9. ACKNOWLEDGEMENTS

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11. APPENDICES

Appendix 1. Depletion of phytoplankton by mussel farms

Given the ability of filter feeding shellfish to filter large volumes of water (up to 15 l/hr/g of soft dry tissue weight, Hawkins et al. 1999) and the high concentrations shellfish within farm sites, models have hypothesised that local depletion of phytoplankton in the water column should be detectable (e.g. Duarte et al. 2008 cites five examples). This depletion may have local impacts on other organisms that utilise phytoplankton or may indicate secondary impacts (e.g. zooplankton larval grazing – Gibbs 2004). Effects of aquaculture on phytoplankton depletion may have a cumulative effect in areas of multiple mussel farms. As a consequence of this, chlorophyll surveys have been introduced into fisheries research assessments (FRIAs) to assess the potential for cumulative impacts of new developments on this issue.

These surveys were conducted by pumping water from 2-3 metres depth through a fluorometer to measure chlorophyll to derive spatial estimates of phytoplankton abundance and see if significant depletion was detectable. Although these surveys were undertaken as quickly as possible in order to try to produce a “snapshot” of chlorophyll distribution, due to the time taken to cover the survey area inevitably the survey represents composite of measurements spanning a period of time, typically 40 to 90 minutes. Surveys were also undertaken in differing conditions, all which may influence the results of the survey, namely:

- The level and age of cultures in existing farms (i.e. different filtration pressures)
- Different times (i.e. different states of the tide and seasons)
- Different locations with differing physical (i.e. flushing rates, stratification and currents) and biological (e.g. natural algal concentrations) regimes
- Naturally occurring levels of spatial “patchiness” within algal populations

Consequently, results from these surveys will be dependent on the conditions under which they were measured, as is undertaken in the site-specific assessments. Nevertheless, a broad-scale synthesis of these surveys is useful for identifying the approximate magnitude and extent of local area depletion by shellfish farms, and whether this is indeed a significant environmental concern. Particularly given mussel farming culture represents the scenario with the highest density of culture (animals per cubic metre) and the amount of water filtered by each animal (as highlighted by the results of Hawkins et al. 1999).

In order to summarise these data, a review of 36 surface chlorophyll surveys was undertaken to assess the efficacy of this method for determining the extent of depletion around existing mussel farm developments. An automated comparison of spatially-interpolated chlorophyll concentration was undertaken, where the chlorophyll concentration of the four closest farms sites within the survey area (regardless of the level of culture) was compared to concentrations outside the farm site (Table 9 and Figures 24 - 27). The results of this analysis show:
• 21 of the 36 surveys had comparatively lower concentrations within the farmed areas of between 0.96% and 14.79%.
• 4 surveys appeared to show no difference between within and outside of the farm areas.
• 8 surveys appeared to have higher concentrations within the farm areas of between 1.41% to 12.77%.
• 3 surveys either had no data or farms within the survey area.

Table 9.  Mean percentage difference of the inside farm chlorophyll concentrations relative to the outside farm chlorophyll concentrations for 18 surveys from Port Underwood and Horseshoe Bay (Pelorous Sound) and 18 surveys from Kenepuru Sound.

<table>
<thead>
<tr>
<th>Location</th>
<th>Survey</th>
<th>NZMG_E</th>
<th>NZMG_N</th>
<th>Mean% Diff +/- SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port Underwood</td>
<td>PU01</td>
<td>2605557</td>
<td>5989004</td>
<td>4.2 +/- 0.1%</td>
</tr>
<tr>
<td></td>
<td>PU02</td>
<td>2605551</td>
<td>5989043</td>
<td>-7.1 +/- 0.2%</td>
</tr>
<tr>
<td></td>
<td>PU03</td>
<td>2605962</td>
<td>5989619</td>
<td>-4.1 +/- 0.2%</td>
</tr>
<tr>
<td></td>
<td>PU04</td>
<td>2605249</td>
<td>5988259</td>
<td>-2.2 +/- 0.2%</td>
</tr>
<tr>
<td></td>
<td>PU05</td>
<td>2605256</td>
<td>5988169</td>
<td>0.0 +/- 0.2%</td>
</tr>
<tr>
<td></td>
<td>PU06</td>
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<td>5989611</td>
<td>-4.9 +/- 0.3%</td>
</tr>
<tr>
<td></td>
<td>PU07</td>
<td>2605931</td>
<td>5987470</td>
<td>0.0 +/- 0.6%</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>5988193</td>
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</tr>
<tr>
<td></td>
<td>PU10</td>
<td>2606613</td>
<td>5988158</td>
<td>1.2 +/- 0.4%</td>
</tr>
<tr>
<td></td>
<td>PU11</td>
<td>2605259</td>
<td>5984908</td>
<td>-1.0 +/- 0.2%</td>
</tr>
<tr>
<td></td>
<td>PU12</td>
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<td>5984896</td>
<td>-1.3 +/- 0.4%</td>
</tr>
<tr>
<td></td>
<td>PU13</td>
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</tr>
<tr>
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<td>PU14</td>
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<td>5.2 +/- 0.9%</td>
</tr>
<tr>
<td>Horseshoe Bay</td>
<td>HB01</td>
<td>2589034</td>
<td>6019808</td>
<td>1.4 +/- 0.3%</td>
</tr>
<tr>
<td></td>
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<td>6019805</td>
<td>-1.6 +/- 0.4%</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>HB04</td>
<td>2589627</td>
<td>6018820</td>
<td>-4.8 +/- 0.4%</td>
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<tr>
<td>Kenepuru Sound</td>
<td>KP01</td>
<td>2597860</td>
<td>6002591</td>
<td>8.4 +/- 0.5%</td>
</tr>
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<td>KP02</td>
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<td>-3.4 +/- 0.3%</td>
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<tr>
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<tr>
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</tr>
<tr>
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<td>5999597</td>
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</tr>
<tr>
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<tr>
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<td>6002235</td>
<td>-8.4 +/- 0.5%</td>
</tr>
<tr>
<td></td>
<td>KP14</td>
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<td>6002333</td>
<td>N/A</td>
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<tr>
<td></td>
<td>KP15</td>
<td>2594709</td>
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<tr>
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<td>KP16</td>
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<tr>
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<td>6003578</td>
<td>-5.7 +/- 0.5%</td>
</tr>
<tr>
<td></td>
<td>KP18</td>
<td>2596541</td>
<td>6003581</td>
<td>-4.9 +/- 0.4%</td>
</tr>
</tbody>
</table>
On the basis of these results alone there is some weak evidence which suggests that local depletion within mussel farming sites is occurring. However, a subjective visual assessment of interpolated survey data shows that there are possibly more sites where down-current depletion may extend outside of the farmed areas (so called “depletion shadows”). Of the 36 surveys inspected, approximately 29 (80%) of the surveys appeared to have depleted regions within or close to existing farms (Figure 24) which provides more evidence for local depletion by farms. However, a lack of control sites for these assessments it is difficult to state conclusively what fraction of the apparent depletion in the sites is a result of natural spatial variation (e.g. Figure 27) and what fraction is driven by the culture.

These results are further confounded by the fact that chlorophyll measurements are taken from a single depth. Vertical aggregations or “patches” of phytoplankton are also common and given the mixing influence of aquaculture structures (e.g. Stevens et al. 2008) it is possible that the apparent depletion shadows are partly an artefact of dilution where lower concentrations of phytoplankton are mixed into the sampling depth. Indeed, the opposite of this mechanism (higher concentrations mixing into the sample depth) may also help explain the appearance of an “enhancement shadow” seen in some of the surveys (Figure 26). Additionally, given one of the goals of the surveys to address the impacts on naturally occurring filter-feeding organisms which are likely to be situated on the seabed. Given the chlorophyll measurements relate to surface waters it is difficult to assess the relevance of these results to organisms at depth.

In order to remove any potential bias and improve the utility of these surveys, the inclusion of control sites and depth-integrated chlorophyll measurements would address the stated issues. However, these may not be practical options given the uniqueness of individual sites would make control site selection extremely subjective and depth-integrated surveys would further increase the time taken by a single vessel or survey costs of additional vessels to assist in undertaking the surveys. Despite the limitations of these surveys, if the results are assumed to be broadly representative of the water column, it appears that local depletion of the water column around mussel farms is likely and is up to about 80% of the mean ambient concentrations (Table 9) although maximum differences may be higher (approximately 50-80%). The relevance of this result to the wider ecosystem is difficult to address given that the response of competing organisms to a reduction in seston supply may not result in a corresponding linear response in growth due to base metabolism overheads. Nevertheless, it seems likely that the energy available for growth and reproduction of organisms close to aquaculture sites could be reduced by up to the 20% observed in the surveys. This would be expected to have some negative influence on the growth, fecundity and abundance of these natural populations proximate to cultured areas.

Due to the limited spatial extent of these surveys, it is difficult to assess wider area implications from these survey results. This is further complicated by the possibility of suggested far-field mechanisms, such as improved phytoplankton recovery from shifts to more bioavailable nutrients (e.g. Nitrate to ammonia) and increase availability of light from increases in water clarity (Broekhuizen et al. 2002). Consequently, the use of biophysical numerical models to assess larger area impacts will continue to prove useful.
In summary, it seems that there is evidence to suggest that local depletion around shellfish farms does occur, with a majority of the studies showing either local depletion within or close to the farm site. A maximum mean percentage decrease of about 15% is observed between the interior of the farm sites and the area outside for an individual survey (KP16 - Table 9) and visual inspection of the data suggests that depletion shadows from farms may lead to localised depletions which are as high as 50% of the maximum ambient concentrations (Figure 24). More rigorous analysis of these datasets could also be undertaken; however this would not necessarily offer greater utility given the underlying systemic issues of this approach, namely a lack of control data and single depth readings. Consequently, the magnitudes seen in these surveys may serve as a guide for estimating local impacts only in a qualitative sense.
Figure 24. Examples of chlorophyll concentration surveys undertaken where results appear to show depletion within or close to farm sites.
Figure 25. Examples of chlorophyll concentration surveys undertaken around existing farm sites where results do not appear to show depletion.

Figure 26. Examples of chlorophyll concentration surveys undertaken around existing farm sites where results appear to show enhancement of chlorophyll.
Figure 27. Examples of chlorophyll concentration surveys undertaken away from existing farm sites showing natural variation in chlorophyll concentrations.
Appendix 2. Diseases and pathology in farmed shellfish and other non-finfish species.

Over a hundred species of mussel parasites/symbionts have been reported in overseas mussel species (Webb 2007) but only the paramyxean protozoan *Marteilia* sp. and putative virally transmitted hemic neoplasia appear to be economically significant in their home waters. That these two have not been reported in New Zealand is fortunate, but it means that we cannot gauge their infectivity or virulence to Greenshell™ mussels and other native bivalves. Overseas reports in bivalves do show, however, that aquaculture-borne transmission from cultured animals to wild stocks is possible and vigilance is required to forestall such risks.

**Greenshell™ mussels**

Hine (1989) reported no disease-associated mortalities in Greenshell™ mussels. Similarly, Hine (1996) included no listed serious or potentially serious pathogens in New Zealand blue mussels *Mytilus* spp. or Greenshells™. A recent review on mytilids with particular emphasis on *P. canaliculus* (Webb 2007) indicates that there are no particularly destructive diseases of mussel species in New Zealand, with the exception of a digestive viral disease. That disease was first noted by Jones et al. (1996) who reported mortalities in cultured Greenshell™ mussels in the outer Marlborough Sounds of 50-100% associated with virus-like particles and digestive tubule damage. They also reported similar infections in mussels from Westport. Subsequent surveys (Hine 2002a; S Webb, pers. obs.) have shown that although present, the disease appears to have receded in importance to sporadic events and low infection levels. The condition also affects scallops and clams in New Zealand and other molluscs elsewhere. Besides being found in other New Zealand molluscs, viruses producing similar digestive tissue effects have been reported in Australia, Scotland, Denmark, and elsewhere (Bower 2001).

Subsequently, Hine (2002a) reported that Greenshell™ mussels examined from the Marlborough Sounds and Coromandel appeared to be in good health overall despite recording low levels of apparent digestive viral infection. Jeffs et al. (1999) echoes these reports when he cites mortality problems associated with an unenveloped RNA virus as of greatest concern in Greenshell™ mussels and that, of several other parasites found, none caused significant mortalities. In addition to the digestive viral disease, a suite of other parasites was also mentioned by Hine (1997) however none was deemed a serious cause of mortality.

Another pathogen that warrants mention is the parasite APX, which is reported from New Zealand only (Diggles et al. 2002; Hine 2002b) and has been found in mussels from the Marlborough Sounds and also occurs commonly in dredge oysters *O. chilensis* (also known as flat oysters) from all around the coast (Diggles et al. 2002; Hine 2002b). In dredge oysters APX can cause a significant condition referred to coccidiosis (Hine & Jones 1994); however, its effect on mussels is less noteworthy.

In summary, cultured Greenshell™ mussels appear to present no major threat to wild mollusces, as wild Greenshell™ stocks appear to harbour all pathogens with the exception of APX. Since
APX is also found in dredge oysters, however, there would remain a reservoir of infection even in the absence of Greenshell™ mussel culture.

**Potential disease risks**

Exotic pathogen threats to Greenshell™ mussels can only be speculated upon. In this category, *Marteilia* spp. and disseminated haemic neoplasia (a molluscan leukemia) were determined by Webb (2007) as the most likely overseas threats. The potential consequences of introduction of hosts with these conditions and possible pathways by which more susceptible hosts could emerge are discussed below.

The greatest potential threat to New Zealand Greenshell™ mussel aquaculture appears to be posed by parasites introduced by invading species of blue mussel (*e.g.* *Mytilus edulis*). These common ship-borne fouling organisms are a likely source of overseas pathogens. Hybridisation of invasive with indigenous blue mussels (*M. galloprovincialis*) presents a further potential pathology hazard by production of a more susceptible reservoir host suitable for these pathogens. Evidence for such a risk is found in Beaumont et al. (2004) who report depressed performance of *M. edulis* x *M. galloprovincialis* hybrids when compared with pure species. In addition, Fuentes et al. (2002) report the lower viability of hybrids challenged by heat shock or infection with *Marteilia refringens*.

The physical coincidence of hybridising mussels and pathogens in New Zealand waters is possible: *Mytilus* species are likely to be arriving regularly in New Zealand and they could be carrying pathogens such as *M. refringens* or *M. maurini*. Some protection might be afforded by the currently remote known range of these *Marteilia* spp. [western Europe and the Mediterranean (Bower 2007)] but this restricted range may be a more a function of survey effort rather than actual distribution. Arrival of *M. edulis* facilitates hybridisation of *M. edulis* x *M. galloprovincialis* and the resulting hybrid, because of its increased susceptibility to *M. refringens*, might provide a more accommodating reservoir host with a consequent increase in numbers of pathogen transmission stages. Hypothetically, if *Mytilus* spp. were to become infected with *Marteilia* and if appropriate intermediate hosts (or local substitutes) were present, then there is the possibility of transmission to Greenshell™ mussels. We note, however, that the pathological threat to Greenshell™ mussels posed by *Marteilia* remains undetermined. Currently, it is not known if the apparent absence of *Marteilia* from Greenshell™ mussels is because of resistance by the mussels, lack of infection opportunity or perhaps because of insufficient geographic range of sampling specifically for *Marteilia*.

Hemic neoplasia may benefit from similar conditions. It is reported in *Mytilus* species and is associated with high mortalities. Hybrids of *M. edulis* and *M. galloprovincialis* (Fuentes et al. 2002) have been reported with elevated prevalences as compared with pure species, and invasion dynamics are likely to produce hybrids contemporaneously with the arrival of the neoplasia. The potential thus exists for production of more infected susceptible hosts and greater water load of transmission stages. It is known that this condition can be transmitted by cohabitation (Bower 2006) thus suggesting a direct life cycle which eliminates the need for a local intermediate host and simplifies transmission. Currently, we cannot say how harmful or
otherwise these two pathologies might be to Greenshell™ mussels. Our only guide is that they are both damaging to *Mytilus* populations.

Overseas, the aquabirnavirus Infectious Pancreatic Necrosis virus (IPNV) has also been detected in *M. edulis* (VPS 2000). It is a common virus of salmonids and is also a suspected clam pathogen in Taiwan. Similarly, Kitamura *et al.* (2007) report finding an aquatic birnavirus (ABV) in *M. galloprovincialis* where the mussel was acting as a reservoir host for infections in the Japanese flounder *Paralichthys olivaceus*. This internationally significant disease of world wide distribution is reported in healthy King salmon (*Oncorhynchus tshawytscha*) returning from the sea on the east coast of South Island, New Zealand (Diggles *et al.* 2002). Although not detected in New Zealand mussels, the possibility of *P. canaliculus* harbouring this virus, at least temporarily, finds support in the reports by Lewis *et al.* (1986) and Greening *et al.* (2001) where polioviruses and enteroviruses have been shown to persist in *P. canaliculus* after experimental exposure. Caution is clearly required in polyculture, as mytilids might harbour viruses with consequent threat to susceptible fish.

**Pacific oysters**

*Crasostrea gigas* was first reported in New Zealand (Dinamani 1971) from Northland; it subsequently appeared (Jenkins & Meredity-Young 1979) in the South Island and since then the parasites of this oyster in New Zealand have received significant attention. There are no OIE listed (OIE 2001) serious parasites/pathogens of *C. gigas* in New Zealand (Diggles *et al.* 2002). Nevertheless several diseases and parasites associated with New Zealand Pacific oysters have been reported, most of which are also globally ubiquitous and pose some commercial threat to oyster production (especially in hatcheries). These include vibriosis (Overseas incidences are documented in Bower 2002), rickettsiosis, and planocerid flatworms (Diggles *et al.* 2002); spionid mud-worms (Handley 1995; Handley & Bergquist 1997) and ostreid herpes virus OsHV-1 (Hine *et al.* 1992), which infects oyster larvae and spat. In the latter case, however, a recent survey (Webb *et al.* 2007) has not detected this virus in *C. gigas*, *Ostrea chilensis* or a range of other New Zealand adult bivalves. Organisms of insignificant impact (Dinamani 1986) include turbellarians, chironomids, nematodes, mudworms and pea crabs. Hine & Jones (1994) mentioned the copepod *Pseudomyicola spinosus* but asserted that it appears to have little effect.

Despite the above occurrences, New Zealand oysters are generally healthy: a survey of 290 Northland oysters failed to detect any significant parasites (Hine 1997). In addition, “with the exception of *Perkinsus olseni* only relatively trivial infections occur in New Zealand commercial bivalves. “...Pacific oysters in particular consistently appeared to be in good health” says Hine (2002a). In the light of the currently known local diseases, it can be inferred that culture of *C. gigas* in New Zealand is unlikely to pose a threat to naturalised *Crasostrea* or other species. Conversely, so far in New Zealand, *C. gigas* has not suffered significant or unexpected effects from indigenous pathogens, which suggests minimal threat from that quarter.
Pathogens in overseas populations of Pacific oysters

- The oyster parasite Bonamia ostreae was introduced into France from California on seed oysters of O. edulis (Carnegie 2005). It has since spread to other European countries and was also introduced to Washington from California (Straus et al. 2008).
- The paramyxean protozoan Marteilia sydneyi is thought to have extended its range by aquaculture of Saccostrea glomerata transplantation (Carnegie 2005; Bower & Kleeman 2007).
- The protozoan parasite Haplosporidium nelsoni introduced in the Pacific oyster C. gigas is implicated in mortalities of native Chesapeake oysters (Torchin & Kuris 2005).
- The bacterial disease nocardiosis, associated with Pacific oysters, originated in Japan and appears to have spread to California, Washington, and British Columbia (Straus et al. 2008).
- The withering syndrome organism, Xenohaliotis californiensis, was probably spread in California by outplanting of infected hatchery reared abalone (Friedman & Finley 2003).

Pacific oysters appear to evade many of the disease issues that beset other oysters (Elston 1993; FAO 2006). For instance C. gigas is partially resistant to infection and disease caused by Perkinsus marinus (Bower 2006a) which affects Crassostrea virginica severely. Bower (2007b) report that, in experimental challenges, C. gigas seems to be more resistant to Mikrocytos mackini than some other oysters.

Despite this, a number of significant pathologies do affect C. gigas. Summer mortalities of oyster seed have been linked to herpes virus in California but a causal association has not been confirmed (Friedman et al. 2005). Recent unpublished work from France has produced more convincing circumstantial evidence linking herpes virus with such mortalities. Oyster velar virus disease, caused by an irido-like virus, can result in near 100% mortality in affected hatchery tanks (Bower 2001d). Hinge ligament disease caused by bacteria of Ctyophaga spp. can affect juvenile oysters (~1cm) especially in warmer water (Bower 2001e). Another pathogenic bacterium Nocardia crassostreae can occur in C. gigas and O. edulis cultivated nearby (Bower 2006b). Nocardiosis, associated with Pacific oysters, originated in Japan and appears to have spread to California, Washington, and British Columbia (Straus et al. 2008). Rickettsia-like and Chlamydia-like organisms (Rickettsiales) are intracellular parasites (Bower 2006c) in which heavy infections in C. gigas are reported to have caused gill lesions and mortalities. Ciliate infections have been associated with mortalities exceeding 50% in oyster seed (Bower 2001c) and similar ciliates have been seen in New Zealand oyster spat (S Webb, pers. obs.). Denman Island Disease Mikrocytos mackini (OIE listed) prospers due to either its increased pathogenicity or the increased susceptibility of Pacific oysters in areas where there may be several months at water temperatures below 10°C (Bower 2007b). The protozoan Haplosporidium nelsoni (OIE listed) introduced in the Pacific oyster C. gigas is implicated in mortalities of native Chesapeake oysters (Torchin & Kuris 2005). Although introduced in C. gigas, H. nelsoni appears to have greater pathological impact on C. virginica (Bower 2007c). Marteilioides chungmuensis can infect C. gigas and impact on marketability by degrading appearance (Bower et al. 2006). M. refringens (OIE listed) is thought to have occurred in C. gigas, but has been confirmed in O. edulis, M. edulis, Cardium edule, C. virginica and Ostrea
Chilensis (Bower 2007a). The fungus Ostracoblabe implexa occurs in O. edulis, C. gigas, Saccostrea cucullata and less severely Crassostrea angulata. It grows through the shell to the inner surface and can elicit production of brown wart-like nodules - water temperatures exceeding 22 °C for more than two weeks favour its development (Bower 2001a). Echinocephalus crassostreati (Nematoda) has insignificant effect on the oyster, but it can cause of human health problems (Bower 2001b). The customary eating of oysters raw facilitates human infection.

Previous oyster introductions or translocations of native species are reported (Ruesink et al. 2005; Carnegie 2005) to be a significant cause of molluscan disease outbreaks. Clearly, a number of diseases mentioned would have significant impact on the New Zealand oyster industry if they were to become established here. In some cases they may also impact on the local molluscan fauna. Good biosecurity practises and surveillance are probably the best way of managing the threat. Specific mitigation factors should be devised from best practice elsewhere should one of these exotic diseases become established.

Ecological factors
Despite being a recently introduced species, New Zealand C. gigas have been reported to show no evidence of reduced genetic variation (Smith et al. 1986). This lack of a founder effect is surprising, but on the positive side it would suggest that the naturally high resistance of this species to many infections has not been degraded by genetic bottlenecks in New Zealand populations. Of course, further work is required to confirm this. Such work would also be an opportunity to ascertain gene flow between naturalised and cultivated New Zealand C. gigas. This might also afford insights into potential pathogen flows between the two populations.

Introduced organisms, even if they do bring parasites with them, could benefit from dislocation of the parasite life cycle. For instance, some parasites such as Marteilia spp. and Haplosporidium spp. appear to have an indirect life cycle requiring intermediate hosts. These hosts may have been left behind and there may be no local substitutes – we currently cannot make informed inferences on this. It is possible that potential intermediate hosts (if present) could be part of the suite of fouling organisms: this would afford opportunities both in progressing life cycle studies and as possible control measures.

The apparent advantage of C. gigas in being more resistant yet not immune has a darker side. It might also serve as an asymptomatic reservoir of pathogens damaging to other more susceptible species - the account above of diseases carried by C. gigas clearly illustrates that a range of other molluscs can be hosts. Although New Zealand may lack the exact species mentioned, congenerics and others of close affiliations do occur in New Zealand waters and could be similarly affected. It follows that should New Zealand C. gigas suffer an incursion by an exotic disease, it is likely that not only will naturalised C. gigas suffer, but so too would other molluscan species. Despite these pessimistic possibilities, the effect of invasive molluscs can be unpredictable. An example is provided by Thieltges et al. (2008) who report on the mitigation of the parasite burden of M. edulis by the presence of introduced Pacific oysters (C.
gigas) and American slipper limpets (*Crepidula fornicata*). It appears that the introduced species diverted the trematodes from their usual hosts, thus reducing infection levels.

The above examples, both optimistic and pessimistic, highlight the range of possible outcomes. Obviously, further studies are required before we can begin to understand the factors that govern these. Until the current paucity of information is remedied any projection must be largely speculative – in which case a precautionary approach should be adopted that assumes a worst case until proven otherwise. Further work will allow us to relax these strictures.

**Flat oysters**
The most significant pathogen found in New Zealand *O. chilensis* is the OIE (2000) listed haplosporidian protozoan *Bonamia exitiosa* (Diggles et al. 2002) which can cause significant to severe mortalities in adult oysters. Second in importance is apicomplexan X (APX); this protozoan is reported from New Zealand only, where it occurs in mussels from the Marlborough Sounds and in flat oysters from all around the coast (Diggles et al. 2002). Its presence is thought to predispose the oysters to *B. exitiosa* infection (Hine 2002). A similar coccidian has been reported from the kidneys at low prevalences (Hine & Jones 1994). A potential problem in hatcheries is the ostreid herpes virus OsHV-1. This virus, of worldwide distribution, has been reported in New Zealand *O. chilensis* (Hine et al. 1998; Diggles et al. 2002) and has been associated with significant larval mortalities. A more recent survey (Webb et al. 2007) has not detected this virus in *O. chilensis* or a range of other New Zealand adult bivalves.

Parasites and pathogens of secondary importance to *O. chilensis* include the following. Mudworm infestations by the spionids *Polydora* spp. and *Boccardia* spp. can cause embrittlement of the shell and stimulate production of thin nacre-covered mud blisters on the inner shell surface (Diggles et al. 2002). This problem occurs as a nuisance to many bivalve species and can also affect paua. *Microsporidium rapuae*, a protozoan, (Hine & Jones 1994; Webb, pers obs.) appears to have no pathological effect. Sporocytes of the digenean trematode *Bucephalus longicornutus* (Jones 1975, Hine & Jones 1994; Webb, pers obs.) are commonly encountered, usually at low prevalences. Although individual oysters are often heavily parasitised the pathogen is at worst a nuisance. In common with its occurrence in other bivalves, the copepod *Pseudomyicola spinosus* (Jones 1975) has minor or insignificant impact on the host. Rickettsia-like organisms (RLOs), although they have been implicated in mass mortalities in scallops, generally produce no gross signs of pathology (Diggles et al. 2002) in *O. chilensis*. Neoplasms, such as hemic neoplasia, germinomas and seminomas are reported by Hine (1997) to occur at prevalences of below 1%.

**Scallops**
Most scallops surveyed for digestive epithelial virosis (DEV) have high prevalence and high relative intensity (Hopkins et al. 2003; Hopkins & Webb 2004; Webb & Hopkins 2005; Webb 2006; Webb & Govier 2006; Webb & Govier 2007). Previous reports on this virus include: Jones et al. (1996); Hine & Wesney (1997); Diggles et al. (2002). The latter authors assert that all scallops examined from around New Zealand have these viruses and that infections may possibly become pathogenic if they reach high levels. Rickettsia-like organisms (RLO)
Infections are ubiquitous and severe infections have been implicated in periodic mass mortalities (Diggles et al. 2002). Surveys over several years have confirmed the high prevalence and intensities of infections (Hopkins et al. 2003; Hopkins & Webb 2004; Webb & Hopkins 2005; Webb 2006; Webb & Govier 2006; Webb & Govier 2007). Surprisingly, other than the gill involvement, there are few signs of pathology. RLOs also occur in kidney, muscle and digestive epithelial tissue at low prevalences and intensities with no apparent health impact (Webb & Govier 2007). Unidentified inclusions were recently found in a survey of Marlborough Sound scallops (Webb & Duncan 2008). The condition consists of many 10-15 µm inclusions at moderate to high prevalences and intensities in the mantle, palps, digestive gland, kidney and gonad tissues. Despite high prevalence and intensity, further work is needed to gauge pathogenicity of this condition.

Other parasites are of minor significance. Prokaryotic mycoplasmas are reported from the blood cells (Diggles et al. 2002). The turbellarian Paravortex occurs (Woods & Hayden 1998) in apparently healthy scallops. Hopkins & Webb (2004) discuss the pathological significance of Paravortex. No pathological effects (S Webb, pers obs.) have been noted in association with any infections. Spionids - annelid worms responsible for mud blisters - occur at low prevalences; no pathological effects were associated with any of these occurrences (Hopkins et al. 2003; Hopkins & Webb 2004; Webb & Hopkins 2005; Webb & Govier 2006; Webb & Govier 2007). The copepods Pseudomyicola sp. and Lichomolgus sp. appear to be innocuous in scallops even at intensities comparable to those in Mytilus spp. where reduction in condition has been noted (Caceres-Martinez et al. 1996). Webb & Govier (2006) discuss the minor pathological threat posed by these copepods. Nematodes (roundworms) occur at low prevalences (usually below 5%) and intensities with no signs of associated tissue changes or damage. They are likely to be fortuitous inclusions rather than dedicated parasites (Webb & Hopkins 2005). Crustacean ostracods are uncommon, and probably commensal or accidental rather than pathogenic (Webb & Govier 2006). The pea crab Pinnotheres sp. has been seen at prevalences below 5% (Hopkins et al. 2003; Webb & Hopkins 2005). Its pathological effect is negligible. Hopkins et al. (2003) review Pinnotheres and its relationship with bivalve hosts. A Nematopsis-like gregarine (Protozoa: Apicomplexa) at low prevalences and intensities has been noted in the mantles of scallops (Webb & Govier 2007; Webb & Duncan 2008). Similar parasites are common in many bivalve hosts and are considered to be of minor pathological significance even in heavy infections (Jones 1975). One example of a larval tetrarhynchidean or lecanicephalidean tapeworm was found encysted in connective tissue under the digestive epithelium of a scallop from Tasman Bay (Webb & Govier 2007). These cestodes usually develop to adults in sharks and rays and thus pose no threat to human health. Similar occurrences have been reported elsewhere (Getchell 1991). Dark structureless granules were found in the nephridia (kidneys) at prevalences from below 10% to over 80%. There were no other signs of pathology or pathogens in the affected scallops. Benninger & Penne (1991) ascribe the granules to phosphate metabolism or for the detoxification of heavy metals. One scallop from Golden Bay contained a metacercarial (Trematoda: Digenea) cyst in the mantle tissues (Hopkins & Webb 2004). Such metacercariae present a pathological threat only when there are many (dozens, hundreds or thousands) present in the host. This occurrence is pathologically insignificant.
Blue mussel (*Mytilus galloprovincialis*)

No mussels in New Zealand have been reported with any pathogens on the Office International des Epizooties (OIE) list of important diseases (Webb 2007). Moreover, few *M. galloprovincialis* parasites of any kind are reported from New Zealand. Jones (1975) and Hine (1997) mention the digenean *Tergestia agnostomi*, the copepod *Pseudomyicola spinosus* and the pea crab *Pinnotheres* sp., of which Hine (1997) asserts that none are apparently pathogenic. In a further work Jones *et al.* (1996) report infections with a digestive epithelial virosis. This paucity of listed *Mytilus* parasites in New Zealand probably reflects its lesser commercial importance rather than any biological propensity. It is likely that the parasite fauna of the blue mussel is comparable to that of the green mussel, but with the data currently available, pathogen resistance differences between New Zealand *Mytilus* and *Perna* cannot be determined.

The greatest potential disease threat posed by local *M. galloprovincialis* is their possible facilitation of establishment by serious exotic diseases such as *Marteilia refringens* or *M. maurini* and hemic neoplasia. Invading species of blue mussel (*e.g. M. edulis*) are common ship-borne fouling organisms that can hybridise with indigenous blue mussels (*M. galloprovincialis*) to produce a susceptible reservoir host suitable for these pathogens. Invading *Mytilus* species could also be carrying the pathogens. See Webb (2007) for details of other significant exotic *Mytilus* pathogens.

Overseas, the aquabirnavirus Infectious Pancreatic Necrosis virus (IPNV) has been detected in *M. edulis* (VPS 2000). It is a common virus of salmonids and is also a suspected clam pathogen in Taiwan. Similarly, Kitamura *et al.* (2007) report finding an aquatic birnavirus (ABV) in *M. galloprovincialis* where the mussel was acting as a reservoir host for infections in the Japanese flounder *Paralichthys olivaceous*. IPNV is an internationally significant disease of worldwide distribution is reported in healthy *O. s tshawytscha* returning from the sea on the east coast of South Island, New Zealand (Diggles *et al.* 2002). Caution is clearly required in polyculture, as mytilids might harbour such viruses with consequent threat to susceptible fish.

Paua (*Haliotis iris*)

A range of disorders have been noted in *H. iris*. Diggles & Oliver (2005) report haplosporidia, epithelial erosion, rickettsial inclusions in gut, protozoa in foot epithelium, bacterial infection (see also Bower 2006a), non-specific necrosis, granuloma-like lesions, haemocytic neoplasia-like inflammation and gregarines (apicomplexans). Diggles *et al.* (2002) report pustule disease caused *Vibrio* bacteria. Paua also exhibit a fungal shell mycosis (Grindley *et al.* 1998) as well the shell boring *Spionid* mud worms *Polydora* and *Boccardia* (Diggles *et al.* 2002; Bower 2006e) that can be a problem in culture. Severe cases of mudworm can cause significant shell embrittlement (S Webb, pers. obs.). In addition to shell damage, there can be loss of condition: *H. iris* infected with *Polydora hoplura* can be underweight and produce abnormal deposits of conchiolin (Diggles & Oliver 2005). Despite the significant nuisance of some, none presents an insurmountable obstacle to the New Zealand abalone industry. Hine (1997) in his review of health in commercially important New Zealand molluscs mentions only fungal shell disease and even that he says is not a cause of significant mortality. Diggles & Oliver
(2005) add to this that the haplosporidian has been associated with mortalities. Potential problems could arise from rickettsia, granuloma-like lesions, inflammatory lesions suggestive of hemocytic neoplasia, mudworm and fungal infections as discussed by Diggles & Oliver (2005). More needs to be done on investigating the husbandry factors influencing these agents.

Since some of these pathogens are apparently found only in New Zealand waters, it is difficult to gauge their potential threat to foreign abalone. In cases such as that of our fungal mycosis (Bower 2006f) there are no extant control methods and prevention is the only option. Rigorous examination and quarantine regimes before introduction to new foreign habitats is essential.

Although apparently uninfected, abalone in New Zealand are potentially at risk from an indigenous parasite, Perkinsus olseni, which currently is reported in the Northland bivalves Austrovenus stutchburyi, Macomona liliana, Barbatia novaeseelandia and Paphies australis (Diggles et al. 2002). It is curious that in the higher water temperatures of Australia the, apparently, same Perkinsus species can infect H. rubra, H. laevigata, H. cyclobates and H. scalaris (Bower 2007). The lack of host specificity shown by this parasite suggests that paua would not be immune in favourable circumstances. Perhaps climate change could be the factor that allows this threat to materialise.

**Exotic abalone pathogens**

Reported exotic abalone pathogens that could impact on New Zealand abalone include, amyotropia (probably viral), withering syndrome from the West Coast of United States of America (Diggles et al. 2002; Bower 2006d); the shell dwelling sabellid Terebrasabella heterouncinata (Bower 2006b); Labyrinthuloides haliotidis a protist in H. kamtschatkana and H. rufescens (Bower & Meyer 2005) and the kidney coccidia Margoliisiella (=Pseudoklossia) haliotis from the West Coast of United States of America (Bower 2006c). A more distasteful but possibly less likely threat comes from the nematode Echinacephalus pseudouncinatus (Bower 2001). It weakens the foot muscle and allows easier detachment from the substratum. The usual final hosts are certain sharks and rays, but human consumption of the live worms in undercooked abalone may allow migration of the larvae through human tissues.

The most immediate exotic threat to New Zealand abalone is from viral ganglioneuritis which now has been reported from farmed Australian H. laevigata and H. rubra (Hooper et al. 2007). It is thought to have come from the Far East where farmed abalone have been reported with similar herpes-like viruses (Wang et al. 2004; Chang et al. 2005). Mortality attributable to this virus has occurred on some farms (Hooper et al. 2007) and there is evidence to suggest that the virus has spread to wild populations causing significant mortality events among abalone, and possibly other gastropods (Hine 2006). As to the hazard facing New Zealand, Hine (2006) concluded that the taxonomy and geographic isolation of H. iris in New Zealand is such that it is likely to be currently free of the virus. Clearly, this virus could have significant impact on New Zealand paua if it became established here.
Appendix 3. Consequences of the movement, mixing and interbreeding of genetically distinct stocks of Greenshell™ mussels in New Zealand

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Rationale
This section deals specifically with the genetic and associated fitness consequences of the movement of Greenshell™ mussels around New Zealand, whether this be via transfer of spat from Kaitaia to aquaculture sites, or via other means such as hatchery-based breeding of mussels for use (transplant out to) aquaculture sites.

Background
The Greenshell™ mussel, *Perna canaliculus* Gmelin 1791, is endemic to New Zealand and is distributed from the far north (approx 34.5° S) to as far south as Stewart Island (approx 47° S). It is not found on offshore islands such as the Chatham Islands or any of the Subantarctic Islands. The mussel is the focus of a significant aquaculture industry, which had an estimated export value in 2007 of $174 million to the New Zealand economy (http://www.seafood.co.nz/greenshell). As outlined earlier on in this report, approximately 80% of the spat (small mussels) used by industry are collected on drift *Sargussum* seaweed on Ninety Mile Beach in the far north (approx 35° S) which is then trucked to three main aquaculture centres – the east coast of the Coromandel Peninsula (approx 37° S), the Marlborough Sounds (approx 41° S), and Stewart Island (approx 47° S) (Hickman 1983). The purpose of this section is to review the possible genetic and fitness consequences of such mass movement of mussels around New Zealand.

Introduction
Evolutionary theory suggests that populations which experience different environmental regimes (temperature, salinity, food availability, sedimentation, wave exposure etc.) as well as differing biological regimes (*e.g.* predation, parasitism, disease etc.) will become genetically adapted to the local conditions via the process of natural selection. Because natural selection acts at the level of the individual and not at the level of the population (at least in this particular case), this localised adaptation allows individual mussels to maximise their fitness (their reproductive contribution to the next generation) throughout their life spans. For the purposes of this argument, this adaptation can be thought of as occurring at small spatial scales, of the order of hundreds to a few thousand metres (*e.g.* all the mussels within one bay or on one stretch of rocky coastline). Neighbouring populations (*e.g.*
those in an adjacent bay) may be adapted to similar environmental conditions because the physico-chemical properties of the two bays are similar or almost identical. Alternatively, the mussels may be differently adapted because the two bays are very different in their environmental properties. For example, one bay may have riparian input which results in fluctuating salinity levels and also in variable particulate food supply (quantity and quality of particulates) over time, whereas the adjacent bay may not have any riparian input and may be less variable in terms of its physico-chemical properties over time. At a larger spatial scale, of the order of hundreds of kilometres, it is expected that New Zealand mussels will be genetically adapted to different environments because the country (the three main islands) spans 13° of latitude, from the warm subtropical north to the cold temperate south. Natural selection is therefore thought to give rise to what are known as “co-adapted gene complexes”. These can be thought of as an assortment of genes which function very efficiently together (i.e. they confer high fitness on the individual) in the given environment. As outlined above, different environments require different genes to confer high fitness – this is the main (but not only) reason why genetic variation is thought to exist among populations.

The difficulty from a technology perspective with the above is that we are only now starting to identify the genes which may be responsible for fitness differences among individuals, whether these are the genes that respond to environmental variation (e.g. heat shock proteins) or those that regulate processes such as growth, reproduction and development. Until very recently this has meant that it has not been technically possible in almost all cases to quantify genetic differences among populations based on the natural selection response of the individuals within that population (historically, we have used surrogate genetic markers to quantify similarities or differences among populations and individuals). Recent technological advances in rapid whole genome sequencing and the ability to process huge amounts of information (e.g. as single nucleotide polymorphisms – SNPs) are now starting to open the door into the realm of a new approach to quantifying and understanding genetic variation, how this is influenced by environmental variability (e.g. via natural selection), and how this contributes to fitness differences among individuals.

The view outlined in the first paragraph of this section reflects the sessile adult life stage, but it must be remembered that mussels reproduce by spawning gametes directly into the sea, where fertilisation is external. Mussels are highly fecund. One large female can produce tens of millions of eggs, with the result that the reproductive output of a single spawning event may give rise to billions of offspring. The planktotrophic mussel larvae (a feeding stage) spend 3-4 weeks in the water column (Hayden 1994), while pediveligers remain planktonic for several weeks if suitable substrate for settlement is not encountered (Buchanan 1994). Although these larvae do have some limited swimming capacity, they are viewed as being passive particles (propagules) in the water column. As such, they are moved around by wind and tide-driven surface water circulation until they are developed enough to settle at a site, where they will undergo metamorphosis and achieve the sedentary adult life style. Thus, adults which are presumed to be adapted to their local environment give rise to offspring (larvae) which are likely to also be adapted to that environment, but which spend sufficient time being moved around in the water column that we can reasonably assume that most newly settling spat at any site are in fact immigrants and did not originate from the population into which they recruit (e.g. Tracey et al. 1975). While there are certainly well documented cases of self-recruitment (reviewed by Swearer et al. 2002) and there is increasing evidence that this phenomenon is not as rare as first thought (e.g. Wood &
Gardner 2007), in the context of the extent and patterns of larval connectivity (= gene flow) among \textit{P. canaliculus} populations, it’s safe to say that self-recruitment is not an important consideration in the present situation. Because of the vast numbers of offspring produced by broadcast spawning species such as \textit{P. canaliculus}, and because of the highly dispersive nature of the larval stage (an evolutionary adaptation to promote gene flow among populations and the colonisation of new environments), it has been reasonably assumed that connectivity among populations is high.

The final point for consideration is the view that the sea (including coastal regions) is large and open and fully connected, without barriers or impediments to the movement of biota. Historically, this idea arose out of the obvious vastness of the sea and the view that all marine regions are connected by seawater. From a marine perspective, it is the land that forms islands which are separated by the sea, and it is the sea that provides a continuous environment, even if the physical, chemical and geomorphological properties of the sea (and its substratum) vary in space and time. This view of the sea as continuous and without boundaries was at least in part reinforced by many population genetic (allozyme) studies from the 1970s onwards which often demonstrated an apparent absence of genetic differentiation, or at least very low levels of genetic differentiation, among populations which were hundreds or even thousands of kilometres apart (\textit{e.g.} Scheltema & Williams 1983; Hunt & Ayre 1989; Creasey \textit{et al.} 1996; Murray-Jones & Ayre 1997; Apte & Gardner 2001). This apparent lack of genetic differentiation was often explained in terms of high levels of gene flow (= high levels of genetic mixing among populations) which in turn resulted in genetic homogeneity among populations (note that theory suggests that one recruit per generation is all that is required to be shared between populations to prevent genetic divergence between those populations). This was viewed as only being achievable because of the connectivity of many or all marine environments by the medium of the sea itself. More recent studies, on both hydrodynamic processes and on genetic connectivity, have shown that this is not the case. Many coastal regions are characterised by distributional discontinuities or barriers to gene flow despite the continuous nature of seawater. Such barriers (which may be variable in time or space) may include features such as upwelling and downwelling (\textit{e.g.} Dahlhoff & Menge 1996; Apte & Gardner 2002), riparian flow with its associated freshwater and sedimentary load (\textit{e.g.} Koehn \textit{et al.} 1980; Gardner & Palmer 1998), gyres which entrain and trap propagules (\textit{e.g.} Chiswell 2000), or may include geomorphological forms such as headlands or long stretches of habitat that are unsuitable to the organism in question. All of these features are now known to contribute to a breakdown of the “continuous nature” of the sea (in particular in coastal regions), with the result that genetic heterogeneity among populations is now known to be much more prevalent than was thought as recently as ten years ago.

\textbf{Genetic variation and genetic connectivity among populations of \textit{P. canaliculus}}

We can only start to assess the fitness consequences of the interbreeding of distinct genetic stocks if such stocks exist and if we know where they occur. Genetic variation, and components of it such as gene flow, can be assessed using a variety of different approaches: each has its own strengths and weaknesses. Interpretation of the data must therefore be understood in this context because different markers are informative at different levels and in different (not necessarily concordant) ways.

The earliest studies of genetic variation involved the use of allozymes, which are biochemical (protein) markers. Traditionally, allozymes are viewed as being selectively neutral (that is, they are
not under the influence of selection or if they are, that selection is so slight as be negligible). Neutral markers have the benefit of not reflecting small-scale or large-scale spatial or temporal variability which may result as a consequence of natural selection (e.g. environmental variability). As such, allozymes can be good tools for estimating gene flow among populations, but they are/were thought to not reflect genetic differences which may exist as a consequence of natural selection. A notable exception to this is work at the LAP locus (one of many different aminopeptidase loci) in blue mussels of the genus *Mytilus*. This locus controls internal cell volume and concentration (i.e. it is associated with osmoregulation) and because of this ecophysiological role it is possible to observe profound differences in individual gene frequencies as a consequence of LAP genotype-dependent selection for/against individuals in low (fluctuating) versus high (constant) salinity environments (e.g. Koehn et al. 1976, 1980; Hilbish et al. 1982; Hilbish & Koehn 1985; Gardner & Kathiravetpillai 1997; Gardner & Palmer 1998).

The first allozyme surveys of New Zealand Greenshell™ mussels reported different findings and interpreted these to imply very different types of population genetic structuring across the country. Smith (1988) reported significant heterogeneity between two northern and four southern populations, which led him to suggest that local hydrography, as well as genetic–physiological adaptation to different thermal environments, might partially isolate mussel populations, which could result in a warm water-adapted northern group and a cold water-adapted southern group, between which there only was limited gene flow. Gardner et al. (1996a) found no evidence of a north–south genetic split and explained the population genetic structuring that they observed by an isolation by distance model (populations which are geographically close share high levels of genetic similarity, populations which are far apart share low levels of similarity). Gardner et al. (1996b) compared allozyme variation between the wild (naturally occurring) mussels from Wellington Harbour (North Island) and mussels from the cultured population at Beatrix Bay, Marlborough Sounds (South Island). Both sites are at approximately the same latitude (~41.5°S). Mussels from the two sites exhibited different patterns of genetic linkage (non-random genotypic frequencies), indicating that the genotypic disequilibrium (the pattern of association between alleles at different loci) was different between the two populations. This can arise as a result of different selection pressures at the two sampling locations which favour different combinations of genotypes. Thus, different non-random genotypic associations are expected to originate and be maintained at different geographic locations because of the differing selection pressures (physical, chemical, and biological) that characterise the different sites. This is the concept of the co-adapted gene complex (see above). Most recently, and in the largest survey to date in terms of both numbers of populations and individuals, Apte & Gardner (2001) examined allozyme variation and found that a model of panmixia (wide spread gene flow resulting in no significant genetic differences among populations) best explained the observed genetic variation among the 35 assayed populations. Thus, while different studies have identified different models of genetic structuring within populations of *P. canaliculus* (a putative north-south split; an isolation-by-distance model; and a panmixia model), all studies have demonstrated high levels of genetic variation within the species, the exact importance of which remains unknown.

The most recent studies of population genetic variation in *P. canaliculus* have employed modern molecular approaches. These studies have examined genetic variation using markers such single-stranded conformational polymorphisms (SSCPs - Apte & Gardner 2002), randomly amplified
polymorphisms (RAPDs – Star et al. 2003) and microsatellites (Wei et al. 2009) in both the mitochondrial and nuclear genomes (these are different and are physically unlinked) of Greenshell™ mussels. There are two significant findings arising from this research.

1. A pronounced genetic discontinuity exists among Greenshell™ mussel populations at ~42°S, such that a northern group can be clearly recognised and differentiated from a southern group of mussels. Within the northern group there is a high degree of genetic homogeneity, presumably resulting from high levels of gene flow among northern populations. Within the southern group there is some degree of differentiation between populations on the east and west coasts of the South Island, but the southern group as a whole is more homogeneous within itself than it is by comparison with the northern group. The location of the genetic discontinuity at ~42°S is consistent with major hydrological features in the region, such as upwelling (which may move larvae offshore and away from suitable habitat) and strong coastal currents. Based on mitochondrial DNA variation data there is evidence of such coastal features acting as a barrier to gene flow, because one mitotype found in the southern group at a frequency of ~20% is not found at all in the northern group. The three different studies, using different molecular approaches and applied to both mitochondrial and nuclear genomes, all indicate that despite the continuous distribution of Greenshell™ mussels throughout New Zealand, there is a profound genetic difference between the northern and southern stocks. Such genetic-based stock differences are likely to be associated with fitness differences.

2. The genetic difference that exists between northern and southern stocks can be used as a signal to track the movement of stocks from Kaitaia (in the northern group) to Stewart Island (in the southern group). Note that at present it is not possible to track the movement of Kaitaia spat to other northern stock sites such as the Coromandel Peninsula or the Marlborough Sounds. The studies of Apte et al. (2003), Star et al. (2003) and Wei et al. (2009) all clearly show that the Stewart Island aquaculture population in Big Glory Bay (derived from Kaitaia spat) shows greatest similarity to the northern group as one would expect, given its northern origins. More significantly however, the wild (natural) mussel population at Horse Shoe Bay which is <20 km from the Big Glory Bay site shows intermediate affiliation with the northern and southern groups, when it should only exhibit affinity to the southern group. This intermediate status is clear evidence of the introgression of northern genes into this wild southern population. For the first time, we have clear evidence of interbreeding and successful recruitment and subsequent development of mussels of mixed northern/southern ancestry. The geographical extent and the fitness consequences of this event are unknown.

**Fitness consequences arising from interbreeding of discrete stocks of *Perna canaliculus***

In the present context, we define a hybrid as an individual of mixed genetic origin – the offspring of a cross between a northern and a southern Greenshell™ mussel.

The classical view (e.g. Mayr 1963; Dobzhansky 1970) has been that hybrids are less fit (i.e. they exhibit hybrid unfitness) than one or both parental types, and reviews of the literature (almost exclusively of the terrestrial hybridisation literature) tended to support this view (e.g. Barton & Hewitt 1985, 1989; Harrison 1990; Arnold 1992). Indeed, in some cases, hybrids have been viewed as evolutionary dead-ends because they were thought to be sterile, making them interesting oddities, but
nothing more than that. As outlined above, the explanation for this hybrid unfitness is that the act of hybridisation (the equal contribution of genes from two different stocks or species) has resulted in the break-up of co-adapted gene complexes, such that the hybrids are not genetically suited to the environment in which they arise or to which they recruit. However, more recent appraisals and reviews have indicated that in fact hybrids are often not less fit than the parental genotypes (e.g. Arnold & Hodges 1995; Gardner 1997). This suggests that not only are hybrids formed by occasional (or sometimes frequent) interbreeding of parental types, but that such hybrids (which would correctly be regarded as F1 (first filial generation) hybrids) are in fact capable of breeding, either with such hybrids (to produce F2 hybrids) or with one or both parental types to produce backcrosses. In turn, this has lead to the suggestion that hybrids may be an important evolutionary step towards the production of new genotypic variants, some of which may have greater fitness than the parental types for reasons such as heterosis (heterozygote advantage – because, by definition, F1 hybrids are the ultimate form of heterozygotes) or simply because they happen to be better fitted to the local environment as a serendipitous consequence of novel and previously untried genotypic combinations that out-perform parental genotypic combinations. Such F1 hybrids may be a bottleneck in the sense that they are rare, and therefore limit the production of new genotypic combinations (Arnold & Hodges 1995), but they may be nonetheless very important in promoting new evolutionary genotypes which enhance fitness of their holders within the environment under consideration.

In terms of the likely fitness consequences of interbreeding of north and south Greenshell™ mussels, there is very little published information on which to base predictions about relative hybrid fitness, and there is no clear indication from the literature about what to expect. The assessments of genetic variation by Gardner and colleagues (Apte et al. 2003; Star et al. 2003; Wei et al. 2009) point to the successful interbreeding of Kaitaia (northern stock) mussels with Horse Shoe Bay (southern wild stock) mussels when the former are transferred to the Stewart Island aquaculture site of Big Glory Bay. The genetically intermediate identity of the Horse Shoe Bay population between the northern and southern stocks strongly suggests that not only have northern genes introgressed into this southern population, but that hybrids and individuals of mixed ancestry (i.e. possible backcrosses) are reproductively active and that holders of novel genotypic combinations are surviving. The exact fitness status of such mussels of mixed ancestry is presently unknown, but is certainly worthy of investigation. Beyond this, it is presently not possible to say anything definitive about the genetic and fitness consequences of the mixing of the two stocks.

It is worth reiterating that our genetic knowledge of stock differences and their geographic locations is only as good as the information provided by the genetic markers. To date, we know of two distinct stocks and we have evidence of interbreeding and introgression of northern mussels at one wild southern site in Stewart Island. The present generation of genetic markers does not allow us to identify genetic differences between Kaitaia mussels and those at aquaculture sites in what is presently recognised as the northern group – i.e. the Coromandel Peninsula and the Marlborough Sounds. It must be born in mind that genetic differences may exist between the mussels of these different regions, but at present it is not possible to detect this. Thus, just because there are presently no apparent differences between these geographically distinct areas in terms of mussel genetic variation does not mean that no such differences exist. It simply means that we cannot detect them. Advances in molecular techniques and a new generation of molecular genetic tools (e.g. whole genome
sequencing and the identification of coding versus non-coding DNA sequence differences, and/or single nucleotide polymorphisms (SNPs) across the genome) will clarify this situation.

**What can we learn from other examples?**

This section provides two different perspectives of the possible consequences of the movement, mixing and interbreeding of genetically distinct stocks of Greenshell™ mussels in New Zealand.

**Scenario I**

Perhaps the best known, and most worrying example of aquaculture enhancement and its profound negative effects on wild populations in terms of decreased fitness involves the salmon industry from the western United States of America, in particular the Snake and Columbia Rivers (reviewed by Knudsen & MacDonald 1999). Salmonids such as sockeye salmon (*Oncorhynchus nerka*) have a highly developed homing instinct (philopatry) and return to their natal lakes, streams or rivers to breed after several years at sea. There is therefore often low or no interbreeding between fish from different natal regions, and over many generations this has resulted in high levels of genetic adaptation to the localised environment (*i.e.* the development of specialised co-adapted gene complexes which are highly environment-dependent) which promotes reproductive isolation, even among fish populations within a single lake (Quinn 1985; Quinn & Dittman 1990; Ramstad *et al.* 2004). The last 100 years has seen most wild salmonid populations in the western United States of America (and elsewhere) come under huge pressures from increasing fishing activity, increasing habitat degradation (*e.g.* increased stream sedimentation), dams being built which prevent the movement upstream of fish to their breeding grounds, and the loss of many streams and rivers as water is taken for crop irrigation (*e.g.* Costello *et al.* 2003). Cumulatively, these and other events have led to the reduction or extinction of localised breeding populations. To counter this, hatchery-based breeding programmes were set up to enhance wild populations. Millions of fry are bred each year and released into streams and rivers with the hope of enhancing or at least buffering wild fish populations against further losses. However, it has become apparent that the mass production of hatchery-produced fry has not had the desired effect. Typically such fish are produced from a relatively narrow genetic base (the parental stock is too small) and the fry are not genetically adapted to any particular localised environment. In short, hatchery production of fry tends to result in the breeding of fish which have high fitness in the hatchery (*i.e.* exhibit co-adapted gene complexes suited to the hatchery), but low fitness in the real world. Most worrying however, is the interbreeding of hatchery-produced fish with wild fish, and the associated impact of introgression of genes from hatchery-produced populations into wild populations which acts to decrease the mean fitness of the wild population. Thus, it may be argued that the hatchery-breeding programme has actually exacerbated the very problem that it was set-up to solve. Wild fish populations now have even lower fitness than before because the introgression of genes from hatchery-produced fish has reduced the mean fitness of wild populations.

This salmonid example is clearly not identical to the present Greenshell™ mussel situation. The primary difference is that the salmon fry are hatchery-produced (usually from wild stock), whereas the Greenshell™ mussel spat which are moved around New Zealand are wild caught (*i.e.* not hatchery produced). Nonetheless, the salmonid example provides an important lesson in the value of genetic variation which is an adaptation to the local environment, and why it is important that it not be disrupted. The concern for New Zealand’s Greenshell™ mussels is that such disruption might be able
to happen here. The interbreeding of northern and southern stocks of mussels as a consequence of the movement of Kaitaia spat around the country during the last ~25 years may lead to an outcome here in which one or more wild (native) populations of mussels has decreased fitness as a consequence of the introgression of northern genes into southern stocks. While evolutionary theory suggests that the genes of less fit individuals will be removed over time from a population because, by definition, they exhibit decreased fitness compared to the genes of other individuals, it is however possible for such genes to be maintained in the population in at least two different ways. First and most likely, the constant influx of northern genes as a consequence of the mass movement of Kaitaia spat may be sufficient to overwhelm the putative removal of such genes from the local (southern) population by the process of natural selection. Second, the introgressed northern genes may be maintained in the recipient southern population because fitness differences between the different genotypic combinations are low or are not subject to natural selection challenges in the present environment. Subsequent changes may expose the true extent of the loss of mean fitness within the population. An example of this sort of case is disease resistance, and the diluting of the genes which confer such resistance by the introgression of non-native and therefore non-adapted genes.

Scenario II

The literature pertaining to hybridisation in the sea has been reviewed by Gardner (1997). A number of different conclusions were reached in this review, the most important of which (in the present context) is that the fitness consequences of hybridisation cannot be generalised. On the basis of the data in the more than 100 papers reviewed by Gardner (1997), some generalities emerge (see below), but there is no single statement that can accurately predict the likely fitness consequences of hybridisation within any one genus. Each instance has to be treated on a case by case basis, as summarised below. In all cases, the study-specific references are given in Gardner (1997).

1. Examples of marine animal hybrid unfitness are plentiful and include slower rates of development (echinoderms, molluscs), decreased fertilisation success (crustaceans, echinoderms), reduced fecundity or complete sterility (crustaceans, echinoderms, flatfish), increased mortality (echinoderms, molluscs), increased susceptibility to gonadal neoplasia (molluscs), increased morphological variability (echinoderms), lower body size or weight (echinoderms), highly skewed sex ratios (fish), and higher rates of parasitism (fish, molluscs).

2. Hybrids are often reported to be morphologically intermediate between the parental types, reflecting the equal contribution of the two differentiated parental genomes. Other examples of intermediate hybrid fitness include development stability and developmental rate (echinoderms, molluscs), growth rate (molluscs), body size (cetaceans, echinoderms), fertility and fecundity (crustaceans, echinoderms, molluscs), viability (crustaceans, molluscs), chromosomal structure (molluscs), allozyme thermostability (molluscs) and resistance to parasites (molluscs).

3. In a smaller number of cases it has been reported that hybrids have increased fitness when compared to both parental types. Examples include feeding ability (echinoderms), growth rate (fish, molluscs), fecundity (fish), and longevity (molluscs).

4. In summary, hybridisation in the sea results in decreased individual fitness (either narrow sense with effect only on reproductive success, or in a wider sense beyond immediate reproductive success) about as frequently as it results in intermediate or increased hybrid fitness compared with parental types. Thus, hybrids are not uniformly unfit.
One final point is worthy of mention in the context of individual fitness, especially as it might be reported from field-based assessments. This point is almost always over-looked and may only receive attention in the context of laboratory or hatchery-based assessments of hybrid fitness. In this scenario, the fieldworker may successfully estimate relative fitness of individual mussels on the shore, but without realising that only a very small subset of hybrid individuals is actually being assessed. This is because it is theoretically possible for a very large proportion of hybrid mussel larvae to be formed from the union of gametes from the two parental types, but for the vast majority of these larvae to be unfit in the sense that they carry genotypic combinations which are non-viable. Such individuals are therefore selected against before they can recruit to the shore population. Thus, they are never seen by the fieldworker who has no knowledge of the massive selection experienced by the majority of hybrid individuals. What the fieldworker observes on the shore is the very small subset of hybrid mussels which carry successful combinations of genes (within the nuclear genome and/or as a combination of the nuclear and the mitochondrial genomes) which permit survival and may even confer some form of hybrid superiority. Thus, while field-based assessments of broad-sense fitness are very important, it is equally important to carry out hatchery-based determinations of fertilisation success and larval development and mortality rates to a time beyond metamorphosis and up to the size/age of successful settlement in the field. In this way the researcher can be confident of having most accurately quantified hybrid fitness across the full range of life history stages. If this sort of scenario does indeed occur when northern and southern Greenshell™ mussel stocks interbreed, then it will result in massive wastage of gametes from the local wild stock. This may cause problems later on in terms of diminished recruitment by local mussels which may open up space on the shore for other species and result in a long-term shift in the local ecological balance.

Recommendations
Given the fact that the mass transfer of *P. canaliculus* spat from Kaitaia to several locations in New Zealand has been going on now for at least two decades, and that this is viewed as being an activity of considerable economic benefit to New Zealand’s Greenshell™ mussel industry, it seems highly unlikely that this transfer will stop (this would be the course of action under the precautionary principle). Based on this assumption, the following recommendations are advanced to help improve knowledge about the fitness consequences (if any) of the interbreeding of *P. canaliculus* stocks resulting from the mass transport of mussel spat from one region (most usually Kaitaia, but it could be elsewhere) to another. These recommendations are not solely focused on the most obvious case of northern and southern stock interbreeding (in Stewart Island), but are framed in the larger geographic context to include the transfer of, for example, northern Kaitaia spat to other northern regions including, but not limited to the Coromandel Peninsula and the Marlborough Sounds.

1. Samples of wild mussels should be collected at various spatial scales (<1 km, 1-10 km, 10-100 km) from regions with a history of receiving mussel transfers from other regions. The most obvious examples will be the Coromandel Peninsula, the Marlborough Sounds and Stewart Island, although increasingly, other regions too may be affected. Samples should be tested with the best set of genetic markers available at the time to ascertain the extent (if any) of introgression of northern genes into southern wild populations.

2. Field-based assessments of fitness (growth rate, longevity, reproductive output, parasite counts, estimates of disease *etc etc*) should be conducted at recipient sites, as well as at wild sites at various distances from the donor site (see Gardner 1994 and Gardner & Thompson 2001) to
quantify individual fitness components. Such field-based work must be complemented by molecular assessments of mussel status (multi-locus genotype).

3. Samples of wild mussels should be collected as soon as possible from donor and recipient locations and archived. Such collections should be carried out routinely on a 5-year basis. Collections should be of ~100 mussels per site, and samples should be stored in alcohol (100% ethanol). Such archive material will be particularly valuable for subsequent analysis with new generations of markers, as these are developed. Once analysed, the archived material will provide historical estimates of the rate of spread (if any) of introgressed genes within each region.

4. Hatchery-based assessments of hybridisation rates between parental mussels from different donor regions should be conducted to quantify interbreeding success at all stages in the life-history of the animals, at least up to settlement stage. This is the only sure way to quantify individual fitness from fertilisation up to settlement, and will provide an indirect estimate of “gamete wastage” of native wild mussels (i.e. gametes lost to unsuccessful hybridisation).

Until these combinations of approaches are carried out to quantify individual mussel fitness at various sites there is no way to know just what impact (if any) that the mass transfer of spat around the country is having on the mean fitness of local stocks. Many of these projects would be highly suited for PhD candidates to conduct and will not be that expensive to run. There is no short-cut to finding the answer to what is, from several different perspectives, a fascinating question. The answer to this question is important to industry because of the possible long-term erosion of localised genetic variation which may provide critical new genotypic combinations for a hatchery-based breeding programme, as well as to regional authorities and the general public because of the implications for local marine communities.