Understanding the Ecology of Dorvilleid Polychaetes in Macquarie Harbour

Response of the benthos to organic enrichment from finish aquaculture

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Executive Summary

The expansion of salmon farming in Macquarie Harbour is contingent upon ecologically sustainable development. Underpinning such development are management regimes designed to regulate and minimise the impact of farming on the benthic environment. The management protocols implemented in Macquarie Harbour were developed based on farming practices and conditions in southern Tasmania and were established with the aim of ensuring that any impacts were not severe, reversible and could be constrained within defined boundaries. However, the response of the benthic invertebrate communities, and most notably Dorvilleid polychaetes, in Macquarie Harbour to enrichment from salmonid aquaculture has been inconsistent with expectations. The benthic communities in Macquarie Harbour, and their response to organic enrichment, differ from that previously observed in southern Tasmanian regions. In particular, the response of the Dorvilleid polychaetes in Macquarie Harbour was quite different to that observed in other polychaete species in southern Tasmania and highlighted the need for focused research on their ecology and behaviour in response to organic enrichment in Macquarie Harbour. This report describes the work conducted by the Institute of Marine and Antarctic Studies (University of Tasmania) to address this knowledge gap. In the first part of the study, the international literature was reviewed to establish the current state of understanding regarding Dorvilleid ecology, and in particular, how they respond to organic enrichment. The second part of the study comprised a targeted field survey at selected leases to identify the relationship between Dorvilleids and sediment condition, and to characterise the environmental conditions associated with major changes in Dorvilleid distribution and abundance. The results were compared with previous surveys (including baseline surveys undertaken throughout the harbour) to investigate whether there have been any broad scale changes in the benthic ecology. The findings are discussed in the context of benthic monitoring requirements for fish farm management in Macquarie Harbour.

Background

Previous research has shown a clear impact gradient associated with cage salmon farming operations, and that presence of bacterial mats (*Beggiatoa spp.*) and proliferation of opportunistic species are features commonly associated with high levels of organic enrichment. In southern Tasmania, Capitellid worms are the key opportunists associated with high levels of organic enrichment (Macleod and Forbes 2004). The understanding that proliferating opportunists represents deteriorating conditions was translated to monitoring protocols in Macquarie Harbour. Although the relationship between opportunists and the level of enrichment was not explicitly tested in this region, video surveys in Macquarie Harbour suggested that in this region Dorvilleid worms rather than Capitellids were the species most indicative of organic enrichment effects. However, recent responses of the benthos in Macquarie Harbour to enrichment from salmonid aquaculture have appeared to be somewhat inconsistent with expectations developed from southern Tasmanian regions. This project was designed to enhance understanding of the ecology of Dorvilleid polychaetes in Macquarie Harbour and their response to organic enrichment from fish farming through a review of literature and a field study.

Objectives

1. Review the international literature to establish the current state of knowledge regarding Dorvilleid ecology, and in particular, their response to organic enrichment. This will include a review of their current use as indicator of the impacts of finfish aquaculture.
2. Carry out targeted field survey at selected leases to identify the relationship between Dorvilleids and sediment condition, characterising the environmental conditions associated with changes in Dorvilleid abundance.
3. Based on the findings of 1. and 2. determine the reliability of Dorvilleids as an indicator of sediment condition in Macquarie Harbour
4. In conjunction with industry and government stakeholders make recommendations on the future use of Dorvilleids in regulatory monitoring of Salmonid aquaculture in Macquarie Harbour

Methodology

Benthic grabs and sediment cores were collected by IMAS to assess the relationship between benthic communities and organic enrichment. Aquenal Pty Ltd conducted concomitant ROV transects at all of the study sites; this provided an important comparison of estimates of Dorvilleid abundance from benthic grabs and ROV footage.

The sites were positioned along the enrichment gradient of each of the four leases sampled using a cross hair design, i.e. 4 transects radiating out from the lease at approximately 90 degrees to each other, with samples collected at 5 positions on each transect (0m, 50m, 100m, 250m and 500m from cages). In addition, 18 external sites were sampled that were at least 1km, but up to 10km, from the leases. The feed input for cages at each of the sampled leases throughout 2014 was made available by the relevant companies and compared with the distribution and abundance of Dorvilleids.

Results/key findings

Both species of Dorvilleids, *Ophryotrocha shieldsi* and *Schistomeringos loveni,* appear to be good indicators of organic enrichment from salmon farming in Macquarie Harbour. However, their presence reflects different levels of enrichment; *O. shieldsi* occurred predominately as colonies directly under stocked cages and was only occasionally observed out to 50m whereas the peak abundance of *S. loveni* was further away from the stocked cages at 50-100m. These results suggest that *S. loveni* is less tolerant of the conditions associated with highly enriched sediments (i.e. as would be found directly adjacent to stocked cages).

Although the distribution of each of the Dorvilleid species was patchy, both within and between leases, there were some broad patterns in their distribution which could be related to feed inputs and farm history. When feed inputs were low, the peak abundance of *S. loveni* was observed to be closer to the cage and where feed input was high, peak abundance of *S. loveni* was at a noticeably greater distance and in this instance both *O. shieldsi* and *Beggiatoa* were more common. These effects appeared to be exacerbated at leases which had been operational for a long time, with *O. shieldsi* and *Beggiatoa* persisting for longer and *S. loveni* reaching higher abundances in these situations.

The response of the sediment chemistry (redox, C and N isotopes and ratios) and bottom water dissolved oxygen levels to organic enrichment were consistent with the "*a priori*" expectations, and highlighted the influence of farm production intensity, farming history and the importance of local conditions in determining the magnitude of this response. For more recently established leases, the results also suggest that sampling sediment parameters to shallower depth is likely to provide a more sensitive measure of the organic matter footprint.

Changes in the composition of benthic communities were broadly consistent with that expected in response to organic enrichment but appear to be occurring at an increased spatial scale in Macquarie Harbour (i.e. at greater distances from the source (cages) than observed in the southern regions). Peak faunal abundance and species richness occurred at 50m and 100m, respectively, while species diversity increased from approximately 100m. At a functional group level burrowing/epibenthic fauna (mostly *S. loveni* and *Nebalia sp.*), were found to dominate out to 100m, but beyond this point tube building species (mostly Sabellid fan worms) dominated.

Comparisons with surveys conducted in 2000 and 2012 indicate that there have been some harbour-wide changes both in the benthic communities and in the overall abundance of Dorvilleids since the onset of large scale farming. There appears to be a general increase in abundance, but this is largely associated with species that respond well to increased levels of organic enrichment. Functionally this is reflected in increases in communities associated with more tube-building suspension feeders and surface-deposit feeders. As might be expected, the changes are more pronounced in areas where farming has occurred, with "farm communities" converging within the central harbour relatively quickly (<2 years). There is also some evidence that the harbour wide changes have occurred in the last 2-3 years. However, it is difficult to resolve the timing and factors behind the changes as the spatial and temporal resolution of the sampling lacks sufficient power.

Estimates of Dorvilleid abundance from the video (ROV) footage were compared with actual measures of abundance from Van-veen grabs and the results proved to be very enlightening. Interestingly the ROV was the most reliable approach for detection of colonies of *O. shieldsi*. This observation is particularly important given *O. shieldsi* appears to be the species most associated with *Beggiatoa* and an indicator of particularly high levels of enrichment. In contrast the ROV was not as reliable for the determination of the abundance of *S. loveni,* as this species would appear to reside both on the sediment surface and deeper in the sediments. It may be that the presence (or absence) of this species on the sediment surface is a function of sediment and bottom water conditions. If this is the case and the relationship can be established, then the ROV footage could be standardised accordingly. However, the behavioural ecology of *S. loveni* demonstrated that the ROV at present cannot provide a reliable indicator of this species' presence or relative abundance.

Finally, it is important to remember, that as comprehensive as this study was, it was conducted at one time of the year. Ultimately, the survey needs to be repeated throughout the year to take into account natural variability and different stages of farm management. Early insight from a survey in May highlighted why this is so important. This work has been funded and is already underway through FRDC project 2015-024.

Implications for relevant stakeholders

The monitoring protocols in Macquarie Harbour were based on the understanding that proliferation of opportunists was representative of deteriorating conditions. Although this relationship was not explicitly tested in Macquarie Harbour, ongoing environmental surveys and initial assessments of farming operations in this region suggested that the relationship held but that it would be Dorvilleid rather than Capitellid worms that would be the relevant indicator species (Department of Primary Industries Water and Environment 2004). The results of the current study show that there are two Dorvilleid worm species, *Ophryotrocha shieldsi* and *Schistomeringos loveni*, in Macquarie Harbour. Although both species are responsive to organic enrichment their distributions reflect different sensitivities. This has implications for their potential utility in regulatory monitoring and what that might mean for assessment of impact.

The findings of the current study would suggest that *O. shieldsi* is perhaps the species that would be most useful as an indicator of "unacceptable impact". This colony forming species was regularly observed in close association with stocked cages and in the presence of the anoxic/ hypoxic bacterial species *Beggiatoa*. This association would suggest that the presence *O. shieldsi* at compliance monitoring sites could be considered as representative of "unacceptable impact". On the other hand, *S. loveni* was found to be less tolerant of highly enriched sediments and interpretation of the presence of this species is more ambiguous. For example, *S. loveni* can occur at similar densities that are likely to reflect different levels of impact. They can be present closer to cages where conditions are deteriorating and relatively poor, and the benthic community might otherwise be considered to be highly disturbed (i.e. where there are few species and these tend to be dominated by burrowing and epibenthic taxa). However, a similar number of *S. loveni* may also be present further from the cages and associated with more moderate levels of enrichment and a more diverse community indicative of improving conditions. As a result, it is suggested that more context would be required when seeking to interpret the presence of *S. loveni* and the level of impact. It is hoped that repeat surveys currently underway through FRDC project 2015-024 will provide greater insight into the processes underpinning the variability and potential usefulness of *S. loveni* as an indicator species.

Notwithstanding the need for further study, it remains that the presence of *S. loveni* is associated with elevated levels of enrichment and that there is a clear response to enrichment in the benthic community in Macquarie Harbour, and that the pattern of response is not inconsistent with the patterns observed in southern Tasmania albeit with some important caveats. The results suggest that the response/ impact in Macquarie Harbour has a greater spatial footprint in that it can be observed at a greater distance from the cages than was the case in southern Tasmania. This has implications for management, as depending on the locations of cages within the lease area it may be possible for the effects to be observed beyond the lease boundaries. Unlike in southern Tasmania cages positioned close to the lease boundary in Macquarie Harbour are more likely to result in benthic effects outside the lease). The lease age and level of feed inputs were also found to have an influence on the spatial footprint in Macquarie Harbour. The lease with the lower feed inputs over the 12 months prior to sampling in the current study having a better (less impacted) benthic community composition close to the cage than the leases with higher feed inputs. In conclusion, this study has shown that changes in benthic community composition and the presence of *S. loveni* may provide valuable insight on the extent of the benthic footprint and changes in response to farm management. This will be explored in greater detail following repeat surveys in the FRDC 2015 024.

The comparison with baseline surveys highlighted a change in the broader benthic ecology over the past 15 years, and arguably mostly in the last 2 years, with a measurable increase in total abundance, species richness and species diversity. These observed changes have also had an influence at a functional level, with a decrease in burrowing taxa and an increase in tube builders (both suspension and deposit feeding). Whilst there could be a range of explanations for this change, such as a recovery from the effects of mining or influx of organic matter associated with changes in the regulation of catchment inflows, it is highly likely that the addition of nutrients and organic matter from fish farming has played some role in stimulating benthic productivity.

This work has clearly highlighted the value of the ROV as a monitoring tool. The full benthic assessment has shown the successional stages and indicators along the organic enrichment gradient but having established those the video footage (as in southern Tasmania) can then provide a quick and easy way to detect the key indicators of highly enriched conditions i.e. the presence of *Beggiatoa* and *O. shieldsi* colonies. The ROV has a distinct advantage over grab sampling in that it can quickly review large areas of the seabed, and thus greatly increase the sensitivity and ability to detect these key indicators in the environment despite the high degree of spatial patchiness observed. Although there are still questions regarding the reliability of the abundance of *S. loveni* as an indicator, it is important to note that the presence of this species on the sediment surface in ROV footage may be an important indicator and it is hoped that the new project FRDC 2015-024 will provide more information about this.

Recommendations

This project provides a detailed snapshot of the distribution of Dorvilleid polychaetes in Macquarie Harbour in January 2015 and has provided useful insights into their ecological role and behaviour within the harbour. The results show that there is a gradient of ecological response to organic enrichment in the harbour and that there are key indicators that can be used to infer impact stages. This study has also provided a good scientific rationale to support the continued use of ROV footage for monitoring both the spatial extent and implications of impacts associated with farming activities in the harbour.

However, there are some important caveats that must be considered when interpreting these findings. Firstly, as the sampling was performed at only one time, temporal changes, particularly seasonal ecological responses and cyclical/ seasonal farm management responses are still largely unknown. The inclusion of some preliminary data from repeated sampling of certain sites in May demonstrates that substantial changes in time occur in Macquarie Harbour and highlights the need for continued monitoring to develop a more complete understanding of the interactions between aquaculture and the benthos – this work is underway and funded under FRDC project 2015-024. In addition, we would recommend further studies to better understand the reproductive strategies, tolerances and environmental preferences of the key indicator species (including Dorvilleids) in this ecosystem.

A key challenge when assessing change in the broader system-wide benthic communities of the harbour was a lack of consistent sampling in space and time. In order to address this, we would recommend that regular sampling of reference sites distant from the farms be conducted.

Finally, the importance of local taxonomic expertise and the application of consistent sampling and processing methodologies across surveys cannot be understated, this is essential to ensure reliable data sets for ongoing assessments.

Keywords

Salmon Aquaculture, *Salmo salar,* benthic fauna, sediments, Macquarie Harbour, organic enrichment, Dorvilleids, polychaetes, *Ophryotrocha shieldsi*, *Schistomeringos loveni*, environmental management

1 Introduction

1.1 Background

Previous research has shown a clear impact gradient associated with cage salmon farming operations, and that presence of bacterial mats (*Beggiatoa spp.*) and proliferation of opportunistic species are features commonly associated with high levels of organic enrichment (Pearson and Rosenberg 1978; Macleod and Forbes 2004). The presence and abundance of these species can be used as an indication of deteriorating environmental conditions (Macleod and Forbes 2004). For example, the presence of numerous annelid opportunists, such as Capitellid worms, 35m outside the boundary of the lease area, may be interpreted as representative of “unacceptable impact” (Crawford 2002). This premise has been validated in south-east Tasmania (Macleod and Forbes 2004) and underpins regulatory monitoring requirements statewide (Department of Primary Industries Water and Environment 2004).

The understanding that proliferating opportunists represents deteriorating conditions was translated to monitoring protocols in Macquarie Harbour, but the relationship between opportunists and the level of enrichment was not explicitly tested in this region. That said, environmental surveys in Macquarie Harbour suggested that in this region Dorvilleid worms rather than Capitellids were the species most indicative of organic enrichment effects (Department of Primary Industries Water and Environment 2004).

However, Macquarie Harbour is ecologically very different from farming areas in southern Tasmania and elsewhere: the sediments in this region are inherently depauperate (O’Connor et al. 1996; Talman et al. 1996; Edgar et al. 1999) with a large component of the natural fauna being epibiotic and spatially patchy. A recent study in Canada has highlighted the need to better understand the relationships and compliance thresholds for established enrichment indicators (i.e. *Beggiatoa spp.* and opportunistic polychaete complexes) in systems where ecological patchiness may occur (Hamoutene et al. 2014); suggesting that where there is significant potential for small scale spatial variability, normal successional responses may not be as reliable.

1.2 Need

Previous research and reporting has addressed the desire for ecologically sustainable development of the Tasmanian Salmonid Industry in Macquarie Harbour over the coming decade. Underpinning such development are management regimes that regulate and minimise the impact of the farms on the benthos, and ensure that the impact is contained (to within 35m of the lease boundary) and reversible or not severe.

Recent responses of the benthos in Macquarie Harbour to enrichment from salmonid aquaculture have appeared to be somewhat inconsistent with expectations developed from southern Tasmanian regions. Concerns from industry were raised over the response of macrobenthic communities, in particular Dorvilleid polychaetes, and thus the appropriateness of Dorvilleids as indicators of enrichment. The limited understanding of the behaviour of Dorvilleid polychaetes in Macquarie Harbour highlighted the need for focused research into this area. Such research will underpin recommendations on the utility of Dorvilleids as an indicator of organic enrichment in regulatory monitoring requirements for fish farming in Macquarie Harbour.

This project was designed to enhance understanding of the ecology of Dorvilleid polychaetes in Macquarie Harbour and their response to organic enrichment from fish farming through a review of literature and a field study.

2 Objectives

The objectives of this study were as follows:

1. Review the international literature to establish the current state of knowledge regarding Dorvilleid ecology, and in particular, their response to organic enrichment. This will include a review of their current use as indicator of the impacts of finfish aquaculture.
2. Carry out targeted field survey at selected leases to identify the relationship between Dorvilleids and sediment condition, characterising the environmental conditions associated with changes in Dorvilleid abundance.
3. Based on the findings of 1. and 2. determine the reliability of Dorvilleids as an indicator of sediment condition in Macquarie Harbour
4. In conjunction with industry and government stakeholders make recommendations on the future use of Dorvilleids in regulatory monitoring of Salmonid aquaculture in Macquarie Harbour

For Objective 2, a sampling regime was designed to address the following four sets of research questions, allowing overlapping sampling.

Part 1: Distribution and abundance of Dorvilleids

- Do Dorvilleids respond to salmon farming enrichment?
- Are there multiple species and do they respond in a similar fashion?
- Is farm derived enrichment more extensive in Macquarie Harbour than elsewhere?

Part 2: Regional and spatial variation in enrichment

- Does the response to organic enrichment vary within the harbour (regionally)?
- Does the extent of the farm enrichment footprint vary within the harbour (regionally)?
- Do regional differences in environmental conditions influence the response to enrichment?

Part 3: Benthic community composition

- How do the communities and sediment condition measured in parts 1 and 2 relate to the broader benthic ecology of the harbour?
- Has the benthic ecology of the harbour changed over recent years?

Part 4: Sensitivity of video assessment

- Does the level of enrichment, source and or environmental conditions influence the behavioural ecology of Dorvilleids?
- How does the behavioural ecology influence the sensitivity of video assessment?

It is important to note that the work program for this study was subsequently expanded at the request of industry and government. A fourth lease was added to the sampling program and an additional 10 external sites. Furthermore, to investigate if there is a shift in behaviour of Dorvilleids with size, one of the three grabs from each site was sieved and sorted down to 0.5mm as well as 1mm. The comparisons with historical surveys (part 3 above) to assess how the harbour has changed has been expanded to form a considerable part of the study.

3 Review of Dorvilleid Polychaetes

This review examines the use of Dorvilleid polychaetes as indicators of enrichment, especially as that relates to finfish aquaculture. It is particularly focused on the two species of Dorvilleid identified in Macquarie Harbour, *Ophryotrocha shieldsi* and *Schistomeringos loveni* examining the life history of these and related species and considers the possible environmental factors that might influence (seasonal) changes in abundance.

3.1 Dorvilleids as indicators of enrichment

Polychaetes as a group frequently account for a large proportion of the benthic macrofauna and often possess characteristics that can make them good indicators of organic enrichment. Many studies have shown that opportunistic polychaetes can be useful indicators of organic enrichment, from the seminal study by Pearson and Rosenberg (1978) and the reviews by Pocklington and Wells (1992) and Levin (2000) to more recent studies focussing specifically on the monitoring and management of impacts from finfish aquaculture (e.g. Weston 1990, Iwama 1991, Hargrave et al. 1997, Macleod et al. 2004, Holmer et al. 2005, Kalantzi and Karakassis, 2006, Holmer et al. 2008a, Hamoutene et al. 2014). Change in abundance of opportunistic species has been used as an indicator of organic enrichment from a range of different sources, such as sewage outfall (Dauer and OÇonnor 1980, Bailey-Brock et al. 2002), pulp mill effluent (Pearson 1975) and aquaculture (Hargrave et al. 1997, Tomassetti and Porrello 2005; Martinez-Garcia et al. 2013). For finfish aquaculture, the abundance of these indicator species generally decreases with distance from cage and these species are often significantly correlated with other known indicators of enrichment and/ or fish farming, i.e. organic content, flocculent matter, evidence of off-gassing, sulfides and also the benthic bacteria *Beggiatoa* (Holmer et al. 2008b, Hargrave 2010, Keeley et al. 2012). Many countries use the presence and abundance of specific polychaetes or opportunistic species as indicators of organic enrichment for environmental assessment, and this is a common feature in environmental assessment and management of aquaculture (Henderson and Ross 1995, Wilson et al. 2009). However, indicator species do not always respond in a consistent manner to enrichment and as such they can be site-specific, therefore any specific management criteria should be developed in the context of the study area (Bustos-Baez and Frid 2003).

Dorvilleid species have been shown to be opportunistic polychaetes; they can tolerate anaerobic sediments and high levels of hydrogen sulphide (Levin et al. 2013), and can be tolerant of pollutants (Cheung et al. 2008; Belan 2003; Taboada et al. 2015) and heavy metals (Davydkova et al. 2005); they often have short life-cycles and display a range of reproduction strategies (Beesley et al.2000), and as such can respond very quickly to changes in enrichment and sediment chemistry (Tomassetti and Porrello 2005).

Dorvilleids can be useful indicators of the impacts of finfish aquaculture (Hall-Spencer et al. 2006; Paxton and Davey 2010; Keeley et al. 2013), with abundance increasing close to cages. In New Zealand it has been suggested that Dorvilleids act as second order opportunists, with the impact gradient progressing from Dorvilleids→Capitellids→Nematodes→Azoic (Keeley et al. 2015).

Similarly, Dorvilleid abundances have also been observed to increase close to mussel farms (Hartstein and Rowden 2004). The relative abundance of *Schistomeringos loveni* and brittle stars was found to reflect the organic enrichment gradient from the mussel lease, with Dorvilleid abundance increasing close to the lease where enrichment was higher and ophiuroids (brittle stars) being more abundant outside the lease area where the organic enrichment levels were low. Water flow had a significant effect on the impact levels from the mussel farms; with little evidence of impact at high energy sites but a clear gradient of effect inside and outside the lease areas at the more sheltered sites. In this study there was a strong correlation between the sediment Carbon: Nitrogen ratio, levels of total organic matter and *S. loveni* abundance at the low energy sites but little correlation between the macrobenthos and these environmental variables at the higher energy sites. These observations might suggest that Dorvilleids may not be as useful as indicators of enrichment where there is higher water flow. Thus, the water movement within Macquarie Harbour may influence the effectiveness of Dorvilleids as indicators of enrichment.

3.2 Life History and Reproductive Strategies of Dorvilleid Polychaetes

In general, Dorvilleids are opportunistic species that colonise a range of enriched environments and their life histories and environmental tolerances reflect this. For example, Massamba-N'Siala et al. (2012) demonstrated the phenotypic plasticity of life history traits and thermal tolerances of the Dorvilleid *Ophryotrocha labronica*, and as such their ability to adapt to the effects of environmental changes in temperature.

While sex determination is generally considered polygenic, some species of *Ophryotrocha* have shown plasticity in sexual expression including: four sexual phenotypes (i.e. pure females, pure males, females with a few sperm and males with a few oocytes); sex determination influenced by the sex of adults in the environment; and some degree of flexibility as adults depending on environmental conditions (Meconcelli et al. 2014; Lorenzi and Sella 2013). There are even two Dorvilleid species, *Parougia bermudensis* and *Parougia albomaculata*, that are obligate asexual reproducers, something which is uncommon among polychaetes (Akesson and Rice 1992). *Ophryotrocha* are particularly flexible, displaying dioecious, simultaneously hermaphroditic and sequentially hermaphroditic reproduction - some species even show a high degree of labile sex expression in response to social conditions (Meconcelli et al. 2014). This level of flexibility allows species to colonise new areas easily and recover quickly when the population size is reduced for any reason.

Some studies have suggested that there may be trade-offs between traits related to male and female function, which may reduce as sexual specialisation evolves (Di Bona et al. 2014). Dorvilleids show a range of fertilisation methods including pseudocopulation in most *Ophryotrocha* species, copulation by hypodermic impregnation in *Dinophilus* and some external fertilisation (Beesley et al. 2000). Some species, particularly the smaller ones, brood their egg masses, whilst others are broadcast spawners and some, for example *S. rudolphi*, are free-spawning with a lecithotrophic planktonic stage (Beesley et al. 2000). Mercier et al. (2014) found that a deep sea species of *Ophryotrocha* guarded juveniles in egg masses until they reached dispersal at the 1-chaetiger stage, after which the parents died. In this case the juveniles took 8-9 months to reach sexual maturity. Life history and reproductive strategy are important factors in determining whether a species can be considered a reliable indicator over the longer term.

3.3 *Ophryotrocha shieldsi*

Ophryotrocha is a large genus with a cosmopolitan distribution covering a range of enriched and sulphidic environments; from polluted harbours in the Mediterranean (Simonini et al. 2010), sewage outfall in the tropics (Bailey-Brock et al. 2002), methane and mud seeps (Thornhill et al. 2012; Levin et al. 2013; Decker et al. 2012), to wood fall and whale fall in temperate and polar regions (Wiklund et al. 2009; Taboada et al. 2013; Wiklund 2009; Wiklund et al. 2012).

A new species, *Ophryotrocha shieldsi,* found in association with fish farms was recently described from Macquarie Harbour (Paxton and Davey 2010). *O. shieldsi* forms aggregations resembling mounds with fragile, often vertically-aligned tubes that appear to move together as a group (Paxton and Davey 2010). From observations of ROV footage, this species occurs in areas immediately beneath or adjacent to the fish cages. This species is small and only infrequently collected in benthic grab samples. The colonies of *Ophrytrocha shieldsi* appear similar to the colonies of *O. cyclops* observed from video footage underneath finfish cages in Newfoundland, Canada (Salvo et al. 2014; Salvo et al. 2015).

O. shieldsi has been located in the 'lobifera' clade of *Ophryotrocha* with *O. craigsmithi*, *O. lobifera* and *O. orensanzi* (Taboada et al. 2013). *Ophrytrocha cyclops*, a recently described species found on both whale fall and in association with finfish aquaculture sites, has close affinities with *O. shieldsi*, *O. lobifera* and *O. craigsmithi* based on phylogenetic analyses (Salvo et al. 2014). All species within the ‘lobifera’ clade have been found in the enriched environments, and in association with bacterial mats (e.g. *Beggiatoa spp*.) and there is some suggestion that they may feed on these (Taboada et al. 2013; Salvo et al. 2014). There have been shown to be direct trophic linkages between *O. cyclops* and fish pellets, with this species apparently consuming both flocculent matter and bacterial filaments (Salvo et al. 2015). Other *Ophryotrocha* species (e.g. *O. maculata*, originally described at pulp mill outfalls), and Dorvilleids more generally have been observed to feed on bacterial mats (Wiklund et al. 2009, Taboada et al. 2013; Salvo et al. 2015).

Simonini et al. (2010) examined the diet of eight species of *Ophryotrocha* in the Mediterranean, representing both the major clades (‘labronica’ and ‘hartmanni’), and found that all were omnivorous with similar dietary requirements. Faecal pellets contained a range of material from sediment and particulate detritus to unicellular microalgae (e.g. diatoms and dinophyceans), to multicellular algae, sponges, and fragments of *Ophryotrocha* and crustaceans. *Ophryotrocha* have also been successfully cultured in laboratory conditions on a diet of spinach (Di Bona et al. 2014; Lorenzi and Sella 2013; Massamba-N’Siala et al. 2012).

Ophryotrocha display a wide range of reproductive strategies and the major clades can be separated based on their gonochronistic or hermaphrodite strategies, though it's not known if hermaphroditism or gonochronism was the ancestral state (Dahlgren et al. 2001). Based on laboratory observations, *O. shieldsi* reproduces sexually, with the male fertilising eggs after they are spawned by the female (Paxton and Davey 2010). However, its reproductive strategy is not fully understood as it was not possible to observe the egg development. While other clades within *Ophryotrocha* are clearly grouped according to gonochronism (‘labronica’ clade) or hermaphroditism (‘hartmanni’ clade) (Dahlgren et al. 2001), the reproductive strategies of the ‘lobifera’ clade are unknown. The development of free trochophores in the ‘lobifera’ clade most resemble *O. maculata and O. natans* (Paxton and Davey 2010), members of the ‘hartmanni’ clade where *O. natans* is a simultaneous hermaphrodite (Taboada et al. 2013; Pfannestiel 1976).

Given the range of life history strategies observed globally for the genus *Ophryotrocha* it might be useful to clarify these characteristics for *O. shieldsi* in Macquarie Harbour. In this context it is important to note that *O. shieldsi* has free swimming trochophores (Paxton and Davey 2010), which could potentially aid dispersal between enriched areas of the harbour and would suggest it might be a reliable indicator. Consequently, clarification of the reproductive strategy and environmental tolerances (i.e. dissolved oxygen and temperature) of both adult and juveniles for *O. shieldsi* specifically may help to explain the observed distribution patterns and environmental relationships.

3.4 *Schistomeringos loveni*

Schistomeringos is not as widely studied as *Ophryotrocha* but the genus also displays a range of habitat and environmental tolerances and reproductive strategies. *Schistomeringos loveni*, the species found in Macquarie Harbour, is widespread throughout Australia from the Great Barrier Reef, along the eastern coast and down into Tasmania. Research conducted on colonisation of *Schistomeringos loveni* at Lizard Island, on the Great Barrier Reef found that this species probably has a relatively short life span, (maximum 2 years), possibly only reproducing once and that breeding generally occurs in late summer but that it may occasionally be extended with some winter recruitment, as such colonisation generally occurred from late summer through to early winter (Hutchings et al. 1992).

In general, *Schistomeringos* species seem to be relatively tolerant of pollution and organic enrichment but the degree of tolerance would seem to vary depending on species, location and the nature of the contamination. For example, *Schistomeringos japonica* appears relatively insensitive to contaminants from municipal and industrial waste water, urban runoff, marine transportation and dredged materials (Belan 2003), and is tolerant of heavy metal pollution (Davydkova et al. 2005), whilst *Schistomeringos rudolphi* was classified as only moderately pollutant tolerant with species of terebellidae, nereididae and capitellidae being deemed more tolerant (Cheung et al. 2008). However, in another study the same species *S. rudolphi*, was classified as a first order opportunist, pioneer, coloniser and species extremely tolerant to hypoxia (Simboura and Zenetos 2002). *S. loveni* has been found in sediments subject to organic enrichment from aquaculture specifically (Bright 2001), and the change in abundance of this species was an important indicator of the communities inside and outside mussel leases (Hartstein and Rowden 2004). Similarly, *Schistomeringos annulata* has been found both at reference stations and underneath cages in a study from British Colombia, but once again it was far more abundant close to the cages than elsewhere (Bright 2001).

Like other Dorvilleids, *Schistomeringos* species have a range of dietary preferences, with some being carnivores while others prefer algae and detritus (Fauchald and Jumars 1979). Species of *Schistomeringos* have been found in tropical seagrass beds in Bermuda (Pocklington and Coates 2010), the Mediterranean (Prevedelli et al. 2005) and in the cold-temperate Sea of Japan (Davydkova et al. 2005; Belan 2003).

Clearly *Schistomeringos* species are not exclusively associated with enriched environments. The life history information that is available for *Schistomeringos* would suggest that there are marked differences in the environmental tolerances of this genera and that there is the potential for particular species to adapt to environmental conditions. Consequently, once again, obtaining a better understanding of the reproductive strategy and environmental tolerances specific to Macquarie Harbour could greatly enhance our potential to interpret the distribution patterns and environmental relationships.

3.5 Seasonal changes in abundance

Numerous studies have found temporal and/ or seasonal changes in abundance of polychaete species, or species that act as indicators of enrichment. This may be due to temperature and other climatic conditions influencing reproductive behaviour or changing competitive relationships between species. For example, Dean (2008) in a review of the use of polychaetes as environmental indicators noted that dominant species (polychaetes) within the community can change both from year to year, and seasonally. In La Spezia, Italy, abundance of Dorvilleid polychaetes changes seasonally with *S. rudolphi* being most abundant during the winter months, January and February (Prevedelli et al. 2005). The authors suggest this could be linked to temperature (Prevedelli et al. 2005), with temperature being recognised as a key driver of both life cycles and abundance (Simonini et al. 2010). An undescribed deep-sea species of *Ophryotrocha*, which should not be affected by the same temperature seasonality as shallower species, was still found to show a cyclical reproductive strategy, only breeding between February and May (Mercier et al. 2014).

Temporal variation in the benthic community, and the response of Dorvilleids to enrichment from marine fish farming has been reported in a number of studies (e.g. Sasaki and Oshino 2004, Tomassetti and Porrello 2005, Koçak and Katağan 2005). For example, Sasaki and Oshino (2004) found that sedimentation from salmon cages changed cyclically concomitant with the production cycle, this in turn changed the organic loads in the benthos and the abundance of benthic indicator species such as *S. japonica* responded accordingly. Abundances were highest in July at harvest time and lowest in February following 7 months of fallowing. The authors also noted that the number of mature *S. japonica* decreased between July and September and by December only juveniles dominated.

4 Field Survey Methodology

4.1 Survey Design

The field survey addressed Objective 2 of this study, and was designed to incorporate the research questions highlighted in section 2 of this report. The 105 sites, details in Appendix 11.5, were selected to evaluate the relationship between Dorvilleids and sediment condition at both farm (research questions - Part 1) and lease scales (research questions - Part 2). Sites external to the active leases were included to allow a comparison between benthic communities associated with farming and the broader benthic ecology of the harbour (research questions - Part 3). These sites were also chosen to maximise overlap with previous benthic assessments and explore any changes in the benthic ecology of the harbour since baseline surveys were conducted in 2000 and 2012 (research questions - Part 3). Benthic grabs and sediment cores were taken by IMAS for the assessment of benthic communities and environmental parameters while Aquenal Pty Ltd conducted concomitant ROV transects at all of the study sites. This provided an important comparison of estimates of Dorvilleid abundance from benthic grabs and ROV footage, which is currently used as a regulatory monitoring tool in Macquarie Harbour (research questions -Part 4). The feed input for cages at each of the sampled leases throughout 2014 was made available by the relevant companies and compared with the distribution and abundance of Dorvilleids to give insight to any variation in response related to feed input (research questions – Parts 1 and 2).

Samples were taken along the enrichment gradient of each of the four leases sampled using a cross hair design, i.e. 4 transects radiating out from the lease at approximately 90 degrees to each other, Figure 4-1. On each transect sampling occurred at 5 positions (0m, 50m, 100m, 250m and 500m from cages). In addition, 18 external sites were sampled that were at least 1km, but up to 10km, from the leases, Figure 4-2. While the lease boundary lay within 500m from the cages, sites along transects are referred to in this report as lease sites. One transect from each of leases 266 and 267 was extended to measure every 250m up to 1250m and 1500m from the cage, respectively, extending into unoccupied lease 213. Dorvilleid polychaetes had been found on ROV footage at this lease, despite the absence of farming.

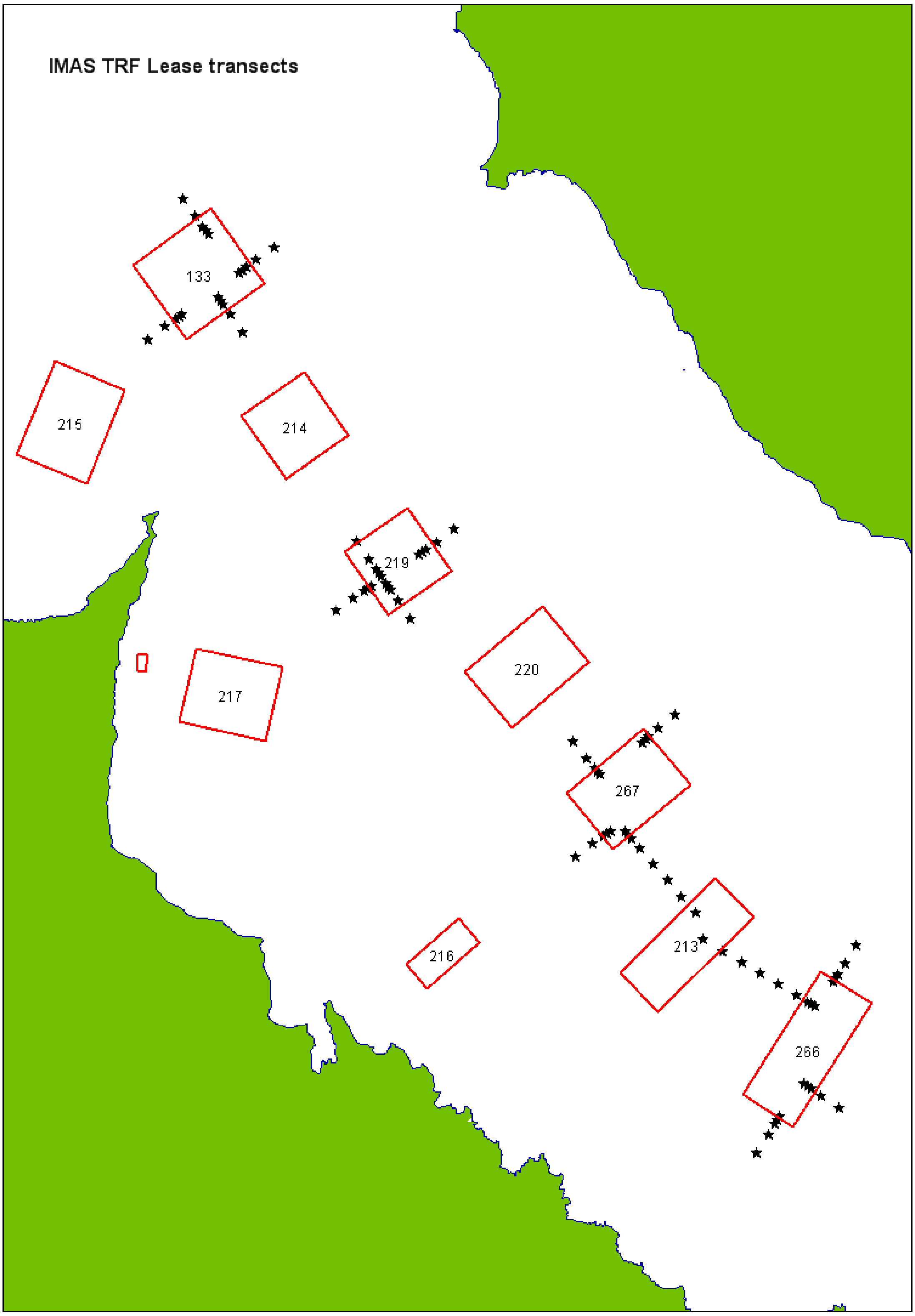

Figure 4-1 Map of Macquarie Harbour showing leases boundaries (red lines) and number with sample sites radiating out in a cross-hair design

Figure 4-2 Map of Macquarie Harbour showing the location of leases and cages (pink lines with black oblongs) and external external sites (numbered red dots).

4.2 Environmental Characteristics

At each site triplicate samples were collected using quad-corer consisting of Perspex tubes (250mm long, 45mm internal diameter) to evaluate sediment redox, organic carbon and nitrogen content and their isotopic composition (δ^{15}N, δ^{13}C). Two cores were used for analysis and the third set aside as a sediment archive (top 2cm).

In the field, a WTW combination oxidation-redox potential probe, calibrated with Zobell's solution was used to measure the redox potential of the sediment from two cores at 3cm depth. Cores were photographed and physical descriptions of the colour and layers of the sediment noted. Readings were taken when the meter displayed constant values for approximately 10 seconds. The top 2cm of each of the cores was then taken and frozen for subsequent analyses. Upon return, the sediment samples from the cores were freeze dried to constant weight. Samples for carbon and nitrogen content and isotopic composition were ground and the sample for carbon analysis was acidified with a dilute HCl solution to dissolve solid carbonates. The Water Studies Centre (Monash University) analysed the samples on an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK). The precision of the elemental analysis was 0.5 µg for both C and N (n = 5). The precision of the stable isotope analysis was ±0.1‰ for ^{13}C and ±0.2‰ for ^{15}N (SD for n=5). Stable isotope data are expressed in the delta notation (δ^{13}C and δ^{15}N), relative to the stable isotopic ratio of Vienna Pee Dee Belemnite standard (RVPDB= 0.0111797) for C and atmospheric N2 (RAir = 0.0036765) for nitrogen.

To help characterise the conditions beyond that of the sediment, the physio-chemical properties of the overlying water column (dissolved oxygen, salinity, pH and temperature) were measured at each site at 5m intervals using a YSI 6600 V2 Multi Parameter Water Quality Sonde with YSI 650 MDS logger.

The environmental indicators used in this study were compared with the distance from the cages to give an overall impression of the degree and extent of enrichment in the sediment. External sites, and extended transect sites were grouped into three distances categories for analyses: 501-1250m, 1251-2000m and >2000m.

4.3 Macrofauna

Benthic infauna were sampled in triplicate at each site using a Van Veen Grab (surface area 0.0675 m^2). All grab samples were wet sieved to 1mm, and one replicate of the three was subsequently sieved to 0.5mm. Samples were preserved in 10% formalin: seawater (4% formaldehyde) in the field, and washed and stored in ethanol after being transported to laboratories in Hobart. Samples were sorted and the infauna identified to the lowest possible taxonomic level and counted.

The abundance of Dorvilleids in the untransformed 1mm grab data was analysed by Analysis of Variance (ANOVA) using R (R Core Team 2014) to detect differences in *S. loveni* abundance between leases, transect direction in the harbour, distance along the transects and interactions between these factors. All factors were included as fixed factors.

Dorvilleid polychaetes in Macquarie Harbour lie at the centre of this study. As such, the analysis adopted has focused on Dorvilleid abundance and distribution throughout the harbour. To complement this, total abundance of all taxa (N), Species richness (S) and Shannon-Wiener diversity index (*H*') were calculated for each site based on both the 1mm and 0.5mm sieved samples in R (R Core Team 2014) using the vegan package (Oksanen et al. 2015). Multivariate community analysis was conducted in Primer-e 6 (Clarke and Gorley 2006). 1mm grab data were averaged by site, and both 1mm and 0.5mm data were square-root transformed and Bray-Curtis dissimilarity matrices created. On these matrices, two permutational CAP (Canonical analysis of principal coordinates) were performed based on the workflow described by Anderson and Willis (2003) to determine differences in the *a priori* groups of distance along transect and lease. Furthermore, a BIO-ENV analysis was conducted to reveal any correlations between community structure and the environmental parameters measured

4.3.1 Functional groups and indices

The infauna were assigned to broad functional groups reflecting their behaviour and function in the sediment, modified from categories in Ross and Macleod (2012): epibenthic, burrowing, tube-building, burrowing/epibenthic, epibenthic/tube-building, unknown. Functional groups can be difficult to assign as some taxa can switch between groups, and others do not fit neatly. In addition, these groups do not reflect the trophic level, feeding guild or mobility or the taxa. For example, some of the burrowing taxa are sessile suspension feeders and others predatory and motile. However, the groups selected represent a level of behaviour that can still shed light on community changes along the impact gradient.

A second community index used to indicate the level of impact present was tested. AMBI, Atzi Marine Biotic Index (Borja et al. 2000; Borja and Muxika 2005), was developed to assess the level of impact at a site based on the proportion of species that are known to be tolerant of or sensitive to stressful conditions. Where possible, species from this study and historical data were matched with species in the AMBI database. Not all taxa could be matched to species level; these were matched to a generic or family level classification in AMBI. Some taxa were matched to a different species of the same genus if that species appeared to belong to a more appropriate ecological group than the genus group, or if no broader taxonomic group was available. Some species, particularly those in very low abundance in the study, were not assigned to an ecological group if no suitable match could be found, or the matching taxon was unassigned. This accounted for, on average, 18% of the total abundance at each site. *Schistomeringos loveni* was not present in the species database, but other members of *Schistomeringos* were, as well as a generic group (*Schistomeringos* sp.). The generic group had an ecological classification of II, species indifferent to enrichment, yet many of the congeners belonged to a higher ecological group, and based on the prior knowledge and information in section 3.4 of this report the EG II ranking was considered misrepresentative of *S. loveni*. Thus, *S. loveni* was matched with *S. annulata* based on the behaviour *S. loveni* from this study and the behaviour of *S. annulata*, which has been identified as an indicator of enrichment at salmon farms in Canada (Bright 2001). A full list of the species, their ecological group and the taxa they were matched with in AMBI can be found in the Appendix 11.4 Species List.

4.3.2 Changes over time

To assess how benthic communities have changed since farming began in Macquarie Harbour, both 1) broadly in the harbour and 2) in direct response to farm derived organic enrichment, the data from this study were compared to baseline surveys of farm and external sites conducted during 1999-2003 and 2012. A challenge with investigating potential changes through time is taking into account concomitant differences in sampling methodology, site locations and the amount and location of farming. Fortunately, the principal sampling techniques have largely remained the same with triplicate Van Veen grabs used to collect fauna at each site, infauna sieved to 1mm and sorted to at least family level. To address differences in site location and farming activity, the analysis was restricted to sites sampled in the same region across surveys. The total number and distribution of sites within each region also varied across surveys given their different purposes, and as such, a similar number of sites with a comparable distribution (e.g. depth range) were selected to minimise any potential biases.

To assess broader changes to the harbour benthic ecology between 2000 and 2015, 16 sites from each survey were selected that were at least 500m from an active farm[1] and in the central harbour region that was sampled in both 2000 and 2015.

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[1] It is important to note that this does not discount the potential for farming to have influenced benthic communities beyond 500m. In fact, if communities have changed significantly through time more broadly in the harbour, increased farming would be one of a number of potential mechanisms driving it.

To investigate benthic community changes due to farming the baseline surveys for leases 133 and 219 conducted in 2000 were compared to the current survey data for these leases. For this comparison, 12 sites from each lease (i.e. at 0, 50 and 100m) in the current survey were compared to 12 randomly selected baseline sites from within the same leases[2] and the 35m site. The comparison of changes at lease 133 and 219 provides an interesting contrast given that lease 219 has been actively farmed for over a decade since the baseline survey whereas 133 wasn't farmed until 2013.

Baseline surveys for new lease sites were conducted in 2012. Considerable differences between the baseline results for the benthic community and earlier baseline data collected in 2000 prompted DPIPWE to sample a sub-set of the external sites. Comparisons between the different surveys can been seen in Figure 4-3. The 2012 baseline survey (referred to here as 2012 baseline #1) found very few individuals at all depths, at either external or new lease sites, compared to the 2012 DPIPWE survey, 2000 baseline survey and the current 2015 data. As a consequence, the 2012 baseline was repeated (2012 baseline #2). It is important to note that for all surveys, with the exception of the 2012 baselines, the same invertebrate taxonomist was involved in the collection, processing and identification of benthic invertebrates.

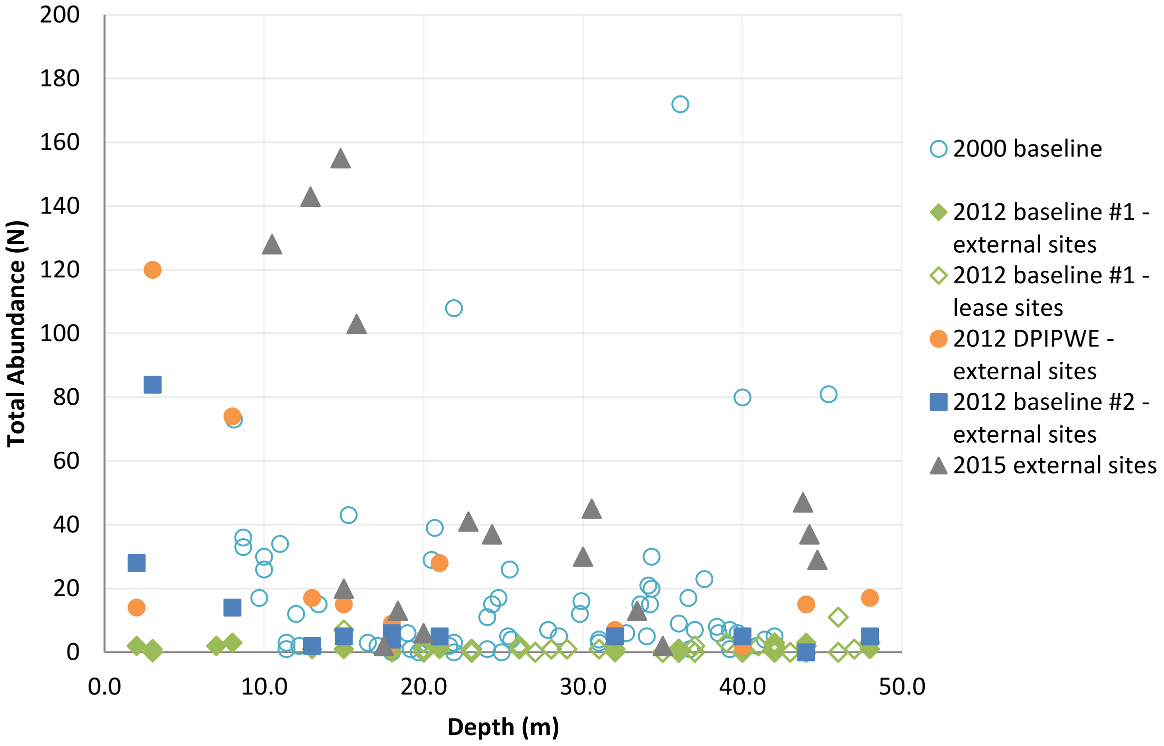

Figure 4-3 Comparison of total abundance data with depth between the 2000 baseline, 2012 baseline surveys (initial #1 and repeated #2 survey, 2012 DPIPWE re-survey of external sites and the 2015 external sites

Given the discrepancies found between the original 2012 baseline community data[3] and the resampled external sites by DPIPWE, only the latter data were used for the comparison of benthic communities between 2012 and 2015; this was restricted to the nine external sites that overlapped both surveys.

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[2] Noting the lease sites are pre-farming

[3] Although the baseline was repeated and abundances clearly higher, there were differences in the species identification, and thus we restricted the analysis to the data that had been proceeded and identified by the same taxonomist

Finally, a comparison was made between benthic community composition across 2000, 2012 and 2015. This comparison was restricted to all external sites sampled in 2015, all external sites sampled by DPIPWE in 2012 and the 2000 sites that broadly overlapped the same region. Although there is not perfect site overlap across the surveys, the sites nonetheless span the same region of the harbour, and as such, will provide further insight into potential longer term harbour changes in benthic ecology. Site details for each of the comparisons are available in appendix 11.6.

These comparisons were conducted with the same parameters measured for the macrofauna in the present survey (abundance of Dorvilleids, *N*, *S*, *H'* and functional groups) with the exception of AMBI and CAP as very sparse samples interfered with accuracy of these measures. Instead of a CAP a PCO analysis was conducted in Primer-e 6 (Clarke and Gorley 2006) to compare the current communities with those from 2000 and 2012.

4.4 Video Assessment

Video assessments of all 105 sites using an ROV were conducted by Aquenal Pty. Ltd. with 3 minutes of footage collected per sampling site. The principal aim of this sampling was to compare Dorvilleid abundance and patterns of distribution estimated from ROVs with benthic grab samples.

The quantitative method of video analysis described by Crawford et al. Macleod (2001) was adopted and modified to the requirements of this study. In particular, scoring categories were expanded for Dorvilleids, which were counted and classified in greater detail, Table 1. Additional fauna or features were noted as appropriate. Scoring of videos was performed by IMAS and DPIPWE staff.

Table 1 Scoring categories for video assessment

Dorvilleids	***Dorvilleid abundance***	***Dorvilleid morphology***	***Dorvilleid distribution***
no	0-30	long	uniform
individuals	30-100	short	patchy individuals
colonies	>100	mixed	patchy individuals/colonies
mixed	>300		
	>1000		
Capitelids	***Sabellids***	***Crustaceans***	***Beggiatoa cover***
no	no	no	patchy
few	few	few	thick patches
many	many	many	thin mat
			thick mat
			Streaming beggiatoa
Gas bubbles	***Sediment***	***Pellets***	***Faeces***
no	normal	no	no
Bubbling on disturbance	black/grey	few	few
Free bubbling		many	many
		spill	

4.5 Comparison with feed input

The volume of fish feed was examined at each lease, as it is the primary source of organic enrichment at salmon cages and in Macquarie Harbour. If Dorvilleids respond to enrichment from fish farms then those leases with more feed input were expected to show a greater abundance of Dorvilleids, perhaps up to a threshold. Data on the amount of feed released at each pen bay throughout 2014 at the leases in this study, and the location of the pen bays (cages), was made available by DPIPWE with permission from the companies. Despite best efforts, not all 0m sites were located on cage edges at the time of sampling. Likewise, one 50m site was located on a cage edge, and another 50m site was located the edge of a pen bay that had been recently stocked.

Two main questions of interest were used with the feed data. The overall amount of feed that went into the whole lease area in the preceding 12 months to sampling and how that related to the overall number of Dorvilleids at that lease (up to 250m along the transects). A more detailed inspection of the feed input for the sites that were located on cages, and other close cages, in relation to the number of Dorvilleids, with the aim of shedding light on how the intensity of feed input affects the location and abundance these polychaetes. This question should be able to help industry understand what measures they can take in terms of stocking cages to ensure that they remain compliant.

5 Results

5.1 Distribution and abundance of Dorvilleids

Two species of Dorvilleid polychaete were found in Macquarie Harbour in this study. Of the 1mm sieved samples, *Schistomeringos loveni* was found at 69 of the 105 sample sites and 78 of the 105 sites in the 0.5mm sieved samples. It was the most abundant of all species counted from both 1mm and 0.5mm sieved samples with a total of 5539 and 2192 individuals, respectively. In contrast, only one individual of *Ophryotrocha shieldsi* was found across all sites in a 0.5mm sieved sample, 267-SW-1, and none in the 1mm sieved samples. In contrast *O. shieldsi* colonies were commonly observed in ROV footage. Therefore, the analysis of the distribution and abundance described below is based on ROV data for *O. shieldsi* and grab data for *S. loveni* (but see section 5.4 for comparison of ROV and grab sampling for estimating *S. loveni* distribution and abundance).

Schistomeringos loveni

The response of *S. loveni* showed a general pattern of decreasing abundance with increasing distance from the fish cages, after a peak 50m from the cage in the 1mm samples, Figure 5-1a. The 0.5mm samples showed a similar trend, however, the peak was at 100m from the cage, Figure 5-1b. The site 219-E-50m, which had extremely high numbers of *S. loveni*, enhances the peak at 50m in the 1mm sample: it had 492, 576 and 476 in the three grabs. With this site removed the pattern remains, but the peak at 50m is at approximately 30 *S. loveni* per grab, rather than approximately 60.

Despite, the clear trend with distance and peak at 50-100m, the pattern was variable between leases as evidenced by the significant interaction between lease and distance (DF= 12, F=2.872, P=0.0012). In other words, the change in *S. loveni* abundance with distance varied between leases.

At lease 133, the peak worm density was 46.83±19.57 per grab was at 50m. At 219 and 266 the peak density was also at 50 m, but with densities of 136.08±62.95and 36.33±8.64 respectively. In contrast the peak density at 267 was at 0 m with 57.58±18.78 per grab, Figure 5-2. The total numbers of worms out to 250m at each lease were 971 at lease 133, 2389 at 219, 911 at 266 and 986 at 267. At the transect scale (within lease) there is also significant spatial variation, both in terms of the pattern of distribution and the abundance, Figure 5-3 to Figure 5-6. For example, the East transect at lease 219 has a much greater abundance at 50m compared to other transects, or indeed other leases, Figure 5-4. While at lease 266, the South transect has a lesser abundance across distances compared to other transects at that lease, Figure 5-5.

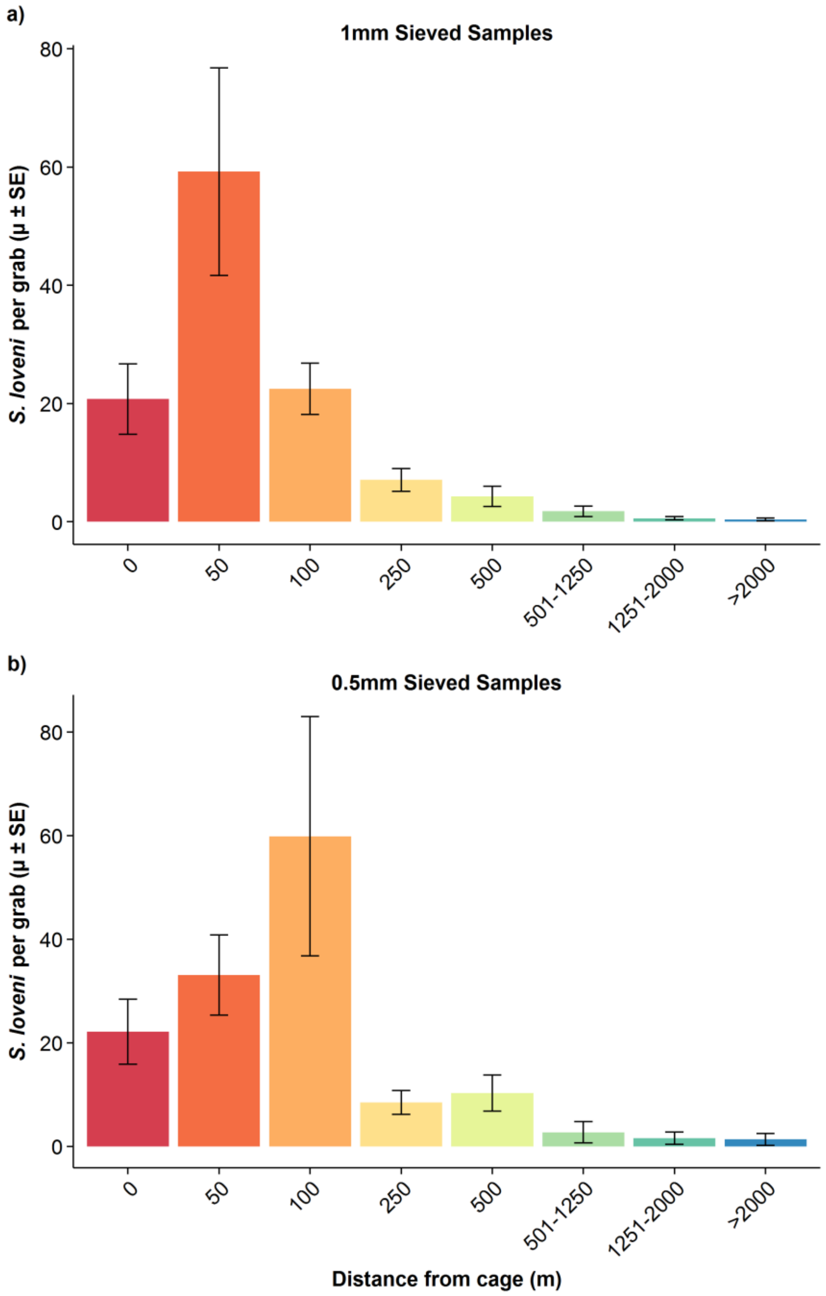

Figure 5-1 Mean abundance of Schistomeringos loveni *in a) 1mm-sieved samples and b) 0.5mm sieved samples at sites increasing distances from salmon cages. Error bars show standard error of the mean.*

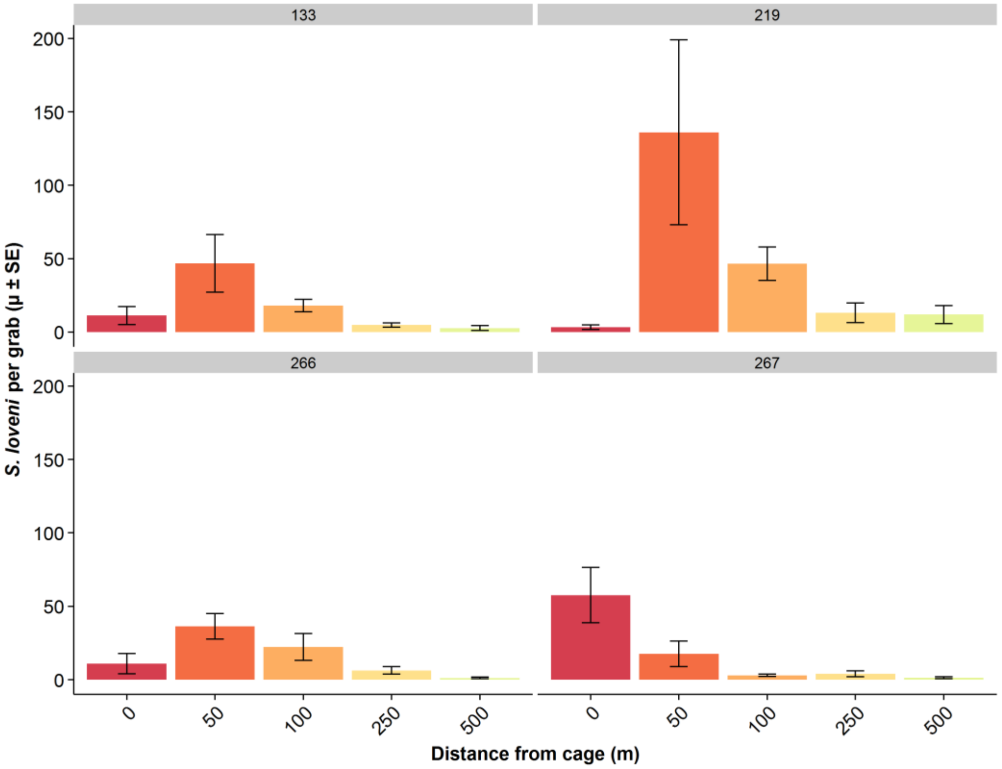

Figure 5-2 Mean abundance of Schistomeringos loveni *in 1mm-sieved samples at Leases 133, 219, 266 and 267 at increasing distances from salmon cages. Error bars indicate the standard error.*

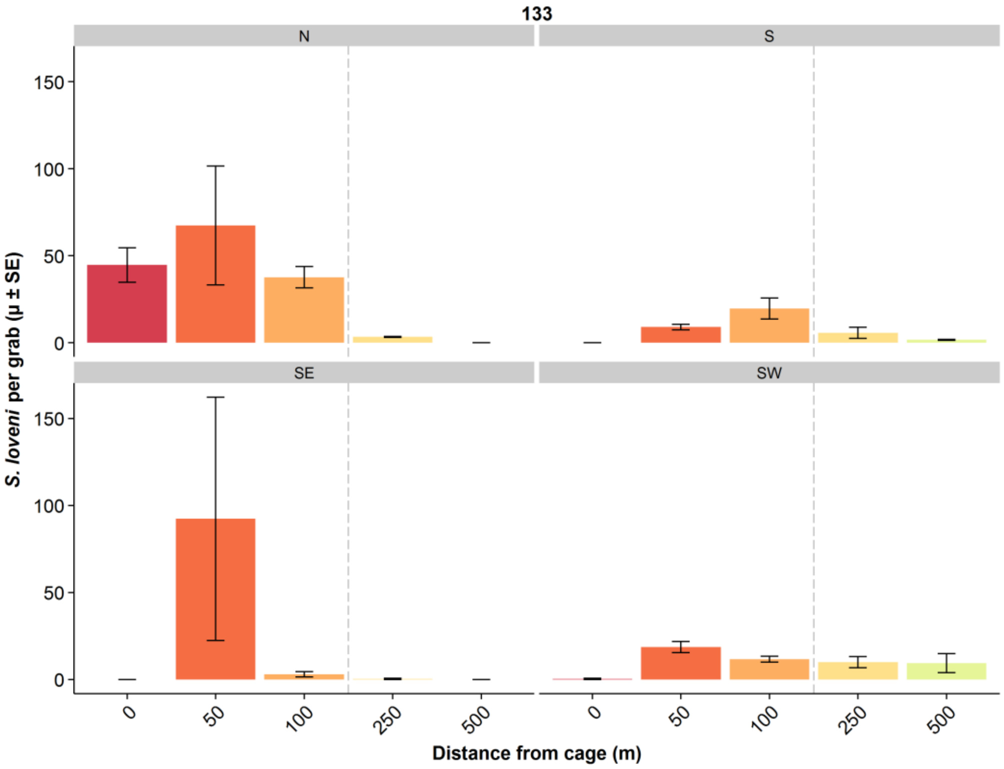

Figure 5-3 Mean abundance of Schistomeringos loveni *in 1mm-sieved samples along each transect at Lease 133, demonstrating variability between transects. Error bars indicate the standard error. Grey dashed line shows the approximate location of the lease boundary.*

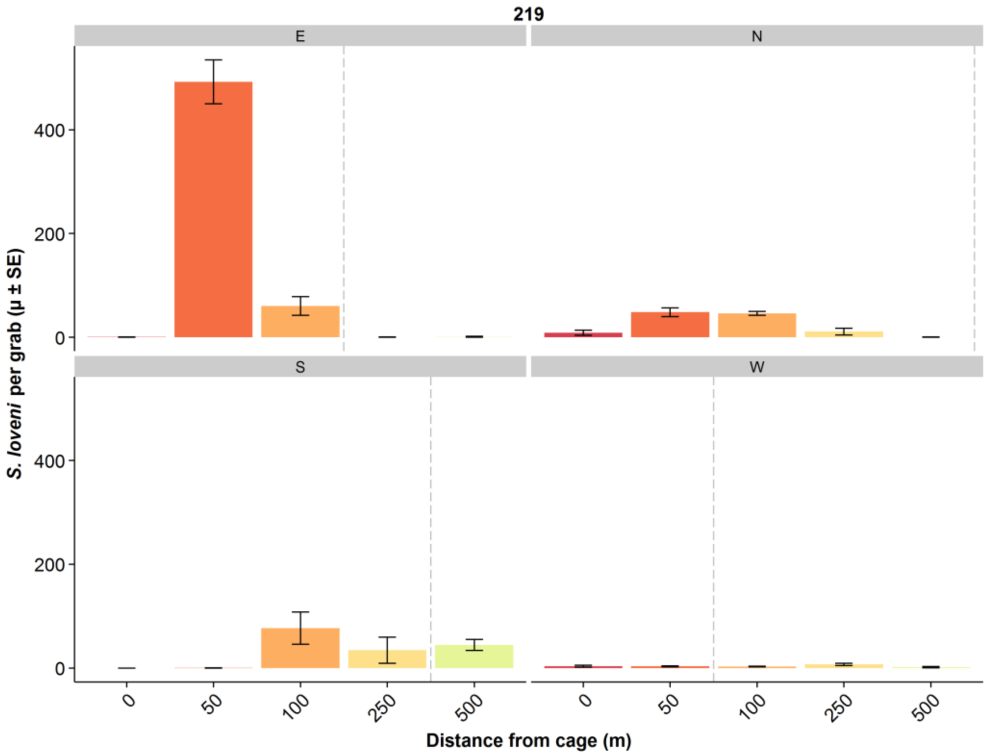

Figure 5-4 Mean abundance of Schistomeringos loveni *in 1mm-sieved samples along each transect at Lease 219, demonstrating variability between transects. Error bars indicate the standard error. Grey dashed line shows the approximate location of the lease boundary.*

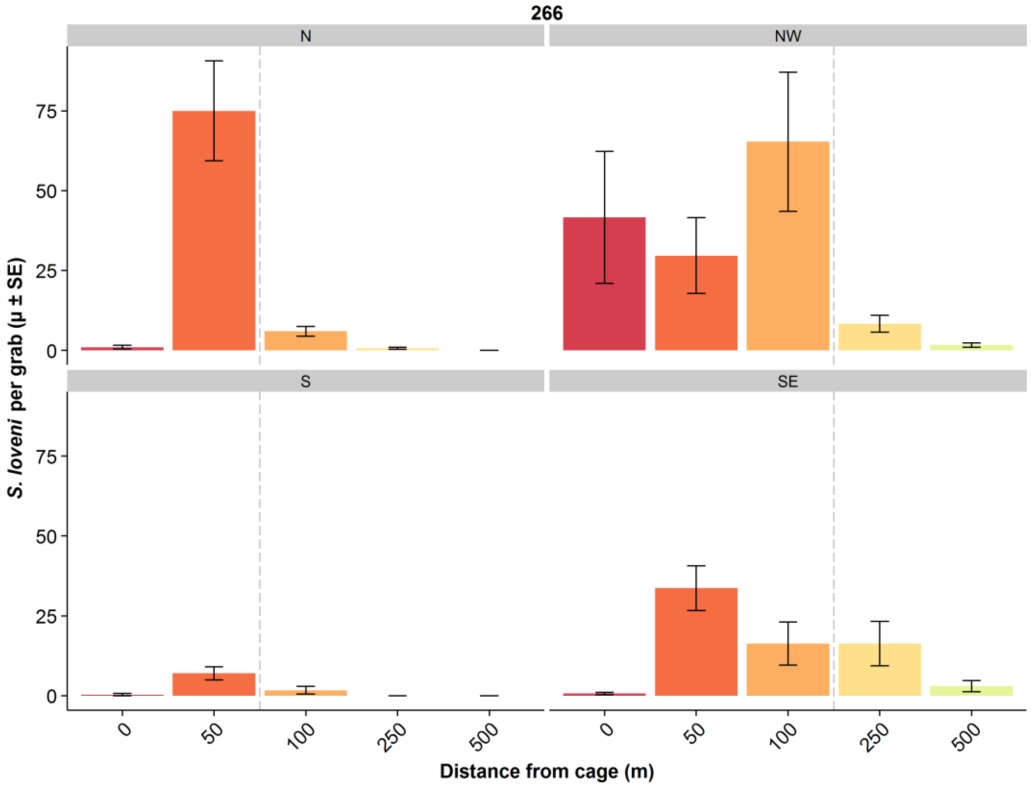

Figure 5-5 Mean abundance of Schistomeringos loveni *in 1mm-sieved samples along each transect at Lease 266, demonstrating variability between transects. Error bars indicate the standard error. Grey dashed line shows the approximate location of the lease boundary.*

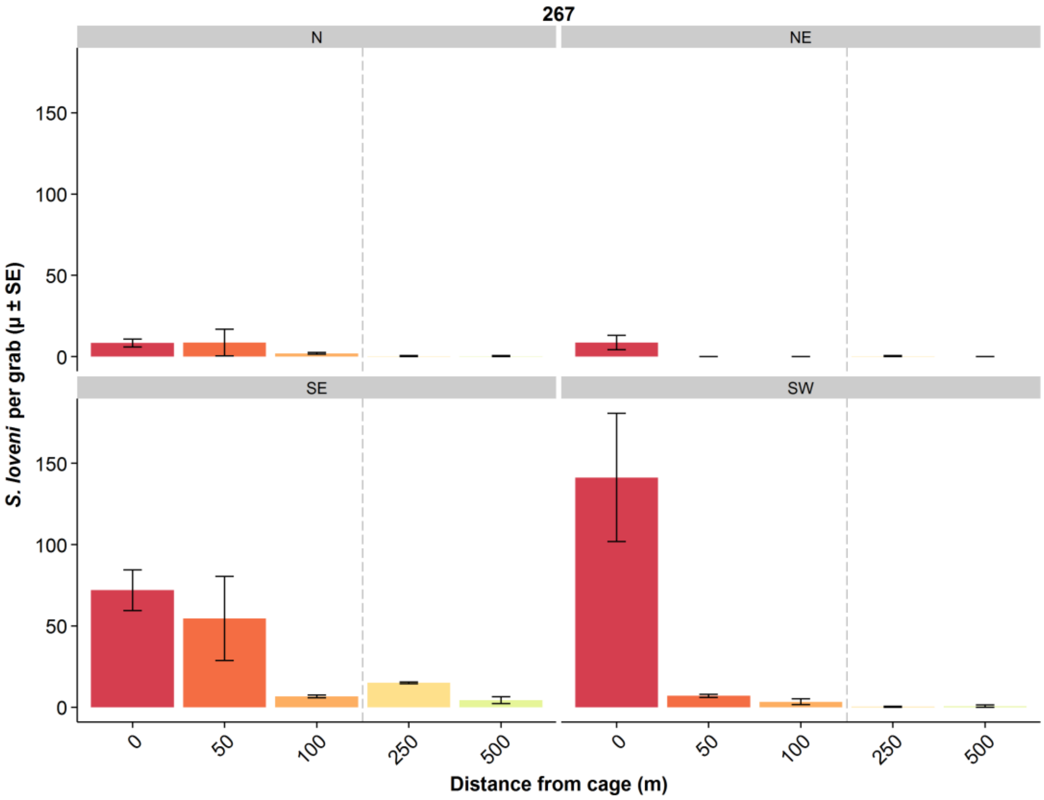

Figure 5-6 Mean abundance of Schistomeringos loveni *in 1mm-sieved samples along each transect at Lease 267, demonstrating variability between transects. Error bars indicate the standard error. Grey dashed line shows the approximate location of the lease boundary.*

Ophryotrocha shieldsi

The response of *Ophryotrocha shieldsi*, though absent from grab samples, could be estimated from the video footage by identifying the colonies they form. These colonies had previously been confirmed as *O. shieldsi* (Paxton and Davey 2010) and were observed in the video footage at the cages and up to 50m away, Figure 5-7. All leases had sites with colonies of *O. shieldsi*, generally at the cage but two sites at 50m had colonies. Lease 267 had one site (267-NE-1) with colonies, while 133 had three (133-S-1, 133-SE-1, 133-SE-2) and both 219 and 266 had four sites each with colonies (219-E-1, 219-N-1, 219-S-1, 219-S-2, 266-N-1, 266-NW-1, 266-S-1, 266-SE-1), Figure 5-8. Thus, there seems to be little broadscale regional variation between leases 133, 219 and 266 in the distribution of *O. shieldsi*, but lease 267 has fewer colonies. No colonies were observed at external sites. *O. shieldsi* was generally associated with *Beggiatoa* bacterial mats: 10 of the 12 sites with colonies also had *Beggiatoa*, and another (219-E-1) possibly had bacterial mats but the footage was unclear. The only site at lease 267 with colonies also had *Beggiatoa*. Eight sites had *Beggiatoa* but no colonies.

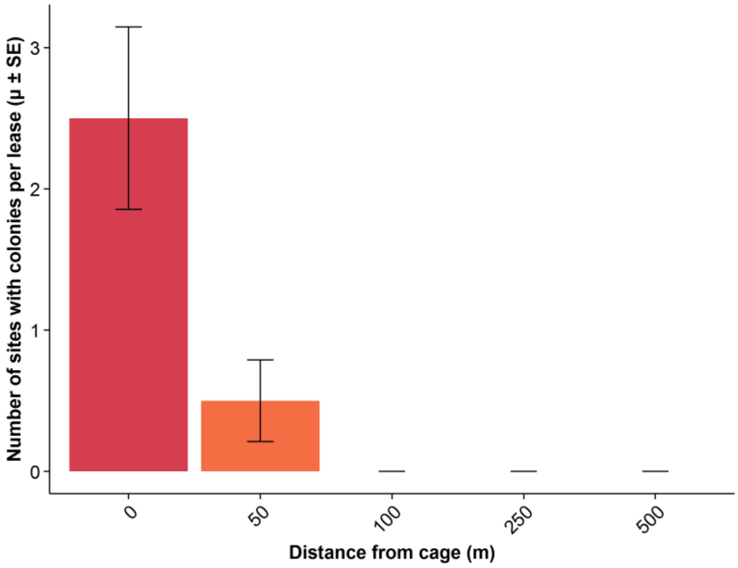

Figure 5-7 Number of sites with colonies of Ophryotrocha shieldsi *per lease (Mean ± S.E).*

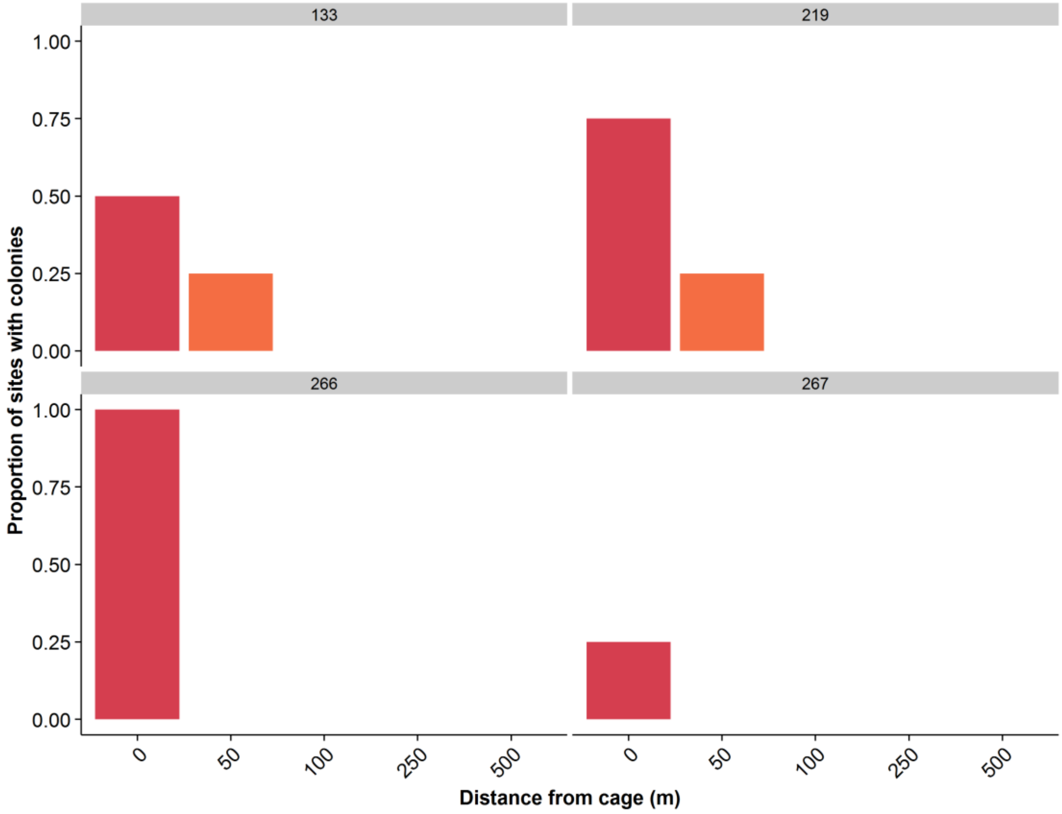

Figure 5-8 Proportion of sites with colonies of Ophryotrocha shieldsi *at distance within each lease. Error bars indicate standard error.*

5.1.1 Comparison with feed input

Feed input in 2014 varied markedly between each leases, Table 2. The variation in yearly input was broadly reflected in the lease-wide input for December 2014. Lease 266 was the most intensively farmed during this period, and 267 was the least intensively farmed. Although relationships with *S. loveni* abundance are difficult to detect at the detailed cage and grid scale, some broader patterns are evident. Firstly, the greatest abundance was found at the lease with the longest farming history, 219. Secondly, the site with the lowest feed input for 2014, lease 267, has the peak abundance close to the cages. Thirdly, the lease with the greatest feed input for 2014, lease 266, has the most sites with *O. shieldsi* colonies and Beggiatoa while lease 267 has the fewest.

Table 2 Feed input (provided as ranges) in December and throughout 2014 for the whole lease and for cages at the start of transects, and Dorvilleid abundance and Beggiatoa *presence. Note, the actual feed input sits within the range presented.*

Lease And Transect	*Feed In December (T)*	*Feed In 2014 (T)*	*S. Loveni Abundance 0m*	*S. Loveni Abundance 50m*	*Colonies Of O. Shieldsi*	*Beggiatoa*
133	**250-500**	**2500-5000**	**135**	**562**	**3**	**3**
133-N	0	10-25	134	202		
133-SE	10-20	100-200	0	277	0m	0m
133-S	10-20	50-100	0	27	0m	0m
133-SW	20-30	200-300	1	56	50m	0m
219	**250-500**	**1500-2500**	**41**	**1700**	**4**	**4**
219-N	0	0	27	145	0m	
219-E	30-40 *at cage in between 0m and 50m	100-200 *at cage in between 0m and 50m	6	1544	0m	50m
219-S	10-20	50-100	0	1	0m, 50m	0m, 50m
219-W	0	0	10	10		0m
266	**500-1000**	**5000-10000**	**131**	**436**	**4**	**6**
266-N	20-30	100-200	3	225	0m	50m
266-NW	10-20	100-200	125	89	0m	0m, 50m
266-SE	20-30	100-200	2	101	0m	0m, 50m
266-S	10-20	200-300	1	21	0m	0m
267	**0-250**	**0-1500**	**691**	**211**	**1**	**1**
267-N	0	1-10	25	26		
267-NE	10-20	25-50	26	0	0m	0m
267-SW	10-20	25-50	424	21		
267-SE	10-20	25-50	216	164		

Not all cages were stocked at the time of sampling and stocked cages varied in the amount of feed received. Lease 133 had cages at three of the four 0m sites, Table 2. The fallowed cage (133-N-1) contrasted with the stocked cages as it had the highest abundance of *S. loveni* at 0m and no colonies of *O. shieldsi*. While the most intensively farmed cage had few *S. loveni* at 0m and colonies of *O. shieldsi* at 50m.

At lease 219 only one of the four cages was stocked at the time of sampling (219-S-1). There had been a stocked cage at the 50m site on this transect in early 2014. This may explain why colonies of *O. shieldsi* and *Beggiatoa* were found out to 50m on this transect. On the eastern transect, there was a cage adjacent to the 50m site, which put the 0m and 50m sites on either side of a stocked cage that received 33.06t of feed in December. The north transect for 219 had been stocked until April 2014 and had *O. shieldsi* colonies, while the 0m site with no colonies was not stocked at all during 2014. This implied that, at least at this older site, colonies of *O. shieldsi* and *Beggiatoa*, can persist for many months after fallowing.

Lease 266 had cages stocked at all four 0m sites, Table 2. The transect with the lowest number of *S. loveni* at 0m corresponded with the highest feed tonnages in 2014, while the transect with the most *S. loveni* at 0m had the lowest tonnage of feed in 2014. Colonies of *O. shieldsi* and Beggiatoa were present at all four 0m sites, but only *Beggiatoa* was found at 2 of the 50m sites.

The peak abundance of *S. loveni* at lease 267 was at 0m, rather than 50m. Whilst there were stocked cages at three out of the four 0m sites, this lease had the lowest lease-wide feed input, as well as the lowest input per cage at 0m sites in December. The lower tonnage of feed at this lease may have fostered conditions under cages more favourable for *S. loveni*.

5.2 Environmental Characteristics

The temperature and salinity of bottom water was similar across all sites and leases, including the external sites (see Appendix, section 11.5 for raw data). The temperature of the bottom water ranged from 14.3-15.1° C and the salinity mostly ranged between 28-31 psu. Site 42, near the Gordon River had a salinity of 22 psu. It is a shallow site and, along with sites 49 and 50, was also warmer at 16 °C.

Dissolved oxygen in the bottom water was higher at the northern end of the harbour, closer to the mouth. Bottom water dissolved oxygen saturation at lease 133 was higher than at the other leases, and all of the external sites with >40% saturation, with the exception of site 51, were located in northern third of the harbour. At the lease scale, there was also a pattern in bottom water dissolved oxygen saturation with distance from cages at two of the leases. Dissolved oxygen at leases 266 and 219 showed a gradient of lower dissolved oxygen saturation at cage sites that increased to background levels by approximately 100m, Figure 5-9. These two leases also had more sites with low (<25%) or extremely low (<5%) bottom water dissolved oxygen. At lease 133 and 267 there was no clear pattern with distance.

Redox potential at 3cm increased with distance at lease 219, but not at leases 133, 266 and 267. The lowest redox values were all observed in samples from lease 219, at 0m and 50m on three transects, while the highest values were found at external sites 11 and 50. Between these two extremes the majority of sites fell within a band of approximately -50mV to 50mV.

The carbon and nitrogen stable isotopes of sediments throughout the harbour at both lease and external sites reflected a typical estuarine gradient. The sites closer to the mouth of the Gordon River, e.g. sites 39, 42, 43, 44, and 45 were more depleted in $\delta^{13}C$ and $\delta^{15}N$ whereas sites closer to Hells Gates (and the marine influence) were more enriched in both $\delta^{15}N$ and $\delta^{13}C$, Figure 5-11. This was also evident when comparing leases, with sediments at lease 267 the most depleted in both $\delta^{15}N$ and $\delta^{13}C$ and lease 133 the most enriched. However, the influence of farming, and the more enriched $\delta^{15}N$ and $\delta^{13}C$ of feed relative to the background harbour signature can also be seen, particularly at the oldest lease, 219, where there is clear gradient with distance from cage, Figure 5-10. The 0m sites at lease 133 are also slightly enriched in $\delta^{13}C$ and $\delta^{15}N$. In contrast, there was no clear pattern with distance at leases 266 and 267 (see later discussion on the influence of sampling methodology).

A similar estuarine gradient was also observed in the C:N ratio of sediment organic matter, with higher ratios closer to terrestrial inputs at the Gordon and King (site 52) and increasingly lower ratios moving towards the marine influence at Hells Gates. Similar to the isotopic signatures, the influence of farm enrichment was also particularly evident at lease 219, with the more depleted C:N ratio typical of feed inputs evident closest to the cages.

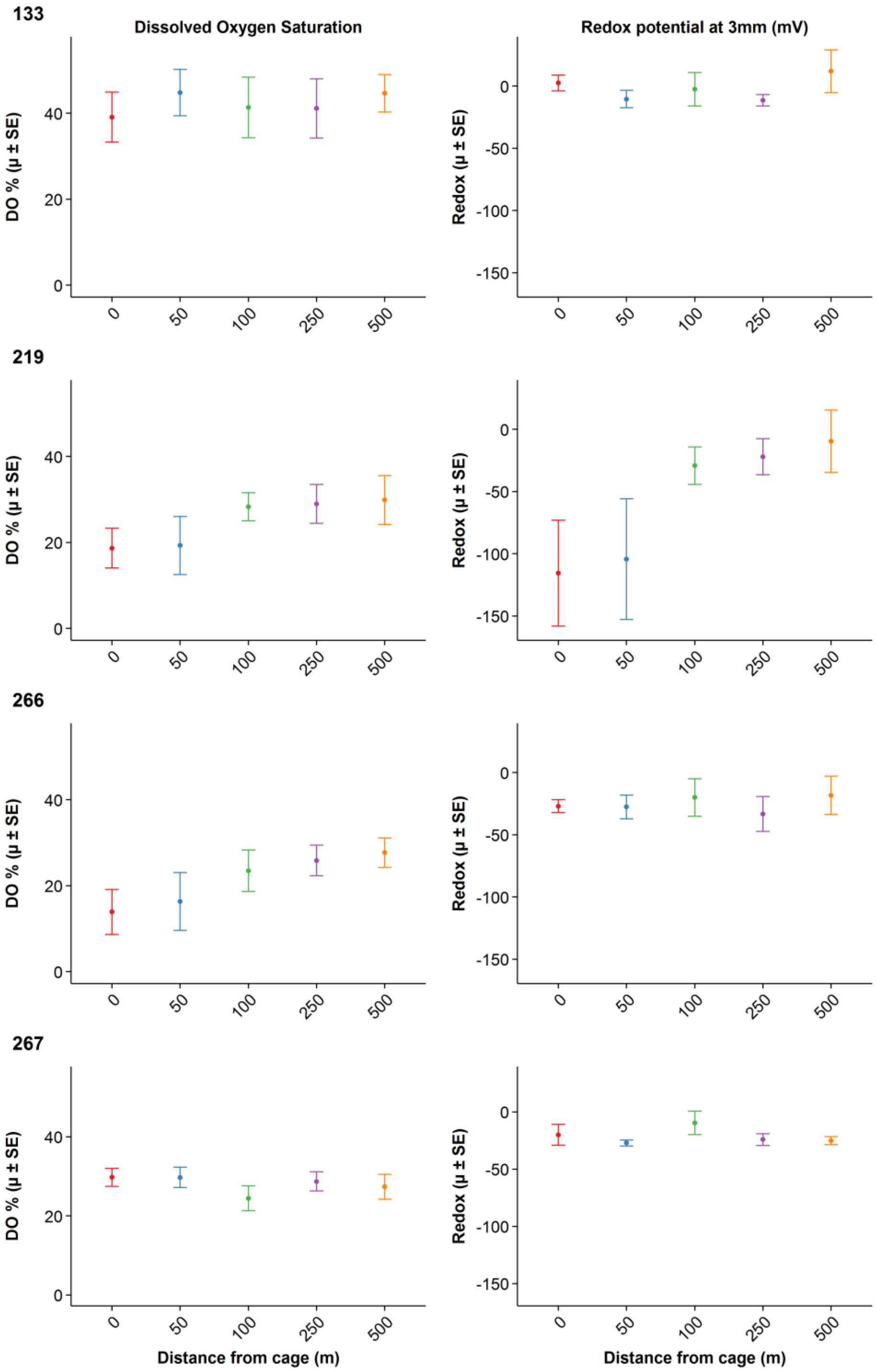

Figure 5-9 Distribution of dissolved oxygen saturation and redox potential at increasing distances from cages at leases 133, 219, 266 and 267. Values are means, with bars indicating standard error.

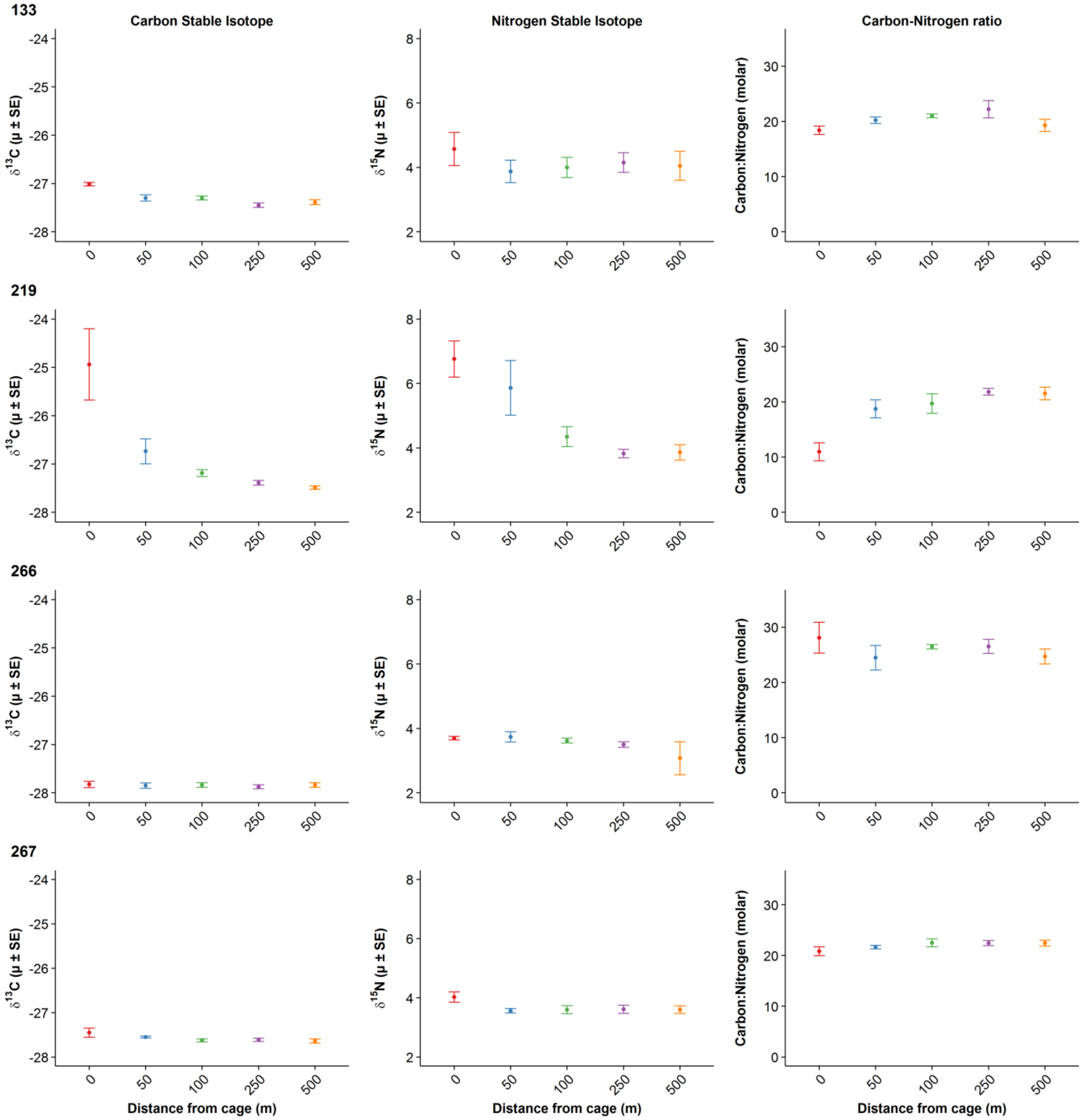

Figure 5-10 Carbon and nitrogen stable isotope values and carbon and nitrogen ratio at increasing distances from cages at each lease. All values are means, error bars indicate the standard error.

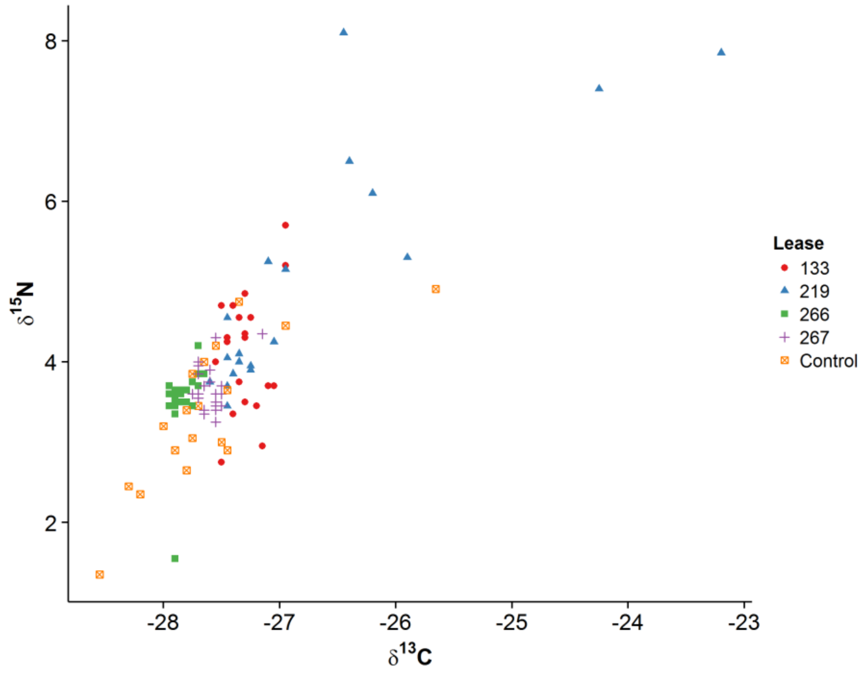

Figure 5-11 Distribution of sites based on carbon and nitrogen stable isotopes. Colours and symbols area indicative of lease.

5.3 Benthic community composition

Benthic community composition from 1mm grab samples showed that lease 267 differed from the other leases, which were all similar, and the external sites were generally quite distinct from lease sites, Figure 5-12. This was driven primarily by the greater presence of species of Sabellid (*Euchone varibilis*) and Flabelligerid polychaetes and heart urchins (*Echinocardium cordatum*) at lease 267. Leases 133, 219 and 266 were characterised by a greater abundance of copepods, terebellids (*Pista* sp) and *Pseudopolydora cf. paucibranchiata*.

The community broadly changed from the cages outwards, Figure 5-13. The drivers of these changes were greater abundance of Nebaliidae, Capitellid polychaetes, amphipods and *S. loveni* at the 0m and 50m sites, an increased abundance of the polychaetes *Pseudopolydora cf. paucibranchiata, Pista sp,* and the larger sabellid, *Branchiomma cf. nigromaculata*, at 100m, 250m and 500m. Some 0m samples appeared quite similar to sites further away from the cages, presumably due to the depauperate nature of both 0m and external sites, albeit for different reasons.

The multivariate analyses of the 0.5mm samples show patterns in the benthic community similar to the 1mm fraction, and the same species largely appear to be the drivers of this change, Figure 5-14 and Figure 5-15, yet a notable difference is the importance of a lyssianasid amphipod at sites closer to the cages in the 0.5mm samples.

The BIO-ENV analysis on the 1mm data found that distance was the most important single environmental variable, with a correlation (Rho) of 0.312. Redox and DO% were the next most important and combined with distance showed a 0.410 correlation (Rho). Depth did not correlate with the benthic community. The BIO-ENV for the 0.5mm grab data found distance and DO% showed the strongest correlation with the community, Rho = 0.342. Yet, overall, these are still weak correlations with any particular environmental variables measured.

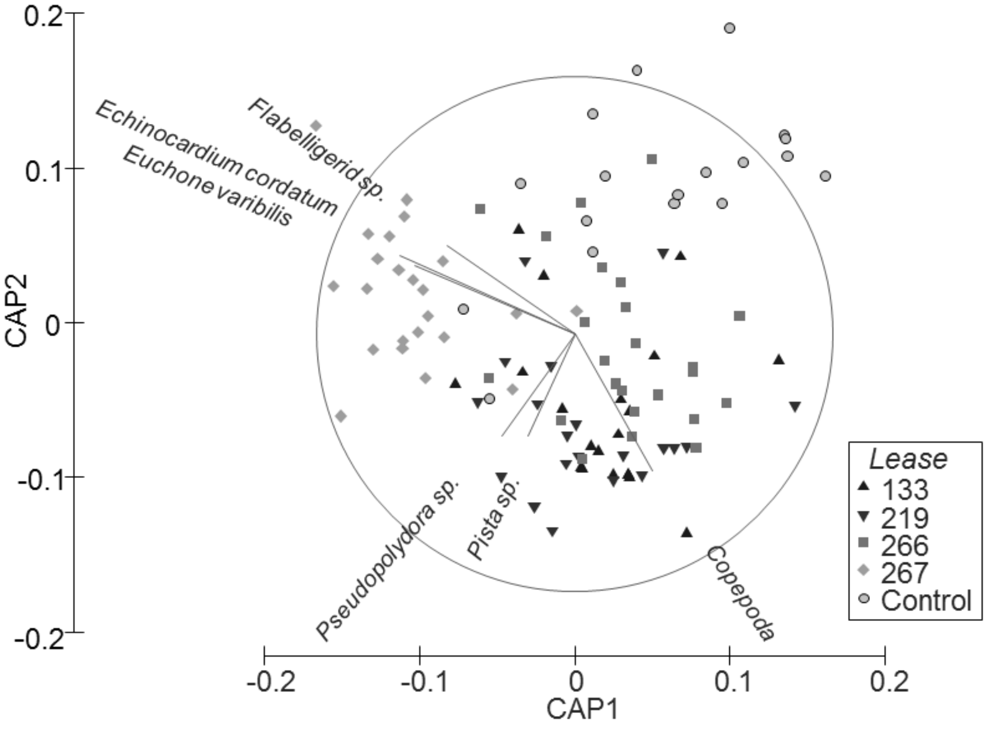

Figure 5-12 Canonical Analysis of Principal coordinates demonstrating differences in the benthic community at lease 267 (out to 500m), from 1mm-sieved grab samples.

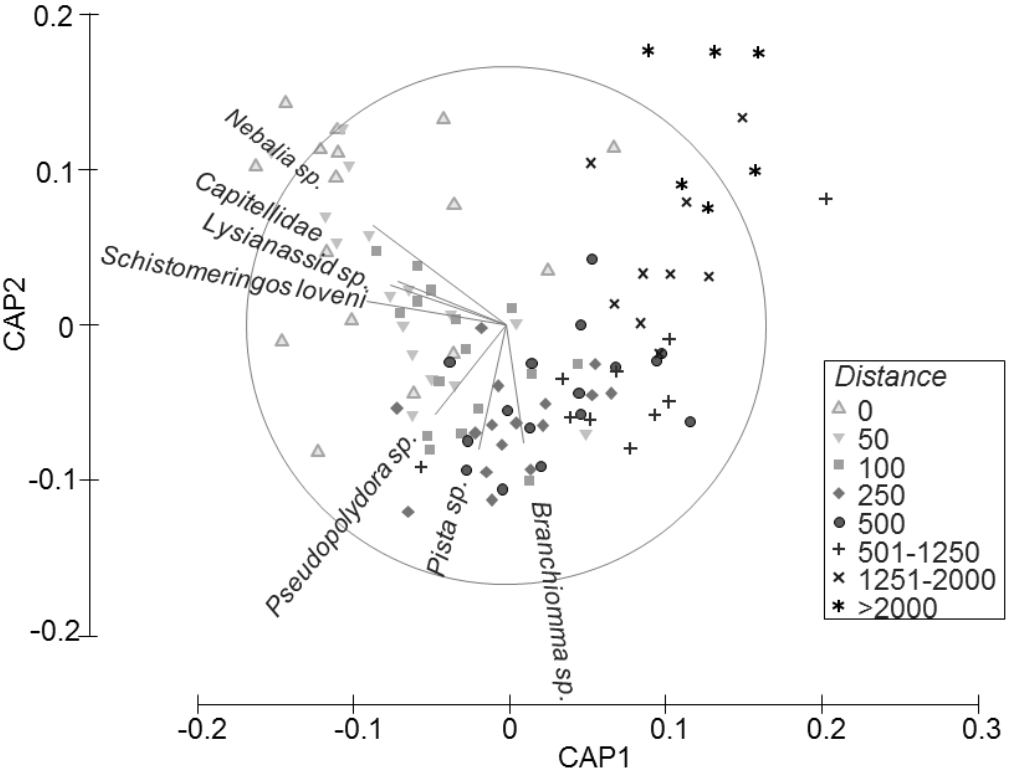

Figure 5-13 Canonical Analysis of Principal coordinates by distance, demonstrating changes in the benthic community with increasing distance from cages, from 1mm-sieved grab samples.

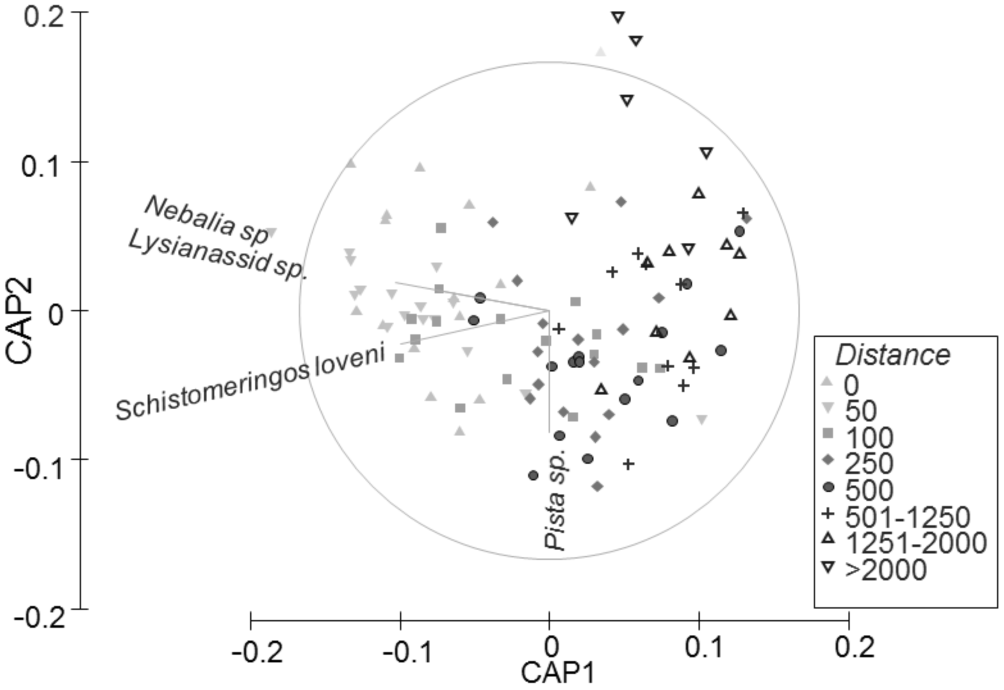

Figure 5-14 Canonical Analysis of Principal coordinates by distance, demonstrating changes in the benthic community with increasing distance from cages, from 0.5mm-sieved grab samples.

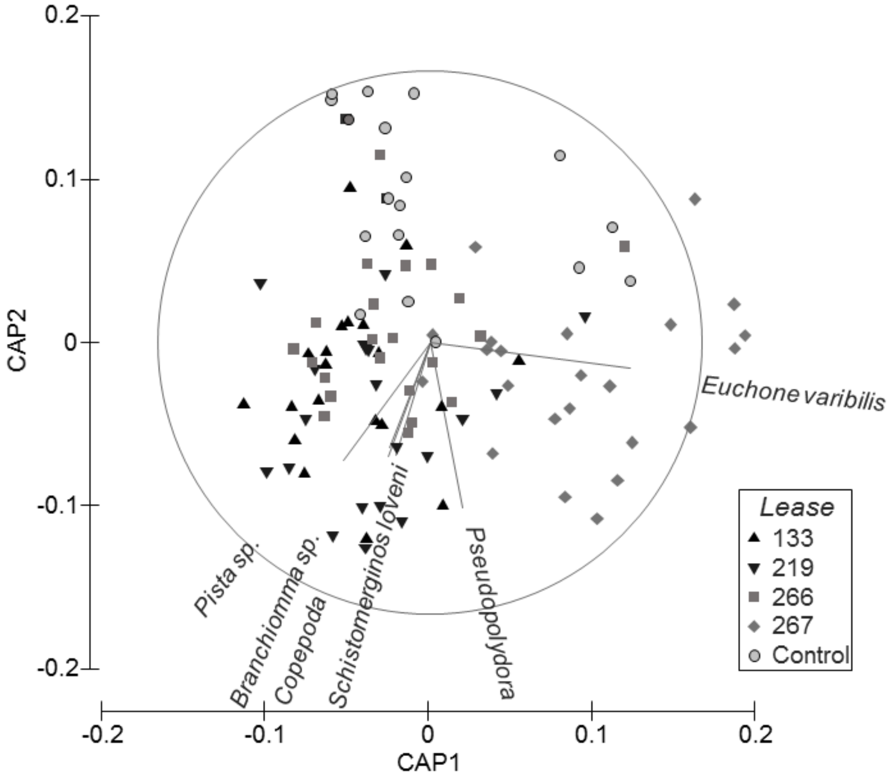

Figure 5-15 Canonical Analysis of Principal coordinates demonstrating differences in the benthic community at lease 267 (out to 500m), from 0.5mm-sieved grab samples.

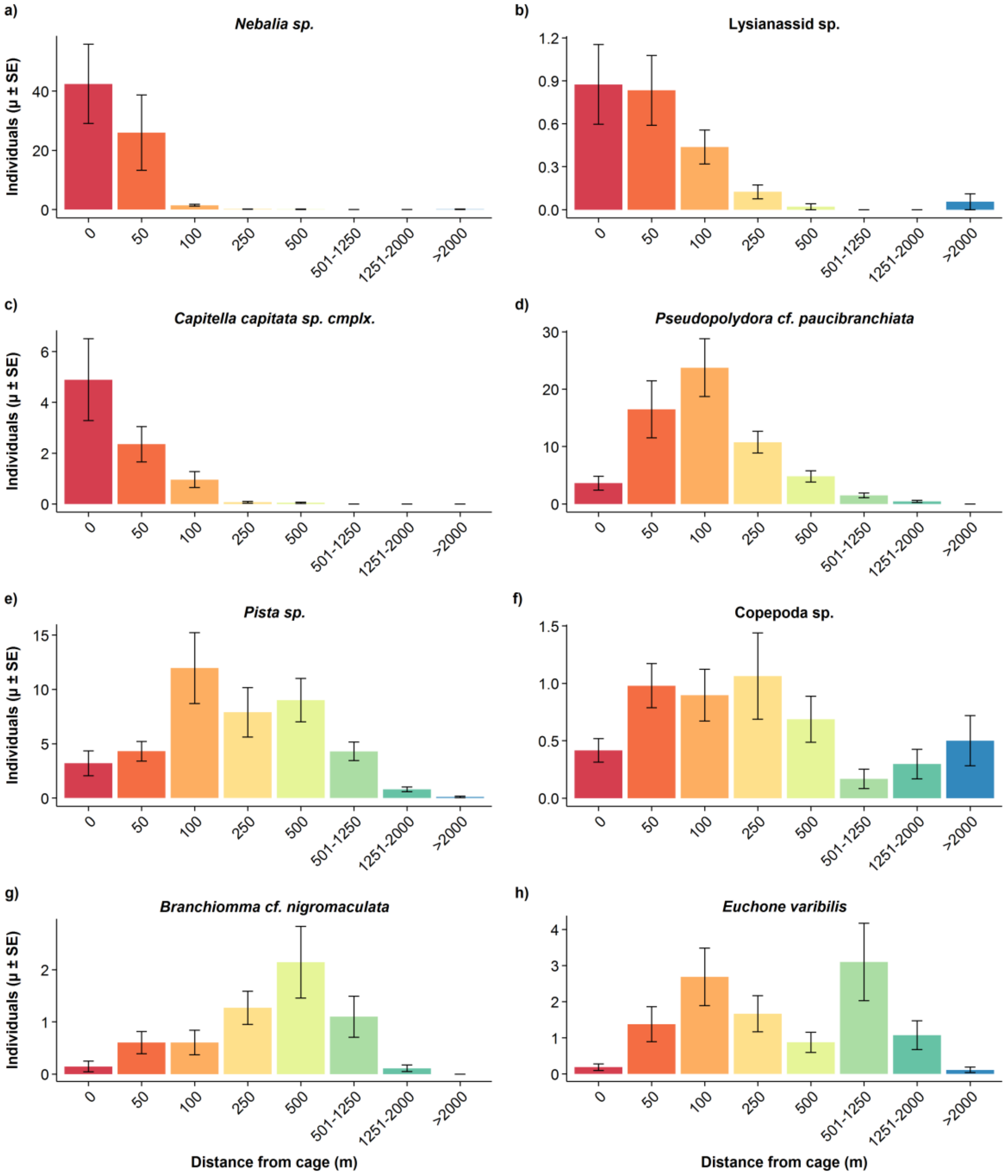

Figure 5-16 Mean abundance of a) Nebalia sp., *b) Lysianassid sp., c)* Capitella capitata *species complex, d)* Pseudopolydora cf. paucibranchiata, *e)* Pista sp., *f) Copepod sp., g)* Branchiomma cf. nigromaculata *h)* Euchone varibilis *in 1mm-sieved samples at sites increasing distances from salmon cages. Error bars show standard error of the mean.*

The distribution of a selection of species, specifically those found to be important in the CAP analysis, demonstrate different responses to enrichment in Macquarie Harbour. *Nebalia sp.* (Figure 5-16a), *Lysianassid* sp. (Figure 5-16b), and *Capitella capitata species complex* (Figure 5-16c) all had peak abundances at cage sites. The terebellid *Pista sp.* (Figure 5-16e) and the spionid *Pseudopolydora cf. paucibranchiata* (Figure 5-16d) had peak abundances at 100m from cages and the Copepoda group was more variable, peaking 50-250m from cages (Figure 5-16f). The two Sabellid fan worms had different distributions. The larger *Branchiomma cf. nigromaculata* steadily increased in abundance to 500m before declining again (Figure 5-16g), while the smaller *Euchone varibilis* showed two peaks, one at 100m and one at 501-1250m (Figure 5-16h). These two peaks were due to the different distributions of this species between leases. *E. varibilis* was common at lease 267, increasing in abundance to a peak at 100m and declining beyond that. Lease 133 also had a peak abundance at 100m, but none at 0-50m.

Very few *E. varibilis* were found at leases 266 and 219 out to 500m. Sites beyond 500m, both external and from the extended transect at leases 266 and 267, caused the second peak at 501-1250m.

The number of species (*S*) found at each site increased with distance from the cage up to 100m, after which it decreased again, Figure 5-17. The total abundance of all taxa (*N)* was greatest at 50m, Figure 5-17. At 50m *S. loveni* accounts for 47% of all individuals and has a considerable influence of the peak at this distance, Figure 5-17.

Four sites had a diversity index (*H'*) value over two: 267-NE-1 and 49-North 1km, 1-North Deep, 133-SW-1. Interestingly these sites included two external sites and two sites located on a cage. 75 sites had a diversity index between 1 and 2, and 26 were less than 1. Figure 5-17 shows that the mean Shannon diversity index generally increased with distance from cage up until the 1251-2000 category, which was more similar to the 50m sites.

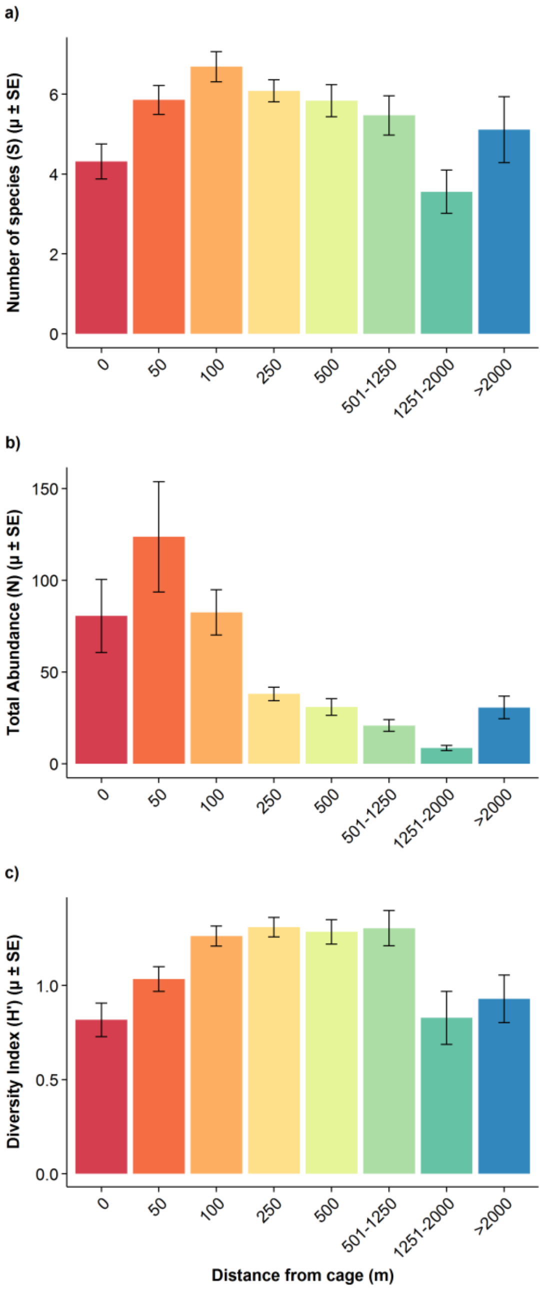

Figure 5-17 Mean a) Number of species, b) total abundance of all taxa, and c) Shannon-Weaver diversity index per 1mm grab sample with increasing distances from cages, including external sites. Values are means, error bars indicate standard error.

5.3.1 Functional groups and indices

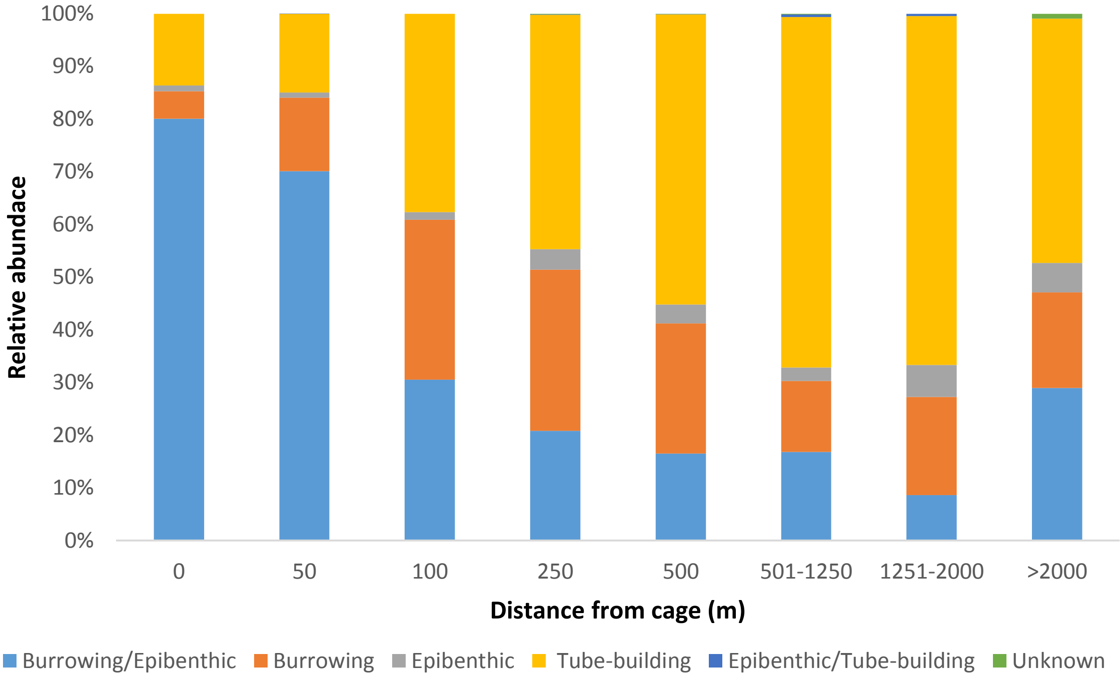

Figure 5-18 Relative abundance of benthic functional groups with distance from cage

The classification of functional groupings revealed a clear decrease in the proportion of burrowing/epibenthic fauna with increasing distance from the cages, while the proportion of tube-building taxa increased, Figure 5-18. The proportion of burrowing taxa was greatest between 100 and 500m from cages. Purely epibenthic and unknown taxa formed a relatively very small proportion of the community at all distances. The full list of species with assigned groupings can be found in Appendix 13.1. The relative abundance of *S. loveni* follows the same pattern over distance as the mean abundance for the species. While it only constituted 26%, a lesser proportion of the 80% Burrowing/Epibenthic group at 0m, it made up 48% of the community at 50m where its functional group represented 70%. *Nebalia* sp. made up a considerable proportion of the remainder of this group with 52% and 21% at 0 m and 50 m, respectively.

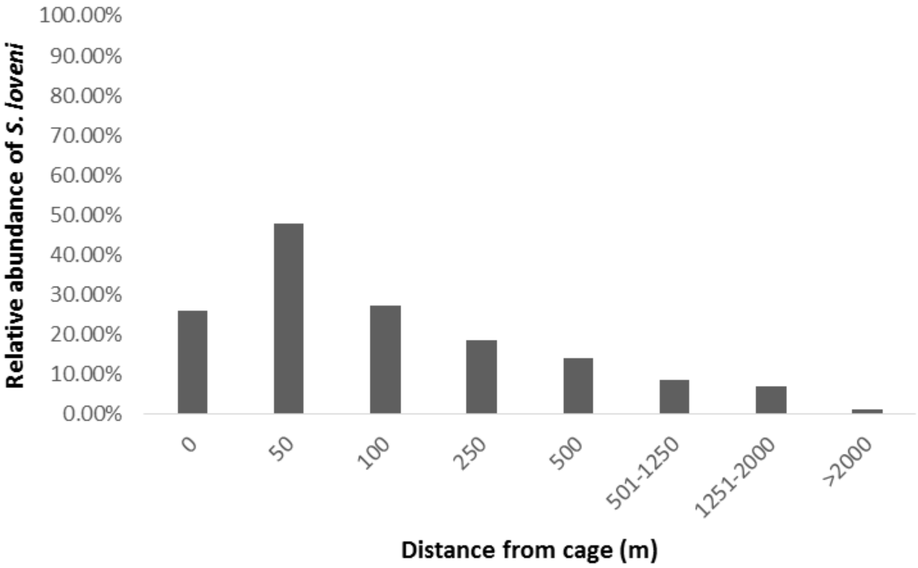

Figure 5-19 Relative abundance of Schistomeringos loveni at each distance from the cages

The AMBI index generally categorised sites as heavily disturbed at the cages, decreasing in impact to slightly disturbed or un-impacted by the end of the transect and external sites were generally slightly disturbed, Figure 5-20 to Figure 5-24. The AMBI is based on the relative contribution of species of differing sensitivity to the total number of species. It aligns well with the patterns in the functional groups identified in this study, as the functional groups broadly align with the ecological groups used in AMBI. The presence of species such as *Nebalia* sp, and copepods which do well in highly enriched environments contribute to the heavily disturbed classification of 0m and 50m sites in the AMBI.

External sites generally fell into undisturbed or slightly disturbed categories, with three classified as moderately disturbed, Figure 5-20. Lease 267, while still heavily impacted at cage sites showed fewer sites than other leases between 50 and 500m that were heavily disturbed, Figure 5-23. It also shows some variation between transects. Lease 266 was heavily disturbed to 50m, and to 100m on one transect, Figure 5-24. The mean 0m AMBI is 5.19 (heavily impacted) and the mean 100m AMBI is 3.56 (moderately impacted).

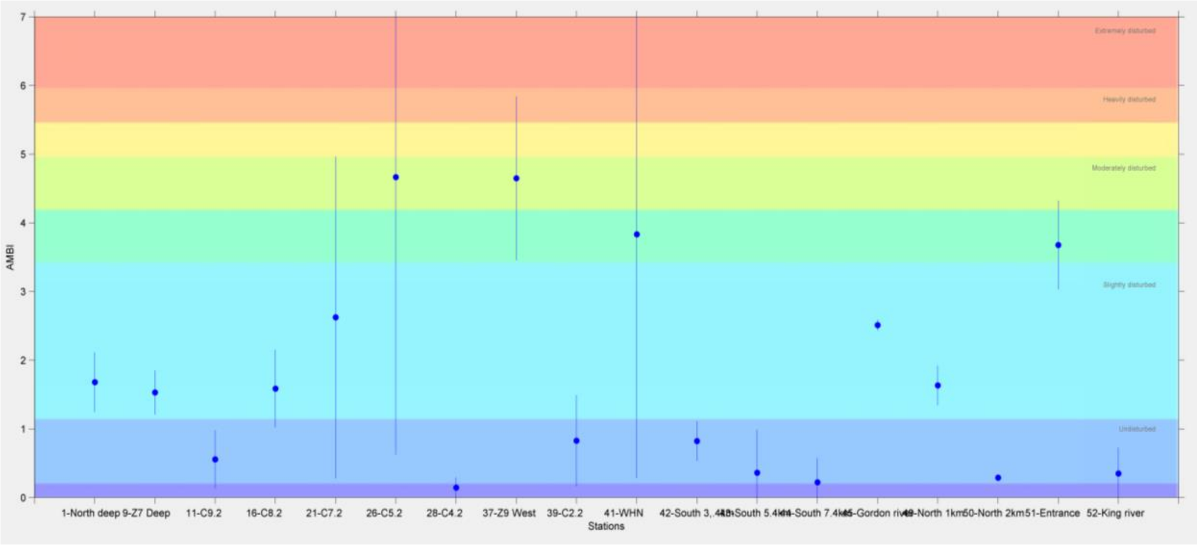

Figure 5-20 AMBI index at external sites

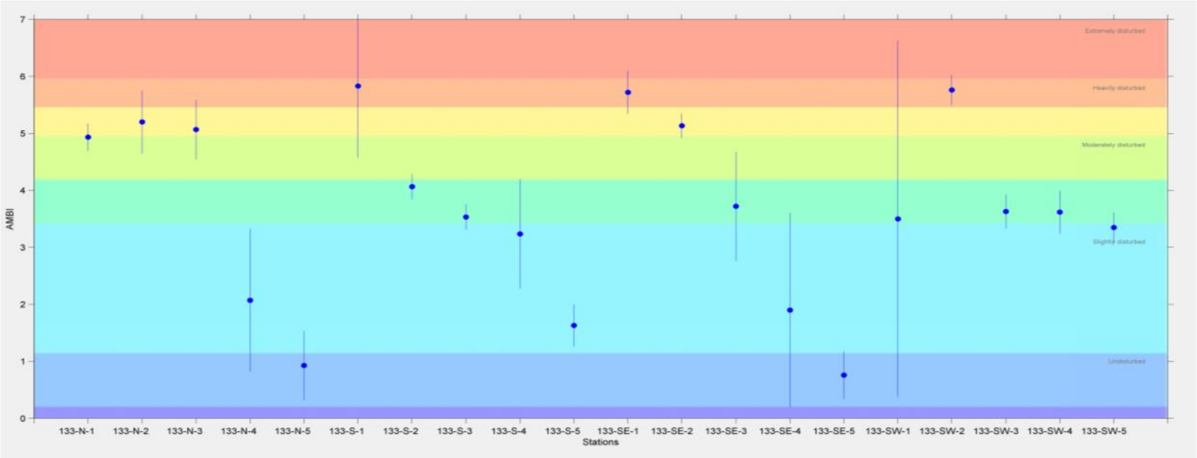

Figure 5-21 AMBI index for all transect sites at lease 133

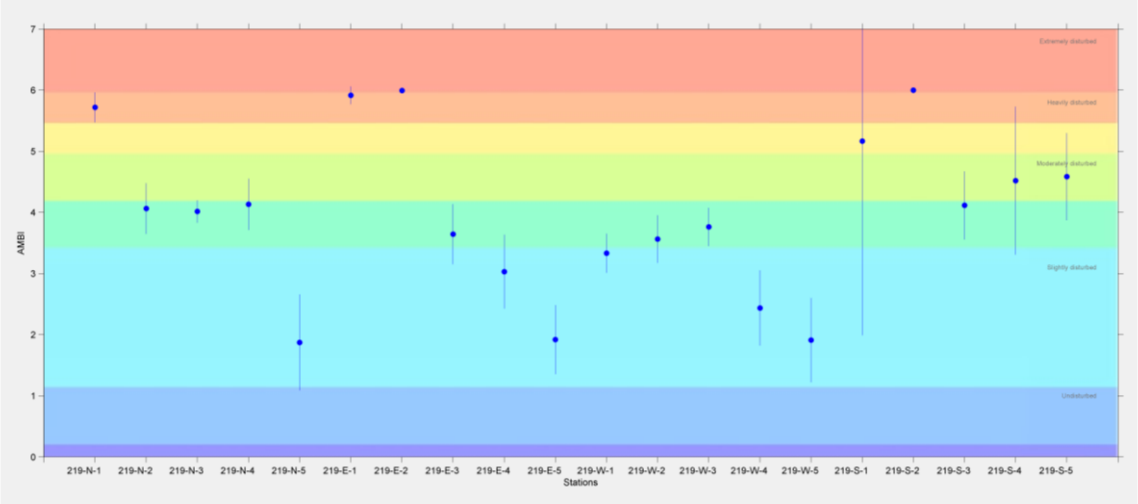

Figure 5-22 AMBI index for all transect sites ate lease 219

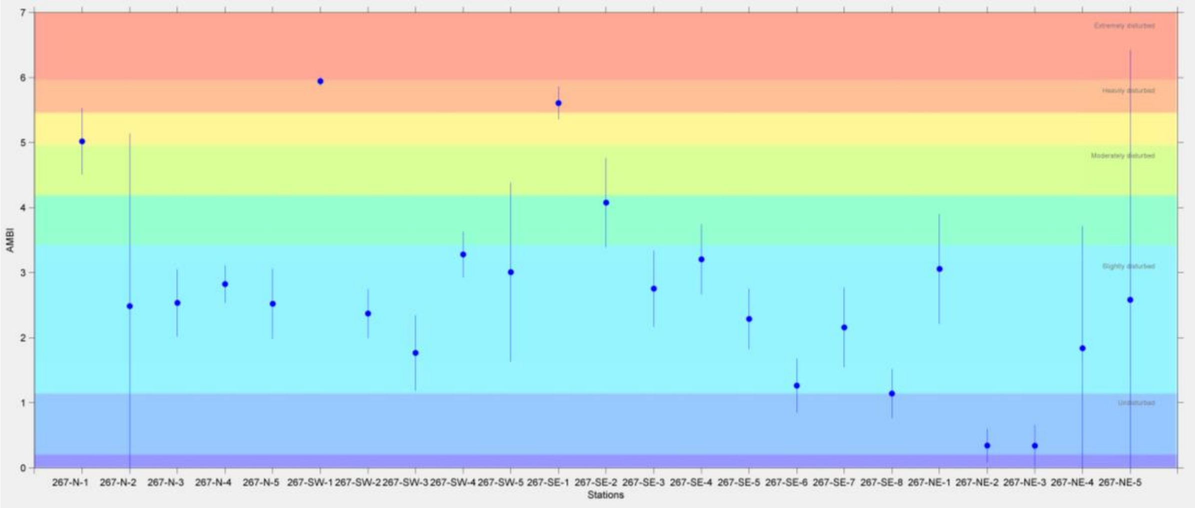

Figure 5-23 AMBI index for all transect sites at lease 267

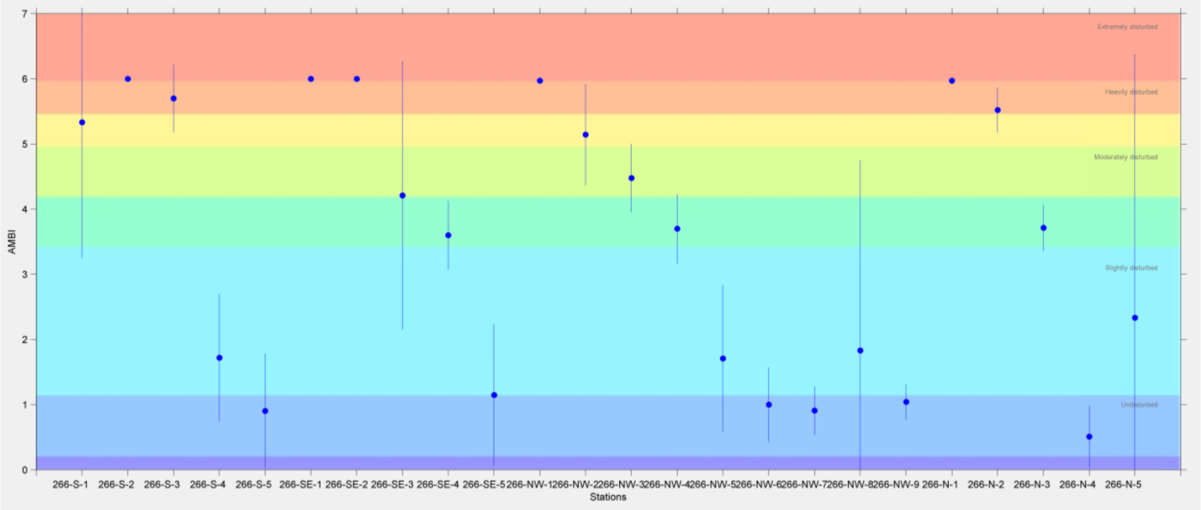

Figure 5-24 AMBI index for all transect sites at lease 266

5.3.2 Changes over time

The benthic community surveyed in the present study shows some substantial differences compared with the community from the baseline surveys in 2000. Notably, only two Dorvilleids were found throughout the entire sample area in the 2000 baseline survey, one individual in lease 219 and one in lease 220. The presence of Dorvilleids became more apparent during 6-monthly video surveys in the 2 years after farming began. It is unclear whether the Dorvilleids observed on video were *S. loveni* and or *O. shieldsi*; they were only reported as Dorvilleids given that the presence of two species was only recently discovered.

2000 baseline vs. 2015 - external sites

There were are a number of other marked changes in the benthic communities at external sites between the baseline surveys and 2015, Figure 5-25. Species richness increased from a mean of 4.38 (±1.14 SE) species per grab in the 2000 baseline compared to 11.25 (±1.09 SE) species per grab in 2015. Similarly, total abundance increased from a mean of 5.48 ± (±1.31 SE) individuals per grab in the 2000 baseline compared to 35.17 (± 7.44 SE) individuals per grab in 2015 and the species diversity index (*H'*) increased from <1 to >1.5 in 2000 compared to 2015. At a functional group level, there is a decrease in burrowing taxa and an increase in tube builders, Figure 5-26.

The multivariate analyses also demonstrate a clear change in community composition between 2000 and 2015 at the external sites from farming, Figure 5-27a. The differences are largely driven by the increased abundance of the leptostracon crustacean *Nebalia*, spionid polychaete *Pseudopolydora* and the terebellid *Pista* sp. The sites from lease 220[4] in the baseline survey were distinct from the other baseline and all 2015 sites. This is probably due to the sandy, rather than silty, sediment throughout much of the lease and shallower sites. This lease was characterised by greater numbers of *Paphies erycinea, Cuspidaria brazier, Chaetozone setosa,* and a Terebellid sp.

2000 baseline vs. 2015 - lease sites

The direct impact of salmon farming on benthic communities was measured by comparing leases 133 and 219 pre-farming in the 2000 baseline and during active farming in 2015, Figure 5-25. Lease 219 was farmed for the duration of the 15-year period while lease 133 was farmed only since 2013. There was a clear increase in species richness associated with farming. In the baseline survey, species richness was similar in leases 133 (3.33 ± 0.45) and 219 (2.25 ± 0.25). In 2015, species richness had increased in both leases (133, 9.17 ± 0.98; 219, 8.25 ± 0.93), despite the very different periods of farming and likely differences in farming practices at the two leases. Similarly, there was a clear increase in the total abundance associated with farming, however, the pattern was somewhat different between leases. In the baseline survey, total abundance was slightly lower in lease 133 (2.36 ± 0.50) compared to lease 219 (4.91 ± 3.52). In 2015, total abundance had increased in both leases, but substantially more in lease 219 (133, 58.97 ± 12.10; 219, 170.83 ± 69.52). In terms of the species diversity, 133 was clearly higher in the baseline survey compared to lease 219, however in 2015, species diversity in 2019 is now similar to that recorded in lease 133. At a functional group level, there has been an increase in burrowing/epibenthic and tube dwelling groups and a decrease in burrowing fauna at the lease sites during farming, Figure 5-26.

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[4] Lease 220 was to the north of current lease No. 133

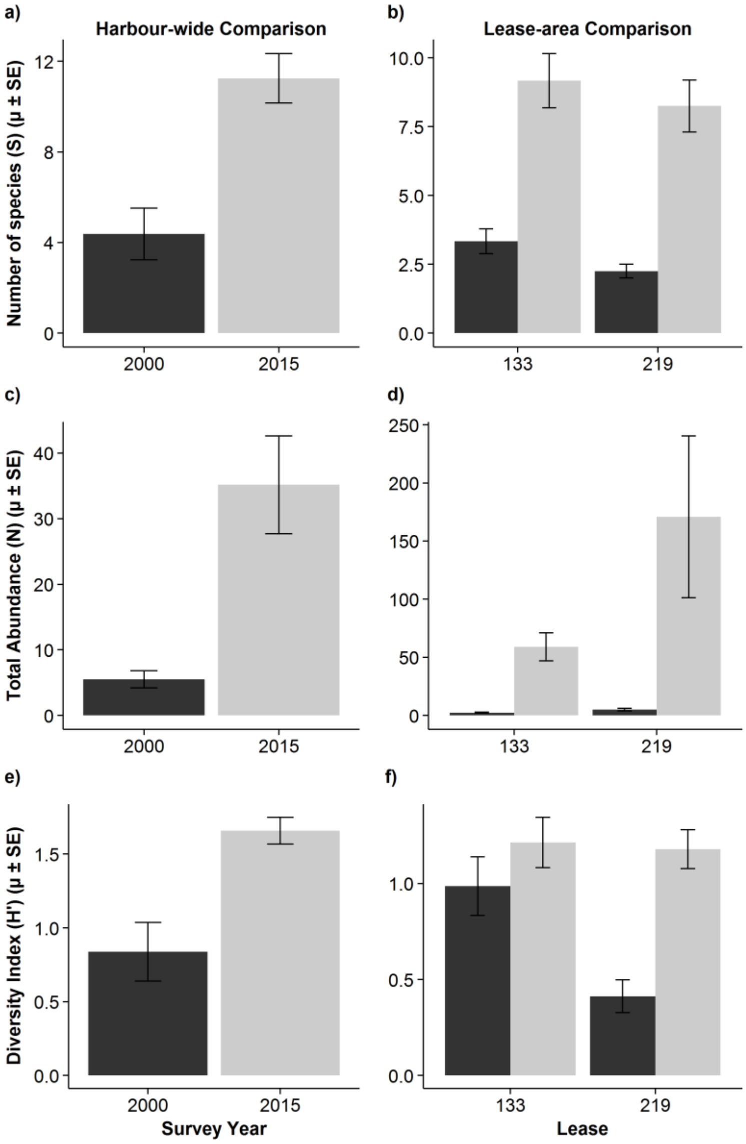

Figure 5-25 Mean a-b) Number of species, c-d) total abundance of all taxa, and e-f) Shannon-Weaver diversity index per 1mm grab sample in the central, unfarmed harbour (a, c, e) and at leases 133 and 219 (b, d, f) in the year 2000 (dark grey) and in 2015 (light grey). Values are means, error bars indicate standard error.

From the multivariate ordination, Figure 5-27b, it is evident that the faunal composition has changed since the onset of farming and become more variable, due to the range of communities found along the distance gradient from cages. A wide range of taxa appear to be driving the differences between the baseline and 2015 surveys, *Phyllodoce* sp., *Paracaprella alata*, *S. loveni*, *Parawaldeckia* sp. and Copepoda more generally associated with the lease sites in 2015, *Pseudopolydora*, *Pista* sp. and an Ampharetid sp. typical of the 50 -100m from cage sites, and *Nebalia* sp., *Xenostrobus secures* and Isaeid amphipods typical of the 0m sites.

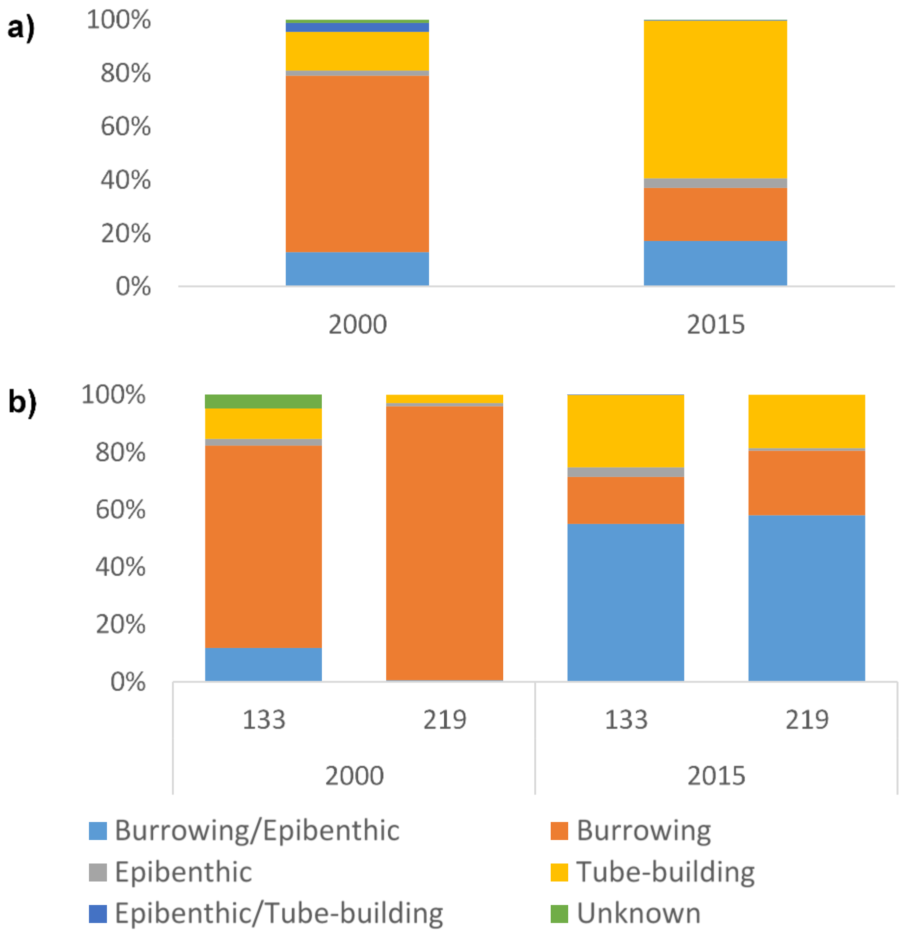

Figure 5-26 Percentage of total fauna of each functional group in a) the broader harbour area in 2000 and 2015, and b) the lease area of leases 133 and 219 in 2000 and 2015

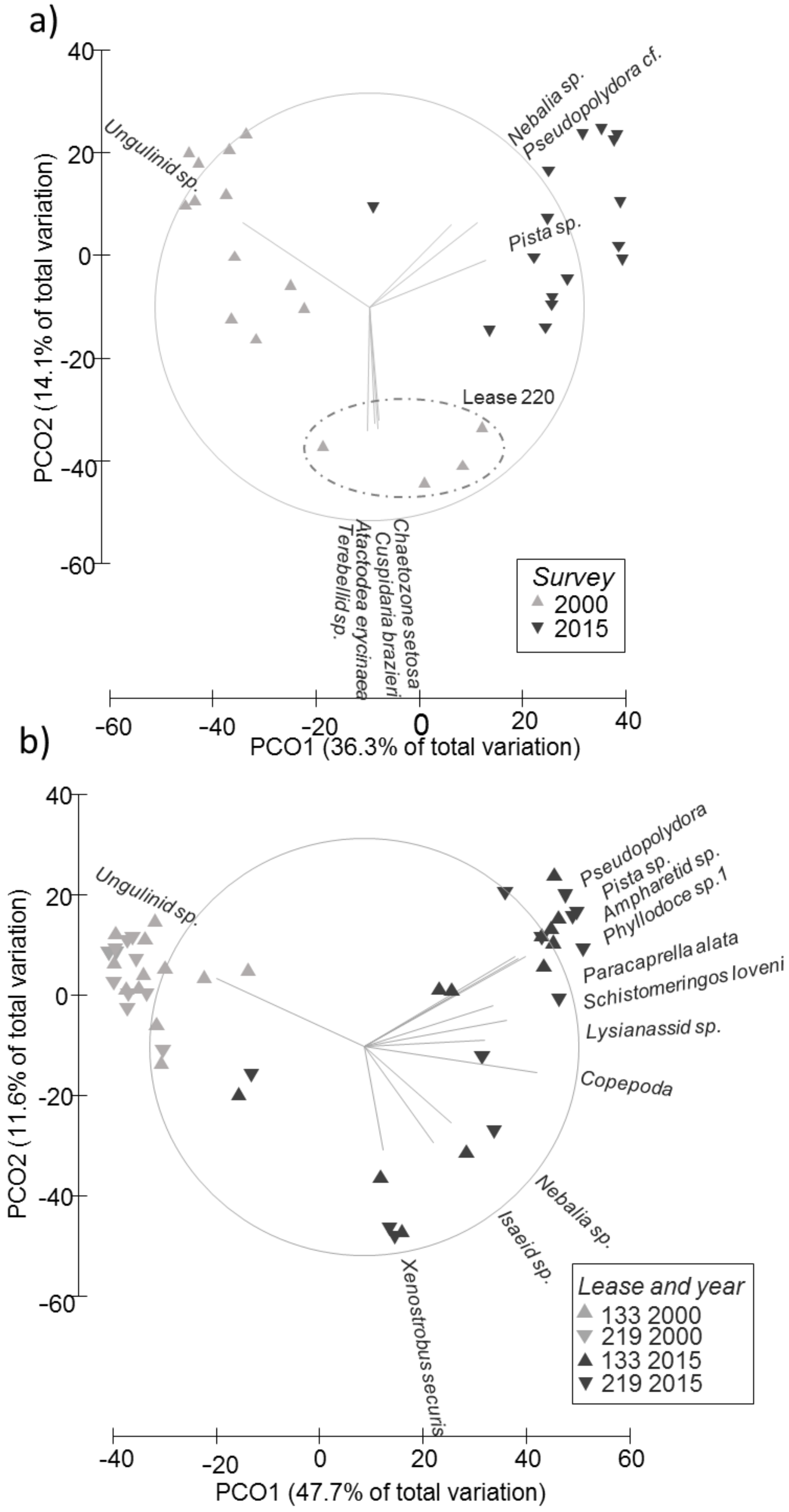

Figure 5-27 Principal Coordinate analysis comparing benthic communities from 1mm-sieved grab before farming in the baseline study (year 2000) and after farming in 2015 at a) unfarmed sites and b) at leases 133 and 219. Species shown have a 0.4 correlation.

Comparison with 2012 baseline external sites (DPIPWE)

Of the external sites sampled by DPIPWE in 2012, eight overlapped with sites in the 2015 survey. In terms of species richness, total abundance and species diversity, there was no clear pattern between surveys, Figure 5-28. For example, in 2015 some sites showed greater species richness was higher, others lower and some similar. However, the PCO revealed that the community in 2015 is by and large different to the community in the 2012 and 2000 surveys, Figure 5-29. This appears to be largely driven by the increased presence of nebaliid and copepod crustaceans and spionid and terebellid polychaetes.

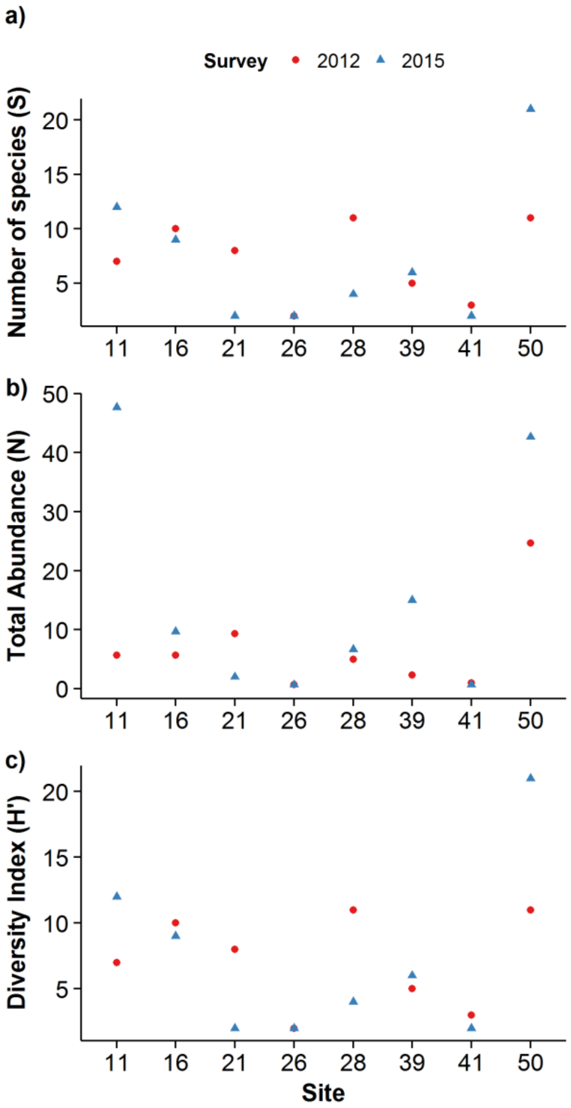

Figure 5-28 Comparison of species richness (S), abundance of all taxa (N), and Shannon-Weaver diversity index (H') at external sites surveyed in 2012 (red dots) and again in 2015 (blue triangles).

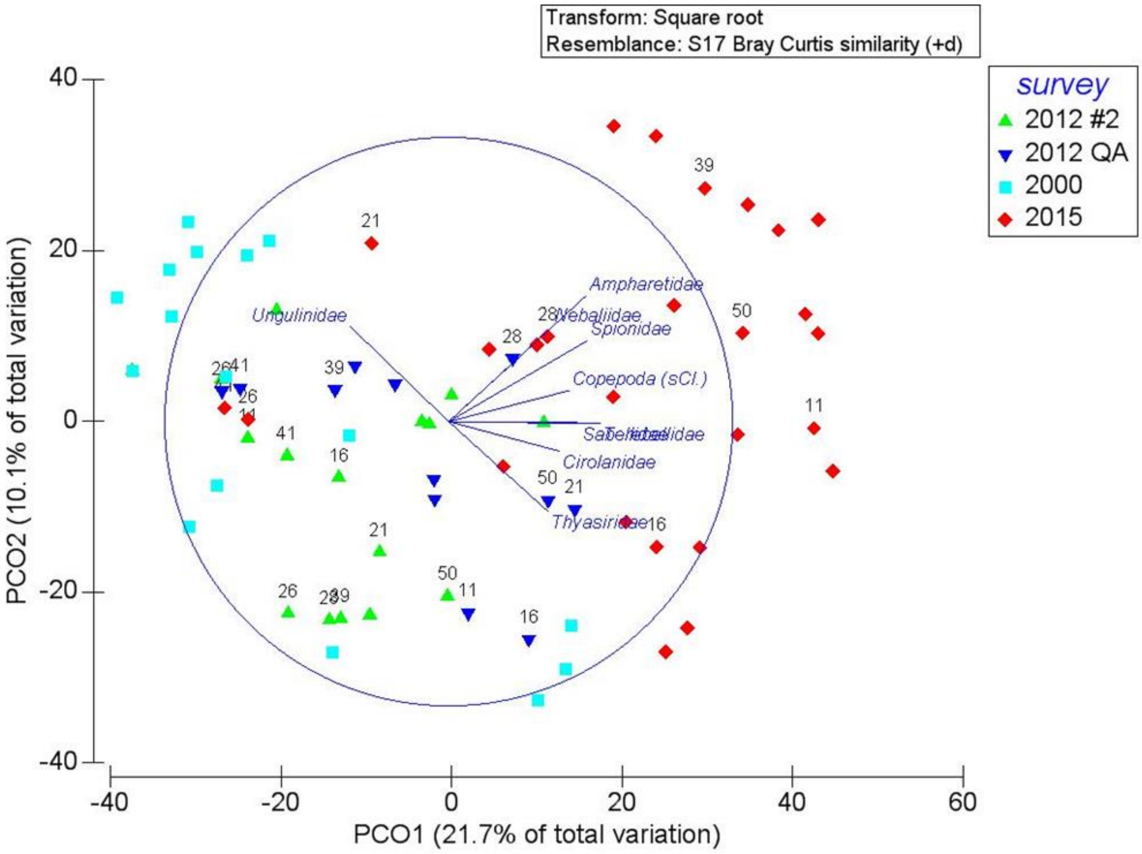

Figure 5-29 Principal Coordinate Analysis of benthic communities from external sites in the 2000 baseline (squares), 2012 baseline #2 (upturned triangles), 2012 DPIPWE survey(down-turned triangles), and this study (diamonds). Numbers reveal sites that were sampled in the two 2012 surveys and in 2015. Vectors show families of fauna driving the differences, with a correlation of 0.4 or greater.

5.4 Sensitivity of video assessment

5.4.1 Comparison with grabs

Dorvilleids were seen in video footage at 9 of the 18 external sites and 75 of the 87 lease sites, thus 84 of the total 105 sites. A decrease in Dorvilleid abundance with increasing distance from the cages is evident from the ROV data, however, unlike the grabs, the greatest abundance of Dorvilleids in the ROV was at 0m, Figure 5-30a. The abundance close to cages in video footage represents *S. loveni* and *O. shieldsi* as both were detected in the ROV. However, when comparing the pattern of distribution of *O. shieldsi* colonies from the ROV, Figure 5-7, and *S. loveni* as determined by grabs, *O. shieldsi* peaks close to cages (0m) and *S. loveni* at 50m.

Unlike grab sampling, ROV analysis provides estimates based on broader groupings because it is difficult (impossible) to count individuals in a colony or at extremely high densities. As such ROV analysis is less sensitive, particularly at higher abundances. The highest grouping, >1000, could be reached in any stage of the video during the 3 minutes of footage, and therefore could encompass quite a wide range of actual abundances. For example, on the south-east transect at lease 266, the Dorvilleids abundance estimated from ROV was >1000 at all sites, Figure 5-30c. yet the grab data documented large differences in abundance between distances, Figure 5-30b; also consistent with the relative numbers seen in the screen images from the ROV, Figure 5-32.

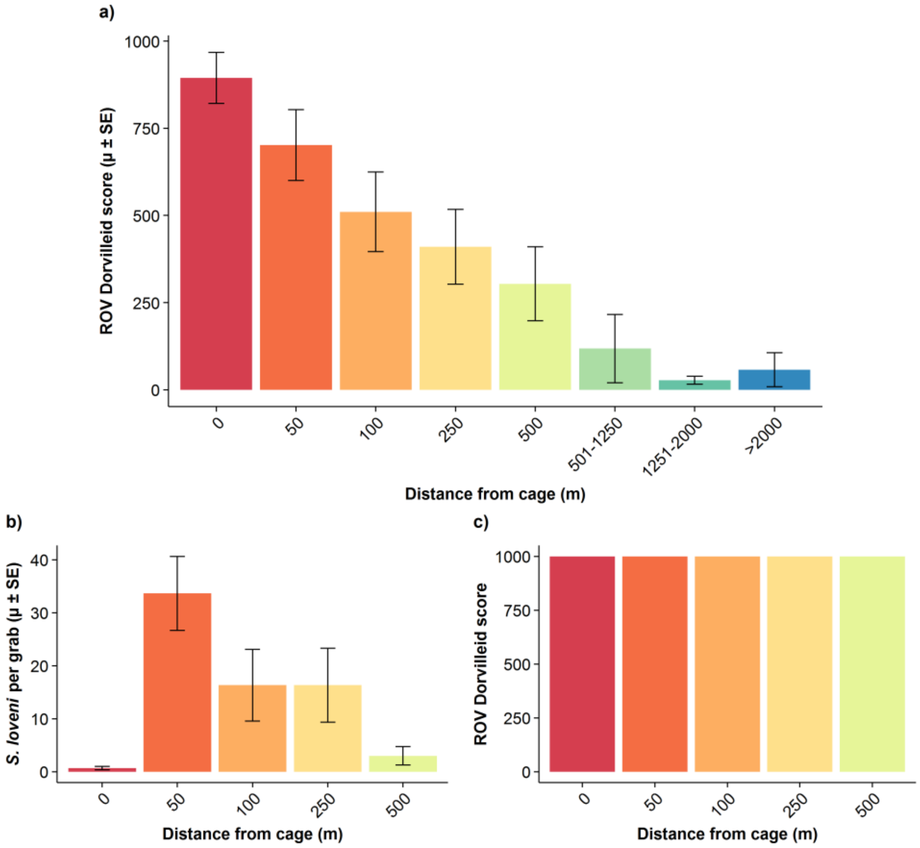

Figure 5-30 a) Approximation of Dorvilleid abundance based on ROV footage for all sites, b) Abundance of S. loveni from grab samples on the 266-SE transect, and c) the approximation of Dorvilleid abundance from the ROV on the 266-SE transect.

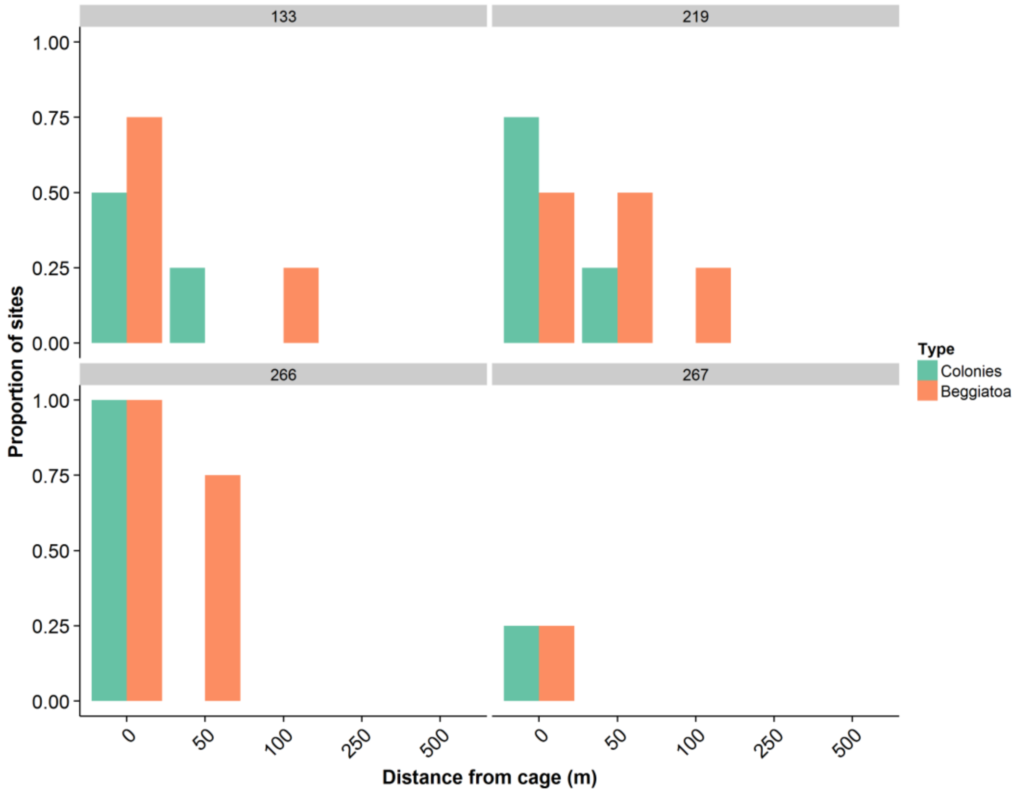

Figure 5-31 Number of sites with colonies of Ophryotrocha shieldsi *per lease (Mean ± S.E).*

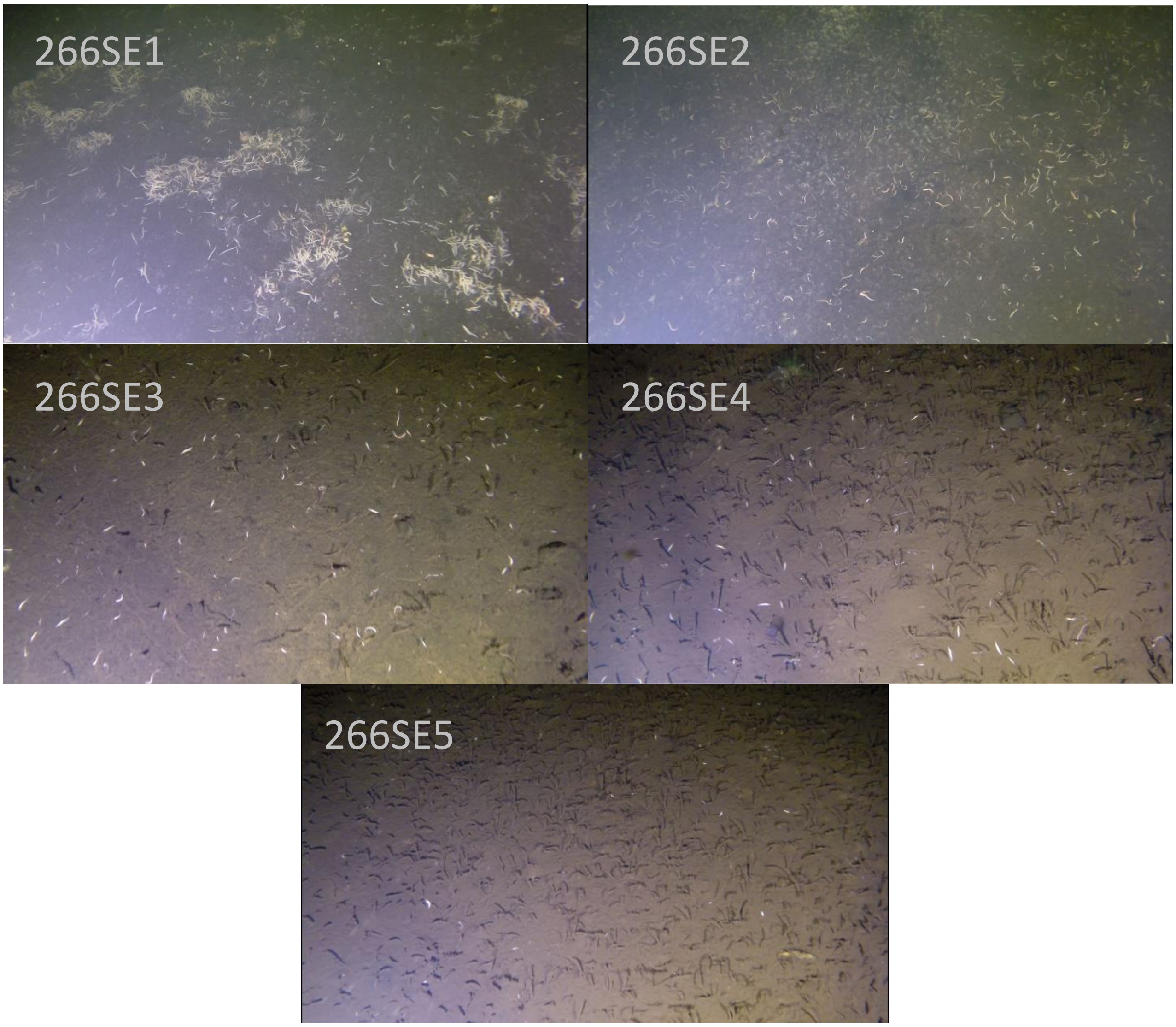

Figure 5-32 Screen captures of GoPro footage from Lease 266, South East transect, demonstrating some of the range of Dorvilleid densities that are classed as >1000. Dorvilleids appear as white flecks or lines. Note the colonies of O. shieldsi *at 266SE1. The rest show only* S. loveni.

Despite the limitations of measuring abundance from video footage, the ROV is able to capture features of the sediment that the grab cannot. Firstly, the area covered by the ROV is much greater than the grab, increasing the chance of detecting features that are patchy or uncommon. Secondly, key features such as the presence of *Beggiatoa*, faeces, feed pellets, off-gassing, and sediment colour are all detectable on video footage. Video footage in this study revealed a frequent association between colonies of *O. shieldsi* and *Beggiatoa*, Figure 5-31. Notably, the prevalence of *Beggiatoa* and colonies was greater at Leases 266, 219, and 133 than at lease 267.

The presence of other fauna, such as Sabellids are also recorded from the ROV data. In line with the benthic community assessment of grab data, Sabellids were more common at lease 267, present at 96% of sites, a similar proportion to the external sites (92% had Sabellids). At lease 266, 219 and 133, 38%, 70% and 70% of sites had Sabellids present, respectively.

5.4.2 Preliminary comparisons with May

To investigate temporal changes in the distribution and abundance of Dorvilleids from both grab and ROV sampling, the core of this survey is currently been repeated quarterly in FRDC 2015-024. The preliminary results for the May 2015 were available at the time of writing this report and they provide interesting insight into the relative performance of grab and ROV data for monitoring Dorvilleid distribution and abundance. Analysis of ROV footage from May 2015 indicates substantial temporal variation in the abundance of Dorvilleids; with Dorvilleid scores significantly reduced in May compared to January across all three leases, Figure 5-33c, and with distance from cage, Figure 5-33d. In contrast, the grab data (from 1mm-sieved samples) shows little difference between the January and May surveys, with some leases and distances more abundant in May and others in January, Figure 5-33 a and b.

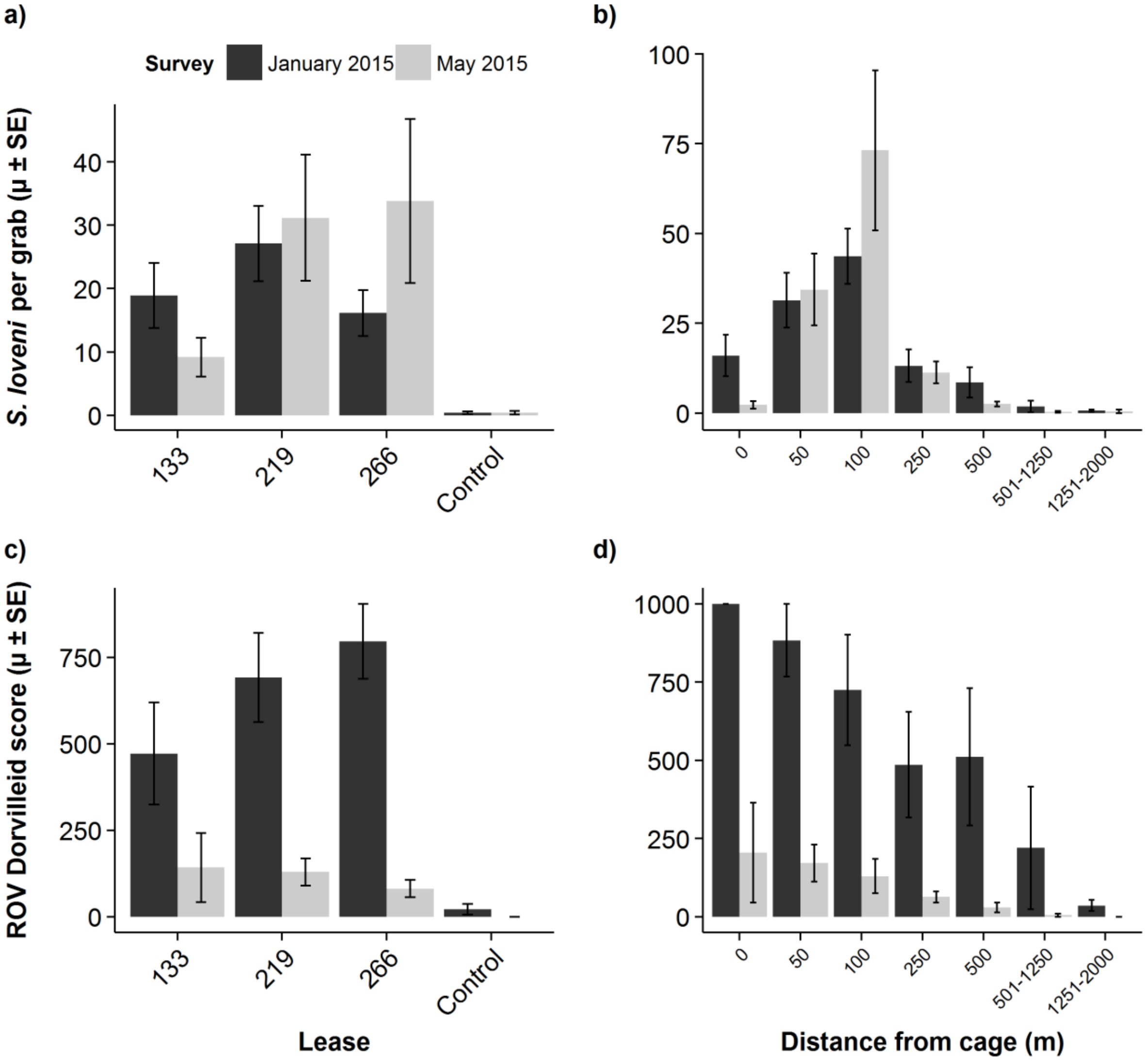

Figure 5-33 Comparison of sites repeated in January 2015 (dark grey) and May 2015 (light grey). Comparisons made at each lease (a, c) and with distance from cages (b, d), both using results from the grab sampling (a, b) and from the ROV (c, d)

6 Discussion

6.1 Distribution and abundance of Dorvilleids

The results of this study show that the two Dorvilleid species identified in Macquarie Harbour respond to organic enrichment from salmon farming. This study confirms these species are opportunists. However, the two species of Dorvilleids differed in their response to enrichment. *Ophryotrocha shieldsi* was predominately found closer to stocked cages (e.g. 0 - 50m) than *Schistomeringos loveni*, suggesting that *S. loveni* is less tolerant of the highly enriched conditions directly adjacent to stocked cages.

O. shieldsi was not dependably sampled using the grab samples, consequently the distribution of this species could only be reliably determined using video footage. However, the footage clearly shows that individuals form colonies that sit above the sediment and appear to correspond with the highest levels of enrichment, with colonies only recorded out to 50m from cages. This species was consistently observed in conjunction with the presence of *Beggiatoa*. The colonies sit above the sediment, and the "loose" nature of these colonies may explain why they were could not be collected in the grab. The association of *O. shieldsi* with *Beggiatoa*-like bacterial mats is consistent with previous observations of a similar species, *O. cyclops*, which also forms colonies at aquaculture sites that are largely associated with *Beggiatoa* (Salvo et al. 2015). Previous studies have suggested that *Ophryotrocha* species may feed on these bacterial mats (Salvo et al. 2015; Taboada et al. 2013), and if this is the case for *O. shieldsi* then it could explain their presence directly under cages, but not further than 50m away. Regardless of whether the *Beggiatoa* is a food source the presence of this species under and adjacent to the cages suggests they are highly adapted to the disturbed sediment and bottom water conditions associated with high levels of organic enrichment.

S. loveni was found in benthic grabs at a number of the external sites throughout the harbour. However, where present at external sites it was in much reduced abundance compared to sites on farm transects. Like *O. shieldsi*, the presence of *S. loveni* was clearly associated with salmon farming, although in this case the peak abundance was at 50 -100m from cage. Many previous studies have suggested that *S. loveni*, and congeners, are opportunistic species. *S. loveni* was found to be up to 30 times more abundant at organically enriched sites under ropes used to propagate mussels than at reference sites in a New Zealand study (Hartstein and Rowden 2004). Whilst sediments under mussel farms may be less enriched than those under salmon farms, the results still show that *S. loveni* responds opportunistically to the enrichment. *Schistomeringos annulata* was one of the top three most abundant species close to fish farm operations (i.e. within 300m) in a Canadian study (Bright 2001) and *Schistomeringos japonica* has been shown to aggregate underneath fish farms in Japan (Sasaki and Oshino 2004). In the current study, *S. loveni* was most abundant 50-100m from stocked cages which suggests that this species potentially has a tolerance window, in which it is enhanced under moderately enriched conditions but sensitive to the highly enriched conditions directly under salmon cages.

Diet

As an adjunct to the current study, the honours project by Hortle (2015) investigated the diet and behavioural ecology of *S. loveni* in Macquarie Harbour. Understanding the diet of Dorvilleids provides further insight into their relationship with organic enrichment, however, there is high degree of variability reported amongst Dorvilleids. For example, *Schistomeringos rudolphi* is believed to have a diet that include detritus and algae while *Schistomeringos neglecta* are believed to prey on small invertebrates (Fauchald and Jumars 1979). Nonetheless, a diet that includes fish feed and or fish faeces has been documented amongst the Dorvilleids. In laboratory trials and in samples collected from under cages at aquaculture sites *Ophryotrocha cyclops* and *Ophryotrocha labronica* were found to mainly consume fish feed (Prevedelli and Vandini 1998; Salvo et al.2015). Similarly, Yokoyama et al. (2006) reported that a *Schistomeringos* sp. (species not documented) was able to utilise fish faeces at farms, while at "clean" sites, it was able to utilise benthic microalgae and phytoplankton based on stable isotope analysis. Stable isotope analysis of *S. loveni* collected on farm transects suggests that *S. loveni* feeds predominantly on salmon feed (and most likely faeces). At a number of sites there was also a good match with epilithic green algae. The latter hypothesised to be associated with net wash (Hortle 2015). The utilisation of fish feed and faeces in the diet of polychaetes in Macquarie Harbour was further demonstrated by White et al. (submitted ms); key fatty acid indicators of farm waste evident in polychaetes to 50-250 m and in sediments to distances of 250 – 500m from farms.

Relationship with feed inputs and farm history

Although there was a clear relationship between the abundance of *S. loveni* and the level of organic enrichment at sites in Macquarie Harbour there was marked variation in abundance both between transects and between leases, and with distance from cages. Although some of this variation may be a function of natural variability a portion can be explained by differences in farming intensity and history.

In the current study feed input within the leases considered ranged from <1,500t to 5,000-10,000t for the 12 months preceding the survey. There was also a marked difference in farming history, with one lease having been farmed for approximately 15 years whilst the remaining three leases had only been operational for less than two years. The greatest abundance of *S. loveni* was observed at the lease with the longest farming history. This lease also showed the clearest signs of benthic enrichment, based on the other sediment and bottom water parameters measured (i.e. redox, C:N, isotopes and bottom water dissolved oxygen). The lease with the lowest feed input, had the smallest footprint of opportunists and the peak in *S. loveni* abundance was close to the cages in this instance. Keeley et al (2015) noted that abundances of Dorvilleids increased at cages during fallowing, and indicated sediment conditions recovering from highly reducing conditions. In this case the assumption is that the enrichment footprint might be expected to be more tightly maintained and closer to the cage under reduced feed inputs. The two other newer leases had markedly higher feed inputs in the preceding year and in each case the peak of abundance for *S. loveni* was further from the cages, at a distance of approximately 50-100m. The AMBI index showed a similar response pattern, supporting this explanation and suggesting that the environmental impact extended further from the cage at these two leases. Interestingly, it is also important to note that at both of these leases reduced feed inputs can be seen to have resulted in a retraction of the peak abundance of *S. loveni,* bringing the footprint of the highest impact closer to cages.

A similar response was observed from ROV footage with both *O. shieldsi* colonies and *Beggiatoa*. The lease with the greatest feed input had the most sites with *O. shieldsi* colonies and *Beggiatoa*, and there were notably fewer colonies and *Beggiatoa* evident at the lease with the lowest feed inputs. The lease with the longest history of farming had *O. shieldsi* colonies and *Beggiatoa* not only at 0m but also at a number of the 50m sites. Interestingly, the lease closest to the harbour entrance to the ocean had conspicuously fewer occurrences of *O. shieldsi* colonies, despite having the second highest feed inputs. One possible explanation for this could be the better bottom water oxygen conditions at this end of the harbour as a result of oceanic recharge of oxygenated seawater. There is also evidence that fallowing at the newer leases improves conditions with *O. shieldsi* colonies and *Beggiatoa* appearing less prevalent adjacent to fallowed cages. This is consistent with studies from British Colombia which showed that *Ophryotrocha cf vivipara* abundance declined during fallowing and after harvest (Brooks et al. 2003). However, at the older lease it appears that *O. shieldsi* colonies and *Beggiatoa* are more persistent, with less evidence of improvement when stocking levels are reduced. Ultimately further surveys will be necessary to fully understand the response of these species to farm management in Macquarie Harbour. It is hoped that the repeated surveys to be undertaken as part of the ongoing FRDC survey 2015-024 will help to clarify the extent to which temporal variability and differences in farming practices might influence the recovery response.

Environmental indicators of enrichment

The sediment chemistry at the study sites was broadly consistent with expectations, with redox values broadly in line with previous research (Wildish 2001; Macleod and Forbes 2004; Hargrave et al. 2008). The majority of sites sampled, both around cages and at external sites, indicated that the sediments were hypoxic at 3cm. This is as might be expected from areas where bottom water dissolved oxygen levels are naturally low. However, lease 219 stood apart from the others sampled with redox levels at five sampling sites (at 0m and 50m) indicating anoxic conditions. This lease had a been farmed longer than any of the other three leases and these findings support the assertion that farming history can influence the enrichment response.

Redox in this study was measured at 3cm depth as per the recommendations of Macleod and Forbes (2004) and consistent with previous monitoring in the harbour. However, the results of this study suggest that 3cm may not provide the level of detail necessary to differentiate change in sediment condition given the naturally low oxygen conditions in Macquarie Harbour. In fact, at the newer leases in the harbour, 3cm may be below the depth where effects on redox potential are likely to be evident and a shallower measurement depth may provide a more sensitive indication of enrichment.

Dissolved oxygen in bottom waters, overlying the sediments, showed marked differences both on a regional and farm scale. Those leases with the longest history of farming and the greatest feed input over the previous 12 months displayed a clear gradient of decline in bottom water dissolved oxygen saturation near the cages (i.e. at 0 and 50m) relative to background levels measured further away. Furthermore, dissolved oxygen in bottom waters was higher at the northern end of the harbour, presumably due to the proximity to Hells Gates and the potential for highly oxygenated oceanic water to more easily replenish this region of the harbour. Lease 133, located in the northern end of the harbour showed no evidence of the farm affecting the bottom water dissolved oxygen measurements (oxygen saturation was generally >40%) despite this site having reasonable feed inputs over the previous 12 months. Lease 267 had the lowest feed inputs for the previous 12 months and there was no evidence of the cages directly affecting bottom water oxygen saturation at this lease either, despite the lower background levels in general in this part of the harbour.

Overall, it is not surprising, and in fact expected that the enrichment under finfish cage will lead to elevated oxygen consumption, and as a consequence, in areas where there is reduced water exchange or high levels of enrichment this could result in localised drawdown of bottom water oxygen concentrations. The results of this study highlight the influence of farm intensity, history and the importance of local conditions in determining the magnitude of this effect.

The stable isotope signatures throughout the harbour reflected a typical estuarine gradient and this was evident in both the farm (lease) and external sites. The $\delta^{13}C$ signature of terrestrial organic matter (25 to -33‰) and freshwater phytoplankton (-25 to -30‰) is typically more depleted compared with marine particulate organic matter (-22 to -18‰) (see Middelburg and Nieuwenhuize, 1998). Similarly, marine organic matter usually has $\delta^{15}N$ signature of 5 - 7‰ indicating it is derived from phytoplankton, whereas terrestrial organic matter generally has $\delta^{15}N$ values < 4‰ (see Middelburg and Nieuwenhuize, 1998). The external sites in the southern part of the harbour generally showed relatively depleted $\delta^{13}C$ and $\delta^{15}N$ values, consistent with higher levels of terrestrial or freshwater inputs, whereas sites closer to Hells Gates (and the marine influence) were more enriched in both $\delta^{15}N$ and $\delta^{13}C$. A similar estuarine gradient was also observed in the C:N ratio of sediment organic matter. Terrestrial organic matter generally has a high C:N (>20) compared to marine organic matter (phytoplankton ~6.6). Consequently, higher ratios (>20) were generally found at those sites closer to terrestrial inputs from the Gordon and King river (site 52), with ratios decreasing (<20) as you move towards the marine influence at Hells Gates.

Despite the distinct estuarine gradient, the influence of farming could be seen in the lease samples where the $\delta^{15}N$ and $\delta^{13}C$ signature was more enriched and the C:N ratio was more depleted relative to the background harbour signature and typical of fish feed (Crawford et al. 2003; Chen et al. 2003; Wang et al. 2013). This was particularly evident at the oldest lease, 219, where there was a clear gradient in the isotopic signatures and C:N ratio with distance from cage. The 0m sites at lease 133 also appeared to be slightly enriched in $\delta^{13}C$ and $\delta^{15}N$. In contrast, there was no evidence of any change within leases 266 and 267. Previous stable isotope studies in Macquarie Harbour had suggested that cage sites could be readily distinguished from external sites based on both carbon and nitrogen ratios and stable isotopes (Ross et al. 2015) however, that study only sampled one lease (lease 219). The current results clearly suggest that farming history and intensity can markedly affect the ability to detect impacts using this approach. In this study, the top 2cm of sediment was sampled and homogenised for sediment analysis, providing an integrated measure of the factors that have influenced this depth horizon. At the older lease (with a 15-year history of farming), it is reasonable to assume that a longer, more intensive history of farming has produced a deeper layer of farm-affected sediment than at the newer leases, with the full 2cm being influenced by farming. In contrast, at the other leases, which were just over a year old, the depth of influence may be much shallower than 2cm. Consequently, it is important to consider the purpose of this comparison, whilst measurement of the full 2cm might provide a meaningful assessment and comparison of enrichment level over the longer term, a shallower sample depth or a profile could provide a more sensitive measure of the enrichment footprint at more recently established leases (see Figure 6-1).

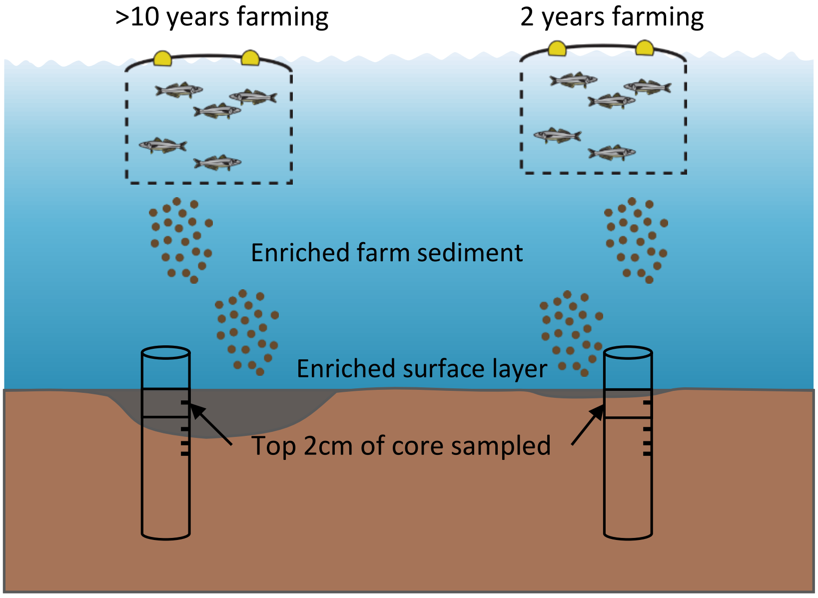

Figure 6-1 Diagram outlying the proposed underlying cause of differences in detectability of sediment properties in samples from older and newer leases. Greater amounts of farm-derived, enriched sediment over time might be expected to form a higher proportion of the sampled sediment at older leases. Symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/)

6.2 Benthic Communities

Polychaetes dominated the benthic infauna throughout the harbour. After *Schistomeringos loveni*, the most abundant polychaetes were Spionids, Terebellids, Ampharetids, Sabellids and Capitellids. Crustacea were also found in high abundance, but predominantly as a result of swarming aggregations of species such as *Nebalia* at sites under cages. The overall benthic community changed discernibly with increasing distance from cages, reflecting the pattern observed in the Dorvilleids. Interestingly, the community at lease 267 was somewhat different from that at the other leases. This potentially reflects the relatively low feed inputs at this lease over the previous year.

The biodiversity metrics, species richness (S), total abundance (N), and Shannon-Weaver Diversity Index (H') showed a response pattern that is generally consistent with both prior research and the guidelines established for the south (Macleod and Forbes 2004) albeit over a greater spatial scale. The number of species and total abundance increased away from the cages with peaks at 100m and 50m respectively. This response broadly aligns with the expectations outlined in the Species-Abundance-Biomass curves produced by Pearson and Rosenberg (1978) and reflects the zone of increased diversity which often occurs some distance from an enrichment source due to the crossover between tolerant and sensitive species. The results show that all sites became more diverse with increasing distance from cages (up to 1250m) this too is in line with previous research and the clear expectation that impacted sites would be less diverse (Keeley et al. 2015; Macleod and Forbes 2004; Pearson and Rosenberg 1978).

However, the naturally depauperate nature of Macquarie Harbour (O'Connor et al. 1996) places it at odds with some of the key premises set out in the monitoring and assessment guidelines established by Macleod and Forbes (2004). For example, under the guidelines a Shannon-Weaver Diversity index value of two was identified as indicative of a relatively unimpacted site, with major degrading effects seen at a diversity index under 1 (Macleod et al. 2004; Macleod and Forbes 2004; Hargrave et al. 2008), however, only four of the sites out of the 105 sampled in the current study had a diversity index (H') value over two and in this case those sites included two external sites and two sites located at a cage. Using the guidelines (Macleod and Forbes, 2004), all of the remaining sites would be considered moderately or majorly impacted. Similarly, impact criteria based on total abundance and species richness established through the guidelines are not likely to be suitable for Macquarie Harbour given the depauperate nature of the background ecology. Whilst, the guidelines recommend using the biotic categorisation in conjunction with a suite of other indicators, and not as standalone thresholds, the results of the current study tend to suggest that the biological impact categorisation and recommendations based on conditions in southern Tasmania may not be directly applicable to Macquarie Harbour.

There are a number of other metrics that have been proposed as useful tools to infer sediment condition using the biological community structure and a number of studies have sought to compare the applicability of these (e.g. Borja 2004, Borja and Dauer 2008). A recent comparison of marine biotic indices (MBIs) that are commonly used to assess organic enrichment gradients associated with finfish aquaculture, but which also consider the specific taxonomic and ecological constraints applicable to Australasia suggested that AMBI was the most applicable tool in this context (Keeley et al. 2012). AMBI uses a database of known species to classify organisms into five ecological groups based on their tolerance or sensitivity to disturbance (see Borja and Franco, 2000; Borja and Muxika, 2005). The index is then calculated based on the proportions of each group. When applied to the dataset in this study (as described in the methods) the index described the transition from impacted to un-impacted sites quite well. In fact, the AMBI appears to provide a better classification than the number of Dorvilleids, aligning well with Dorvilleid abundances except at the under cage sites (0m). The under cage sites were clearly heavily impacted but frequently only had very few *S. loveni*. If abundance of *S. loveni* was the sole criteria for condition assessment then these sites may appear similar to sites much further away, but by incorporating other taxa and their sensitivities AMBI was better able to distinguish the real gradient of impact. AMBI was developed in Europe and the database and consequent classifications have not been verified for the Tasmanian (or Australian) fauna. As a result, many species had to be left unassigned or matched to a different species or taxonomic level, consequently these results should be viewed with caution until the assumptions are locally verified. However, the preliminary assessment is very promising and broadly consistent with the observations of Keeley et al. (2012).

Whilst assessment of species diversity and abundance can be used to provide an understanding of the major changes in the overall community structure associated with organic enrichment and farming, understanding the functional ecology and how this changes can provide insight into how the broader ecology and ecosystem function might have changed. Functional group assessment characterises the different types of fauna found at different sites according to the key role they play in the ecosystem.

Burrowing/Epibenthic fauna tends to dominate under/ around the cages, whilst Tube-building and filter/ suspension feeding fauna tend to be more common where conditions are slightly less depositional. Although Burrowing/Epibenthic fauna can comprise a range of species, for most sites in this study it consisted almost entirely of *S. loveni* and *Nebalia* sp. This group is characterised by their ability to move through the water column as well as burrow into the sediment, and these species are often able to alter their behaviour in response to changes in the physicochemical properties of the sediment. While 80% of individuals at the 0m sites were Burrowing/Epibenthic taxa, only 26% were *S. loveni*, 52% were *Nebalia* sp. *Nebalia* sp. have been shown elsewhere to be prolific opportunists, and are frequently found underneath salmon cages (Sasaki and Oshino 2004; Brooks and Mahnken 2003; Keeley et al. 2012). While *O. shieldsi* was not detected in grabs and therefore could not be included in the functional group analysis, from the video footage its distribution appeared similar to that of *Nebalia* sp. and *Capitella capitata* sp. cmplx. In previous studies *Nebalia* sp. and *Capitella capitata* sp. cmplx. have been shown to proliferate and have a strong affinity for salmon farm enrichment with significantly elevated abundances in the areas under and around cages, acting as key indicator species (Pearson and Rosenberg, 1979, Brooks and Mahnken 2003; Macleod and Forbes, 2004; Dean 2008; Keeley et al. 2015). Although not common in Macquarie Harbour, *C. capitata* were also associated with the highly enriched cages sites with peak abundances at 0m.

The Tube-building group is comprised of sessile suspension or surface deposit-feeders, with little capacity to move in and out of areas as the sediment quality changes. These functional feeding types are generally more sensitive to organic enrichment and deterioration of environmental condition than their more mobile counterparts. At a distance of 250-500m from the cages this group was the dominant functional group, with the key species being Sabellid fan worms, the Terebellid, *Pista sp.*, and Ampharetids. This spatial pattern of succession is aligned with the spatial transition from deposit to suspension feeders associated with increasing distance from cages identified in previous research (Macleod and Forbes 2004; Brooks and Mahnken 2003).

In conclusion, the suite of community and ecological metrics used in this study all indicate that the direct impact of farming is greatly reduced by about 100-250m from the cages, and suggest that the final distance category (>2000) may be representative of a different type of community. The successional response to enrichment seems to either reverse or stop at sites beyond 2000m from leases. The few sites in the study that were greater than 2000m from leases are more heavily influenced by the Gordon and King Rivers based on the carbon and nitrogen results, and as such, this may explain the different communities.

6.2.1 Changes over time

Comparison of the data from this survey with baseline data from sites collected in 2000 and 2012 suggests that there has been a considerable change in the unfarmed areas throughout the harbour in the 15-year period. This component of the study was specifically focused on assessing changes in the unfarmed area of the harbour since farming commenced, but also considered the farmed areas associated with leases 133 and 219. To eliminate bias with respect to differences in site location and farming activity between surveys, comparisons were restricted to sites from the same region and for comparisons of harbour-wide changes only sites greater than 500m from an active farm were considered.

The findings of the comparisons between 2000 and 2015 suggest that species diversity, overall abundance and abundance of Dorvilleids have all increased, and that this effect appears to be most pronounced where farming has occurred. The functional analysis suggests that the proportion of burrowing taxa has declined, this would seem to be predominantly as a result of a decline in bivalve numbers and a concomitant increase in tube building species. Comparison with 2012 surveys was not straightforward due to concerns regarding compatibility of the sampling approach and identifications in 2012. These concerns were raised at the time (i.e. in 2012) and a number of sites were resampled to be consistent with the methods used in 2000 (and 2015). The results from these resampled sites indicated a response similar to that observed in the longer-term comparison, with the benthic communities having changed over time and an overall increase in abundance[5]. The challenges with this temporal comparison notwithstanding, it is clear that the benthic community in the central harbour outside lease has changed substantially since 2000 and that arguably, the greatest change occurred between 2012 and 2015.

Comparison of change over time “on-farm” suggests that although the two sites assessed (leases 133 and 219) had very different farming histories, in that lease 133 had only been farmed for approximately 2, whilst lease 219 had been farmed for the full 15 years, the benthic community composition (both at the species and functional levels) were very similar in 2015. This tends to suggest that the benthic community change to one better adapted to organic enrichment happens fairly rapidly and that this community once established will be maintained throughout the operation of the lease.

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[5] Note, as a consequence the full 2012 baseline sampling of benthic infauna was conducted again later in 2012.

While the community change at leases 133 and 219 can be attributed directly to the effects of organic enrichment from farming, the underlying cause of the harbour-wide change is not so clear. There a number of factors, over and above the farming activity in the harbour which must be considered. Macquarie Harbour has been subject to run-off from the Mt Lyell copper mine for many years and given the footprint that was established for those inputs (O'Connor et al. 1996; Talman et al. 1996) it is likely that the benthic community is still influenced by that. Hydro-electric power production has resulted in significant regulation of the river flow into the harbour, and this will influence both the nature and timing of the delivery of the terrestrial and river derived organic matter and sediments. The decline in bottom water oxygen conditions reported in recent years is likely to have also played a role in defining the broader benthic community, and interestingly the increased faunal biomass would in turn likely increase demand on bottom-water oxygen. However, whilst there are a number of other potential factors that could mitigate/ aggravate the benthic response clearly the increase in fish farming within the harbour, particularly in the last few years, has provided a significant source of organic enrichment to the system, and it is likely that the infauna throughout the harbour would respond and take advantage of that additional nutrient source.

6.3 Sensitivity of video assessment

Due to the relative ease and affordability, video assessment has become an important tool for monitoring benthic impacts of salmon aquaculture, not just in Tasmania but worldwide (Janowicz and Ross 2001; Hamoutene et al. 2013; Hamoutene et al. 2015; Mabrouk et al. 2014). Research conducted in southern Tasmania tested video against a range of more traditional and detailed measurements of sediment condition and found that quantitative video assessment could accurately detect major environmental changes around fish farms (Crawford et al. 2001; Crawford et al. 2002; Macleod et al. 2004; Macleod and Forbes 2004). This research supported the continued use of video monitoring in Tasmania and supported using video footage as part of an adaptive management framework in conjunction with the surveys using benthic grabs and sediment chemistry measurements. One of the goals of this study was to provide more detail on the relationship in Macquarie Harbour between the video footage and benthic community assessments, which are generally considered the benchmark method for evaluating the impact of salmon farming.

While the video assessment broadly aligned with the infauna, two notable differences were observed. Firstly, the video in Macquarie Harbour was able to detect colonies of *Ophryotrocha shieldsi*, which were not sampled using the benthic grabs. The reason for this is assumed to be that the colonies are very fragile and the delicate mucus masses are easily disturbed and broken up when using the grab sampler. Consequently, video would in this case appear to give a better indication of the ecological status than the sediment samples. This is important as *O. shieldsi* are commonly found under/ around the cages and in association with *Beggiatoa* and as such would appear to be a valuable indicator of high levels of enrichment.

The second major difference between the video and grab results is the ability to differentiate between sites with high abundances of Dorvilleids. It is important to keep in mind that what appears to be relatively few Dorvilleids per grab may be the equivalent of >1000 in the video assessment. For example, 10 individuals per grab are approximately 148 per m^2, assuming even coverage. Even if the ROV covers 10 m^2 that is still an assessment of >1000 and much of the differences between sites in terms of *S. loveni* abundance occurs above this threshold. In other words, an ROV estimate of >1000 this could be equivalent to anything between ~6-7 and 100 (or more) per grab. This distinction is important ecologically when differentiating where a site sits with respect to the peak abundance and impact gradient from the source. This was clearly demonstrated in Figure 5-30b&c, with the density of Dorvilleids as determined by grabs showing a distinct drop in density with distance from cage compared with the ROV estimates of >1000 at all sites. Of course, the accuracy of density estimates could be improved with greater standardisation of video techniques (e.g. field of view, tow speed etc.). It is also important to recognise that the greater area covered by the ROV is advantageous when detecting patchiness of the Dorvilleids, which sometimes aggregate around food sources; this is difficult to discern from grab samples.

There is also potential for the roles to be reversed and for the ROV to underestimate abundance. This was observed in May 2015 when the ROV footage suggested a major drop in the abundance of *S. loveni* but the grabs indicated little change between the January and May sampling. The most likely explanation in this instance is that the worms had migrated down into the sediments in May. Whilst initially this might suggest that grab sampling would be the more useful approach for monitoring, their presence (or absence) on the sediment surface in ROV footage may provide valuable insight into the ecological / sediment conditions. In this instance the bottom water oxygen concentrations in January were much lower than in May, and oxygen penetration into the sediments is likely to have been reduced. Consequently, the presence of worms on the sediment surface may reflect this change in the sub-surface biogeochemistry. Clearly, without the actual measurements of the sediment oxygen conditions this is purely supposition, and there are any number of other factors that may be responsible for the apparent change in behaviour, but the video footage provides a valuable trigger to initiate those questions. Ultimately, a longer time series of monitoring along with further behavioural studies are required to better understand the significance of the observed changes in Dorvilleid behaviour for benthic monitoring in Macquarie Harbour.

7 Conclusions

In conclusion, it is important to remember that this study was intended to provide an improved understanding of Dorvilleid ecology, and in particular their response to organic enrichment; identify the relationship between Dorvilleids and sediment condition and determine the reliability of this species as an indicator of sediment condition in Macquarie Harbour; and on that basis make recommendations as to the future use of Dorvilleids in regulatory monitoring of Salmonid aquaculture in Macquarie Harbour.

There are two species of Dorvilleid in Macquarie Harbour, *Ophryotrocha shieldsi* and *Schistomeringos loveni*, and their presence reflects different levels of enrichment. *O. shieldsi* occurs predominantly as colonies under stocked cages, with occasional extension out to 50m, whereas *S. loveni* was less tolerant of highly enriched sediments and was most abundant away from stocked cages, generally associated with sites and conditions 50-100m from the cages.

Although the distribution of both Dorvilleid species was patchy both within and between leases there were some broad patterns consistent with feed inputs and farm history. When feed inputs were low, *S. loveni* peak abundance was closer to the cage and where feed input was high, *S. loveni peak* abundance was further out and *O. shieldsi* and *Beggiatoa* were more common. The results also suggest that these effects may be exacerbated at older leases with *O. shieldsi* and *Beggiatoa* persisting for longer and *S. loveni* reaching higher abundances.

Changes in the composition of the broader benthic communities were consistent with previously described responses to organic enrichment. In this instance peak faunal abundance and species richness occurred at 50m and 100m respectively, and species diversity was noticeably increased from approximately 100m. At a functional group level, the Burrowing/Epibenthic group (mostly *S. loveni* and *Nebalia sp.*), were dominant out to 100m, beyond which the Tube Building group (mostly Sabellid fan worms) dominated. Whilst these changes are consistent with those previously observed in the south the spatial scale of the response would appear to be greater – i.e. the transition between impact stages is occurring further from the source (cages) than would be predicted in southern Tasmania.

The response of sediment (redox, C and N isotopes and ratios) and bottom water (i.e. bottom water dissolved oxygen) environmental parameters to organic enrichment were also consistent with expectations, highlighting the influence of farm intensity, history and the importance of local conditions in determining the magnitude of this response. For more recently established leases, the results also suggest that sampling sediment parameters to shallower depth is likely to provide a more sensitive measure of the organic matter footprint.

Temporal comparison with surveys conducted in 2000 and 2012 suggests that there has been a harbour-wide change in the benthic communities since the inception of farming, with an increase throughout the harbour in species tolerant of moderate levels of organic enrichment, and a concomitant functional change in the ecology with a greater presence of tube-building suspension and surface-deposit feeders. These changes have been more pronounced in farming areas. Where farming has been initiated the communities have tended to become more similar and reflect a greater tolerance to organic enrichment, this would appear to occur relatively quickly (<2 years) after farming commences. Unfortunately, it is difficult to resolve the specific causal factors behind the harbour-wide changes given that there have been a number of inconsistencies in sampling structure.

The ROV assessments showed that video was the most reliable approach to detect colonies of *O. shieldsi;* important given *O. shieldsi* (in conjunction with *Beggiatoa*) appears to be the most effective indicator of particularly high levels of enrichment. In contrast the ROV was not as reliable for the determination of the abundance of *S. loveni*, as this species would appear to reside both on the sediment surface and deeper in the sediments. The presence (or absence) of this species on the sediment surface may in itself be a useful indicator of sediment and bottom water conditions, but further work is required before this can be determined.

Finally, it is important to remember, that as comprehensive as this study was, it reflects the situation at only one time of the year and we would recommend that the assessments be repeated at a timescale that will take into account both natural variability and different stages of farm management. This work has been funded and is already underway through FRDC project 2015-024.

8 Implications

Previous research has shown a clear successional impact gradient associated with cage salmon farming operations, and that there are a number of key ecological features that can be used to identify stages along that gradient (spatially and temporally); the presence of bacterial mats (*Beggiatoa spp*.) and proliferation of opportunistic species are features commonly associated with high levels of organic enrichment (Pearson and Rosenberg 1978; Macleod and Forbes 2004). The presence and abundance of these “indicator species” can be used as a sign of deteriorating environmental conditions (Macleod and Forbes 2004). The presence of numerous annelid opportunists, such as Capitellid worms, 35m outside the boundary of the lease area, may be interpreted as representative of “unacceptable impact” (Crawford et al. 2002). This premise has been validated in southern Tasmania (Macleod and Forbes 2004) and underpins the regulatory monitoring requirements statewide (Department of Primary Industries Water and Environment 2004).

The understanding that proliferating opportunists represents deteriorating conditions was translated to monitoring protocols in Macquarie Harbour. Although the relationship between opportunists and the level of enrichment was not explicitly tested in this region, environmental surveys in Macquarie Harbour suggested that in this region Dorvilleid worms rather than Capitellids were the species most indicative of organic enrichment effects (Department of Primary Industries Water and Environment 2004).

The results of this study confirmed the presence of two Dorvilleid species, *Ophryotrocha shieldsi* and *Schistomeringos loveni* in Macquarie Harbour. The distribution and abundance of both species was clearly associated with organic enrichment, but they have different sensitivities. This has implications for their potential utility in regulatory monitoring and the interpretation of impact. *O. shieldsi* is a colony forming species that was closely associated with stocked cages and the presence of *Beggiatoa*. The association with very high levels of organic enrichment, indicates that the presence *O. shieldsi* at compliance monitoring sites could be classified as representative of “unacceptable impact”.

On the other hand, *S. loveni* appears less tolerant of the highly enriched sediments directly adjacent to stocked cages and interpretation of the presence of this species is more ambiguous. For example, the presence of *S. loveni* at similar densities either side of their peak abundance is likely to reflect different levels of impact. Closer to the cage, their presence may be associated with deteriorating conditions and a highly disturbed benthic community with few species dominated by burrowing and epibenthic taxa. In contrast a similar number of *S. loveni* beyond the peak abundance is likely to reflect more moderate levels of enrichment and a more diverse community with more tube building taxa present. Thus, more information is required when interpreting the presence of *S. loveni* and the level of impact. Repeat surveys currently underway through FRDC project 2015-024 will provide greater insight into the importance of natural temporal variability and the influence of different stages of farm management on the presence of *S. loveni*, and thus, their utility as an indicator species.

Notwithstanding the need for further study, it remains that the presence of *S. loveni* is associated with elevated levels of enrichment and the response to enrichment in the benthic community is also clear, with a number of key species defining the process. In a naturally depauperate system like Macquarie Harbour these changes are more easily identified, and the results suggest that the benthic response is occurring at greater distances from the cages than observed in the southern Tasmania. This has implications for positioning of cages within the lease area; cages positioned close to the lease boundary are more likely to lead to benthic effects outside of the lease. The results also indicate that farming history (lease age and feed inputs) will influence the spatial extent of any impact with lower feed inputs resulting in a more constrained zone of influence and higher feed inputs and longer farming history resulting in a greater zone of influence (footprint). As such, the presence of *S. loveni* and changes in benthic community composition can provide valuable management insight on the extent of the benthic footprint and how that footprint might respond to changes in farm management.

The comparison with baseline surveys highlighted a change in the broader benthic ecology of the harbour, with the greatest effect arguably being evident mostly in the last 2 years, with an increase in total abundance, species richness and species diversity harbour-wide. At a functional group level, this has corresponded to a decrease in burrowing taxa and an increase in tube builders. Whilst there are a range of explanations for this change, such as a recovery from the effects of mining or influx of organic matter associated with changes in the regulation of catchment inflows, it is highly likely that the addition of nutrients and organic matter from fish farming has played a role in stimulating benthic productivity.

In terms of assessment techniques, this work again highlighted the utility of ROV for detecting key indicators that are associated with highly enriched conditions (i.e. the presence of *Beggiatoa* and *O. shieldsi* colonies). The major advantage of the ROV is that it can cover a large area, and thus is able to detect these indicators over a much larger area and is particularly useful where there is a high degree of patchiness. Although the abundance of *S. loveni* does not appear to be as reliably detected by ROV, their presence on the sediment surface in ROV footage may be an important indicator of benthic conditions. This will be further assessed in FRDC 2015-024.

9 Recommendations

This project provides a detailed snapshot of the Dorvilleid polychaetes in Macquarie Harbour in January 2015 and has provided useful insights into their distribution and behaviour within the harbour. However, as the sampling was performed at only one time period, the potential for temporal changes, particularly on seasonal cycles and in response to farm management, is still largely unknown. The inclusion of some preliminary data from repeated sampling of certain sites in May demonstrates that substantial changes can occur over time in Macquarie Harbour and highlights the need for continued monitoring to develop a more complete understanding of the interactions between aquaculture and the benthos – this work is underway and funded under FRDC project 2015-024. Culturing Dorvilleids could give an important insight into their reproductive strategies, tolerances and preferences and would add value to the field surveys and further research in these areas is recommended.

A key challenge when documenting the change in benthic communities more broadly in the harbour was a lack of consistent sampling in space and time; consequently, it is recommended that regular sampling of external sites be conducted. The study also highlighted the importance of local taxonomic expertise and the application of consistent sampling and processing methods across surveys to ensure a robust data set for assessment.

9.1 Further development

As described in the recommendation, further development of the project is currently underway and funded under FRDC project 2015-024

10 Extension and Adoption

This project provides the salmonid aquaculture industry and Tasmanian State Government with an improved understanding of the relationship between Dorvilleids and sediment condition and farm input. This information will be used to assist discussions on the use of Dorvilleids as an indicator of organic enrichment in regulatory monitoring requirements for fish farming in Macquarie Harbour. The findings of the research may assist farm management practices (e.g. stocking and fallowing regimes) by providing an improved understanding of the sediment footprint and the ecological response to farm derived enrichment in Macquarie Harbour.

The project findings have been provided to government and industry during regular meetings over the course of the study and will be provided in the form of this report. The results of this study were also presented in a public seminar during the National Estuaries Network (NEN) Meeting in Hobart, November 2015 and will be presented at the Estuarine Coastal Sciences Association conference in Bremen, Germany, September 2016.

This project will provide benefits not just to the ongoing management of the salmon industry in Macquarie Harbour but also for monitoring programs associated with organic enrichment in other coastal ecosystems; this will be achieved through associated research publications. This research will improve our understanding of Dorvilleid ecology and their role as an indicator of organic enrichment. This knowledge will be used to help refine existing management strategies to ensure that salmon farming is managed sustainably in Macquarie Harbour. This knowledge transfer will occur via the project final report and meetings with the key stakeholders, industry and government. The final report will be made available on the FRDC and IMAS websites along with associated research publications.

11 Appendices

11.1 Researchers and Project staff

Dr Jeff Ross – Principal Investigator, IMAS

Dr Catriona Macleod – Co-Investigator, IMAS

Adam Davey - Research Assistant, IMAS

Arlie McCarthy – Research Assistant, IMAS

Andrew Pender – Technical Assistant, IMAS

Geoff Endo –Technical Assistant, IMAS

James Hortle - Technical Assistant/Honours Student, IMAS

Samuel Gray – Technical Assistant, IMAS

11.2 Intellectual Property

There is no specific IP associated with this project.

11.3 Project Coverage

There has been no media coverage of the results or findings to date nor have the findings been published. The results of this study were presented in a public seminar during the National Estuaries Network (NEN) Meeting in Hobart, November 2015 and will be presented at the Estuarine Coastal Sciences Association conference in Bremen, Germany, September 2016.

11.4 Species list

List of taxa used in this study, from the field survey conducted in January 2015 and baseline surveys in 2000. Each taxon is shown to the level at which it was identified and the function group to which it was assigned. For the taxa used in the AMBI index the ecological group they were assigned is indicated, as well as whether they needed to be changed to match a group in the database, and what they were changed to. Not used = from historical or 0.5mm data set that were not used for the AMBI analysis, ignored=from 1mm data set but excluded from AMBI analyses, Not assigned=included in AMBI analyses but not assigned an ecological group.

Phylum	Class	Family	Species	Functional Group	AMBI ecological group	AMBI Changed to	Code
Crustacea	Amphipoda	Ampeliscidae	*Byblis mildura*	Burrowing/Epibenthic	I	*Byblis sp.*	CAB1
		Corophiidae	*Paracorophium sp.*	Burrowing/Epibenthic	III		CAC1
		Paracalliopiidae	*Paracalliope sp.*	Burrowing/Epibenthic	Not used		CAF1
		Isaeidae	*Isaeid sp.*	Burrowing/Epibenthic	Not assigned		CAI1
		Lysianassidae	*Parawaldeckia sp.*	Burrowing/Epibenthic	Not used		CAL_IM13
			Lysianassid sp.	Burrowing/Epibenthic	I	Lyssianassidae	CAL1
		Liljeborgidae	*Liljeborgid sp.*	Burrowing/Epibenthic	I	*Liljeborgia sp.*	CAM1
		Melitidae	*Melita sp.*	Burrowing/Epibenthic	I		CAM2
		Phoxocephalidae	*Limnoporeia yarrague*	Burrowing/Epibenthic	I	Phoxocephalidae	CAP1
			Brolgus tattersalli	Burrowing/Epibenthic	Not used		CAP_IM31
		Oedicerotidae	Oedicerotidae?	Burrowing/Epibenthic	I	Gammaridae	CAX2
			Oedicerotid sp.	Burrowing/Epibenthic	Not used		CAO_IM1
		Caprellidae	*Paracaprella alata*	Epibenthic	I	*Paracaprella tenuis*	CC1
		Amphipoda	cf. Phoxocephalidae v Urohaus	Burrowing/Epibenthic	Not used		CAX3
		Amphipoda	Amphipod	Burrowing/Epibenthic	Not assigned		CAX1
	Decapoda	Grapsidae	Grapsidae	Epibenthic	Not assigned		CBG2
		Hymensomatidae	*Amarinus laevis*	Epibenthic	Not used		CBH1
		Palaemonidae	Palaemonidae ?	Epibenthic	I	*Palaemon sp.*	CD1
		Axiidae	Axiidae	Epibenthic	Not assigned		CD2
		Palaemonidae	Palaemonidae	Epibenthic	I	*Palaemon sp.*	CD3

			Processa sp.	Epibenthic	Not used		CD_IM11
	Copepoda	Copepoda (sCl.)	Copepoda (sCl.)	Epibenthic	Not assigned		CF1
	Isopoda	Cirolanidae	*Cirolanid sp.*	Epibenthic	III	*Cirolana sp*	CIC1
		Anthuridae	*Anthurid sp.*	Unknown	I	Anthiuridae	CIN1
	Mysida	Mysidae	*Mysid sp.*	Epibenthic	II	Mysida	CM1
	Leptostraca	Nebaliidae	*Nebalia sp.*	Burrowing/Epibenthic	V		CN1
	Ostracoda	Ostracod	Philomedidae	Burrowing/Epibenthic	II	*Philomedes sp.*	CO2
		Ostracod	Myodocopida sp.2	Burrowing/Epibenthic	not assigned	Ostracoda	CO3
		Ostracod	Myodocopida	Burrowing/Epibenthic	not assigned	Ostracoda	CO1
	Malacostraca	Tanaidacea	Colletteidae	Burrowing	Not used		CTL1
			Leptognathia sp.	Burrowing	Not used		CTL_IM2
		Cumacean	*Cumacean sp.*	Burrowing/Epibenthic	I	Cumacea	CU1
Porifera			*Haliclonissa sp.*	Burrowing	Not used		DSN_IM1
Echinodermata		Loveniidae	*Echinocardium cordatum*	Burrowing	I		EE1
		Synaptidae	*Leptosynapta dolabrifera*	Unknown	Not used		EH1
		Ophiuridae	Ophiuridae	Burrowing/Epibenthic	II		EO1
			Amphiura sp.	Burrowing/Epibenthic	Not used		EO_IM5
Arthropoda		Chironmidae	*Chironmid sp.*	Epibenthic	Not used		IC1
Mollusca	Gastropoda	Hydrobiida	*Tatea rufilabris*	Epibenthic	III	Hydrobiidae	MGH1
			Ascorhis tasmanica	Epibenthic	III	Hydrobiidae	MGH2
		Retusidae	*Retusa pelyx*	Burrowing/Epibenthic	Not used		MGR_IM13
		Dorididae	Dorid sp.	Epibenthic	Not used		MGW_IM11
		Philinidae	*Philine angasi*	Burrowing/Epibenthic	II	*Philine sp.*	MGW1
		Opisthobranchia	c.f. Limapontid sp.	Epibenthic	Not used		MGW2
	Bivalvia	Mesodesmatidae	*Atactodea erycinaea*	Burrowing	Not used		MPE_IM2
		Mytilidae	*Xenostrobus securis*	Epibenthic			MPM1
		Lucinidae	*Lucinoma euclia*	Burrowing	I	*Lucinoma sp.*	MPL1
		Nuculidae	*Nuculidae*	Burrowing	I	*Nucula sp.*	MPN1
		Lasaeidae	*Arthritica semen*	Burrowing	III	*Arthritica bifurcata*	MPX3

			Ungulinid sp.	Burrowing	Not used		MPX_IM60
		Thyasiridae	Thyasiridae	Burrowing	II	*Thyasira sp.*	MPX1
			Cuspidaria brazieri	Burrowing	Not used		MPX_IM75
			Bivalve sp.	Burrowing	Not used		MPX_IM76
		Bivalve	Bivalve sp.	Burrowing	ignored		MPX2
Platyhelminthes		Platyhelminthes	Platyhelminthes (P)	Unknown	Not used		PL1
Spinuncula			Sipunculan sp.	Burrowing	Not used		SI_IM4
Cnidaria		Ceranthidae	Ceranthidae	Epibenthic/Tube-building	I	*Cerianthus sp.*	TA1
		Edwardsiidae	Edwardsiidae	Epibenthic/Tube-building	II		TA2
Chordata		Ophidiidae	Ophidiidae	Unknown	ignored		VI1
		Gobiidae	Gobiidae	Unknown	ignored		VI2
		Pleuronectidae	*Rhombosolea tapirina*	Unknown	Not used		VIF_IM2
Hemichordata			*Enteropneust sp.*	Burrowing	Not used		WB_IM3
Nermertea		Nemertea	Nemertean sp.1	Unknown	III	Nemertea	WN1
			Nemertean sp.2	Unknown	III	Nemertea	WN2
Nematoda		Nematoda	Nematoda	Unknown	Not used		WN3
Annelida	Clitellata		*Piscicolid sp.*	Unknown	Not used		WH_IM3
	Oligochaeta	Oligochaeta (sCl)	Oligochaeta (sCl)	Unknown	Not used		WO1
	Polychaeta	Phyllodocidae	*Phyllodoce sp.1*	Burrowing/Epibenthic	II	*Phyllodoce sp.*	WPB1
			Phyllodoce sp.2	Burrowing/Epibenthic	II	*Phyllodoce sp.*	WPB2
		Capitellidae	Capitellidae	Tube-building	V		WPC1
			Heteromastus sp.	Tube-building	Not used		WPC_IM54
		Dorvilleidae	*Ophryotrocha shieldsi*	Epibenthic	Not used		WPD2
			Schistomeringos loveni	Burrowing/Epibenthic	V	*Schistomeringos annulata*	WPD1
		Flabelligeridae	*Flabelligerid sp.*	Burrowing	Not assigned	*Flabelligera sp.*	WPF1
		Goniadidae	*Goniada sp.*	Burrowing	II	*Goniada sp.*	WPG1
		Glyceridae	*Glycera sp.*	Burrowing	Not used		WPG_IM63

		Hesionidae	*Hesionid sp.*	Epibenthic	II	*Hesionides sp.*	WPH1
		Sabellidae	*Branchiomma cf. nigromaculata*	Tube-building	I	*Branchiomma sp.*	WPI1
			Sabellid sp.	Tube-building	Not used		WPI2
			Euchone varibilis	Tube-building	III	*Euchone sp.*	WPI3
		Lumbrineridae	*Lumbrineris sp.*	Tube-building	Not used		WPL_IM12
		Maldanidae	Maldanid sp.1	Tube-building	I	Maldanidae	WPM1
			Maldanid sp.2	Tube-building	I	Maldanidae	WPM2
		Nereididae	Nereidid sp.	Burrowing/Epibenthic	Not used		WPN1
			Australonereis ehlersi	Burrowing/Epibenthic	Not used		WPN3
		Orbiniidae	*Leitoscoloplos bifurcatus*	Burrowing	IV	*Leitoscoloplos sp.*	WPO1
			Scoloplos simplex	Burrowing	Not used		WPO_IM23
			Scoloplos normalis	Burrowing	I	*Scoloplos sp.*	WPO2
		Spionidae	Spionid sp.	Burrowing	Not used		WPP_AD_IM8
			Prionospio multipinulata	Burrowing	Not assigned	*Prionospio sp.*	WPP3
			Pseudopolydora cf. paucibranchiata	Burrowing	IV	*Polydora sp.*	WPP1
			Prionospio cf. tatura	Burrowing	Not assigned	*Prionospio sp.*	WPP2
			Polydora cmplx.	Burrowing	IV	*Polydora sp.*	WPP4
		Cirratulidae	*Cirratulid sp.*	Burrowing	IV	*Cirratulus sp.*	WPQ1
			Chaetozone setosa	Burrowing	Not used		WPR_IM70
		Syllidae	*Exogone sp.*	Burrowing/Epibenthic	II		WPS1
			Syllid sp.	Burrowing/Epibenthic	Not used		WPS_IM55
		Terebellidae	*Terebellid sp.*	Tube-building	Not used		WPT_IM6
			Pista sp.	Tube-building	Not assigned		WPT1
			Amaena sp.	Tube-building	Not assigned		WPT2
			Terebellidae	Tube-building	I	*Terebellides sp.*	WPT3
		Trichobranchidae	*Terebellides sp.*	Tube-building	I		WPU1
		Ampharetidae	*Ampharetid sp.*	Tube-building	I	*Ampherete sp.*	WPV1

		Polynoidae	Polynoidae	Epibenthic	Not assigned		WPW1
		Polychaete	Polychaete uid	Unknown	ignored		WPX1
		Scalibregmatidae	*Scalibregmid sp.*	Burrowing	Not used		WPZ_IM1

11.5 2015: Site information and environmental data

List of detailed information for each site sampled in 2015, including location, depth, and environmental data. Coordinates given according to the datum Map Grid of Australia 1994 (MGA 94) Zone 55. Distances for external sites are to the edge of the nearest lease not cage. Some values missing for the pH data because water quality at some sites was recorded by hand and pH omitted. Salinity values marked with * are likely to be erroneous.

									Bottom water properties			
Lease	*Site ID*	*Easting*	*Northing*	*Depth (m)*	*Distance from cage**	*Cage stocked at sampling*	*Redox (mV) (corrected 3cm)*		Dissolved Oxygen (%)	Temperature	pH	Salinity
133	133-N-1	361861	5320418	38.9	0	No	17.3844	12.3844	39.8	14.6	7.3	30.9
133	133-N-2	361833	5320459	40.4	50		8.616	-2.364	50.6	15.0	7.9	25.1*
133	133-N-3	361803	5320500	41.2	100		7.3302	-13.0098	54.0	15.1	7.8	28.4*
133	133-N-4	361716	5320622	40.5	250		-1.6698	1.3302	47.0	14.9	7.5	30.2
133	133-N-5	361571	5320826	33	500		-1.384	17.2618	44.9	14.8	7.7	30.1
133	133-S-1	361980	5319680	20.9	0	Yes	11.3844	3.0102	25.4	14.3	7.5	29.4
133	133-S-2	362009	5319639	20.8	50		-4.384	4.2702	31.0	14.5	7.6	29.7
133	133-S-3	362038	5319599	21	100		43.9986	27.3044	25.1	14.3	7.6	29.6
133	133-S-4	362125	5319477	21.1	250		-11.444	-7.764	25.1	14.4	7.6	29.8
133	133-S-5	362270	5319273	26.7	500		121.7186	-2.9956	35.1	14.5	7.7	30.1
133	133-SE-1	362223	5319969	40	0	Yes	9.9496	-4.7646	53.8	15.2	7.9	30.5
133	133-SE-2	362264	5319998	48	50		-16.3904	-25.1046	55.6	15.2	7.9	30.9
133	133-SE-3	362304	5320027	49	100		-24.6762	-22.3362	52.3	15.1	7.8	31.1
133	133-SE-4	362426	5320116	45	250		-19.9962	-23.7104	56.9	15.2	7.8	31.2
133	133-SE-5	362627	5320264	44	500		-15.282	-29.4246	56.1	15.2	7.8	31.1
133	133-SW-1	361562	5319486	26	0	Yes	2.9018	-32.3092	37.4	14.6	7.2	29.8
133	133-SW-2	361522	5319456	26	50		-23.724	-24.384	42.0	14.8	7.4	30.1
133	133-SW-3	361481	5319426	26	100		-16.3498	-22.0982	34.0	14.7	7.6	30.1
133	133-SW-4	361360	5319338	26.4	250		-24.4382	-3.4382	35.5	14.6	7.4	30.0
133	133-SW-5	361157	5319190	27.2	500		6.2702	-0.104	42.5	14.6	7.2	30.2

219	219-E-1	364318	5316682	26.3	0	Yes	-124.887	-194.414	29.1	14.9	7.5	30.7
219	219-E-2	364359	5316711	26	50	Yes	-206.414	-220.584	32.2	14.9	7.6	31.0
219	219-E-3	364400	5316740	25.7	100		-45.5843	-31.9414	35.4	15.0	7.6	31.0
219	219-E-4	364524	5316826	26.3	250		-38.9414	-62.9414	40.0	15.0	7.7	31.1
219	219-E-5	364728	5316970	26.5	500		-64.9414	-48.9414	44.3	15.1	7.7	31.2
219	219-N-1	363876	5316422	36	0	No	-152.422	-158.119	7.8	14.3	7.5	30.3
219	219-N-2	363847	5316463	37	50		-61.1188	-38.003	22.4	14.3	7.5	30.3
219	219-N-3	363818	5316504	37.9	100		-37.003	-95.173	24.2	14.3	7.5	30.4
219	219-N-4	363732	5316627	39.9	250		-33.5301	-45.8872	23.9	14.3	7.5	30.4
219	219-N-5	363589	5316831	42.3	500		-42.0572	65.9428	20.6	14.3	7.5	30.4
219	219-S-1	363927	5316343	27.2	0	Yes	-149.834	-168.976	15.0	14.4	6.8	27.9*
219	219-S-2	363956	5316302	28.2	50		-137.405	-170.119	0.3	14.5	7.1	27.1*
219	219-S-3	363985	5316261	29.1	100		-15.4588	-15.833	32.0	14.5	7.5	30.1
219	219-S-4	364071	5316138	32	250		1.4528	-21.5472	32.4	14.5	7.6	30.3
219	219-S-5	364214	5315934	37.6	500		-42.8872	-45.8872	33.8	14.7	7.5	30.6
219	219-W-1	363764	5316312	23	0	No	13.2286	11.2286	22.8	14.3	7.4	30.0
219	219-W-2	363723	5316284	21.6	50		15.2286	-15.9414	22.3	14.3	7.4	30.1
219	219-W-3	363682	5316255	21.6	100		-10.2985	17.7015	21.6	14.3	7.5	30.1
219	219-W-4	363559	5316169	20.2	250		49.0586	-22.9414	19.6	14.3		30.1
219	219-W-5	363354	5316025	18.2	500		48.0586	54.0586	20.8	14.4		30.1
266	266-N-1	369148	5311699	34.4	0	Yes	-48.404	-33.404	26.9	14.8	7.4	30.0
266	266-N-2	369174	5311742	34.3	50		-27.5911	-28.5911	30.9	14.7	7.5	30.1
266	266-N-3	369201	5311784	34.2	100		-35.5911	-31.5911	31.1	14.7	7.5	30.1
266	266-N-4	369281	5311911	33.4	250		-63.7782	NA	31.4	14.7		30.1
266	266-N-5	369414	5312122	34.7	500		-51.9692	-49.9521	33.3	14.7		29.1
266	266-NW-1	368936	5311409	36.8	0	Yes	-19.6356	-26.2785	1.5	14.5	7.6	22.8*
266	266-NW-2	368893	5311434	36.4	50		-38.6356	-43.6356	0.7	14.6	7.9	26.9*
266	266-NW-3	368850	5311459	36.1	100		-47.6356	-53.6356	31.0	14.7	7.6	29.2
266	266-NW-4	368721	5311537	35.2	250		-43.6356	-42.6356	32.2	14.7	7.6	29.4
266	266-NW-5	368506	5311665	34.4	500		-37.6356	-38.6356	33.8	14.8	7.6	29.6

266	266-NW-6	368291	5311793	34.1	750		-36.3327	-37.3327	35.5	14.8	7.6	29.7
266	266-NW-7	368076	5311922	34.5	1000		-29.7885	-30.9585	36.5	14.8	7.5	29.8
266	266-NW-8	367862	5312050	34.7	1250		-58.1456	-42.3156	36.0	14.8	7.6	29.8
266	266-NW-9	367636	5312193	35.9	1500		-26.6014	-27.9585	37.1	14.8	7.6	29.9
266	266-S-1	368525	5310117	25.9	0	Yes	-15.5369	-17.7069	14.5	14.4	7.6	28.6
266	266-S-2	368498	5310075	25.9	50		3.1231	-3.8769	10.4	14.4	7.5	29.5
266	266-S-3	368472	5310033	25.8	100		20.596	19.596	11.0	14.4	7.4	29.6
266	266-S-4	368392	5309906	25.8	250		-30.404	-28.404	17.7	14.2	7.4	29.3
266	266-S-5	368259	5309694	25.6	500		13.4089	6.596	23.0	14.4	7.4	29.7
266	266-SE-1	368800	5310500	28.8	0	Yes	-23.853	-30.9072	12.7	14.4	7.5	30.4
266	266-SE-2	368842	5310473	28.7	50		-18.023	-63.7372	23.4	14.4	7.5	29.4
266	266-SE-3	368884	5310445	28.6	100		-13.9072	-17.9072	20.8	14.4	7.7	30.5
266	266-SE-4	369009	5310363	28.3	250		7.6199	-0.9072	22.2	14.4	7.6	30.5
266	266-SE-5	369220	5310225	27.7	500		23.2628	-11.4514	20.6	14.4	7.5	30.4
267	267-N-1	366432	5314109	41	0	No	-36.3498	-49.3498	31.4	14.9	7.8	27.3*
267	267-N-2	366400	5314147	41	50		-28.7069	-36.7069	31.6	14.9	7.6	29.4
267	267-N-3	366368	5314186	40.8	100		-16.7069	-4.7069	28.9	14.9	7.6	29.8
267	267-N-4	366272	5314300	41	250		-28.234	-32.234	28.8	14.9	7.6	29.9
267	267-N-5	366111	5314492	41	500		-19.234	-27.234	26.5	14.8	7.5	30.0
267	267-NE-1	366919	5314490	42.5	0	No	3.8744	-0.4827	34.4	14.9	7.5	30.0
267	267-NE-2	366958	5314523	42	50		-17.4827	-25.0098	35.6	14.9	7.4	30.2
267	267-NE-3	366996	5314555	42	100		-19.0098	-26.0098	16.0	14.9	7.9	26.5*
267	267-NE-4	367111	5314652	40.5	250		-16.1798	-13.1798	34.1	14.8	7.6	30.1
267	267-NE-5	367302	5314814	38.6	500		-26.1798	-24.1798	34.8	14.8	7.6	30.2
267	267-SE-1	366726	5313446	39	0	No	-13.9385	-20.3498	29.6	14.8	7.7	28.5*
267	267-SE-2	366759	5313407	39	50		-29.4485	-20.3327	28.2	14.8	7.6	29.6
267	267-SE-3	366792	5313369	39.2	100		-15.9756	-34.3327	29.5	14.8	7.5	29.8
267	267-SE-4	366890	5313256	39.6	250		-35.1456	-36.1456	29.7	14.8	7.6	29.9
267	267-SE-5	367054	5313067	40	500		-27.6727	-41.0298	28.7	14.8	7.5	30.0
267	267-SE-6	367217	5312877	39.8	750		-10.9585	-6.9585	35.9	14.9	7.6	25.8*

267	267-SE-7	367382	5312687	38.6	1000		11.0415	5.3986	34.9	14.9	7.6	28.6*
267	267-SE-8	367545	5312498	37.1	1250		-2.6014	0.3986	32.2	14.8	7.5	29.2
267	267-SW-1	366550	5313455	37	0	No	-20.6527	-22.6527	23.6	14.8	7.5	30.0
267	267-SW-2	366509	5313425	36	50		-33.0098	-27.0098	23.5	14.7	7.5	30.0
267	267-SW-3	366469	5313395	35.5	100		28.9902	10.9902	23.4	14.7	7.5	30.1
267	267-SW-4	366348	5313306	33	250		-12.0098	-20.3669	22.3	14.5	7.5	30.0
267	267-SW-5	366147	5313157	28	500		-19.5369	-15.5369	19.5	14.5	7.3	29.9
External	1-North deep	362143	5322104	44.2	1736		-19.9243	-23.9243	44.5	14.9	7.7	29.7
External	9-Z7 Deep	364549	5318950	43.8	1529		4.0757	-18.0943	44.9	15.0	7.7	29.9
External	11-C9.2	361995	5316895	12.9	1193		144.0757	141.0757	20.2	14.3	7.4	28.1
External	16-C8.2	365528	5317239	44.7	1323		-29.9243	-32.9243	44.2	15.0	7.6	27.8
External	21-C7.2	364200	5313682	20	1708		9.6092	-46.7479	17.5	14.5		27.4
External	26-C5.2	368282	5315063	35	1415		-21.7479	-13.275	1.5	14.6		28.0
External	28-C4.2	365619	5311429	15	1037		33.0415	39.0415	21.1	14.2	7.5	28.3
External	37-Z9 West	367604	5309219	18.4	1267		35.895	23.895	1.0	14.3		27.4
External	39-C2.2	370237	5309976	30.54	1491		-51.683	7.7899	24.1	14.4	7.6	30.4
External	41-WHN	370850	5312281	17.5	1746		-2.595	-4.935	18.8	14.2		28.5
External	42-South 3,.4km	371977	5308865	22.8	3574		-8.7988	14.0312	18.0	14.2	7.5	30.0
External	43-South 5.4km	373483	5307571	24.3	5510		-77.4417	-84.7988	17.9	14.2	7.6	30.0
External	44-South 7.4km	374640	5305921	15.8	7427		-35.513	-39.4417	20.4	14.3	7.6	28.6
External	45-Gordon river	375845	5303913	7	9666		58.487	41.2012	54.5	16.2	7.7	22.3
External	49-North 1km	361017	5321054	14.8	1025		87.0757	95.0757	93.6	16.1	8.0	28.8
External	50-North 2km	360390	5321847	10.5	2028		24.0757	171.0757	93.3	16.0	8.0	28.3
External	51-Entrance	360199	5319942	30	1061		5.7928	23.0786	34.6	14.7	7.8	29.0
External	52-King river	361474	5325891	33.4	>2000		-44.0943	-38.0943	50.9	15.0	7.8	30.0

11.6 Historical surveys: Site information and environmental data used in this report

Details of sites sampled in baseline surveys in 2000 and 2012 including the various codes that have referred to the sites in different surveys, location and which of the comparisons with 2012 each site was included in.

Current lease name	Lease/Zone when sampled	Site ID when sampled	Current Site ID (if overlapping)	Easting	Northing	Type	Year sampled	Depth	Harbour-wide comparison	Lease comparison	Historical comparison with 2012
old 220	220	220-1				Lease	1999	11.4	yes		
old 220	220	220-2				35m	1999	11.4			
old 220	220	220-3				35m	1999	19			
old 220	220	220-4				Lease	1999	12.2			
old 220	220	220-5				35m	1999	13.4			
old 220	220	220-6				Lease	1999	9.7			
old 220	220	220-7				35m	1999	11			
old 220	220	220-8				Lease	1999	15.3			
old 220	220	220-9				35m	1999	20.5			
old 220	220	220-10				35m	1999	12			
old 220	220	220-11				Lease	1999	8.7	yes		
old 220	220	220-12				35m	1999	8.7			
old 220	220	220-13				Lease	1999	10			
old 220	220	220-14				35m	1999	10			
old 220	220	220-15				External	1999	20.7	yes		
old 220	220	220-16				External	1999	8.1	yes		
133	218	218-1				Lease	1999	38.5		yes	
133	218	218-2				35m	1999	40		yes	
133	218	218-3				35m	1999	27.8		yes	
133	218	218-4				Lease	1999	24			
133	218	218-5				35m	1999	24		yes	

133	218	218-6				Lease	1999	25.3	yes	yes	
133	218	218-7				35m	1999	24.9		yes	
133	218	218-8				Lease	1999	25.5		yes	
133	218	218-9				35m	1999	24.7		yes	
133	218	218-10				35m	1999	28.5		yes	
133	218	218-11				Lease	1999	36	yes	yes	
133	218	218-12				35m	1999	36.6		yes	
133	218	218-13				Lease	1999	37.6			
133	218	218-14				35m	1999	38.4		yes	
133	218	218-15				External	1999	42	yes		
133	218	218-16				External	1999	31	yes		
214	214	214-1				Lease	2000	36.7			
214	214	214-2				35m	2000	36.1			
214	214	214-3				35m	2000	45.4			
214	214	214-4				Lease	2000	40			
214	214	214-5				35m	2000	37	yes		
214	214	214-6				Lease	2000	21.9			
214	214	214-7				35m	2000	21.9			
214	214	214-8				Lease	2000	18			
214	214	214-9				35m	2000	18			
214	214	214-10				35m	2000	17.1			
214	214	214-11				Lease	2000	19.7			
214	214	214-12				35m	2000	19.2	yes		
214	214	214-13				Lease	2000	21.9			
214	214	214-14				35m	2000	21.6			
214	214	214-15				External	2000	16.5	yes		
214	214	214-16				External	2000	31	yes		
219	219	219-1				Lease	2000	39.7	yes	yes	
219	219	219-2				35m	2000	40.9		yes	
219	219	219-3				35m	2000	41.4		yes	

219	219	219-4				Lease	2000	39.2		yes	
219	219	219-5				35m	2000	39.2		yes	
219	219	219-6				Lease	2000	34		yes	
219	219	219-7				35m	2000	34.1			
219	219	219-8				Lease	2000	32.7		yes	
219	219	219-9				35m	2000	34.2		yes	
219	219	219-10				35m	2000	24.3	yes	yes	
219	219	219-11				Lease	2000	29.9		yes	
219	219	219-12				35m	2000	25.4			
219	219	219-13				Lease	2000	34.3		yes	
219	219	219-14				35m	2000	34.3		yes	
219	219	219-15				External	2000	29.8	yes		
219	219	219-16				External	2000	33.6	yes		
External	C	c1	45-Gordon river			External	2012	7			yes
External	C	c2	39-C2.2			External	2012	32			yes
External	C	c3	41-WHN			External	2012	18			yes
External	C	c4	28-C4.2			External	2012	15			yes
External	C	c5	26-C5.2			External	2012	40			yes
External	C	c6				External	2012	18			yes
External	C	c7	21-C7.2			External	2012	21			yes
External	C	c8	16-C8.2			External	2012	48			yes
External	C	c9	11-C9.2			External	2012	13			yes
External	C	c10				External	2012	44			yes
External	C	c11				External	2012	2			yes
External	C	c12	50-North 2km			External	2012	8			yes
External	C	c13	52-King river			External	2012	36			yes
External	C	c14				External	2012	3			yes
External	C	c15				External	2012	7			yes
External	C	c16				External	2012	3			yes
External	C (QA)	c2	39-C2.2			External	2012	32			yes

External	C (QA)	c3	41-WHN			External	2012	18			yes
External	C (QA)	c4	28-C4.2			External	2012	15			yes
External	C (QA)	c5	26-C5.2			External	2012	40			yes
External	C (QA)	c6				External	2012	18			yes
External	C (QA)	c7	21-C7.2			External	2012	21			yes
External	C (QA)	c8	16-C8.2			External	2012	48			yes
External	C (QA)	c9	11-C9.2			External	2012	13			yes
External	C (QA)	c10				External	2012	44			yes
External	C (QA)	c11				External	2012	2			yes
External	C (QA)	c12	50-North 2km			External	2012	8			yes
External	C (QA)	c16				External	2012	3			yes

11.7 References:

Akesson, Bertil, and Stanley a Rice. 1992. “Two New Dorvillea Speices (Polychaeta, Dorvilleidae) with Obligate Asexual Reproduction.” *Zoologica Scripta* 21 (4): 351–62.

Anderson, Marti J., and Trevor J. Willis. 2003. “Canonical Analysis of Principal Coordinates: A Useful Method of Constrained Ordination for Ecology.” *Ecology* 84 (2): 511–25. doi:10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2.

Bailey-Brock, J. H., B Paavo, B. M. Barrett, and J Dreyer. 2002. “Polychaetes Associated with a Tropical Ocean Outfall: Synthesis of a Biomonitoring Program off O’ahu, Hawai'i.” *Pacific Science* 56: 459–79. doi:10.1353/psc.2002.0042.

Beesley, P L, G J B Ross, and C J Glasby, eds. 2000. *Polychaetes & Allies: The Southern Synthesis. Vol 4A Polychaeta, Myzostomida, Pogonophora, Echiura, Spinuncula*. Fauna of Australia. Melbourne: CSIRO Publishing.

Belan, T. A. 2003. “Benthos Abundance Pattern and Species Composition in Conditions of Pollution in Amursky Bay (the Peter the Great Bay, the Sea of Japan).” *Marine Pollution Bulletin* 46: 1111–19. doi:10.1016/S0025-326X(03)00242-X.

Borja, A., J. Franco, and V. Perez. 2000. “A Marine Biotic Index to Establish the Ecological Quality of Soft-Bottom Benthos Within European Estuarine and Coastal Environments.” *Marine Pollution Bulletin* 40 (12): 1100–1114.

Borja, A. 2004. “The biotic indices and the Water Framework Directive: the required consensus in the new benthic monitoring tools.” *Marine Pollution Bulletin*, 48(3), 405-408.

Borja, A., and I. Muxika. 2005. “Guidelines for the Use of AMBI (AZTI’s Marine Biotic Index) in the Assessment of the Benthic Ecological Quality.” *Marine Pollution Bulletin* 50 (7): 787–89. doi:10.1016/j.marpolbul.2005.04.040.

Borja, A., & Dauer, D. M. 2008. “Assessing the environmental quality status in estuarine and coastal systems: comparing methodologies and indices.” *Ecological indicators*, 8(4), 331-337.

Bright, D. A. 2001. *RE-ANALYSIS OF RELATIONSHIPS BETWEEN SEDIMENT CHEMISTRY AND INFAUNAL MACROBENTHIC COMMUNITY RESPONSES, BASED ON BROOKS (2001) DATA*.

Brooks, Kenneth M., and C. V W Mahnken. 2003. *Interactions of Atlantic Salmon in the Pacific Northwest Environment. II. Organic Wastes*. *Fisheries Research*. Vol. 62. doi:10.1016/S0165-7836(03)00064-X.

Brooks, Kenneth M., Annette R. Stierns, C. V W Mahnken, and Dale B. Blackburn. 2003. “Chemical and Biological Remediation of the Benthos near Atlantic Salmon Farms.” *Aquaculture* 219 (1-4): 355–77. doi:10.1016/S0044-8486(02)00528-8.

Bustos-Baez, Silvana, and Chris Frid. 2003. “Using Indicator Species to Assess the State of Macrobenthic Communities.” *Hydrobiologia* 496: 299–309. doi:10.1023/A:1026169520547.

Chen, Y S, M C M Beveridge, T C Telfer, and W J Roy. 2003. “Nutrient Leaching and Settling Rate Characteristics of the Faeces of Atlantic Salmon (Salmo Salar L.) and the Implications for

Modelling of Solid Waste Dispersion." *Journal of Applied Ichthyology* 19 (2): 114–17. doi:10.1046/j.1439-0426.2003.00449.x.

Cheung, S. G., N. W Y Lam, R. S S Wu, and P. K S Shin. 2008. "Spatio-Temporal Changes of Marine Macrobenthic Community in Sub-Tropical Waters upon Recovery from Eutrophication. II. Life-History Traits and Feeding Guilds of Polychaete Community." *Marine Pollution Bulletin* 56: 297–307. doi:10.1016/j.marpolbul.2007.10.019.

Clarke, KR, and RN Gorley. 2006. "PRIMER v6: User Manual/Tutorial." Plymouth: PRIMER-E.

Crawford, C. M., I. M. Mitchell, and C. K. A. Macleod. 2001. "Video Assessment of Environmental Impacts of Salmon Farms." *ICES Journal of Marine Science* 58: 445–52. doi:10.1006/jmsc.2000.1042.

Crawford, Christine M, Catriona K.A. Macleod, and Iona M Mitchell. 2003. "Effects of Shellfish Farming on the Benthic Environment." *Aquaculture* 224 (1-4): 117–40. doi:10.1016/S0044-8486(03)00210-2.

Crawford, Christine, Catriona Macleod, and Iona Mitchell. 2002. *Technical Report Series Number 8: Evaluation of Techniques for Environmental Monitoring of Salmon Farms in Tasmania*.

Dahlgren, Thomas G., Bertil Akesson, Christoffer Schander, Kenneth M. Halanych, and Per Sundberg. 2001. "Molecular Phylogeny of the Model Annelid Ophryotrocha." *Biological Bulletin* 201 (2): 193–203.

Dauer, D. M., & Conner, W. G. (1980). "Effects of moderate sewage input on benthic polychaete populations." *Estuarine and Coastal Marine Science*, 10(3), 335-346.

Davydkova, I. L., N. P. Fadeeva, L. T. Kovekovdova, and V. I. Fadeev. 2005. "Heavy Metal Contents in Tissues of Dominant Species of the Benthos and in Bottom Sediments of Zolotoi Rog Bay, Sea of Japan." *Russian Journal of Marine Biology* 31 (3): 176–80. doi:10.1007/s11179-005-0064-z.

Dean, Harlan K. 2008. "The Use of Polychaetes as Indicator Species of Marine Pollution: A Review." *Revista de Biología Tropical* 56 (4): 11–38.

Decker, Carole, Marie Morineaux, Saskia Van Gaever, Jean Claude Caprais, Anna Lichtschlag, Olivier Gauthier, Ann C. Andersen, and Karine Olu. 2012. "Habitat Heterogeneity Influences Cold-Seep Macrofaunal Communities within and among Seeps along the Norwegian Margin. Part 1: Macrofaunal Community Structure." *Marine Ecology* 33: 205–30. doi:10.1111/j.1439-0485.2011.00503.x.

Department of Primary Industries Water and Environment. 2004. *A Review of the Tasmanian Finfish Farming Benthic Monitoring Program*. Hobart, Tasmania.

Di Bona, V., C. Minetti, V. Trotta, G. Sella, and M.C. Lorenzi. 2014. "A Trade-off between Traits That Contribute to Male and Female Function in Hermaphrodites." *Ethology Ecology & Evolution* 27 (1): 79–92. doi:10.1080/03949370.2014.880162.

Edgar, G. J., N. S. Barrett, and D. J. Graddon. 1999. *A Classification of Tasmanian Estuaries and Assessment of Their Conservation Significance Using Ecological and Physical Attributes , Population and Land Use*. Hobart, Tasmania.

Fauchald, Kristian, and Peter A Jumars. 1979. “The Diet of Worms: A Study of Polychaete Feeding Guilds.” *Oceanography and Marine Biology. An Annual Review* 17: 193–284.

Hall-Spencer, Jason, Nicola White, Ewan Gillespie, Katie Gillham, and Andy Foggo. 2006. “Impact of Fish Farms on Maerl Beds in Strongly Tidal Areas.” *Marine Ecology Progress Series* 326: 1–9. doi:10.3354/meps326001.

Hamoutene, D, G Mabrouk, L Sheppard, C Macsween, E Coughlan, and C Grant. 2013. “Validating the Use of Beggiatoa Sp . and Opportunistic Polychaete Worm Complex (OPC) as Indicators of Benthic Habitat Condition at Finfish Aquaculture Sites in Newfoundland.” *Canadian Technical Report of Fisheries and Aquatic Sciences* 3028.

Hamoutene, D., L. Sheppard, J. Mersereau, V. Oldford, T. Bungay, F. Salvo, S. Dufour, and G. Mabrouk. 2014. “Applicability of the Use of Visual Indicators [presence of Beggiatoa And/or Opportunistic Polychaete Complexes (OPC)] to Identify Benthic Changes due to Aquaculture on Various Substrates.” DFO Can. Sci. Advis. Sec. Res. Doc. 2014/063.

Hamoutene, Dounia, Flora Salvo, Terrence Bungay, Gehan Mabrouk, Cyr Couturier, Andry Ratsimandresy, and Suzanne C. Dufour. 2015. “Assessment of Finfish Aquaculture Effect on Newfoundland Epibenthic Communities through Video Monitoring.” *North American Journal of Aquaculture* 77 (2): 117–27. doi:10.1080/15222055.2014.976681.

Hargrave, B. T., Phillips, G. A., Doucette, L. I., White, M. J., Milligan, T. G., Wildish, D. J., & Cranston, R. E. 1997. “Assessing benthic impacts of organic enrichment from marine aquaculture. In The Interactions Between Sediments and Water (pp. 641-650).” Springer Netherlands.

Hargrave, B. T., M. Holmer, and C. P. Newcombe. 2008. “Towards a Classification of Organic Enrichment in Marine Sediments Based on Biogeochemical Indicators.” *Marine Pollution Bulletin* 56 (5): 810–24. doi:10.1016/j.marpolbul.2008.02.006.

Hargrave, B. T. 2010. “Empirical relationships describing benthic impacts of salmon aquaculture.” *Aquacult Environ Interact*, 1, 33-46.

Hartstein, Neil D., and Ashley A. Rowden. 2004. “Effect of Biodeposits from Mussel Culture on Macroinvertebrate Assemblages at Sites of Different Hydrodynamic Regime.” *Marine Environmental Research* 57: 339–57. doi:10.1016/j.marenvres.2003.11.003.

Henderson, A. R., & Ross, D. J. 1995. “Use of macrobenthic infaunal communities in the monitoring and control of the impact of marine cage fish farming.” *Aquaculture Research*, 26(9), 659-678.

Holmer, M., Wildish, D., & Hargrave, B. 2005. “Organic enrichment from marine finfish aquaculture and effects on sediment biogeochemical processes.” In Environmental effects of marine finfish aquaculture (pp. 181-206). Springer Berlin Heidelberg.

Holmer, M., Hansen, P. K., Karakassis, I., Borg, J. A., & Schembri, P. J. 2008. “Monitoring of environmental impacts of marine aquaculture (pp. 47-85).” Springer Netherlands.

Hortle, James. 2015. “Understanding the Drivers of *Schistomeringos Loveni* (Kinberg, 1865) Distribution in Macquarie Harbour and the Importance of Salmon Farm Enrichment.” University of Tasmania.

Hutchings, P. a., W. E. Kiene, R. B. Cunningham, and C. Donnelly. 1992. “Spatial and Temporal Patterns of Non-Colonial Boring Organisms (polychaetes, Sipunculans and Bivalve Molluscs) in Porites at Lizard Island, Great Barrier Reef.” *Coral Reefs* 11 (1992): 23–31. doi:10.1007/BF00291931.

Iwama, G. K. 1991. “Interactions between aquaculture and the environment.” *Critical Reviews in Environmental Science and Technology*, 21(2), 177-216.

Janowicz, M, and J Ross. 2001. “Monitoring for Benthic Impacts in the Southwest New Brunswick Salmon Aquaculture Industry.” *ICES Journal of Marine Science* 58: 453–59. doi:10.1006/jmsc.2000.1024.

Kalantzi, I., & Karakassis, I. 2006. “Benthic impacts of fish farming: meta-analysis of community and geochemical data.” *Marine Pollution Bulletin*, 52(5), 484-493.

Keeley, Nigel B., Barrie M. Forrest, and Catriona K. Macleod. 2013. “Novel Observations of Benthic Enrichment in Contrasting Flow Regimes with Implications for Marine Farm Monitoring and Management.” *Marine Pollution Bulletin* 66 (1-2). Elsevier Ltd: 105–16. doi:10.1016/j.marpolbul.2012.10.024.

———. 2015. “Benthic Recovery and Re-Impact Responses from Salmon Farm Enrichment: Implications for Farm Management.” *Aquaculture* 435. Elsevier B.V.: 412–23. doi:10.1016/j.aquaculture.2014.10.007.

Keeley, Nigel B., Catriona K. MacLeod, and Barrie M. Forrest. 2012. “Combining Best Professional Judgement and Quantile Regression Splines to Improve Characterisation of Macrofaunal Responses to Enrichment.” *Ecological Indicators* 12 (1). Elsevier Ltd: 154–66. doi:10.1016/j.ecolind.2011.03.022.

Keeley, N. B., Forrest, B. M., Crawford, C., & Macleod, C. K. 2012. “Exploiting salmon farm benthic enrichment gradients to evaluate the regional performance of biotic indices and environmental indicators.” *Ecological Indicators*, 23, 453-466.

Koçak, Cengiz, and Tuncer Katağan. 2005. “İzmir Körfezi (Ege Denizi, Türkiye)’nde Yer Alan Üç Balık Çiftliğinin Makrofauna Üzerine Etkilerinin Karşılaştırılması.” *Ege Journal of Fisheries & Aquatic Sciences* 22: 3–4.

Levin, L. A., 2000. “Polychaetes as environmental indicators: response to low oxygen and organic enrichment.” *Bull. Mar. Sci.* 67: 668–678.

Levin, Lisa A., Wiebke Ziebis, Guillermo F. Mendoza, Victoria J. Bertics, Tracy Washington, Jennifer Gonzalez, Andrew R. Thurber, Brigitte Ebbe, and Raymond W. Lee. 2013. “Ecological Release and Niche Partitioning under Stress: Lessons from Dorvilleid Polychaetes in Sulfidic Sediments at Methane Seeps.” *Deep-Sea Research Part II: Topical Studies in Oceanography* 92. Elsevier: 214–33. doi:10.1016/j.dsr2.2013.02.006.

Lorenzi, M. Cristina, and Gabriella Sella. 2013. “In between Breeding Systems: Neither Dioecy nor Androdioecy Explains Sexual Polymorphism in Functionally Dioecious Worms.” *Integrative and Comparative Biology* 53 (4): 689–700. doi:10.1093/icb/ict043.

Mabrouk, G, T Bungay, D Drover, and D Hamoutene. 2014. *Use of Remote Video Survey Methodology in Monitoring Benthic Impacts from Finfish Aquaculture on the South Coast of Newfoundland (Canada)*.

Macleod, Catriona, and Susan Forbes. 2004. *Guide to the Assessment of Sediment Condition at Marine Finfish Farms in Tasmania*.

Macleod, Catriona K., Christine M. Crawford, and Natalie a. Moltschaniwskyj. 2004. "Assessment of Long Term Change in Sediment Condition after Organic Enrichment: Defining Recovery." *Marine Pollution Bulletin* 49 (1-2): 79–88. doi:10.1016/j.marpolbul.2004.01.010.

Martinez-Garcia, Elena, Pablo Sanchez-Jerez, Felipe Aguado-Giménez, Pablo Ávila, Alejandro Guerrero, Jose Luis Sánchez-Lizaso, Victoria Fernandez-Gonzalez, et al. 2013. "A Meta-Analysis Approach to the Effects of Fish Farming on Soft Bottom Polychaeta Assemblages in Temperate Regions." *Marine Pollution Bulletin* 69: 165–71. doi:10.1016/j.marpolbul.2013.01.032.

Massamba-N'Siala, Gloria, Piero Calosi, David T. Bilton, Daniela Prevedelli, and Roberto Simonini. 2012. "Life-History and Thermal Tolerance Traits Display Different Thermal Plasticities and Relationships with Temperature in the Marine Polychaete Ophryotrocha Labronica La Greca and Bacci (Dorvilleidae)." *Journal of Experimental Marine Biology and Ecology* 438. Elsevier B.V.: 109–17. doi:10.1016/j.jembe.2012.09.008.

Meconcelli, S., M. C. Lorenzi, and G. Sella. 2014. "Labile Sex Expression and the Evolution of Dioecy in Ophryotrocha Polychaete Worms." *Evolutionary Biology* 42: 42–53. doi:10.1007/s11692-014-9297-0.

Mercier, Annie, Sandrine Baillon, and Jean François Hamel. 2014. "Life History and Seasonal Breeding of the Deep-Sea Annelid Ophryotrocha Sp. (Polychaeta: Dorvelleidae)." *Deep-Sea Research Part I: Oceanographic Research Papers* 91. Elsevier: 27–35. doi:10.1016/j.dsr.2014.05.007.

Middelburg, Jack J., and Joop Nieuwenhuize. 1998. "Carbon and Nitrogen Stable Isotopes in Suspended Matter and Sediments from the Schelde Estuary." *Marine Chemistry* 60 (3-4): 217–25. doi:10.1016/S0304-4203(97)00104-7.

O'Connor, N A, F Cannon, B Zampatti, P Cottingham, and M Reid. 1996. *Mount Lyell Remediation: A Pilot Biological Survey of Macquarie Habour, Western Tasmania*. Barton, ACT, Australia.

Oksanen, Jari, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, L. Gavin Simpson, Peter Solymos, M. Henry H. Stevens, and Helene Wagner. 2015. "Vegan: Community Ecology Package."

Paxton, Hannelore, and Adam Davey. 2010. "A New Species of Ophryotrocha (Annelida: Dorvilleidae) Associated with Fish Farming at Macquarie Harbour, Tasmania, Australia." *Zootaxa* 61 (May): 53–61.

Pearson, T. H. 1975. "The benthic ecology of Loch Linnhe and Loch Eil, a sea-loch system on the west coast of Scotland. IV. Changes in the benthic fauna attributable to organic enrichment." *Journal of Experimental Marine Biology and Ecology*, 20(1), 1-41.

Pearson, T.H., and Rutger Rosenberg. 1978. “Macrobenthic Succession in Relation to Organic Enrichment and Pollution of the Marine Environment.” *Oceanography and Marine Biology Annual Review* 16: 229–311. doi:10.1111/j.1540-5834.2012.00707.x.

Pfannestiel, H. D. 1976. “Ist Der Polychaet Ophryotrocha Labronica Ein Proterandrischer Hermaphrodit?” *Marine Biology* 38 (2): 169–78. doi:10.1007/BF00390770.

Pocklington, P., & Wells, P. G. 1992. “Polychaetes key taxa for marine environmental quality monitoring.” *Marine Pollution Bulletin*, 24(12), 593-598.

Pocklington, Patricia, and Kathryn A Coates. 2010. “Three New Species of Polychaetes (Annelida: Polychaeta) from Bermuda.” *Proceedings of the Biological Society of Washington*. doi:10.2988/09-24.1.

Prevedelli, Daniel, G. Massamba N’Siala, and R. Simonini. 2005. “The Seasonal Dynamics of Six Species of Dorvilleidae (Polychaeta) in the Harbour of La Spezia (Italy).” *Marine Ecology* 26: 286–93. doi:10.1111/j.1439-0485.2005.00067.x.

R Core Team. 2014. “R: A Language and Environment for Statistical Computing.” Vienna, Austria: R Foundation for Statistical Computing.

Ross, D. Jeff, Neil Hartstein, Catriona M. Macleod, M. Auluck, V. Lucieer, P. Cook, and J. Valentine. 2015. *Characterising Benthic Pelagic Interactions in Macquarie Harbour - Organic Matter Processing in Sediments and the Importance for Nutrient Dynamics*. FRDC Final.

Ross, Jeff, and Catriona Macleod. 2012. *Evaluation of Broadscale Environmental Monitoring Program (BEMP) Data from 2009-2012*.

Salvo, Flora, Dounia Hamoutene, and Suzanne C. Dufour. 2015. “Trophic Analyses of Opportunistic Polychaetes (Ophryotrocha Cyclops) at Salmonid Aquaculture Sites.” *Journal of the Marine Biological Association of the United Kingdom* 95 (4): 713–22. doi:10.1017/S0025315414002070.

Salvo, Flora, Helena Wiklund, Suzanne C Dufour, and Dounia Hamoutene. 2014. “A New Annelid Species from Whalebones in Greenland and Aquaculture Sites in Newfoundland: Ophryotrocha Cyclops, Sp. Nov. (Eunicida: Dorvilleidae)” 3887 (5).

Sasaki, Ryo, and Akio Oshino. 2004. “Environmental Conditions Relevant to Aggregative Distribution of Macrobenthos below Coho Salmon Culture Cage.” *Bulletin of Fisheries Research Agency* Supplement (1): 19–31.

Simboura, N, and a Zenetos. 2002. “Benthic Indicators to Use in Ecological Quality Classification of Mediterranean Soft Bottom Marine Ecosystems , Including a New Biotic Index.” *Mediterranean Marine Science* 3: 77–111.

Simonini, R., V. Grandi, Gloria Massamba-N’Siala, M. Pia Martino, Alberto Castelli, and D. Prevedelli. 2010. “Diversity, Habitat Affinities and Diet of Ophyrotrocha Species (Polychaeta, Dorvilleidae) Living in Mediterranean Harbour Habitats.” *Vie et Milieu - Life and Environment* 60 (1): 27–38.

Taboada, Sergi, Maria Bas, and Conxita Avila. 2015. “A New Parougia Species (Annelida, Dorvilleidae) Associated with Eutrophic Marine Habitats in Antarctica.” *Polar Biology* 38 (4): 517–27.

Taboada, Sergi, Helena Wiklund, Adrian G. Glover, Thomas G. Dahlgren, Javier Cristobo, and Conxita Avila. 2013. "Two New Antarctic Ophryotrocha (Annelida: Dorvilleidae) Described from Shallow-Water Whale Bones." *Polar Biology* 36: 1031–45. doi:10.1007/s00300-013-1326-4.

Talman, Sonia, Nicholas O'Connor, Brenton Zampatti, and Frances Cannan. 1996. *Mount Lyell Remediation: Monitoring of Benthic Invertebrates in Macquaire Harbour, Western Tasmania*. Barton, ACT, Australia.

Thornhill, Daniel J., Torsten H. Struck, Brigitte Ebbe, Raymond W. Lee, Guillermo F. Mendoza, Lisa A. Levin, and Kenneth M. Halanych. 2012. "Adaptive Radiation in Extremophilic Dorvilleidae (Annelida): Diversification of a Single Colonizer or Multiple Independent Lineages?" *Ecology and Evolution* 2: 1958–70. doi:10.1002/ece3.314.

Tomassetti, P, and S Porrello. 2005. "Polychaetes as Indicators of Marine Fish Farm Organic Enrichment." *Aquaculture International* 13: 109–28. doi:10.1007/s10499-004-9026-2.

Wang, X, K Andresen, a Handå, B Jensen, Ki Reitan, and Y Olsen. 2013. "Chemical Composition and Release Rate of Waste Discharge from an Atlantic Salmon Farm with an Evaluation of IMTA Feasibility." *Aquaculture Environment Interactions* 4 (2): 147–62. doi:10.3354/aei00079.

Weston, D. P. 1990. "Quantitative examination of macrobenthic community changes along an organic enrichment gradient." *Marine Ecology Progress Series* 61(3), 233-244.

Wiklund, Helena. 2009. "Evolution of Annelid Diversity at Whale-Falls and Other Marine Ephemeral Habitats."

Wiklund, Helena, Iris V. Altamira, Adrian G. Glover, Craig R. Smith, Amy R. Baco, and Thomas G. Dahlgren. 2012. "Systematics and Biodiversity of Ophryotrocha (Annelida, Dorvilleidae) with Descriptions of Six New Species from Deep-Sea Whale-Fall and Wood-Fall Habitats in the North-East Pacific." *Systematics and Biodiversity*. doi:10.1080/14772000.2012.693970.

Wiklund, Helena, Adrian G. Glover, and Thomas G. Dahlgren. 2009. "Three New Species of Ophryotrocha (Annelida: Dorvilleidae) from a Whale-Fall in the North-East Atlantic." *Zootaxa* 56: 43–56.

Wildish, D. 2001. "Cost-Effective Monitoring of Organic Enrichment Resulting from Salmon Mariculture." *ICES Journal of Marine Science* 58 (2): 469–76. doi:10.1006/jmsc.2000.1030.

Wilson, A., Magill, S. and Black, K.D. 2009. "Review of environmental impact assessment and monitoring in salmon aquaculture. In FAO. Environmental impact assessment and monitoring in aquaculture." F*AO Fisheries and Aquaculture Technical Paper*. No. 527. Rome, FAO. pp. 455–535

Yokoyama, Hisashi, Katsuyuki Abo, and Yuka Ishihi. 2006. "Quantifying Aquaculture-Derived Organic Matter in the Sediment in and around a Coastal Fish Farm Using Stable Carbon and Nitrogen Isotope Ratios." *Aquaculture* 254 (1-4): 411–25. doi:10.1016/j.aquaculture.2005.10.024.