Translocation of *Evechinus chloroticus* in the SUR 5 Fishery and the reproductive potential of large urchins

Samuel Frederick Thomas

A thesis submitted for the degree of
Master of Science at the University of Otago, Dunedin,
New Zealand
September the 8th 2010

Abstract

Evechinus chloroticus (commonly known as Kina) is a commercially important species found in coastal New Zealand waters. Their gonad quality is, however, poor in many regions and thus the areas with high quality gonads tend to be overfished. Feeding *E. chloroticus* on enhanced food diets is a common method to improve the roe quality but limited success has been achieved in improving the gonad quality to acceptable market standards. Translocation of *E. chloroticus* in the wild as a method for commercial gonad enhancement is examined in this thesis within the South Coast urchin fishing area (SUR5) to assess if gonad quality could be improved. Approximately 1000kgs of urchins were translocated from an area with poor quality gonad and high urchin population densities (Passage Island) to an area of high gonad quality and low urchin population densities (Sealers Bay) in Chalky Inlet a commercially fished area. The urchins were left for five months and sampled three times within this period. Changes in gonad index (GI) were measured to assess whether translocating *E. chloroticus* can be effective.

Significant differences occurred after the translocations with increased gonad growth in both the translocated samples compared to the Passage Island samples which had lower gonad indices. There were also differences in gonad index over the five month study length as a result of spawning before the last sample was taken, which resulted in significant changes in gonad index. Aristotle's lantern index increased in two translocated samples over the five month period.

Evechinus chloroticus fished from the Southern Coast SUR5 fishing area are of a large test diameter (TD) >120mm. Questions have been raised about the reproductive potential of these large urchins, thus the opportunity was taken to test

whether these large urchins were reproductively senile in the SUR5 fishery and if they do produce viable gametes would their larvae develop normally. Six large urchins >150mm TD were spawned and the fertilization rates were measured, with the embryos then cultured through to an 8 arm larval stage. Postoral arm length was measured over the larvae development to assess whether normal development occurred.

Fertilization success in the large urchins was high (99-100%) in all replicate samples. Larvae development was normal with increasing postoral arm length (µm) over the culture period, and no abnormal larvae were observed.

Translocation of *E. chloroticus* would provide an effective method of gonad enhancement over a short time frame (five months) that could be more cost effective than intensive enhanced feeding methods in improving gonad for commercial sale.

Large *E. chloroticus* >150mm TD, produce viable gametes and larvae that contribute to the larval pool and therefore could be a residual breeding stock in overfished areas around the coast.

Acknowledgements

Firstly, thanks to Mike Barker, my supervisor, for helping guide me through the thesis and providing advice where needed. Thanks to all the staff at both the Portobello Laboratory and Castle Street department, especially to Bev Dickson and Rene Van Baleen for helping set up experiments and also for general help around the Laboratory. Also, I would like to thank Caroline Wills for teaching me an amazing scone recipe to help become a great scone maker at the laboratory, a good pressure release activity. To Miles Lamare and Chris Hepburn I would like to thank you for helping me out with some important and often impromptu questions.

For making the thesis possible, I would like to thank Campbell McManaway for providing use of the commercial boat F.V. San Nicholas, and for providing information on the sea urchin (Kina) industry and the areas where gonad quality was low and high. I would also like to thank the crew of the F.V. San Nicolas (Ryan, Weeman, Jethro, Glen, Rowan Keys, and Tim) for helping move the 1000kgs of urchins.

I would like to mention thanks to my classmates and flatmates for being there to spin some yarns with, when a break was needed. Especially to Tom MacTavish, Rob Major, and Sarah Cumming for helping me out with questions and some statistics programmes.

Mum and Dad I would like to thank you for providing support when I needed during times of stress occurring here and there.

Finally I would like to thank my girlfriend Katerina Kelblova for putting up with me and supporting me throughout the time, as well as helping out with formatting my master's work.

Table of contents

List of Tables

List of Figures

1 General introduction

1.1 Biology and reproduction

New Zealand's most common sea urchin is *Evechinus chloroticus* (Kina) (Barker 2007; James 2007). Not only is *E. chloroticus* the most common sea urchin it is also the largest, with sizes of up to 190mm in test diameter (TD) (Barker 2007; McShane *et al*. 1994).

Both the gonads and the gut of *E. chloroticus* are used as storage organs, with nutrients stored in the gonad and the gut if excess nutrients are available (Andrew 2003; Barker 2007; Lamare *et al*. 2002; Wing *et al*. 2003). During times of low food supply *E. chloroticus* will reabsorb nutrients from its gonads in order to survive, with larger gonads occurring in areas of high food availability (Andrew 2003; Lamare *et al*. 2002; Minor & Scheibling 1997; Wing *et al*. 2001). The ability of *E. chloroticus* to reabsorb its gonads and the way in which its resources are allocated allows populations of *E. chloroticus* to persist even in areas of very low productivity (Andrew 2003; Lamare *et al*. 2002; McShane & Anderson 1997; Wing *et al*. 2001). *E. chloroticus* are a stenohaline species, with both male and female individuals present in populations at a ratio of about 1:1 which is typical of gonochoric species (Barker 2007; Dix 1970a; Franke 2005; Wing *et al*. 2001). *E. chloroticus* like similar marine organisms is a broadcast spawner with males and females releasing eggs and sperm into the water column where fertilisation takes place externally (Andrew 2003; Franke 2005; James 2007; Mladenov *et al*. 2007).

The size at which *E. chloroticus* reaches sexual maturity varies between populations, with some reaching sexual maturity at sizes of 35-45mm TD, while other

populations do not spawn until sizes of up 50-60mm TD (Barker 2007; Dix 1970a; McShane *et al*. 1997; James 2007). Food availability has been shown to be a major factor in determining size at sexual maturity. In areas with high food availability *E. chloroticus* reach sexual maturity between 3-4 years of age (Brewin *et al*. 2000; Dix 1970a; James 2007).   The spawning season of *E. chloroticus* ranges from November through to March (Andrew 2003; Barker 2007; Dix 1970a; Mladenov *et al*. 1997). Spawning is however very sporadic occurring at different times at different areas throughout the country (Andrew 2003; Brewin *et al*. 2000; Dix 1970a; Franke 2005; Mladenov *et al*. 1997). Once spawning occurs the fertilised eggs develop into free swimming pluteus larvae, which metamorphose through 4, 6, 8 arm echinopluteus larvae stages which settle on the bottom and metamorphose into juvenile urchins (Andrew 2003; Lamare & Barker 1999; James 2007). Once the juvenile *E. chloroticus* has settled on the bottom it grows into the adult stage (Andrew 2003; Barker 2007; James 2007).

1.2   Habitat, geographic distribution and ecology

E. chloroticus is found throughout all of the mainland New Zealand coastal waters and around most offshore Islands including the Three Kings, Snares and Chatham Islands (Andrew 1988; Barker 2008; James 2007). *E. chloroticus* is however not present around the Sub Antarctic Islands such as the Auckland and Campbell Islands, it is also absent from the Kermadec Islands in the north (Andrew 1988).

E. chloroticus is found from the sea surface down to 60 meters in some recorded cases, however its most commonly found at depths of less than 15m (Andrew 2003; Barker 2007; James 2007; Wing *et al*. 2001). The common habitat is hard rocky substrate, rock pools and crevices in the rocks, occasionally to a lesser

extent on sandy/mud and shingle habitats (Andrew 1988; Barker 2007; Dix 1970a). Densities increase with increasing wave exposure up until a certain point, where a decrease in densities will occur in areas of extreme wave exposures (Dix 1970a). *E. chloroticus* often occur in 2 different levels of abundance along the coast either in dense aggregations or "patches" sometimes up to 50 individuals per meter squared or in very sparse patches with very few individuals (Andrew 1988; Barker 2007; Dix 1970a). Distances between dense aggregations and sparsely aggregated *E. chloroticus* can be very short even when the habitats appear to be similar (Andrew 1988; Barker 2007).

E. chloroticus is a very opportunistic species, feeding on most algae species, as well as encrusting algae and some sessile animals (Barker 2007; Andrew 2003; Dix 1970a; Walker 1981). *E. chloroticus* removes portions of the food item using its Aristotle's lantern, and also the tube feet are sometimes used to trap drift algae (Andrew 2003; Barker 2007; McShane *et al* 1994; Walker 1981). The preferred food is large brown fucoid algae such as *Ecklonia radiata* and *Macrocystis pyrifera* (Andrew 2003; Barker 2007; James 2007; Walker 1981).

Sea urchins have a large effect on the diversity and biomass of algae in their habitat when present in large densities (Dix 1970b; Scheibling 1986). In medium densities urchin populations can cause the diversity of algae communities to increase. High densities of urchins rather than promoting diversity, cause the destruction of large areas of standing macrophtye algae species reducing areas to "barrens", large areas of Coralline flats (Andrew 1988; Barker 2008; Wing *et al*. 2003). In areas with high densities preferred algae are almost entirely removed through grazing as densities are too high for sufficient food to be obtained through drift feeding (Andrew 1988, 2003; Barker 2007). This pattern of barren formation during high densities is

common of urchin species around the world (Scheibling 1986). Where food is abundant urchins in large densities will remain relatively stationary consuming both drift and standing algae. When food levels are low urchins will move around in large groups consuming all standing seaweed in their path (Barker 2007; Dix 1970b). *E. chloroticus* has been shown to move in relation to food, with little to no movement occurring when food is high, and small amounts of individual movement occurring in low food conditions (Andrew 1988; Barker 2007; Dix 1970a). Movement in *E. chloroticus* is therefore concluded to be very dependent on food supply; there is however no recorded large scale feeding fronts occurring in New Zealand (Barker 2007; Dix 1970b).

Predation on *E. chloroticus* is very high during the juvenile stages right through to when the urchins are adults of around 70mm in diameter (Andrew 2003; Andrew & MacDiarmid 1999; Wing *et al*. 2003). Predators of *E. chloroticus* include many rock fish species such as *Parapercis colias* (blue cod), *Notothenia angustata* (Maori chief), *Psduedolabrus fucicola* (banded wrasse) and many other rock fish species as well as starfish species particularly *Astrostole scabra* and the rock lobster (*Jasus edwardsii*). All predators consume juvenile *E. chloroticus*, with predation decreasing as the size of the urchin increases with only large rock lobsters and starfish able to prey upon large urchins (90mm +), (Andrew 1988, 2003; Andrew & MacDiarmid 1999; Barker 2007; Wing *et al*. 2003). The full effect which predation plays regulating *E. chloroticus* populations, is not fully known, but will vary from area to area (Andrew 1988; Andrew & MacDiarmid 1999; Barker 2007).

1.3 World fisheries for sea urchins

Sea urchins have been harvested around the world for many centuries, in both traditional and now more commonly commercial fishing. The gonad (roe) from the sea urchin is a sought after commodity in many countries with large scale fishing targeting sea urchins to meet the demand of world markets. Japan is the largest market for gonad, accounting for 80% of the world's consumption, with the second largest consumer being France (Andrew *et al*. 2002; Williams 2002). The major fisheries of sea urchins during the 1970s were the areas in the Pacific North West (Japan and Korea). The fishery has now expanded around the Pacific, and on the west and east coasts of Canada and the USA, with the biggest producing area the Chilean urchin fishery (Andrew *et al*. 2002; Williams 2002; Explorations Unlimited Inc 2006). Sea urchin production is however declining in almost all of the fisheries around the world, with a peak catch of 113,654 tonnes in 1995 (Williams 2002). This decline is due to fishing down of the urchin biomass in each area, and poor management strategies (Andrew *et al*. 2002; Williams 2002). This strategy of fishing down the biomass of accumulated urchins in different areas leads to "boom and bust" cycles of urchin production (Andrew *et al*. 2002; Williams 2002). The further decline in overall production is only being maintained by an increase in production from Chile. This however, is not likely to continue as the extra production from Chile is a result of the expansion of the fishery into previously unfished areas, rather than through good management practises (Andrew *et al*. 2002; Williams 2002).

Sea urchin species which have dominated the world market in the past have been of the genus *Strongylocentrotus*, however this genus has been in decline since 1990 (Andrew *et al*. 2002). In 1998 the *Strongylocentrotus* catch made up only 39% of the total world production with the majority made up by the urchin *Loxechinus*

albus from the Chilean fishing industry. Other popular species of urchin which are sought after by the Japanese market are *S.franciscanus* on the West coast of the USA and *S.droebachiensis* on both the West coast of the USA and North into European waters (Andrew *et al*. 2002; Explorations Unlimited Inc 2006). The continued demand for high quality gonad from the Japanese market means that urchin stocks worldwide are being kept under continuous pressure. This pressure has led to many new strategies being trialled in order to meet the consumer demand as well as protect the urchin stocks, strategies such as aquaculture and closure of fishing grounds during spawning (Andrew *et al*. 2002; Williams 2002). In Japan one of the main reasons why the sea urchin fishery has survived is due to large amounts of reseeding of juvenile urchins, with around 70-85 million juveniles released each year (Andrew *et al*. 2002; Explorations Unlimited Inc 2006).

1.4   New Zealand's urchin fishing industry

The New Zealand sea urchin fishery, customary catch, and recreational take are based on the fishing of *E. chloroticus*. *Evechinus chloroticus* was only introduced into the Quota Management System (QMS) in 2002 in the South Island and 2003 in the North Island (James 2007; Williams 2002; Woods *et al*. 2002). Ten Fishery Management Areas (FMAs) were created around New Zealand following the introduction of the QMS. These FMAs have been divided up into sections so there are 5 QMAs in the South Island and 7 in the North Island each one known as SUR (Sea urchin) (Fig 1.1), (Andrew & Naylor 2002; James 2007 McShane 1997; McShane *et al*. 1994; Kina (SUR), 2009).
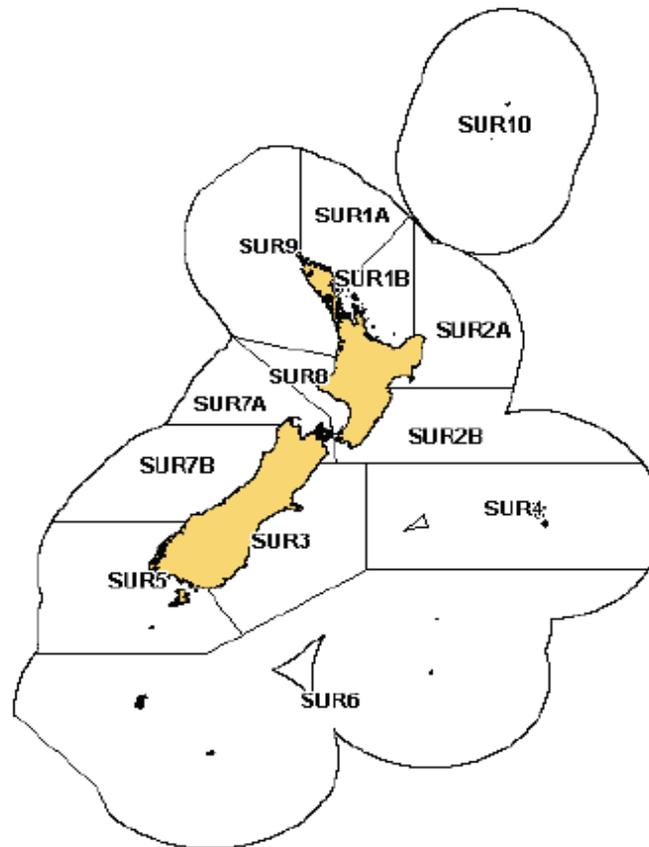
Figure 1.1 New Zealand's Sea urchin Fishery management areas (SUR) (fs.fish.govt.nz/Doc/ 21739/42_SUR_09.pdf.ashx)

A Total Allowable Commercial Catch (TACC) is allocated to each SUR area based on previous years fishing catches and some surveys, as standing biomass stocks of *E. chloroticus* aren't well known (Table 1.1), (James 2007; McShane 1997; McShane *et al*. 1994; Kina (SUR), 2009). There is however very little information on the standing biomass of *E. chloroticus* in most areas making accurate allocations of quota difficult (fs.fish.govt.nz/Doc/21739/42 _SUR_09.pdf.ashx). For the 2009/2010 fishing year the TACC was set 1147 metric tonnes (Kina, 2010). It is unknown whether the current level of fishing is allowing for a sustainable yield or whether there is overfishing in certain SUR areas occurring (fs.fish.govt.nz/Doc/21739/42_ SUR_09.pdf.ashx).

Table 1.1 The TACCs (t) and the reported landings of urchins for the fishing years 2007-08 for all fishing areas in New Zealand (SUR) (Kina (SUR), 2009)

Fishstock	QMA	2007–08 Actual TACC (t)	2007–08 Reported landings (t)
SUR 1A	Auckland (East - North)	40	31.7
SUR 1B	Auckland (East - South)	140	140.4
SUR 2A	Central (East - North)	80	18.0
SUR 2B	Central (East - South)	30	< 1
SUR 3	South-East (Coast)	21	2.1
SUR 4	South-East (Chatham),	225	147.4
SUR 5	Southland	455	276.2
SUR 6	Sub-Antarctic	None	0
SUR 8	Central (Egmont)	1	0
SUR 9	Auckland (West)	10	5.8
SUR 7A	Challenger (North)	135	134.6
SUR 7B	Challenger (South)	10	6.5
Total		1 147	762.9

Catches have varied over the past 20 years with a peak in 1993 of 1032 tonnes, since then the catch has declined to the current levels with of between 600 to 1000 tonnes a year (Table 1.2) (James 2007; Williams 2002; Woods *et al*. 2005; Kina (SUR), 2009).

Table 1.2 Total reported catch by all fishing methods for the Fishery management areas around New Zealand (SUR) (Kina (SUR), 2009).

Year	SUR 1	SUR 1A	SUR 1B	SUR 2	SUR 2A	SUR 2B	SUR 3	SUR 4	SUR 5	SUR 6, 8, & 9	SUR 7	SUR 7A	SUR 7B	Total
1983	66.2	–	–	33.0	–	–	4.8	11.3	0.5	3.6	26.3	–	–	157
1984	81.4	–	–	180.3	–	–	14.4	4.0	0.9	0.3	55.1	–	–	342
1985	64.5	–	–	83.8	–	–	4.0	7.4	4.6	0.9	99.6	–	–	275
1986	72.0	–	–	139.1	–	–	6.2	52.7	0.2	2	86.6	–	–	360
1987	52.1	–	–	142.6	–	–	2.4	28.4	4.3	0.1	52.6	–	–	283
1988	22.1	–	–	154.1	–	–	1.7	76.5	2.3	–	175.6	–	–	432
1989	35.5	–	–	92.8	–	–	0.8	216.6	19	1.5	6.2	–	–	372
1990	10.0	–	–	282.4	–	–	4.1	190.0	13.4	6.5	41.5	–	–	548
1991	71.5	–	–	87.2	–	–	21.3	35.3	166.9	4.4	56.3	–	–	443
1992	78.7	–	–	37.3	–	–	15.8	192.9	272.2	5	114.4	–	–	717
1993	89.7	–	–	170.4	–	–	9.9	21.8	*530.3	–	210.2	–	–	1 032
1994	150.7	–	–	176.7	–	–	8.8	55.3	327.2	2.3	98.2	–	–	820
1995	155.9	–	–	129.7	–	–	7.1	100.7	342.9	89.5	149	–	–	975
1996	174.5	–	–	41.2	–	–	6.0	99.5	446.4	0.1	142.2	–	–	910
1997	161.6	–	–	49.9	–	–	5.4	225.7	171.6	0.2	121.7	–	–	736
1998	134.8	–	–	36.5	–	–	3.8	303.1	91.2	1.4	144.7	–	–	716
1999	201.4	–	–	20.2	–	–	38.4	168.2	120.6	0.5	113.9	–	–	663
2000	297.4	–	–	14.5	–	–	50.4	396.5	106.3	0.1	87.9	–	–	956
2001	184.5	–	–	11.4	–	–	11.2	472.6	69.8	3.1	80.1	–	–	832
2001–02	237.0	–	–	3.0	–	–	5.2	368.0	184.9	–	31.7	–	–	829.7
2002–03	211.2	–	–	30.4	–	–	0.3	167.3	132.5	0.9	1.3	63.2	0	607.4
2003–04	1.7	26.9	111.0	0	14.5	4.6	0.3	114.8	199.1	3.8	0	85.4	0	562.3
2004–05	–	20.9	131.1	–	6.5	1.4	.5	91.7	350.4	0.9	–	101.3	–	704.7
2005–06	–	41.0	138.6	–	22.1	0.2	<0.1	70.2	473	4	–	72.1	5.3	826.5
2006–07	–	37.1	147.3	–	13.8	< 0.1	3.2	108.3	423	8.6	–	117.3	9.2	868
2007–08	–	31.7	140.4	–	18	< 0.1	2.1	147.4	276.2	5.8	–	134.6	6.5	762.9

Data from 1989 and 1990 are combined from the FSU and CELR databases. – indicates no recorded catch. Data for the period 1983 to 1999 are from Andrew (2001), and have been groomed. Catch estimates for 2000 and 2001 are taken directly from MFish. * includes 133 t caught in Dusky Sound experimental fishery. Catches from SUR 6, 8, and 9 have been pooled because too few permit holders recorded catches in these FMAs to report them singly.

The largest producing areas for commercial harvest are, SUR 1 (north east North Island), SUR 4 (Chatham Island), SUR 5 (Southland), and SUR 7 (West coast of the South Island), (James 2007; Kina (SUR), 2009; McShane 1997; McShane *et al*. 1994) (refer to Table 1.1). Maori customary catch accounts for a high percentage of the catch in some areas such as SUR7A were 80% of the catch is customary (James 2007). Harvesting is undertaken by breath holding divers and dredging has occurred in the past in some areas, predominantly in the Marlborough Sounds within SUR 7 were 10% of the total catch in 1998-99 was dredged. There is no minimum legal size for harvesting of *E. chloroticus* either recreationally or commercially, however, the average size of urchins taken commercially is between 90-130mm in the South Island, with much smaller urchins fished in the North Island. The recreational catch is 50 per person per day (fs.fish.govt.nz /Doc/21739/42_SUR_09.pdf.ashx; McShane *et al*. 1996).

The quality of gonad produced by each urchin varies markedly throughout the country with many areas being of poor gonad quality and unable to be fished. This is due to the urchins having small gonads (low Gonad Index GI percentage) and poor colour and taste often associated with this low quality gonad (Barker 2007; James 2007; Woods *et al*. 2005; Williams 2002). The percentage GI for kina when fished commercially is calculated as the percentage wet weight of gonad out of a tonne of urchins. The minimum GI percentage in the North Island before it becomes economically non viable is 6% and in the South Island 10% however in the South Island ideally 16% is the baseline (James 2007; C.McManaway, pers comm.). Urchins are brought into port alive then processed, with the gonad being removed and put into pottles for sale. The remainder of the animal is waste. Gonad is almost entirely sold on the domestic market to predominantly Maori and Polynesian consumers (James

2007; C.McManaway, pers comm.). Retail prices for gonad range from $20 to $70 per kilo however in supermarkets gonad is sold in 200 to 400gram pottles, which sell for around $13 to $20.81 per 200grams for good quality gonad (James 2007, C.McManaway, pers comm.; Woods *et al*. 2005). There are no strict regulations on colour or texture for gonad sold on the domestic market, with quantity being the main factor to be considered, only the darker brown or blackish coloured gonad are downgraded to lower price levels (James 2007; C.McManaway pers comm.; Woods *et al*. 2005; Woods *et al* 2008). This is in stark contrast to the international market (especially the Japanese market), which has strict quality attributes which gonad has to reach. Export markets demand fine textured small and yellow or orange coloured gonad (James 2007; McShane 1997 Woods *et al*. 2005; Woods *et al* 2008).

The appearance, texture taste and odour are other factors which influence the value of gonads on the international market. Gonad from *E.chloroticus* is variable in these attributes, with the male gonad often having a sweet taste with a dairy flavour, compared to the female roe which often has a bitter and sour taste, with a slightly metallic aftertaste (Phillips *et al* 2009; Phillips *et al* 2009; Woods *et al* 2008). The taste of the gonad however varies over the season in both male and female urchins (Phillips *et al* 2009; Phillips *et al* 2009). These other factors of taste, texture, appearance and odour add to the difficulty of producing gonad of an export standard.

A trial fishery was established in Dusky Sound in order to try and expand into the export market, however, due to poor gonad quality only 130t of the permitted 1000 tonnes per annum was harvested (McShane 1993; McShane 1994; McShane 1997). Poor gonad quality is holding back the New Zealand sea urchin industry as the lucrative Japanese and international markets have high quality standards (Barker 2007; James 2007; Woods *et al*. 2005; Williams 2002). This has led to the overfishing

of *E. chloroticus* in areas where gonad quality is high enough for the domestic market (Andrew & MacDiarmid 1999; James 2007; Williams 2002). Areas of high gonad quality are patchily distributed along the coast line, with these areas often being unreliable in producing high quality gonad annually (Andrew & MacDiarmid 1999).

There are opportunities available for gonad enhancement for both domestic and export markets. There have been a number of trails and experiments looking into improving quality of gonad through both feeding natural and artificial diets, with most of these trials being undertaken in either land based or sea cages (Barker *et al.* 1998; Barker 2007; James 2006; James 2007; Woods *et al*. 2005; Woods *et al*. 2008). These feeds are used to promote a rapid and fast increase in the gonad quality (Barker 2007; Woods *et al.* 2005; Woods *et al* 2008). At present most of these feeds are experimental and there has been very limited success in achieving gonad up to the quality of the domestic market let alone the international market (Barker 2007; Woods *et al*. 2005). Large amounts of effort are still being put into investigating ways of improving the quality and yield or gonad in order to improve the gonad for the domestic markets and meet the requirements of the international market (Andrew & MacDiarmid 1999; Barker 2007; James 2007). Other options for gonad enhancement that have been considered involve moving wild urchins from areas where the gonad quality is known to be poor to areas that have produced better gonad, this has been trailed with some promising results so far in a study by James (2009).

A pilot study (Thomas 2009) where wild kina were translocated from areas where gonad quality was low to areas were gonad quality was high, showed that translocation was a viable option to use as a gonad enhancement technique. The study showed the opportunity available to transplant urchins on a large commercial scale was viable and could prove to be an effective enhancement method.

1.5   Objectives of the present study

The purpose of the present study was to test whether commercial scale translocations of *E. chloroticus* in the Southern fishery SUR5 from areas which are unfished due to poor gonad quality to areas which are fished (high gonad quality) causes a change in the quality of gonad. The gonad index and colour are the main factors assessed to gauge whether there is a positive or negative effect on the urchin as a result of translocation. The broader effects on ecology and on the fishing industry will be discussed in relation to these findings. The reproductive output of old large urchins (140mm+) was investigated in order to determine whether they contribute to the larvae pool or whether they are reproductively senile. Fertilization rates and the development of the larvae from these embryos through to an 8 arm stage were measured to assess the reproductive contribution of these urchins. These results are discussed in relation to the effects on the biology of *E. chloroticus* and the impacts on the fishing industry.

2 Translocation of *Evechinus chloroticus*

2.1   Introduction

Sea urchin gonads are made up of 3 main cell types, nutritive phagocytes (cells which store nutrients), gametogenic cells or developing gametes and mature gametes (Barker 2007; James 2007; Explorations Unlimited Inc 2006). Nutritive phagocytes store necessary nutrients such as proteins, carbohydrates and lipids that are essential in gametogenesis. Nutritive phagocytes are also the tissue which is largely responsible for the flavour and focus of the gonad as a product for human consumption (Barker 2007; James 2007; Explorations Unlimited Inc 2006). The

percentage of all 3 cell types in the gonad varies seasonally, with the reproductive cycle and food availability affecting this as well as gonad growth. Greater amounts of nutritive phagocytes can lead to an increase in gonad index with a higher reproductive output (Gonor 1972; James 2007; James & Heath 2008; Explorations Unlimited Inc 2006).

The reproductive cycle of *E. chloroticus* follows a normal pattern similar to other sea urchins species. Nutrient reserves start increasing within the nutritive phagocytes, in autumn and early winter, followed by the onset of gametogenesis. Gonads then ripen from October to November, with spawning occurring from December to March (Barker 2007; James 2007; McShane *et al* 1994; Mladenov *et al*. 1997). During the reproductive cycle the gonad index varies according to the reproductive stage. During the same time period oocyte size changes occur in response to the different reproductive stages (Gonor 1972; James 2007; Lamare *et al*. 2002).

Food availability has a large effect on somatic and reproductive growth of *Evechinus chloroticus*. Nutrients are stored in 3 major body components: the gonad, gut (secondary storage) and the body wall, with the amount of food available affecting each of the 3 components (Andrew 2003; Barker 2007; Fell 2002; McShane 1997). High food availability allows for more nutrients to be stored in all components resulting in larger urchins with higher gonad and gut indices (Fell 2002; McShane 1997; James & Heath 2008). The opposite effect occurs with less nutrients available, smaller gonad, gut and urchin size occurs (Fell 2002; James & Heath 2008; James & Herbert 2009; McShane 1997; McShane & Anderson 1997). Food availability affects other urchin species in the same way, e.g. in *Strongylocentrotus spp* resulting in lower growth and reproduction (Minor & Scheibling 1997; Tsdua *et al*. 2006). Available

energy can also be allocated to reproduction at the expense of somatic growth when food is scarce (Gonor 1972; Minor & Scheibling 1997). Sea urchins of all species have a large capacity for plasticity, with differing resources allocated to reproduction or somatic growth depending on food availability and quality. The Aristotle's lantern is the feeding apparatus of the sea urchin. Aristotle lantern size also shows plasticity in size, in relation to food availability, with larger lanterns under low food conditions, and the opposite occurring under high food availability (Minor & Scheibling 1997; McShane 1997; Russell 1998; Wing *et al*. 2001).

Quality of food plays an important part in gonadal, gut and somatic growth. Consumption of algae with a higher nutritional value allows for more nutrients to be made available for growth (Fell 2002; McBride 1997; Russell 1998; Vadas *et al*. 1999). The different nutritional values of algal species is thought to lead to a preference in feeding in sea urchins, with some algal species preferred over others such as fleshy brown algae over coralline algae (Andrew 1986; Andrew 1988; Schiel 1982; Vadas *et al*. 1999). Higher gonad indices occur when more protein is present in the algae (Vadas *et al* 1999). The gut as a secondary storage organ is not only affected by food availability but also to a large extent by quality of food, with excess nutrients being stored in the gut. It is therefore thought the gut does reflect the nutritional condition of a sea urchin (Fell 2002; Russell 1998).

Population density of urchins is an important factor affecting almost all forms of growth in a sea urchin, as varying numbers of urchins affect the food availability and diversity of algae present in an area (Andrew 1988; McShane & Anderson 1997; Vadas *et al* 1999; Villouta *et al*. 2001). With low urchin numbers higher food availability occurs, along with increased algae diversity, although competition may lower algae diversity in some areas, with the opposite effect happening with high

urchin numbers (Andrew 1988; McShane & Anderson 1997; Vadas *et al* 1999; Villouta *et al*. 2001).

Gonad colour is another major factor affecting the demand for the product when sold on the international market. Most factors affecting the growth of urchins also play a part in affecting the colour of the gonad. Colour is directly related to what the urchin eats with pigments from the algae creating the differing colours (Agatsuma *et al*. 2005; Fell 2002 Robinson *et al*. 2002; Woods *et al*. 2005). A key pigment providing the yellowish-orange colour required and preferred by the international market is beta carotene, and other carotenoids such as astaxanthm synthesised from ingested carotenoids by the urchin (Agatsuma *et al*. 2005; Robinson *et al*. 2002). Macro algae are the key food providing carotenoid pigments giving the gonad a yellowish-orange colour, with each algae species containing differing amounts of pigments (Fell 2002; Robinson *et al*. 2002; Agatsuma *et al*. 2005). Another factor affecting gonad colour is urchin size with older larger urchins often having darker coloured gonad unsuitable for sale (McShane *et al*. 1994). The reproductive cycle is thought to possibly effect the colouration of the gonad; however it is not fully known yet how dominant a role this plays (Agatsuma *et al*. 2005).

Many studies have looked into the improvement of gonad quality through enhanced feeding with both natural and artificial feeds in *Evechinus chloroticus* include Barker *et al*. 1998, Fell 2002, James 2006, James 2007, James & Heath 2008 and Woods *et al*. 2005. These studies have had mixed success with some gonad quantity and colour improvements occurring, however, much more research is needed to perfect an enhancement technique to consistently produce gonad of high quality. Studies have also been undertaken looking at enhanced feeding through natural and artificial diets in other species of urchins around the world especially on

Strongylocentrotus spp a highly valued species in Japan, some of these studies include Lawrence *et al*. 1997, Meidel & Scheibling 1999, Minor & Scheibling 1997, McBride 1997, McBride *et al*. 2004 and Robinson *et al*. 2002. There has been a higher success rate in many of these studies compared to *Evechinus chloroticus*.

Another method of gonad enhancement which has been considered is the translocation of urchins from areas where food resources are low to where food availability is high to allow for enhancement of the gonad. In Japan this practise involves moving urchins from deeper low algal density areas to shallow kelp beds for about 3 months for a rapid increase in gonad quality and quantity before sale (Explorations Unlimited Inc 2006). This method has also been trialed in studies using *E. chloroticus* in the Coromandel and Doubtful Sound with the method showing a high success rate in the Coromandel and that it is viable in Doubtful Sound (James & Herbert 2009; Thomas 2009).

The lack of, and patchy distribution of urchins with high quality gonad around New Zealand has meant that the *E. chloroticus* fishery has been heavily exploited in some places. Fishermen concentrate on the areas with the good quality gonad resulting in overfishing and low urchin densities in these areas (Andrew & MacDiarmid 1999; James 2006; James 2007; Williams 2002). This has occurred in fishery SUR 5 on the south coast of Fiordland in Chalky Inlet. Currently fished areas contain *E. chloroticus* in low numbers from fishing pressure, while areas near by with low quality gonad have urchins present in high densities. In the present study the translocation of *E. chloroticus* from areas with low quality gonad and high density to areas known to produce urchins with high quality gonad but where density is low due to fishing pressure, will determine whether translocating would be a viable tool for commercial fishermen to consider in this specific area.

The gonad index and the colour of the gonad where the main factors measured for assessing the effects of translocating urchins from the poor gonad fishing area at Passage Island to the good gonad quality site at Sealers Bay. Gut index, Aristotle's lantern index and oocyte size change was measured to determine if these factors were affected as a result of translocation the urchins. The density of urchins and the algal biomass and diversity was also determined to assess the role these factors had on the urchins overall growth of body components.

2.2   Materials and methods

Collection and translocation of *E. chloroticus* was undertaken using the vessel San Nicholas which is owned and operated by Campbell McManaway of Cando Fishing out of the port of Bluff. Campbell. McManaway holds one of the larger fishing quotas for *E. chloroticus* in SUR5, and using a commercial fishing operation was a convenient way to move the quantity of *E. chloroticus* required in a commercial scale translocation, as well as allowing for access to remote Chalky Inlet. Campbell. McManaway provided the information about the sites where *E. chloroticus* of a low gonad quality occurred, and as a consequence were not fished, and areas where gonad quality was high and where the San Nicholas currently fishes. Weather and fishing schedules of C. McManaway made sampling dates unpredictable meaning some of the sampling was done later than would have been ideal. All post harvest processing of the urchins and laboratory work was undertaken at the Otago University Portobello Marine Laboratory on Otago Peninsula.

2.2.1 Site description

Two sites were used for the translocation of the urchins both of these sites are located in Chalky Inlet on the South coast of Fiordland in the fishing area SUR5. The

area where the urchins were known to have poor quality gonad, and where they were not fished was on the northern side of Passage Island (S46°01.319, E166°32.967), (Fig 2.1). The reef at this site is relatively shallow with depths ranging from 2 to 5 meters. Waves and currents are relatively slight in this area, however during strong North East winds, wind chop and swell can be large. *Carpophyllum flexuosum* is the most common algae with distinct bands formed from the surface down to about 2 meters, however, below this where the urchins are located, algae is sparse with distinct urchin barrens present (pers obs).

Sealers Bay (S 46°02.478 E166°31.791) was the site where urchins of a higher quality gonad had previously been fished, and where the urchins from the poor gonad quality site where translocated to (Fig 2.1). Sealers Bay has been fished frequently for urchins, but as a consequence of fishing pressure now has low urchin densities of 2.7 urchins per m^2 across the Bay. The rocky reef at this site is mainly composed of large boulders and runs from the shore out to 50- 90m from Chalky Island. This reef is relatively shallow (1 to 3m), but drops to a broken reef (3 to 8m) depth. Large brown fucoid algae dominate the reef, with *Ecklonia radiata*, *Marginariella boryana* and *Carpophyllum flexuosum* the most dominate species present. The current is small and wave exposure slight in the area apart from strong W and NW wind/swell on occasions.
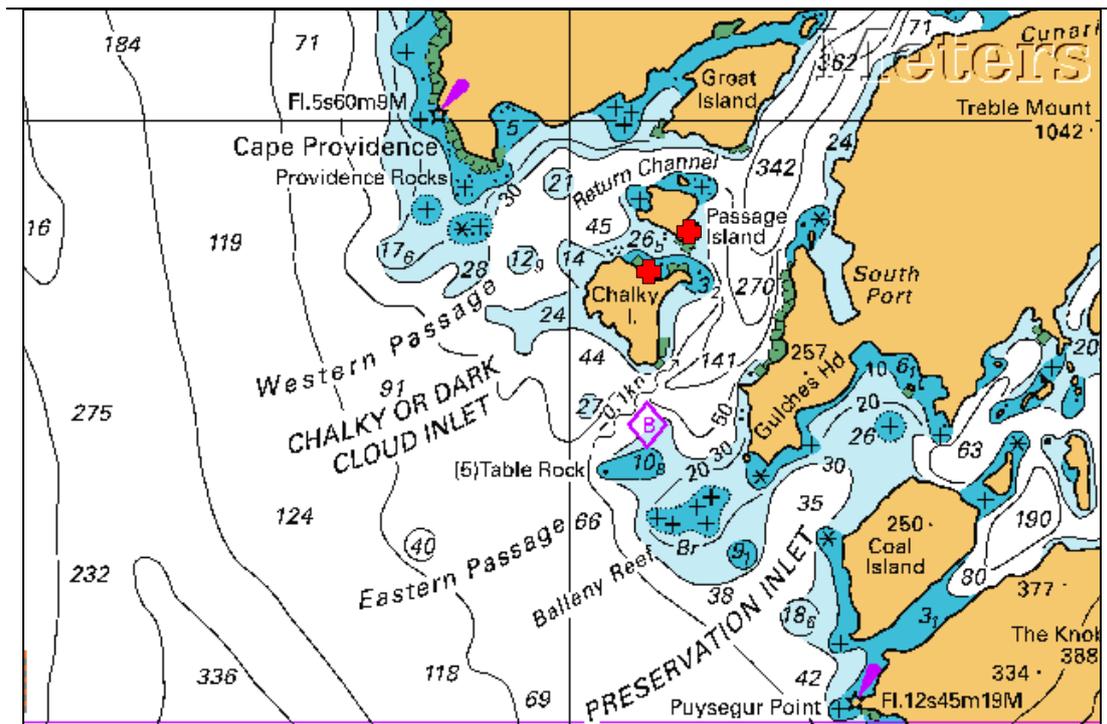
Figure 2.1 Map of Chalky inlet showing Passage Island (site of poor quality gonad), and Chalky Island where Sealers Bay is found on the North East side (site of good quality gonad).

2.2.2 Collection and translocation of the urchins

Approximately 1000kg of 105 -135mm test diameter (TD) urchins were collected from Passage Island, by 3 breath holding divers working from the commercial fishing boat San Nicholas. This occurred from the 30th October to the 2nd November 2009. The 1000kg of urchins were transported in fish bins to Sealers Bay by dingy. The urchins were dropped into the water in a 50m^2 area (S 46°02.478 E166°31.791) in front of a rocky outcrop clearly visible above the water surface in the middle of the Bay. No tags were put into the urchins or any other form of identification. Results from a pilot study (Thomas 2009), showed that tagging caused death of urchins and other forms of identification such as spine clipping was not particularly successful. Prior removal of urchins through fishing in Sealers Bay had left the area with low numbers (2.7 per m^2), so when urchins were sampled at later dates in the area it could be assumed that the majority of urchins gathered were from

the translocated sample. It was not possible to clear away all the urchins at Sealers Bay due to the time constraints of working in with a Commercial fisherman. No tanks were permitted onboard for diving so to clear all the urchins in the area where the translocation was undertaken could not be achieved. Also because the research was undertaken on a commercial vessel the fisherman didn't have time to spare for this process to be done. A reciprocal transplant was not undertaken from Sealers Bay to Passage Island, due to the high density of urchins at Passage Island (pers. obs.), meaning urchins could not be identified as either translocated or resident stock.

At both sites, Passage Island and Sealers Bay, twenty urchins were gathered at the time of the experimental translocation to test for quantity and quality of gonads. Only twenty urchins were gathered because of the time constraints cutting/processing up 20 large urchins at a time, also as mentioned above working in with the commercial fisherman meant there wasn't the time to stay at the spots longer to collect more urchins. These urchins were kept in a tank in flowing seawater on the boat deck during the trip back to Bluff (11 hours). From there they where transported in buckets containing seawater to Portobello, where they were then put into holding tanks. The urchins were kept in seawater because of there large size, as they died rapidly once removed. Even while kept in flowing seawater on the boat trip back to Bluff some of the urchins died, as well as during the transportation to Portobello. On the first trip (30th October to 2nd November) the urchins gathered from Passage Island, through human error were accidently discarded by the crew of the San Nicholas so only the twenty Sealers Bay urchins were processed. Two further trips to sample urchins were made. On the 19th of January 20 urchins were collected from Passage Island, 20 from the previously translocated urchins in Sealers Bay and 20 from Sealers Bay at the Eastern end remote from the translocation site. This latter site at

Sealers Bay was far enough away (about 20 meters) from the translocation site that no movement between these populations was likely to occur. A further collection of urchins was collected during the 8th to the 10th of March, 20 urchins from each of the three sites: Passage Island, Sealers Bay and from the translocation site, and transported to the Portobello Laboratory. The study was undertaken at this time of the year, as this was the only time in which it was possible to work in with the commercial fisherman to get to these sites as fishing for *E.chloroticus* doesn't occur at other times of the year in this area.

Figure 2.2 Timeline of sample collection. At the beginning of the study (SB initial), after 13 weeks (SB 13, PI 13, TL1) and after 21 weeks (SB 21, PI 21, TL2). SB = Sealers Bay, PI = Passage Island and TL = Translocated sample.

2.2.3 *E. chloroticus* and algal density

A $0.5m^2$ quadrat was used to determine the density of urchins. The quadrat was randomly dropped at each site from the surface to depths between 2-3 meters, all the urchins within the quadrat were counted and recorded, and this was repeated twenty times. *E. chloroticus* density was recorded at the Sealers Bay translocation site and at Passage Island before the translocations occurred (start) and at the termination of the experiment.

At both sites before translocation and at the end of the experiment, 5 randomly placed $1 m^2$ quadrats were photographed. Photographs were taken at each site so 20 photographs were collected in total 10 from the beginning of the study and 10 after the study had been completed. Algae biomass with a $0.5m^2$ quadrat was determined at both sites at the start of the experiment and again at the completion of the experiment.

The quadrat was randomly dropped and all algae within the quadrat was scraped and cut off the rocks. The algae was then put into rubbish bags which were labelled and transported back to the Portobello Laboratory and frozen to be processed at a later date.

The bags were taken out of the freezer the night before work was undertaken to make sure all the algae had defrosted and any excess water had melted and drained away. The contents of each bag were then weighed and recorded. The algae was sorted into separate species with all species present identified and the total combined weight of each species for each transect was recorded. One whole plant of each algal species found at each site was weighed and then put into onion bags to dry in the sun for 3 days and any excess water evaporated. The algal plants were put in a drying oven at 60˚C and left until all moisture was evaporated. The dry weight of each algae species was recorded. The above procedure was undertaken for each sample and site. Algae identification was carried out using Seaweeds of New Zealand by Nancy M. Adams, and the help of a local algal specialist Dr. Chris Hepburn.

2.2.4 Photoquadrat analysis

Photoquadrats were analysed using the program CPCe (Coral point count with Excel extensions). Each image was opened and size was manually set around the outside of the quadrat. Fifty points were set to randomly appear on each quadrat, and then all the species under the points were identified. Diversity and a percentage algal cover were calculated through CPCe for algae per m^2. The diversity and percentage cover was input into Minitab, where a t-test was undertaken. T-tests were undertaken twice initially with coralline algae and then without coralline algae as it was so dominant at Passage Island. Diversity tests looked at the differences between Sealers Bay diversity and percentage of algal cover at the

start of the experiment compared to Passage Island, with the same comparison at the completion of the experiment.

2.2.5 Urchin dissection and processing methods

Collected urchins were put in holding tanks without food, to allow as much faecal matter to be excreted as possible before dissection. The diameter (0.1mm) of each urchin was measured three times using Vernier callipers, and the three measurements were then averaged to give test diameter in millimetres. The urchins were weighed using a digital balance (0.01g). Dissection was carried out by cutting the urchin around the circumference of the test using scissors. The gonads were removed using a spoon and drained to remove excess moisture. The gut was removed with tweezers, and any excess faeces were washed out in seawater, then the gut was drained and weighed. Once the gonads and gut were removed the Aristotle's lantern was removed using scissors. The water vascular system (mainly ampullae) was cut with scissors to release all water. The gonads, gut, Aristotle's lantern and the empty test were all individually weighed. These weights were used to calculate the gut and gonad index. Draining all water from the separate organs of the urchins is important to ensure accurate gonad and gut index calculations. The calculation of the gonad index in this study differs from that used in a lot of previous studies as it uses the dissected and drained weights of organ components and doesn't just remove excess water from the urchin and calculate the index which is the common method of commercial fishermen. One section of gonad from each urchin was put in a labelled jar of 10% seawater buffered formalin for preservation until histological processing. The rest of the gonads from each urchin were wrapped in tinfoil, labelled and then put on ice, and transported to the Food Science Department for colour readings to be taken.

The sex of each urchin was determined from a smear of gonad under the compound microscope, or through examination of the gonad after histology. Pieces of gonad were sectioned and cut to a thickness of 7µm, and stained with haematoxylin and eosin.

2.2.6 Gonad colour readings

Gonad colour readings were taken with a Minolta CM2500D Miniscan XE spectrophotometer (Hunter Laboratory Associates inc. Virginia U.S.A), under the standard lighting condition of D_{65}. A piece of gonad from each urchin was placed into a crucible, which was placed underneath the photo lens/bulb of the spectrophotometer. Three colour readings of L* (Lightness), a* redness (+) and b* yellowness (+) was determined for each piece of gonad, for more information on the spectrophotometer used refer to Woods *et al* 2008. These three colour readings were averaged to give a colour reading for the gonad of each urchin. The spectrophotometer was used to remove any bias colour results from human observations using another colour method such as the Maine colour chart (James 2007).

2.2.7 Histological analysis

Ten stained slides from each sample were analysed under a compound microscope linked to a computer, using the programme analySIS LS Research. The kina were sexed, and then the maturity stage of each gonad was determined. The six stages of maturity of both oogenesis and spermatogenesis range from stage 1 (recovery stage) to stage 6 (spent stage). All the stages used in this study were as described by Byrne (1990).

For each ovary 50 oocytes were measured using the polygon tool in the analySIS LS Research package. A line was drawn around the outside of each oocyte present, this generated an area for each of the oocytes measured. The oocyte area was transformed to diameter using the formula $r=\sqrt{(area/\pi)}^{*2}$. The mean diameter of 50 oocytes was determined for at least 3 female urchins from each sample. An oocyte size frequency distribution was calculated for each site. Oocyte size frequencies were examined by contingency analysis using the statistical programme JMP to compare differences in oocyte size frequency between samples and sites. Graphs of mean oocyte frequency for each sample were created using Microsoft Excel.

2.2.8 Statistical comparisons

- Sealers Bay Start (SB Initial) versus Passage Island middle (PI 13)
- Sealers Bay Start (SB initial) versus Sealers Bay Middle (SB 13) versus Sealers Bay End (SB 21)
- Passage Island middle (PI 13) versus Passage Island End (PI 21)
- Sealers Bay End (SB 21) versus Passage Island End (PI 21)
- Sealers Bay End (SB 21) versus 2nd Translocated sample (TL2)
- Passage Island End (PI 21) versus 2nd Translocated sample (TL2)
- Sealers Bay Start (SB Initial) versus Translocated 1st sample (TL1)
- Passage Island middle (PI 13) versus Translocated 1st sample (TL1)
- Sealers Bay Middle (SB 13) versus Translocated 1st sample (TL1)
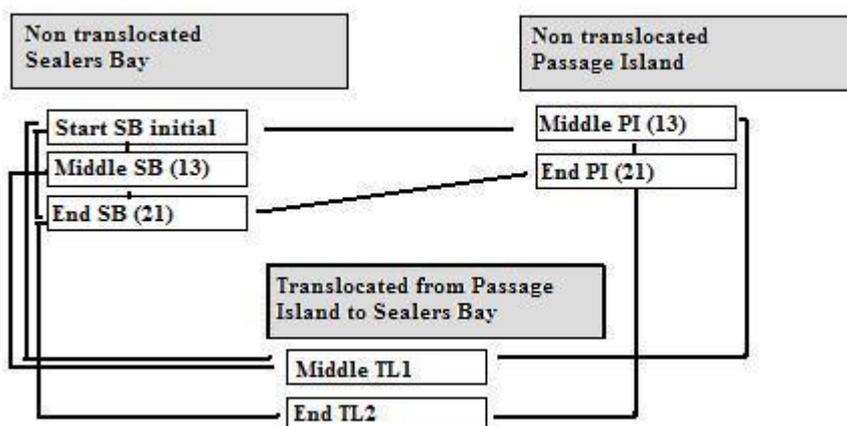
Figure 2.3 Overview of statistical comparison of samples

2.2.9 Statistics analyses

The following calculations were used to analyse the gonad, gut, and Aristotle's lantern index of all the urchins from each sample.

Gonad index (calculated from drained weights) was calculated using the following formula:

Gonad index (%) = (total weight of gonad (g) / total drained weight (g)) * 100

The total drained weight is the combined weight of gut, test, and Aristotle's lantern. The Gonad index (GI) data was converted to a proportion and Arcsine transformed in Minitab. A one way ANOVA was undertaken on the arcsine transformed data comparing gonad index between the different samples.

The same calculation as above was used to obtain the gut and Aristotle's lantern index. Mean gonad, gut and Aristotle's index were then graphed using Excel comparing all the samples against each other.

A Chi squared test was performed using Excel to determine whether there was any difference in the sex ratio within each site and overall with all sites pooled. The

Number of urchins per $0.5m^2$ was multiplied by 2 to get urchins per $1m^2$ before any statistics was undertaken. Density comparisons of urchins between sites were done using T-tests, equal variance was assumed if there was little variation between the samples. The comparisons undertaken were as follows:

- Passage Island start vs. Sealers Bay Start

- Passage Island end vs Sealers Bay end

- Sealers Bay start vs Sealers Bay end (equal variance assumed)

- Passage Island start vs Passage Island end (equal variance assumed)

- Algal biomass between the sites was compared with a T-test.

The average colour readings for each site and each colour L*, a*, b* were compared using a one-way ANOVA. The mean colour readings for each site and each colour L*, a* and b* were graphed using Excel.

2.3 Results

The *E. chloroticus* translocated and sampled from each site during the experiment were of a commercial size for the Chalky Inlet area (between 105-135mm TD). The average TD of the urchins was 124.3mm (SD ± 9.4).

2.3.1 Sex ratio

Table 2.1 Raw data of the expected and observed values for sex ratio at both Passage Island and Sealers Bay, and the p-values and Chi squared values (n= 10 for both samples).

Passage Island	Male	Female
Observed	5	5
Expected	5.5	4.5
Chi Squared value	0.045455	0.05555556
P-Value	0.750621	-
Sealers Bay	Male	Female
Observed	6	4
Expected	6.05	4.95
Chi Squared value	0.000413	0.18232323
P-Value	0.669032	-

There was no significant difference in the ratio of male to female urchins at Passage Island or Sealers Bay (Chi squared p-value = 0.594) (Table 2.1), with both the sites having an almost equal number of male and female urchins.

2.3.2 Gonad index

Differences in gonad index between sexes were compared at each of the sites. No significant difference was found between the factors sex and, GI ($P > 0.05$), and therefore the GIs were pooled for all comparisons.

When sampling commenced the GI at Sealers Bay was 23.09. This was lower than the Passage Island sample (26.5) after 13 weeks, although these differences were not significant (one-way ANOVA, p-value 0.159) (Fig 2.4). Thirteen weeks after translocation the mean GI of translocated urchins (34.6) was significantly higher than either Passage Island after 13 weeks ($P = 0.017$) or Sealers Bay ($P = 0.003$) at the start of the experiment (Fig 2.4). The sample from Sealers Bay (SB 13) collected 13 weeks after translocation had the highest GI (42.45) of all the samples. This sample

was significantly higher than the translocated sample (TL1) collected at the same time ($P < 0.001$) (Fig 2.4). After 21 weeks, at the completion of the study, there was a major drop in the GI of Sealers Bay (SB 21, 20.4), Passage Island (PI 21, 10.5), and the translocated urchins (TL2, 16.8) (Fig 2.4), indicating that spawning had occurred. The drop in GI after 21 weeks resulted in a significant difference between all the Sealers Bay samples (at the beginning of the experiment, after 13 weeks (SB 13) and after 21 weeks (SB 21) ($P < 0.001$)). The drop in GI after 21 weeks also caused a significant difference between the two Passage Island samples ($P < 0.001$), with the GI after 13 weeks from Passage Island (26.5) higher than the Passage Island sample after 21 weeks (10.5) (Fig 2.4). At the end of the study, the translocated urchin (TL2) sample (16.8) had a significantly lower GI than that of the Sealers Bay sample (SB 21, 20.4) at the end of the study ($P < 0.001$, Fig 2.4). The Passage Island sample (PI 21) was significantly lower ($P < 0.001$), than the translocated sample (TL2) at the end of the study. After 21 weeks the GI from the Sealers Bay sample (SB 21, 20.4) was significantly higher than the Passage Island sample (PI 21, 16.8).
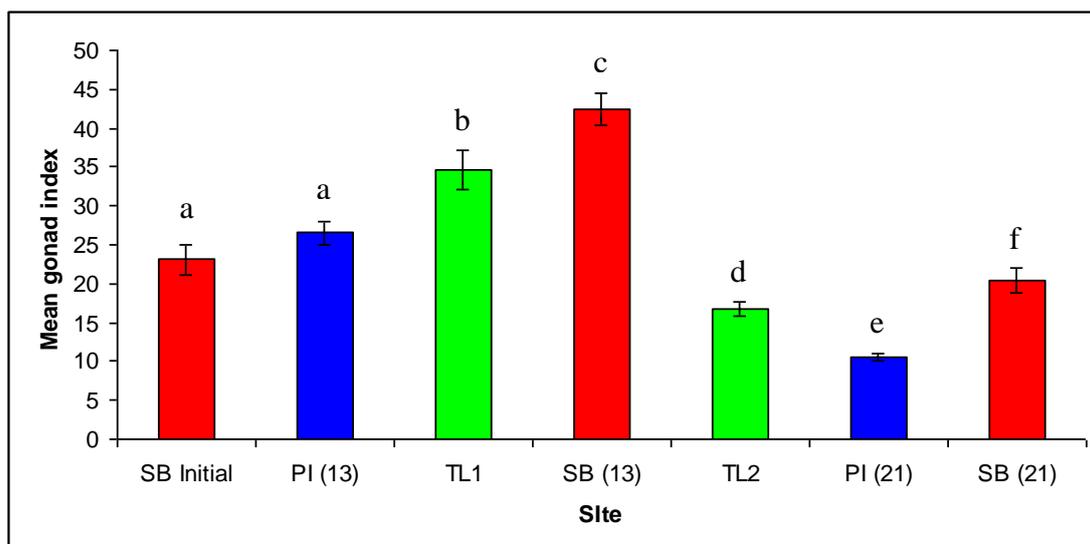
Figure 2.4 The mean gonad index of urchins from Passage Island middle (PI 13, n=10), Translocated 1st sampling date (TL1, n=10), Sealers Bay start (SB Initial, n=10), Sealers Bay middle (SB 13, n=10), Sealers Bay end (SB 21, n=11), Translocated 2nd sampling date (TL2, n=20) and Passage Island end (PI 21, n=20). Letters above a bar show post-hoc analysis from ANOVA. Treatments that share a letter are not significantly different from one another (P<0.05)

2.3.3 Gut index

No significant difference was found when male and female gut indices were compared (One-way ANOVA $P > 0.05$) indicating that sex had no effect on the gut index. Gut indices were therefore pooled for statistical analysis.

There was less variation in mean gut indices compared to gonad indices. Samples from Sealers Bay (SB Initial 7.37) at the start of the study had a significantly ($P < 0.001$) higher mean gut index than urchins from Passage Island after 13 weeks (PI 13 4.42) (Fig 2.5). The translocated sample collected after 13 weeks (TL1 6.86) had a significantly lower ($P < 0.001$ and 0.015) gut index than either Passage Island after 13 weeks (PI 13 5.42) or Sealers Bay (SB Initial 7.37) at the beginning of the study (Fig 2.5). Gut indices from Sealers Bay were significantly higher ($P < 0.001$) after 21 weeks (SB 21 6.69) than the two other sampling times at the start of the study (SB Initial7.37) and after 13 weeks (SB 13 4.35) (Fig 2.5). After 21 weeks the gut index at Passage Island (PI 21 7.31) was significantly higher ($P < 0.001$) than at the beginning of the experiment (PI 13 5.42) (Fig 2.5). There was no significant difference between the gut indices in any of the other comparisons ($P > 0.05$) (Fig 2.5).
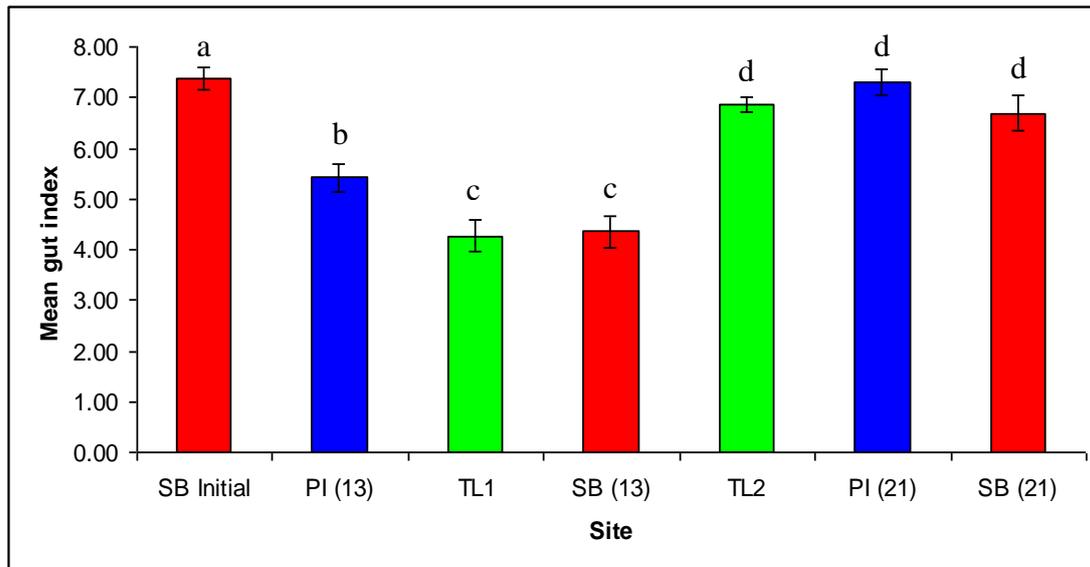
Figure 2.5 The mean gut index of urchins from Passage Island middle (PI 13, n=10), Translocated 1st sampling date (TL1, n=10), Sealers Bay start (SB Initial, n=10), Sealers Bay middle (SB 13, n=10), Sealers Bay end (SB 21, n=11), Translocated 2nd sampling date (TL2, n=20) and Passage Island end (PI 21, n=20). Letters above a bar show post-hoc analysis from ANOVA. Treatments that share a letter are not significantly different from one another (P<0.05).

2.3.4 Aristotle's Lantern Index

The Aristotle's lantern index at Passage Island (PI 13 5.0) after 13 weeks was significantly higher (One-way ANOVA $P = 0.022$) than that of the urchins from Sealers Bay (SB Initial 4.3) (Fig 2.6). After 13 weeks of translocation the translocated urchins (TL1 4.1) had a significantly higher ($P = 0.021$) Aristotle's lantern index than the urchins sampled at Sealers Bay (SB 13 3.88) after 13 weeks (Fig 2.6), which was an unexpected result. Twenty-one weeks after translocation the Aristotle's lantern index of the translocated urchins (TL2 3.68) was significantly lower ($P < 0.001$) than the urchins from Passage Island (PI 21 5) after 21 weeks (Fig 2.6). At the conclusion of the experiment after 21 weeks Passage Island (PI 21 5) urchins had a significantly higher ($P < 0.001$) Aristotle's lantern index than urchins sampled from Sealers Bay (SB 21 3.79) (Fig 2.6). There were no significant differences ($P > 0.05$) in the Aristotle's lantern index in any of the other comparisons (Fig 2.6).
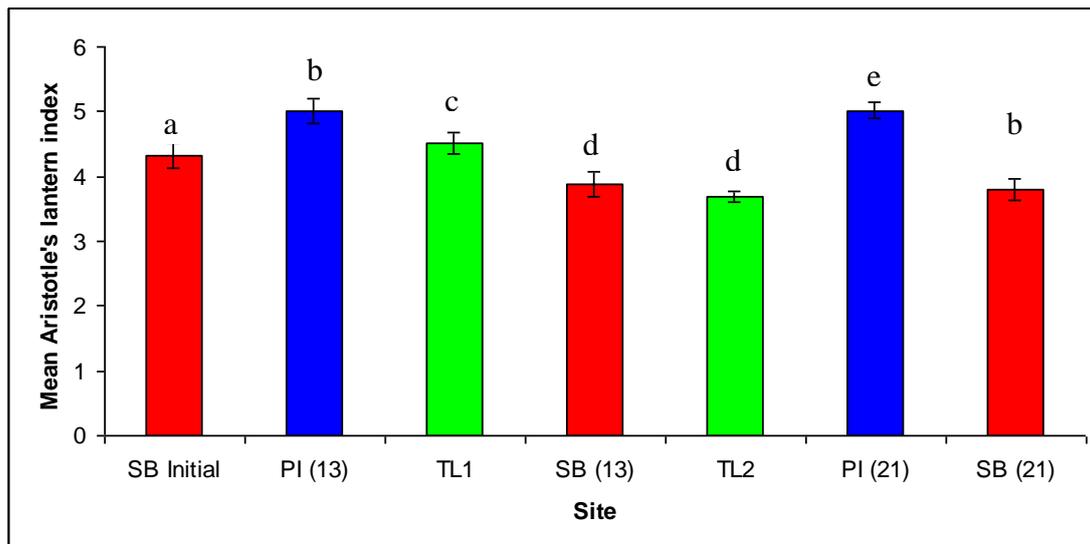
Figure 2.6 The mean Aristotle's Lantern index of urchins from Passage Island middle (PI 13, n=10), Translocated 1st sampling date (TL1, n=10), Sealers Bay start (SB Initial, n=10), Sealers Bay middle (SB 13, n=10), Sealers Bay end (SB 21, n=11), translocated 2nd sampling date (TL2, n=20) and Passage Island end (PI 21, n=20). Letters above a bar show post-hoc analysis from ANOVA. Treatments that share a letter are not significantly different from one another (P<0.05).

2.3.5 Density of *Evechinus chloroticus*

There was a clear and significant (T-test: p-value <0.001) difference in the mean density of urchins at the start of the experiment with a higher density at Passage Island (14.7 per m^2), than Sealers Bay (2.7 per m^2) (Fig 2.7). There remained a clear difference in mean density of urchins per m^2 at the end of the experiment between the two sites, even after approximately 1000kgs of urchins had been translocated to Sealers Bay. Passage Island had significantly a higher (T-test: p-value <0.001) mean density of urchins per m2 (10.8) than Sealers Bay (2.1) (Fig 2.7). Density of urchins per m2 changed slightly at each site with Sealers Bay at the start of the experiment (2.7) having a slightly higher mean density per m^2 than Sealers Bay at the end of the experiment (2.1). This same pattern of changing density per m^2 occurred at Passage Island with large density change of 14.7 urchins per m^2 at the start of the experiment to 10.9 per m^2 at the end of the experiment. Both the differences in density per m^2 at each site were not significant (T-test: p-value >0.05).
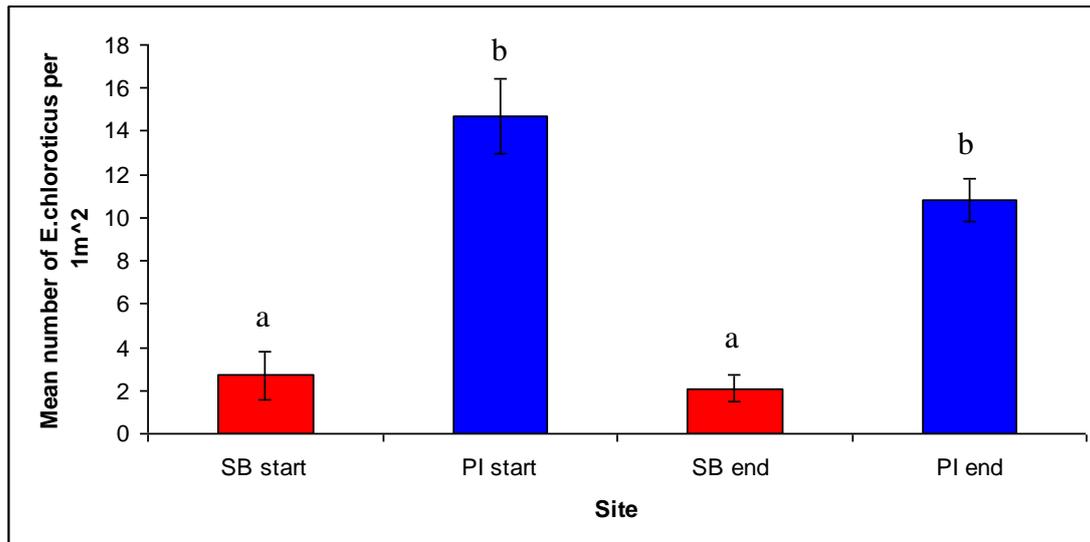
Figure 2.7 Mean density of urchins per 1m2 at Sealers Bay start (SB start), Passage Island start (PI start), Sealers Bay end (SB end) and Passage Island end (PI end) n= 20. Error bars represent the individual variation of urchin density at each site (SE ±). Letters above a bar show post-hoc analysis from ANOVA. Treatments that share a letter are not significantly different from one another (P<0.05).

2.3.6 Gonad colour

Colour was compared between sexes, and it was found that for all 3 colours L*, a*, and b* that the factor sex had no significant effect on colour ($P > 0.05$), so all colour results for both sexes were pooled at each site.

The mean lightness (L) in the gonad showed a significant difference between Passage Island and Sealers Bay (one-way ANOVA: $P < 0.001$). The lightness of the gonads was also significantly different between the 2 sites at the end of the experiment ($P = 0.030$). Only two of the samples from the translocated urchins showed any significant differences in lightness as a result of translocation. These were Sealers Bay (SB Initial) at the beginning of the experiment compared with the translocated urchins after 13 weeks (TL1) ($P = 0.001$) and Passage Island (PI 21) after 21 weeks compared with the translocated urchins (TL2) after 21 weeks of translocation ($P < 0.001$). There was a significant difference in gonad lightness between all three samples from Sealers Bay ($P = 0.004$). All other comparisons of

gonad lightness showed no significant difference when translocations had not occurred and even after translocations had occurred ($P > 0.05$).

Mean gonad redness (a*) varied little between sites and samples irrespective of whether translocations had occurred. The only significant differences in redness occurred between Passage Island (PI 13) after 13 weeks compared to Sealers Bay (SB Initial) at the start of the experiment ($P = 0.006$) and Passage Island (PI 21) after 21 weeks compared to Passage Island (PI 13) after 13 weeks ($P = 0.033$).

Gonad yellowness (b*), was significantly higher in Sealers Bay (SB Initial) at the start of the experiment compared to Passage Island (PI 13) after 13 weeks ($P = 0.001$, Fig 2.8). Significant differences were found in gonad yellowness between Passage Island urchins (PI 13) after 13 weeks compared to Passage Island urchins after 21 weeks (PI 21) ($P = 0.001$), and between all three Sealers Bay samples ($P = 0.006$, Fig 2.8). The only translocated sample which showed a difference in yellowness of the gonads was the urchins from Sealers Bay (SB Initial) at the start of the study when compared against the translocated urchins (TL1) after 13 weeks ($P = 0.001$). All other comparisons showed no significant difference in the yellowness of gonads even after translocation had occurred ($P > 0.05$, Fig 2.8).
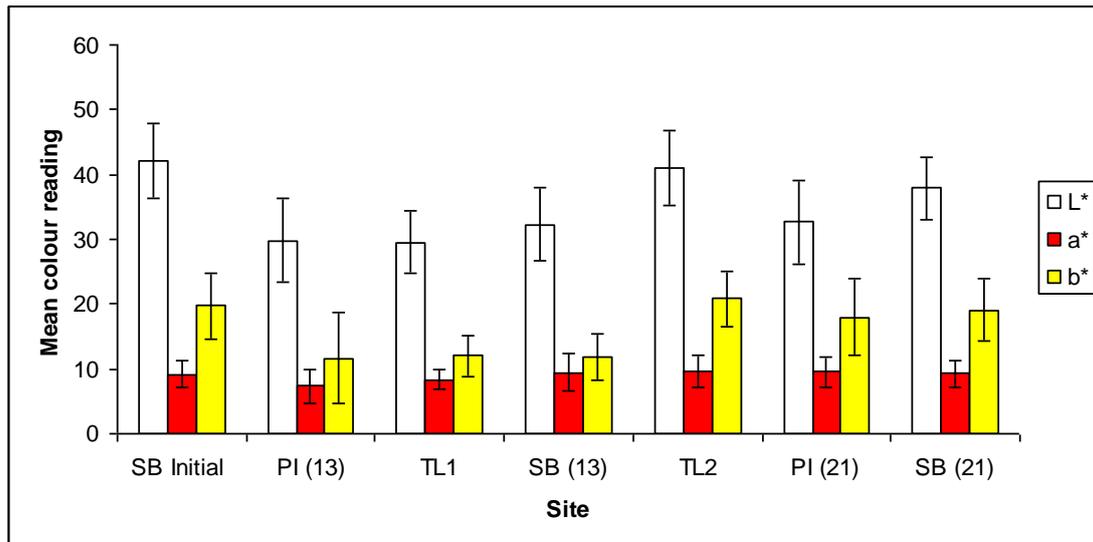
Figure 2.8 Mean whiteness (L*), redness (a*) and yellowness (b*) colour readings from urchins at each site Sealers Bay start (SB Initial), Passage Island middle (PI 13), Translocated 1st sample (TL1), Sealers Bay middle (SB 13), Translocated 2nd sample (TL2), Passage Island end (PI 21), Sealers Bay end (SB 21) Error bars (SE ±).

2.3.7 Algae biomass

At the beginning of the study there was a significantly higher biomass of algae per m^2 at Sealers Bay (2577g) compared to Passage Island (675g) (one-way ANOVA: $P = 0.030$) (Fig 2.9). Upon completion of the study the same difference in the algae biomass per m^2 occurred between the two sites, with Sealers Bay (5491g) having a significantly ($P < 0.001$) higher mean biomass per m^2 than Passage Island (855g) (Fig 2.9). A slight increase in algae biomass per m^2 occurred at Passage Island over the duration of the study from 675g to 855g per m^2 (Fig 2.9), but this was not significant ($P = 0.714$).
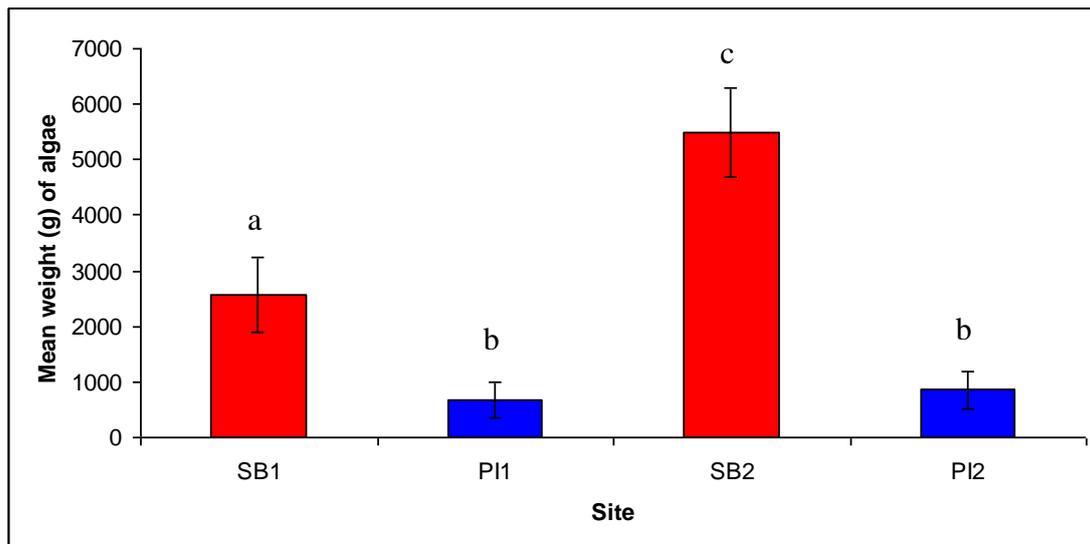
Figure 2.9 Mean algae abundance per m2 at the two sampling sites from the start and at the end of the experiment: Sealers Bay start (SB Initial), Passage Island start (PI 13), Sealers Bay end (SB 13) and Passage Island end (PI 21). Error bars show the individual variation in algal abundance per m2 at each site (SE ±). Letters above a bar show post-hoc analysis from ANOVA. Treatments that share a letter are not significantly different from one another (P<0.05).

2.3.8 Algae species

The mean percentage cover of algae between Sealers Bay (84.1%) and Passage Island (68.5%) was not significantly different (2-way T-test P = 0.195). Passage Island had significantly (P < 0.001) more coralline algae (% cover m^2) of 56.3% than Sealers Bay only had 8.7%. There was a greater species diversity of algae at Sealers Bay compared to Passage Island as can be clearly seen with only 3 different algae species found at Passage Island, compared with 7 different species at Sealers Bay (Table 2.2, Fig 2.10). At Passage Island only *C. flexuosum* and coralline algae were found in the photoquadrats, compared to 7 species at Sealers Bay. Removing coralline algae from the diversity test showed that there was a significant difference (P = 0.003), in species richness/diversity with Sealers Bay having a mean of 5.6 and Passage Island only 0.81.

Table 2.2 List of all Algae Species found at Sealers Bay and Passage Islands from both the start and the end of the experiment

Site	Species
Sealers Bay	*Macrocystis pyrifera*
	Marginariella boryana
	Ecklonia radiate
	Carpophyllum flexuosum
	Dictyota kunthii (formerly Glossphora kunthii)
	Landsburgia quercifola
Passage Island	*Xiphophora gladiate*
	Marginariella boryana
	Carpophyllum flexuosum
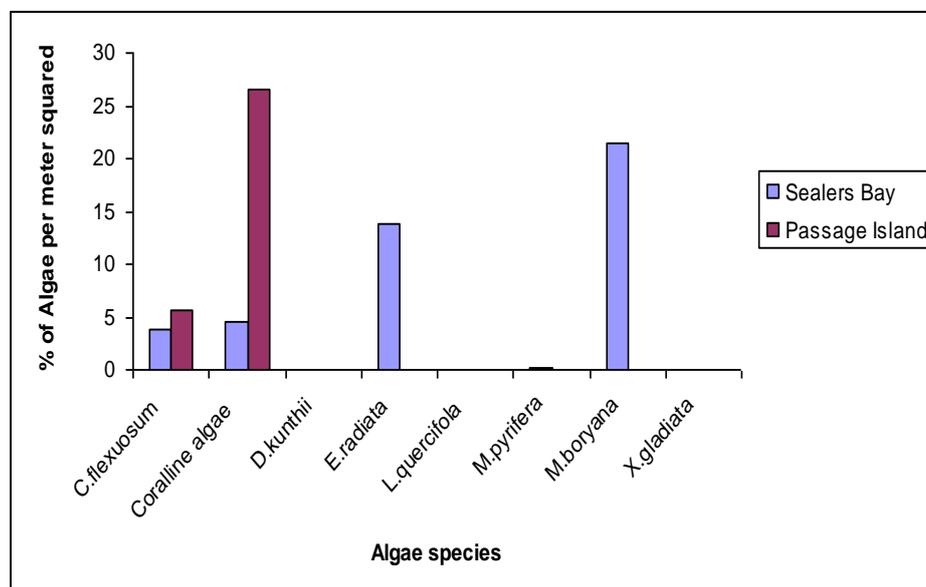
Figure 2.10 Percentage of each algae species per m2 at each site overall for the whole experiment

2.3.9 Oocyte size and gametogenesis

The reproductive stages of the urchins from all sites over the length of the study were very similar to those described by Byrne 1990. Both male and female urchins at the beginning of the experiment at Sealers Bay (SB Initial) had ovaries and testis at stage 3 the premature stage. After 13 weeks the ovaries and testis of all samples (Passage Island, Sealers Bay and the translocated urchins) were at stages 4-5,

mature and partially spawned stages. The ovaries and testis from the samples after 21 weeks (PI 21, TL2 and SB 21) were all at stage 6 to 1 the spent and recovery stages.

Oocyte size frequencies in the samples from PI 13, SB Initial, TL1 and SB 13 all have the highest mean oocyte size frequencies within the 60-89μm size range (Figs 2.11 a-d). The size range agrees with the reproductive stage (3-5) that the urchins are at during the present study. After 23 weeks at the conclusion of the experiment it is clear that spawning has occurred, as there is a dramatic drop in oocyte size with no large oocytes found (Figs 2.11 a-f) Urchins from PI 21, TL2 and SB 21, all had the highest frequency of oocytes within the oocyte size range of 20-29μm, and were in the post spawning stage (Figss 2.11 e-f). Passage Island (PI 13) after 13 weeks had a significantly higher (Pearson Chi sq $P < 0.001$) oocyte size frequency than the urchins from Passage Island (PI 21) after 21 weeks (Fig 2.11 a-f). There was also a significant ($P < 0.001$) difference between all 3 Sealers Bay samples with the Sealers Bay (SB Initial) urchins from the beginning of the experiment and after 13 weeks (SB 13) having larger oocytes than the Sealers Bay urchins after 21 weeks (SB 21). No other comparisons showed any significant differences in oocyte size frequencies ($P < 0.001$).
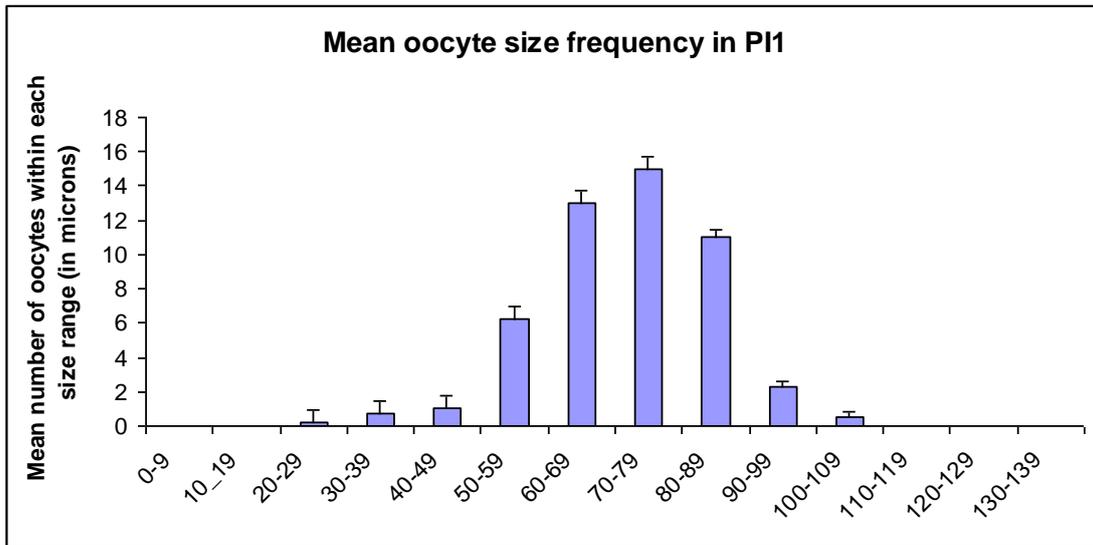
Figure 2.11a
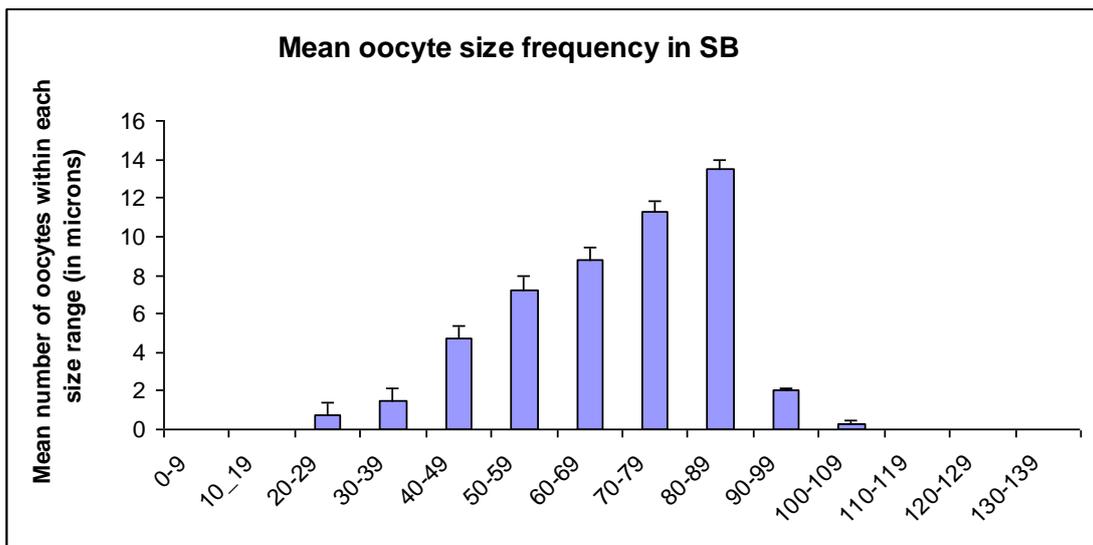
Figure 2.11b
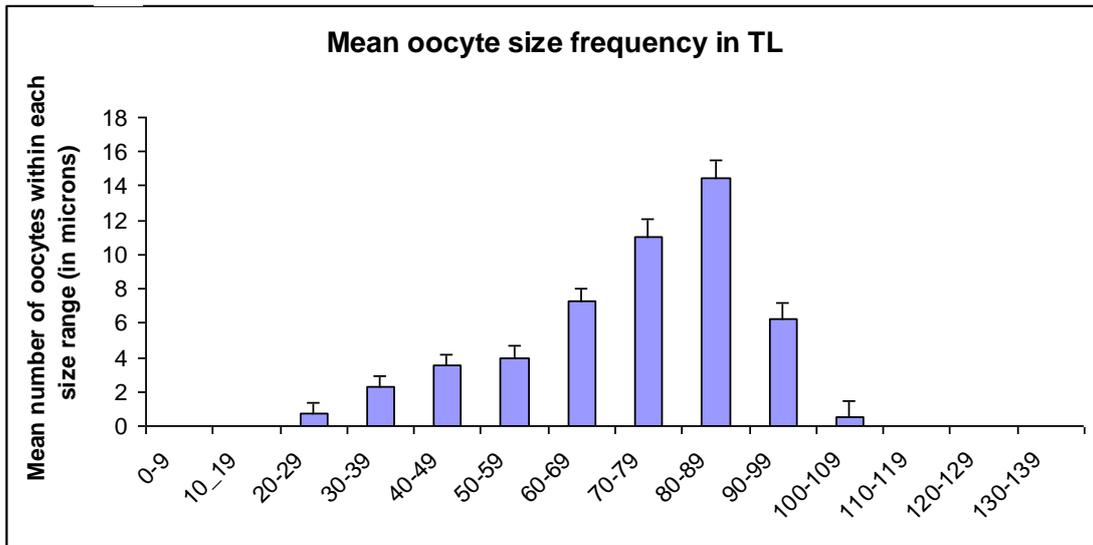
Figure 2.11c
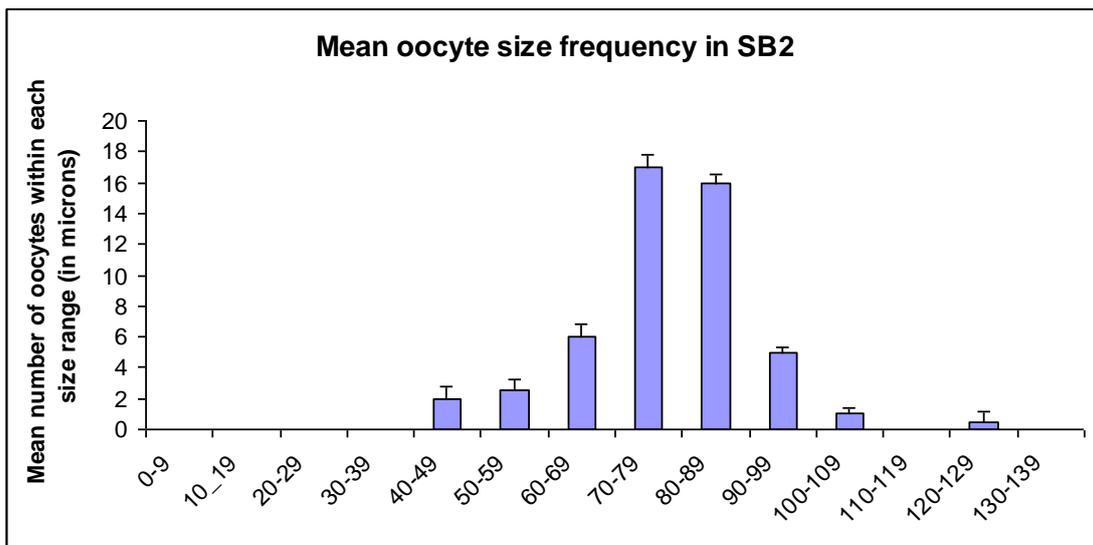
Figure 2.11d
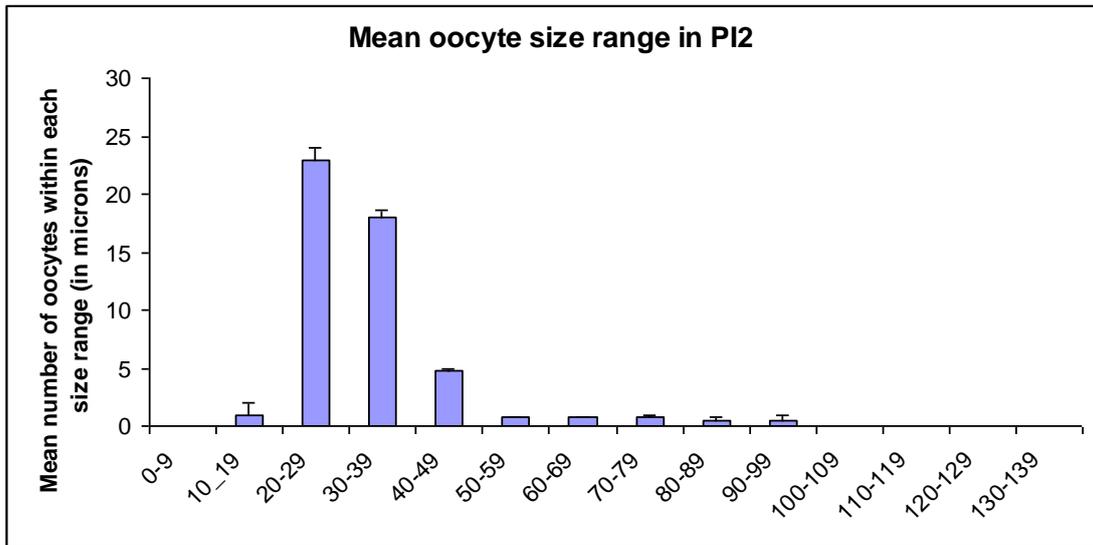
Figure 2.11e
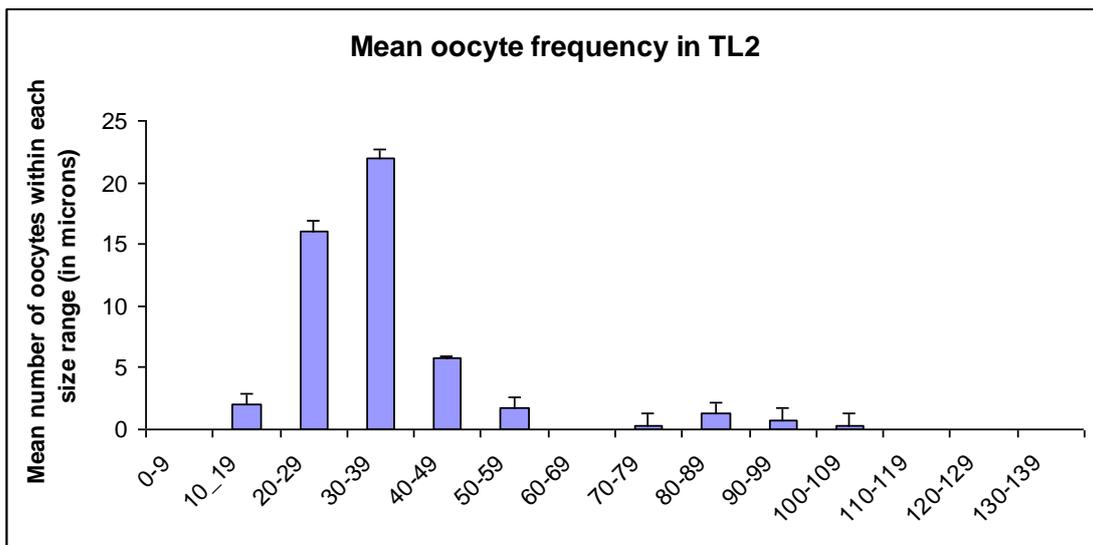
Figure 2.11f
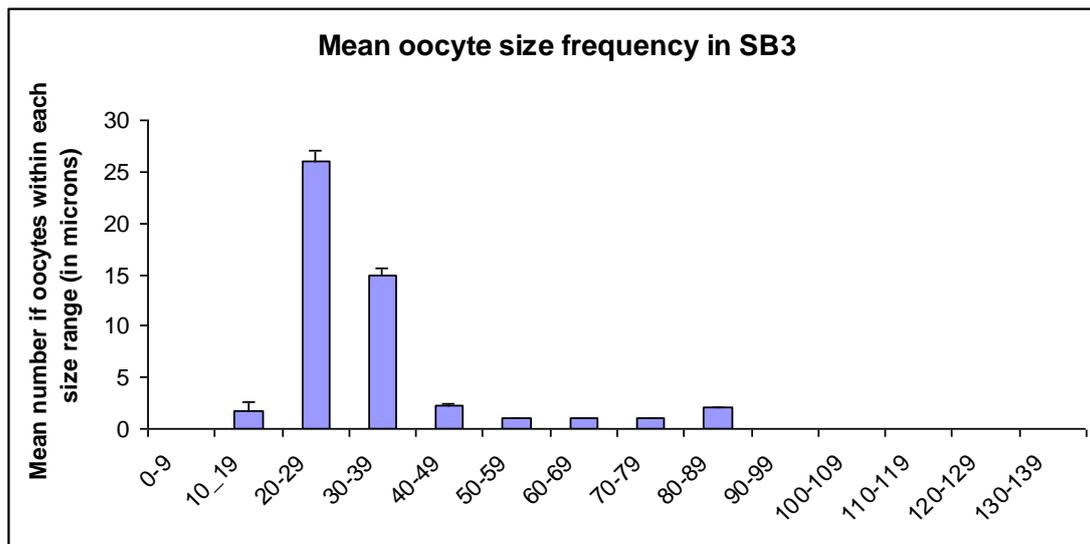
Figure 2.11g

Figure 2.11 a-g Mean oocyte size frequency of sea urchins for each site, Fig a (PI 13 = Passage Island middle), b (SB = Sealers Bay start), c (TL = Translocated 1st sample), d (SB 13 = Sealers Bay middle), e (PI 21 = Passage Island end), f (TL2 = translocated 2nd sample), g (SB 21 = Sealers Bay end) Error bars (SE ±)

2.4   Discussion

Evechinus chloroticus populations sampled from Chalky Inlet at both Passage Island and Sealers Bay, showed no difference in the male to female sex ratios at each site (Table 2.1). Equal sex ratios were expected as *E. chloroticus* is a gonochoric species with a normal sex ratio of 1:1 and similar approximately equal sex ratios have been found in other studies (Barker 2007; Lamare *et al*. 2002; McShane 1994). The reproductive cycle of *E. chloroticus* in Chalky inlet is similar to that of other urchin populations in the southern area of the country (Lamare *et al*. 2002; McShane 1994).

The gametogenic cycle of urchins from all the sites followed the expected pattern of reproductive stage as described for *Paracentrotus lividus* by Byrne 1990. The stages of gonads in the samples from the beginning of the experiment and after 13 weeks were between stages 3-5 indicating that the urchins were spawning or were about to spawn. After 21 weeks the urchins from the end of the experiment had

gonads at stage 6 (spent stage) which indicated that spawning had occurred. The urchins had the same reproductive stages as described in many other studies of *E. chloroticus*, as well as having the same reproductive stages/season as those in Dusky Sound close to the urchins of Chalky Inlet and in Chalky Inlet itself (Lamare *et al*. 2002; McShane *et al*. 1994; Keys 2008).

2.4.1 Gonad and gut indices

There was no significant differences found between sex and gonad or gut indices over the study. Gonad index was not expected to be different between the sexes, with other studies of *E.chloroticus* finding no differences between gonad index and sex (Brewin *et al*. 2000; Keys 2008; Thomas 2009).

Gonad index at the start of the study at the two sites Passage Island and Sealers Bay was expected to be different, as Passage Island is not fished because of poor quality gonad and Sealers Bay is currently fished due to good gonad quality. When compared after 13 weeks the mean gonad index was slightly higher at Passage Island (Fig 2.4), however, no significant difference was found between these two samples. The lack of a difference between the two original samples may have been due to the Passage Island sample not being collected until 13 weeks later. Due to human error by crew members throwing the urchins that were collected on the first trip overboard. Therefore, it is likely that gonad growth would have occurred in the Passage Island urchins over this time period. Translocation of the urchins to a location with greater available nutrients caused an increase in gonad growth. When the translocated urchins were compared after 13 weeks against both the Sealers Bay and Passage Island urchins the mean gonad index was significantly higher (Fig 2.4). The highest mean gonad index occurred at Sealers Bay (42.5) in the sample collected 13 weeks after the start of the experiment (Fig 2.4), with these urchins having a

significantly higher gonad index than the translocated urchins collected after 13 weeks. High gonad indices after 13 weeks from Sealers Bay and the translocated urchin samples could be the result of the samples were being collected in January just before spawning so the gonads would have been fully ripe, therefore having a peak GI (Fig 2.4). The higher gonad index at Sealers Bay compared to the translocated urchins could be due to the Sealers Bay urchins having a higher gonad index in the beginning, compared to the translocated urchins which would be likely to have had a low gonad before they were translocated.

The samples from the end of the study after 21 weeks (SB 21, PI 21, and TL2) showed a clear drop in the mean gonad index (Fig 2.4), most likely due to the urchins having spawned as they were sampled in March. This can clearly be seen in the corresponding decrease in oocyte sizes (Fig 2.11a-f). The majority of oocytes from the samples after 21 weeks (SB 21, PI 21, and TL2) were in the 20-29μm range, compared to the samples from experiments beginning and after 13 weeks (SB Initial, SB 13, TL1 and PI 13) which have the highest frequency of oocytes within the 60-89μm range. Spawning had a clear effect on the comparisons. The Sealers Bay sample (42.45) after 13 weeks had the highest gonad index of the Sealers Bay samples, followed by the sample from the experiment beginning (23.09), with the sample after 21 weeks having the lowest gonad index (20.4), with these differences being significant (Fig 2.4). The Passage Island (10.5) sample after 21 weeks had a lower gonad index than the Passage Island (26.5) sample after 13 weeks which is most likely attributed to the urchins having spawned, with the difference being significant (Fig 2.4). Even though spawning occurred, it is apparent that translocating urchins increases the gonad index. The translocated (16.8) sample after 21 weeks had a higher gonad index than the Passage Island (10.5) sample after 21 weeks with this difference

being significant. Sealers Bay (20.4) urchins after 21 weeks had a higher gonad index at the end than both Passage Island (10.5) and the translocated urchins (16.8), with these differences both being significant (Fig 2.4). Increased gonad index as a result of urchin translocations even after spawning would suggest that higher food availability could be a major factor affecting the growth of gonad at each site.

The gonad index followed a similar trend in the current experiment to that described by Keys (2008) for Chalky Inlet, with the first samples from November having high gonad index, with this increasing in the samples taken in January (pre spawning), followed by a decrease in the final samples taken in March (post spawning) (Fig 2.4).

The gut index was affected by translocating urchins, but not to the same extent as the gonad index. The gut index at Sealers Bay at the beginning of the experiment was higher than that at Passage Island after 13 weeks (Fig 2.5). This difference could be due to higher food availability at Sealers Bay than Passage Island, allowing for more nutrients to be stored in the gut and therefore producing a larger gut index. A decrease in the mean gut index occurred in the translocated urchin sample after 13 weeks, with the gut index being lower than both the Passage Island sample after 13 weeks and the Sealers Bay sample from the beginning of the experiment (Fig 2.5). This difference could be due to seasonal changes. All the nutrients could have been used up during gametogenesis before spawning as the translocated sample was taken in January before spawning occurred. This would also explain why the Sealers Bay sample after 13 weeks has a low mean gut index (Fig 2.5). The change in gut index within each site over the study duration could be a result of a seasonal change due to the reproductive cycle, lower gut indices prior to spawning, and higher gut indices after spawning when nutrients are being accumulated for the next reproductive cycle.

Both the gonad and gut index were altered over the duration of the study, with the gonad index altered more by the translocation of the urchins than the gut index. Food availability is the most likely reason there was a change in both gonad and gut index. The gonad and gut are both storage organs, with the gut storing excess nutrients when they are available (Andrew 2003; Barker 2007; Lamare *et al*. 2002), therefore differing food availability will affect both indices, but not equally. It is also thought that the gut index is a good indicator of nutrition in *E.chloroticus*, with gut indices most likely decreasing as a response to lower nutrition (Fell 2002). Studies in *Strongylocentrotus purpuratus* and *S. franciscanus* have found that the size of the gut varies with season and nutrition, with gut size decreasing in lower nutrient conditions and increasing in higher conditions (Russell 1998).

High densities of urchins at Passage Island have led to a low algae biomass and low quality of algae at this site. Under high densities coralline algae flats are formed due to overgrazing of fleshy and standing algae. This leads to areas of low food supply and availability (Andrew 1998; Barker 2007; James 2007; Tsuda *et al*. 2006; Vadas *et al*. 1999). The low quality and biomass of algae is likely to account for the low gonad and gut index found at Passage Island, which would account for the changes in gonad and gut index after translocations of urchins to Sealers Bay took place. Sealers Bay had a significantly higher algae biomass than Passage Island, with a significantly lower density of urchin's therefore higher food availability. Many studies have shown that higher amounts of food lead to higher gonad indices in most sea urchin species, such as *Strongylocentrotus spp* (James 2008; Minor & Scheibling 1997; Fell 2002; Tsuda *et al*. 2006). Algal biomass affects the gut index in a similar way, with higher food availability causing an increase in gut size. Higher gut indices were found in *E.chloroticus* during the summer period and then decreased from

February through to May (Fell 2002). In the current study there was a seasonal change in algae biomass this being highest in the samples taken at the studies completion in January, the height of summer (Fig 2.9). The larger gut indices from the end of the study were associated with higher food availability, meaning excess nutrients can be stored in the gut (Fig 2.5). Gonad index was affected in the same way with higher indices found at Sealers Bay due to the lower urchin densities and higher algal biomass.

Over a 24 week experiment, Minor & Scheibling 1997 found that barrens with low kelp densities could support somatic growth and some gonadal growth in *Strongylocentrotus droebachiensis,* but the gonad index remained low. Urchins in these barrens persisted through feeding on drift algae. Urchins were then fed on either low or high rations of algae and the gonad index increased significantly, with nutritive phagocytes far more abundant in higher fed urchins than those under low food rations. Other studies involving *S. droebachiensis* have shown that increasing the amount of food for lengths of time (as little as 3 weeks in one experiment) can lead to a rapid increase in gonad index, due to the increase in nutrients available to be converted to nutritive phagocytes and therefore gonad mass (Russell 1998; Vadas *et al*. 1999; Tsuda *et al*. 2006). McBride 1997 found that gonad index can increase by as much as 1% per week in the red sea urchin *S. franciscanus* with continuous feeding of prepared algae diets. Gonad index is directly related to food availability (James *et al*. 2007; James 2008). Under low food conditions it has been shown with *Strongylocentrotus spp*, that little energy is available for growth or reproduction resulting in low gonad indices (Tsdua *et al*. 2006; Vadas *et al*. 1999). Similar studies with *E.chloroticus* at high population densities have found that gonad index remains low (Andrew 1988; James 2007; James & Herbert 2009). In a similar study it was

found that increased food availability was the likely cause of the increased gonad index of *Parcentrotus lividus* when urchins where translocated from non fished areas in France with high densities of urchins to sites with low densities (San Martin 2002). High urchin densities have a similar effect on gut size. As a secondary storage organ, little nutrition is likely to be stored in the gut when food is low. This is because most nutrition will be used in gonad growth for reproduction resulting in smaller gut sizes (Fell 2002; Vadas *et al*. 1999). Gut indices from samples within the spawning season (November to March) are likely to be lower, as energy is used for reproduction rather than being stored (Andrew 2003; Dix 1970a; Fell 2002; Gonor 1972). Only the study by San Martin 2002, mentioned above, involved wild urchin translocations but all of them showed increased gonad index with higher food. It can therefore be assumed, based on the current studies results that the same applies to *E.chloroticus* once they are moved from barrens of low food availability to high food availability.

The diversity of algae species was significantly higher at Sealers Bay than at Passage Island when coralline algae were removed from the comparison calculation (Fig 2.10, Table 2.2). This could also help account for the gonad index increase of translocated urchins at Sealers Bay as a greater diversity of species could provide better nutrition for gonad growth. *Evechinus chloroticus* has been shown to have a preference for the algae species *Ecklonia radiata* and *Macrocystis pyrifera* over other species such as Carpophyllum *spp* (Schiel 1982; Fell 2002). In a study by Andrew (1986) it was found that *E.chloroticus* at low densities and fed on, *E.radiata*, had gonads that were larger than urchins fed on less preferred species such as *Carpophyllum maschalocarpum* and *C. angustifolium*. At higher densities of *E.chloroticus* (20 per m^2) there was no significant difference in gonad size even with urchins feeding on more preferred algae species (Andrew 1986). A species specific

effect has also been found in other species including *Strongylocentrotus spp* with certain algae species of higher nutritional value resulting in a higher gonad index than that of less preferred species (Tsuda *et al*. 2006; Minor and Scheibling 1997; Vadas *et al*. 1999). In a study by Vadas *et al*. (1999), *P.palmata*, a red algae species with high nutritional content, caused greater amounts of gonad growth than that of lower ranked nutritional species. A higher diversity of algae species is thought to also have an effect on gut index. Fell 2002 found that *E.chloroticus* fed on *Macrocystis pyrifera* had higher gut indices than urchins fed on less desired species and starved urchins. Schiel (1982) has shown that it is possible that *Evechinus chloroticus* has a selective preference for certain species of algae such as *Ecklonia radiata* (which was the most preferred species found in Schiel's experiment). Nutritional testing of algae was not undertaken in the current study, however, with more species of algae at Sealers Bay, including preferred algae species, it could be assumed that this species diversity helped to contribute in the gonad and gut index increases. Further studies could look into the effects of nutritional content of algae on gonad and gut growth in *E.chloroticus*.

A similar experiment translocating *E.chloroticus* from an area where urchins occurred in high densities with low gonad index Needle Rock (36° 43.768'S / 175° 50.190'E) and Wigmore Pass (36° 50.282'S / 175° 48.932'E) to an area of low density and high gonad index Middle Island (36° 38.261'S / 174° 51.540' E) and Flat Island (36° 57.225'S / 176° 03.400'E) was undertaken recently in the Coromandel by James & Herbert (2009). During the seven month period after the urchins were translocated to the area with higher food abundance, an increase in the gonad index occurred. High increases in gonad index occurred in the non translocated urchins at the original poor site after urchins were transferred due to the lower densities of urchins remaining

(James & Herbert 2009). Overall increased yields of approximately 50% occurred in the translocated urchins and up to almost 100% in the non translocated urchins in the food poor areas after lowering urchin density (James & Herbert 2009). It can be assumed that the increased gonad index in the current experiment is based on higher food availability under lower urchin densities as a result of translocation. The movement of 1000kgs was not great enough to change the urchin densities at Passage Island so effects on gonad index of lowered densities on non-translocated urchins at poor gonad sites could not be detected. In the study by James & Herbert 2009, the effect of translocations on the gut index was not looked at, however, from the current study it is apparent that translocations had very little effect on the gut index of *E. chloroticus*, as no comparisons with the translocated urchins after 21 weeks showed any significant differences. The time period of the experiment may also be too short to detect any significant differences in gut index even with urchins from Passage Island translocated into a food rich area. It was found that after intensive feeding on high quality food there was no change in gut indices of *S. droebachiensis* over a 6 week study, but during longer studies ranging from 16, 32 and 64 weeks gut index changed in *S. purpuratus* and *S. franciscanus* (Lawrence *et al* 1965; Russell 1998). Further research could look into longer timescale translocation effects on gut size.

2.4.2 Aristotle's lantern index

The Aristotle's lantern index was significantly different between the sample from Sealers Bay at the start of the study and Passage Island after 13 weeks, as well as in the comparisons between these sites at the completion of the study. In both comparisons Passage Island has a higher mean Aristotle's lantern index compared to that of the urchins at Sealers Bay, which was expected as there is less food at Passage Island, so urchins are more likely to have a larger Aristotle's lantern to optimise

feeding. Lack of mobility in urchins of all species has meant that plasticity of resources in urchins is very high, with most energy directed into reproduction, storage of nutrients in the gut and then into the feeding apparatus (Aristotle's lantern) under low food conditions (Lamare & Mladenov 2000; Minor & Scheibling 1997; McShane 1997; Russell 1998; Wing *et al*. 2001). *Evechinus chloroticus* in other studies has been found to follow a trend of decreasing jaw/lantern size with increasing food availability, with the reverse happening in food poor areas. A study by McShane 1997 involving *E. chloroticus* within Dusky Sound (high food availability) and Arapawa Island (low food) found that smaller jaws/lantern occurred in Dusky Sound urchins compared to larger jaws of the Arapawa Island populations. Other studies on *E. chloroticus* have found similar results, with Wing *et al* 2001 and Lamare & Mladenov 2000 finding small jaw sizes in urchins from areas with high food availability and larger jaw sizes in food poor areas.

The plasticity in resources in urchins could also account for the significant difference in lantern size between the translocated urchins after 13 weeks and the Sealers Bay urchins after 13 weeks (Fig 2.6). However, only one other translocated sample of urchins after 21 weeks compared against Passage Island after 21 weeks showed significant difference in lantern size, with the translocated urchins having a lower mean lantern index than the Passage Island sample (Fig 2.6). Changing lantern size as a result of the translocation is very surprisingly over such a short time scale, and was not expected to occur. This change in lantern size could also be in part due to the small sample sizes in the study. The poorer food at Passage Island could have contributed to this difference, as mentioned above, poor food causes differences in lantern sizes, however, none of the other translocated samples show any significant differences. It is hard to account for such marked differences in a 20 week period. In

studies of *S.purpuratus* and *S. franciscanus*, and *S. droebachiensis* it was found that lantern changes were only apparent after time periods of 16, 32 and 64 weeks, with only other body organs such as gonads changing rapidly over shorter time periods (Lawrence *et al* 1965; Russell 1998). More time would have been expected to be needed for significant changes in the lantern size to become apparent, which could explain why not all of the translocated urchin samples showed any significant difference in lantern index. An experiment of a longer time frame with larger sample sizes is needed to draw accurate assumptions of the plasticity of lantern size in relation to translocation of urchins.

2.4.3 Gonad Colour

The two colours of the gonad which are affected the most throughout the experiment are the lightness (L*) and yellowness (a*) parameters, with redness (b*) only having a minor change due to translocations (Fig 2.8). Yellowness and lightness could be linked as often yellower gonads are lighter. Lightness and yellowness are also high on the list of qualities required for gonad in the Japanese market, with high Lightness, and an almost pumpkin coloured gonad being desired (James 2007: Woods *et al*. 2005). Even with translocations or with gonad from the Sealers Bay (high quality gonad) site the highest mean lightness reading was only 42.13 (Fig 2.8), far below that of the lowest level required in the Japanese market based on "acceptable gonad colours" Table from the Nippon seafood company L* = 63.95 (Table 2.3).

Significant increases occurred in only 3 of the translocated urchin comparisons across all 3 colours, with lightness differences only in the Sealers Bay sample at the beginning of the experiment compared with the translocated urchins after 13 weeks. The other significant difference was found in the Passage Island sample after 21 weeks compared to the translocated urchins sampled after 21 weeks. Yellowness was

the other colour to show a significant difference from a translocated sample. The lack of significant difference in gonad colour after translocations is surprising, considering the significant differences between the site comparisons (SB Initial and PI 13) at the beginning and end of the experiment. Sealers Bay has a higher mean colour reading in all colours (Fig 2.8), compared to Passage Island. Colour differences between the non-translocated urchins at Sealers Bay and Passage Island, could be due to differing food availability and algal species composition between Passage Island and Sealers Bay. There was, however, only a significant difference between SB 21 and PI 21 in lightness.

Studies of other sea urchin species around the world have found that the orange-yellowish colour of gonad is caused by carotenoids, with the pigment echinome, synthesised in the urchin from beta-carotene having the largest effect on colour (Robinson *et al*. 2002; Agatsuma *et al*. 2005). Sea urchins obtain the carotenoids from fleshy macroalgae in the diet (Agatsuma *et al*. 2005; Matsuno and Tsushima 2001), which could account for the higher colour readings at Sealers Bay due to the higher diversity and algae biomass present (Fig 2.8). *Hemicentotus pulcherrimus* fed on green algae was found to have more preferable gonad colour than when feed on brown or calcareous algae (Agatsuma *et al*. 2005).

McShane *et al*. 1994 found that *E. chloroticus* in Dusky Sound had gonad colours ranging from light brown to a dull orange colour, with larger urchins above >130mm having smaller blackish/brown coloured gonad. The urchins in the current study were between 105-135mm TD from a typical commercial size range fished in Chalky Inlet (pers. obs.). Different size classes were, however, not compared separately in the present study to look at size effects on colour, but a similar colour variation occurred in the gonad from the urchins in this experiment (Fig 2.6, pers.

obs.). Agatsuma *et al*. 2005 found that gonad production is related to colour with decreasing gonad growth leading to a brownish colourisation. Lower gonad production at Passage Island could account for the difference in colour when compared against Sealers Bay at the start, but the same differences in gonad colour should be expected in the end comparisons for all colours. Some of the urchins from the Passage Island, translocated sample and Sealers Bay sample all taken after 13 weeks were dead upon arrival to Bluff and before processing due to the way they had been stored/ transported to Bluff by the fishermen. The death of the sea urchins before processing could therefore account for the lack of significant difference in some of the comparisons, and account for the significant difference found when the Sealers Bay samples were compared against each other. Once the urchin dies the colour is likely to be altered (Barker pers comm.), it is therefore likely that this could have influenced the results.

It was expected that translocating urchins should have improved gonad colour once urchins had more food available at Sealers Bay. The lack of this noticeable difference could be due to the death of some urchins before processing. In future experiments more live urchins and more comparisons could be undertaken to more accurately assess colour changes from translocating, due to the large importance placed on colour in the export market.

Table 2.3 The lightness (L*), redness (a*) and yellowness (b*) of roe colours which are "acceptable" for sale in Japan, based on the Japanese Seafood company Nippon Suisan Kaisha Ltd (James 2007).

Maine colour chart reference number	L* reading	a* reading	b* reading
104	87.15	-5.17	43.86
105	84.01	-4.26	56.03
106	77.76	6.20	48.44
107	82.17	-2.11	67.97
108	76.86	4.28	61.03
109	69.19	15.69	36.46
110	71.57	13.19	49.85
111	69.20	17.92	40.22
112	63.95	17.31	34.77

3 Gamete quality in larger *Evechinus chloroticus*

3.1 Introduction

Sea urchins mature at different ages and sizes depending on the species, location and food availability (Kato & Schroeter 1985; Kenner & Lares 1991; Williams 2002). *Evechinus chloroticus* is no different to other species with size at maturity varying according to the food availability in the area (Barker 2007; Dix 1970a; McShane 1997 McShane *et al*.1994). Average maturity of *E. chloroticus* is reached around 3-4 years of age in most populations, with the size varying depending considerably on location (Andrew 2003; Barker 2007; Dix 1970b). Size at sexual maturity can be 35-45mm test diameter (TD) (e.g. urchins from Kaiteriteri) or up to 55-75mm in urchins found at Kaikoura (Dix 1970b). On average the size range of

mature urchins is between 35 to 90mm TD, with urchins from food poor sites attaining maturity at a smaller size than those from food rich sites (Dix 1970b; McShane 1997; McShane *et al* 1994). After reaching sexual maturity urchins continue to grow, with sizes of 170 to 180mm TD recorded, and ages from 30 to 50+ years reached in large urchins as the growth rate slows with age (Barker 2007; Dix 1970a; Keys 2008; Lamare & Mladenov 2000; McShane *et al* 1994; McShane 1997). Recent studies which are yet to be published suggest that the age of some *E. chloroticus* could be as high as 100 years (Barker pers comm.).

Size and age has a large impact on the gonad quality and quantity in sea urchin species around the world. Upon reaching sexual maturity the size of the gonad starts to increase and continues to increase until the largest size class is reached. After reaching the larger size class, the quantity of gonad gradually decreases, which is normally associated with a corresponding decrease in quality (Dix 1970a; Kato & Schroeter 1985; Moore *et al*. 1963; McShane 1997). The red sea urchin *Strongylocentrotus franciscanus* was found to have an increase in gonad growth and size upon reaching sexual maturity, with the gonad increasing in size until the urchin reaches 95mm TD, then a decrease in gonad size occurs (Kato & Schroeter 1985). Fecundity along with gonad growth also decreases with larger sizes, these, however, are two different phenomena with both affected separately. Increasing fecundity was found in *Lytechinus variegitus* after sexual maturity right through until the largest size class of +50mm TD was reached, where upon a considerable decrease occurred (Moore *et al*. 1963).

Similar effects occur in *E. chloroticus* with gonad size decreasing with age and increasing TD. Urchins larger than 120mm TD produce smaller gonads than smaller age and size class urchins (Dix 1970a; McShane 1997; McShane *et al* 1994;

McShane *et al* 2010; Woods *et al*. 2008). Gonads of large old urchins are often poor in quality, being small and of a darkish coarse texture (McShane 1997; McShane *et al* 1996). It is also thought that fecundity decreases in old large *E. chloroticus* (McShane *et al* 1994). The higher metabolic costs of maintaining a large body size >120mm TD, are thought to be the reason why large *E. chloroticus* produce smaller gonads than urchins in smaller size classes (McShane *et al* 2010; Woods *et al* 2008).

Food availability along with old age and large size are other factors which affect the quantity and quality of the gonad. If food availability is low then a corresponding low gonad index occurs, compared to high food availability which allows for higher gonad index (Brewin *et al* 2000; James 2007) and the present study (chapter 2). A higher gonad index means more nutrients are available for gametogenesis, so in larger urchins both maturation and gonad growth in relation to food availability affect their reproductive output (Brewin *et al* 2000; Meidel & Scheibling 1999; McShane 1994; Woods *et al* 2008). A higher gonad index in *E. chloroticus* is associated with an increase in reproductive potential compared to that of a lower gonad index urchin (Andrew 1986; Barker 2007).

Larvae of *E. chloroticus* are, like many other echinoderms, planktotrophic, with larvae developing through pluteus and echinopluteus stages while in the plankton (Andrew 2003; Barker 2007; Keys 2008; Lamare & Barker 1999). High mortality occurs in planktotrophic larvae over the period of development through to the juvenile urchin stage, with many factors including both predation and food availability affecting this (Barker 2007; Lamare & Barker 1999). During larval development the amount of food a larva receives affects how fast it grows and develops (George *et al* 1990; Lamare & Barker 1999; Poorbagher *et al*. 2010). Higher food availability results in faster growth and development of larvae, with low food availability

resulting in slower growth and in some cases halted development (George *et al* 1990; Lamare & Barker 1999; Poorbagher *et al*. 2010). Parental nutrition has also been found to affect egg quality and the subsequent development of larvae, with higher quality eggs producing larvae which develop faster than larvae from lower quality eggs (George *et al*. 1990; Poorbagher *et al*. 2010).

Originally it was thought (Dix 1970a) that older large *E. chloroticus* might be reproductively senile, and the gametes would not be viable. Later studies of large *E. chloroticus* (>120mm TD) by McShane *et al* 1994 found that fertilization was successful, proving that these large urchins can produce viable gametes. In a later study it was found that viable gametes were found in larger urchins (>140mm TD), with successful spawning and rearing of larvae through to an 8arm stage occurring (Keys 2008).

In the current study the viability of gametes in large *E. chloroticus* (>150mm TD) from the South Cape of Stewart Island was examined by measuring fertilization success rates. Fertilized embryos were then cultured to allow larvae to develop to determine whether the larvae from these large urchins are likely to contribute to the larval pool. Gonad index was also measured in the female urchins to assess whether the urchins were of a commercial quality. The implications on the fishing of large *E. chloroticus* are then discussed in relation to whether they contribute to the larval pool and the quality of their gonads.

3.2   Materials and methods

Urchins for the reproduction experiment were collected using the same vessel as described in Chapter 2, the San Nicholas operated out of Bluff by Campbell McManaway. Urchins were collected by breath holding divers and brought back to

the boat with a dingy. The urchins were collected from Mokonui Island, located of the South cape of Stewart Island (S47°03.039 E167°24.522). Broken rocky reef protruded seaward out from the Island down to a depth of around 15 meters, the urchins were gathered from depths of around 5 meters. Dense large brown algae stands dominated the area with sparsely distributed aggregations of *E. chloroticus* within the algal stands (pers. obs.). The area is currently commercially fished for urchins, with urchins present in low densities.

Ten of the largest urchins found were used for the study. Once on the boat the urchins were stored in a tank with flowing water for the journey back to Bluff. These urchins were transported in buckets of seawater from Bluff to the Portobello Marine Laboratory and stored in tanks with flowing water. *Macrocystis pyrifera* was fed to the urchin's until spawning was undertaken. Urchins were collected on the 15th to 17th of February 2010.

3.2.1 Spawning and fertilisation of the urchins

Six urchins were placed on top of beakers filled with filtered seawater (to 1.0 μm) so their aboral gonopores were immersed. Because of the urchin's large size 10mls of 0.5 M of KCL was injected through the peristomial membrane into the coelom of each urchin, with a 23-25 gauge hypodermic syringe. The urchin was lightly shaken to allow the KCL to be dispersed throughout the urchin's body. Male urchins were put on top of a petri dish sitting on ice after they started releasing sperm so the sperm would remain inactive until required. Once female urchins started releasing eggs, all eggs were pipetted from the spawning beaker into a second beaker of fresh filtered seawater (to 1.0 μm). The sample consisted of three female and three male urchins.

Sperm from all 3 males was mixed with seawater and counted through a particle counter (Elzone XY 180) until a concentration of 100 000 sperm per ml was achieved. The three female urchins spawned were labelled A, B and C. After fertilisation these three jars were divided into three replicate samples for each female so there were nine replicates in total (A1, A2, A3, B1, B2, B3, C1, C2, and C3). Eggs were counted using a Bogorov counting tray and dissecting microscope, in order to obtain a high enough concentration of eggs, so each of the cultures had 6000 eggs per 3 litre culture jar.

Once the sperm was added the jars were then left for 30 minutes to ensure that fertilization had occurred. A 20ml sample was taken with a 25ml pipette from all 9 replicates and fixed with 5mls of 10% seawater buffeted formalin.

Fertilisation rates were calculated from the nine 20ml samples, by counting the number of fertilized and unfertilized eggs in 3 ml of sample on a Bogorov counting tray. An egg was counted as fertilised if a fertilisation membrane had formed around the outside of the egg, this was easily visible as a pale line around the egg cytoplasm.

Urchins which were spawned had their diameter measured, they were then cut open in the same way as in chapter 2 with all body components measured so a gonad index could be calculated, as described in chapter 2. Photographs of the gonad were taken to gauge colour, as to whether it was poor or of an acceptable commercial colour.

3.2.2 Larval culture and sampling

Once fertilised, the eggs were put into the jars at a concentration of two eggs per ml, they were kept suspended in the water column, throughout the development from an embryo to an 8arm larva. The suspension in the water column was done with a standard paddle system which moves at 10 strokes per min^{-1} (Strathmann 1987) (Fig

3.2). Larvae were sampled on the following 2 days after fertilization, by collecting a 20ml sample taken mid water, not on the bottom so debris were not picked up. This sample was taken to monitor progress, and had 3ml of formalin added. The embryos were left to develop for 5 days after fertilisation before a 50ml sample was taken (day 5). The jars were stirred before each sample was taken to ensure that enough larvae were suspended in the water column. It was expected that 4 arm larvae would develop after 5 days based on the normal growth pattern of juvenile *Evechinus chloroticus,* however this is very dependent on culture conditions and food concentration (Barker 2007, Table 3.1). Twenty five ml samples were collected on days 5, 9, 16, 21, 28 and 33. On day 29 a 50ml sample was taken. This sample was collected during jar cleaning so the larvae were concentrated to ensure enough larvae were sampled for postoral arm analysis. Population data of larvae from day 33 were not used in the population statistics due to the larvae being concentrated from sampling.

Larvae were first fed on day 1 when they reached the 4 arm stage. After this cultures were fed 20ml of the green algae *Dunaliella tertiolecta* and *Dunaliella sp* every 2 days. The jars containing the larvae were cleaned every 3 to 4 days from day 5 onwards. This was done by filtering the larvae into new seawater and then refilling the jars with fresh seawater and returning the larvae to the jars.

Table 3.1 The chronological order of development of *Evechinus chloroticus* from the fertilised egg to the settlement stage when fed *Rhodomonas lens* (Barker 2007).

| Stage of development	Time
Fertilization	0
Late blastulae/early gastrula	24 hours
Prism. Skeltal rods present, gut complete.	48 hours
4 armed feeding pluteus	4-5 days
6 armed pluteus	9-10 days
8 armed pluteus, epaulettes forming	12 days
8 armed pluteus, epaulettes complete	14 days
Early echinopluteus, pedicellaria forming	19 days
Mature echinopluteus, tube feet present	21 days
Settlement when pipetted onto Coralina	24-28 days
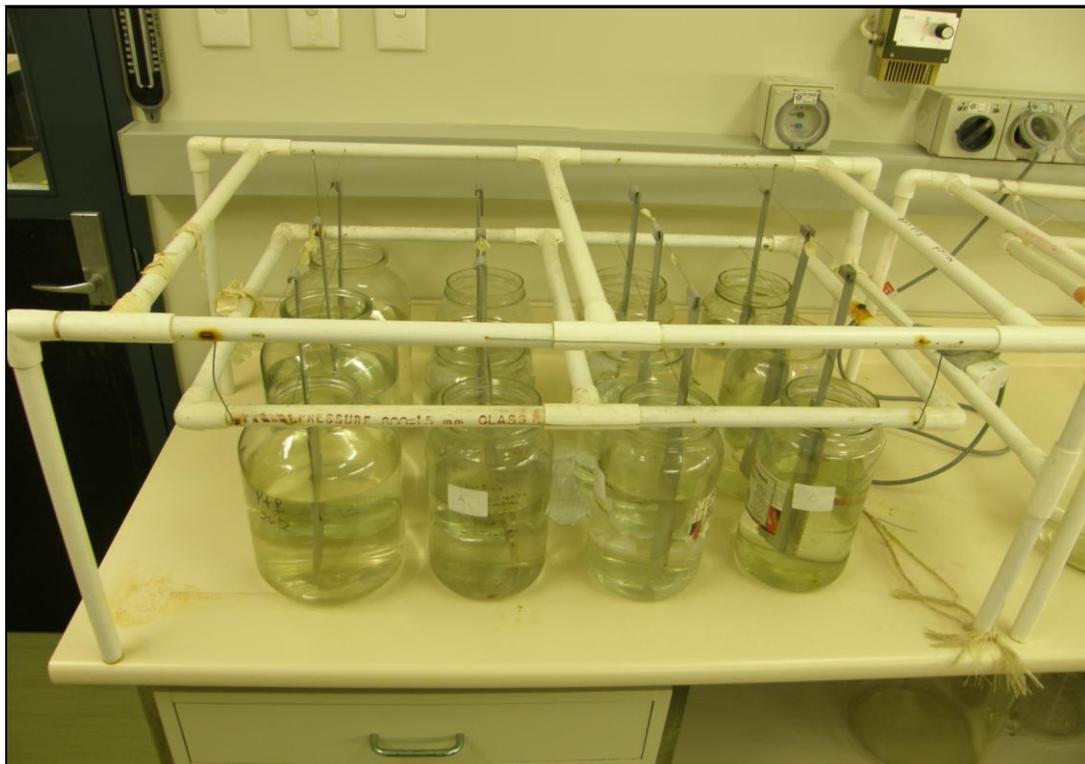
Figure 3.1 Larval cultures set up with the paddle stirring system

3.2.3 Photographing and measuring larvae

The number of larvae in each of the 9 larvae culture samples taken on each sampling date was counted on a Bogorov tray. The first 10 larvae found in each sample were also put onto a cavity slide individually and then photographed using the program Qcapture. The first samples from day 5 and day 9 were photographed under 12× magnification. The remaining samples from all other days were photographed under 15× magnification. At each magnification a photo was also taken of the 100μm scale bar, to provide a scale when measuring the larvae.

The photographed larvae were analysed using the programme imageJ. The length of the postoral arms and the anterolateral arms was measured, using the straight line measuring tool on imageJ. When the larvae had developed into 8arm larvae the posterodorsal arms were also measured, refer to Poorbagher *et al* 2010 for details of the larval arms measured.

3.2.4 Statistics

Percent fertilization of each female urchin where compared by one way ANOVA. Abundance of larvae per ml was analysed with a 2-way ANOVA comparing time and larvae per ml, with a Tukey's post hoc test. For arm length analysis only postoral arm length was used as it was the only arm length which had measurements for all cultures and dates and is the largest arm in pluteus larvae and provides a very reliable measure of larval size (Poorbagher *et al*. 2010; Strathmann & Strathmann 1994). The postoral arm length was analysed using a 2-way ANOVA with a Tukey's post hoc test. The mean postoral arm length from larvae from day 9 was also compared against the mean 4 arm larvae measurements of smaller adult urchins (80-105mm TD), reared in separate study (Cumming 2010). A repeated measures

ANOVA was tried but even though the larvae were from the same culture each time, the larvae were individually different each time so therefore a repeated measures ANOVA could not be used and a 2-way ANOVA was used.

3.3 Results

3.3.1 Fertilization success

The percentage of fertilization from each of the spawned individuals was very high with the lowest mean fertilization rate 99% from urchin A and urchin B. The individual variation is not large within the replicates from each of the urchin's, as can be seen by the small error bars (Fig 3.2). No significant difference was found between the fertilization rates from individual urchins (ANOVA: p-value >0.05).
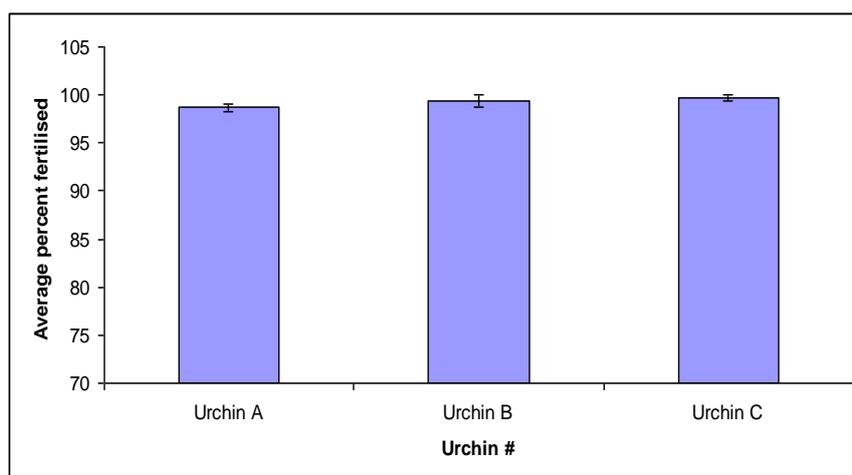
Figure 3.2 Mean fertilization success of the 3 spawned urchins A, B and C. Error bars ($\pm$ SE) represents the individual variation in fertilization percentage amongst replicates.

3.3.2 Parameters of the spawned urchins

The diameter of all the urchins spawned in the experiment was large, with the smallest diameter of 148mm test diameter (TD) from one of the male urchins (Table 3.2). The largest female was 156mm TD, also with highest gonad index 25.16, with the largest male having a TD of 156mm and a total weight of 1463.7g (Table 3.2).

The colour of the gonad from the 3 females was dark orange-yellowish colour, with females A and C having slightly yellower colouring than B which has a slight brownish tinge (Fig 3.3, a-c).

Table 3.2 The diameters and gonad index of females A-C, and the diameter and total weight of the 3 males used in the spawning experiment.

	Diameter (mm)	Gonad index	Weight of urchin (g)
Female A	156	23.38	1150
Female B	157	25.16	1239
Female C	154	24.23	1702
	Diameter (mm)	**Gonad index**	**Weight of urchin (g)**
Male 1	153		845
Male 2	148		1121
Male 3	156		1464

Figure 3.3a

Figure 3.3b

Figure 3.3c

Figure 3.3a-c Roe from the 3 female urchins in the spawning experiment.

3.3.3 Larval survival

The number of larvae per ml changed over the time period the larvae were cultured (Fig 3.4). There was a significant difference in the number of larvae per ml over time (2-way ANOVA: $P < 0.0001$). More larvae were found on day 5 than on days 16, 21 and 28 with this difference in number of larvae per ml over time being significant ($P < 0.0001$) (Fig 3.4). There was no difference between the two first sampling days, 5 and 9 in larvae numbers with similar survival rates. A higher number of larvae per ml was found on day 9 compared to day 28 (Fig 3.4) with this difference being significant ($P < 0.0002$). There was little variation in the survival of larvae per ml over time in all the other sampling days, with this variation being not significant ($P > 0.05$) (Fig 3.4).

Larval survival also varied between female urchins with females B and C having a higher per ml survival rate (Fig 3.4). A significant difference was found between females and larval survival ($P < 0.002$). Female A had a lower number of larvae per ml in most of the sampling periods when compared with females B and C, with this difference being significant ($P < 0.0056$, and 0.0049) (Fig 3.4). There was no significant difference in survival between female B and C.
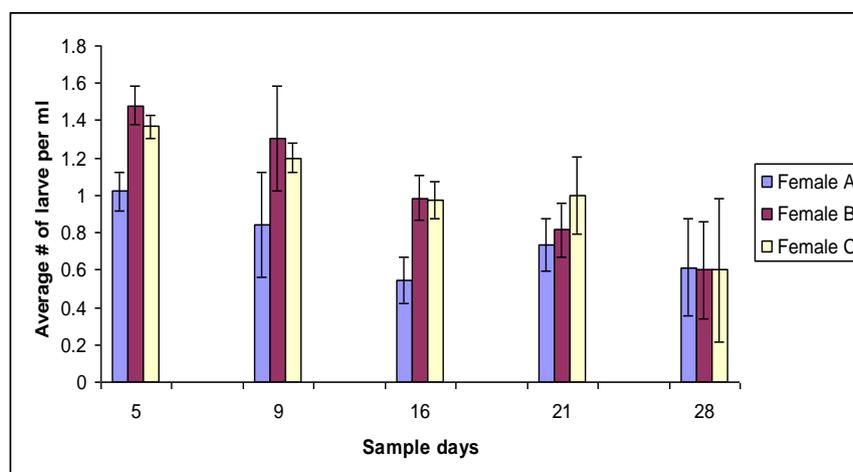

Figure 3.4 Average number of larvae per ml compared over time and between females. The error bars (± SE) represent the variation in arm length between the larvae within each culture.

3.3.4 Postoral arm growth

The development of larvae was the same for all the cultures, with each culture producing larvae that developed into an 8 arm pluteus stage (Table 3.3).

Postoral arm length varied over time, with smaller arms at the start of the experiment and a gradual increase until a drop in arm length occurred on day 28, with a slight increase of arm length after this on day 33 (Fig 3.5). The difference in postoral arm length over time was significant (2-way ANOVA: $P < 0.0001$). Arm length didn't vary between the two first samples on days 5 and 9. When days 5 and 9 were compared against the other sampling days, a significant difference occurred between postoral arm length over time as arm length started increasing ($P < 0.0001$) (Fig 3.5). On sampling day 28 there was a drop in postoral arm size that was significantly smaller than all other sampling days ($P < 0.0001$) (Fig 3.5) No other differences occurred between the other sampling dates and arm length.

Pluteus from female C had significantly longer postoral arm length ($P < 0.0071$) than larvae from female A which had smaller arms when all the days were pooled (Fig 3.5). Postoral length didn't vary between the larvae of other females ($P > 0.05$).

Table 3.3 Larval developmental stages over the course of sampling during the experiment

| Days	Larval Stages
5	4 arm pluteus
9	4 arm pluteus
16	6 arm pluteus
21	8 arm pluteus
28	8 arm pluteus
33	8 arm pluteus
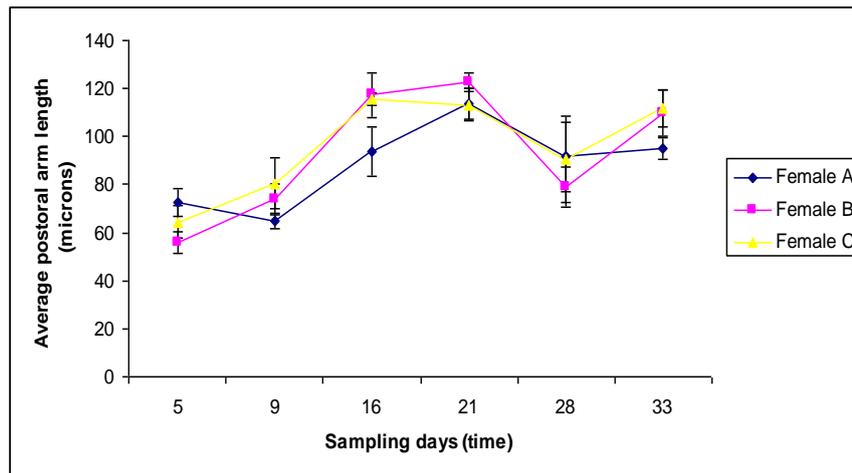
Figure 3.5 Average postoral arm length (µm), comparing the female urchins against each other over. Error bars represent the individual variation in postoral length within each culture.

The 4 arm larvae sampled on day 9 used in this study had smaller mean postoral arms than larvae of a similar age from urchins supplied by Cumming 2010, with a test diameter of 80-105mm TD. The mean arm length of the larvae from smaller urchins was 46.5µm compared to 36.3µm the mean arm length of the larvae from all 3 large urchins, used in the present study.

3.4 Discussion

3.4.1 Fertilization

The percentage of eggs fertilized was extremely high for all the females in the study, with mean fertilization of 99 percent through to 100% fertilization (Fig 3.2). The high fertilization rate was unexpected even under laboratory conditions in such large and old urchins. The high % fertilization does not support the suggestion by Dix 1970a, that large older urchin's may be reproductively senile and no longer contribute to the larval pool. Large urchins >150mm TD can produce viable gametes even when both the female and male urchin's involved are large and old (Table 3.2).

McShane *et al* 1994 also found that older larger urchins still produced viable gametes, but that the gonads of these large urchins >130mm TD was often small

compared to the total weight of the urchin and the gonads were brownish/blackish and generally poor in quality. In a similar comparison of fertilization there was no difference found across 4 size classes (<70mm, 90-110mm, 110-130mm and >130mm) in the fertilization which varied between 57 and 69% (McShane *et al* 1994), although these were much lower than the % fertilization found in the present study. These urchins were gathered from Dusky Sound where they would not normally have been fished due to the large size and poor gonad quality. In another study viable gametes were also produced from older large urchins when spawned, with the embryos being raised through to an 8 arm stage (Keys 2008). These urchins were fished from SUR5 on the southern Fiordland coast line, and were from an area commonly fished for commercial sale due to better gonad quality.

Food availability is most likely the reason old urchins can still produce viable gametes. With a constant or high food supply enough nutrients could be obtained to maintain the daily metabolic costs of the urchin as well as providing enough nutrients to use in gonad growth and therefore reproduction. Larger older urchins are thought to require far more nutrition to compensate for higher metabolic costs (McShane *et al* 1994; Woods *et al* 2008). Since viable gametes are still produced by older larger urchins it means that enough food is obtained in their environment to both maintain metabolic costs and reproduction. The urchins from Dusky Sound had small darker gonad which was of little commercial value (McShane *et al* 1994), whereas the urchins in the present study were from commercially harvested populations. The gonad was large and of an orange-yellowish colour, with the only slightly darker coloured gonad from female C (Figs 3.3a-c). The gonad index was high in these urchins as it was calculated from drained weight, with the highest (female B 25.16), which is high for *E chloroticus* of any size with maximum gonad indices reported for

E. chloroticus between 25-29 from the Marlborough Sounds and Doubtful Sound urchins (Barker 2007). The high gonad index could account for the high fertilization rates found within these large urchins, compared to the lower rates found in the urchins from Dusky Sound (4-16% whole wt). More nutrients would be available for gametogenesis so a higher reproductive output can be assumed, than from similar large urchins with poor gonad. Food is therefore likely the driver behind poor gamete quality and therefore fertilization/reproduction rather than age and size.

Sea urchins of many species such as *Lytechinus variegates*, *Strongylocentrotus franciscanus* and also *E. chloroticus*, upon reaching maturity have an increasing reproductive output as size increases until the largest size class (>120 TD for *E.chloroticus*), where there is a sudden decrease (Ebert & Russell 1992; Kato & Schroeter 1985; McShane *et al* 1994; Moore *et al*. 1963). In a study by Moore *et al*. 1963 on *Lytechinus variegates* it was found that the spawning output decreased from around 40% in urchins of a small diameter (40-50mm) down to 8% in urchins above 60mm TD. In *Strongylocentrotus intermedius* age was found to be a more important factor affecting reproduction rather than size (Kenner & Lares 1991).

Fecundity wasn't determined during the present experiment, but neither the female or male urchin released large amounts of eggs or sperm when injected with KCL (pers. obs.). Future tests into fecundity of large *Evechinus chloroticus* would be interesting to determine whether large urchins contribute proportionally larger numbers of larvae to the larval pool as small urchins. Even though large old urchins produce viable gametes, low fecundity could mean that as a breeding stock they might contribute few larvae.

Age was not determined in these urchins but large *E.chloroticus* urchins >130mm TD are thought to be of an age of 30 to 50 yrs (Dix 1970a; McShane *et al*

1994; McShane 1997). Other urchin species such as *Lytechinus variegates* and *Strongylocentrotus franciscanus* and many other species have shown the same relationship between increasing size and old age, with the largest urchins being the oldest (Moor *et al* 1963; Kato & Schroeter 1985). The urchins in the present study were assumed to be old and at the age where they would have a low reproductive value if any. In further studies age could be measured and then more age classes could be tested for viable gametes and reproductive potential.

Due to the complications of working with a commercial fisherman the experiment was only done once as more urchins couldn't be collected to replicate the whole procedure again. More large older urchins could be looked at from different fishing areas and other locations around the country to assess effects of location and age in further research.

3.4.2 Survival of larvae and postoral arm length

Survival of larvae varied over the experiment with more larvae present in the cultures at the start than at the end, which is expected as mortality of larvae occurs over time.

Larvae survival was expected to be similar between the individual female urchins as they all had high fertilization rates. Female A, however, had lower survival than females B and C (Fig 3.4). The difference between female A and females B and C could be due to the lower gonad index found in female A compared to the other 2 females (Table 3.3). A lower gonad index could mean that the larvae from female A might not have as many nutrient reserves per egg, causing larvae to start dying faster than the other female larvae. In a study of *Pseudechinus huttoni* by Poorbagher *et al* 2010 it was found that parental nutrition or larval food quality had no effect on the mortality of larvae in the experiment. A lower gonad index could also mean that the

best eggs had previously been spawned, which could make a difference in the larvae survival. Differing conditions in the culture jars is the most likely reason that survival of larvae differed between females.

Postoral arm length of urchins is expected to increase with time as the larvae develops and grow additional arms. This was shown with the significant difference between time and post oral arm size. Differences in time and post oral arm length occurred as expected in relation to development as there was no difference present between days 5 and 9, with both of these larvae at the 4arm developmental stage (Table 3.3). The arm length gradually increased over time, with natural development throughout the larval stage likely to account for these differences (Fig 3.5). The differences in postoral arm length between the 3 females is small (Fig 3.5), and any differences between the them is likely to be due to differing culture conditions rather than biological factors such as egg quality.

After comparing the mean postoral arm size of the large urchins >150mm TD (36.3μm) they were found to be shorter than the mean postoral arm size of the smaller urchins (80-105mm TD, 46.5μm). This could be due to a higher gonad index in the smaller urchins, allowing for higher quality eggs to be produced resulting in larvae with larger postoral arms. Culture conditions of the larvae could also account for the differences in postoral arm size between the small and large urchins. Further studies need to be done between postoral arm size between small and large urchins to draw more accurate conclusions, but on first comparison it appears larger urchins produce smaller larvae than younger small urchins.

The development of larvae through to the 8 arm stage shows that not only are older larger urchins >150mm TD producing viable gametes, but that the larvae are contributing to the larval pool and therefore recruitment. It can be assumed that if the

larvae reach the 8arm stage they will metamorphosis into juvenile urchins and grow to adults (Barker pers comm.). Large urchins could therefore possible provide recruits into stock within fishing areas, however, if the fecundity of large urchins is low then these urchins should be fished preferentially as they wouldn't provide as many larvae to the larval pool. Small sized urchins could then be left as breeding stock and not fished if they had a higher fecundity. The importance of large urchins in contributing to the larval pool and therefore recruitment into fished areas can be assessed further after differences in fecundity have been more accurately assessed.

4 General discussion

This study looked into the effectiveness of translocating *E. chloroticus* from an area of poor gonad quality Passage Island to an area, Sealers Bay, where the gonad quality of urchins previously fished was high (Chapter 2). The change in gonad index was the major factor assessed to determine any differences associated with translocations. At Passage Island urchins with low quality gonad were found in high densities (14.7 and 10.8 per m^2), while at Sealers Bay urchins containing high quality gonad were present in low densities (2.7 and 2.1 per m^2) due to high fishing pressure. The study was conducted over 5 months which is considered to be a short term enhancement period. Movement of urchins was found to be a successful method of enhancing the gonad to a level which is acceptable for commercial fishing. Once urchins were moved from Passage Island to Sealers Bay an increase in the gonad was apparent (Fig 2.3).

The density of the urchins and algal species present at each location are the main factors which affect the quality of gonad. Population density of *E. chloroticus* is linked to the amount/biomass of algae in each location. Quality of the algal species present is also affected, with a decrease in both the biomass and quality of algae when high densities of urchins are present. Algae quantity and quality affect the gonad index. Sealers Bay had a higher density and higher diversity of algae than Passage Island, which meant that Sealers Bay urchins had a gonad index higher than those at Passage Island. The gonad index after translocation was also higher than urchins from Passage Island, due to increased food availability, but remained smaller than in urchins located in Sealers Bay. In the current study the number of urchins removed from Passage Island was not enough to create a difference in the remaining density of

urchins. Moving more urchins in a future study would allow for density effects to become apparent at this site and greater insight into the effects on gonad quality of reducing the density of urchins in sites where they are performing poorly.

In a similar study James & Herbert 2009 also found that the gonad of urchins could be improved by translocating from areas in the Coromandel containing high population densities to areas with lower population densities, with increased yields of up to 50%. It was found that density and related changes in food availability were the major driver behind the increase in gonad index in the Coromandel urchins, with urchins from the poor site (unmoved urchins) having a larger increase in gonad index than translocated urchins. This increase in gonad index of urchins at the poor site was linked to reduced densities of urchins after the removal of urchins for translocation (James & Herbert 2009). The study was conducted over a 7 month period so was of a longer duration than the current study. However, the results of the current study showed that even a relatively shorter period of 5 months allows for increased gonad growth when urchins were moved to areas where food availability was high and presumably of a higher quality due to low densities of urchins. During the current study the food quality of algae was not looked at, but could be an area of future research in relation to translocating urchins, to see whether certain algae species have a significant impact on gonad growth.

An unexpected result in the current study although still possible based on results by Lawrence *et al.* 1965 and Russell 1998, was the increase in the Aristotle's lantern index which was caused by translocating urchins. This was found in two different translocated samples. The plasticity of growth in sea urchins, including *E. chloroticus* is high with resource allocation to different body components based on the food available to the urchin. However, changes in the growth of lantern from

improved food supply usually take a longer time span than 5 months, with studies on *S.purpuratus*, S.*franciscanus*, and *S.droebachiensis* by Lawrence *et al*. 1965 and Russell 1998 showing lantern index changes after periods of 16 (which is similar to the present study), 32 and 64 weeks. The considerable change in food availability (algae biomass) after the urchins were translocated to Sealers Bay from Passage Island (Fig 2.8) could account for changes in the lantern index, however, not all translocated samples showed a difference in lantern index. Further studies of a longer duration and replicates are required to validate whether changes in lantern index commonly occur after translocation for short time periods in *E. chloroticus*.

Colour is a quality factor which affects whether gonad is accepted on the international market and is often not met by any gonad from *E. chloroticus* caught around the New Zealand coast line. Translocating the urchins did not increase the colour to an acceptable international level, but the colour was acceptable for the domestic market from the Sealers Bay urchins as the colour is not strictly regulated and didn't change over the study.

The difficulties of working with a commercial fisherman led to one delivery of dead urchins, and therefore alterations of the gonad colour before spectrophotometer readings were undertaken. More research on the colour changes needs to be undertaken with urchins that are alive before dissection to gauge a more accurate understanding of colour changes in relation to translocating urchins.

It is clear that translocating *E. chloroticus* affects gonad growth in a study of short term duration, with gonad growth increasing as a result of higher food availability and due to lower urchin densities. Translocating urchins can therefore be used as a viable tool in the fishing industry to achieve a short term increase in gonad size to a level which is acceptable to be fished, especially when there are high

densities of locally unfished populations because of poor gonad quality. Intensive feeding of artificial or natural diets to *E.chloroticus* in land or sea based cages (aquaculture) is a costly method to improve gonad index. Translocating urchins from poor gonad areas to high quality gonad areas is a more cost effective method of gonad enhancement over short durations. Future research looking into a combination of translocating urchins in the wild for short term periods to improve gonad size followed by holding urchins for a period on enhanced diets to improve gonad colour could be investigated to assess the probability of achieving internationally acceptable gonad. A combination of both aquaculture and translocating wild urchins would allow for a more cost effective option of obtaining gonad to meet export standards, than aquaculture on its own.

Larger scale translocations need to be studied to assess further effects on the ecology of an area so long term impacts of this type of urchin population manipulation as a fisheries tool can be obtained. If this method was used frequently throughout the country to enhance gonads, then the areas which had high densities and poor gonad could become overfished because of large scale removal of urchins. Greater areas of the coast would then become overfished if this method was used without control it would mean areas high in urchin numbers, which most likely provide larvae into the larval pool would be overfished themselves reducing the residual breeding stock. Caution and further study is needed to look at possibilities of using this method in conjunction with reseeding, to ensure sustainable urchin stocks in New Zealand waters.

Viability of gametes and the larvae of large old *E. chloroticus* urchins >150mm TD were investigated to assess whether these urchins contribute to the larval pool and what this means for the fishing industry (Chapter 3). Fertilization success

rates were very high with 99 % the lowest mean rate (Fig 3.2) recorded in the experiment, showing that older large urchins are not reproductively senile and produce viable gametes. The embryos were then cultured through development until the 8 arm larvae stage, with the larvae growing normally without any abnormalities (pers observ). It could be assumed that once the larvae reached the 8 arm stage, they would continue to develop until settling as juveniles if left in culture. Therefore old large (>150mm TD) *E. chloroticus* produce viable gametes and contribute to the larval pool. Studies by McShane *et al*. 1994 and Keys 2008 both found that old larger urchins (>120mm TD) produced viable gametes and in the study by Keys 2008, fertilized embryos developed into 8 arm larvae. Fecundity was not investigated during the present study, but the volume of eggs and sperm released from KCL induction was low (pers. obs.). Further research needs to be undertaken on the fecundity of these large old urchins, and then comparisons of fecundity should be compared across different size classed urchins, to find the size class which has the highest fecundity levels.

Large old urchins could provide a breeding stock for the fishing industry, however, the urchins used in the present study were from a commercial catch and had large gonads, with larger urchins often targeted by fishermen (pers. obs.). In a study by McShane *et al* 1994 the large urchins (>120mm TD) produced viable gametes but the gonad was of poor quality and below the standard required to be commercially fished. In areas where large urchins have poor quality gonad they could remain as breeding stock when smaller urchins are taken, but in areas where their gonad is high quality they will be fished. It would therefore be valuable to know which size class produces the greatest numbers of larvae so then restrictions can be put in place on

urchin sizes that contribute the greatest amount to the larval pool to maintain healthy breeding stocks preventing overfishing.

Test diameter size should also be investigated when undertaking future translocations, as smaller urchins could have faster gonad growth than large ones, and by leaving the larger urchins a breeding stock will remain in the area. However, if large urchins have fast gonad growth, then smaller urchins could be left for breeding stock and larger urchins moved. Further studies should concentrate on different size classes, fecundity, and rate of gonad growth to understand translocation effects on the biology of *E. chloroticus* so informed decisions for the fishing industry to maintain sustainability can be made.

Working with a commercial fisherman led to limitations in the present study. The time frame of the study could have been longer to allow for more data to be collected over time, however, this wasn't possible due to the seasonal nature of commercial sea urchin fishing. A big limitation of the study was the crew throwing back the control sample of urchins from Passage Island on the first trip. This could have led to biased results in the statistical comparisons as the gonads would have grown more before the first Passage Island sample could be taken. The reliability of the fisherman's schedule meant that I was unable to get replicate samples of urchins for spawning which limited the amount of data and therefore reliability of the results.

4.1   Overall conclusion

Translocating urchins has a positive effect on gonad growth, with translocated urchins having a higher gonad index. The method of translocating urchins can be used as a biologically viable and a possibly cost effective tool in the fishing industry for gonad enhancement. Often older larger urchins >120mm TD are targeted when

fishing due to larger gonads, and originally they were thought to possibly be reproductively senile. Older larger urchins >150mm TD produce viable gametes, that once fertilized produce embryos capable of developing through to an 8 arm larval stage and therefore presumably into juvenile urchins. These large old urchins could provide a residual breeding stock in certain areas around the country.

References

Agatsuma, Y. Sato, M. Taniguchi, K. (2005) Factors causing brown-colored gonads of the sea urchin *Strongylocentrotus nudus* in northern Honshu, Japan. *Aquaculture* 249: 449-458

Andrew, N.L. (1988) Ecological aspects of the common sea urchin, *Evechinus chloroticus*, in northern New Zealand: a review *New Zealand Journal of Marine and Freshwater Research* 22: 415-426

Andrew, N.L. (1986) The interaction between diet and density in influencing reproductive output in the echinoid *Evechinus chloroticus* (Val.). *Journal of Experimental Marine Biology and Ecology* 97: 63-79

Andrew, N.L. and MacDiarmid, A.B. (1999) Sea urchin fisheries and potential interactions with a kina fishery in Fiordland. Conservation Science Notes No. 266, department of conservation Wellington

Andrew, N.L. Agatsuma, Y. Ballesteros, E. Bazhin, A.G. Creaser, E.P. Barnes, D.K.A. Botsford, L.W. Bradbury, A. Campbell, A. Dixon, J. D. Einarsson, S. Gerring, P.K. Hebert, K. Hunter, M. Hur, S.B. Johnson, C.R. Juinio-Menez, M.A. Kalvass, P. Miller, R.J. Moreno, C.A. Palleiro, J.S. Rivas, D. Robinson, S.M.L. Schroeter, S.C. Steneck, R.S. Vadas. R.L. Woodby. D.A. Xiaoqi. Z. (2002). Status and management of world Sea Urchin Fisheries. Oceanography and Marine Biology: an Annual Review, 40, 343-425

Andrew, N. (2003) Kina. In: Andrew, N. Francis, M ed. The Living Reef, The Ecology of New Zealand's Rocky Reefs. New Zealand, Craig cotton pub Pp 108-114

Barker, M.F. Keogh, J.A. Lawrence, J.M. Lawrence, A.L. (1998) Feeding rate, absorption efficiencies, growth and enhancement of gonad production in the New Zealand sea urchin *Evechinus chloroticus*, Valenciennes (Echinoidea: Echinometridae) fed prepared and natural diets. *Journal of Shellfish Research* 17: 1583-1590

Barker, M.F. (2007) Recent advances in sea-urchin aquaculture in New Zealand and Australia. *Bulletin of the Aquaculture Association of Canada*. 108 pp 10-17

Barker, M.F. (2007) Ecology of *Evechinus chloroticus*. In: Lawrence, J.M. ed. Edible sea urchins: Biology and Ecology. Amsterdam, Elsevier. Chap 16 319-337

Brewin, P.E. Lamare, M.D. Keogh, J.A. Mladenov, P.V. (2000) Reproductive variability over a four-year period in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinodermata) from differing habitats in New Zealand. *Marine Biology* 137: 543-557

Byrne, M. (1990) Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Marine Biology* 104: 275-289

Cumming, S. (2010) Larval rearing and reseeding of New Zealand sea urchin *Evechinus chloroticus*, Unpublished Thesis, University of Otago, New Zealand

Dix, T.G. (1970a) Biology of *Evechinus chloroticus* (Echinoidea: Echinometridae) from different localities. 1. General. *New Zealand Journal of Marine and Freshwater Research* 4:91-116

Dix, T.G. (1970b) Biology of *Evechinus chloroticus* (Echinoidea: Echinometridae) from different localities. 3 Reproduction. *New Zealand Journal of Marine and Freshwater Research* 4: 385-405.

Ebert, T.A. and Russell, M.P. (1990) Growth and mortality estimates for red sea urchin *Strongylocentrotus franciscanus* from San Nicolas Island, California. *Journal of Experimental Marine Biology and Ecology* 141: 107-118

Explorations Unlimited Inc (2006). Pacific urchin harvesters association West Coast Green urchin association – Sea urchin fishery profiles

Fell, J. (2002) Gonad enhancement of *Evechinus chloroticus* (Val.) using artificial and macro-algae diets in Otago Harbour Dunedin, New Zealand. Unpublished thesis, Otago University, New Zealand pp. 1-95

Franke, E.S. (2005) Aspects of Fertilization Ecology in *Evechinus chloroticus* and Coscinasterias muricata. Unpublished PHD thesis, University of Auckland, New Zealand pp. 1-50

George, C. Cellario, C. Fenaux, L. (1990) Population differences in egg quality of *Arbacia Zixula* (Echinodermata: Echinoidea): proximate composition of eggs and larval development. *Journal of Experimental Marine Biology and Ecology* 141: 107-118

Gonor, J.J. (1972) Gonad growth in the sea urchin *Strongylocentrotus purparatus* (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. *Journal of Experimental Marine Biology and Ecology* 10: 89-103

James, P.J. (2006) A comparison of roe enhancement of the sea urchin *Evechinus chloroticus* in sea-based and land based cages. *Aquaculture* 253: 290-300

James, P. (2007) The effects of environmental factors and the husbandry techniques on roe enhancement of the New Zealand sea urchin *Evechinus chloroticus*. Unpublished thesis, University of Victoria of Wellington, New Zealand.

James, P.J. Heath, and M.J. Unwin, (2007). The effects of season, temperature and initial gonad condition on roe enhancement of the sea urchin *Evechinus chloroticus*. *Aquaculture* 270: 115-131

James, P.J. and Heath, P.L. (2008) Long term roe enhancement of *Evechinus chloroticus*. *Aquaculture* 278: 89-96

James, P. and Herbert, P. (2009) Kina roe enhancement by translocation. Seafood Innovations Ltd, NIWA Client Report

Kato, S. and Schroeter, S. (1985) Biology of the Red Sea Urchin, *Strongylocentrotus franciscanus*, and Its Fishery in California. Marine Fisheries Review 47(3)

Kenner, M.C. and Lares, M.T. (1991) Size at first reproduction of the Sea urchin *Strongylocentrotus purpuratus* in a central California kelp forest. *Journal of Marine Ecology Progress Series* 76: 303-306

Keys, R. (2008) Reproductive cycle and Settlement of *Evechinus chloroticus* in the SUR 5 Fishery. Thesis, University of Otago, New Zealand

Kina (SUR) (2009) (*Evechinus chloroticus*) Kina 1. Fishery Summary retrieved December 5th 2009 from, fs.fish.govt.nz/Doc/21739/42_SUR_09.pdf.ashx

Kina (2010) retrieved January the 15th , from http://seafoodindustry.co.nz/ Default.aspx?id=1244&area=246

Lamare, M.D. and Barker, M.F. (1999) In situ estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). *Journal of Marine Ecology Progress Series* 180: 197-211.

Lamare, M.D. Brewin, P.E. Barker, M.F. Wing, S.R. (2002) Reproduction of the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. *New Zealand Journal of Marine and Freshwater Research* 36: 719-732

Lamare, M.D. and Mladenov, P.V. (2000). Modelling somatic growth in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinometridae). *Journal of Experimental Marine Biology and Ecology* 243(1): 17-43.

Lawrence, J.M. Lawrence, A.L. Holland, N.D. (1965) Annual Cycle in size of Gut of purple Sea Urchin *Strongylocentrotus purpuratus* (Stimpson). *Nature* 205: 1238-&

Lawrence. J.M. Olave, S. Otaiza, R. Lawrence, A.L. Bustos, E. (1997) Enhancement of Gonad Production in the Sea Urchin *Loxechinus albus* in Chile Fed Extruded Feeds. *Journal of the World Aquaculture Society* 28: 91-96

Matsuno, T. and Tsushuma (2001) Carotenoids in sea urchins. In, Edible Sea Urchins: Biology and Ecology, Elsevier 32: 115-138

McBride, S. (1997) Aquaculture boosts urchin roe production. *California Agriculture* 51 (4): 30-31

McBride, S.C. Price, R.J. Tom, P.D. Lawrence, J.M. Lawrence, A.L. (2004) Comparison of gonad quality factors: colour, hardness and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. *Aquaculture* 233: 405-422

McShane, P.E. and Anderson, O.F. (1997) Resource allocation and growth rates in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinometridae). *Marine Biology* 128: 657-663

McShane, P.E. Naylor, J.R. Anderson, O. Gerring, P. Stewart, R. (1993) Pre-fishing surveys of kina (*Evechinus chloroticus*) in Dusky Sound, southwest New Zealand. New Zealand Fisheries Assessment Research Document 93/11

McShane, P. E. Anderson, O. Gerring, P. Stewart, R. Naylor, J.R. (1994) Fisheries biology of kina (*Evechinus chloroticus*). New Zealand Fisheries Assessment Research Document 94/7.

McShane, P.E. (1997) A summary of commercial catch data and biological information for kina (*Evechinus chloroticus*). New Zealand Fisheries Assessment Research Document 97/6.

McShane, P.E. Gerring, P.K. Anderson, O.A. Stewart, R.A. (1996) Population differences in the biology of *Evechinus chloroticus* (Echinoidea: Echinometridae). *New Zealand Journal of Marine and Freshwater Research* 30: 333-339

Meidel, S.K. and Scheibling, R.E. (1999) Effects of food type and ration on reproductive maturation and growth of the sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* 134: 155-166

Minor, M.A. and Scheibling, R.E. (1997) Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* 129: 159-167

Mladenov, P.V. Allibone, R.M. Wallis, G.P. (1997) Genetic differentiation in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) *New Zealand Journal of Marine and Freshwater Research* 31 261-269

Moore, H.B. Jutare, T. Bauer, J.C. Jones, J.A. (1963) The Biology of Lytechinus Variegatus. *Bulletin of Marine Science* 13: 23-53

Naylor, J.R. and Andrew, N.L. (2002) Relative abundance and size frequency of kina (*Evechinus chloroticus*) in the Chatham Islands in the 2001-2002 fishing year. MFISH, New Zealand fishing assessment report, 2002/35

Phillips, K. Miimi, J. Hamid, N. Silcock, P. Delahunty, C. Barker, M. Sewell, M. Bremer, P. (2009). Sensory and volatile analysis of sea urchin roe from different geographical regions in New Zealand. LWT – *Food science and Technology* 243: 202-213

Phillips, K. Hamid, N. Silicock, P. Delahunty, C. Barker, M. & Bremer, P. (2009). Effect of season on the sensory quality of the seas urchin (*Evechinus chloroticus*) roe. *Journal of Food Science* 75(1): S20-30

Poorbagher, H. Lamare, M.D. Barker, M.F. Rayment, W. (2010) Relative importance of parental diet versus larval nutrition on development and phenotypic plasticity of *Pseudechinus huttoni* larvae (Echinodermata: Echinoidea). *Marine Biology* Research 6: 302-314

Robinson, S.M.C. Castell, J.D. Kennedy, E.J. (2002) Developing suitable colour in the gonads of cultured green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture* 206: 289-303

Russell, M.P. (1998) Resource allocation plasticity in sea urchins: rapid, diet induced, phenotypic changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller). *Journal of Experimental Marine Biology and Ecology* 220: 1-14

San Martin, G.A. (2002) Transplantation of Large *Parcentrotus lividus*: A restocking strategy. Edible Sea Urchins, Lancaster PA Destech Publications 74-83

Scheibling, R. (1986) Increased macroalgal abundance following mass mortalities of sea urchins (*Strongylocentrotus droebachiensis*) along the Atlantic coast of Nova Scotia. Oecologia (Berlin) 68:186-198

Schiel, D.R. (1982) Selective Feeding by the Echinoid, *Evechinus chloroticus*, and the Removal of Plants from Subtidal Algal Stands in Northern New Zealand. *Oecologia* 54: 379-388

Sea Urchin Fishery Profiles (2003) Pacific urchin harvesters associations and West coast Green urchin associations. A Background document produced by Explorations unlimited Inc

Strathmann, M.F. and Strathmann, R.A. (1994) Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei, *Journal of Limnology and Oceanography* 39 (1) 84-98

Thomas, S. (2009) Improving the Roe Quality of Wild Kina (*Evechinus chloroticus*) by Transplantation of Urchins. Unpublished thesis proposal. University of Otago, New Zealand

Tsuda, F. Hoshikawa, H. Agatsuma, Y. Taniguchi, K. (2006) Gonad production of the Sea urchin *Strongylocentrotus nudus* in relation to algal vegetation in shallow waters of Japan, Southwestern Hokkaido, Japan. Sci. Rep. Hokkaido. Fish. Exp. Stn. 71: 63-68

Vadas, R.L. Beal, B. Dowling, T. Fegley, J.C. (2000)Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. *Aquaculture* 182: 115-135

Villouta, E. Chadderton, W.L. Pugsley, C.W. Hay, C.H. (2001) Effects of sea urchin (*Evechinus chloroticus*) grazing in Dusky Sound, Fiordland, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 35: 1007-1024

Walker, M.T. (1981) Influence of season on growth of the sea urchin *Evechinus chloroticus*. *New Zealand Journal of Marine and Freshwater Ecology* 15: 201-205

Williams, H. (2002) Sea urchin fisheries of the world: A review of their status, management strategies and biology of the principal species. Draft background paper, Marine resources. Department of Primary Industries, Water and Environment Tasmania.

Wing, S.R. Vasques, J. Lamare M.D. (2001) Population structure of sea urchins (*Evechinus chloroticus*) along gradients in benthic productivity in the New Zealand fjords. Echinoderms, Barker (ed)

Wing, S.R. Gibbs, M.T. Lamare M.D. (2003) Reproductive sources and sinks within a sea urchin, *Evechinus chloroticus*, population of a New Zealand fjord. *Marine Ecology Progress Series* 248: 109-123

Woods, C. Moss, G. Wright, J. (2005) Kina roe enhancement and the development of enhancement diets. *New Zealand Aquaculture* 8: 4-5

Woods, C. James, P. Moss G.A. Wright, J. Siikavuopio, S. (2008) A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus*. *Journal of Aquaculture* 16:49–68