Population genetics of *Liothyrella neozelanica* in Breaksea Sound

By
Erik Suring

Supervisor Dr. Steve Wing

A research project submitted in partial fulfillment of the requirements for the Degree of Postgraduate Diploma in Marine Sciences at the University of Otago, Dunedin, New Zealand

1 November, 2001

Table of Contents

Abstract

The population genetics of the articulate brachiopod *Liothyrella neozelanica* in Breaksea Sound, Fiordland, New Zealand were examined using allozyme electrophoresis. Genetic differentiation between inner and outer fiord locations measured by Wright's F_{st} was 0.031, significantly different from zero ($p<0.05$). *L. neozelanica* have brooded, lecithotrophic larvae with low dispersal potential and an aggregated distribution. These traits may be responsible for apparently greater within fiord differentiation in *L. neozelanica* than in *Terebratella sanguinea*, a brachiopod with a more even distribution. Fiord circulation and bathymetry may also play a role in controlling gene flow in Fiordland. This study shows there is potential for using genetic markers to understand the interactions between the physical and biological processes controlling gene flow and population differentiation in Fiordland.

Introduction

The deep water fiords in southwest New Zealand are characterized by rich and diverse assemblages of unique and fragile benthic marine organisms (Grange et al. 1981, Smith and Witman 1999). Inundated for around 10,000 years (Pickrill et al. 1992) and with a mean estuarine circulation (Gibbs et al. 2000) the fiords can act as a natural laboratory to study the interactions between life history characteristics, oceanography, and gene flow.

Gene Flow and Larval Dispersal

Molecular genetic techniques can provide insights into the evolutionary response of organisms to environmental change, the evaluation of speciation models, and the development of population structure and function. While terrestrial models of animal speciation have focused on allopatric processes (Mayr 1942) these processes may be less important in marine systems as there are fewer geographic barriers and larval dispersal can increase gene flow (Palumbi 1992).

In sessile marine organisms larval dispersal is the only mechanism for genetic exchange. The amount of larval dispersal and concomitant gene flow is controlled by a combination of life history characteristics and geographic features. Larval features such as planktonic duration, larval type, and settlement characteristics have the greatest influence on gene flow. The duration of the larval planktonic phase is the most consistent proxy for the distance of dispersal (Palumbi 1995). Extended planktonic dispersal can genetically homogenize populations across ocean basins (Scheltema 1972). Extended larval periods are more often associated with feeding planktotrophic larvae than lecithotrophic larvae. The type of larvae, swimming or passive, can also affect the extent of dispersal. Swimming larvae, while they may not be strong enough to overcome currents, can control

their position in the water column and thus which currents they will be carried in. Gregarious settlement can cause patchy distributions and form population "islands." Organisms with more even distributions may maintain better genetic connections between populations in a stepping stone fashion. Adult life history characteristics can also play a role. If spawning time is different in separate populations there is less chance for genetic exchange. Adult depth distributions can also affect which current regime will transport passive larvae.

Geographic and oceanographic factors can create barriers or corridors for gene flow. Genetic markers may record differentiation that occurs not only because of present but also past geographic factors. A change in the presence of barriers because of sea level change has been implicated in the genetic differentiation of Indonesian reef populations (Barber et al. 2000). The near total isolation caused by emergence of the Isthmus of Panama has resulted in a large divergence between formerly connected populations (Bermingham and Lessios 1993). Less complete isolation caused by headlands and protected embayments can still lead to genetic breaks at physical barriers (Edmands et al. 1996). In New Zealand fiord hydrography and geography may play an important role in limiting larval dispersal and gene flow in benthic marine organisms (Mladenov et al. 1997, Sköld et al. in prep).

There are a variety of biological and physical controls on the extent of larval dispersal and genetic exchange in marine organisms. The interactions between these effects will define the population structure, evolutionary response, and speciation mechanisms for marine species. The presence of genetically structured species without larval dispersal constraints found in areas such as oceanic basins with no apparent physical

barriers (Miya and Nishida 1997) indicates as yet not understood processes may shape marine gene flow.

Fiord Dynamics

Fiordland's marine environment is subdivided into fiords with hydrographic characteristics that may create barriers to dispersal. The fiords are long, narrow and have shallow sills at the entrance and where most branch inlets join a major fiord (Stanton and Pickard 1981). A low salinity layer (LSL) at the surface created by high rainfall and high relief flows seaward creating a special type of estuarine circulation pattern. Saltwater exchange may be limited by the shallow sills and may only move out of the fiord entrained in the LSL (Stanton and Pickard 1981). Salt balance is maintained by seawater flow into the fiord below the LSL (figure 1). Planktonic larvae may be retained within their natal fiord by this inward flow (Lamare 1998). Planktonic larvae from the fiord may also only be transported from the fiord entrained in the LSL, but osmotic stresses are thought to prevent this. Genetic data from sea urchins, *Evechinus chloroticus*, and sea stars, *Coscinasterias muricata*, suggests hydrographic features of the fiords may limit between fiord gene flow (Mladenov *et al.* 1997, Sköld *et al.* in prep., Perrin unpublished data). However the majority of species that make up the diverse rocky cliff assemblages in Fiordland have short living lecithotrophic larvae (Smith and Witman 1999). The genetic structure of a sessile organism with lecithotrophic larvae, such as the articulate brachiopod *Liothyrella neozelanica* has not been well described in this system.

Brachiopods

Brachiopods are lophophorate sessile filter feeders with two hardened valves (Brusca and Brusca 1990). The brachiopod fossil record stretches back nearly 600 million years. At their peak there were over 3000 genera of brachiopods and they were an

abundant and diverse component of coastal shelf ecosystems (Brusca and Brusca 1990). Brachiopods were hard hit by the Permian extinction, though, and then bivalve mollusks replaced them in many niches. Today there are about 335 extant species of brachiopods left, mostly inhabiting marginal habitats such as areas of high current velocity or cold temperatures (Brusca and Brusca 1990). For modern oceans Fiordland, with eight different brachiopod species, has relatively high abundance and diversity of brachiopods (Ryan and Paulin 1998).

Liothyrella neozelanica Thomson 1918 (Terebratulacea, Articulata, Brachiopoda) is found throughout the southern fiords and is reported as far north as Doubtful Sound at depths below 20 meters (Ryan and Paulin 1998). It is found attached, often to overhangs, and has a patchy, clustered distribution with individuals settling near and on top of each other (figure 2, Richardson 1981). *L. neozelanica* has lecithotrophic, brooded larvae, which may be brooded until the pre-settlement phase (Chuang 1994).

Genetic Differentiation

When gene flow is interrupted between two populations directed processes such as selection and random processes such as mutation and drift lead to genetic differences in those populations (Hartl 1988). The genetic structure of populations reflects the effective movement of individuals or their genes. Greater structure indicates populations have diverged genetically due to little gene flow. Allozyme polymorphisms can be used as genetic markers to quantify the divergence between populations. Despite advances in other molecular techniques allozyme electrophoresis remains a powerful tool for most taxa (Parker et al. 1998). The statistic F_{st} can be used as a measure of between population differentiation (Wright 1978, Weir and Cockerham 1984) to estimate the degree of population segregation. F_{st} estimates the reduction in heterozygosity from

Hardy-Weinberg equilibrium that results from population subdivision. F_{st} values range from zero, indicating no genetic differences between populations, and one, indicating fixed allelic differences between populations. Waples (1987) demonstrated that F_{st} can be robust to variations in the number and type of loci and species used.

Aims

While other studies have examined population differentiation between fiords or between Fiordland and other New Zealand locations (Mladenov *et al.*, 1997, Sköld *et al.*, in prep), this study examines within fiord population structure by addressing the following questions: 1) Is the population structure of *L. neozelanica* at an inner fiord site different from an outer fiord site in Breaksea Sound? 2) Is the genetic differentiation between inner and outer fiord populations different between brachiopod species?

Methods

Sample Collection

In 1999 divers collected animals from an inner location in Vancouver Arm and an outer location near the conjunction with Acheron Passage in Breaksea Sound (figure 3). Animals were collected at 30 to 40 meters from aggregations of *L. neozelanica*. There are several interior sills, including at the entrances to Vancouver Arm and Acheron Passage (figure 3). Based on sill depth and sea level change it is estimated Breaksea Sound has been inundated with seawater for about 16,000 years (Smith 2001). Lophophore tissue was removed from each animal, frozen in liquid nitrogen in the field, and stored at -80^{o} C until used for electrophoresis.

Allozyme Electrophoresis

Tissue was homogenized with an equal volume of buffer (2 ml Tris-HCl (pH 8.0), 0.1 ml mercaptoethanol, 10 g sucrose, and 25 mg NADP per 100 ml H_2O – adjusted to pH 7.2 with HCl). Electrophoresis was performed on cellulose acetate plates (Titan III Helena Laboratories). I focused on six different enzyme systems that were polymorphic and consistently resolvable in *Terebratella sanguinea* (Ostrow et al. 2001). The enzymes that were used for the analysis are (abbreviation and enzyme commission number in parentheses): hexokinase (HK, E.C. 2.7.1.1), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), peptidases with GL and LGG as substrates (PEP, E.C. 3.4.11/13.-), mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8), and 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44).

Tris-glycine (pH 8.5) was used as the running buffer for *Hk, Mpi, Pep,* and *Pgi.* Tris-malate (pH 7.8) was used as the running buffer for *6Pgd* (see Richardson et al. (1986)

for buffer recipes). Enzyme stains were modified from Hebert and Beaton (1989). Relative electrophoretic mobilities were confirmed by numerous side-by-side comparisons of electromorphs. Alleles were assigned number codes with "1" representing the slowest allele (traveling the least during an electrophoretic run) at a locus.

Data Analysis

The statistical analysis of allozyme electrophoresis is well documented compared to some newer techniques (Parker et al. 1998). The program GENEPOP (version 3.1b, Raymond and Rousset 1995) was used to test for linkage disequilibrium, conduct exact tests for deviations from Hardy-Weinberg equilibrium, and calculate allele frequencies. Linkage disequilibrium tests that each loci is independent of the others and ensures there is no linkage bias. If a population is in Hardy-Weinberg equilibrium then allele frequencies are expected to remain the same in subsequent generations. Deviations of genotype frequencies from Hardy-Weinberg equilibrium are shown as values of H_D, where $H_D=(H_0-H_E)/H_E$. H_0 is the observed number and H_E is the expected number of heterozygotes. Differences in allele frequencies between populations may indicate evolutionary processes are working independently in each population.

Genetic correlations among populations were described using F_{st}, calculated with the program FSTAT (Goudet 1995). The significance of pairwise comparisons of F_{st} between populations was tested using 1000 permutations. Bootstrap analysis and jackknifing to calculate F_{st} confidence intervals and standard error could not be conducted with these data though, as there were only two populations and three loci present.

Results

Of the six loci examined only four could be consistently resolved, *Hk, Pep,* and *Pgi.* *Pep* with GL as a substrate was monomorphic. *Pep* with LGG as a substrate was the most variable with eight total alleles, with one allele found only in the inner population and two alleles found only in the outer location. *Hk* and *Pgi* had only two alleles each (table 1). Strong deviations from Hardy-Weinberg equilibrium were found at *Pep* and *Pgi* at both locations. While *Hk* was in equilibrium at both locations as a whole both populations were significantly out of equilibrium (table 1). The pairwise differentiation test yielded an F_{st} of 0.031, which was significantly different from zero ($p < 0.05$).

Discussion

This study examined genetic variation at the intrafiord level in a sessile marine species with low larval dispersal, the brachiopod *Liothyrella neozelanica*. The results show small but significant genetic differentiation, F_{st}, at the within fiord level. These findings confirm that *L. neozelanica* has low larval dispersal and indicate the possibility of interactions between gene flow, species distribution and fiord oceanography.

Larval Dispersal

Species with extended larval dispersal generally exhibit panmixia or little genetic divergence, even over distances greater than 1000 km, with F_{st} = 0.004 to 0.03 (Hellberg 1996, Waples 1987, Mladenov et al. 1997, Edmands et al. 1996). A long planktonic phase is correlated with genetic homogeneity over large geographic scales in many species (Palumbi 1995). The inverse of this situation occurs in organisms with no or low larval dispersal. In comparative studies genetic differentiation has been higher in species with lower dispersal potential (Hellberg 1996, Duffy 1993, Hunt 1993). Waples (1987) demonstrated that F_{st} and estimated dispersal ability can be highly correlated despite differences in species and in the number and type of allozyme loci used. Previous studies in Fiordland have not focused on intrafiord genetic structure but data from Sköld et al. (in prep.) show that for a starfish, *Coscinasterias muricata*, with long dispersal potential within fiord differentiation in Doubtful and Dusky Sound is minimal. However in a similar study of a species with little dispersal, the black coral *Anthipates fiordensis*, Miller (1997) found no differentiation even in separated fiords. This species is long lived and has both sexual and asexual reproduction, though, and Miller (1997) has suggested that populations have not come to genetic equilibrium since the colonization of the fiords. A significant

genetic difference between intrafiord populations in *L. neozelanica* confirms that it has limited larval dispersal.

Individual Distribution Characteristics

In addition to larval dispersal potential gene flow in *L. neozelanica* may be affected by species distribution characteristics. Ostrow et al. (2001) conducted allozyme analysis of another articulate brachiopod with lecithotrophic larvae, *Terebratella sanguinea*. It is the most widespread brachiopod in Fiordland (Ryan and Paulin 1998) and in contrast to *L. neozelanica* is has a more even, less aggregated adult distribution (Richardson 1981). The F_{st} between inner and outer locations at George Sound was a non significant –0.0067, whereas the F_{st} between inner and outer locations in Dusky Sound was a significant 0.0227 ($p<0.01$). These values compare to the F_{st} for *L. neozelanica* found in this study of 0.031. These results suggest that there may be greater within fiord differentiation in *L. neozelanica* than in *T. sanguinea*.

Differences in individual distributions between species may explain some of this variation. Though both species have low larval dispersal the more even distribution of *T. sanguinea* may allow gene flow to occur in a stepping stone fashion (figure 4). Even though *T. sanguinea* larvae only disperse short distances it is enough to put them in contact with other individuals and homogenizing gene flow could occur across a small area each generation. The aggregated distribution and gregarious settlement pattern of *L. neozelanica*, combined with short dispersal, would make gene flow less common unless clusters were close enough together (see Toonen and Pawlik 1994). Hydrographic factors may also play a role in this interspecies comparison. The *L. neozelanica* used in this study were collected at depths of 30 to 40 meters, where currents in the fiord are weak, whereas *T. sanguinea* may occur as shallow as five meters (Ryan and Paulin 1998) where dispersal

aiding currents may be stronger. However, much of the difference between F_{st} values between the species may be due to among fiord differences and parallel studies of the two species are needed.

Hardy Weinberg Equilibrium

Two loci, especially peptidase, were not in Hardy Weinberg equilibrium. This is not necessarily uncommon in marine systems though, and there are a variety of possible reasons. Banding patterns may be the result of isozyme variants that don't reflect allelic variation or banding may have been improperly scored (Richardson et al. 1986). Natural selection, inbreeding, Wahlund effect, or small sample size could also result in disequilibrium. Numerous side-by-side comparisons of electromorphs should reduce misscoring error. *Hk* was in HW equilibrium, indicating that factors that would affect all loci such as the Wahlund effect and inbreeding may not be as important in this case. We do not have enough ecological data to suggest what selective pressures are working at the two locations or whether the two locations might differ enough to explain why one population shows an excess of heterozygotes and the other a heterozygote deficit at the *Pep* locus. It is difficult to make further conclusions about the reasons for disequilibrium without further tests, though; the overall disequilibrium probably results from several small effects, each working together to create significant disequilibrium.

Allozyme Electrophoresis

These results do have to be considered with some caution as they are primarily based on a single allozyme locus. Allozyme electrophoresis is an indirect gauge of genetic variation as it measures phenotypic changes caused by allelic differences (Richardson et al. 1986). Though most electrophoretic changes are assumed to be selectively neutral there is evidence that selection can act directly on allozymes or linked traits (Parker et al. 1998).

Selection may cause clinal variation in allozyme frequencies along ecological gradients or balancing selection may homogenize frequencies throughout an area of similar ecology. There are indications from comparative studies of DNA sequence and allozyme electrophoresis that balancing selection may have widespread effects (Burton and Lee 1994, Endo et al. 2001). We do not have any ecological data such as differences in salinity, current, temperature, or food availability patterns to suggest inner and outer populations experience different selective regimes, though balancing selection could be at work to reduce apparent differentiation. Parker et al. (1998) suggest 10-20 polymorphic loci are required to provide statistical confidence overcome issues like natural selection. However it can be difficult to screen enough loci and some organisms may simply lack the necessary allozyme variation.

Allozyme electrophoresis quantifies broader scale genetic variation than some other markers. It does not register variation in non-coding areas, in redundant codons that code for the same amino acid, or amino acid substitutions than do not change the electrophoretic nature of the enzyme. The genetic variation that allozymes measure may take much longer to accumulate, making allozymes an inappropriate marker to resolve the genetic variation of some species in the fiords. The fiords have been inundated with seawater for around 10,000 years (Pickrill et al. 1992), which may not be long enough for meaningful allozyme differences to accumulate even if gene flow was restricted. Individual allozyme loci may change at very different rates though (Bermingham and Lessios 1993). In work with another brachiopod, *Lingula anatina* DNA sequences have revealed genetic structure not seen with allozymes (Endo et al. 2001)

Further Work

This study shows there are several possibilities for further work in examining the interactions between life history and oceanographic effects on gene flow. A marker with that can quantify the genetic change that occurs over smaller time scales may be more useful for studies of populations within Fiordland. Other genetic markers such AFLP and DNA sequencing are being analyzed in *L. neozelanica* and *T. sanguinea* by Gigi Ostrow. Increasing the number of sample sites would bolster the conclusions of this study. Sampling more inner and outer location replicates in different fiords would allow is to evaluate if the pattern of within fiord differentiation holds throughout Fiordland. It would also allow us to compare how the different oceanographic factors in each fiord, such as current patterns and interior sills, play a role in gene flow. Sampling more locations within Breaksea Sound could elucidate the patterns of gene flow with the fiord. It could be interesting to make direct comparisons to species with planktotrophic larvae to tease out the effects of different life history characteristics. Such work is underway with *Evechinus chloroticus*, *L. neozelanica*, and *T. sanguinea* by Gigi Ostrow and Cecile Perrin.

Conclusions

Fiords may be important habitats for genetic differentiation. Our data show significant population structure in the brachiopod *Liothyrella neozelanica* (F_{st} = 0.031, $p<0.05$) from an inner and outer location in Breaksea Sound. There are also suggestions that *L. neozelanica* may have greater within fiord differentiation than *Terebratella sanguinea* because of the more aggregated distribution of *L. neozelanica*. This study shows the importance of both life history characteristics and oceanographic effects on gene flow in sessile marine organisms. Further study of the interactions between these factors in Fiordland is promising.

Acknowledgements

I would like to thank Gigi Ostrow for her help at all stages of this project especially lab work and data analysis, Dr. Stephen Wing for his supervision, insight, and sample collection, and Dr. Michael Roy for help and laboratory facilities.

References

Barber, P.H., Palumbi, S.R., Erdmann, M.V. and Moosa, M.K., 2000. A marine Wallace's line? Nature, 406(6797): 692-693.

Bermingham, E. and Lessios, H.A., 1993. Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. Proceedings of the National Academy of Sciences, 90(7): 2734-2738.

Brusca, R.C. and Brusca, G.J., 1990. Invertebrates. Sinauer Associates, Sunderland, Massachusetts, 922 pp.

Burton, R.S. and Lee, B.N., 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus.* Proceedings of the National Academy of Sciences, 91(11): 5197-5201.

Chuang, S.H., 1994. Observations on the reproduction and development of *Liothyrella neozelanica* Thomson 1918 (Terebratulacea, Articulata, Brachiopoda). Journal of the Royal Society of New Zealand, 24(2): 209-218.

Duffy, J.E., 1993. Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. Marine Biology, 116(3): 459-470.

Edmands, S., Moberg, P.E. and Burton, R.S., 1996. Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus.* Marine Biology, 126(3): 443-450.

Goudet, J., 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. Journal of Heredity, 86(6): 485-496.

Grange, K.R., Singleton, R.J., Richardson, J.R., Hill, P.J. and Main, W.d.L., 1981. Shallow rock-wall biological associations of some southern fiords of New Zealand. New Zealand Journal of Zoology, 8(2): 209-227.

Hartl, D.L., 1988. A primer of population genetics. Sinauer Associates, Sunderland, Massachusetts, 305 pp.

Hellberg, M.E., 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution, 50(3): 1167-1175.

Hunt, A., 1993. Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patiriella calcar* and

P. exigua. Marine Ecology Progress Series, 92(1-2): 179-186.

Lamare, M.D., 1998. Origin and transport of larvae of the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. Marine Ecology Progress Series, 174: 107-121.

Miller, K.J., 1997. Genetic structure of black coral populations in New Zealand's fiords. Marine Ecology Progress Series, 161: 123-132.

Miya, M. and Nishida, M., 1997. Speciation in the open ocean. Nature, 389(6653): 803-804.

Mladenov, P.V., Allibone, R.M. and Wallis, G.P., 1997. Genetic differentiation in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). New Zealand Journal of Marine and Freshwater Research, 31(2): 261-269.

Palumbi, S.R., 1992. Marine speciation on a small planet. Trends Ecol. Evol., 7(4): 114-118.

Palumbi, S.R., 1995. Using genetics as an indirect estimator of larval dispersal. In: L. McEdward (Editor), Ecology of Marine Invertebrate Larvae. CRC Press, Boca Raton, Florida, pp. 369-388.

Scheltema, R.S., 1972. Dispersal of larvae as a means of genetic exchange between widely separated populations of shoal-water benthic invertebrate species. In: D.J. Crisp (Editor), Forth European Marine Biology Symposium. Cambridge University Press, Cambridge, pp. 7-28.

Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C. and Fuerst, P.A., 1998. What molecules can tell us about populations: Choosing and using a molecular marker. Ecology, 79(2): 361-382.

Pickrill Richard, A., Fenner Juliane, M. and McGlone Matthew, S., 1992. Late Quaternary evolution of a fjord environment in Preservation Inlet, New Zealand. Quaternary Research, 38(3): 331-356.

Richardson, B.J., Baverstock, P.R. and Adams, M., 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, San Diego, 410 pp.

Richardson, J.R., 1981. Distribution and orientation of six articulate brachiopods species from New Zealand. New Zealand Journal of Zoology, 8(2): 189-196.

Ryan, P. and Paulin, C.D., 1998. Fiordland underwater: New Zealand's hidden wilderness. Exisle Publishing, Auckland, NZ, 192 pp.

Smith, F. and Witman, J.D., 1999. Species diversity in subtidal landscapes: Maintenance by physical processes and larval recruitment. Ecology, 80(1): 51-69.

Smith, F., 2001. Historical regulation of local species richness across a geographic region. Ecology, 82(3): 792-801.

Waples, R.S., 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution, 41(2): 385-400.

Ostrow, D.G., S.R. Wing, P.V. Mladenov, and M.S. Roy. 2001. Genetic differentiation of Terebratella sanguinea in the New Zealand fiords: a dispersal barrier in the marine environment? In: Brunton, Cocks, and Long (Editors), Brachiopods: past and present. Taylor and Francis Books, London.

Mayr, E., 1942. Systematics and the origin of species. Columbia University Press, New York.

Hebert, P.D., and Beaton, M.J., 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis: A practical handbook. Helena Laboratories, Beaumont, Texas.

Endo, K., Ozawa, T., Kojima, S., 2001. Nuclear and mitochondrial gene sequences reveal unexpected genetic heterogeneity among northern Pacific populations of the brachiopod *Lingula anatina*. Marine Biology, 139: 105-112.

Gibbs, M.T., Bowman, M.J., Dietrich, D.E., 2000. Maintenance of near-surface stratification in Doubtful Sound, a New Zealand fjord. Estuarine, coastal, and shelf science, 51(6): 683-704

Raymond, M., and Rousset, F., 1995, GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248-249.

Sköld, M., Wing, S.R., and Mladenov, P.V., In Prep., Genetic subdivision of the sea star Coscinasterias muricata in the fiords of New Zealand.

Stanton, B.R., and Pickard, G.L., 1981, Physical oceanography of the New Zealand fjords. New Zealand Oceanographic Institute Memoir, 88, 1-37.

Weir, B.S. and Cockerham, C. C., 1984, Estimating F-statistics for the analysis of population structure. Evolution, 38,1358-1370.

Wright, S., 1978, Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

Tables

Table 1. Average allele frequencies and heterozygote deficit for each locus and population.

Loci	Allele	Population	
		Breaksea Inner (n=32)	Breaksea Outer (n=27)
Pep	1	0.062	0.077
	2	0.281	0.269
	3	0.000	0.173
	4	0.031	0.000
	5	0.031	0.077
	6	0.359	0.351
	7	0.031	0.000
	8	0.203	0.096
	H_D	0.15 (p=0.43)	-0.1 (p=1)
Hk	1	0.328	0.333
	2	0.672	0.667
	H_D	-0.16 (p<0.001)	0.09 (p<0.001)
Pgi	1	0.078	0.000
	2	0.859	0.962
	3	0.062	0.038
	H_D	0.64 (p=0.002)	-1.00 (p=0.019)

Figures

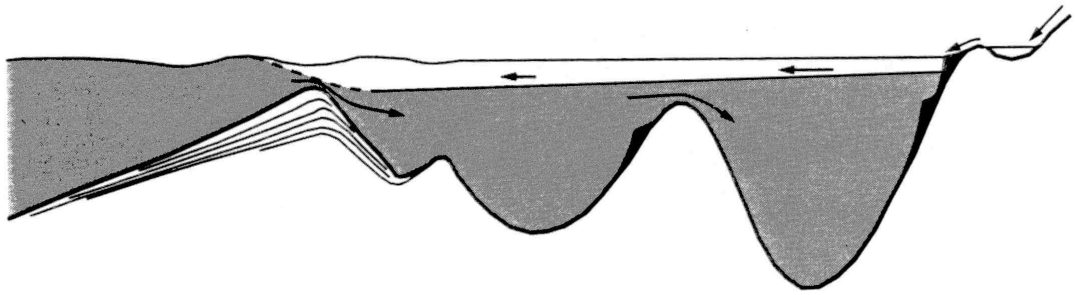

Figure 1. High rainfall and steep topography create a low salinity layer (LSL, light area). The LSL flows towards the entrance of the fiord. Though some saltwater becomes entrained in the LSL the depth of the fiord prevents friction induced mixing and the LSL remains distinct. Salt balance is maintained by input of ocean water over the entrance sill below the LSL (dark area). Interior sills may also have an effect on fiord circulation (from Pickrill et al. 1992).

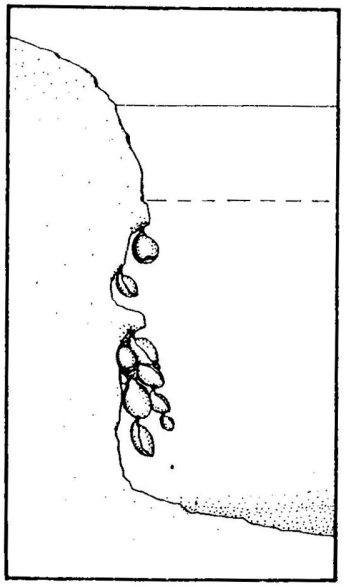

Figure 2. *Liothyrella neozelanica* has an aggregated distribution; recruitment occurs next to and on top of established adults. The dotted line indicates the lowest tidal level. In Fiordland *L. neozelanica* is commonly found from 20 to 40 meters (from Richardson 1981).

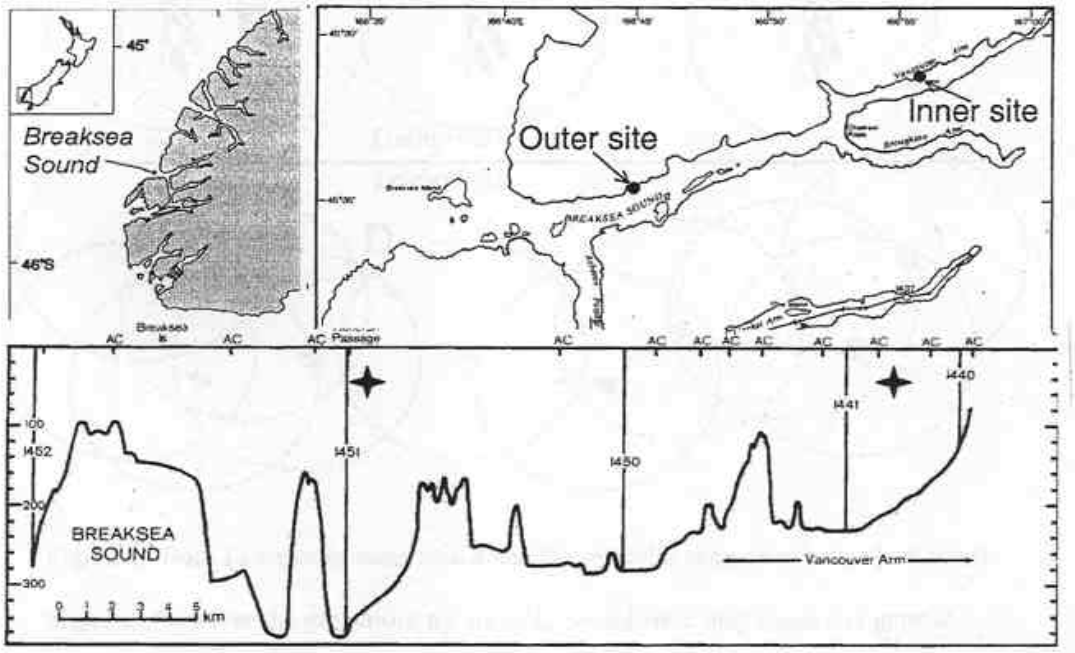

Figure 3. Location of inner and outer sampling sites from Breaksea Sound, Fiordland, New Zealand. Bathymetric map shows sills that separate the fiord from the open ocean and the study sites from each other (adapted from Stanton and Pickard 1981)

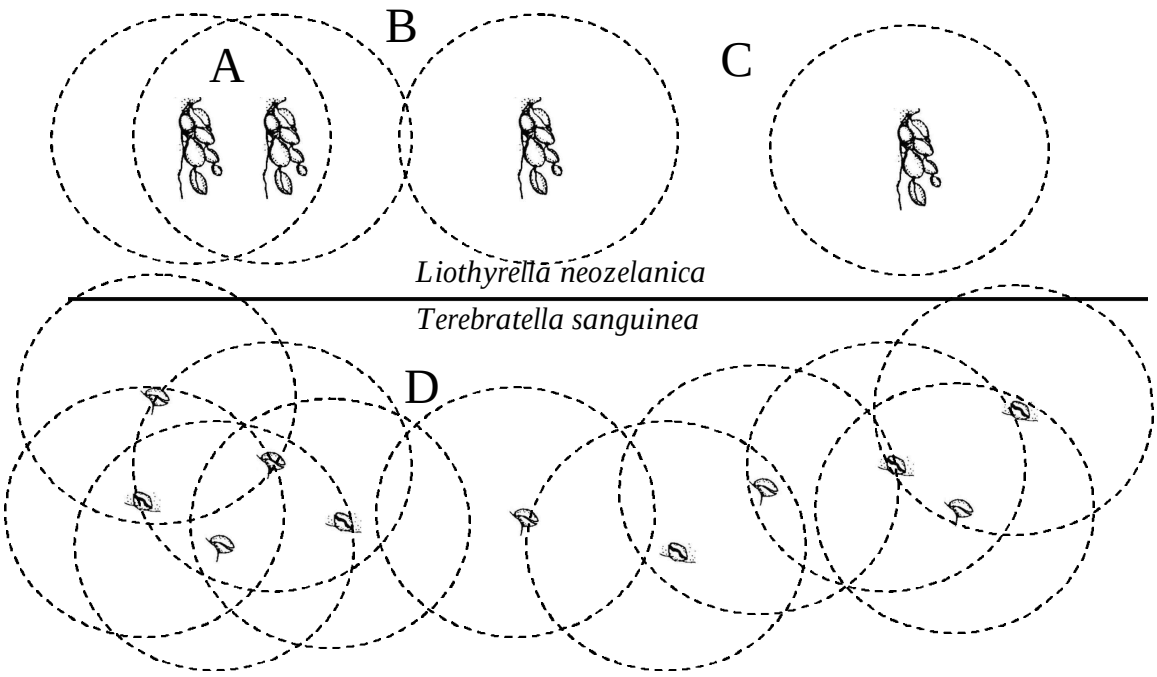

Figure 4. Both *Liothyrella neozelanica* and *Terebratella sanguinea* have short larval dispersal, however the gregarious nature of *L. neozelanica* may cause less genetic exchange between populations. Groups of organisms are shown with a dotted circle indicative of the potential larval dispersal. In (A) we expect that gene flow occurs between the two populations because larvae from one population can disperse and settle at the other. In (B) the area of potential larval dispersal overlaps but gene flow would be rare because the only conspecifics within the dispersal range are from the source population. Likewise in (C) genetic exchange would be small as larval settlement characteristics would prevent the establishment of intermediate colonies. In (D) the even distribution of *T. sanguinea* allows stepwise gene flow to occur across the range and intermediate colonies can be created.

Appendix

Table 2. Allozyme data for *Liothyrella neozelanica* from inner and outer locations of Breaksea Sound, Fiordland, New Zealand. Alleles are numbered in order according to their electrophoretic mobility, with "01" being the slowest electromorph. Individuals with the same number twice were scored as homozygotes.

Population	Individual	Loci		
Breaksea Inner		*Hk*	*Pgi*	*Pep*
	BS01,	0102	0102	0505
	BS02,	0101	0202	0309
	BS03,	0102	0202	0107
	BS04,	0102	0202	0309
	BS05,	0102	0102	0909
	BS06,	0202	0202	0309
	BS07,	0202	0202	0111
	BS08,	0202	0202	0111
	BS09,	0102	0202	0107
	BS10,	0102	0102	0309
	BS11,	0202	0202	0909
	BS12,	0202	0202	1313
	BS13,	0102	0202	0913
	BS14,	0202	0202	0909
	BS15,	0202	0202	1313
	BS16,	0202	0202	1313
	BS17,	0101	0202	0309
	BS18,	0202	0202	0913
	BS19,	0202	0102	0309
	BS20,	0102	0202	0309
	BS21,	0202	0202	0309
	BS22,	0102	0202	0309
	BS23,	0102	0202	0309
	BS24,	0202	0202	0309
	BS25,	0102	0202	0309
	BS26,	0102	0303	1313
	BS27,	0102	0202	0309
	BS28,	0102	0202	0303
	BS29,	0102	0303	1313
	BS30,	0102	0102	0303
	BS31,	0102	0202	0913
	BS32,	0202	0202	0309
Breaksea Outer				
	BSO01,	0202	0202	0909
	BSO02,	0102	0202	0309
	BSO03,	0102	0202	0309
	BSO04,	0102	0202	0309

BSO05,	0102	0202	0107
BSO06,	0102	0202	0309
BSO07,	0101	0202	0107
BSO08,	0102	0202	0309
BSO09,	0101	0202	0309
BSO11,	0000	0202	0404
BSO12,	0202	0202	0413
BSO13,	0101	0202	0413
BSO14,	0102	0202	0107
BSO15,	0202	0202	0404
BSO16,	0202	0202	0309
BSO17,	0202	0202	0309
BSO18,	0102	0202	0309
BSO19,	0202	0202	0309
BSO20,	0102	0202	0107
BSO21,	0000	0202	0413
BSO22,	0102	0202	0309
BSO23,	0202	0202	0413
BSO24,	0202	0202	0413
BSO25,	0202	0303	0309
BSO26,	0202	0202	0309
BSO27,	0202	0202	0309