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Effects of Low Salinity on *Evechinus chloroticus* Valenciennes.

Carolyn R. Antonie

A thesis submitted for the degree of
Master of Science
At the University of Otago,
Dunedin, New Zealand.

December 2003
The salinity tolerance of a species has been shown to differ among ages and in different stages of the life cycle. The sea urchin *Evechinus chloroticus* (Val.) is a common grazer of the intertidal and subtidal areas around New Zealand, including Doubtful Sound. A persistent surface layer of low salinity in Doubtful Sound overlies deep basins in which adult *E. chloroticus* live. The planktotrophic larvae of *E. chloroticus* are the main dispersive stage of this species.

Three questions were posed in this study: do *E. chloroticus* larvae vertically distribute themselves differently in Doubtful Sound due to the Low Salinity Layer (LSL); what affect does low salinity have on *E. chloroticus* larval development; and do embryos and adult *E. chloroticus* from geographically separated populations within Doubtful Sound have differing tolerances to low salinity?

The first question was investigated through examination of the vertical distribution of *Evechinus chloroticus* larvae in Doubtful Sound. The second question was explored through a series of experiments analyzing the development of *E. chloroticus* embryos and larvae reared in salinities ranging from 5-35‰. Resolving the third question required the observation of the embryonic development of *E. chloroticus* in salinities from 5-35‰, as well as monitoring the reactions of *E. chloroticus* adults placed in diluted seawater (25-34.6‰). The purpose of this study was to investigate whether *E. chloroticus* was tolerant to reduced salinities as the environment in which certain populations live (Fiordland, Doubtful Sound) contains low salinity conditions which may be detrimental to survival and development.

Larvae of *Evechinus chloroticus* were never found within the LSL. Larvae were found in abundance from 6-8m depth, a layer close to the halocline. Experimentally, development of *E. chloroticus* was incomplete in salinities lower than 27.5‰. Embryos lysed in salinities similar to the LSL indicating extreme sensitivity at this critical life-stage. Larval growth experiments revealed that even higher salinities (27.5‰ and 30‰) were detrimental. Adult *E. chloroticus* were less tolerant of low salinities than their larvae. Prolonged exposure to 25‰ and 27.5‰ caused mortality of all adults. The location from which a subject was either taken (adult experiments) or derived (egg experiments) was not a significant factor in its salinity tolerance. The later stages of the *E. chloroticus* larvae are proposed to be the most
tolerant to reduced salinities, lending themselves well to dispersal by means of the LSL.
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Chapter 1

General Introduction

Salinity and Echinoderms

A number of abiotic and biotic factors have been implicated in contributing to the distribution of species. In the marine realm, salinity, wave action, food availability, and predation are considered significant in influencing the distribution of species and populations (Kinne 1964a, Tegner and Dayton 1981, Himmelman et al. 1983, Balch and Scheibling 2000, Charmantier et al. 2001, Wing et al. 2001). Salinity is fairly consistent throughout the world's ocean, with typical values ranging from 32%o to 35%o depending on temperature and location. The salinity regimes in coastal areas, estuaries, and fiords, are often quite dynamic. Salinity is an important influence on benthic marine invertebrates, as often the adult is a relatively sedentary animal. In echinoderms, salinity is even more important as they lack a differentiated osmoregulatory organ (Yaroslavtseva and Shirmunskii 1978). Low salinity has been shown to structure adult populations of echinoderms (Gezelius 1964, Stancyk and Shaffer 1977, Himmelman et al. 1983, 1984, Sarantchova 2001) and other invertebrates (Lagerspetz and Mattila 1961, Dybern 1967, Drouin et al. 1985, Charmantier et al. 2001, Macrellis 2001).

The salinity tolerance of a single species has been shown to differ between ages and in different stages of the life cycle (Kinne 1964b, Vernberg and Vernberg 1975, Drouin et al. 1985). In many species a narrow salinity range is tolerable for gametes and embryos, tolerance to fluctuating salinity then increases during late larval stages or juvenile development, and finally the adult, again, has a narrow tolerance (Kinne 1964b, Dybern 1967, Vernberg and Vernberg 1970, Binyon 1972). Echinoderms have been identified as having particularly constricted salinity tolerances during development (Fenchel 1965, Kasenko 1992, 1998, Roller and Stickle 1993).

Early stages of Echinoderms have been observed to be sensitive to salinity. Gametes and embryos of various echinoderms have been shown to be vulnerable to lowered salinities (Thorson 1950, Gezelius 1964, Dybern 1967, Hendler 1977, Greenwood and Bennett 1981, Kasenko 1992, 1998, Roller and Stickle 1993).

The stages of development most vulnerable to low salinity vary among species, but generally the critical stage is in the early life history. Gonads of *Psammechinus miliaris* lysed when adults were transferred from oceanic salinities to 15‰ (Gezelius 1964). Kashenko (1992) identified the stages from fertilization to the hatching of the blastula as the development stages of *Stichopus japonicus* most sensitive to reduced salinity. Watts et al. (1982) found that many larvae of *Echinaster modestus* failed to fully develop mouth-frame spines or the terminal spines of the rays. The authors proposed that salinity may affect the deposition of carbonate material required for spine formation (Watts et al. 1982). Low salinities affect many stages of early development in echinoderms.

In the field, early planktonic stages of echinoderms such as zygotes and embryos cannot alter their vertical distribution, or avoid low salinities. Larvae of echinoderms, on the other hand, possess swimming ability and have been observed to avoid and swim actively out of low salinity surface layers (Metaxas 1998, Metaxas and Young 1998). Mileikovsky (1973) investigated the swimming ability of various larvae of marine bottom invertebrates, including echinoderms. The author suggested that in nearshore marine and estuarine waters, most larvae of echinoderms (excluding an ophioplutei) possessed strong enough swimming ability to alter their vertical distribution even in the presence of tidal currents, although local water stratification may inhibit movement (Mileikovsky 1973). The swimming ability of later developmental stages, therefore, may affect the vertical distribution of larvae, possibly causing variation in larval dispersal and recruitment dynamics.

When adult Echinoderms living normally in full-strength seawater are experimentally placed in diluted seawater, a number of effects are noticeable. There is usually swelling (Kinne 1964a), weight gain (Himmelman et al. 1984), loss of function (e.g. righting (Lawrence 1975, Shirley and Stickle 1982, Stickle and Diehl 1987), reduction in the extension of tube feet (personal observation), and a decrease in efficiency or reduction in metabolism (Kinne 1964a, Sabourin and Stickle 1981,
Shirley and Stickle 1982). These affects eventually result in death. Some animals are able to survive short periods in diluted seawater, and when returned to full-strength seawater the animal regains normal function and turgor (Binyon 1972).

**Doubtful Sound**

The fiords of the west coast of the South Island contribute heavily to the New Zealand coastline. In the deep basins of the fiords, *Evechinus chloroticus* (Valenciennes), a regular echinoid lives in the oceanic layer. In Doubtful Sound, a Low Salinity Layer (LSL) (typically 1-5‰) several meters thick overlies the denser oceanic water of the basin (Stanton 1984). This LSL in Doubtful Sound is a result of heavy rainfall in the region (>7m per year) and the additional influence of the Manapouri Power Station outflow at Deep Cove, which effectively doubles the amount of freshwater entering the fiord (Gibbs 2001). As a result of the freshwater input, Doubtful Sound has classic estuarine circulation; the LSL progresses seaward from the head of the fiord, entraining seawater and suspended particles from below (Stanton and Pickard 1981).

*Evechinus chloroticus*

*Evechinus chloroticus* is common in the intertidal and subtidal areas of the New Zealand coast and outlying islands (McRae 1959), with greatest abundances above 10m depth (Wing *et al.* 2001). Juveniles of this species generally inhabit shallower waters than adults and keep cover under rocks and in crevices (Dix 1970a, Barker 2001). As a dioeciously spawning echinoderm, *E. chloroticus* releases gametes into the water, where they are fertilized. Development from the embryo, through planktotrophic larvae, into a competent larva and settled juvenile requires from 18 to 63 days (Dix 1969, Walker 1984, Lamare and Barker 1999). The LSL in Doubtful Sound has been suggested as the dominant influence structuring both echinoderm and bivalve populations (Macrellis 2001, Perrin 2002, Wing *et al.* 2003).

In Doubtful Sound, *Evechinus chloroticus* is a prolific member of the benthic community and has been observed in greatest abundance from 3-10m depth (Lamare 1997, Wing *et al.* 2003) just below the LSL. In a model of *E. chloroticus* dispersal in Doubtful Sound by Wing *et al.* (2003), a low salinity limit of 28‰ was employed as the lowest salinity of larval tolerance, indicating the belief that low salinities are
intolerable for this species. *E. chloroticus* have been observed spawning directly into the boundary layer or LSL itself (Miles Lamare, Steve Wing, personal communications), directly affecting the gametes and developing stages. In fiords, larval stages could be entrained into the LSL and swept seaward. If larvae cannot tolerate low salinity, mortality will be high in the LSL and larvae will fail to survive and recruit. Wing *et al.* (2003) suggest that the estuarine circulation in Doubtful Sound is the main distributive force for *E. chloroticus* larvae, leading to variation in recruitment.

The estuarine circulation in the fiords of New Zealand has also been implicated as the main force influencing genetic differences in *E. chloroticus* populations throughout Fiordland (Perrin 2002). Perrin (2002) discovered two genetically distinct groups of *E. chloroticus* in Fiordland, one group consisting of samples collected from the inner fiords, while the other group contained samples collected from the outer fiords and the open coast. The author suggested that a restriction of larval dispersal due to fiord hydrography has caused the differentiation between the populations (Perrin 2002).

**Recruitment Dynamics**

Recruitment has been advocated as one of the most important events structuring invertebrate populations (Underwood and Fairweather 1989). Variation in recruitment can be divided into three broad categories:

1. Pre-settlement processes,
2. Settlement processes, and

Pre-settlement processes include, but are not limited to, reproductive variability of adults (Ebert 1983, Brewin *et al.* 2000), mortality of larvae in the plankton (Thorson 1950, Tegner and Dayton 1981, Roughgarden *et al.* 1988), larval behavior (Vasquez and Young 1996, Metaxas 2001), and dispersal of larvae due to hydrographic processes (Cameron and Rumrill 1982, Scheltema 1986, Epifanio *et al.* 1988, Roughgarden *et al.* 1988, McEdward and Miner 2001). Variation in environmental conditions such as salinity (Scarratt and Raine 1967, Metaxas and Young 1998), temperature (Young and Chia 1987), light (Thorson 1964, Pennington and Emlet 1986), and food (Metaxas and Young 1998, Meidel *et al.* 1999) are regularly
influential on pre-settlement processes. Settlement processes include differential settlement of metamorphosed individuals due to habitat (Tegner and Dayton 1981, Balch and Scheibling 2000) and selection of substrates by metamorphosing individuals (Chia 1989, Pechenik 1990, McEdward and Miner 2001). Predation of newly settled and juvenile urchins is a well-known post-settlement process (Tegner and Dayton 1981, Rowley 1990), as well as general mortality of settlers due to poor habitat choice (Cameron and Rumrill 1982). Analysis of genetic heterogeneity can provide an indirect means of assessing the scale of realized larval dispersal and whether or not populations are demographically open or closed over a given range (Todd 1998).

Salinity has been shown to adversely affect many stages of the reproductive cycle including fertilization (Thorson 1950, Greenwood and Bennett 1981), development of the embryo (Davis and Calabrese 1964, Hendler 1977, Kashenko 1992, 1998), and successful development of the larva (Vernberg and Vernberg 1970, Roller and Stickle 1994, Metaxas 1998). Later in ontogeny, lowered salinities have been implicated in the direct mortality or exclusion of juveniles and small adults from habitats (Himmelman et al. 1983, Drouin et al 1985, Raymond and Scheibling 1987, Chen and Chen 1993).

Salinity, therefore, has the ability to influence recruitment processes by affecting every stage of the life cycle of *Evechinus chloroticus*. It is unknown what stages of *E. chloroticus* are most affected by low salinity. It is also unknown how developing stages *E. chloroticus* are affected.

This thesis is organized into 4 main chapters and a general discussion.

Firstly, do larvae distribute themselves differently in the field due to the Low Salinity Layer? Either the vertical distribution of *E. chloroticus* will not be influenced by the LSL, therefore larvae will be found evenly distributed throughout the water column. Or conversely, *E. chloroticus* larvae distributions will be influenced by the LSL with distributions being patchy throughout the water column, below the LSL.

- **Chapter 2** presents the distribution of *E. chloroticus* larvae at three stations in Doubtful Sound from January to March 2003.

Secondly, does low salinity affect the early development of *Evechinus chloroticus*? Either lowered salinities may not affect the development of *E. chloroticus*, or embryos and larvae may be detrimentally affected by lowered salinities.
• **Chapter 3** investigates the tolerance of *E. chloroticus* embryos to lowered salinities.

• **Chapter 4** examines the low salinity tolerance of *E. chloroticus* larvae. Thirdly, does low salinity detrimentally affect adult *Evechinus chloroticus* from Doubtful Sound? Either adult *E. chloroticus* will not be affected by lowered salinities, or lowered salinities may alter the reactions of adults in differing salinities.

• **Chapter 5** inspects whether adults of *E. chloroticus* were sensitive to lowered salinities.

These investigations analyze four critical stages in determining the possible variation of *Evechinus chloroticus* recruitment and population structure. By investigating tolerance to low salinity in the laboratory, detrimental affects that are observed may help to explain the distribution in the wild. Since larval distribution equates to connectivity of populations, investigating tolerance of early life stages to influential environmental conditions may advance understanding of recruitment dynamics.
Chapter 2. Vertical Distribution of *Evechinus chloroticus* larvae in Doubtful Sound with respect to the Low Salinity Layer

2.1. Introduction

The distribution of larvae in the field can be influenced by numerous biotic and abiotic factors. Salinity, temperature, food availability, and hydrodynamic processes are regarded as especially influential in determining the distribution of larvae (Greenwood and Bennett 1981, Cameron and Rumrill 1982, Rothschild and Osborn 1988, Fenaux *et al.* 1994, Metaxas and Young 1998, Meidel *et al.* 1999). Of particular interest, salinity has the ability to affect the growth, survival, and distribution of larvae through critical ranges of survivability in areas where salinity is variable.

Development rate of echinoderm larvae was directly related to salinity in a number of studies (e.g. Vernberg and Vernberg 1970, Watts *et al.* 1982, Roller and Stickle 1985, 1993, 1994, Metaxas 1998). Roller and Stickle (1994) found that development rates and survival to metamorphosis of larvae of the sea urchins *Strongylocentrotus droebachiensis* and *S. pallidus* were significantly affected by salinity. Larval development was incomplete in both species below 20‰. In experiments on the sea urchin *Echinometra lucunter*, Metaxas (1998) found that mortality of developing larvae was significantly increased in treatments of lowered salinity (15‰-24‰) as compared to higher salinity treatments (33‰ and 27‰). Larvae in the 33‰ treatments were able to complete development to the 8-arm stage, while those larvae in the lower salinity treatments failed to develop further than the 4-arm stage (Metaxas 1998). Lower larval survival has also been observed in asteroids (Hendler 1977, Watts *et al.* 1982, Sarantchova 2001), the Japanese Sea Cucumber *Stichopus japonicus* (Gavrilova and Mokretsova 1983, Kashenko 1993, 1998), and other marine invertebrates (e.g. Scheltema 1965, Johns 1981, Laughlin 1983, Anger 1985, Zimmerman and Pechenik 1991, Charmantier *et al.* 2001). Salinity was the dominant factor affecting the development and growth of *Echinaster* larvae, with high and low salinities inhibiting spine development (Watts *et al.* 1982).

Studies have shown that larvae will alter their vertical distribution in the water column due to differences in salinity. Metaxas and Young (1998) investigated the
responses of larvae of two sea urchins, *Echinometra lucunter* and *Arbacia punctulata*, finding that the gradient of the halocline significantly affected the vertical position of larvae of both species. In the absence of a halocline, larvae of both species swam to the surface of the experimental cylinders, while in experiments with haloclines, larvae of both species congregated at the halocline, but moved across the discontinuity layer in proportion to the salinity in the upper layer. Less larvae traveled into the surface layers when the salinity was low compared to salinities tested that were closer to the salinity in the lower layer (Metaxas and Young 1998). Low salinities (10-16‰) actively hindered the upward swimming of all compound ascidian larvae studied by Vazquez and Young (1996). Vigorously swimming newly hatched *Homarus americanus* larvae rarely passed salinity discontinuities into lower salinity water, but if so, often reacted quickly, actively swimming down into the higher salinity water (Scarratt and Raine 1967). Larvae of other invertebrates have been shown to aggregate at salinity discontinuities (Mann *et al.* 1991, Raby *et al.* 1994, see review Metaxas 2001).

The distribution of larvae in fiords can be studied due to the special hydrographic dynamics found within them. The New Zealand fiords have typical fiord features: they are narrow bodies of water with steep sides plunging into deep basins often hundred of meters deep (Stanton 1984). The net transport of water is out of the fiord in the surface layer, which is often characterized by lowered salinity. Coastal water of oceanic salinity is drawn over the shallow sill into the deep basins to replace water mixed into the outflowing layer. Doubtful Sound is a representative member of the New Zealand fiords experiencing extensive rainfall (>7m) through the year. Unique to Doubtful Sound, it also receives additional freshwater outflow from the Manapouri Hydroelectric Power Station (Stanton and Pickard 1981).

Due to the extensive freshwater input into Doubtful Sound, a persistent low salinity layer (LSL) floats above denser ocean water. LSLs are common in New Zealand fiords (Grange *et al.* 1991) and other fiord systems (Pickard 1961, Stickle and Denouix 1976, Farmer and Freeland 1983, Kaartvedt and Aksnes 1992). The LSL in Doubtful Sound typically has a salinity of 1-5‰ (Stanton 1984), while surface salinity is typically 34-35‰ off the West Coast of New Zealand (Heath 1985). The LSL generally is thicker at the head of the fiord compared to the sill (Walls 1995, McCully 1996). Classic estuarine circulation takes place in Doubtful Sound, the LSL
progresses seaward while coastal seawater is drawn into the fiord and displaces bottom waters (Stanton and Pickard 1981). As the LSL flows seaward, it entrains higher salinity water and suspended particles from below, slowly increasing in salinity towards the sill (Walls 1995, Bowman et al. 1999, Macrellis 2001).

Due to the shallow sill, narrow entrance, and the pattern of circulation inside the fiord, larvae have been shown to be retained in significant numbers inside Doubtful Sound (Lamare 1998, Lamare and Barker 1999, Wing et al. 2003). Since larvae are retained, their vertical distribution throughout the water column can be studied, as they are not quickly advected out of the system. In this study the vertical distribution of *Evechinus chloroticus* larvae in the water column of Doubtful Sound was monitored.

Adult *Evechinus chloroticus* (Valenciennes) are common throughout New Zealand, including Doubtful Sound. Inhabiting waters of the intertidal through subtidal, *E. chloroticus* generally has greatest abundances above 10 meters depth (Wing et al. 2001), but individuals have been found in deeper waters (27-55m) where favorable food and habitat conditions exist (Dix 1970a). Recently, Lamare (1997, 1998), Lamare and Barker (1999), and Wing et al. (2003) have studied the distribution of *E. chloroticus* larvae in Doubtful Sound. Larval supply has been implicated as a significant factor structuring adult *E. chloroticus* populations in Doubtful Sound (Wing et al. 2003). Do *Evechinus chloroticus* larvae distribute themselves differently in Doubtful Sound due to the Low Salinity Layer? Either the vertical distribution of *E. chloroticus* will not be influenced by the LSL and larvae will be found evenly distributed throughout the water column, or on the contrary, *E. chloroticus* larvae distributions will be influenced by the LSL with distributions being patchy throughout the water column, below the LSL. It is hypothesized that *E. chloroticus* larvae will distribute themselves differently due to the Low Salinity Layer. Assuming *E. chloroticus* larvae are intolerant to low salinities (Chapter 4, current study), larvae from inner-fiord sites may not recruit into other populations as they cannot escape via the LSL. However, larvae contributed by populations “upstream” as it were, would be entrained into Head-ward moving, oceanic salinity water, and could contribute their offspring to Head populations.
2.2 Materials and Methods

Plankton Sampling

Doubtful Sound is situated approximately midway along the Fiordland Coast, on the west coast of the South Island of New Zealand (Figure 2.1). The field component of this study took place from January 2003 until March 2003. Trips were made nearly weekly throughout this period. A total of seven sampling trips were successful. The first trip on 14 January was excluded as only one site was sampled prior to the damage of the plankton net. Plankton samples were taken from three sites (LS-1 to LS-3) approximately equidistant along the main channel of Doubtful Sound (Figure 2.2). The RV Niad was anchored at each site and then vertical tows were made with a UNESCO WP2 (UNESCO 1968) plankton sampling net with a 50cm diameter opening and fitted with 100μm mesh. This net was used on all trips except 21 January 2003 when a UNESCO WP2 net with a 46cm diameter opening and 100μm mesh was used due to damage of the 50cm opening net the previous week. Both nets were fitted with a closing mechanism to allow for discreet depth samples. Three replicate tows were taken from 25-20m, 20-15m, 15-10m, 10-8m, 8-6m, 6-4m, and 2m-surface. Each tow was lowered to the appropriate depth and hauled vertically at a rate of approximately 30cm/s until the shallower depth was reached, the closing mechanism was then activated, and the net hauled to the surface. This was the standard procedure except for 2m-surface samples where the opening of the net was hauled above the surface, the net washed down, and the sample collected from the cod end. All samples were sieved with a 100μm mesh sieve and immediately preserved in 50% isopropyl alcohol.

Oceanographic Information

A SeaBird SBE-19 Conductivity-Temperature-Depth (CTD) probe with an attached WET labs WET Star miniature chlorophyll fluorometer was deployed at each site on each sampling day. The CTD was positioned in the water column until it was just submerged, then allowed to run for two minutes to cleanse the system. The CTD was then lowered to approximately 25-30m at a rate of 30cm/s to obtain a vertical profile of salinity, temperature, and fluorescence. Measurements were taken every half-second.
Figure 2.1 Map of New Zealand (a) and location of Doubtful Sound denoted by the X (45° 18’00”S, 166° 58’45”E), and a map of Doubtful Sound (b), with Deep Cove and the inner (The Gut) and outer sills (Hares Ears) identified by dotted lines.
Figure 2.2. Locations of plankton sampling in Doubtful Sound. (LS-1= Hall Arm, Head site, LS-2= Crooked Arm, Middle site, LS-3= Grono Bay, Sill site).
Analysis

Oceanographic data was compiled and summed over the depths in which larvae were sampled (for example 0-2m, 10-15m) to obtain average values for salinity and fluorescence at each depth strata for statistical analysis.

Previous analysis of the UNESCO WP2 net found that the net had a mean catch efficiency of 94% in oligotrophic waters, such as Doubtful Sound, if the tows were less than 16 minutes in duration (Henroth 1987). All tows taken for this study were less than two minutes in duration, therefore catch efficiency was assumed to be 100%.

At the Portobello Marine Lab, samples were emptied into a dish and sorted using an Olympus SZ30 dissecting microscope under 40X power. Positive identification of *Evechinus chloroticus* larvae in the samples was accomplished by comparing field specimens to lab-reared *E. chloroticus* larvae. For each sample depth, the total numbers of *E. chloroticus* larvae were determined, and each larva assigned a stage of development (Table 2.1, Figure 2.3).

The total numbers of larvae in each replicate were then pooled for each depth to give a total number of larvae/depth. This procedure was undertaken before all analyses due to the low numbers of larvae captured in the current study in any one replicate. The pooled larval counts at each depth were then converted into a density of larvae/m³. These densities were then normalized using the natural log of the density plus one (ln (larval number + 1)). These data were spatially and temporally compared using one-way and two-way ANOVAs using the statistical programs SPSS® (SPSS, Inc., Chicago, IL, USA) and MINITAB® (MINITAB, Inc., State College, PA, USA). Statistical significance was inferred if the p-value was less than or equal to 0.05.
Table 2.1. Classification of *Evechinus chloroticus* larval stages (adapted from Lamare 1997). Also see Figure 2.3.

<table>
<thead>
<tr>
<th>Larval Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastula</td>
<td>No Arms</td>
</tr>
<tr>
<td>4 Armed</td>
<td>Having both postoral and anterolateral arms</td>
</tr>
<tr>
<td>6 Armed</td>
<td>Having postoral, anterolateral, and postdorsal arms</td>
</tr>
<tr>
<td>Early 8 Armed</td>
<td>Having fully formed postoral, anterolateral, postdorsal, and forming preoral arms</td>
</tr>
<tr>
<td>Late 8 Armed</td>
<td>Having fully formed postoral, anterolateral, postdorsal, and preoral arms</td>
</tr>
</tbody>
</table>

Figure 2.3.(over page) Stages of *Evechinus chloroticus* larval development classified in this study. The larvae were lab reared. Unhatched and blastula viewed using an Olympus BH-2 compound microscope under 200X power. All others observed under 31X power using a Wild-Heerbrugg dissecting microscope. (A = unhatched, undamaged embryo, B = prism larva, just hatched, C = early 4 armed, D = late 4 armed, E = early 6 armed, F = late 6 armed, G = early 8 armed, H = late 8 armed.) Scale bar represents 100μm.
2.3 Results

Vertical Distribution of Larvae

*Evechinus chloroticus* larvae were found during only the first five of the seven successful sampling trips from January to March 2003. Temporal changes in larval abundance and developmental stage can be seen in Figure 2.4. The depth of the LSL (taken as the 30% isobar) is illustrated in Figure 2.5. Tows taken on 10 March and 17 March 2003 failed to capture any *Evechinus chloroticus* larvae.

**All Trips - Total (Figure 2.6)**

All developmental stages of *Evechinus chloroticus* larvae were collected during this study. At the Grono Bay (LS-3), early 8-Armed larvae were absent from all tows collected. Larvae at this site distributed themselves throughout the 25m sampled, while at Hall Arm (LS-1) and Crooked Arm (LS-2), *E. chloroticus* larvae were absent from the upper 2m of the water column. Greater total numbers of larvae were collected at Grono Bay (200.2 larvae/m³) than either Crooked Arm (145.6 larvae/m³) or Hall Arm (39.7 larvae/m³). At all three sites, greatest total numbers of larvae were collected in the 6-8m tows, although the average depths of the Low Salinity Layer (LSL) differed greatly among sites. At Hall Arm the LSL was on average 5.2m deep, while at Crooked Arm the LSL was shallower, at an average depth of 3.7m deep. Grono Bay had the shallowest LSL, measuring on average of only 1.3m deep.

**21 January 2003 – Trip A (Figure 2.7A)**

*Evechinus chloroticus* larvae were found in the water column at the Head (LS-1) and Middle (LS-2) sites, but not at the Sill (LS-3) site. Slightly greater total numbers of larvae were found at Crooked Arm (26.5 larvae/m³) than at Hall Arm (22.9 larvae/m³). Crooked Arm counts comprised all stages of *E. chloroticus* larvae, while at Hall Arm, late 8-Arm larvae were absent. Greatest numbers of larvae were found from 10-15m (7.2 larvae/m³) at Hall Arm, while at Crooked Arm, greater numbers were found shallower, at 8-10m (9.0 larvae/m³). At both sites, larvae were absent from the upper 2m of the water column. This result is not surprising as LSL was 5.4m deep at Hall Arm and 3.5m deep Crooked Arm during this sampling period (figures 2.5, 2.7A).
Figure 2.4. Temporal variation in density of *Evechinus chloroticus* larvae at three sites Hall Arm (LS-1), Crooked Arm (LS-2), and Grono Bay (LS-3) through the sampling period. The numbers of larvae in each developmental stage is indicated by the key. Density is displayed as pooled *E. chloroticus* larvae/m$^3$. Note the difference among the scales of the y-axis among the graphs. Confidence intervals have been excluded for clarity.

*Evechinus chloroticus* larval developmental stages

- 4-Arm
- 6-Arm
- early 8-Arm
- late 8-Arm
Figure 2.5. Changes in the Low Salinity Layer depth between the three sites over the sampling period from January until March 2003, as well as the overall depth distribution of the LSL. The distance along the x-axis is in reference to Grono Bay (LS-3).
Figure 2.6. (over page) Cumulative vertical distribution of *Evechinus chloroticus* larvae (pooled larvae/m³) between 0 and 25m depth at three sites, Hall Arm (LS-1), Crooked Arm (LS-2), and Grono Bay (LS-3) from January to March 2003. Shown to the right are Salinity and Fluorescence profiles for all sites throughout the sampling period. Note the difference among the scales of the y-axis among the larval graphs. Confidence intervals have been excluded for clarity.

*Evechinus chloroticus* larval developmental stages

- 4-Arm
- 6-Arm
- early 8-Arm
- late 8-Arm

Oceanographic key

- Salinity (psu)
- Fluorescence (mg/L)
4 February 2003 – Trip B (Figure 2.7B)

A great decrease in the total numbers of *Evechinus chloroticus* larvae were collected on this trip than during the 21 January trip. Again, *E. chloroticus* larvae were found only at the Head (LS-1) and Middle (LS-2) sites, and not at the Sill (LS-3) site. Total numbers of larvae at both sites were quite low. At Crooked Arm, only 2.0 larvae/m$^3$ were found, and at Hall Arm, a slightly larger number of 6.1 larvae/m$^3$ were recovered. At both sites, early 8-Armed larvae of *E. chloroticus* made up the entirety of the larvae retrieved, a large shift from the diversity of developmental stages recovered from the 21 January trip. At Hall Arm, greater numbers of larvae were retrieved from the 4-6m (5.1 larvae/m$^3$) tows, with the remainder of larvae (1.0 larvae/m$^3$) collected at 15-20m. At Crooked Arm, larvae were found only in the 15-20m depth tows (2.0 larvae/m$^3$). During this trip, the LSL was very similar between the two sites where larvae were captured. The LSL at Hall Arm was 2.6m deep, while at Crooked Arm it was 2.5m deep.

10-11 February 2003 – Trip C (Figure 2.7C)

The third successful trip compromised the first trip in which *Evechinus chloroticus* larvae were found at all sites. A single cohort of 4-Armed larvae made up the samples retrieved from the Sill Site (LS-3). Crooked Arm tows collected the greatest diversity of *E. chloroticus* developmental stages, retrieving 4-, 6-, and late 8-Armed larvae, while Hall arm tows retrieved only late 8-Armed larvae. Greatest numbers of *E. chloroticus* larvae were obtained from Grono Bay (39.7 larvae/m$^3$), with lower numbers of larvae at Crooked Arm (27.0 larvae/m$^3$) and lowest numbers of larvae from Hall Arm (2.5 larvae/m$^3$). The *E. chloroticus* larvae at Grono Bay distributed themselves all through the 25m sampled, but most densely in the 6-8m tows (12.7 larvae/m$^3$) and in the tows from 2m to the surface (10.2 larvae/m$^3$). At Crooked Arm larvae were densest at 8-10m (17.8 larvae/m$^3$), while at Hall Arm, the only larvae collected were in the 6-8m tows (2.5 larvae/m$^3$). Again the LSL depth at Hall Arm and Crooked Arm was quite similar, being 4.6m and 4.7m deep, for the two sites respectively. At Grono Bay the LSL was much shallower, being only 1.2m deep.
18 February 2003 – Trip D (Figure 2.7D)

Distributions of *Evechinus chloroticus* larvae at the three sites were different on this fourth successful trip. Great numbers of *E. chloroticus* larvae were collected from Grono Bay (159.4 larvae/m³) and Crooked Arm (90.1 larvae/m³) sites, four- and three-fold increases from the previous sampling trip, respectively. Much fewer larvae were collected at Hall Arm (8.1 larvae/m³) compared to Grono Bay and Crooked Arm, but this was still a three-fold increase from the 10 February trip. At all sites, 4- and 6-Armed *E. chloroticus* larvae compromised the entire tow. Four-Armed larvae were the dominant developmental stage at all sites (Grono Bay (128.3 larvae/m³), Crooked Arm (82.5 larvae/m³), and Hall Arm (4.6 larvae/m³)). At Crooked and Hall Arms, *E. chloroticus* larvae were not retrieved in the surface two meters of the water column, and larvae were densest at 8-10m, with 17.8 and 5.1 larvae/m³ collected from these sites, respectively. At Grono Bay, larvae were densest in the 6-8m tows (50.9 larvae/m³). The depth of the LSL at the three sites differed greatly from the previous weeks sampling. At Hall Arm the LSL was very deep, the 30‰ isobar appearing 8.1m beneath the surface. At Crooked Arm the depth of the LSL was 5.4m, while at Grono Bay, it again was quite shallow, being only 1.5m deep.

4 March 2003 – Trip E (Figure 2.7E)

One *Evechinus chloroticus* larva was retrieved from the Grono Bay (LS-3) site during this trip. This single collection of a single late 8-Armed *E. chloroticus* larvae was collected from 10-15m. This collection was a 100-fold decrease from the collection two weeks prior, during the 18 February trip. The LSL at Grono Bay on this trip was much deeper than in previous samplings, being 2.5m below the surface.

10 and 17 March 2003 – Trips F and G

Both of these later trips failed to collect any *Evechinus chloroticus* larvae at any site and at any depth.
Figure 2.7. (over pages) Vertical distribution of *Evechinus chloroticus* larvae (pooled larvae/m³) from 21 January 2003 until 4 March 2003. Larval distribution between 0 and 25m depth at three sites, Hall Arm (LS-1), Crooked Arm (LS-2), and Grono Bay (LS-3). Shown to the right are Salinity/Fluorescence profiles for all sites throughout the sampling period. Note the axes among graphs may differ.

*Evechinus chloroticus* larval developmental stages

- □ 4-Arm
- □ 6-Arm
- □ early 8-Arm
- □ late 8-Arm

Oceanographic key

- — Salinity (psu)
- — Fluorescence (mg/L)
A. 21 January 2003

**Hall Arm**

- **Pooled larvae**
  - Depth (m): 0-2, 4-6, 6-8, 8-10, 10-15, 15-20, 20-25
  - Fluorescence (mg/L): 0-2, 4-6, 6-8, 8-10, 10-15, 15-20, 20-25

**Crooked Arm**

- **Pooled larvae**
  - Depth (m): 0-2, 4-6, 6-8, 8-10, 10-15, 15-20, 20-25
  - Fluorescence (mg/L): 0-2, 4-6, 6-8, 8-10, 10-15, 15-20, 20-25

**Grono Bay**

- **Pooled larvae**
  - Depth (m): 0-2, 4-6, 6-8, 8-10, 10-15, 15-20, 20-25
  - Fluorescence (mg/L): 0-2, 4-6, 6-8, 8-10, 10-15, 15-20, 20-25
C. 10 February 2003

Pooled Larvae / m³

Hall Arm

Salinity (psu)

Crooked Arm

Grono Bay

Fluorescence (mg/L)

Depth (m)
D. 18 February 2003

Pooled Larvae / m^3

Salinity (psu)

Fluorescence (mg/L)

Depth (m)

Pooled Larvae / m^3

Salinity (psu)

Fluorescence (mg/L)

Depth (m)

Pooled Larvae / m^3

Salinity (psu)

Fluorescence (mg/L)

Depth (m)

Hall Arm

Crooked Arm

Grono Bay
E. 4 March 2003

- **Hall Arm**
  - Pooled Larvae / m³
  - Salinity (psu)
  - Depth (m)

- **Crooked Arm**
  - Pooled Larvae / m³
  - Salinity (psu)
  - Depth (m)

- **Grono Bay**
  - Pooled Larvae / m³
  - Salinity (psu)
  - Depth (m)
**Statistical Analyses**

**All Trips - Total**

One-way ANOVA of all larval stages combined for all trips showed that the Site ($F_{(2,167)}$ 2.64, $P = 0.074$), Depth ($F_{(6,167)}$ 1.34, $P = 0.243$), average Salinity ($F_{(23,167)}$ 0.86, $P = 0.650$), and average Fluorescence ($F_{(13,167)}$ 1.27, $P = 0.235$) were not significant factors affecting the distribution of *Evechinus chloroticus* larvae in Doubtful Sound over the entire sampling period. The distribution of larvae in reference to the Low Salinity Layer, however, was very significant ($F_{(1,167)}$ 11.34, $P = 0.001$), as well as the factor of Julian Day, ($F_{(9,146)}$ $P < 0.001$).

Breaking down the analyses by developmental stage, provided slightly different results (Table 2.2). Late 8-Armed *E. chloroticus* larvae showed no significant factors, while the other developmental stages were all significantly different with Julian Day. Four Armed larvae were also significantly different among the sites ($P = 0.026$) throughout the sampling period.

<table>
<thead>
<tr>
<th></th>
<th>Julian Day</th>
<th>Depth</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Armed</td>
<td>$F_{(9,146)}$ 17.13, $p&lt;0.001$</td>
<td>$F_{(6,146)}$ 0.47, $p=0.826$</td>
<td>$F_{(2,146)}$ 3.73, $p=0.026$</td>
</tr>
<tr>
<td>6-Armed</td>
<td>$F_{(9,146)}$ 4.70, $p=0.001$</td>
<td>$F_{(6,146)}$ 0.90, $p=0.494$</td>
<td>$F_{(2,146)}$ 0.72, $p=0.487$</td>
</tr>
<tr>
<td>Early 8-Armed</td>
<td>$F_{(9,146)}$ 2.61, $p=0.008$</td>
<td>$F_{(6,146)}$ 0.52, $p=0.794$</td>
<td>$F_{(2,146)}$ 1.98, $p=0.142$</td>
</tr>
<tr>
<td>Late 8-Armed</td>
<td>$F_{(9,146)}$ 1.24, $p=0.276$</td>
<td>$F_{(6,146)}$ 0.55, $p=0.773$</td>
<td>$F_{(2,146)}$ 0.16, $p=0.856$</td>
</tr>
</tbody>
</table>

**Table 2.2. One-way Analysis of Variance for overall *Evechinus chloroticus* larval numbers. Statistical significance**

**Individual trips**

Analyses were undertaken for each individual trip to tease out any between-trip, between-site, and within-site differences. For the total *Evechinus chloroticus* larvae collected during Trip A, average Fluorescence ($P = 0.031$) and Site ($P = 0.002$) were the overall factors that were significant (Table 2.3a). Analyses were also broken down among developmental stages (Table 2.3a). Within Trip A, early 8-Armed larvae were significantly correlated to the average Fluorescence ($P = 0.002$). All
other factors were not significant for the other developmental stages. Statistical analysis of Trip B failed to result in any significance among the factors analyzed (Table 2.3b). During Trip C, total numbers of *E. chloroticus* larvae were significantly related to Site (*P* = 0.006), and other significant factors were determined among developmental groups (Table 2.3c). Site (*P* = 0.006) was again significant for predicting total larval distribution during Trip D, as was the distribution of larvae in respect to the Low Salinity Layer (*P* = 0.014). Several factors were significant among the different developmental groups (Table 2.3d). Trip E yielded only one *E. chloroticus* larva, so analyses within the tip were not performed.
Table 2.3 One-way Analysis of Variance for overall *Evechinus chloroticus* larval numbers for individual trips. LSL refers to the distribution of larvae in waters under 30‰ (inside the LSL) or waters above 30‰. **Statistical significance**

Table 2.3a One-way Analysis of Variance for overall *Evechinus chloroticus* larval numbers for Trip A – 21 January 2003.

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth</th>
<th>Salinity</th>
<th>Fluorescence</th>
<th>LSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>All larvae</td>
<td>8.73</td>
<td>0.76</td>
<td>1.33</td>
<td>4.23</td>
</tr>
<tr>
<td>4-Armed</td>
<td>0.002</td>
<td>0.061</td>
<td>0.312</td>
<td>0.031</td>
</tr>
<tr>
<td>6-Armed</td>
<td>2.64</td>
<td>0.53</td>
<td>0.44</td>
<td>2.84</td>
</tr>
<tr>
<td>Early 8-Armed</td>
<td>2.19</td>
<td>1.15</td>
<td>0.137</td>
<td>0.90</td>
</tr>
<tr>
<td>Late 8-Armed</td>
<td>1.00</td>
<td>0.38</td>
<td>0.270</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 2.3b One-way Analysis of Variance for overall *Evechinus chloroticus* larval numbers for Trip B – 4 February 2003. Results are identical for all larvae and Early 8-Armed larvae as other developmental stages were absent from tows.

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth</th>
<th>Salinity</th>
<th>Fluorescence</th>
<th>LSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>All larvae</td>
<td>1.04</td>
<td>1.30</td>
<td>0.11</td>
<td>1.65</td>
</tr>
<tr>
<td>4-Armed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6-Armed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Early 8-Armed</td>
<td>1.04</td>
<td>1.30</td>
<td>0.11</td>
<td>1.65</td>
</tr>
<tr>
<td>Late 8-Armed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2.3c One-way Analysis of Variance for overall *Evechinus chloroticus* larval numbers for Trip C – 10-11 February 2003. Analyses for Early 8-Armed larvae are absent as this developmental stage was absent from all tows.

<table>
<thead>
<tr>
<th></th>
<th>Site</th>
<th>Depth</th>
<th>Average Salinity</th>
<th>Average Fluorescence</th>
<th>LSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>All larvae</td>
<td>$F(2,20)$ 7.04,</td>
<td>$F(6,20)$ 0.27,</td>
<td>$F(7,20)$ 0.91,</td>
<td>$F(7,20)$ 1.28,</td>
<td>$F(1,20)$ 2.39,</td>
</tr>
<tr>
<td></td>
<td>$p=0.006$</td>
<td>$p=0.940$</td>
<td>$p=0.529$</td>
<td>$p=0.334$</td>
<td>$p=0.138$</td>
</tr>
<tr>
<td>4-Armed</td>
<td>$F(2,20)$ 16.09,</td>
<td>$F(6,20)$ 0.23,</td>
<td>$F(7,20)$ 1.40,</td>
<td>$F(7,20)$ 3.02,</td>
<td>$F(1,20)$ 1.83,</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
<td>$p=0.958$</td>
<td>$p=0.284$</td>
<td>$p=0.040$</td>
<td>$p=0.192$</td>
</tr>
<tr>
<td>6-Armed</td>
<td>$F(2,20)$ 1.00,</td>
<td>$F(6,20)$ 1.00,</td>
<td>$F(7,20)$ 0.09,</td>
<td>$F(7,20)$ 0.27,</td>
<td>$F(1,20)$ 0.10,</td>
</tr>
<tr>
<td></td>
<td>$p=0.387$</td>
<td>$p=0.463$</td>
<td>$p=0.998$</td>
<td>$p=0.957$</td>
<td>$p=0.755$</td>
</tr>
<tr>
<td>Early 8-Armed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Late 8-Armed</td>
<td>$F(2,20)$ 0.58,</td>
<td>$F(6,20)$ 0.86,</td>
<td>$F(7,20)$ 0.98,</td>
<td>$F(7,20)$ 6.78,</td>
<td>$F(1,20)$ 0.20,</td>
</tr>
<tr>
<td></td>
<td>$p=0.572$</td>
<td>$p=0.548$</td>
<td>$p=0.483$</td>
<td>$p=0.002$</td>
<td>$p=0.664$</td>
</tr>
</tbody>
</table>

Table 2.3d One-way Analysis of Variance for overall *Evechinus chloroticus* larval numbers for Trip D – 18 February 2003. Analyses for Early and Late 8-Armed larvae are absent as these developmental stages were absent from all tows.

<table>
<thead>
<tr>
<th></th>
<th>Site</th>
<th>Depth</th>
<th>Average Salinity</th>
<th>Average Fluorescence</th>
<th>LSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>All larvae</td>
<td>$F(2,20)$ 6.80,</td>
<td>$F(6,20)$ 1.19,</td>
<td>$F(9,20)$ 1.20,</td>
<td>$F(7,20)$ 2.18,</td>
<td>$F(1,20)$ 7.25,</td>
</tr>
<tr>
<td></td>
<td>$p=0.006$</td>
<td>$p=0.367$</td>
<td>$p=0.379$</td>
<td>$p=0.107$</td>
<td>$p=0.014$</td>
</tr>
<tr>
<td>4-Armed</td>
<td>$F(2,20)$ 8.18,</td>
<td>$F(6,20)$ 1.01,</td>
<td>$F(9,20)$ 1.10,</td>
<td>$F(7,20)$ 2.52,</td>
<td>$F(1,20)$ 6.76,</td>
</tr>
<tr>
<td></td>
<td>$p=0.003$</td>
<td>$p=0.457$</td>
<td>$p=0.431$</td>
<td>$p=0.071$</td>
<td>$p=0.018$</td>
</tr>
<tr>
<td>6-Armed</td>
<td>$F(2,20)$ 2.15,</td>
<td>$F(6,20)$ 1.02,</td>
<td>$F(9,20)$ 1.06,</td>
<td>$F(7,20)$ 1.29,</td>
<td>$F(1,20)$ 2.39,</td>
</tr>
<tr>
<td></td>
<td>$p=0.146$</td>
<td>$p=0.452$</td>
<td>$p=0.455$</td>
<td>$p=0.327$</td>
<td>$p=0.139$</td>
</tr>
<tr>
<td>Early 8-Armed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Late 8-Armed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
2.4 Discussion

The vertical distribution of *Evechinus chloroticus* larvae in Doubtful Sound was examined in the present study. Larvae were captured at all depths at the Grono Bay (Sill) site, where the LSL was generally thin, on average 1.3m deep. At Crooked Arm (Middle) and Hall Arm (Head), the inner fiord sites, the LSL was generally deeper (5.2m and 3.7m deep, respectively), and *E. chloroticus* larvae were absent from tows taken from 2m to the surface. This observation lends evidence to my hypothesis that *E. chloroticus* larvae distribute themselves differently in Doubtful Sound due to the Low Salinity Layer. Statistical analysis, however, revealed that most of the factors tested were not significant. The numbers of larvae in reference to the LSL, whether they were in waters less than 30‰ or in waters greater than 30‰, was significantly related (P = 0.001). Lending more evidence to the hypothesis that larvae distribute themselves differently due to the LSL. The only other factor that was overall statistically significant was trip (P = 0.032). This result shows that there was a temporally significant difference in the total numbers of *E. chloroticus* larvae captured.

The overall concentration of *Evechinus chloroticus* larvae in the 6-8m tows at all three sites is an interesting result. At Hall Arm and Crooked Arm, this layer would have been very close to the halocline, and larvae and other planktonic organisms have been shown to gather at discontinuities in the water column. Experimentally, Metaxas and Young (1998) found the larvae of *Echinometra lucunter* and *Arabacia punctulata* gathered at haloclines, while in cylinders without haloclines, larvae of both species swam and aggregated at the surface. Ascidian larvae studied by Vasquez and Young (1996) also congregated at haloclines, and the authors suggest that this behavior in ascidian larvae may retain larvae in estuarine situations. If *E. chloroticus* larvae react similarly to haloclines as other echinoderm or ascidian larvae do, they may aggregate around the halocline between the LSL and high salinity, deeper waters in Doubtful Sound. Lamare (1998) collected plankton in tows of a similar fashion as in this study, but collected plankton at six depth strata, including 10m to the 33‰ isocline (taken as demarking the bottom of the LSL) and from the bottom of the LSL to the surface. Highest densities of *E. chloroticus* larvae were recovered from the 10m to 20m tows, deeper than in this study, but still in the vicinity of the halocline. Lamare (1998) also
found that larvae of *E. chloroticus* were absent from the LSL, and the author suggests the vertical distribution of larvae within Doubtful Sound may be associated with the stratification of the water column (Lamare 1998). In this study, depths were not segregated, therefore some tows included the halocline defined by Lamare (1998). Still, similar results on the vertical distribution of *E. chloroticus* in Doubtful Sound were obtained. At Hall Arm and Crooked Arm larvae were absent from the 2m to surface tows, depths which always contained the LSL. The observation that *Evechinus chloroticus* larvae were absent from obviously low salinities may indicate that they have similar avoidance behavior of low salinities as larvae of other euryhaline echinoderms and invertebrates (e.g. Stickle and Diehl 1987, Mann et al. 1991, Raby et al. 1994, review Metaxas 2001).

The breakdown of the study by trips allowed for comparison between sites on specific dates. At this level, *Evechinus chloroticus* larvae were found to be significantly correlated with site and the average fluorescence on several occasions. The result of the significance of site is not surprising since larval numbers varied greatly among sites over the sampling period. The statistical significance of average fluorescence, a proxy for chlorophyll, and therefore phytoplankton abundance or food availability, at several times during the sampling is interesting, though compounding effects may be at work. On 21 January, one of the occasions in which average fluorescence was statistically significant in the distribution of *E. chloroticus* larvae, fluorescence values were fairly homogeneous at the two sites at which larvae were recovered. In another instance, in which 4-Armed larvae from Trip C, 10-11 February were significantly correlated with average fluorescence, the great numbers of 4-Armed larvae at Grono Bay and the high average fluorescence at this site may be skewing the results. On two occasions, Trip A and Trip C, late 8-Armed larvae were significantly associated with average fluorescence, but larvae were only found at one depth strata, consequently making average fluorescence a factor in their distribution.

Growth of sea urchin larvae has been shown to be linked to food availability (Fenaux et al. 1994). Larval and plankton distribution has been shown to be coupled with food abundance (Banse 1964, Cameron and Rumrill 1982, Lopez et al 1998, Folt and Burns 1999), but salinity is probably a more important factor influencing the distribution and survival of *E. chloroticus* larvae in Doubtful Sound. Larvae of *E. chloroticus* are very sensitive to lowered salinities (Chapter 4, current study).
Exposed to sustained low salinities, larvae react quite quickly, aberrations in form developing and cohorts having great mortality in only a few days. Starvation of invertebrate larvae with natural concentrations of food has never been demonstrated, possibly indicating that phenotypic and developmental plasticity is available depending on whether food is abundant or scarce (Fenaux et al. 1994).

All larval stages of *E. chloroticus* were found, although not at all sites. Early 8-Armed larvae were absent from the Grono Bay site, while all larval stages were captured at Crooked Arm and Hall Arm. In general, later staged *E. chloroticus* larvae were found in shallower depths, a similar result to Lamare (1998). This response of later stage larvae being shallower is common in intertidal invertebrates (Pennington and Emlet 1986, Epifanio et al. 1988).

The majority of larvae at Grono Bay were 4-Armed (168.1 larvae/m³) from a single cohort captured on 18 February 2003, while at Crooked Arm and Hall Arm, there was greater diversity of *Evechinus chloroticus* developmental stages over the entire sampling period. The great abundance of larvae collected at Grono Bay on 18 February may have corresponded to a spawning event just prior to the sampling, as a total of only 39.7 larvae/m³ were collected during the previous sampling on 11 February. Unfortunately, this cohort did not appear at Grono Bay or either of the inner fiord sites at the following sampling on 4 March. It is possible these larvae were lost to the open coast and not transported into the fiord in the head-ward moving seawater layer. Although larval abundance generally increased over time at both Crooked Arm and Grono Bay, this pattern was not observed at Hall Arm. These weak temporal patterns observed in this study fail to conclusively support the thought that larvae may travel up-fiord in deeper Head-ward moving waters, supplying Deep Cove and Hall Arm *E. chloroticus* populations with recruits.

Previous work by Wing et al. (2003) suggests that the overall *E. chloroticus* population in Doubtful Sound consists of several reproductive sources and sinks. Using models, the authors observed that the weak mean estuarine circulation within Doubtful Sound functioned to disperse particles (larvae) that were seeded at Espinosa Point (mid-fiord) throughout the fiord. Greatest densities were encountered after 30 days at mid-fiord though, with lower densities at the sill, and much lower densities at the head of the fiord. This model closely matched field observations (Wing et al. 2003). Work done by Perrin (2002) on population genetics of *E. chloroticus* in
Fiordland indicated that there were two groups of populations: the first group included outer fiord sites and the open coast, the second group contained samples collected from inner fiord sites. The author suggested that these genetically different groups are kept separate by habitat type (Perrin 2002). Interestingly though, genetic results also indicated that there was dispersal of larvae from inner fiord sites to other inner fiord sites (Perrin 2002), refuting the thought that larvae produced in Hall Arm or Deep Cove were incapable of dispersing seaward.

Statistical analysis of the vertical distribution of *Evechinus chloroticus* larvae in Doubtful Sound failed to result in significant links between salinity and the vertical distribution of larvae. Direct observations of the distributions though allude to the significance of salinity as a factor. Larvae at Hall Arm and Crooked Arm were always found below the LSL and greatest abundances at these sites were from 6m-8m, just below the LSL. Fluorescence/food abundance was a significant determinant of larval distribution on a few occasions, but compounding affects with other factors and possible sampling artifacts may account for its statistical significance. It is probable that these results are only applicable to fiord populations, as *E. chloroticus* larvae are positively phototaxic (personal observation) in culture and therefore would probably be found in surface layers of unstratified water columns. Further work could build upon this work study including a site completely beyond the sill of Doubtful Sound, as the Sill site (Grono Bay) used in this study was between the main basin sill (the Gut) and the outer sill at Hare’s Ears and therefore possibly not a true representation of coastal *E. chloroticus* larval production. Sampling every week through the spawning period may also uncover temporal and spatial patterns not discernable in this study due to patchiness and low overall recovery of *E. chloroticus* larvae.
Chapter 3. Effects of Salinity on *Evechinus chloroticus* embryos

3.1 Introduction

Echinoderms have been conventionally considered a solely marine phylum (Nichols 1964, Binyon 1966). They lack a differentiated excretory organ, and are therefore regarded as osmoconformers (Yaroslavtseva and Shirmunskii 1978). However, in regions where salinity has been noted to be relatively stable, but not necessarily oceanic in salinity, Echinoderms are found. In fact, species of Echinoderms have been observed inhabiting waters ranging from 0.5-80%o (Kinne 1964b, Binyon 1966). Although species are found at these salinity extremes, even these species have a range of salinities tolerable for life. A number of studies have shown that salinity tolerances of single species may differ between populations inhabiting diverse salinity regimes (Gezelius 1964, Dybem 1967, Stancyk and Shaffer 1977, Sarantchova 2001). Gezelius (1964) studied the sea urchin *Psammechinus miliaris*, as it exists in two phenotypes having differing salinity tolerances depending on the salinity regime in which it inhabits. The author observed that when urchins were directly transferred from their normal salinity regime to either a regime with a mean higher or lower salinity, that mortality was increased in the treatments compared to controls (Gezelius 1964).

The salinity tolerance of a single species has been shown to differ between ages and in different stages of the life cycle (Kinne 1964b, Vernberg and Vernberg 1975, Drouin et al. 1985). In many species a narrow salinity range is tolerable for gametes and embryos, tolerance to fluctuating salinity then increases during late larval stages or juvenile development, and finally the adult, again, has a narrow tolerance (Kinne 1964b, Dybem 1967, Vernberg and Vernberg 1970, Binyon 1972). Echinoderms have been identified as having particularly constricted salinity tolerances during development (Fenchel 1965, Kashenko 1992, 1998, Roller and Stickle 1993).

Salinity has the ability to affect many variables prior to the development of the larva, therefore it is an especially influential environmental factor. Greenwood and Bennett (1981) found that ova of the sea urchin *Parechinus angulosus* are unable to osmoregulate in cases where salinity differs from that of normal seawater. Low salinity contributed to mortality of the ova at salinities below 15‰ (Greenwood and
Spermatozoa of echinoderms also have been found to require rather high salinities in order to be functional (Thorson 1950, Kashenko 1998). Gezelius (1964) observed that gonads of *Psammechinus miliaris* cytolized and there was no regrowth of the gonad when adults were transferred from their normal habitat of salinities fluctuating between 16%o and 32%o into aquaria at a constant 15%o.

Normal development of embryos has been shown to be significantly altered by lowered salinity in echinoderms (Gezelius 1964, Dybem 1967, Hendler 1977, Kashenko 1992, 1998, Roller and Stickle 1993) and bivalve species (Davis and Calabrese 1964, Dos Santos and Nascimento 1985, Madrones-Ladja 2002). Roller and Stickle (1994), working with the sea urchins *Strongylocentrotus droebachiensis* and *S. pallidus*, found that the greatest mortality due to osmotic shock occurs during early embryogenesis at salinity extremes, but abnormal development occurs later in larval life at slightly higher salinities. Hendler (1977) found that *Amphiopius abditus* embryos reared in salinities ranging from 5-15%o had higher mortality than their siblings reared at higher salinities. The author suggested that salinities ranging from 25-40%o were necessary for the survival of the embryo (Hendler 1977). Working with the Japanese sea cucumber, *Stichopus japonicus*, Kashenko (1992, 1998) observed that fertilization took place in salinities from 18-32%o, but only salinities greater than 26%o allowed for normal development of greater than 50% of the embryos.

The sea urchin *Evechinus chloroticus* is a common grazer endemic to the New Zealand coast, offshore reefs, and the neighboring Three Kings, Chatham, and Snares Islands (McRae 1959, Pawson 1965, Dix 1970a). Throughout Fiordland animals may be exposed to reduced salinities due to extensive rainfall (>7m) in the region. In Doubtful Sound, additional input from the Manapouri Power Station effectively doubles the freshwater entering the fiord (Gibbs 2001).

The copious amount of rainfall and additional freshwater outflow contributes to a persistent Low Salinity Layer (LSL) in Doubtful Sound. The LSL in Doubtful Sound varies in thickness and is typically 1-5%o (Stanton 1984), while surface salinity is generally 34-35%o off the West Coast of New Zealand (Heath 1985). The LSL contributes to classic estuarine circulation in Doubtful Sound, the LSL progresses seaward while coastal seawater is drawn into the fiord and displaces bottom waters (Stanton and Pickard 1981). As the LSL flows seaward, it entrains higher salinity...
water and particles from below, slowly increasing in salinity towards the sill (Walls 1995, Bowman et al. 1999, Macrellis 2001).

The estuarine circulation in Doubtful Sound has the ability to structure *Evechinus chloroticus* populations, as this circulation is the main distributive force for larvae, leading to variation in recruitment (Wing et al. 2003). Perrin (2002) implicated the circulation in Doubtful Sound as a factor influencing the genetic heterogeneity of *E. chloroticus* populations through the fiord. The author found two genetically distinct groups, one consisting of samples collected from the outer fiords and open coast, the other comprising samples collected from the inner fiords. Restricted larval dispersal due to fiord hydrography was suggested as the cause of the differentiation between the groups (Perrin 2002).

Recruitment of larvae has been proposed to be a significant process in determining invertebrate population structure (Underwood and Fairweather 1989) and in *Evechinus chloroticus* (Lamare and Barker 1999). Variation in recruitment can be divided into three broad areas, pre-settlement processes, settlement processes, and post-settlement processes (Todd 1998, McEdward and Miner 2001). Salinity is a significant pre-settlement process as it can alter the development and survival of developing stages (e.g. Vernberg and Vernberg 1970, Watts et al. 1982, Roller and Stickle 1985, 1993, 1994, Kashenko 1998, Metaxas 1998, Sarantchova 2001), as well as their vertical distribution (Scarratt and Raine 1967, Metaxas and Young 1998).

The salinity tolerance of *Evechinus chloroticus* embryos is unknown. As the estuarine circulation of Doubtful Sound is driven by the LSL, its seaward travel is the main dispersive force of developing stages from the head in this fiord. *E. chloroticus* is a dioeciously spawning echinoderm, directly releasing its gametes into the water column. Direct spawning of *E. chloroticus* into the boundary layer or directly into the LSL has been observed (Miles Lamare, Steve Wing, personal communications). If embryos and larvae of *E. chloroticus* cannot tolerate lowered salinities, their ability to recruit from their parental population to others may be affected. Recruitment of *E. chloroticus* into populations throughout Doubtful Sound may, therefore, be greatly influenced by the LSL. The LSL is generally thicker at the head of the fiord compared to the sill, therefore there may be differences in the salinity tolerance of developing stages resulting from spawning at the separated locations. Identification of decreased survival due to low salinity and the influence of salinity on development
and hatching of *E. chloroticus* may lead to greater understanding of the recruitment dynamics of this species within Doubtful Sound and other systems with similar features. Therefore, the affects of low salinity on development and growth of *Evechinus chloroticus* embryos was investigated.
3.2 Materials and Methods

General Methods

The laboratory portion of this study took place from January 2003 until April 2003. Divers collected adult *Evechinus chloroticus* (100-140mm Test diameter) from Deep Cove and Causet Cove in Doubtful Sound (Figure 3.1) to represent Head and Sill populations, respectively. These adults were kept separate and transported in seawater-filled 15L buckets to the Portobello Marine Laboratory on the Otago Peninsula. Adults were then placed into 50L flow-through aquaria awaiting experiments. Water flowing into the aquaria was from the Otago Harbor at ambient salinity and temperature. Otago Harbor water generally is between 15°C and 12°C from January to April, respectively. Salinity in the harbor during this period is essentially oceanic (33-35‰). Adults were fed *ad libitum* weekly with *Macrocystis pyrifera* (Linnaeus) a natural food for *Evechinus chloroticus* (Barker et al. 1998). Standard methods were followed for the artificial spawning of adults (Dix 1970b, Leahy 1986). Individuals were induced to spawn with a 3-5mL injection of 0.5mol/L KCl into the body cavity. The injection of KCl causes muscular contraction of the gonads and mature gametes are expelled through the gonopores. After injection, individuals were inverted over a 200mL beaker filled with one-micron (1µm) bag-filtered (Filter Media (NZ) LTD. Auckland) seawater (hereafter referred to as “filtered seawater”). Eggs that were shed were allowed to settle, then rinsed 3-5 times with filtered seawater. Eggs from 2-5 females were allowed to settle individually, then mixed. Sperm was collected “dry” from two males (approximately 5mL dry sperm from each male) and resuspended in 200mL filtered seawater, then 10mL of this sperm suspension was added to the mixed eggs, and the contents of the container stirred. The mixture was allowed to settle for five minutes, then rinsed with filtered seawater. This procedure was repeated twice to reduce polyspermy. A sample of eggs was observed with an Olympus BH-2 compound microscope under 200X power to determine percent fertilization. All fertilizations resulted in greater than 95% fertilization (96-99%, n=4). A 10mL sample of fertilized eggs was also observed with an Olympus SZ30 dissecting microscope under 40X power to determine the number of fertilized eggs per milliliter. Fertilized eggs were stirred and about 15 fertilized
Figure 3.1 Locations of adult *Evechinus chloroicus* collection in Doubtful Sound for salinity experiments, Causet Cove and Deep Cove, representing sill and head populations, respectively.
eggs/mL were added to each of the experimental jars. There were three experimental jars per salinity treatment and jars were filled with 3L of experimental seawater.

Experimental seawater was filtered and either concentrated through evaporation for the 35% treatments, or diluted with 1μm Milipore® filtered freshwater to obtain the required dilution. All experiments were conducted in a temperature-controlled room. The temperature in this room fluctuated between 13-15°C throughout the duration of the egg and larval experiments and was under a 12:12 light/dark cycle.

**Egg Experiment 1**

In the first egg experiment, eggs were fertilized with sperm from the same location. Embryos were kept separated by location, either head or sill. Salinities from 5% to 35% at 5% increments were tested in this first experiment. Embryos were allowed to develop and 50mL of the treatment water and fertilized eggs were sampled at 2, 7, 12, and 18 hours after fertilization. Samples were fixed in 50% isopropyl alcohol and the number of damaged and undamaged embryos counted. Treatments were then monitored for hatched larvae. Samples were taken from the just the Head embryos at 42 hours and both the Head and Sill embryos and 72 hours to determine the number of hatched and unhatched embryos.

**Egg Experiment 2**

In the second egg experiment, gametes from individuals were not separated by location, but mixed. Salinities from 25% to 35% at 2.5% increments were tested in the second experiment. Eggs were allowed to develop and 50mL sampled at 24, 48, 54, 60, 66, and 72 hours after fertilization. Samples were fixed in 50% isopropyl alcohol and the number of hatched and unhatched embryos counted at these intervals. All samples were observed using an Olympus BH-2 compound microscope under 200X power. Eggs were classified as hatched or unhatched for all treatments.

**Statistical Analysis**

These data of damage and hatching were arcsine (√(x/100)) transformed prior analysis. Tests of Analysis of Variance (ANOVA) were performed to determine whether damage and hatching were affected by salinity and time (and in the first
experiment, location as well) using the statistical program MINITAB® (Minitab, Inc., State College, PA, USA). Statistical significance was inferred if the p-value was less than or equal to 0.05.
### 3.3 Results

**Egg Experiment 1**

Eggs showed signs of damage after only two hours. General distension and lysing of embryos in lower salinities was common, as well as abnormal development (Figure 3.2). Damage was much greater in lower salinities than in the 35%o control (Figure 3.3). Over the 18 hours of monitoring damage, ANOVA results indicated that location was not a significant factor \((P = 0.404)\), while both salinity \((P < 0.001)\), time \((P < 0.001)\), and the combination of salinity*time \((P < 0.001)\) were all very significant factors (Table 3.1). Two-way ANOVA of the damage data at the different sampling times showed that at two hours, salinity \((P < 0.001)\), as well as location \((P = 0.033)\), where the parents originated, were both statistically significant. Damaged eggs in the following hours continued to show significance with salinity, but location became an insignificant factor. Interestingly, at 18 hours, the interaction of salinity*location was significant \((P = 0.004)\), although location was not significant \((P=0.063)\) (Table 3.2). Although variations in the per cent of embryos damaged was observed, the general pattern was for embryos in lower salinities to become damaged earlier and at higher frequencies than those embryos in salinities closer to the 35%o control.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq. SS</th>
<th>Adj. SS</th>
<th>Adj. MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>6</td>
<td>2.361601</td>
<td>2.361601</td>
<td>0.393600</td>
<td>70.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>0.547059</td>
<td>0.547059</td>
<td>0.182353</td>
<td>32.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>0.003919</td>
<td>0.003919</td>
<td>0.003919</td>
<td>0.70</td>
<td>0.404</td>
</tr>
<tr>
<td>Salinity*Time</td>
<td>18</td>
<td>1.676836</td>
<td>1.676836</td>
<td>0.093158</td>
<td>16.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity*Location</td>
<td>6</td>
<td>0.014812</td>
<td>0.014812</td>
<td>0.002469</td>
<td>0.44</td>
<td>0.849</td>
</tr>
<tr>
<td>Location*Time</td>
<td>3</td>
<td>0.033214</td>
<td>0.033214</td>
<td>0.011080</td>
<td>1.99</td>
<td>0.120</td>
</tr>
<tr>
<td>Salinity<em>Time</em>Location</td>
<td>18</td>
<td>0.108988</td>
<td>0.108988</td>
<td>0.006055</td>
<td>1.09</td>
<td>0.376</td>
</tr>
<tr>
<td>Error</td>
<td>112</td>
<td>0.624864</td>
<td>0.624864</td>
<td>0.005579</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>5.371321</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 Three-way ANOVA results for overall damage of *Evechinus chloroticus* embryos. **Statistical Significance**
Table 3.2 Two-way ANOVA results for damaged *Evechinus chloroticus* embryos over 18 hours. **Statistical Significance**

<table>
<thead>
<tr>
<th>Time</th>
<th>Salinity</th>
<th>Location</th>
<th>Salinity*Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>$F(6,41)\ 6.46$,</td>
<td>$F(1,41)\ 5.05$,</td>
<td>$F(6,41)\ 1.33$,</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.033</td>
<td>p=0.276</td>
</tr>
<tr>
<td>7 hours</td>
<td>$F(6,41)\ 1239.82$</td>
<td>$F(1,41)\ 2.22$,</td>
<td>$F(6,41)\ 0.48$,</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.147</td>
<td>p=0.821</td>
</tr>
<tr>
<td>12 hours</td>
<td>$F(6,41)\ 16.64$,</td>
<td>$F(1,41)\ 1.35$,</td>
<td>$F(6,41)\ 0.71$,</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.255</td>
<td>p=0.646</td>
</tr>
<tr>
<td>18 hours</td>
<td>$F(6,41)\ 16.38$,</td>
<td>$F(1,13)\ 3.75$,</td>
<td>$F(6,41)\ 4.20$,</td>
</tr>
<tr>
<td></td>
<td>p=0.000</td>
<td>p=0.063</td>
<td>p=0.004</td>
</tr>
</tbody>
</table>

No *Evechinus chloroticus* embryos hatched in salinities less than 27.5%. In the first experiment embryos had not hatched by 42 hours post-fertilization. After 72 hours, all embryos in the 30% and 35% treatments had hatched.
Figure 3.2 Common damage among *Evechinus chloroticus* embryos in lowered salinities compared to a normally developing embryo (A). All embryos observed under 200X power using an Olympus BH-2 compound microscope. Scale bar represents 100μm.
Figure 3.3 Per cent damage (+/- SE) (n = 3) to *Evechinus chloroticus* embryos over time at tested salinities for the Head and Sill. Key is identical for all times. The * represents a statistically significant difference.
**Egg Experiment 2**

The second experiment was monitored from 24 hours until 72 hours post-fertilization. The hatching of *Evechinus chloroticus* embryos is illustrated in Figure 3.4. Embryos reared in 35% hatched earlier than those reared at lower salinities. None the less, both egg experiments showed identical results for hatching embryos, with Salinity (P < 0.001), Time (P < 0.001), and the interaction Salinity*Time (P < 0.001) all being significant factors (Table 3.3). Although the first experiment used embryos which were separated by location (Table 3.3a), the results were identical to the second experiment which used embryos of mixed parentage (Table 3.3b).

Table 3.3. Two-way ANOVA overall hatching of *Evechinus chloroticus* embryos.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>6</td>
<td>14.0835</td>
<td>2.3472</td>
<td>9.7*10⁻⁴</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>2.8279</td>
<td>2.8279</td>
<td>2.3*10⁻⁵</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity*Time</td>
<td>6</td>
<td>7.0418</td>
<td>1.1736</td>
<td>9.7*10⁻⁴</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>49</td>
<td>0.0006</td>
<td>0.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>23.9538</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3a. Two-way ANOVA overall hatching of *Evechinus chloroticus* embryos from separated locations (Egg Experiment 1). *Statistical Significance*

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>4</td>
<td>9.5504</td>
<td>2.3876</td>
<td>576.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>5</td>
<td>14.2233</td>
<td>2.8447</td>
<td>686.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity*Time</td>
<td>20</td>
<td>6.7092</td>
<td>0.3355</td>
<td>80.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>0.2487</td>
<td>0.0041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>30.7318</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3b. Two-way ANOVA overall hatching of *Evechinus chloroticus* embryos from mixed parentage (Egg Experiment 2). *Statistical Significance*
Figure 3.4 Hatching of *Evechinus chloroticus* embryos over time at the different salinities tested ($n = 3$). Error bars have been excluded for clarity.
3.4 Discussion

In Doubtful Sound, a persistent Low Salinity Layer overlies higher salinity water in which adult *Evechinus chloroticus* live. The dynamics of this LSL have been implicated as the dominant dispersive force for *E. chloroticus* in Doubtful Sound (Wing et al. 2003). *Evechinus chloroticus* is a broadcast spawner, therefore the chances of its gametes, fertilized eggs, and embryos encountering waters of reduced salinity in Doubtful Sound is quite possible. If trapped the LSL, development and survival may be affected. Therefore, the affects of low salinity on *Evechinus chloroticus* early development and hatching were investigated.

Previous studies of embryonic development in other invertebrates have indicated that development is influenced by low salinity (eg. Gezelius 1964, Hendler 1977, Roller and Stickle 1993, Madrones-Ladja 2002). In the present study, normal development of *Evechinus chloroticus* embryos was significantly influenced by lowered salinity. *Evechinus chloroticus* failed to develop in all salinities lower and including the 25%o treatments. After only two hours, embryos in the lower salinity (5-15%o) treatments were showing signs of distress, including lysis and water retention. All salinity treatments except the 35%o control contained small amounts of damaged embryos. Figure 3.3 illustrates the effect of extremely low salinities on *E. chloroticus* embryos. In the 5%o and 10%o treatments, a considerable percentage of developing embryos were damaged at the 7 hour and 12 hour samplings. Although there were instances in which there were great differences between the per cent of damaged embryos from the Head and Sill, these differences were only statistically significant at the two hour sampling (Table 3.2).

As the LSL is generally 1-5%o (Stanton 1984), significant numbers of embryos would be damaged if they were swept into and trapped in the LSL. Throughout the experiment, the number of damaged embryos was always significantly related to salinity. The overall result of the interaction of salinity*time also indicates that some sort of dose effect is taking place with the two factors. Lower (15-25%o) than oceanic salinities were well tolerated by the embryos, as damage in these salinities was rarely greater than 10%, but *E. chloroticus* embryos failed to hatch in these salinities. Curiously, the percentage of damaged embryos observed decreased at the 18 hour sampling. This observation may be an artifact of the sampling technique. Although the jars were gently stirred before sampling, severely damaged embryos may not be as
buoyant, may have dissolved, or could have settled at the bottom of the container. Therefore, these embryos were not sampled, leading to the apparent decrease in the amount of damaged embryos.

The location from which the parents originated was only a significant factor concerning the damage of the embryos at the first sampling. This statistical insignificance of location in these analyses may indicate that, although the adult *E. chloroticus* populations in Deep Cove may experience periodic exposure to lowered salinities and tolerate them, this tolerance is not passed to the offspring. Dybern (1967) observed among populations of the ascidian *Ciona intestinalis* living under different salinity regimes that their offspring had differing salinity tolerances. Gezelius (1964) observed that the salinity tolerance of adult *Psammechinus miliaris* was lower (16-34‰) than the tolerance for either its gametes or embryos (27-32‰). The salinity tolerance of *E. chloroticus* gametes was not tested in these experiments, but it would be interesting to establish whether they have similar salinity tolerances as embryos.

Along with the normal development of *Evechinus chloroticus* embryos, the hatching of these embryos into free-swimming blastulae is another critical phase in development. These blastulae contribute to the total larval pool able to recruit back into the population or to disperse to other populations. At 24 hours post-fertilization, *E. chloroticus* embryos in all treatments were still developing, and had not reached the free-swimming blastula stage. At 48 hours, greater than 25% of embryos had hatched in the 35‰ treatments, while none had hatched in any of the other treatments. Through time, (Figure 2.4) embryos in each of the treatments greater than 27.5‰ hatched. As *E. chloroticus* embryos in the 35‰ and 32.5‰ treatments hatched in greater numbers earlier than the lower salinity treatments, this lag in hatching between *E. chloroticus* in the higher (32.5 and 35‰) and lower salinity (27.5 and 30‰) treatments may relate into a lag in development time. Kashenko (1998) found similar results in the delay of hatching. The author observed that blastula development of *Stichopus japonicus* was significantly delayed in those reared in 26‰ and 24‰ compared with their siblings reared in 28‰ and 32‰ for two temperatures. The author also noted that the majority of those blastulae hatching from those low salinities were abnormal (Kashenko 1998).
Although investigations into the ability of *Evechinus chloroticus* embryos to recover from short-term exposure to lowered salinities were not undertaken, some significant conclusions can be drawn. Low salinities did not result in extreme numbers of damaged *E. chloroticus* embryos until 7 hours post-fertilization, therefore, it may be possible for embryos to survive some degree of exposure to lowered salinities in the wild, but they cannot complete development at these low salinities. Consequently, favorable development of *E. chloroticus* is not possible in the LSL as it is generally less saline than the salinities in which normal development occurred. The chances of an *E. chloroticus* embryo surviving in the LSL are also probably quite small. Thus successful dispersal in the LSL from the head of Doubtful Sound to the sill is probably impossible, as the LSL has been estimated to require 6-10 days to travel from Deep Cove to the sill (Bowman et al. 1999). Nearer the sill, though, where salinities in the LSL are more oceanic, embryos of *E. chloroticus* may be able to survive some dispersal through the LSL. The failure of *E. chloroticus* embryos to hatch in salinities less than 27.5%o indicates that oceanic salinities are required for successful embryonic development.

The extreme influence that salinity plays on the normal development and hatching of *Evechinus chloroticus* may help refine our understanding of the pattern of recruitment observed for this species in Doubtful Sound. The embryonic development and hatching of *Evechinus chloroticus* was significantly influenced by the salinity in which embryos were reared in this study. Embryos of *E. chloroticus* in the lower salinity treatments became damaged earlier and in greater numbers than their siblings in higher salinities. Relatively high salinities were required for the successful hatching of *E. chloroticus* blastulae. As the lowest salinities investigated are commonly observed in the LSL of Doubtful Sound, these would likely be encountered by *E. chloroticus* embryos if they were swept into the LSL. Therefore, the recruitment dynamics of this species are probably immensely influenced by the Low Salinity Layer. Investigations into the ability of *E. chloroticus* embryos to recover from short-term exposure to low salinities would advance the understanding of whether *E. chloroticus* dispersal by means of the LSL is possible at any location along Doubtful Sound.
Chapter 4. Effects of Salinity on *Evechinus chloroticus* larvae

4.1 Introduction

Commonly considered a solely marine phylum, Echinoderms are regarded as being stenohaline (Nichols 1964, Binyon 1966). This concept is slowly being re-evaluated, as many species have been found living in extremely low (0.5‰) and extremely high (80‰) salinities (Kinne 1964b, Binyon 1966). Although various species of Echinoderms tolerate extreme salinities, even these species live within certain tolerance ranges. Studies have shown that populations of single species inhabiting differing salinity regimes often possess differing salinity tolerances (Gezelius 1964, Dybem 1967, Stancyk and Shaffer 1977, Sarantchova 2001). Working with *Ophiothrix angulata* (Say), Stancyk and Shaffer (1977) found that animals from a population inhabiting a higher mean salinity (30‰) estuary were less tolerant of lowered salinities than animals sampled from a population living in an estuary with slightly lower mean salinity (25‰). The authors attributed this difference in salinity tolerance to the differences in the length of time in which salinities in the estuaries fluctuated considerably. At the higher mean salinity estuary, periods of reduced salinity lasted a relatively short time, a few hours, while at the lower mean salinity estuary, periods of reduced salinity commonly lasted from a few days to weeks (Stancyk and Shaffer 1977).

The salinity tolerance of a species may even differ between ages and in different stages of the lifecycle (Kinne 1964b, Vernberg and Vernberg 1975, Drouin et al. 1985). Larvae are, by definition, very different from adult animals. In many species, salinity tolerance is most narrow during early ontogeny, then increases somewhat during late larval stages or juvenile development, and finally decreases again in the adult (Kinne 1964b, Dybem 1967, Vernberg and Vernberg 1970, Binyon 1972). Echinoderms have been identified as having particularly constricted salinity tolerances during development (Fenchel 1965, Kashenko 1992, 1998, Roller and Stickle 1993).

Salinity can act on many variables before the development of the larva. Gametes of Echinoderms have been shown to be detrimentally affected by lowered salinity (Thorson 1950, Gezelius 1964, Hendler 1977, Greenwood and Bennett 1981, Kashenko 1998). Correct embryonic development directly affects the pool of larvae.
available to recruit into the population. Normal development of embryos has been shown to be significantly altered by lowered salinity in echinoderms (Hendler 1977, Kashenko 1992, 1998, Roller and Stickle 1993).

Development rate of larvae was directly related to salinity in a number of studies (e.g. Vemberg and Vemberg 1970, Watts et al. 1982, Roller and Stickle 1985, 1993, 1994, Metaxas 1998). Roller and Stickle (1994) found that development rates and survival to metamorphosis of larvae of the sea urchins *Strongylocentrotus droebachiensis* and *S. pallidus* were significantly affected by salinity. Larval development was incomplete in both species below 20‰. Their results also indicated that the greatest mortality due to osmotic shock occurs during early embryogenesis at salinity extremes, but abnormal development occurs later in larval life at slightly higher salinities (Roller and Stickle 1994). Metaxas (1998), experimenting with the sea urchin *Echinometra lucunter*, found that mortality of developing larvae was significantly decreased in 33‰ and 27‰ treatments as compared to lower salinity treatments (15‰-24‰). Larvae in the 33‰ treatments were able to complete development to the 8-arm stage, while those larvae in the lower salinity treatments failed to develop further than the 4-arm stage (Metaxas 1998). Lower larval survival has also been observed in asteroids (Hendler 1977, Watts et al. 1982, Sarantchova 2001), the Japanese Sea Cucumber *Stichopus japonicus* (Gavrilova and Mokretsova 1983, Kashenko 1993, 1998) and other invertebrates (e.g. Scheltema 1965, Johns 1981, Laughlin 1983, Anger 1985, Zimmerman and Pechenik 1991, Charmantier et al. 2001).

The sea urchin *Evechinus chloroticus* is a common member of the intertidal and subtidal community in the Fiordland region of New Zealand (McRae 1959, Pawson 1965, Dix 1970a, Wing et al. 2001). The abundance of *E. chloroticus* in Doubtful Sound has been suggested as influencing the structure of algal communities through their vertical distribution and destructive grazing, leading to less productive coralline-dominated barrens (Wing et al. 2001). Throughout Fiordland animals may be exposed to reduced salinities due to extensive rainfall (>7m) in the region. In Doubtful Sound, additional input from the Manapouri Power Station effectively doubles the freshwater entering the fiord (Gibbs 2001).

The copious amount of rainfall and additional freshwater outflow contributes to a persistent Low Salinity Layer (LSL) in Doubtful Sound. The LSL in Doubtful
Sound varies in thickness and is typically 1-5%o (Stanton 1984), while surface salinity is generally 34-35%o off the West Coast of New Zealand (Heath 1985). The LSL contributes to classic estuarine circulation in Doubtful Sound; this LSL progresses seaward while coastal seawater is drawn into the fiord and displaces bottom waters (Stanton and Pickard 1981). As the LSL flows seaward, it entrains higher salinity water and particles from below, slowly increasing in salinity towards the sill (Walls 1995, Bowman et al 1999, Macrellis 2001). The estuarine circulation in Doubtful Sound has the ability to structure *Evechinus chloroticus* populations, as this circulation is the main distributive force for larvae, leading to variation in recruitment (Wing et al. 2003).

Recruitment of larvae has been proposed to be a significant process in determining invertebrate population structure (Underwood and Fairweather 1989) and in *Evechinus chloroticus* (Lamare and Barker 1999). Variation in recruitment can be divided into three broad areas, pre-settlement processes, settlement processes, and post-settlement processes (Todd 1998, McEdward and Miner 2001). Salinity is a significant pre-settlement process as it can alter the development and survival larvae (eg. Vernberg and Vernberg 1970, Watts et al. 1982, Roller and Stickle 1985, 1993, 1994, Kashenko 1998, Metaxas 1998, Sarantchova 2001), as well as their vertical distribution (Scarratt and Raine 1967, Metaxas and Young 1998).

The salinity tolerance of *Evechinus chloroticus* larvae is unknown. The estuarine circulation of Doubtful Sound, and hence *E. chloroticus* larval dispersal, is driven by the LSL. If larvae cannot survive and develop within salinities similar to the LSL, they will not be able to travel in the LSL and use it as a dispersal mechanism. Only larvae inhabiting deeper waters of higher salinities would be able to successfully recruit into other populations. Identification of aberrations in development due to low salinity and the influence of salinity on development rate of *E. chloroticus* may lead to greater understanding of the recruitment dynamics of this species within Doubtful Sound and other systems with similar features. Therefore, the affects of low salinity on development and growth of *Evechinus chloroticus* was investigated.
4.2 Methods

General Methods

The laboratory portion of this study took place from January to April 2003. Divers collected adult *Evechinus chloroticus* (100-140 mm Test diameter) from Deep Cove and Causet Cove in Doubtful Sound to represent Head and Sill populations, respectively (see Figure 3.1). Adults from these two locations were kept separate and transported in seawater-filled 15L buckets from Doubtful Sound to the Portobello Marine Laboratory on the Otago Peninsula. Adults were then placed into 50L flow-through aquaria awaiting experiments. These aculds were utilized for both the embryo experiments (Chapter 3) and these experiments. Therefore, holding conditions were identical for all adults used in the experiments. Methods for artificial spawning of *E. chloroticus* and determination of successful fertilization were directly duplicated as in Chapter 3.

Experimental seawater was bag-filtered (1µm) and either concentrated through evaporation for the 35% treatments, or diluted with 1µm Millipore® filtered freshwater to obtain the required dilution. Reserve dilutions were held in 10L plastic buckets with lids to discourage evaporation and retained in the experimental room to keep water a uniform temperature. Salinity was checked using a standard salinometer (Horiba, Ltd. Japan, model # U-10). All experiments were conducted in the same temperature-controlled room as experiments in Chapter 3.

Fertilized eggs were stirred and approximately 15 fertilized eggs/mL added to each of the experimental glass jars. There were three jars per treatment and jars were filled with 3L of experimental seawater. Developing larvae were stirred by a mechanism of motor-driven paddles at a rate of approximately 10 strokes per minute. Paddles were activated after three days of development (to allow for hatching) and stirred continuously until the completion of the experiment. Experimental jars were sampled (50mL of the culture) every Monday, Wednesday, and Friday throughout the experiment. Larval samples were fixed in 50% isopropyl alcohol and placed into appropriately labeled jars. Samples contained between 0 and approximately 100 *E. chloroticus* larvae. After sampling, 10mL each of the phytoplankton *Rhodomonas lens* and *Dunaliella tertiolecta* in the exponential phase of growth (approximately 8000 cells/mL in the larval culture) were added to the experimental jars to feed
remaining larvae. These volumes and types of algae were deemed appropriate to allow for *ad libitum* feeding of larvae (Miles Lamare, personal communication). Algae were cultured in 1.5L Erlenmeyer flasks using standard techniques (Keller, *et al.* 1988). Water in each of the experimental jars was replaced once a week using a siphon with mesh that retained larvae. Jars were then refilled with clean water of the appropriate dilution. On days when water was replaced, feeding took place after siphoning.

**Larval Experiment 1**

During the first larval growth experiment, fertilized eggs were separated by parental location, either head or sill. In this first experiment, due to the lack of containers, embryos were allowed to develop in ambient salinity (33%) water for the first three days of the experiment. Embryos were then added to their experimental treatments; salinities ranging from 25% to 35% at 5% increments.

**Larval Experiment 2**

In the second larval growth experiment, fertilized eggs were placed directly after fertilization into their experimental containers. Salinities in the second larval growth experiment mirrored salinities used in the second embryonic development experiment, 25, 27.5, 30, 32.5 and 35%. After these differences, larval culture methods were identical for both of the experiments. Embryos were not grown in salinities less than 27.5% as previous experiments (Chapter 3, current study) revealed that embryos failed to complete normal development in those lower salinities.

**Analysis**

Samples were observed under 31X power using a Wild-Heerbrugg dissecting microscope with video camera attached. The camera was connected to an 8600/200 Power Macintosh computer with NIH Image 1.61 software (National Institutes of Health, Bethesda, Maryland, USA). Larvae were viewed and total larval length body length and arm length were measured in micrometers (µm) using the software (Figure 4.1). Larvae were also categorized depending on developmental stage (see Table 2.1, Figure 2.3). Where obvious morphological aberrations had taken place, larvae were still measured and staged, but their measurements flagged, and classified as
“deformed.” Deformed larvae included larvae with extra arms, fused arms, missing arms, shrunken arms, and arms with exposed spicules. The percentage of normally developing larvae was arcsine transformed prior to analyses. All measurements were transformed using the natural log of the measurements. Analyses were carried out on all data and then the data after the removal of the “deformed” larval measurements. ANOVA were utilized for these data using the statistical programs SPSS® (SPSS, Inc., Chicago, IL, USA) and MINITAB (Minitab, Inc., State College, PA, USA). Statistical significance was inferred if the p-value was less than or equal to 0.05.
Figure 4.1 Morphometric measurements taken on *Evechinus chloroticus* larvae: postoral arm length (AL) and total larval body length (BL) (adapted from Lamare 1997).
4.3 Results

Evechinus chloroticus larvae failed to grow in any of the 25% treatments in either of the experiments.

Larval Experiment 1

The first larval experiment, in which the Evechinus chloroticus larvae were segregated by the location in which their parents lived, failed to result in any significant numbers of growing larvae. A total of 108 larvae were captured and measured. All of these larvae grew in the 35% treatments. Consequently, analysis of the affect of lowered salinities on larval growth was impossible.

Larval Experiment 2

The second larval growth experiment consisted of Evechinus chloroticus larvae resulting from gametes mixed from the two sampling locations.

Morphometrics

At three days post-fertilization, Body Length of Evechinus chloroticus larvae was not significantly affected by Salinity (P = 0.625). ANOVA results also indicated that the ratio of Arm Length to Body Length was significantly influenced by the treatment salinity (P < 0.001). At five days, salinity was now a very significant factor in the Body Length growth of the larvae (P < 0.001). ANOVA results continued to indicate that the ratio of Arm Length to Body Length was significantly different (P < 0.001) among the four successful treatments at five days of age. At seven days Body Length of E. chloroticus larvae were significantly affected by salinity (P < 0.001). ANOVA results indicated that again the Arm Length/Body Length ratio was a statistically significant (P < 0.001) among the four treatments in which larvae grew. An analysis of this first weeks worth of data revealed that Age (P < 0.001), Salinity (P < 0.001), and the interaction of Age*Salinity (P < 0.001) were all very significant factors influencing the Body Length of a larva. Salinity continued to be a significant factor in the growth of E. chloroticus larvae on most days throughout the experiment (Table 4.1). The change between the relationships of Arm Length to Body Length can be seen in Figure 4.2.
Table 4.2 Results of one-way ANOVA tests for *Evechinus chloroticus* larvae. The first column is ANOVA for the ratio of Arm Length/Body Length to salinity, the other columns relate to the Body Length of larvae to the different factors. Develop. Stage refers to the developmental stage of the *E. chloroticus* larvae in the treatments, whether they were 4, 6, or 8 armed. Statistically insignificant value.

<table>
<thead>
<tr>
<th>Day</th>
<th>Salinity</th>
<th>Arm Length/Body Length</th>
<th>Age (Time)</th>
<th>Salinity</th>
<th>Age x Salinity</th>
<th>Develop. Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>F(3,577)</td>
<td>919.27, p&lt;0.001</td>
<td>-</td>
<td>F(3,47)</td>
<td>0.59, p=0.625</td>
<td>F(3,577) 1303.52, p&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>F(3,515)</td>
<td>43.03, p&lt;0.001</td>
<td>-</td>
<td>F(3,55)</td>
<td>25.04, p&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>F(3,562)</td>
<td>25.93, p&lt;0.001</td>
<td>-</td>
<td>F(3,50)</td>
<td>16.78, p&lt;0.001</td>
<td>F(3,562) 65.34, p&lt;0.001</td>
</tr>
<tr>
<td>3-7</td>
<td>F(3,1756)</td>
<td>917.72, p&lt;0.001</td>
<td>F(2,1536) 102.91, p&lt;0.001</td>
<td>F(3,1556) 12.46, p&lt;0.001</td>
<td>F(3,1536) 14.32, p&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>F(3,501)</td>
<td>8.81, p&lt;0.001</td>
<td>-</td>
<td>F(3,42)</td>
<td>24.28, p&lt;0.001</td>
<td>F(3,501) 65.20, p&lt;0.001</td>
</tr>
<tr>
<td>12</td>
<td>F(3,477)</td>
<td>13.28, p&lt;0.001</td>
<td>-</td>
<td>F(3,42)</td>
<td>11.36, p&lt;0.001</td>
<td>F(3,477) 47.27, p&lt;0.001</td>
</tr>
<tr>
<td>14</td>
<td>F(3,571)</td>
<td>14.88, p&lt;0.001</td>
<td>-</td>
<td>F(3,52)</td>
<td>14.13, p&lt;0.001</td>
<td>F(3,571) 27.15, p&lt;0.001</td>
</tr>
<tr>
<td>10-14</td>
<td>F(3,1551)</td>
<td>26.92, p&lt;0.001</td>
<td>F(2,1374) 30.55, p&lt;0.001</td>
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<td>F(6,1374) 2.13, p=0.047</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F(3,529)</td>
<td>5.86, p=0.001</td>
<td>-</td>
<td>F(3,458)</td>
<td>19.02, p=0.000</td>
<td>F(3,529) 30.95, p=0.000</td>
</tr>
<tr>
<td>19</td>
<td>F(3,769)</td>
<td>3.46, p=0.016</td>
<td>-</td>
<td>F(3,755)</td>
<td>84.22, p=0.000</td>
<td>F(3,769) 38.16, p=0.000</td>
</tr>
<tr>
<td>21</td>
<td>F(3,514)</td>
<td>12.38, p&lt;0.001</td>
<td>-</td>
<td>F(3,493)</td>
<td>11.89, p=0.000</td>
<td>F(3,517) 13.53, p=0.000</td>
</tr>
<tr>
<td>17-21</td>
<td>F(3,1817)</td>
<td>2.62, p=0.050</td>
<td>F(2,1706) 89.83, p=0.000</td>
<td>F(3,1706) 73.11, p=0.000</td>
<td>F(6,1706) 14.34, p=0.000</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2-continued Results of one-way ANOVA tests for *Evechinus chloroticus* larvae. Develop. Stage refers to the developmental stage of the *E. chloroticus* larvae in the treatments, whether they were 4, 6, or 8 armed.

Statistically insignificant value †

<table>
<thead>
<tr>
<th>Day</th>
<th>Salinity</th>
<th>Arm Length/Body Length</th>
<th>Age (Time)</th>
<th>Salinity</th>
<th>Age x Salinity</th>
<th>Develop. Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>F(3,323)</td>
<td>3.73, p=0.012</td>
<td>F(3,297)</td>
<td>1460, p&lt;0.001</td>
<td>F(3,323) 6.06, p=0.001</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>F(3,310)</td>
<td>1.79, p=0.149†</td>
<td>F(3,310)</td>
<td>8.02, p&lt;0.001</td>
<td>F(3,310) 0.80, p=0.494†</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>F(3,302)</td>
<td>6.49, p&lt;0.001</td>
<td>F(3,302)</td>
<td>2.76, p=0.042</td>
<td>F(3,302) 2.50, p=0.060†</td>
<td></td>
</tr>
<tr>
<td>24-28</td>
<td>F(3,937)</td>
<td>4.98, p=0.002</td>
<td>F(2,911)</td>
<td>17.92, p&lt;0.001</td>
<td>F(6,911) 4.23, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>F(1,269)</td>
<td>0.47, p=0.706†</td>
<td>F(3,269)</td>
<td>3.81, p=0.11</td>
<td>F(3,269) 0.34, p=0.793†</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>F(3,176)</td>
<td>2.26, p=0.083†</td>
<td>F(3,176)</td>
<td>2.27, p=0.082†</td>
<td>F(3,176) 0.78, p=0.508†</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>F(3,86)</td>
<td>0.44, p=0.723†</td>
<td>F(3,86)</td>
<td>0.42, p=0.737†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td>F(3,533)</td>
<td>0.72, p=0.539†</td>
<td>F(2,533)</td>
<td>1.22, p&lt;0.001</td>
<td>F(5,533) 1.65, F(6,533) 1.02, p=0.409†</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>F(3,55)</td>
<td>2.98, p=0.039</td>
<td>F(3,55)</td>
<td>1.26, p=0.298†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>F(3,48)</td>
<td>6.14, p=0.001</td>
<td>F(3,48)</td>
<td>1.13, p=0.347†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>F(2,34)</td>
<td>1.49, p=0.240†</td>
<td>F(3,34)</td>
<td>0.08, p=0.920†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>38-42</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>F(2,8)</td>
<td>0.26, p=0.776†</td>
<td>F(2,8)</td>
<td>0.59, p=0.590†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>F(3,127)</td>
<td>3.52, p=0.017</td>
<td>F(3,127)</td>
<td>1.06, p=0.368†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>45-47</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.2 (over pages)  Development over time between Ln Arm Length and Ln Body Length in *Evechinus chloroticus* larvae in four salinity treatments. Note axes are not identical among graphs.

Salinity treatments

- □ 35psu
- ◇ 32.5 psu
- ○ 30 psu
- △ 27.5 psu
Development rate

After three days, one-way ANOVA showed that salinity was a very significant factor in the developmental stage of the larvae ($F_{(3,57)} = 1303.52, P < 0.001$). Larvae in 30, 32.5, and 35% treatments were all 4-Armed after three days, while many of the larvae growing in 27.5% were still swimming blastulae without defined growing arms. At the five day sampling, all *E. chloroticus* larvae were 4-Armed. After seven days, some 6-Armed *E. chloroticus* larvae were found in the 30% treatments and above, but all larvae in the 27.5% treatments remained at the 4-Armed stage. Salinity was very significant in predicting the developmental stage ($P < 0.001$) of *E. chloroticus* larvae at this time. Development stage of the *E. chloroticus* larvae remained significantly affected by salinity through the experiment (Table 4.2). In general, *E. chloroticus* larvae in the lower salinity treatments appeared to grow more slowly than their siblings in the 35% and 32.5% treatments.

Deformations

Overall analysis of all larvae measured is included in Table 4.3. Interestingly, ANOVA results were identical for statistics computed using all the larval data (including the deformed measurements) (Table 4.3a) and the larval data with the deformed measurements removed (Table 4.3b). Figure 4.3a illustrates the variation in the percentage of normal *E. chloroticus* larvae in a treatment through the experiment. The LD50 for *E. chloroticus* larvae is illustrated in Figure 4.3b. The *E. chloroticus* larvae growing in the 27.5% and 30% became deformed earlier and in greater numbers than their siblings growing in the 32.5% and 35% treatments. Two-way ANOVA statistics on the percentage of normal *E. chloroticus* larvae in a treatment indicated that for overall, larval age was very significant ($P < 0.001$) as well as salinity ($P < 0.001$) (Table 4.4). The larvae in the 27.5% and 30% treatments appeared to have greater variation of deformities, while those in the higher salinity treatments tended to only have shrunken arms or exposed spicules. Figure 4.4 illustrates common deformities of *E. chloroticus* larvae.
Table 4.3 Results of ANOVA for all *Evechinus chloroticus* larvae measured.

Table 4.3a Results of ANOVA for all *Evechinus chloroticus* larvae measured, including deformed measurements.

For this analysis, $R^2 = 0.974$

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected</td>
<td>600.689</td>
<td>78</td>
<td>7.701</td>
<td>3231.766</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>53.873</td>
<td>1</td>
<td>53.873</td>
<td>22607.755</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LnArm</td>
<td>69.407</td>
<td>1</td>
<td>69.407</td>
<td>29126.293</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>7.859</td>
<td>19</td>
<td>0.414</td>
<td>173.579</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.204</td>
<td>3</td>
<td>6.814E-2</td>
<td>28.569</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age*</td>
<td>0.785</td>
<td>55</td>
<td>1.428E-2</td>
<td>5.993</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>15.978</td>
<td>6705</td>
<td>2.383E-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18028.912</td>
<td>6784</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected</td>
<td>616.666</td>
<td>6783</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3b Results of ANOVA for all *Evechinus chloroticus* larvae measured with deformed measurements removed.

For this analysis, $R^2 = 0.981$

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected</td>
<td>497.793</td>
<td>40</td>
<td>12.445</td>
<td>6433.877</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>31.268</td>
<td>1</td>
<td>31.268</td>
<td>16165.534</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LnArm</td>
<td>33.813</td>
<td>1</td>
<td>33.813</td>
<td>17481.121</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>2.972</td>
<td>9</td>
<td>0.330</td>
<td>170.725</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.521</td>
<td>3</td>
<td>0.174</td>
<td>89.840</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age*</td>
<td>0.625</td>
<td>27</td>
<td>2.315E-2</td>
<td>11.966</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salinity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9.431</td>
<td>4876</td>
<td>1.934E-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13820.833</td>
<td>4917</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected</td>
<td>507.225</td>
<td>4916</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4 Two-way ANOVA analysis of the per cent of normally developing *Evechinus chloroticus* larvae over the experiment. **Statistically Significant**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval Age</td>
<td>19</td>
<td>67.2921</td>
<td>3.5416</td>
<td>3122.00</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Salinity</td>
<td>3</td>
<td>8.5012</td>
<td>2.8337</td>
<td>2497.89</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Larval Age*Salinity</td>
<td>57</td>
<td>4.7354</td>
<td>0.0831</td>
<td>73.23</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Error</td>
<td>160</td>
<td>0.1815</td>
<td>0.0011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>80.7093</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.4 Common deformities of *Evechinus chloroticus* larvae encountered in the present study included, (A) development of extra arms, (B) fused arms, (C) arm shrinkage, and (D) spicule exposure. Scale bar represents 100μm.
4.4 Discussion

In Doubtful Sound, a persistent Low Salinity Layer overlies higher salinity water in which adult *Evechinus chloroticus* live. The dynamics of this LSL are the dominant dispersive force for *E. chloroticus* larvae in Doubtful Sound (Wing et al. 2003). As echinoderm larvae generally swim to the surface early in development (Metaxas 1998, personal observation), *E. chloroticus* larvae exhibiting this behavior will encounter lowered salinities in the wild. If trapped in the LSL, development and survival of the larvae may be affected. Therefore, the affects of low salinity on *Evechinus chloroticus* larvae were investigated in the present study.

As in previous work (Chapter 3) and these larval growth experiments, *Evechinus chloroticus* failed to develop in the 25%o treatments. Unfortunately, only the second *E. chloroticus* larval experiment resulted in sufficient numbers of larvae in the treatments to warrant statistical analyses. The deformation of *E. chloroticus* larvae in 35%o treatments before full competency (Figure 4.3), though indicates that a problem occurred, therefore after about day 30, results are unreliable. The results obtained from the second *E. chloroticus* larval growth experiment, however, are extremely valuable. The results clearly show that larvae of *E. chloroticus* cannot develop normally salinities lower than 30%o. After 24 days of growing in 27.5%o treatments, all larvae observed were deformed, and 60% of larvae in 30%o treatments were deformed. These deformities, as illustrated in Figure 4.4, included the development of extra arms, fused arms, arm shrinkage, and exposure of the skeleton due to epithelial contraction. Obviously, deformities of the arms of *E. chloroticus* would have detrimental effects on the feeding efficiency or swimming capability of the larvae. Therefore, deformed larvae probably have a higher mortality rate than those larvae that continue on a course of normal development. It is unknown whether these deformities are reversible.

It is interesting to note that deformities occurred in all salinities, but to a greater extent in the 27.5%o and 30%o treatments. Therefore, other factors besides salinity may play a role in the deformation of *E. chloroticus* larvae. As 30%o-33%o is often used to demarcate the bottom of the LSL in Doubtful Sound (Lamare 1998, Chapter 2, current study), *E. chloroticus* larvae inhabiting these salinities for significant periods of time would be at a greater risk of developing abnormally than
their siblings inhabiting deeper waters of greater salinity. A dose effect was observed between salinity and the normal development of larvae. As time proceeded, the percent of normal *E. chloroticus* larvae in the samples decreased (Figure 4.3A). This dose effect indicates that the longer an *E. chloroticus* larva is exposed to a lower salinity, the greater the detrimental affect.

At three days of development, Salinity was not a significant factor affecting the growth of the *Evechinus chloroticus* larvae, but was having a very significant affect on the development of the larvae. *E. chloroticus* larvae were developing more slowly in the 27.5‰ treatments after 3 days than their siblings in higher salinities. This lag in development continued throughout the experiment for the 27.5‰ treatments and came to include the 30‰ treatments as well. Similar results of sluggish development of larvae grown in lowered salinities have been observed in other sea urchins (Roller and Stickle 1993, 1994, Metaxas 1998), Echinoderms (Watts *et al.* 1982, Kashenko 1992), and invertebrates (Costlow *et al.* 1960, Davis and Calabrese 1964, Diaz and Bevilacqua 1986, Anger 1991, Medrones-Ladja 2002 ). These slower development rates of larvae growing in the 27.5‰ and 30‰ treatments would contribute to a longer planktonic phase in the wild, resulting in a longer period exposed to predation.

After five days of development, and for the remainder of the experiment, salinity was a very significant factor affecting both the growth and development of *Evechinus chloroticus* larvae. A clear dose effect was observed, as the interaction of Larval Age*Salinity being very significant in the body length growth during the normal development period. This result indicates that the longer *E. chloroticus* larvae are exposed to a lowered salinity, the greater the affect that salinity has on its development and growth. Also, the ratio of Arm Length to Body Length was significantly affected by the treatment salinity. Therefore, if a larva is swept into the LSL in Doubtful Sound, it is most certainly going to undergo stress, as the salinity in the LSL is generally 1-5‰ (Stanton 1984). If *an E. chloroticus* larva is mixed out of the LSL into waters of a salinity greater than 30‰ after only a short period of time, it may be able to continue normal development. If a larva cannot escape the LSL, it will certainly undergo osmotic stress and possibly die before abnormal development would occur. Investigations into the ability of *E. chloroticus* larvae to recover from exposure to lowered salinities were not investigated, but as salinity was not a
significant factor in the development of larvae until 5 days into the experiment, it may be possible for larvae to survive some degree of exposure to lowered salinities in the wild, if they can swim out or are mixed out of the LSL. Gavrilova and Mokretsova (1983), working with *Stichopus japonicus*, found that if exposure to extremely lowered salinities was limited, when larvae were returned to higher salinity water, they were capable of surviving. Metaxas (1998) also observed that short-term exposure to lowered salinities had less adverse effects on the survival and development of *Echinodera lucunter* larvae.

The fate of developing larvae can have direct consequences for populations. Deformation of significant numbers of *Evechinus chloroticus* larvae exposed to low salinities, and delayed development in 27.5% and 30% treatments indicates that long-term survival and development in the LSL is impossible. The chances of survival of an *E. chloroticus* larva are probably quite small in a voyage in the LSL from the head of Doubtful Sound to the sill, as the LSL has been estimated to require 6-10 days to travel from Deep Cove to the sill (Bowman *et al.* 1999). The extreme influence that salinity plays on the normal development and growth of *E. chloroticus* larvae may explain the pattern of recruitment observed in Doubtful Sound. Wing *et al.* (2003) modeled the estuarine circulation in Doubtful Sound and seeded the larval pool at Espinosa Point (mid-fiord). Seeding at mid-fiord resulted in an outcome that closely resembled field observations (Wing *et al.* 2003). The populations near the head of Doubtful Sound could recruit locally, but probably have diminished genetic influence elsewhere in the fiord as significant numbers cf their larvae possibly cannot survive the long term exposure to the LSL required to travel from the head to the sill of Doubtful Sound. This population at mid-fiord may also be the main influence keeping the population genetics of *E. chloroticus* similar to inner-fiord populations throughout Fiordland. Genetic analyses performed by Perrin (2002) indicated that there were two genetically distinct groups of *E. chloroticus* in Fiordland. One group included samples collected from inner-fiord sites, while the other composed samples recovered from outer-fiord and coastal sites (Perrin 2002). *E. chloroticus* larvae produced by the population at Espinosa Point could probably survive the short distance in the LSL to the sill and the open coast, thus distributing their genetic information to other fiords.
The growth and development of *Evechinus chloroticus* larvae were significantly influenced by the salinity in which they were grown in this study. Development of *E. chloroticus* larvae in the lower salinity treatments lagged behind their siblings in the 32.5‰ and 35‰ treatments. Normal development was also significantly influenced by salinity, with the lower salinity treatments having greater per cent deformed larvae through the experiment compared with the higher salinity treatments. As even lower salinities would be encountered by *E. chloroticus* larvae in the LSL of Doubtful Sound, the recruitment dynamics of this species are probably immensely influenced by the Low Salinity Layer. Investigations into the ability of *E. chloroticus* larvae to recover from short-term exposure to low salinities would advance the understanding of whether larval dispersal by means of the LSL is possible at any location along Doubtful Sound.
Chapter 5. Effects of Salinity on Adult *Evechinus chloroticus*

### 5.1 Introduction

Generally regarded as exclusively marine, Echinoderms are thought to be a stenohaline phylum tolerating little salinity change in their environment as they lack a differentiated excretory organ (Nichols 1964, Binyon 1966, Yaroslavtseva and Shirmunskii 1978). Without this excretory organ, in an environment with fluctuating salinities, the animal is unable to regulate osmosis or the diffusion of ions into and out of the body (Binyon 1972). However, various species of Echinoderms are provoking reevaluation of the concept that the phylum is solely marine. In regions where seawater has been gradually diluted over geological epochs, the time scale appears to have been slow enough for several species of Echinoderms to adapt to lowered salinities (Binyon 1966, Pagett 1981). Also, in locations where salinity fluctuates notably, but predictably, Echinoderms have been found (Sabourin and Stickle 1981). In fact, species of Echinoderms are known to occur in waters ranging from 0.5-80‰ (Kinne 1964b, Binyon 1966).

Populations of Echinoderm species have been shown to distribute themselves differently due to gradients in salinity. In the Saint Lawrence Estuary, Canada, Himmelman *et al.* (1983) attributed the population structure of the sea urchin *Strongylocentrotus drobachiensis* to the salinity gradient within the estuary. Near the head of the estuary where salinity was low, urchin numbers were low, and no urchins were found near the surface. Population structure here was suggested to be due to the periodic drops in surface salinity that could be lethal to the urchins. At the seaward end of the estuary, urchins were abundant and small urchins were abundant near the surface and the salinity was relatively stable (Himmelman *et al.* 1983). A further experiment by Himmelman *et al.* (1984) in the same area demonstrated that periodic exposure to lowered salinities caused high mortality of small (5-10mm) urchins and acclimation to lowered salinity decreased mortality. Estuarine populations of *Strongylocentrotus drobachiensis* seemed to have higher tolerances to lowered salinity than urchins from more constant conditions (Himmelman *et al.* 1984). Communities of Echinoderms were found to be significantly different between an area of dynamic salinity and an area of relatively steady salinity (Drouin *et al.* 1985).
Drouin et al. (1985) attributed the variation in the communities to difference in salinity regimes at the two sites studied.

Salinity tolerances between populations of single species are often different depending on the salinity regime in the environment in which a population resides (Kinne 1964a, Dybem 1967, Vernberg and Vernberg 1975, Stancyk and Shaffer 1977). Several authors have found that populations inhabiting an environment with highly variable salinities have greater ranges of tolerance than their conspecifics inhabiting a more stable salinity regime (Gezelius 1964, Dybem 1967, Stancyk and Shaffer 1977). For example, Stancyk and Shaffer (1977) working with *Ophiothrix angulata* (Say), found that animals from an estuary with a higher mean salinity (30‰) were less tolerant of lowered salinities than animals from an estuary with slightly lower mean salinity (25‰). The authors attributed this contrast in salinity tolerance to the differences in the length of time in which salinities in the estuaries fluctuated considerably. At the higher mean salinity estuary, episodes of reduced salinity lasted a relatively short time, usually a few hours, while at the lower mean salinity estuary, periods of lowered salinity typically lasted from a few days to weeks (Stancyk and Shaffer 1977).

When Echinoderms living normally in full-strength seawater are experimentally placed in diluted seawater, a number of effects are noticeable. There is usually swelling (Kinne 1964a), weight gain (Himmelman et al. 1984), loss of function (e.g. righting (Lawrence 1975, Shirley and Stickle 1982, Stickle and Diehl 1987), reduction in the extension of tube feet (Lawrence 1987, personal observation), and a decrease in efficiency or reduction in metabolism (Kinne 1964a, Sabourin and Stickle 1981, Shirley and Stickle 1982), these affects eventually resulting in death. Some animals are able to survive short periods in diluted seawater and when returned to full-strength seawater the animal regains normal function and turgor (Binyon 1972).

Several reactions of the Echinoidea are helpful in the determination of well-being. The tube feet in Echinoderms act as both a means of locomotion and respiration (Lawrence 1987), and are very permeable to water and ions (Nichols 1962, Binyon 1966). The tube feet and spines are sensitive to touch, and when prodded, a defensive reaction causes the animal to locally point its spines towards the area of stimuli (Binyon 1972). The “righting response” has been used by various numbers of
authors to measure the general health of the animal and the degree to which it is stressed under the experimental conditions (e.g., Lawrence 1975, Himmelman et al. 1984, Stickle and Diehl 1987, Stickle et al. 1990, Clarke 2001). When a healthy urchin is inverted, it uses its spines and tube feet to right itself. As this requires a high degree of neuromuscular coordination, it reflects the general health and physiological state of an urchin (Himmelman et al. 1984).

Two very important factors have been recognized in the assessment of salinity tolerance: the duration of exposure to the salinity and the rate at which the salinity was reduced (Vernberg and Vernberg 1975, Stickle and Diehl 1987). Studies have indicated that the duration of exposure is often more important than the rate of exposure (Sabourin and Stickle 1981, Roller and Stickle 1994). Turner and Meyer (1980), observed that gradual exposure to lowered salinities gave results similar to those obtained when animals were exposed to the low salinity directly. Salinity gradients may also alter the reproductive success of populations of Echinoderms living in brackish water (Kinne 1964a, 1966, Binyon 1972, Vernberg and Vernberg 1975). Kinne (1964a, 1966) observed that reduced salinity caused a decrease in the production of gametes or complete sterility among several species from the Baltic Sea, including Asterias rubens.

The sea urchin Evechinus chloroticus is a common regular echinoid that is endemic to the New Zealand coast, including the Fiordland region. Found extensively in the intertidal and subtidal areas, E. chloroticus generally has greatest abundances above 10 meters depth (Wing et al. 2001). Juveniles and small individuals are often cryptic, and inhabiting shallower waters than full grown adults (Dix 1970a, Barker 2001). In Fiordland, shallow dwelling E. chloroticus may be exposed to diluted seawater due to substantial rainfall (>7m annually) in the region. In Doubtful Sound, additional freshwater output from the Manapouri Power Station effectively doubles the freshwater entering the fiord (Gibbs 2001).

The copious amount of rainfall and additional freshwater outflow contributes to a persistent Low Salinity Layer (LSL) in Doubtful Sound. The LSL in Doubtful Sound varies in thickness (3-7m at Deep Cove, 1-4m at the Sill) and is typically 1-5‰ (Stanton 1984), while surface salinity is generally 34-35‰ off the West Coast of New Zealand (Heath 1985). The LSL contributes to classic salt-balance circulation in Doubtful Sound; as the LSL progresses seaward, coastal seawater is drawn into the
fiord and displaces bottom waters (Stanton and Pickard 1981). As the LSL flows seaward, it entrains higher salinity water and particles from below, slowly increasing in salinity towards the sill (Walls 1995, Bowman et al 1999, Macrellis 2001). The estuarine circulation in Doubtful Sound has the ability to structure *Evechinus chloroticus* populations, as this circulation has been suggested as the main distributive force for larvae, leading to variation in recruitment (Wing et al. 2003).

Salinity has the ability to affect all stages of the recruitment process. Importantly, if adults cannot tolerate a dynamic salinity regime, they will die, and therefore be unable to contribute to the larval pool able to recruit into populations. This dichotomy of reproductive success in a variable environment could then contribute to differences in gene frequencies throughout a population. In fact, two genetically dissimilar groups of *Evechinus chloroticus* have been identified in Fiordland (Perrin 2002). Adult *E. chloroticus* sampled from inner-fiord habitats compromised one group, while the other group consisted of *E. chloroticus* collected from outer fiord sites and the open coast (Perrin 2002). The dispersive nature of *E. chloroticus* larvae was suggested by Perrin (2002) to be the force behind the genetic differentiation. But before *E. chloroticus* larvae are able to disperse, their parents must be able to survive.

The ability to which adult *Evechinus chloroticus* tolerate low salinities is unknown. In Doubtful Sound, the tidal range is about 1m, and variations in the depth of the LSL can be considerable, therefore the chances of an adult in the intertidal being exposed to waters of reduced salinity are considerable. As any detrimental effects of low salinity on the adult could result in variations in reproductive fitness, and therefore larval recruitment, the affect of low salinity on *Evechinus chloroticus* adults was examined.
5.2 Materials and Methods

General Methods

Experiments investigating the effects of low salinity on adult *Evechinus chloroticus* were conducted from 20 July to 23 July 2003 at the University of Otago field laboratory in Deep Cove, Doubtful Sound. Adult *E. chloroticus* were removed from Causet Cove to represent sill populations and Deep Cove to represent head of fiord populations, respectively (see Figure 3.1). Adults were collected in 15-20L plastic buckets at ambient salinity. Buckets were covered to prevent the intrusion of freshwater into the buckets during removal through the Low Salinity Layer (LSL). Adults were held in ambient salinity seawater until the start of the experimental period.

Dilutions of seawater were made with 1μm bag filtered (Filter Media (NZ) LTD. Auckland) seawater and freshwater. Treatments tested in this experiment mirrored those salinities tested on larvae; 25, 27.5, 30, and 32.5‰. The only exception was in inclusion of ambient salinity seawater (34.6‰) treatment, which replaced the 35‰ control treatments. Treatments were carried out in either 15L or 20L plastic buckets bubbled with air throughout the duration of the experiment.

Experimental Design

Initial reactions of adult *Evechinus chloroticus* to inversion and stimuli, prodding with a pencil and adherence/extension of tube feet, were recorded for ten adults from the sill and ten adults from the head. These reactions were taken as a general reaction for the sample of adults from each location. Five urchins from Deep Cove were placed in each of the treatment buckets. A plastic cage was then wedged above these urchins in each of the buckets. Adults from Causet Cove were then placed in the cage. Due to the dichotomy in size of the urchins from each locale (adults from Deep Cove were generally smaller (90-110mm test diameter) than those from Causet Cove (100-140mm test diameter)), between three and four adult urchins from Causet Cove were placed in each treatment, rather than five in each treatment. Buckets were covered with black plastic to diminish the potential effects of daylight and discourage evaporation from the treatments.
Three *Evechinus chloroticus* adults from each treatment and locale were sampled every 12 hours after the initial test for 72 hours. Adults were tested after 12 hours of treatment to determine their righting response and response to stimuli. Adults were initially given a maximum righting time of 10 minutes, which was later lengthened to 20 minutes. However, after the initial testing and 12 hours of exposure, adults even in the higher salinity treatments failed to right themselves. The testing of the righting response of the adults was therefore discontinued, and the response of the adults to prodding and the observation of clinging or extended tube feet were used as an indicator of stress. In treatments where adults failed to respond to stimuli, their tube feet were no longer extended, and discolored holding water and obvious discharge of coelomic fluid had taken place, these treatments were discarded from the experiment.

Three *Evechinus chloroticus* adults from each treatment and location were observed at each sampling period (12-72 hours). Urchins were observed *in situ* to determine the presence or absence of clinging or extended tube feet, then removed from the treatment and prodded with a pencil. A positive reaction to prodding was the movement of spines inward towards the area of prodding. A 1 was recorded if the animal positively reacted or had its tube feet extended, while a 0 was recorded if the animal failed to respond or did not have its tube feet extended. The reactions were tabulated and the proportion responded determined over each of the replicates for each location.

**Analysis**

These proportions were then compared using binary logistic regressions. Comparisons were made to determine whether location, salinity, and time had affects on the response to prodding and whether an animal had its tube feet extended. Statistical analysis was carried out using the statistical program MINITAB® (Minitab, INC, Stage College, PA, USA). Statistical significance was inferred if the p-value was less than or equal to 0.05.
5.3 Results

Descriptive

*Evechinus chloroticus* adults in this experiment did not tolerate low salinities well. After 24 hours in the 25%o and 27.5%o treatments, adults from both Causet Cove and Deep Cove were failing to respond to stimuli and were drooping their spines. After 36 hours, the water in these treatments had a distinctly purplish hue, indicating that urchins were expelling celomic fluid. At this time, urchins in these treatments were also beginning to drop their spines, indicating severe stress. After 48 hours of treatment, all adult *E. chloroticus* in the 25%o and 27.5%o treatments had dropped their spines, the water was a dark purple and foamy, and urchins were obviously dead. As these urchins were dead and not responding, these treatments were discarded from the experiment. For statistical analysis, no responses were recorded for urchins in the 25%o and 27.5%o treatments for the remainder of the experiment, in the analyses they were treated as missing values. Adult *E. chloroticus* in the higher salinity treatments (30, 32.5, and 34.6%o) remained responsive and healthy-looking throughout the experiment. The holding water in these treatments remained clear, unlike the water in the lower salinity treatments.

Prodding Response

The variation in the prodding response for the Sill and the Head is illustrated in Figure 5.2. Overall salinity was not significant (P = 0.784) and neither was location (P = 0.768). Time was significant, though (P = 0.028), as well as the interaction of time*salinity (P = 0.033). The likelihood that the slopes for all salinities tested were zero was very significant, P < 0.001 (Table 5.1). Breaking down the analysis revealed several interesting results (Table 5.2). After 12 hours of exposure, Table 5.2 shows that no factors were significant (P = 0.998 for all factors). The chances of all the slopes being zero, was also not significant, P = 0.257 after 12 hours. The prodding response did not vary significantly with any factors after 24 hours, but the probability that all the slopes were zero remained very significant (P < 0.001-0.008). Analyses were not performed for 48 and 60 hours as all surviving adults had identical reactions. Location was not significant at any of the times analyzed.
Table 5.1 Results of binary logistic regression analysis for the overall response of adult *Evechinus chloroticus* to prodding. **Statistically Significant**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>-2.19</td>
<td>0.028</td>
</tr>
<tr>
<td>Salinity</td>
<td>-0.27</td>
<td>0.768</td>
</tr>
<tr>
<td>Location</td>
<td>-0.30</td>
<td>0.768</td>
</tr>
<tr>
<td>Time*Salinity</td>
<td>2.13</td>
<td>0.033</td>
</tr>
<tr>
<td>Salinity*Location</td>
<td>0.24</td>
<td>0.809</td>
</tr>
<tr>
<td>Location*Time</td>
<td>0.85</td>
<td>0.398</td>
</tr>
<tr>
<td>Salinity<em>Time</em>Location</td>
<td>-0.82</td>
<td>0.412</td>
</tr>
</tbody>
</table>

Test that all slopes are zero: $G = 185.922$, DF = 7, $P < 0.001$

Table 5.2 Results of binary logistic regression analysis for the response of adult *Evechinus chloroticus* to prodding. **Statistically Significant**

<table>
<thead>
<tr>
<th>Time</th>
<th>Salinity</th>
<th>Location</th>
<th>Slopes = 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 hours</td>
<td>$Z_{(0.00)}$, $p=0.998$</td>
<td>$Z_{(0.00)}$, $p=0.998$</td>
<td>$G_{(3)} = 4.039$, $p=0.257$</td>
</tr>
<tr>
<td>24 hours</td>
<td>$Z_{(0.01)}$, $p=0.991$</td>
<td>$Z_{(0.01)}$, $p=0.992$</td>
<td>$G_{(3)} = 94.908$, $p&lt;0.001$</td>
</tr>
<tr>
<td>36 hours</td>
<td>$Z_{(1.57)}$, $p=0.117$</td>
<td>$Z_{(0.06)}$, $p=0.949$</td>
<td>$G_{(3)} = 42.455$, $p&lt;0.001$</td>
</tr>
<tr>
<td>48 hours</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60 hours</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hours</td>
<td>$Z_{(0.00)}$, $p=0.998$</td>
<td>$Z_{(0.00)}$, $p=0.998$</td>
<td>$G_{(3)} = 11.902$, $p=0.008$</td>
</tr>
</tbody>
</table>
Figure 5.2 (over page) Variation in response to prodding (+/- SE) (n = 3) of adult *Evechinus chloroticus* over time.

□ Head

□ Sill
Tube Feet Response

Overall, the tube feet response was more variable than the prodding response, as is illustrated in Figure 5.3. The tube feet response also showed greater sensitivity, as illustrated by the LD50 for the tube feet response (Figure 5.3b). Generally, no factors were overall significant in the tube feet response. The possibility that all the slopes were zero had a p-value of less than 0.001 (Table 5.3).

After 12 hours exposure, the responses of the urchins were not significantly correlated with any factor (Table 5.4). The likelihood that the slopes of the lines for the treatments were zero, though, was very significant ($P < 0.001$) and remained very significant at all times, except at 60 hours, over the course of the experiment (Table 5.2).

Table 5.3 Results of binary logistic regression analysis for the overall tube feet response of adult *Evechinus chloroticus*. Statistically Significant

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>-0.18</td>
<td>0.854</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.59</td>
<td>0.557</td>
</tr>
<tr>
<td>Location</td>
<td>-1.70</td>
<td>0.089</td>
</tr>
<tr>
<td>Time*Salinity</td>
<td>0.19</td>
<td>0.849</td>
</tr>
<tr>
<td>Salinity*Location</td>
<td>1.81</td>
<td>0.071</td>
</tr>
<tr>
<td>Location*Time</td>
<td>1.49</td>
<td>0.135</td>
</tr>
<tr>
<td>Salinity<em>Time</em>Location</td>
<td>-1.63</td>
<td>0.104</td>
</tr>
</tbody>
</table>

Test that all slopes are zero: $G = 276.154$, DF = 7, $P < 0.001$
Table 5.4 Results of binary logistic regression analysis for the tube feet response of adult *Evechinus chloroticus*. **Statistically Significant**

<table>
<thead>
<tr>
<th>Time</th>
<th>Salinity</th>
<th>Location</th>
<th>Slopes = 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 hours</td>
<td>$Z_{(0.01)}$, p=0.996</td>
<td>$Z_{(0.01)}$, p=0.995</td>
<td>$G_{(3)} = 89.579, p&lt;0.001$</td>
</tr>
<tr>
<td>24 hours</td>
<td>$Z_{(0.83)}$, p=0.407</td>
<td>$Z_{(0.33)}$, p=0.406</td>
<td>$G_{(3)} = 73.636, p&lt;0.001$</td>
</tr>
<tr>
<td>36 hours</td>
<td>$Z_{(1,11)}$, p=0.269</td>
<td>$Z_{(0.59)}$, p=0.555</td>
<td>$G_{(3)} = 59.617, p&lt;0.001$</td>
</tr>
<tr>
<td>48 hours</td>
<td>$Z_{(0.00)}$, p=0.997</td>
<td>$Z_{(0.00)}$, p=0.997</td>
<td>$G_{(3)} = 30.867, p&lt;0.001$</td>
</tr>
<tr>
<td>60 hours</td>
<td>$Z_{(-0.66)}$, p=0.506</td>
<td>$Z_{(1.16)}$, p=0.246</td>
<td>$G_{(3)} = 4.399, p=0.221$</td>
</tr>
<tr>
<td>72 hours</td>
<td>$Z_{(0.00)}$, p=0.998</td>
<td>$Z_{(0.00)}$, p=0.998</td>
<td>$G_{(3)} = 10.626, p=0.014$</td>
</tr>
</tbody>
</table>
Figure 5.3a Variation in adult *Evechinus chloroticus* tube feet responses (+/-SE) (n = 3) over time.
Figure 5.3b. Adult *Evechinus chloroticus* LD50 for the tube feet response.
5.4 Discussion

In Doubtful Sound, a Low Salinity Layer (LSL) persistently overlies higher salinity water in which adult *Evechinus chloroticus* live. The dynamics of this LSL have been implicated as the dominant dispersal force for *E. chloroticus* larvae in Doubtful Sound (Wing et al. 2003). Detrimental affects of low salinity on survival and reproductive potential have been observed in Echinoderms (Kinne 1964a, 1966) and other species (Binyon 1972, Vernberg and Vernberg 1975). As fluctuating salinities may be encountered by adult *E. chloroticus* in Doubtful Sound, it is important to understand whether adult *E. chloroticus* also exhibit increased mortality when exposed to low salinities. Survival of the adult precedes reproductive success and larval production, therefore supply to the larval pool can be inferred as deceased adults cannot contribute. The present study of the potential of *E. chloroticus* adults to tolerate low salinities may further our understanding of recruitment dynamics of this species.

Low salinities were extremely harmful to adult *Evechinus chloroticus*. After only 12 hours of exposure, *E. chloroticus* adults in the lowest salinities investigated, 25%o and 27.5%o, were clearly stressed, as all urchins observed failed to have their tube feet extended. After 24 hours, urchins in these treatments still did not have their tube feet extended. These *E. chloroticus* adults also had drastically reduced responses to prodding as compared with their conspecifics in the higher (30, 32.5, 34.6%o) salinity treatments. Complete mortality of adult *E. chloroticus* was observed in these treatments after 48 hours indicating that even these higher salinities are intolerable. Previous studies have indicated that for animals normally inhabiting stable salinity conditions, experimental reduction of the salinity causes morphological, functional, and cellular changes (Kinne 1964a,1966, Lawrence 1975, Shirley and Stickle 1982, Sabourin and Stickle 1981, Himmelman et al. 1984, Stickle and Diehl 1987). The functional well being of *Strongylocentrotus droebachiensis* was significantly decreased among sea urchins acclimated to 15%o compared to those in 30%o treatments (Sabourin and Stickle 1981). Shirley and Stickle (1982) observed greater mortality of *Leptasterias hexactis* in their 10%o treatments as compared to their 30%o treatments. Therefore, although animals were able to survive lowered salinity conditions in most cases, the animals would not be as fit as their conspecifics in the higher salinities.
As lowered salinity conditions in Doubtful Sound exist due to LSL, and the fact that this layer is typically 1-5‰ (Stanton 1984), the ability for adult *Evechinus chloroticus* to survive prolonged exposure to these low salinities is highly unlikely. Variation in the depth of the LSL has been attributed to wind stress and precipitation (Gibbs 2001). Gibbs, *et al.* (2000) illustrated using a model that the LSL significantly deepens at the head of Doubtful Sound (Deep Cove) when strong wind and rain events occur in concert. Gibbs (2001) noted that near the head, the salinity at 1m was nearly always <15‰, while at 9m, salinities were mostly oceanic, but the LSL intruded to this depth occasionally. Therefore, shallow dwelling *E. chloroticus* may be exposed to lowered salinities on a fairly regular basis. Although one hundred per cent mortality of adult *E. chloroticus* was observed after 48 hours exposure to 25‰ and 27.5‰, an exposure of this duration is highly unlikely in the wild. The result that *E. chloroticus* adults generally reduce the extension of their tube feet well before the loss of total well being, as illustrated by Figure 5.3, is possibly life-saving. When an *E. chloroticus* adult is exposed to the low salinity of the LSL, the short-term response of the animal would probably be to retract its clinging tube feet. In many areas, this would cause the animal to detach from the steep rock wall, and slide down the wall, into waters of higher salinity.

It is clear that adult *Evechinus chloroticus* cannot tolerate prolonged exposure to reduced salinity. Although analyses into the consequences of lowered salinities on the reproductive potential were not conducted due to the timing of the present investigation, previous studies have shown that gametes of Echinoderms are extremely sensitive to low salinities (Thorson 1950, Gezelius 1964, Greenwood and Bennett 1981, Kashenko 1998). If *E. chloroticus* reacts similarly, the reproductive fitness of an individual exposed to reduced salinities would be greatly decreased compared to an unexposed individual. This decrease in reproductive fitness could lead to a dichotomy in larval production among individuals and even populations. The populations of *E. chloroticus* inhabiting the head of Doubtful Sound where the LSL is generally thicker and more variable could therefore contribute little to the total larval pool of Doubtful Sound. This possibility could help explain the larval recruitment dynamics of *E. chloroticus* in Doubtful Sound modeled by Wing *et al.* (2003). The model was run with seeding of the larval pool at several locations along the fiord, but the seeding at Espinosa Point (mid-fiord) most closely resembled actual
recruitment patterns observed in the field (Wing et al. 2003). Therefore, exposure of adult *E. chloroticus* to low salinities is extremely likely to cause variation in competence and contribution potential of the individual to the larval pool able to recruit to other populations throughout Doubtful Sound and along the coast.

Salinity was a major factor in the overall well-being of adult *Evechinus chloroticus* exposed to lowered salinities. Effects due to lowered salinity ranged from complete mortality of adult *E. chloroticus* in the lowest salinities tested, 25%o and 27.5%o, to reduced exposure of tube feet in lowered salinities and declines in the response to prodding in the higher salinity treatments. The tube feet response is very subtle, the large differences seen between the salinities tested suggests a shift in functionality of an *E. chloroticus* adult even at higher salinities. There were not significant differences in salinity tolerance between the two locations tested, indicating that adult adaptation to a more dynamic salinity regime in Deep Cove has not taken place among that population of *E. chloroticus*.

Even the lowest salinities in the current study are well above the typical salinity values for the LSL in Doubtful Sound. Complete mortality was observed among *E. chloroticus* adults in these salinities, therefore detrimental affects on the overall competence of the adult and possibly reproductive output, in particular, are inevitable when an adult has prolonged exposed to the LSL. Extreme alterations in reproductive output could therefore contribute to possible reproductive dichotomies between populations of adult *E. chloroticus* along Doubtful Sound. These dichotomies leading to variable recruitment of *E. chloroticus* along Doubtful Sound. Therefore, exposure of the adult to lowered salinity has the ability to affect the larval recruitment of *E. chloroticus*. This experiment involved a single, prolonged exposure to lowered salinities, it would be interesting to investigate whether frequent exposures to salinities fluctuating between LSL salinities and oceanic values, which are probably more realistic conditions, have similar affects for *E. chloroticus* adults as a single, prolonged exposure to reduced salinity.
General Discussion

The effects of low salinity on *Evechinus chloroticus* (Valenciennes) were examined in the present study. Three questions were posed: do *E. chloroticus* larvae distribute themselves differently in Doubtful Sound due to the Low Salinity Layer (LSL); what affect does low salinity have on *E. chloroticus* development; and do adult *E. chloroticus* from geographically separated populations within Doubtful Sound have differing tolerances to low salinity?

The first question was addressed through monitoring the upper 25m of the water column of Doubtful Sound from January to March 2003. Larval *Evechinus chloroticus* were absent from the 0-2m tows at both of the inner fiord sites, depths which always included the LSL. Although larval numbers were not significantly associated with depth, the finding that larvae were never found in surface waters of the inner fiord sites, clear areas of low salinity, suggests an avoidance of low salinity by *Evechinus chloroticus* larvae in the field. Lamare (1998) also sampled *E. chloroticus* in Doubtful Sound. Tows were taken below the 33‰ isohaline, and from the 33‰ isohaline to the surface, *E. chloroticus* were absent from all LSL tows. The author suggested that the stratification of the water column in Doubtful Sound may structure the vertical distribution of *E. chloroticus* (Lamare 1998). The observation that *E. chloroticus* larvae were absent from obviously low salinities may indicate that they have similar avoidance behavior of low salinities as larvae of other euryhaline echinoderms and invertebrates (eg. Stickle and Diehl 1987, Mann *et al.* 1991, Raby *et al.* 1994, review Metaxas 2001). Over the sampling period, there was a trend for *E. chloroticus* larvae to be concentrated in the 6-8m tows, a layer that would have been very close to the halocline at the inner fiord sites. These larvae may have accumulated in this layer due to the often higher levels of phytoplankton (implied through fluorescence) at these depths and estuarine circulation of seawater up-fiord.

In the laboratory, the development of *Evechinus chloroticus* was significantly affected by lowered salinities. The embryonic development of *E. chloroticus* was incomplete in salinities lower than 27.5‰. There was delayed hatching of *E. chloroticus* blastulae in lowered salinities, the time required for hatching was
significantly related to the salinity in which embryos were reared. *E. chloroticus* embryos developing in salinities similar to the LSL found in Doubtful Sound (5-10‰) experienced greater amounts of damage, such as lysing and abnormal development, than their siblings in higher salinities. The amount of damage was evident after only two hours of exposure to lowered salinity, indicating extreme sensitivity at this early stage of development. Interestingly, results indicated that the location from which the parents were harvested never had a significant effect on the number of embryos damaged. This result suggests that although adult populations in Deep Cove, at the head of Doubtful Sound, may occasionally experience periods of reduced salinity, tolerance is not passed to the offspring. Dybern (1967) observed among populations of the ascidian *Ciona intestinalis* living under different salinity regimes that their offspring had differing salinity tolerances. Gezelius (1964) observed that the salinity tolerance of adult *Psammechinus miliaris* was lower (16-34‰) than the tolerance for either its gametes or embryos (27-32‰). The salinity tolerance of *E. chloroticus* gametes was not tested in these experiments, but it would be interesting to establish whether they have similar salinity tolerances as embryos.

Investigations into the growth and development of *Evechinus chloroticus* larvae revealed that these stages are also not tolerant to reduced salinities. Larvae did not grow in the 25‰ treatments, while significant numbers of deformed *E. chloroticus* were found in the 27.5‰ and 30‰ treatments. After 5 days, only 64% of larvae sampled from the 27.5‰ treatments were developing normally. Deformities of the arms as observed in this study could obviously contribute to a decrease feeding efficiency or swimming ability of the larvae. This result clearly indicates an intolerance of *E. chloroticus* larvae to reduced salinities. The treatments tested did not simulate actual conditions in the LSL, though, and it is unknown whether *E. chloroticus* larvae could withstand limited exposure to those extremely reduced (5-10‰) salinities.

The fate of developing stages of *Evechinus chloroticus* can have direct consequences for populations. The significant numbers of damaged embryos exposed to low salinities similar to the LSL (5-10‰) indicates that even short-term exposure to reduced salinities is detrimental. The occurrence of significant amounts of deformed larvae in the 27.5‰ and 30‰ treatments indicates even these higher salinities are not well tolerated by *E. chloroticus*. These results indicate that long-
term survival and development within the LSL is impossible. Dispersal through the LSL is probably therefore confined to the later larval stages. Still, the chances of survival within the LSL for the larvae of *E. chloroticus* traveling from Deep Cove to the Sill of Doubtful Sound is probably quite small. Bowman *et al.* (1999) estimated the LSL requires 6-10 days to travel from Deep Cove to the Sill, a time frame which deformed all *E. chloroticus* larvae in this study.

The extreme influence salinity has on *Evechinus chloroticus* developing stages may help explain the pattern of recruitment observed in Doubtful Sound. Wing *et al.* (2003) modeled the estuarine circulation in Doubtful Sound and seeded the larval pool at Espinosa Point (mid-fiord). Seeding at mid-fiord resulted in an outcome that closely resembled field observations (Wing *et al.* 2003). The populations near the head of Doubtful Sound could recruit locally, but probably have diminished genetic influence elsewhere in the fiord. Significant numbers of their larvae may not survive the exposure to the extremely low salinities of the LSL in the time required to travel from the head to the sill of Doubtful Sound. This population at mid-fiord may also be the main influence keeping the population genetics of *E. chloroticus* similar to inner-fiord populations throughout Fiordland. Genetic analyses performed by Perrin (2002) indicated that there were two genetically distinct groups of *E. chloroticus* in Fiordland. One group included samples collected from inner-fiord sites, while the other composed samples recovered from outer-fiord and coastal sites (Perrin 2002). *E. chloroticus* larvae produced by the population at Espinosa Point could probably survive the short distance in the LSL to the sill and the open coast, thus distributing their genetic information to other fiords.

It is important to understand the effects of low salinity on adults, as any detrimental effects of the adult could result in variations in reproductive fitness, directly influencing the larval pool. The dichotomy of reproductive success could lead to differences in gene frequencies throughout a population, as observed by Perrin (2002). Adult *Evechinus chloroticus* from two geographically separated populations in Doubtful Sound reacted similarly to low salinities. The lowest salinities tested (25‰ and 27.9‰) caused extreme stress in all adults tested. After 12 hours, adult *E. chloroticus* in these treatments did not have their tube feet extended and after 24 hours these subjects had drastically reduced responses to prodding, compared to their conspecifics in the higher (30, 32.5, 34.6‰) salinity treatments. Continued exposure
resulted in complete mortality of adult *E. chloroticus* from both habitats after 48 hours. An interesting result, although a duration of exposure unlikely to occur in the field. Previous studies have shown that for animals normally inhabiting stable salinity conditions, experimental reduction of the salinity causes morphological, functional, and cellular changes (Kinne 1964a, 1966, Lawrence 1975, Shirley and Stickle 1982, Sabourin and Stickle 1981, Himmelman *et al.* 1984, Stickle and Diehl 1987). The lowest salinities tested were well above LSL levels, therefore, prolonged exposure to the LSL would certainly have detrimental affects on adult *E. chloroticus*.

To summarize, *Evechinus chloroticus* was significantly affected by lowered salinity at all stages tested. Embryos of *E. chloroticus* cannot survive and hatch in salinities lower than 27.5‰, and certainly cannot tolerate salinities as low as those found in the LSL. This stage of *E. chloroticus* development is the most sensitive to lowered salinities. Larvae of *E. chloroticus* can survive and develop in higher salinities, but even at 27.5‰ and 30‰ severe deformities are found. Deformities which could lead to increased mortality in the plankton. Field studies, however, indicate that larvae of *E. chloroticus* distribute themselves below the LSL, in salinities >30‰. Adult *E. chloroticus* tolerate low salinities less than their larvae, with complete mortality of adults in 25‰ and 27.5‰ after 48 hours. Throughout this study, the location from which subjects were either taken (adult experiments) or were derived from (egg experiments) was never a significant factor in their response to low salinity. Therefore, although adults at the Head of Doubtful Sound may occasionally be exposed to low salinities, it does not impart any increased salinity tolerance to their offspring.

A greater understanding of recruitment dynamics of *Evechinus chloroticus* in Doubtful Sound can be gleamed from this study. Developing stages of *E. chloroticus* cannot survive or develop long-term in the Low Salinity Layer. Short distance dispersal through the LSL may be possible, although only for larvae of *E. chloroticus*. Investigations into the ability of *Evechinus chloroticus* to withstand short-term exposure to LSL salinities (1-10‰) would further insight into possible dispersal through the Low Salinity Layer, resulting in greater awareness of recruitment dynamics of this species.
References


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