microalgae: *Isochrysis, Pavlova, Tetraselmis, Thalassiosira Weissflogii & Thalassiosira Pseudonana*. The amount between each tank was standardized and mixed with filtered sea water using a magnetic stirrer (20ml concentration + 200ml of seawater). The mixture was then added to the tank and left for one hour. After that, all the water from each of the tanks was dumped out and clean filtered sea water was added to the system (50ml per tank). Once a week each of the pottles were removed and measured using the same method previously described, this was done to report the any growth amongst the replicates over time. During this time the sediment was rinsed with sea water and the surfaces of the tanks and racking systems were wiped clean. Mortality rates mean weight and mean length are the parameters that would be used to document changes between the treatments over the trial.

$$\frac{(weight_{initial} - weight_{final})}{weight_{initial}} \times 100$$

$$\frac{(length_{final} - length_{initial})}{length_{initial}} \times 100$$

Figure 14: Formulas used to calculate the weekly percentage weight and length change of the treatments for standardized comparison of experiment 2.

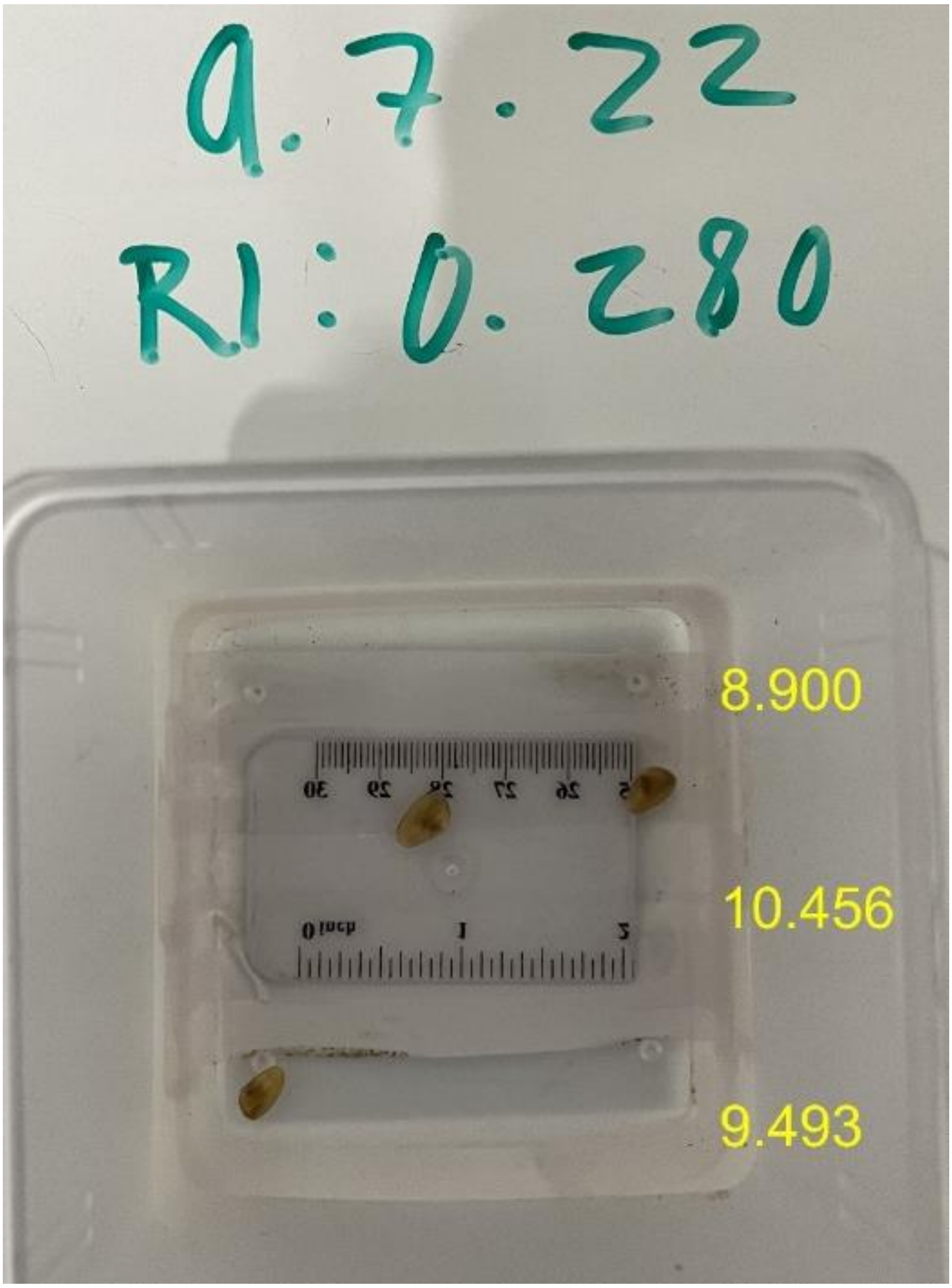


Figure 15: Example of measurement method used to determine growth of toheroa spat. The image recorded weight was processed using Image J to identify accurate record length of the individuals.

## 8.4 Results

The percentage change in weight was calculated for each week using the formulae discussed in the method. Comparing them against the treatments of substrate which include sand and rack showed a difference between the treatments. Replicates containing sand showed a 36.9% increase of men weight over the 8 weeks when compared to replicates within the mesh racks only having a 7.7% increase in mean weight. A single factor ANOVA was used to compare the amount of mean weight gained for each of the replicates. The ANOVA produced a p value = > 0.0023, which shows a highly significant difference between the treatments. The ANOVA testing showed that there was a significant weight increase for both replicates when compared to the time of the trial and the initial weight with a p-value 0.043. Which combined shows that both treatments did increase in weight over the trial however individuals in sand increased more.

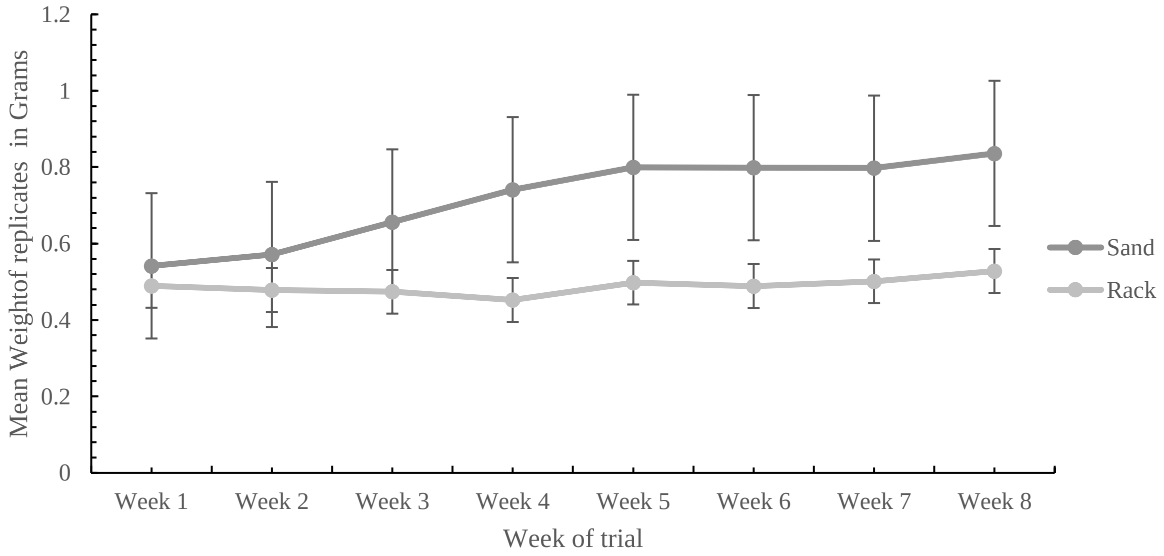
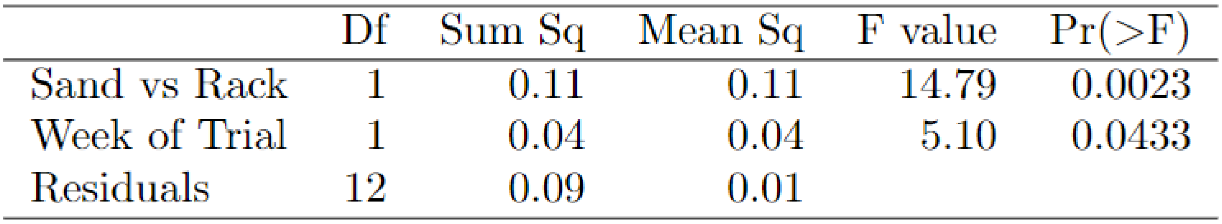


Figure 16: Plot describing the mean weight in grams of the different treatments of substrate over the course the trial. (Error = SE).

*Table 2: Descriptive statistics for Comparing the mean weight % change of toheroa spat of different treatments of substrate over the course the trial.*



| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Sand vs Rack | 1 | 0.11 | 0.11 | 14.79 | 0.0023 |
| Week of Trial | 1 | 0.04 | 0.04 | 5.10 | 0.0433 |
| Residuals | 12 | 0.09 | 0.01 | | |

Mean shell length was recorded as each week and then calculated into a percentage change coming from the initial length. The initial mean length was the same at the beginning of the trial as the individuals chosen for the trial were the same size class for comparison. Over the trial both treatments showed a percentage increase on mean shell length. The replicates inside the sand showed a 10.73% increase whereas the replicates within the Rack showed a 2.62% increase A Multifactorial ANOVA compared the percentage length change over the course of the trial between the replicates. This testing showed a highly significant difference between the two treatment with a p-value of 0.0165. The testing also showed that there was an increase in mean shell length for both treatment over the course of the trial with a p-value of 0.003. This means that both treatments showed a statistically significant increase over the trial however the replicates containing sand had a higher increase.

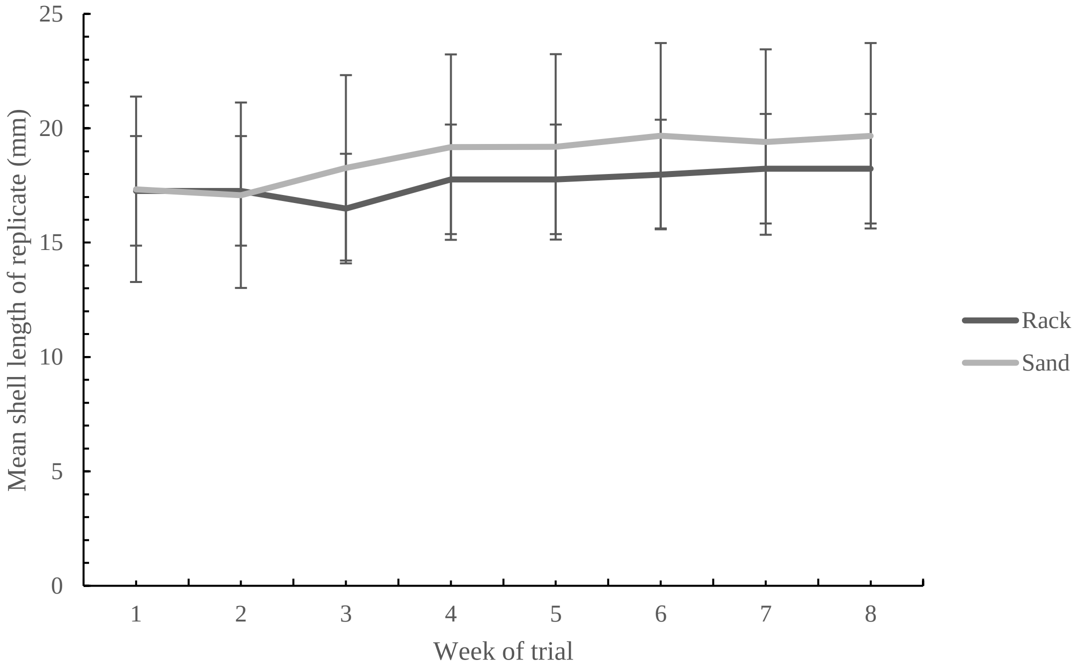


Figure 17: Plot describing comparing the mean length gained of toheroa spat of different treatments of substate over the course the trial. (Error bar= SE)

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*Table 3: Descriptive statistics for Comparing the mean length gained of toheroa spat of different treatments of substate over the course the trial.*

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Sand vs Rack | 1 | 5.02 | 5.02 | 7.76 | 0.0165 |
| Week of Trial | 1 | 8.47 | 8.47 | 13.10 | 0.0035 |
| Residuals | 12 | 7.76 | 0.65 | | |

Mortality rates where done by calculating the percentage of the population remaining after death with the population of the replicates were observed and removed. Both treatments showed an initial sharp increase in mortality between week 1 and week 4 before leveling out. At week 4 the Sand treatment had dropped to 61% where the Rack treatment had dropped to 32%. At the end of the trial the sand treatment had 55% of its population remaining whereas the rack treatment contained 22%. The testing showed a highly significant difference between the two treatments with a p-value of 0.01. The testing also showed that over the course of the trial there was a statistically significant decline in percentage for both treatments.

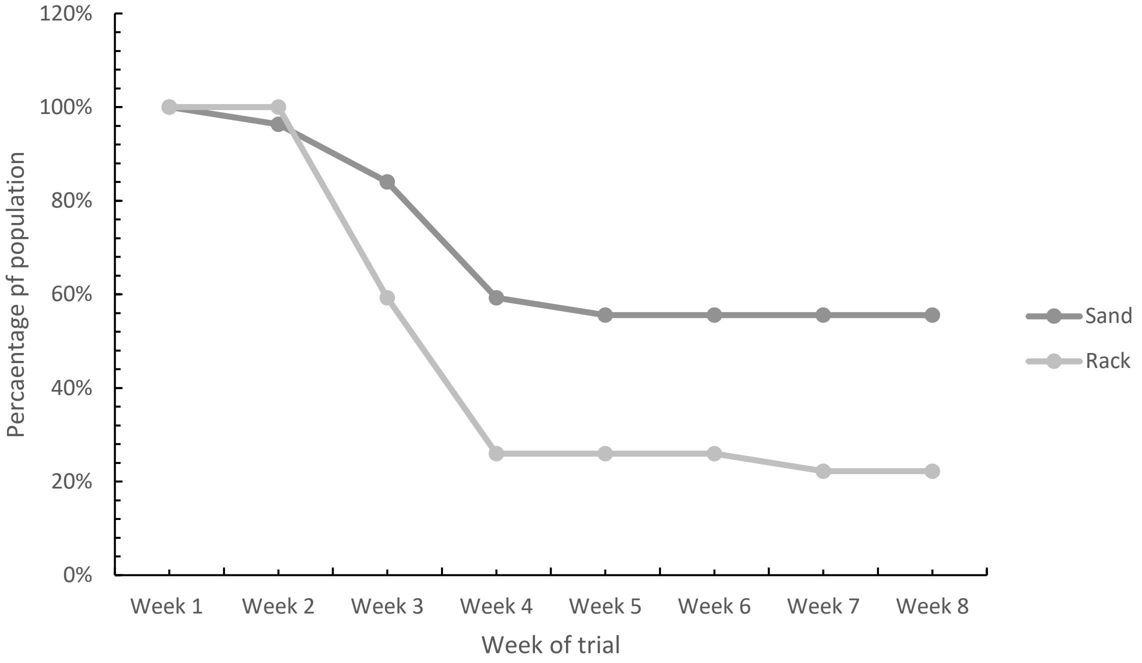

Figure 18: Plot detailing percent survial of the populations of the treatment over the course the trial.

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*Table 4: Descriptive statistics for Comparing the mortality percentage of the populations of the treatments of substrate over the course of the trial*

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Sand vs Rack | 1 | 2153.00 | 2153.00 | 19.87 | 0.0012 |
| Week of Trial | 7 | 6697.17 | 956.74 | 8.84 | 0.0001 |
| Substrate vs Week | 7 | 2356.75 | 336.68 | 3.11 | 0.0137 |
| Residuals | 8 | 1059.50 | 132.44 | | |

## 8.5: Discussion

The results of this study clearly shows that in this trial replicates containing sand performed better in a larval rearing system of toheroa. Both treatments appear to have positive growth rates showing increases in both mean length and weight. However, the animals within the sand treatment grew much faster than the ones in the racks. Differences in mortality rates between the two treatments were even greater.  Both treatments suffered mortality, which is to be expected from taking of wild spat (Degremont et al., 2007). Both treatments saw the highest mortality around in the early stages of the trial with relatively few mortalities after week four in comparison. the racked replicates had almost over 80 percent mortality within the population over the trial. Whereas the sediment replicates began to plateau at 30 percent reduction at week four. The substrate in which toheroa were housed clearly indicated its success within the system demonstrated through growth and mortality rates.

Toheroa behavior also differed between treatments. In sand, after a few weeks all the animals all the toheroa began showing their siphons. It was clear to see both inhalant and exhalent siphons protruding from the top of the sand. This was notably even more apparently, when the lights of the laboratory were switched off, indicating a light response (Kobak, & Ryńska, 2014), something that is being investigated in the MSc thesis of Ethan Russell. When compared to the animal lying in racks, they almost never had siphons or feet extended. The main behavior of the animals within the rack was that their shell was entirely closed. The likely reason for this was that they were not orientated horizontally like the animals within the sand. This means that the animal was not likely to be as affected at feeding when compared to the animals which had their siphons extended (Kondo & Stace, 1998). Toheroa in sand were able to have their foot fully extended moving up and down the sediment. This would have likely aided in their health and growth, using muscles that the animals in the racks did not (Gallardi, 2014).

Another interesting observation which could potentially pose bias within the results was the replicates containing racks appeared to have a major problem with fouling. The mesh that was used to hold the toheroa appeared to be an ideal media for bacteria film to develop within. This film sometimes began developing on the toheroa themselves and required regular cleaning. This film is surprising due to large amount of mechanical and biofiltration used in the system and the daily exchange of water. The mesh appeared to trap material for the film to develop. The mesh was selected to mimic potential commercial operations of toheroa aquaculture. However, future recommendation of trials could try other materials with larger mesh size. Fouling did not appear on the replicates with the sand in the same way, however, they did appear to have a problem with food accumulating within the lips off the pottles on top of the sediment. This did not appear to affect the animals within as their siphons could be seen extending through it.  This was likely caused through the method of feeding and tank design. The tanks were fed from the top and drained from the bottom, this was meant to provide a rapid exchange of water like they would experience in the wild. However, this does give the ability for food to be trapped within the replicates. Future redesigns of this system should take this into account and accommodate it in a commercial sense. In both cases for the treatments, rapid cleaning and water exchanges were done to minimize potential impact on the animals and bias with the results.

What does this mean regarding the potential commercial aquaculture of this species? Firstly, to maximize the survival rate and growth of spat with a system they need to be encompassed within sand. This could be to do with the sediment itself or to do with orientation. This poses extra cost and concern for any potentially business operator. If sand could have been excluded, it would be far simpler with a cheaper, easy to maintain system (Snow et al., 2012). Spat could have been taken from the wild and simply placed into large racks with feeding tanks and wait for them to grow into adulthood, which is done with other bivalves such as oysters in flip cages. However, this trial proves that is not the case, at least for juvenile toheroa. Excluding sediment could be possible for housing acclimatized adults as they are more robust, however, further research needs to be conducted to make that claim. Using sand can create a variety of issues, firstly, the hardware of a RAS system must be able to accommodate sediment loads. UV filter, chillers, pumps sock filters, biofilters would all be having to be rated to accommodate this (Mavraganis et al., 2020). Clogging of accumulated sediment at any point of the system could cause the system to fail (Zhang et al., 2011), potentially causing mass mortality events and expensive repairs for the operator. A secondary issue with sediment is the aim of any aquacultural business to rapidly grow your animals to a marketable size (Zhang et al., 2011). This requires using a large amount of food (Badiola, Mendiola & Bostock, 2012). In most aquaculture operations most of the food is wasted and not consumed by the animal (Badiola, Mendiola & Bostock, 2012). This is particularly the case with toheroa as they are primary suspension feeders, taking only a small number of phytoplankton out of the water column through their siphons (Redfern, 1982). To compensate for this, this feeding method needs to include high cell density for prolonged periods of time to ensure the animal has consumed the food. Excess food within a RAS system can usually be controlled with biofiltration before water quality issues can occur (Naylor et al., 2009). However, sand within the systems can trap excess food before reaching the filtration system (Badiola, Mendiola & Bostock, 2012). Within the sand, the food breaks down and feeds primary production causing a wide array of water quality issues (Granada et al., 2016). It can cause ammonia spikes within the water which can cause mass mortality events within the system (Granada et al., 2016). Within the sediment itself, it could promote bacteria colonization, which can cause the sediment to become anoxic with a black coloration (Holmer, Wildish & Hargrave, 2005). This is troublesome if the cultured species resides within the sediment such as toheroa. Within the course of the trial, due to the routine removal of the animals, we mitigated this issue. The removal and cleaning process aerated the sediment preventing it from becoming anoxic. However, in the instance of a large-scale operation constantly aerating the sediment could be impractical, as well as harmful to the health of the toheroa (Boyd et al., 2005). Lastly, the physical structure of the sediment and potential biological interaction would require further research. Grain size is a critical aspect of the ecology of all benthic macro and micro fauna (du Châtelet et al., 2009). Further research would have to be conducted into the optimal sediment type for survival within a RAS system. The microbial properties of the sediment have been shown in ecological research to directly affect the bacteria composition of the toheroa and by extension its health (Bennion et al., 2022). The ecological research details the change of bacterial composition over time (Bennion et al., 2022), likely due to the change of land use effecting freshwater input into the sediment habitat of the toheroa (Bennion et al., 2022). This research points to the direct link between environmental variables within the sediment and the health of the animal (Bennion et al., 2022). Yet these environmental variables are not well understood but would need to be able to fabricate an optimal environment for toheroa within a RAS System. Also, it has been proven that variables of sediment that toheroa are exposed to has a direct impact on the coloration of their shells (Cassie, 1955). Some populations of toheroa show heavy black rings, whereas others show orange on the shell from the iron washed down from the hills. Some populations possess bacterial abnormality on the surface of the shell (Bennion et al., 2022; Cassie, 1955). These may just be aesthetic qualities and pose little effect on the health of the animal. However, aesthetic qualities are important in the marketability of the product animal (Bennion et al., 2022). This clearly shown with the paua industry within New Zealand. Within the Bream Bay facility in Northland, a company called Moana produce paua commercially (Mohammadi, 2017). Due to the high degree of filtration and selective feeds, the cultured paua produce an unnaturally blue shell (Phillips, 2016). This means they can be sold at a premium price to domestic and overseas markets (Phillips, 2016). Protecting and maximizing the integrity of the condition of the shell would be an important aspect of a toheroa aquaculture industry. This could only be done by careful control and understanding of the substrate of which it is reared (Phillips, 2016). Although, the essential need for sand within a system provides a variety of issues, the benefits of having on land RAS systems is that they can be extremely versatile (Llorente & Luna, 2016). With further research, these issues can be solved and through engineering and protocol changes, a RAS system can be easily modified to accommodate the needs of the cultured animals.

# Chapter 6: Experiment Three: Comparing size classes against mortality of toheroa spat.

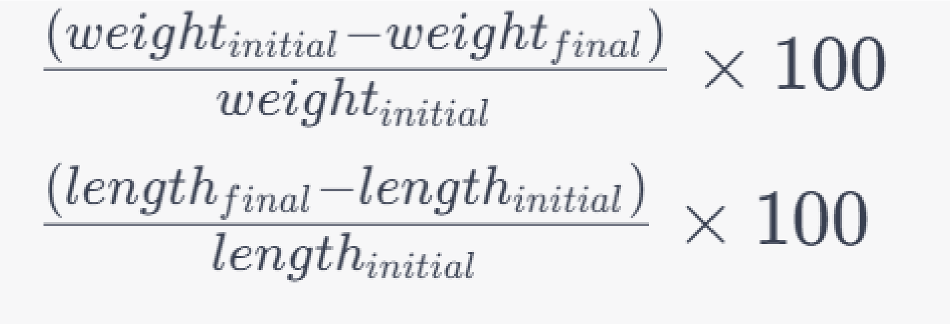
## 6.1 Introduction

Reducing mortality in wild harvested spat is an ongoing challenge in the aquaculture industry. Underlining that wild spat collected will always result in deaths (Alfaro, McArdle & Jeffs, 2010). This is because of the reproductive strategy of these animals which is expected to have mortality (Cassie, 1955). With broadcast spawning, the actual population requirement will have a very low percentage with most fertilized gametes dying (Alfaro, McArdle, & Jeffs, 2010). This is done by many species and is a highly successful breeding strategy within the wild (Hudspith, Reichelt-Brushett, & Harrison, 2017). When compared to mammals for example which have a small number of offspring, which require huge amounts of energy and time to safeguard their survival (Bronson, 1985). Toheroa produce millions of offspring and after spawning have no more involvement in the lifecycle of the offspring (Cassie, 1955). From the initially spawn the potentiality for survival of an individual toheroa juvenile is extremely low. When in their planktonic stage of their lifecycle they could be eaten by any creature which feeds on the planktonic (Hudspith, Reichelt-Brushett, & Harrison, 2017). The ocean current drags them out to sea or taken into shore where the coastal habitat is not favorable (Cassie, 1955). Even after the successful settlement, there are a multitude of factors which could cause a toheroa spat to perish. Predation, food availability sediment type, grain size desiccation, competition for availably habitat and even motor vehicle traffic are some of the variables that juveniles must contend with (Ross et al., 2019). The small size means that these animals are extremely fragile and must be much shallower in the sediment to feed (o Te Ao Tūroa, 2009).

This shallow depth means that they are more exposed to the stressors when compared to the deeper adult populations (Cope 2018; Kondo & Stace, 1998). Being situated at the right tidal height has been reported to be a key component of spat success (Ross et al., 2018; Gadomski, & Lamare, 2015; Greenway 1969; Cassie 1955; Rapson, 1952), reporting then that the highest density of juveniles operated in the upper intertidal. Regarding aquaculture when taking spat from the wild this must be taken to into account. Any potential industry of toheroa aquaculture would have to start with wild harvest of toheroa from the beach (Alfaro et al.,, 2010). Millions of tiny toheroas wash up on the beaches of Northland every year, with most of them destined to perish. Harvesting a small percentage of that would likely have minimal effect on the successful wild population (Cassie, 1955). Breeding in captivity would mitigate any impact on the wild stock and has been successfully completed with this species before (Janssen et al., 2018). However, laboratory breeding conditions are very expensive to build the facilities needed as well as time consuming and it would be more cost effective to skip the larval phase (Marzano & Brizzi, 2009). Wild harvest would eliminate these costs which would further promote the growth of the industry initially (Alfaro, McArdle, & Jeffs, 2010). Wild harvest of spat however does pose a variety of issues. Firstly, how to ensure that the spat that has been gathered will survive in captivity. It would be very costly for industries to harvest the spat on mass only to have them die sometime after within an aquacultural system (Alfaro, McArdle, & Jeffs, 2010). This is especially risky as the entire breeding strategy of this animal accounts for the overwhelming majority to perish. Also, it is very difficult to assess the animal's fitness onsite. Because of the multitude of environmental variables previously discussed, the spat harvested could be already dying at the point of harvest (Friedman & Southgate, 1999). They could be starving, desiccating, or injured and the stress of harvest and transport could be too much for them to recover from (Friedman & Southgate, 1999). From observations, it is also very difficult to assess the health of toheroa on site. When handled they close their shell and retract their foot and siphons, only showing their external shell. A healthy toheroa and a dying toheroa can look identical externally which is a common problem with bivalve aquacultural species (Wijsman et al., 2019). A potential solution to this problem is assessing the size of the animal. Size is an important parameter regarding health as it shows that the animal has been eating and growing (Wijsman et al 2019). It is also the only main distinguishing feature amongst the spat. It is also important to note that the spawning events of toheroa are synchronized so a cohort of spat would be approximately the same age (Gadomski & Lamare, 2015). It should be expected that larger individuals would have been more successful in the environment and would be more resilient with transport and aquacultural success (Wijsman et al 2019). Using knowledge such as this would give harvesters the tools to potentially only harvest animals with a maximum chance of success within a system (Wijsman et al 2019). Conversely, if there is no effect on mortality rates and size of individual collected, this would mean that harvesting could be less selective. In this case, harvesting could be done more efficiently and reduce costs to the industry This trial will be conducted to understand if there is correlation between the initial size of the spat and their rate of mortality within the system.

## 6.2 Methods

The methods for this experiment are very similar to the methods used for experiment two within this thesis. minimize any bias amongst the replicate the animals used for each experiment all belonged to a similar size class. This size classes that were selected were 0 to 10 millimeters, 10 to 20 millimeters and 20 to 30 millimeters. They were assessed on site to encompass most of the toheroa spat populations which were present at that time. The animals required for this trial were then placed in a large refrigerated chilly bin, containing sand and aeration for transport. The sediment was drained slightly to minimize sloshing around during the journey and disrupting the animals. The animals were transported to the University of Waikato's Coastal Marine Field Station to be placed within specially designed tanks for the experiment. After the transport, the animals were assessed again for fitness using the same method to ensure that the transportation had not compromised them. For this experiment there was allocated room for 24 replicates with 12 replicates all of which contained the same about of sediment taken from the beach. The replicates consisted of three induvial toheroas inside a pottle jar. Before being entered into the pottles, all three toheroas where patted dry with paper towels and weighed together. The weighing was done on scientific balance which was calibrated before the experiment began to ensure accurate results. The individuals were then placed on a white board to record the date with a ruler and a photograph was taken. This was done so that the induvial spat could be accurately measured using a soft wear called Image J. The software calculates the amount length within a known distance within the image (the ruler) and then this can be applied to measuring the toheroa. Using this method, it can standardize the measuring of every toheroa ensuring accurate reading throughout the trial. The animals where then placed within the 3D printed racking systems designed to house the pottles and then placed within the tanks. The tanks were exposed at the top where the water came in and drained from the bottom. Having the exposed top gave the study the ability to observe the toheroa spat from above without having to disturb them. The daily requirements required the observation to check for mortalities and remove them from the system due to water quality issues. Mortalities were then measured and recorded (date and time). Other daily requirements included testing water parameters, temperature, and cleaning filtration socks to safeguard against mortality events. This was done to ensure bias potential control. During the first week on the trial, it was allowed that any individual that had died could be replaced within the replicate as the death would likely be due to the stressor of removal and transportation. This also allowed time for the replicates to acclimatize properly to the system and begin feeding. After this time the trial could officially start with full healthy replicates. The trial consisted over eight weeks with two feeds every day. The feeds consisted of shutting off the water circulation of water to the tanks then adding a formulated shellfish diet. Shellfish Diet 1800® is a unique mix of five marine microalgae: *Isochrysis, Pavlova, Tetraselmis, Thalassiosira weissflogii* & *Thalassiosira Pseudonana*.). The amount between each tank was standardized and mixed with filtered sea water using a magnetic stirrer. The mixture was then added to the tank and left for one hour. After that, all the water from each of the tanks was dumped out and clean filtered sea water was added to the system. Once a week each of the pottles were removed and measured using the same method initially described. This was done to report the any growth amongst the replicates over time. During this time the sediment was rinsed with sea water and the surfaces of the tanks and racking system was wiped clean. Mortality rates, mean weight and mean length are the parameter which would be used to document changes between the treatments over the trial.



$$\frac{(weight_{initial} - weight_{final})}{weight_{initial}} \times 100$$

$$\frac{(length_{final} - length_{initial})}{length_{initial}} \times 100$$

Figure 19: Formulas used to calculate the weekly percentage weight and length change of the treatments for standardized comparison of experiment 3.

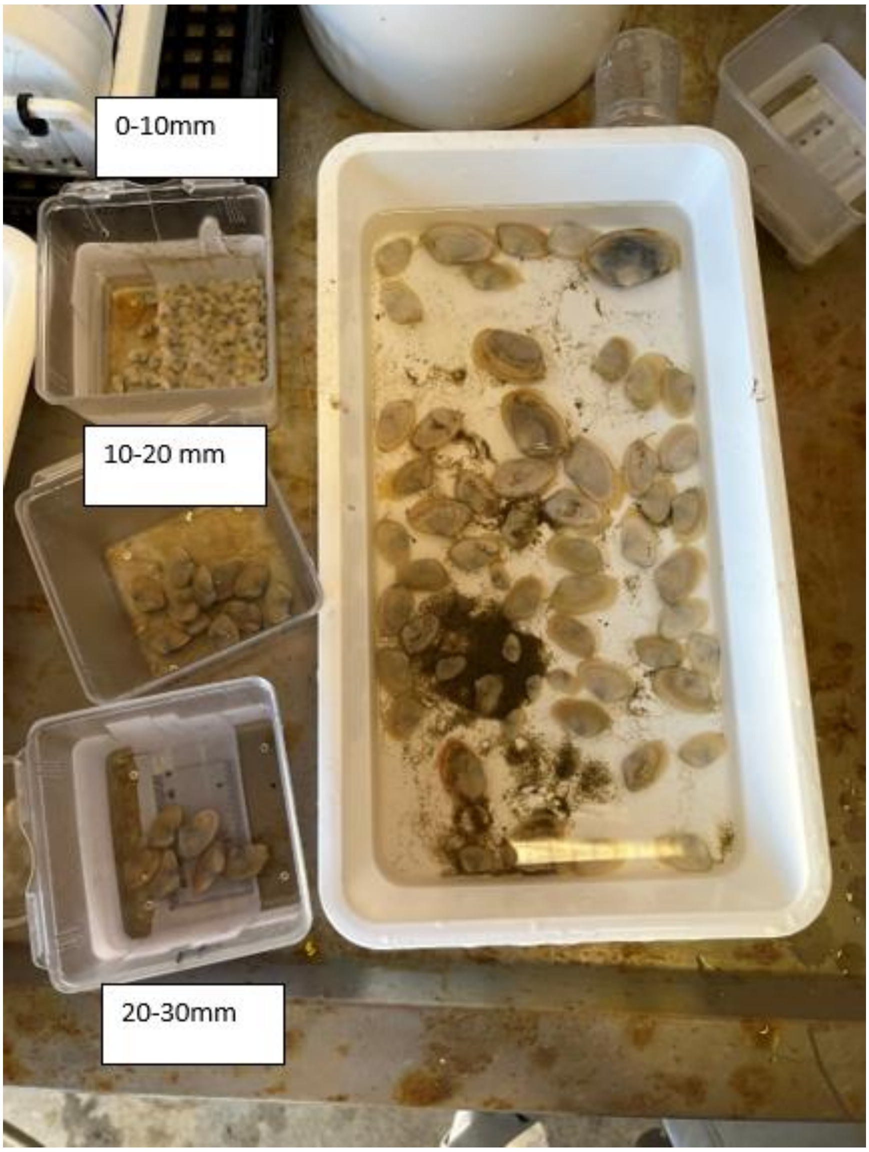


Figure 20: Photo detailing the method of which the animals were sorted into their various size classes treatments for analyses.

## 6.3 Results:

Comparing the weight change of the treatments of size classes was done using by calculating the percentage weight gained from the initial mean weight as previously discussed in the methods. This showed that all the treatment size classes did increase in weight. Through using this formula showed that 0 – 10 mm toheroa spat has a 23.57% increase in weight over the trial. The size class 10-20 had a 43.33% increase in weight and 20-30mm toheroa had a 36.18%. This relationship between percentage weight gain was tested using an ANOVA to understand variance and significant of the differences. The testing showed that there was a highly significant difference between the size class group comparing percentage weight change producing a pvalue of 0.01. However when comparing to see if the time of the trial had an impact on the percentage weight change of the replicates, this showed a none significant relationship with a pvalue of 0.44.

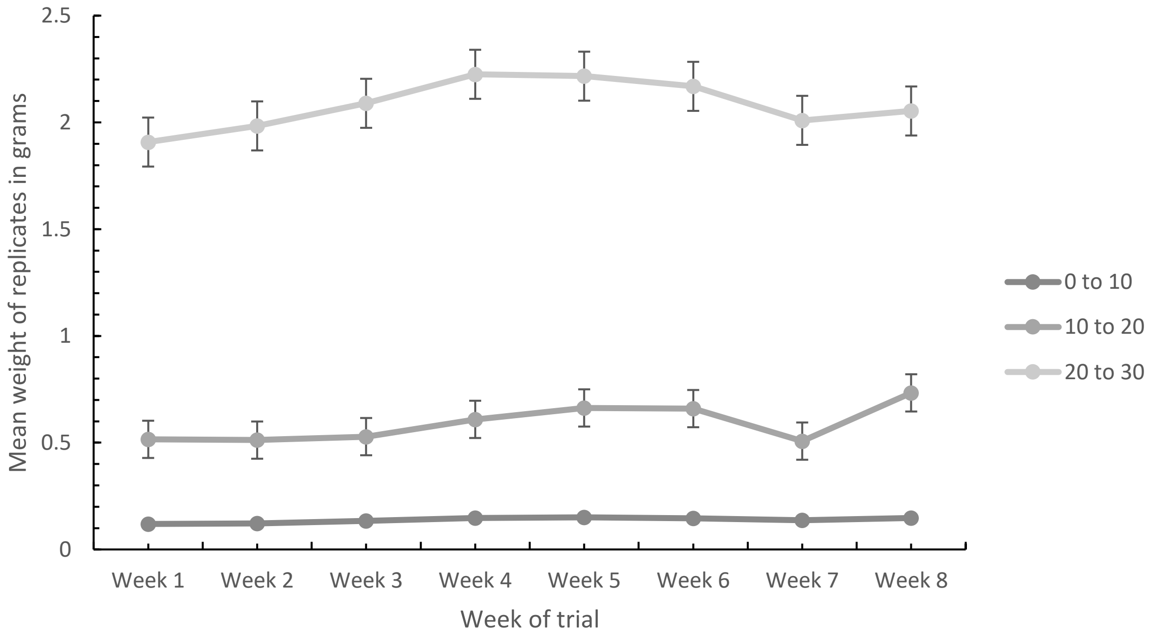


Figure 21: Plot describing detailing the Mean weight in grams of the different size classes over the course the trial. (Error bar= SE)

*Table 5: Descriptive statistics Comparing the Mean weight gained of toheroa spat of different size classes over the course the trial.*

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Week of Trial vs Percentage Weight Change | 1 | 0.03 | 0.03 | 0.60 | 0.4472 |
| Size Class vs Percentage Weight Change | 2 | 13.75 | 6.87 | 120.94 | 0.01 |
| Residuals | 20 | 1.14 | 0.06 | | |

Comparing the length change of the treatments of size classes was done using by calculating the percentage of shell length gained from the initial mean shell length as previously discussed in the methods. All the treatments showed an increase in mean shell length over the course of the trial. 0 to 10mm toheroa showed a 23.58% increase, 10-20 showed a 13.49% increase and 20-30mm showed a 5.76% increase. Through testing using a multifactorial ANOVA Showed a significant difference between all replicates in regard to percentage change over the trial. When comparing the treatments the testing produced a p-value of 0.01 which describes a highly significant difference.

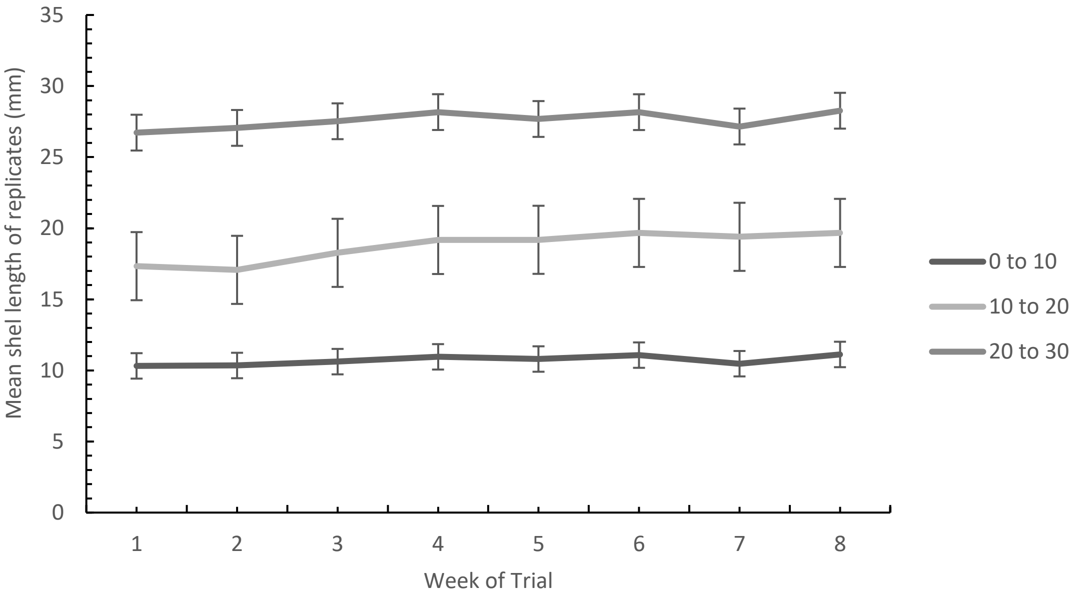


Figure 22: Plot describing mean length recorded each week in mm of different treatments of toheroa size classes over the course the trial. (Error bar= SE)

*Table 6: Descriptive statistics for multifactor analysis of variance (ANOVA) Comparing percentage length change of toheroa spat of different treatments over the course the trial.*

|  | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Week of Trial vs Percentage Length Change | 1 | 5.45 | 5.45 | 31.20 | 0.01 |
| Size Class vs Percentage Length Change | 2 | 1140.74 | 570.37 | 3263.24 | 0.011 |
| Residuals | 18 | 3.15 | 0.17 |  |  |

However, the smallest size class experienced the lowest mortality rate and maintained a healthy population after week four, whereas the middle range size class displayed the highest mortality rate, sharply decreasing the entire span of the trial. 0-10mm manages to retain 81% of its population which as 10-20% managed to retain 56% and 20-30 retained 78%. To confirm a difference multifactor analysis of variance (ANOVA) was used to compare the mortality rates across the size classes. This confirmed the difference amongst the treatments producing a p value = > 0.0001. The effect on time within the trial did have an significant effect on the all treatments producing a p-value of 0.0003.

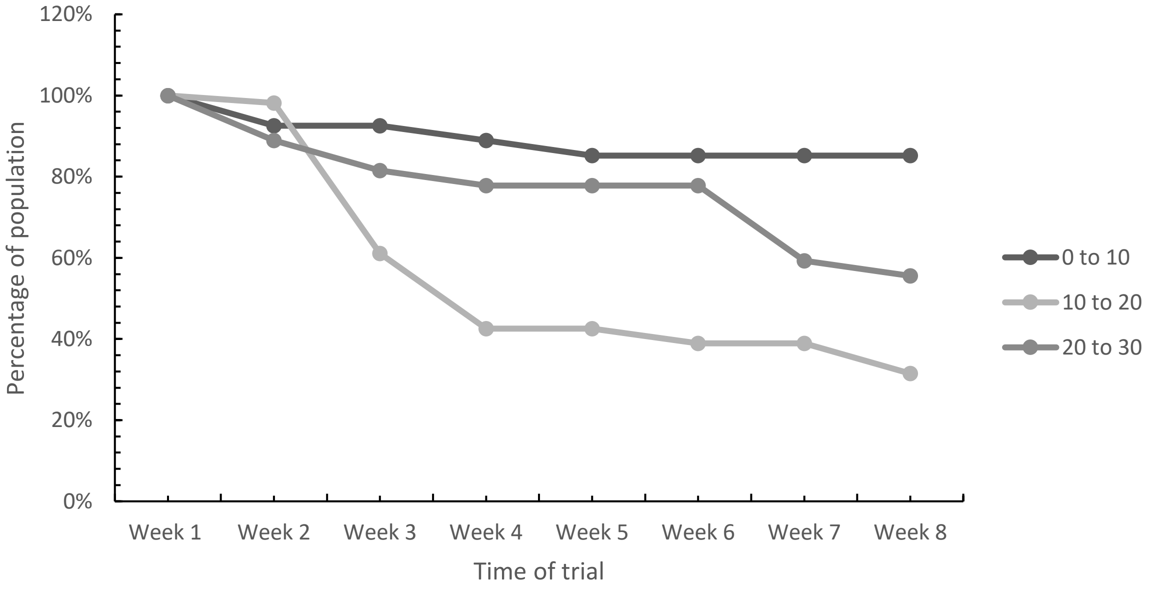


Figure 23: Plot describing mortality rates of the different treatments of size classes over the course the trial.

*Table 7: Descriptive statistics for comparing the mortality rates of the different treatments of size classes of toheroa spat over the course the trial.*

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Week of the Trial vs Population Percentage | 1 | 1679.37 | 1679.37 | 18.72 | 0.0003 |
| Size Class vs Population Percentage | 2 | 1768.58 | 884.29 | 9.86 | 0.0010 |
| Residuals | 20 | 1793.88 | 89.69 | | |

## 6.4 Discussion

The calculated weight percentage gained each week was used to standardize the parameter amongst replicates of different size classes for accurate comparison, which showed the larger class of toheroa to appear to grow slightly faster than the smaller classes. However, potential problems arose with the methodology when reaching conclusions on the weight gained within this trial. Firstly, growth rates were low and a better understanding of how these animals grow would have been better served by a longer trial. The purpose of this trial was not to detail the growth rates of toheroa spat. The purpose of recording theses parameters was to use them as an indicator of health and success within the systems. The next issue found with the methodology was that the size classes provided a wide amount of variance within the model. Due to the weight of difference individuals within a size class varying considerably. Mortality within a size class can begin to lower the validity of the model and skew the results, as they can no longer be recorded within the mean and length recorded weight. Recommendation to eliminate theses issue in future trials is to use smaller size classes. Smaller classes would be more accurate in determined difference and make the treatments more resistant to mortality without skewing the results. The growth rates of the toheroa spat were very small over the trial and difficult to make clear conclusions on difference between the replicates. However, difference amongst the treatments become more defined when analyzing the mortality rates. Initially, it was hypothesized that the larger classes of toheroa spat would have had a higher chance of survival within the system. This was assumed due to using size as a proxy for success within its environment. However, the mid-ranged and the largest size classes populations declined far more rapidly than the smallest. This trend is interesting as it could be explained through observations over the trial and potential inadequate methodology. All the treatments appear to have had an initial period of peak mortality. With the first three weeks showing rapid population loss before relatively leveling out around week four. The initial methodology allowed for an extra week to account for transport and acclimatization stress within the system before the trial began. Mortalities within this time were disregarding and replaced as they would be deemed comprised by the translocation or their initial condition. However, one week appeared to be not enough with the sharp decline across all treatments in the early weeks. This could have been mitigated by extending the period to ensure that all specimens in the trial are fully acclimated and healthy before starting trial (Bromley et al., 2016). The next observation which became apparent is that the larger toheroa appeared to expel themselves from the sediment and then rebury again. Whereas the smallest size class appeared to never expel themselves. This is interesting as expelling from the sediment is not beneficial to the animal's health. This is because it requires a large amount of metabolic energy to expel and then rebury. It would also restrict the animal ability to feed being in a horizontal position, when compared to being upright within the sediment. Expelling in this case was described in the literature as being a stress response to adverse conditions (Damodaran, 2020; Ross et al., 2019). In the wild if conditions were not ideal a toheroa would expel, and the surf and the tide would move it around the beach to a different area (Damodaran, 2020; Ross et al., 2019). However, in the lab the conditions were kept identical among the treatments, with the smaller toheroa thriving and feeding. The only variable that differed was the size, which coincidently caused an issue with the method. The jars used to house the toheroa within the sand were the same size across the treatment, with three individuals within each. Larger toheroa had less available space relative to their size than the smaller ones, so were at a higher relative stocking density. This could explain the reason for their expulsion as they are attempting to be moved to a less crowded area. High stocking densities of similar species have been shown to drastically effect health (Hue et al., 2017; Marshal et al., 2014). Geoducks are a heavily aquaculture species in China and research indicates that mortality rates and growth rates become worse when stocking density increases (Hue et al., 2017; Marshal et al., 2014). This is because competition for food availability and space for growth become restricted (Le et al., 2017). Toheroa can change locations in the wild conditions like this happen (Ross et al., 2018). In a RAS system they are confined, and this behavior could mean the animals constantly keep reburying themselves, expending energy and not feeding. This is purely speculation of observations and future research would have to be conducted into the reasoning for continuous behavior. Although, this trial does suggest further research is needed in optimal stocking density in an aquaculture setting.

Regarding wild harvest however, it appears from this trial that smaller toheroa can survive as well if not better than larger ones, once harvested from the beach. This means that a potential aquaculture industry would not have to be as selective when harvesting wild spat. This trial does show that large mortality rates after collection are to be expected, however this not related to the initial size range of collection. As all size classes experienced mortality events at the beginning of the trial. Mortality is more likely to be the reason due to acclimatization and transportation stress. Within this trial this issue began to level out at around week four, and further research would have to be done to mitigate this issue (Iwama et al., 1998). Other long-term stressors within systems would require further research as they would likely be the cause of mortality after this period (Iwama et al., 1998). Some of these stressors could include stocking densities, food availability, water parameters, sediment type and others (Iwama et al., 1998). The main aim of any successful toheroa industry is to make all juvenile toheroa reach adulthood where they can be consumed. To reach that goal, all parameters need to be isolated to understand the ideal environment for long term survival and fast growth.

Figure 24: Photographic evidence of food accumulation on of the substrate of the replicates containing sand. Also, photographic evidence of expulsion behavior of larger toheroa spat from the sediment.

# Chapter 7: General Conclusion

The purpose of this thesis was to answer a few of the questions surrounding toheroa spat survival in an aquaculture environment. Firstly, to gain a better understanding of its biology and ecology and secondly, to see if the species has the potential to be used within the aquaculture sector. If successful, an industry of toheroa aquaculture would provide jobs and add to local rural economies (Dudding & Ryan, 2000; Pambudi & Clough 2017; Wyatt, 2011). It would relax pressure on harvest of wild adult stocks (Parks, Pomeroy & Balboa, 2003) and allow local Iwi to enjoy a taonga species without fear of it over harvesting (Ross et al.,2019). Aquacultural interest would provide scientists with more of the resources needed to understand the biology of toheroa and aid in the recovery in the wild (Diana, 2009). Toheroa do have the potential to be a fully cultured species. However, there are a multitude of questions which need to be answered before that can become a reality. This thesis only managed to address one. How can farmers maximize the survival of collected toheroa spat in an aquacultural setting? This is a multiparameter problem but within the experimental chapters, the trial attempted to isolate the main three. Optimal environment with the wild (experiment one), optimal substrate with a RAS system (experiment two) and optimal of size of collected spat (experiment three). The aim of these experiments was to fill knowledge gaps within the literature to add to a potential framework for rearing survival of spat in land-based RAS systems. Experiment one sought to identify a new parameter within the sedimentary zone of which juvenile toheroa find themselves in. Chlorophyll was discovered in far higher abundance within this zone than surrounding areas. This parameter was used as a proxy for the presence of microphytobenthos. Being that this was a preliminary trial in cannot be overstated that it is unclear if this affects the survival of juvenile toheroa spat. Although other studies of suspension feeding bivalves have shown that the presence of microphytobenthos is essential for the species success (Needham et al, 2011; Waska & Kim, 2010: Kiørboe & Mohlenberg 1981; Prins et al. 1991; Kang et al., 1999; Kamerman, 1994). The aim of the experiment to identify a new parameter in the environment and raise the question so that further research can confirm or deny its role in the toheroa's lifecycles. Understanding the optimal wild sediment dynamics is key to attempt to mimic those within an aquacultural setting. To truly identify causal link would require extensive biochemical and physiological work (Prins et al. 1991), which is beyond the scope of this thesis. The purpose of experiment to was to compare two methods of housing spat, to understand if sediment was needed in the rearing of collected spat at all. As an exclusion of sediment would bring the cost and risk of developing rearing facilities down (Marzano & Brizzi, 2009). The results clearly show that it cannot be excluded. Growth and mortality were directly tied to the treatments of substrate used with growth rates reduced and mortality rates increased with the absence of sand. However, with sediment confirmed to be needed within a larval rearing system, it raises far more research questions. Sediment poses many issues within aquaculture systems which would have to be eliminated before an industry would be commercial scalable (Holmer, Wildish & Hargrave, 2005). However, the observations made within this trial could give future research an insight into the issues which needs addressing. Experiment three uncovered that a potential toheroa aquaculture industry can collect a various size range of toheroa spat and it will not negatively affect mortality rates. This is beneficial to a potential industry as small toheroa spat can be taken at higher numbers with lower effort. It also makes the process of collection more efficient and puts less stress on larger, more established toheroa. The trial did uncover some unintended observations around the ejecting behavior of the larger toheroa. This would have to be further investigated as possible issues around stocking densities might have large scale effects of long-term effects on the rearing of toheroa. In conclusion, these experiments answered a few questions needed in the development of a larval rearing practice. However, in the process of answering these questions, the discoveries raised many more questions which would have to be resolved before an industry is commercially viable. Toheroas are a wonderfully unique species, with a rich cultural history (Ross et al., 2018). The Aquaculture of this species could mean the start of a new industry and jobs for generations to come (Pambudi & Clough, 2017), while sharing something truly unique with the world. Also, aquaculture could mean the safeguarding of this species for future generations (Diana, 2018), so that it could forever be enjoyed for cultural purposes. As for many Iwi around New Zealand, toheroa have a direct connection to their history and cultural identity (Ross et al., 2018). Both outcomes of toheroa aquaculture would be beneficial for Iwi Hapu and communities that would be involved. However, there are many scientific questions to be answered before this can become reality.

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