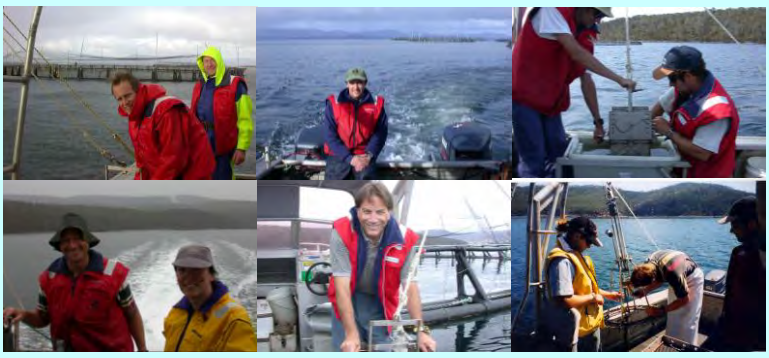
Final Report

DEVELOPMENT OF NOVEL METHODS FOR THE ASSESSMENT OF SEDIMENT CONDITION AND DETERMINATION OF MANAGEMENT PROTOCOLS FOR SUSTAINABLE FINFISH CAGE AQUACULTURE OPERATIONS

Catriona Macleod, Andrew Bissett, Chris Burke, Susan Forbes, Danny Holdsworth, Peter Nichols, Andrew Revill and John Volkman

August 2004

Aquafin CRC Project 4.1
(FRDC Project No. 2000/164)



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1. NON TECHNICAL SUMMARY

CRC Project 4.1 Development of novel methods for the assessment of sediment condition and determination of management protocols for sustainable finfish cage aquaculture operations.

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OBJECTIVES:
1. To assess the potential for progressive degeneration of sediments in association with cage aquaculture operations.
2. To adapt and develop novel combinations of monitoring techniques (identified by TAFI and CSIRO) to facilitate evaluation of sediment degradation associated with ongoing marine cage aquaculture operations.
3. To incorporate these techniques into farm management protocols as tools for the evaluation and management of sediment condition in order to promote sustainable aquaculture production.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

- This research showed that although finfish aquaculture significantly affected sediments, under certain production scenarios (dependent on stocking level and baseline environmental condition) the sediments recovered after 3 months fallowing to a degree that enabled cages to be restocked. However, under intensive production regimes, the present results indicated that there was potential for progressive sediment degeneration, consequently environmental status should be considered as part of production planning.
- A clear relationship between farm management practices and level of impact was established and a series of 9 distinct stages of sediment condition were characterised. Several field based techniques have been recommended which will enable farmers to easily classify sediment condition. With this information farmers will be able to gauge the environmental status of the sediments within their lease and make appropriate management decisions.
- The value of these research findings has been acknowledged by stakeholders (industry and government) through their support for the development of a field-guide, data analysis package and associated training workshops; ensuring that the research outputs are incorporated into management practices as quickly as possible.

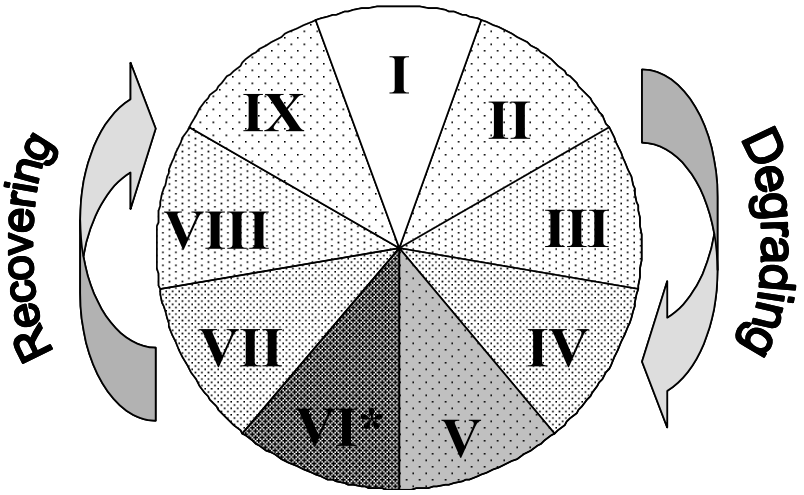
This project constituted a 3-year multidisciplinary study of the changes in sediment condition associated with commercial finfish culture and was undertaken at the instigation of and with the collaboration of the Tasmanian Salmon Aquaculture industry, the Tasmanian Department of Primary Industry, Water and Environment (DPIWE) and the Fisheries Research and Development Corporation (FRDC). It was recognised that if the industry was to be economically sustainable it needed to be environmentally sustainable, and that in order to do this it needed to have a clearer understanding of the relationship between farming practices and environmental conditions. It is well recognised that organic enrichment of the sediments is one of the most significant impacts from caged fish farming. However, the effect that differing farming practices, such as rotational farming/fallowing, have on the level of impact, or the effect that different background environmental conditions may have on overall impact was less clearly understood. This project was initiated to assess the rate of recovery associated with fallowing practices, to determine if current farming practices were sustainable and to develop novel approaches for farm-based monitoring of environmental condition.

Changes in the geochemical processes and benthic infaunal and microbial ecology were studied over two complete farming cycles at two farm sites in Tasmania (Creeses Mistake on the Tasman Peninsula and Stringers Cove on the Huon Estuary) with very different background environmental conditions. The results indicate that at both sites there were clear spatial and temporal impact gradients. Initially, unimpacted conditions at each of the sites were biologically and chemically distinct, but as organic enrichment of the sediment increased the chemistry and ecology of the two systems became more similar. Although there was significant recovery at the end of the study, neither site recovered completely to pre-farming/reference conditions (i.e. some measures always differed). However, sediment recovery was likely to be sufficient to enable re-use of the site for fin-fish aquaculture. Although assessment of the regenerative capacity of sediments associated with intensive cage aquaculture indicated the potential for progressive deterioration, unfortunately the duration of this project was insufficient to establish conclusively whether progressive deterioration does in fact occur.

The rate of recovery differed both between sites and with differing stocking intensities, but clear impact levels were discernible and comparable between the sites. Several of the methods examined (eg. lipid biomarkers) showed a rapid and sensitive response to changes in organic enrichment, but benthic infaunal evaluation proved to be the most useful indicator of both degradation and recovery. We were able to characterise the degree of impact on the sediment condition at each of the study sites according to the benthic infaunal community changes. Nine impact stages were defined, encompassing both degradation and, importantly, the recovery phases. Potential monitoring techniques and differing farming intensities were subsequently related to this scale.

A range of techniques was evaluated to assess their suitability for industry-based management of sediment condition. Benthic infaunal evaluation was used as the basis for judging the usefulness of other approaches. Several established environmental monitoring approaches were found to be poor indicators of sediment recovery, although useful measures of sediment degradation. However, other techniques such as video assessment were found to be very reliable. Semi-quantitative video assessment was determined to be the most effective approach for simple farm-based assessment

of sediment condition. This approach was capable of discerning the broadest range of impact stages and was particularly useful in evaluating recovery over time. It is simple, rapid and cost-effective and can easily be undertaken by fish farmers, thus providing an immediate evaluation of sediment condition. When linked with farm data, the condition of a lease can be reviewed in a management context and informed management actions undertaken. Furthermore, when video footage is assessed with farm data it is possible to categorise the sediment condition to a particular stage and also predict the likely future classification on the basis of the proposed farming schedule.



* Indicates conditions not observed in this study
Suggest stage IX is sufficiently recovered for restocking

STAGE – Category		STAGE – Description	
I	- Unimpacted	I	- No evidence of farm impact
II	- Minor Effects	II	- Slight infaunal & community change observed
III	- Moderate Effects	III	- Clear change in infauna & chemistry
IV	- Major Effects (1)	IV	- Major change in infauna & chemistry
V	- Major Effects (2)	V	- Bacterial mats evident, outgassing on disturbance
VI*	- Severe Effects	VI*	- Anoxic/ abiotic, spontaneous outgassing
VII	- Major Effects	VII	- Monospecific fauna, major chemistry effects
VIII	- Moderate Effects	VIII	- Fauna recovering, chemistry still clearly effected
IX	- Minor Effects	IX	- Largely recovered, although slight faunal/ chemical effects still apparent

Figure 1.1. Impact and recovery stages.

The pattern of response in microbial biomass was very similar to that of the infauna, but infaunal assessment was more useful for farm management purposes. Some parameters (i.e. evaluation of the oxic zone and pore water ammonia), although not very sensitive to fluctuations in production levels, demonstrated that once farming commenced an impact was always detectable irrespective of fallowing protocols. Geochemical biomarkers (e.g. lipids and sterols) were identified for key components of the benthic infauna found in both impacted and unimpacted conditions. They were shown to be powerful tools for elucidating the biogeochemical processes occurring in the sediments and for identifying sources of organic matter, but it is unlikely that they would be useful for farm-based monitoring because the techniques for identifying and quantifying biomarkers are complex and the comparative costs are high.

Relating the results from the scientific studies to farm production data has shown that changes in stocking levels (feed input and fish biomass) and/or duration of the fallow period can have a major effect on sediment recovery. Lengthening the fallow period led to increased recovery at one farm (Creeses Mistake), and reduction in stocking density also resulted in marked improvements in the rate and extent of recovery. These environmental benefits are both measurable and predictable, and therefore production intensity can be established as a variable (incorporating the rate and stage of recovery) in ongoing farm management protocols.

The study also established that farm operations produce a generalised residual impact throughout the farm lease. Consequently, any evaluation of recovery at "fallowed" positions within the lease should take into account the likely effects of adjacent operational cages. It is not appropriate to determine effectiveness of fallowing just by the time that an area has been without a cage. Our results demonstrate that reliable information on sediment condition, used in conjunction with feed rate and stocking density, can assist farmers to manage their lease areas to obtain the best economic and environmental outcomes.

This study has also greatly increased our understanding of the processes involved in organic enrichment, degradation and recovery as well as the effect that different background environmental conditions can have on the recovery process. At Stringers Cove, although the amount of organic carbon added to the sediments over the stocked period increased markedly, there was no change in the proportion of organic carbon in the sediments. We propose that the significantly increased macrofaunal and microbial biomass in the sediments at this time was able to keep pace with carbon inputs, remineralising and assimilating this labile carbon. In contrast, at Creeses Mistake the increase in the faunal and microbial communities was not as great and labile organic carbon in the sediments did increase over the stocking period, suggesting that Creeses Mistake is not as well adapted for increased carbon loading and has a greater potential to be overwhelmed. "A priori" it was anticipated that the more exposed site (Creeses Mistake) would be the more resilient to impact, but these results and the changes in the benthic community structure suggest that this may not in fact be the case, and that because of a natural pre-disposition to organic enrichment at the more sheltered site (Stringers Cove) the benthic infauna more effectively cope with higher levels of organic carbon.

The findings identified and techniques developed in this study can be applied to other areas of research and environmental assessment. Our simple video and photographic assessment approaches would be applicable to other sources of organic enrichment or other sources of contamination. Several local and state government organisations have already expressed interest in these project outcomes. Stakeholders have been regularly updated on progress and results throughout this study and they have shown their support of the findings by their willingness to respond to the outcomes and support the field guide extension project. Both industry and government representatives have indicated their wish to be involved in training workshops, to ensure that the project recommendations are rapidly incorporated into management operations.

KEYWORDS: environmental impact, salmonid aquaculture, recovery, monitoring, benthic fauna, microbiota, geochemistry, sediments.

2. ACKNOWLEDGEMENTS:

The authors would like to thank the funding agencies, the Co-operative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC) and the Fisheries Research and Development Corporation (FRDC), for supporting this research. We would also like to acknowledge the support of the Tasmanian Aquaculture Industry both collectively through the Tasmanian Salmon Growers Association (TSGA), as well as the individual support of Tassal Pty Ltd. In particular we would like to recognise the help of Mick Hortle, Dan Fisk, Sean Tiedemann and Matt Finn in providing farm support and production information.

Several other people have made important contributions to this research:
Bob Connell was an integral part of the field team and made a significant contribution to the field sampling programme;
Perran Cook was responsible for the sediment respiration studies at Stringers Cove (Section 5.4.8);
John Gibson made a significant contribution to the polar lipid fatty acids and ether lipids study, with Mark Rayner and Stephane Armand also providing laboratory assistance (Section 5.5.2);
Iona Mitchell, Stewart Dickson, Regina Magerowski, Dirk Welsford, Julian Harrington and Damian Trinder all provided help at some time with field sampling or laboratory processing of samples;
Dean Thomson and Ros Watson carried out many of the nutrient analyses.

Finally we would like to acknowledge the support of the two collaborative research institutions, the University of Tasmania and CSIRO Marine Research.

3. INTRODUCTION

3.1 Background to study

This study was undertaken at the instigation and with the collaboration of the Tasmanian Salmon Aquaculture industry, the Department of Primary Industry, Water and Environment (DPIWE) and the Fisheries Research and Development Corporation (FRDC). The salmon aquaculture industry recognised that to be economically sustainable it needs to be environmentally sustainable, and that to do this it needed to have a clearer understanding of the relationship between farming practices and environmental conditions. It is well recognised that one of the most significant impacts from caged fish farming is the organic enrichment of the sediments (Iwama, 1991, Black et al., 2001). What is less clearly understood is the effect that differing farming practices, such as rotational farming/fallowing, have on the level of impact or the effect that different background environmental conditions may have on overall impact. Consequently, this project was initiated to assess the rate of recovery associated with fallowing practices, to determine if current farming practices were sustainable and to develop novel approaches for farm based monitoring of environmental condition.

3.2 Previous research on sediment recovery

There is a considerable body of research examining the impacts of organic enrichment but much of this has focussed on the degradation response rather than recovery. One of the most significant studies to date is that of Pearson & Rosenberg (1978). Pearson & Rosenberg identified a series of macrobenthic successional stages in relation to an increasing organic enrichment gradient which have subsequently been supported by many others (eg. Brown et al., 1987; Ritz et al., 1989; Weston, 1990; Holmer & Kristensen, 1992; Findlay et al.,1995; Cheshire et al., 1996; Hargrave, et al., 1997, Wildish et al., 2001, Macleod et al. 2002, Brooks et al., 2003). Several of these have compared the infaunal categories defined by Pearson and Rosenberg to other physical-chemical and biological parameters (Brown et al., 1987, Weston, 1990, Holmer & Kristensen, 1992, Findlay et al., 1995, Cheshire et al., 1996, Hargrave et al. 1997, Wildish et al., 2001, Macleod et al. 2002) and have suggested a direct relationship between the chemical status of the sediment and the infaunal community structure. However, more recent research in Tasmania has suggested that the correlation levels indicated in the northern hemisphere studies may not be applicable to temperate Australian waters (Macleod, 2000; Macleod et al., 2002, Macleod et al., in press). We examined a broad range of physical, chemical and biological parameters, comparing the levels of sediment and benthic recovery to determine the most accurate but cost effective approaches for farm based monitoring in Tasmania.

Relatively few studies have been undertaken to evaluate recovery of marine finfish farms, but results suggest that recovery is relatively rapid (6-12 months) compared with other sources of organic pollution (Johannessen et al., 1994, Brooks et al, 2003). However, in any comparison it is important that the context in which recovery is judged is equivalent. The rate of recovery will be strongly influenced by the prevailing environmental conditions. Many environmental studies have shown that

site characteristics, such as water depth, particle size, current velocities and tidal effects play an important role in determining the rate and extent of both degradation and recovery of sediments. In the aquaculture context, farm management criteria (i.e. cage size, stocking density/biomass, feed input and timing/duration of stocked/fallow period) will also be critical factors in determining impact/recovery level. What is measured is also extremely important in obtaining a realistic evaluation of recovery. Some measures are much more sensitive to sediment impact/recovery than others. For example, at fish farms in British Columbia, Canada, physical-chemical parameters at cage sites returned to reference conditions within a few weeks, whilst the macrofauna took more than 6 months to recover (Brooks et al., 2003). In Tasmania, the physical and chemical properties of sediments showed that fish farm-derived organic matter levels (identified through fatty acid profiles) remained elevated at cage sites 12 months after the cages were emptied, despite redox potential indicating a return to reference conditions (McGhie et al., 2000). In another Tasmanian study, sulphide concentration returned to reference conditions within 6 months of the lease being vacated whilst the infaunal community structure was still significantly different after 36 months (Macleod et al., in press). In the current study, recovery was evaluated for several key criteria (physical, chemical and biological) at two farm sites with very different environmental conditions and the results compared to determine the effect of location on overall recovery performance.

It is well recognised that benthic infaunal evaluation is amongst the most sensitive of approaches for evaluation of sediment condition. Consequently full community assessment will be the benchmark against which all other evaluations of recovery are judged. However, it is also recognised that for sustainable management of sediments within marine farm leases it may not be necessary for the system to recover to pristine condition. A further aim was to evaluate the level of recovery necessary for sustainable operation, and which does not result in progressive deterioration of the sediments.

3.3 Need for Research

3.3.1 Environmental Significance

Marine finfish cage culture can result in organic enrichment of the underlying sediments as a result of the deposition of waste food and faeces. Typically, flora and fauna of impacted sediments adapt to utilise this new nutrient source, resulting in changes in benthic community structure. However, if the sediment's capacity to assimilate organic inputs is exceeded, the sediment may become anoxic, the sediment biogeochemistry will be altered towards a system dominated by anaerobic microbiota, and toxic degradation products can be released into the environment affecting farm production and aquatic ecosystem health. To overcome this, it is usual for farmers to leave areas of seabed free from farming activities for a period of time to enable them to recover, a process commonly referred to as fallowing. However, the level to which this recovery occurs is currently unknown and thus it is important from the perspective of both farm management and ecosystem protection to better understand the rate of change of sediment condition. To date, studies of the environmental impacts of cage aquaculture have not addressed whether progressive sediment deterioration occurs, whether any management practices or other factors exacerbate sediment degradation or whether the aquaculture industry can monitor and mitigate

such effects. In particular, although there have been many studies of the organic enrichment of sediments under cages, (e.g. Brown et al., 1987; Ritz et al., 1989; Weston, 1990; Holmer & Kristensen, 1992; Findlay et al., 1995; Cheshire et al., 1996; Hargrave, et al., 1997), there is much less literature pertaining to the usefullness of fallowing. Generally, the focus has been on changes over relatively broad spatial and/or temporal scales and has attempted to define the distinction between reference sites exhibiting background environmental conditions and farm conditions (e.g. Gowen et al., 1988; Lumb, 1989; Ritz et al., 1989; Johannessen, 1994; Lu & Wu, 1998). As a consequence, as both Braaten (1991) and Cheshire et al. (1996) point out, there is a lack of information on the changes in the sediment conditions associated with fallowing of fish cages.

The development of guidelines for fallowing of sediments beneath marine fish cages requires further information on the changing sedimentary conditions over smaller spatial and temporal scales than is currently available. It also requires that environmental parameters be assessed in relation to conditions prior to each stocking as well as at reference locations. The literature regarding the length of time required for complete sediment recovery is inconclusive. Lumb (1989) and Johannessen (1994) both found significant residual effects 12 months after cessation of farming, whereas Ritz et al (1989) and Wu & Lu (1998) observed what appeared to be more rapid recovery rates. However, all studies considered recovery to be a return to control conditions, which are representative of areas unaffected by farming. With regard to farm sustainability, it may be more appropriate to determine whether sediments have recovered sufficiently such that they can withstand further inputs without undergoing any cumulative deterioration. If fallowing protocols fail to return sediments to this condition, then there is a danger of long-term additive deterioration of the sediment, which may eventually lead to sediment degeneration to such an extent that farming operations become unviable and the ecological function is significantly impaired.

3.3.2 Economic importance

Atlantic salmon (*Salmo salar*) culture is an economically important industry in Tasmania. Figures for 2001/2 indicated that salmon aquaculture was worth more than $110 million annually from a total production of approximately 14,000 tonnes (Love and Langenkamp, 2003).

Both State and Commonwealth governments in Australia strongly appreciate the need for ecologically sustainable development. A guiding principle of the Coastal Policy initiated by the Commonwealth government and developed by the States, is that the coast shall be used and developed in a sustainable manner. The Tasmanian government recognises the economic and social benefits associated with a productive aquaculture industry and is highly supportive of its further development. In 1997, in the Tasmanian Premier's direction statement, sustainable aquaculture development was listed as one of the highest priorities. To this end the Tasmanian State government is actively engaged in facilitating development by ensuring that sufficient areas of state water are made available to accommodate industry expansion.

Similarly, the finfish aquaculture community is acutely aware of its reliance on the environment and is keen to ensure that future development is sustainable. Salmon farming industry representatives have recently identified an urgent need for clear information on the effectiveness of fallowing as a means of rehabilitating sediments.

This information is vital for the optimal management of lease areas and to ensure that production is sustainable.

3.4 Aims & Scope of the study

This project was developed as an integrated multidisciplinary investigation of the changes in sedimentary processes associated with current salmon farming practices in Tasmania. The project involved assessment of the chemical, microbiological and biological responses of sediments under Atlantic salmon (*Salmo salar*) cages at two locations in southern Tasmania. One site was relatively exposed and subject to greater water flows and wave action than the other, which was more sheltered.

The original project had three principal objectives:

1. To assess the potential for progressive degeneration of sediments in association with cage aquaculture operations.
2. To adapt and develop novel combinations of monitoring techniques (identified by TAFI and CSIRO) to facilitate evaluation of sediment degradation associated with ongoing marine cage aquaculture operations.
3. To incorporate these techniques into farm management protocols as tools for the evaluation and management of sediment condition in order to maximise sustainable aquaculture production.

The project was amended in January 2004 to include 2 additional objectives:

4. To develop a training package (field guide and multimedia cd) for the aquaculture industry to simply explain the techniques proposed in CRC project 4.1.
5. To conduct a series of workshops in Tasmania to instruct farm personnel in the field sampling requirements, analysis and interpretation of the techniques recommended in CRC project 4.1 and in the field guide.

3.5 Study Context & Design

3.5.1 Selection of Study Sites

3.5.1.1 Site locations

Salmon farming in Tasmania is largely focused in the south-east, with farms occupying the areas of Port Esperance and the Huon River, the D'Entrecasteaux Channel and the Tasman Peninsula (highlighted in Fig. 3.5.1.1.1). Two of these regions were selected for this study, Port Esperance and the Tasman Peninsula. In selecting sites, it was planned that the outcomes of the study would be applicable to marine finfish farms state wide. To do this, it was essential that different background environmental conditions were examined.

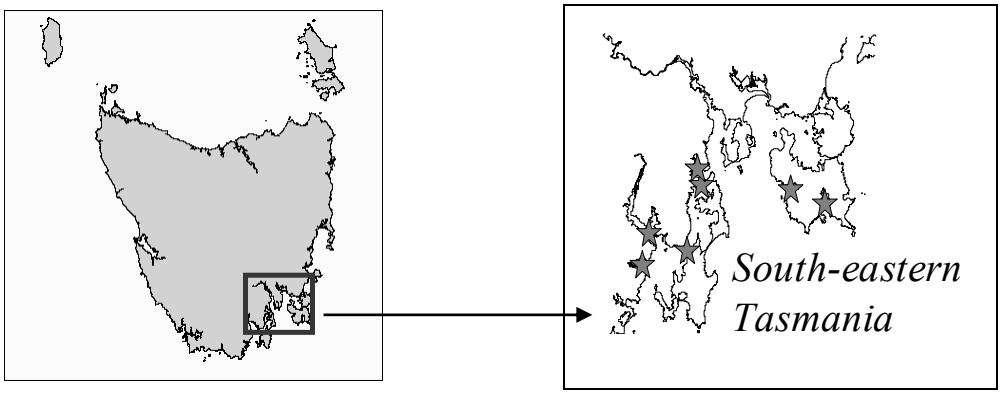


Fig. 3.5.1.1.1. Map of Tasmania with South-eastern Tasmania enlarged. The 3 main farming regions are indicated by stars.

In each of the two selected regions a lease was chosen that would adhere to a specified production regime for two whole cycles. These were Creeses Mistake at the Tasman Peninsula, and Stringers Cove at Port Esperance. The Creeses Mistake lease is located off the northern shore of Wedge Bay, an open bay adjacent to Storm Bay (Fig. 3.5.1.1.2a). The Stringers Cove lease is located approximately 150m off the south-west shore of Port Esperance (Fig. 3.5.1.1.2b).

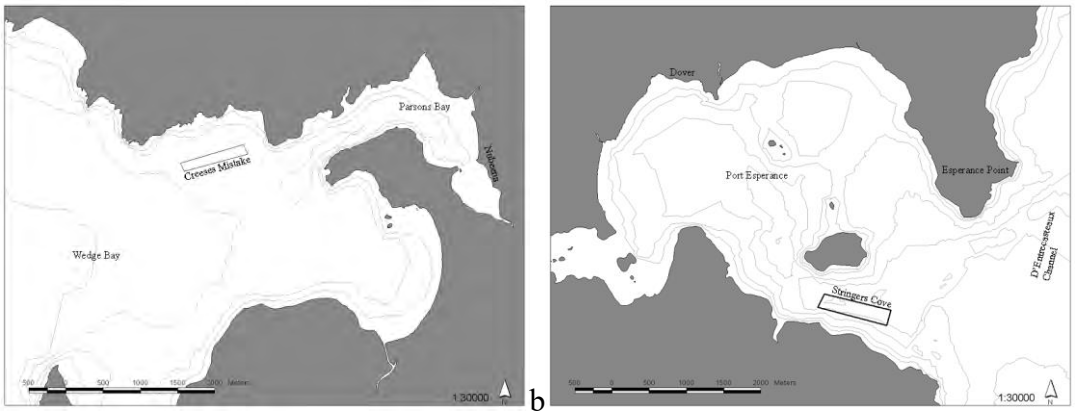


Fig. 3.5.1.1.2. Selected leases in South-eastern Tasmania a) Creeses Mistake and b) Stringers Cove.

3.5.1.2 Natural environmental conditions at the study sites

The background environmental conditions at each site differed considerably. The depth range of sites at Creeses Mistake is 15-20m, compared to 35-40m at Stringers Cove. Creeses Mistake is entirely a marine environment, exposed to westerly and southwesterly winds. The site is also strongly influenced by wave action and ocean swell. Sediments are predominantly fine sands, with a low percentage of silt-clays. Stringers Cove, although essentially marine, is subject to freshwater runoff during heavy rain periods. The lease is exposed to the northwest, but is protected from most wave action and ocean swells. Sediments are predominantly silt-clays.

3.5.1.3 Site history

The original Creeses Mistake lease was granted in 1993. The lease was 15ha, and was stocked with salmonids in May 1995. In the period between 1995 and 2000, the lease was stocked for at least 8 months each year, with fallow periods (whole lease) of between 1 and 4 months. In 1999 the farm lease area was extended to 30 ha, with annual production increasing from 394 tonnes in 1998 to 1336 tonnes in 2001.

Two cages were randomly selected from within the Creeses Mistake lease (Fig. 3.5.1.3.1). Position 5 was first stocked in 1997 for five months, then for three months in 1999. In 2000 position 5 was only stocked towards the end of the year with a break of two months before being restocked in the current study. Position 8 was also first stocked late in 1997 but was not used again until 2000 when it was occupied from July until December, with a fallow period of two and a half months before being restocked as part of this study.

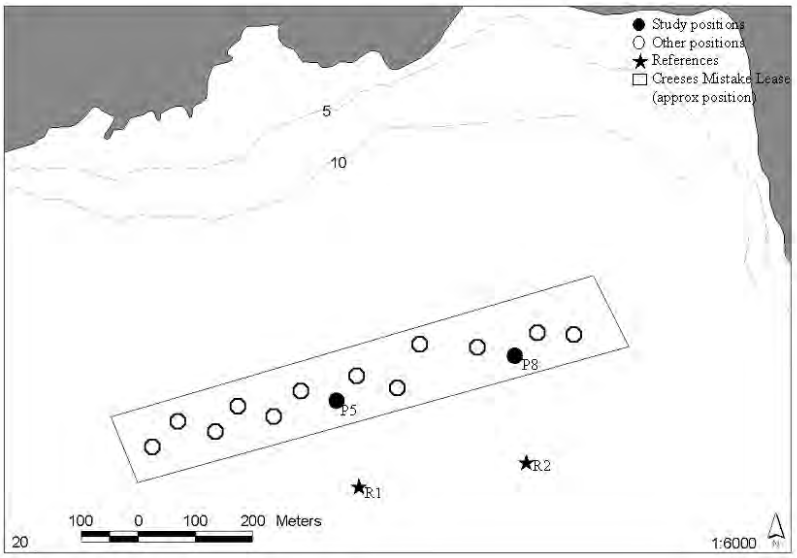


Fig. 3.5.1.3.1. Lease location and pen positions at Creeses Mistake, Tasman Peninsula (5m contour then 10m contour intervals).

The original Stringers Cove lease was first stocked in 1989 with an area of approximately 5 ha. Between 1989 and 1999 there were several expansions to this lease and the position and stocking levels varied markedly. In 1999 the lease was extended to 24.8 ha, although production (tonnes) did not markedly increase from the previous year. Four study cages were originally identified at this site (positions 1, 2, 1A and 2A), all were in the area granted as a lease extension in 1999 and had not previously been farmed (Fig. 3.5.1.3.2). Shortly after the start of the study it was suggested that impact and recovery rates might differ between sediment new to farming and that which had previously been farmed. Consequently, two previously farmed pen positions were added (3A and 4A) to the study. These two positions were located within the original lease area, and have therefore been subject to ongoing farming of varying intensities since 1989 (Fig. 3.5.1.3.2).

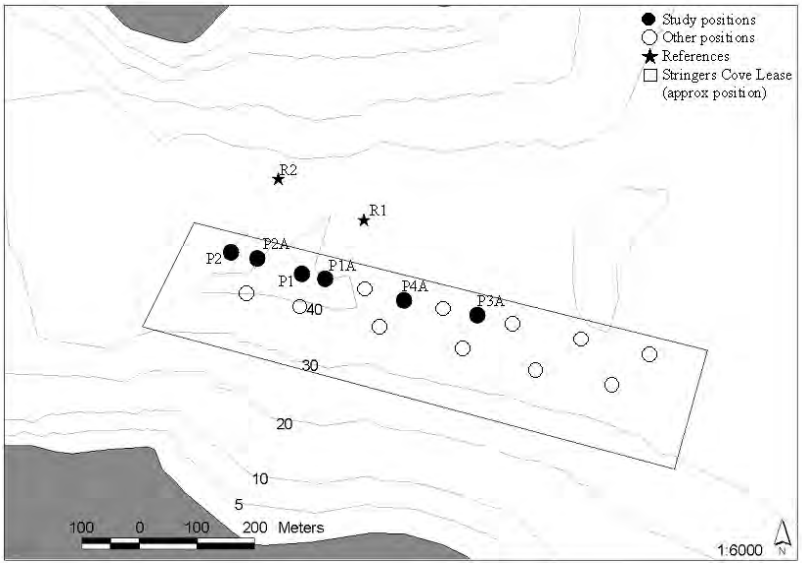


Fig. 3.5.1.3.2. Lease location and pen positions at Stringers Cove, Port Esperance (5m contour then 10m contour intervals).

3.5.2 Basic Sampling DesignSpatial Analysis

At Creeses Mistake two cages (cages 5 and 8) were randomly selected within the lease (Fig. 3.5.1.3.1), with two references located 150m away from the edge of each study cage. The references were within the same depth contour as the cages, and had the same particle size distribution. It was proposed that the cages would be sampled at regular intervals over two farm production cycles. Each production cycle involved a 9 month stocking period and three month fallow period, with the second production cycle starting immediately after the completion of the first cycle's fallow period. Unfortunately, the farm was unable to adhere to this schedule at one of the study cages, and at position 8 the first fallow period continued for 4.5 months. This position then returned to the proposed production scenario and was stocked for 9 months and fallowed for 3 months. This meant that in the second cycle the two cages could no longer function as treatment replicates. In order to obtain as much information as possible from this study it was decided to extend the fallow period of the other cage (position 5) in the second production cycle to compare the effects of the extended recovery period (4.5 months) between the two cycles. A further difficulty at Creeses Mistake was that the farming intensity declined markedly after the first production cycle. In production cycle 2, feed input and fish biomass in the study cages were halved compared to production cycle 1.

At Stringers Cove, two study cages were randomly selected within each of three different farming scenarios/treatments (Fig.6.5.1.3.2), giving a total of six study cages. The first scenario included cages above sediment that had not been previously farmed. These cages (cages 1 and 2) were stocked in the first production cycle and fallowed for the subsequent 15 months. The second scenario (cages 1A and 2A) had also not previously been farmed, but was only stocked in the second cycle (i.e. fallowed over the first production cycle (the first 13 months), then stocked for 9 months and fallowed for 3 months). The third scenario (cages 3A and 4A) was subject to the same stocking regime as treatment two, but these positions had been exposed to previous farming activity. Two reference positions located 150m from the edge of cages 1A and 2A were also studied. Once again there were problems in maintaining

equivalence between the two production cycles and feeding levels were markedly reduced in the second production cycle.

3.5.2.1 Temporal Analysis

Sampling at both sites followed a BACI design (Before, After, Control, Impact). Samples were collected prior to stocking, during stocking (after 4.5 and 6 months), at the end of stocking / start of fallowing (9 months) and during the fallow period. In the first production cycle at Stringers Cove, samples were collected fortnightly over the fallow period to determine the rate of recovery, whilst at Creeses Mistake samples were only collected at the start and end of the fallow period. The extra information gained through fortnightly sampling at Stringers Cove proved to be most valuable in interpreting recovery rates, and as a result fortnightly sampling was undertaken at both Stringers Cove and Creeses Mistake over the fallow period of the second production cycle. The dates that sampling occurred, and the stocking /destocking dates for the study cages, are shown in Fig. 3.5.2.2.1 (a) Creeses Mistake, b) Stringers Cove).

The proposed stocking/destocking schedule was disrupted at Creeses Mistake in the first production cycle, with the duration of the fallow period differing at the end of the first production cycle (the fallow period at cage 8 was extended from 3 months to 4.5 months). As a result, the cages could not follow equivalent production scenarios in the second cycle (they were 6 weeks out of alignment). In order to examine the full 3 month recovery period in the second cycle for both cages, the study was extended to April 2003, when the final sampling was undertaken at cage 8 after its 3 month fallow period. In addition, the fallow period of cage 5 in the second cycle was extended to 4.5 months so as to be equivalent to that of cage 8 in the first cycle, and determine if changes resulting from the additional fallowing time were consistent over the two farming cycles.

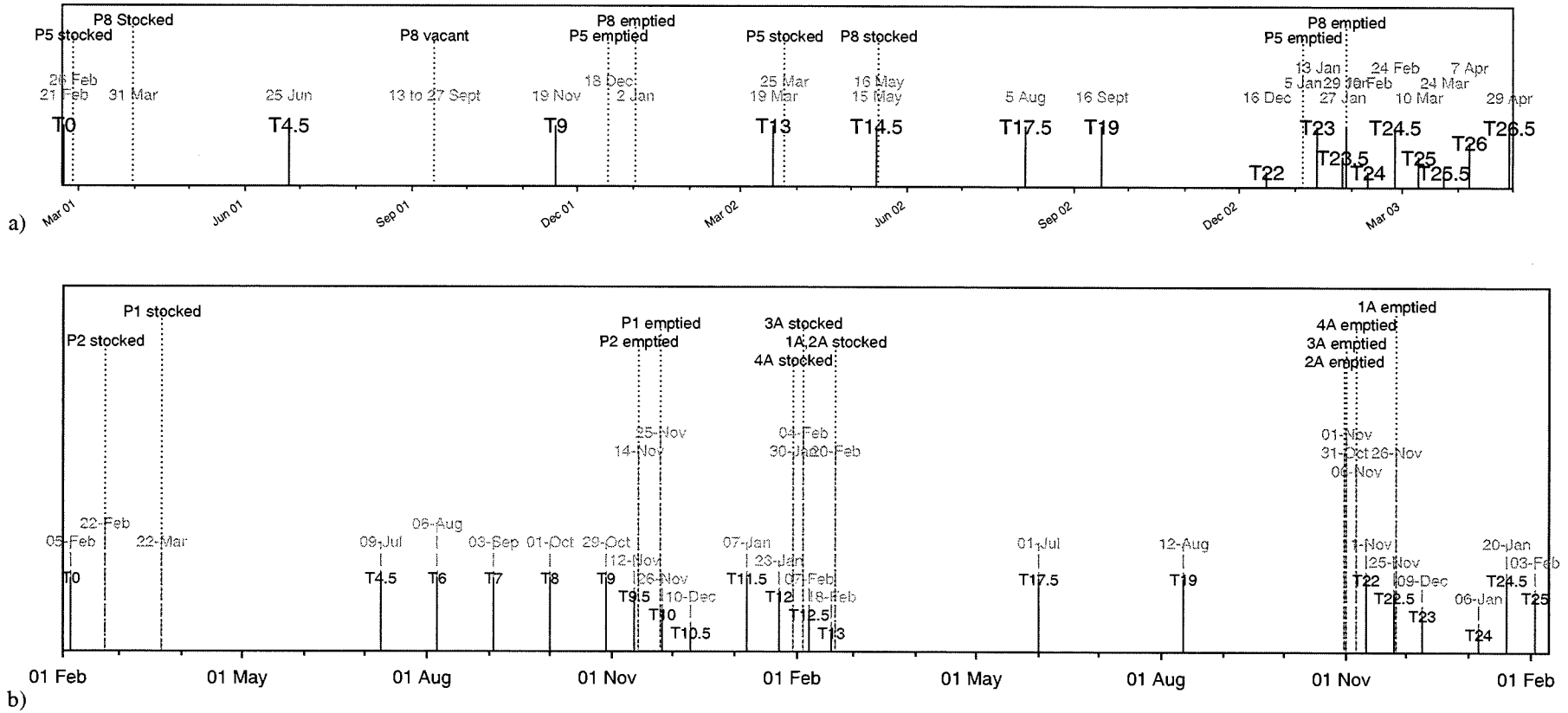


Fig 3.5.2.2.1. Sampling dates and stocking / destocking dates for a) Creeses Mistake and a) Stringers Cove.

4. METHODS

4.1 Positioning equipment

Prior to sampling, each lease was mapped using a Garmin 135 GPS Map unit coupled with a Racal differential unit. Depth and positional information were collected for all cages present on the lease at the time. In addition, reference locations, within the same depth range, but 150m away from the edge of selected study cages, were located using the depth contours and GPS.

4.2 Water column sampling

Current flows were measured using an Acoustic Doppler Current Profiler (ADCP), which measured current speed and direction throughout the water column (from 3m above the sediment surface to the water surface) every 10 seconds. These readings were then averaged over 1 minute. Current data was collected continually for a 1 month period, in summer and winter of the first production cycle at both Stringers Cove and Creeses Mistake. To detect any interannual variation both farms also had an additional summer deployment of the ADCP in the second production cycle.

Temperature and salinity profiles of the water column were recorded on each sampling occasion using a Digital Data Logger (PSW-CTD02). Turbidity was recorded every 10 metres at all sites (until September 2002) using a Turbidimeter (using Nephelometric units, or NTU, for turbidity). Two water samples were taken at each sampling location and the turbidity recorded as the average of these readings. Dissolved oxygen was recorded in % saturation, using a WTW meter and probe. DO was initially recorded every 10 metres throughout the water column at each site. Early analyses showed no difference with depth and therefore measurements were only taken from the water immediately above the sediment surface.

4.3 Faunal sampling

Five replicate samples were collected from each station using a Van Veen Grab (surface area – 0.0675m^2). Grab contents were transferred to mesh bags (mesh size 0.875mm) and rinsed. Samples were then wet seived to 1mm and the retained material preserved in a solution of 10% formalin:seawater (4% formaldehyde). Samples were transferred to the laboratory for sorting and the infauna identified to the lowest possible taxonomic level and enumerated.

4.4 Redox & Sulphide Assessment

Three replicate cores (perspex tubes 250mm length x 45mm internal diameter) were taken at each station using either a multicorer (Stringers Cove) or Craib corer (Creeses Mistake) for measurement of redox potential and sulphide concentration. Redox was measured at 1cm, 2cm, 3cm, 4cm and 5cm using a WTW Redox Probe. Sulphide was also measured at 1cm, 2cm, 3cm, 4cm and 5cm using a Cole-Parmer 27502-40 silver/sulfide electrode according to the method described by Wildish *et al*. (1999). Sub-samples (2ml) were taken at each depth and 2ml of anti-oxidant buffer was added prior to measurement. Temperature of both the sediment and overlying water were recorded at the time of measurement.

After the redox and sulphide measurements were obtained, the top 5cm from each of the three replicate cores at each site was extruded and cut in half. These core halves were retained for granulometry analysis and organic matter determination, any remaining core halves were collected and frozen in sealed plastic bags. Some of these have subsequently been processed for metals analyses and foraminiferal community assessment.

4.5 Granulometry and Organic Matter Determination

For particle size analysis each sub-sample was dried at 30°C and the weight recorded. The samples were then passed wet through a series of sieves (4mm, 2mm, 1mm, 500µm, 250µm, 125µm and 63µm), and the sediment retained on each sieve was collected, dried at 30°C and weighed. The proportion of sediment retained on each sieve was then calculated as a percentage of the total sample weight. The proportion of sediment smaller than 63µm was determined by calculating the difference between the total sample weight and the summed weight of each retained fraction.

For selected samples (Stringers positions 1, 1A, 3A, R1 at 0, 9, 13, 22 and 25 months) the fraction less than 63µm was further analysed using the pipette method (Holme and MacIntyre, 1984).

Total organic matter was determined by modification of the loss on ignition technique (Greiser & Faubel, 1988). Samples collected from the top 5cm of each core were homogenised and a subsample of approximately 2-5 grams was taken. In order to remove excess carbonate, sediments were sieved to remove large shell fragments and any remaining carbonate was neutralised by acidification with 1N HCl. The samples were oven dried for 24 hours at 60°C before being transferred to a muffle furnace for 4 hours at 500°C. The weight of organic material was calculated as the difference between the oven dried and final furnace ashed weights.

4.6 Assessment of Sedimentation Rate

Sediment traps were purpose built to hold three replicate, cylindrical canisters (with an aspect ratio (height:diameter) of 6.25). Canister openings were 1m above the seabed and 0.25m apart (in a triangular formation). A gimbaled arrangement was built into the traps to ensure that the canisters were held upright at all times.

Sediment traps were deployed monthly in the first production cycle for a period of 24 hours at three fixed positions at each farm; i) immediately adjacent to a stocked cage site, ii) at a farm site (in between stocked cages) and iii) at a reference site (corner marker of the farm lease).

Sediment trap canister contents were allowed to settle, and the excess supernatant decanted before being filtered under vacuum using Whatman GF/C glass fibre filters. Filters had previously been ashed at 500°C for 24 hours to remove impurities. To determine the sedimentation rate, filters were dried at 30°C for 24 hours, allowed to cool in a dessicator and the dry weight recorded. Where ‘swimmers’ (large fauna) and algal matter were evident in samples, these were removed prior to drying and their presence noted. Organic matter content (% organic matter and grams organic matter deposited per day) was obtained by ashing filters at 500°C for 4 hours and recording the weight lost.

4.7 Stable Isotope Evaluation

4.7.1 Sample Collection

Sediment samples were collected from adjacent to cages, in-between cages, and reference sites using a Van-Veen grab. Replicate sub samples were collected from two grabs taken at each site and were stored in glass jars and frozen until analyzed for lipid biomarkers, carbon and nitrogen content and stable isotopes (^{13}C and ^{15}N).

4.7.2 Sediment Extraction

A 15- 20 g aliquot of wet sediment was extracted in a 250ml separating funnel using a modified Bligh and Dyer (1959) methanol / DCM / water mixture (2:1:0.8 by volume). A synthetic C_{24} sterol (5β-cholan-24-ol) was added at this stage as an internal standard.The samples were shaken every hour for 3 hours and then left overnight to extract. Phases were separated the next day by addition of DCM and water to bring the final mixture ratio to 1:1:0.9 methanol/DCM/water by volume. The total solvent extract was obtained by rotary evaporation at 40°C of the lower phase. The total extract samples, made up in DCM, were then stored in glass vials, refrigerated ready for saponification.

4.7.3 Fauna Extraction (Stringers only)

A sample, typically 3-5 g of bulk fauna / grit was extracted in a 250 ml separating funnel using the same method described above. Samples of individual fauna, 0.5-1.0 g, were transferred to a large centrifuge tube along with 20 ml of the Blyer and Dyer methanol / DCM / water mix described above. This mixture was vortexed for 2 min, sonicated for 10 mins, and then centrifuged for 10 mins at 1500 rpm, before being transferred to a 250 ml separatory funnel. This procedure was repeated 4 times for each sample. Phases were separated the next day by addition of DCM and water such that the final mixture ratio was 1:1:0.9 methanol / DCM / water by volume. Total extracts were obtained and stored as described for sediments.

4.7.4 Stable isotopes and % organic carbon and nitrogen

Sediment samples for stable isotope analysis were dried in an oven overnight at 60°C, before being ground with a mortar and pestle. Sediment samples were weighed into tin cups (Elemental Microanalysis Ltd., Okehampton, UK) for nitrogen analysis. For the analysis of carbon, samples were weighed into aluminium cups and then acidified using sulphurous acid to remove any mineral carbonates. Samples were then analysed for nitrogen and carbon content, $\delta^{15}N$ and $\delta^{13}C$ using a Carlo Erba NA1500 CNS analyser interfaced *via* a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1090ºC and reduction at 650ºC. Samples were analysed at least in duplicate. Results are presented in standard δ notation:

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ ‰$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon was VPDB limestone and the standard for nitrogen was atmospheric N_2. The reproducibility of the stable isotope measurements was ~0.2‰ for C and 0.5‰ for N.

4.8 Biomarkers

4.8.1 Sample Collection.

Sediment samples were collected as per stable isotope technique described above. Samples of the benthic infauna were obtained by sieving sediment grab samples though a 0.875mm mesh bag. The samples were then immediately sorted and the fauna collected transferred to glass jars and stored frozen until later analysed for fatty acid and sterol biomarkers. Bulk fauna were collected at cage site P2 and ref site 2 at 9.5 months during the first production cycle, and at 4.5 and 6.0 months during the second cycle. An aliquot of the sediment remaining after sieving though the mesh bag was also collected and stored in glass jars ready for analysis.

In the second cycle the most significant species from the reference and cage sites were separated from the bulk fauna / shell grit samples at 4.5 month and 6.0 month and analysed separately. These species were *Capitella sp*, *Nebalia long*, *Corbula gibba* and *Neanthes cricognatha* from the cage samples and *Amphiura elandiformis*, *Nassarius nigellus* and *Thyasira adelaideana* from the reference site.

4.8.2 Fatty acid and Sterol analysis.

A 40% aliquot of the total solvent extract (TSE) from the sediment samples, and 80% from the individual fauna TSE, was saponified with potassium hydroxide in methanol, 5% wt./vol, under nitrogen for 2 hours at 80°C. Non saponified neutral lipids were then extracted into hexane / DCM (4:1 by vol, 3×2 ml) and transferred to sample vials ready for analysis by GC and GC-MS after derivatisation with 100 µl of BSTFA solution at 60°C for 1 hour. Following acidification of the remaining aqueous layer using hydrochloric acid to pH2, total fatty acids (TFA) were obtained and methylated to form their fatty acid methyl esters (FAME) using methanol / hydrochloric acid / DCM (10:1:1 by volume; 80°C, 2 hours). A 19:0 or 23:0 FAME internal injection standard was added to the TFA fractions before GC and GC-MS analysis.

4.8.3 Gas chromatography (GC)

GC was performed on a Varian 3410 gas chromatograph equipped with an 8100 autosampler, flame ionisation detector (FID) and septum-equipped programmable injector (SPI). The GC oven was fitted with an HP5 ultra 2 capillary column (50 m; 0.32 id; 0.17 µm film thickness). Samples were injected at an oven temperature of 45°C; after 1 min the temperature was raised at 30°C/min to 140°C and then by 3°C/min to 300°C where the oven temperature was held for 5 min for analysis of fatty acids. The final oven temperature was increased to 312° C and the final time to 10 mins for the sterol analysis. The SPI was programmed at 180°C/min from 45°C to 310°C immediately after injection and held at 310°C for 30 min before cooling. The detector temperature was held at 310°C. Data were acquired and plotted using Millenium software (Waters Corp.)

4.8.4 Mass spectrometry (MS).

GC-MS analysis of the FAME and non-saponified neutral lipid fractions were performed with a Finnigan GCQ Plus GC-MS system fitted with on-column injection. Samples were injected using an AS2000 auto sampler into a 15 cm 0.52 mm id deactivated capillary retention gap attached to a HP 5 Ultra2 50 m, 0.32 mm id, and 0.17.μm film thickness column, using helium as the carrier gas. Typical mass spectrometer operating conditions were: EV, 70 eV; Emission current 250 μA, transfer line 310°C, source temperature 240°C, 0.8 scans /sec and mass range 40-650 Dalton.

4.9 Visual assessment techniques

Video footage was taken using a digital underwater camera system linked by an umbilical to a digital recorder on the surface. A minimum of 2 minutes footage was taken from each sample station.

Digital photographs were taken of each sample prior to sorting and post-sorting. Specific features deemed to be indicative of environmental condition (identified through the full analysis of these samples) were then assessed in each photograph and scores assigned to these features (Table 4.9.1). Scores were then weighted depending on the level of impact indicated by each feature. A positive weighting indicated better environmental indicators and a negative weighting indicated features symptomatic of impact. The scores were then summed with the total score reflecting the environmental status of that sample and enabling a semi-quantitative comparison between samples.

Table 4.9.1. Visual characterisation of benthic fauna features used at A) Creeses Mistake (Site 1); *and* B) Stringers Cove (Site 2) with weighting and category indicated. All other features scored as presence (1)/absence (0) data.

Feature	Density score	Site 1	Site 2	Weighting	Category
Corbula gibba	0,1,2	x	x	1	-ve
Capitellid worm	0,1,2,3	x	x	2	-ve
Other worm	0,1,2	x	x	1.5	+ve
Brittle star	0,1	x	x	2	+ve
Heart Urchin	0,1,2	x	x	1	+ve
Side gilled sea slug	0,1,2	x	x	1	-ve
Mussel shell	0,1,2	x	x	1	-ve
Nassarid gastropod	0,1,2	x	x	1	-ve
Other invertebrates	0,1,2	x	x	1	+ve

Video was scored according to key features determined to be indicative of impacted or unimpacted conditions, as defined by previous research (Crawford *et al*., 2001; Macleod *et al*., 2002), background environmental information and benthic community structure information collected during this study (Table 4.10.2). As with the benthic photographs, each feature/variable was weighted according to its sensitivity to detect impacts or no impacts. For example, *Beggiatoa* is a well recognized indicator of environmental degradation (GESAMP, 1996), and therefore received a high weighting. Evidence of gas bubble emission received the highest weighting, as this suggests a highly degraded system. The score for each feature could be either positive or negative, depending on whether the variable represented a positive or negative effect. Categorising the variables in this manner means that features indicative of good environmental conditions will tend to increase the final score, whilst those suggesting an impact would reduce the overall score. Therefore the higher the

summed score, the better the sediment condition. The resulting scores can either be analysed using univariate techniques (i.e. summed as a single score for each station/time) or can be set up as a matrix for multivariate analyses.

Table 4.10.2. Video features used at A) Creeses Mistake (Site 1); *and* B) Stringers Cove (Site 2) with weighting and category indicated. All fauna were scored as density estimates (ie. 1= sparse, 2 = dense). *Beggiatoa* scored by thickness of mat (patchy =1, thin = 2, thick = 3). % Algal cover scored as sparse (1), moderate (2) or dense (3), as was Burrow Density. All other features scored as presence (1)/absence (0).

Feature	Site 1	Site 2	Weighting	Category
Gas bubbbles	x	x	10	-ve
Black/grey sediment	x	x	1	-ve
Beggiatoa	x	x	1.5	-ve
Pellets and faeces	x	x	1	-ve
Farm-derived debris	x	x	1	-ve
Nassarid gastropod	x	x	1	-ve
Side gilled seaslug	x	x	1	-ve
Heart urchin	x	x	1	-ve
Squat Lobster		x	1	-ve
% Algal cover	x		1.5	+ve
Burrow density	x	x	1.5	+ve
Worm tubes/casts	x	x	1	+ve
Faunal tracks	x	x	1	+ve
Brittle star	x	x	1.5	+ve
Maoricolpus roseus	x	x	1	+ve
Other Echinoderms	x	x	1	+ve
Crustaceans	x	x	1	+ve
Echiurans & Annelids	x	x	1.5	+ve
Fish	x	x	1	+ve
Planktonic Crustacean		x	1	+ve

4.10 Metals Analyses

Sediment samples were removed from the freezer and dried at 104°C. Each sample was ground to a particle size <2mm and digested using Aqua regia (HCl/HNO_3). Digests were analysed by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrophotometer) for concentrations of Arsenic, Cadmium, Cobalt, Chromium, Copper, Manganese, Nickel, Lead and Zinc (mg/kg DMB).

4.11 Microbiological and Porewater Nutrient sampling

Triplicate sediment cores were collected from each site for bacterial enumeration. Duplicate cores were collected for microelectrode studies and nutrient analyses. Cores (at least 100mm min length) were collected using polyethylene tubes (45 mm diameter) and a Craib corer. After collection cores were stored in an esky filled with ambient water until transfer to the laboratory.

Samples were obtained from:

- Stringers Cove -
  April 24, October 31 and November 1, 2001 and February 7 and 20, July 1 and 3, November 7 and 14, 2002 and February 4 and 6 2003.

- Creeses Mistake -
  April 27, June 28, November 20, 2001 and March 19, August 6, September 16 and December 17, 2002 and January 28, March 11 and April 29, 2003.

4.11.1 Porewater Nutrients

The top layers of sediment were carefully extruded so that slices at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 cm could be collected into 50ml plastic syringes. A 0.45 μm filter was attached to the bottom of the syringe, which was then centrifuged at 40000 rpm for 5 minutes at 5°C. The filtrate was stored frozen at –20°C until being assayed for ammonia on a Technicon Autoanalyser. All glass and plastic were acid washed and rinsed in distilled water.

4.11.2 Bacterial enumeration

Cores for bacterial counts were sliced at three depths (0-2 mm, 2-5 mm and 5-10 mm). Samples were then fixed in 4% formalin in 0.2μm filtered seawater and stored at 4ºC until bacterial dispersions were performed. Bacterial dispersions were carried out after Epstein and Rossel (1995). Samples were sonicated (Misonix, "microson XL") four times for 20s, with at least 30s on ice between sonications. Samples were then washed four times and the supernatants pooled. An appropriate dilution was then chosen to ensure countable cell densities prior to staining.

Semi-quantitative counts were made of *Beggiatoa* spp. on formalin-fixed samples. For each sample, *Beggiatoa* filaments in 5 transects of a wet mount were counted and the totals for each site (all depths and replicates) at each time were compared.

4.11.3 Cell staining and microscope slide preparation

Bacterial suspensions were stained, in the dark, for 20 min. with SYBR Green I nucleic acid stain (molecular probes). A 25 mm glass filter holder (Millipore) was then used to filter the stained sample through a 0.02 μm pore size Al_2O_3 Anodisc 25 membrane filter (Whatman), backed by a 0.8 μm cellulose mixed ester membrane (Millipore type AA). The Anodisc filter was mounted on a glass slide with a drop of antifade (50% glycerol, 50% phosphate buffered saline (0,05 M Na_2HPO_4, 0.85% NaCl, Ph 7.5) with 0.1% *p*-phenylenediamine). Samples were then submitted to the image analysis system. A subsample was also counted manually to confirm the accuracy of automated counts. Ten fields of view were randomly selected, and at least 200 cells counted by image analysis, on a Leica DMRBE microscope with 100x objective (Leica PL Fluotar), under blue excitation. Samples were analysed blind to reduce operator bias (Gough and Stahl, 2003). Images were acquired with a Leica DC 300F couple-charged device (CCD) camera using Leica IM50 software. All pictures were recorded as 8-bit images with a resolution of 1300 by 1030 pixels per μm.

4.11.4 Microbial Counts - Image Analysis

Image analysis was performed using Reindeer graphics' Fovea Pro and Adobe Photoshop 7.0 by employing the following steps. Images were converted to grey-scale and subjected to a Laplacian 5x5 filter to enhance cell boundaries. A Gaussian 5 x 5 filter was then applied to remove any noise created by the Laplacian filter. Images

were thresholded as described by Viles and Sieracki (1992) using the global visual threshold. Cells were then counted and measured.

4.11.5 Microelectrode measurements

Core samples were transported to the laboratory (within 5 h of sampling) they were then submersed in an aquarium filled with water collected from the field site and cooled to temperature equivalent to that at the field site. If necessary the mud in the core was pushed up so that there was only about 1 to 2 cm of gap to the top of the plastic core. This ensured that there was good mixing of water above the mud in the core. Aquarium water was air bubbled to maintain 100% saturation of dissolved oxygen. Oxygen microelectrodes (Clark type with a guard cathode (Revsbech, 1989) from Unisense A/S Aarhus, Denmark) were positioned vertically above the sediment surface on a hydraulic computer-controlled micromanipulator. The micromanipulator and associated software were developed by Island Electronics (Tasmania, Australia). The manipulator was used to take profiles of oxygen across the sediment-water interface to the bottom of the oxic zone typically at intervals of 100 µm. A horizontally mounted stereomicroscope was used to observe the microelectrodes in relation to the sediment surface. The physical arrangement of the microelectrodes and associated electrical circuitry were as described in Revsbech and Jørgensen (1985).

Outputs (mV) were automatically logged and later converted to micromolar concentrations of dissolved oxygen by comparison to Winkler titrations of aquarium water for which the temperature, salinity and electrode output were also recorded. The depth of penetration of oxygen was determined and in some cases profiles were mathematically modelled according to Rasmussen and Jørgensen (1990) to calculate removal rates of oxygen. This included bacterial consumption of oxygen via aerobic respiration of organic carbon, plus biological oxidation of reduced inorganic species plus chemical oxidation of reduced inorganics. Where possible, the calculated parameters were determined as the means of 6 profiles per core, with cores duplicated.

For some of the early sediment cores it was necessary to conduct profiling and data logging manually. In this case, a hand-operated micromanipulator was used to position the microelectrode and the output from a Keithley picoammeter recorded.

A nitrous oxide (N_2O) microelectrode (Unisense A/S, Aarhus Denmark) was calibrated and profiles of the concentration of N_2O measured as it accumulated in acetylene-poisoned cores. The acetylene inhibits the final enzymatic reduction of N_2O to N_2, thus N_2O accumulates in the sediment. From the profile of concentration a rate of formation can be inferred and therefore a rate of denitrification.

4.11.6 Bacteriological monitoring of sedimentary organic carbon.

This component of the research project isolated bacteria from Creeses Mistake sediments that could potentially be used as a tool for monitoring available carbon in the sediment pore water. The rationale being that, as organic carbon increases in the sediment, it will support more bacterial growth. By keeping all other variables constant, except for the concentration of organic carbon, then it is possible to test for the amount present at any one time. The method is described below.

- Facultative heterotrophs were isolated on Anacker and Ordals Medium (with 3% NaCl) from sediment.

- Isolates were partially phenotypically identified.
- Optimal growth conditions were determined for pure cultures in mineral salts medium with single carbon sources, initially glucose and then acetate.
- Organic carbon was then omitted from the mineral salts medium.
- Soluble organic carbon was extracted from sediment by centrifugation.
- The extract was filter-sterilised (0.2 □m) to remove naturally occurring bacteria.
- The sediment extract was serially diluted in mineral salts medium and inoculated with log phase cultures of the isolates.
- The diluted extracts were then incubated under appropriate conditions for the particular isolate (usually aerobic, 20 °C for 2 to 5 days).
- The endpoint of growth was determined by turbidity. This gave a measure of available organic carbon from degree of dilution that was still able to support growth.

Unless otherwise stated, cultures were grown on or in Anacker and Ordal's medium with 3% salinity at 20 °C.

4.12 Phospholipid Fatty Acid (PFLA) and Ether Lipid (EL) Profiling

Samples for phospholipid fatty acid (PLFA) and ether lipid (EL) profiling were obtained from the Stringers farm site, and included two experimental fish cage sites and two reference sites. Each sample set consisted of four samples taken at the initiation of the experiment (0 months), and after periods of 4.5, 9 and 13 months, ie. Covering the first fallowing period only. The samples available were:

Site 17-1 (cage)	0 (PLFA only), 4.5, 9, 13 months
Site 17-2 (cage)	0, 4.5, 9, 13 months
Reference Site 1	4.5, 9, 13 months
Reference Site 2	0, 4.5, 9 (EL only), 13 months.

Two additional samples were collected at 22 months for site 17A; sediment from this site was observed to be ‘out-gassing’, and consequently these samples were included for comparative purposes.

Unsieved sediment samples were dried, and the lipids in a weighed subsample extracted using the single-phase modified Bligh-Dyer method (Bligh and Dyer 1959, White et al. 1979 a&b) defined earlier. Lipids in 20% of the TSE were partitioned into neutral, glycolipid and phospholipid fractions by passage through a chromatography column containing 1 g silica, with elution by chloroform (10 ml), acetone (20 ml) and methanol (10 ml) respectively (Guckert et al. 1985). The methanol fraction (containing the PLFA) was saponified with methanolic KOH, acidified and extracted with 4:1 hexane:CH_2Cl_2. The released fatty acids were methylated with methanolic HCl and treated with BSTFA to convert any hydroxy acids to their more volatile trimethylsilyl derivatives.

PLFA were quantified by GC analysis performed with a HP1 capillary column (0.17 µm film thickness) and a flame ionisation detector fitted in a HP5890 GC using helium as the carrier gas. Samples were injected into a split/splitless injector system at 50°C. After 1 minute the oven temperature was increased from 50°C to 150° C at a rate of 30°C min^{-1}, and then at 2°C min^{-1} to 250°C and finally 5°C min^{-1} to 300°C, whereupon the temperature was maintained for 15 minutes. Individual fatty acids were identified by comparison of retention times to commercial and/or laboratory

standards. Selected samples were also analysed by GC-MS for confirmation of component identifications. An internal injection standard mixture (methyl esters of the fatty acids 19:0 and 23:0) was added to the samples prior to GC analysis. A representative chromatogram is shown in Figure 4.12.1.

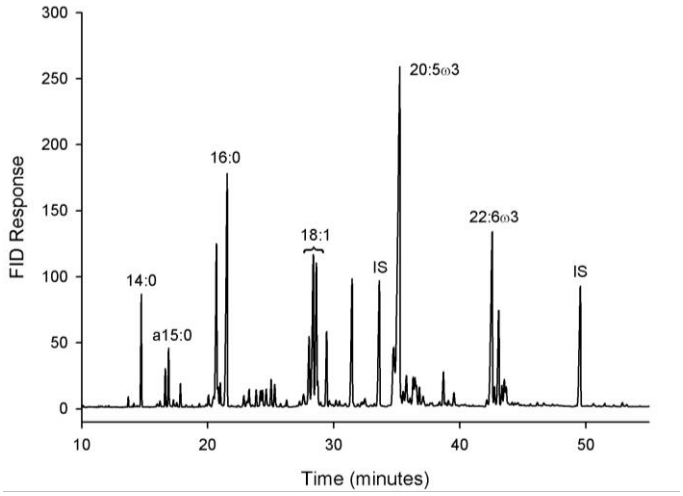


Fig. 4.12.1. Partial gas chromatogram of PLFA (as methyl esters) extracted from a Dover farm site sediment. IS denotes internal standard, and selected PLFA are noted

A further portion of the TSE was treated with methanolic HCl to convert phospholipid ether lipid to free ether lipid. The ether lipids were extracted into 4:1 hexane:CH_2Cl_2, which was removed under a stream of nitrogen. Internal injection standard (1,2-di-*O*-hexadecyl-*rac*-glycerol) was added, and the sample again blown down. BSTFA was added, and the samples heated at 60°C overnight to convert the ether lipids to their more volatile trimethylsilyl derivatives. Excess BSTFA was removed under nitrogen, and the samples taken up in 50 µl chloroform.

The concentrations of the derivatised ether lipids were determined using a 2 m x 0.32 mm i.d. BPX5 capillary column (SGE, Australia) with a stationary phase of 0.25 µm film thickness installed in a Hewlett-Packard 5890 GC (Nichols et al. 1993). Structures for selected ether lipids are provided in Figure 4.12.2. Samples were injected manually on-column, with the injector set at 50°C. The oven temperature was held at 50°C for 2 minutes, and then increased by 15°C min^{-1} to 350°C, and then by 1°C min^{-1} to 380°C. The GC was equipped with a flame ionisation detector maintained at 385°C.

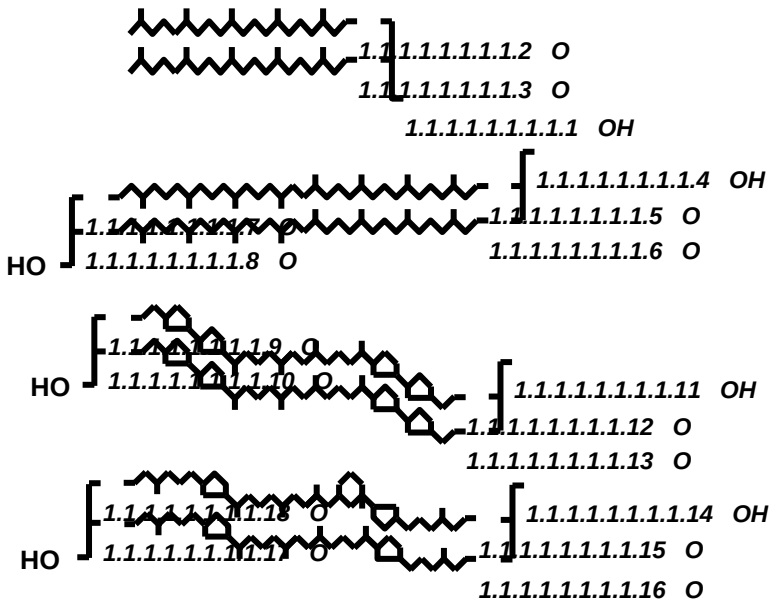


Fig. 4.12.2. Ether lipid structures: archaeol, caldarchaeol (GDGT0), GDGT8 and crenarchaeol (top to bottom).

Due to overlap in the broad GDGT peaks (Figure 4.12.3) and in many cases a descending baseline, and therefore difficulty in accurate integration, GC response data were exported to the software package PeakFit. The peaks were accurately modelled as a suite of tailed Gaussian curves, which allowed precise determination of peak areas. Four peaks attributable to GDGTs were typically observed in chromatograms; these were identified as caldarchaeol (GDGT0), GDGT1, GDGT2 and crenarchaeol. A representative partial gas chromatogram is shown in Figure 4.12.3.

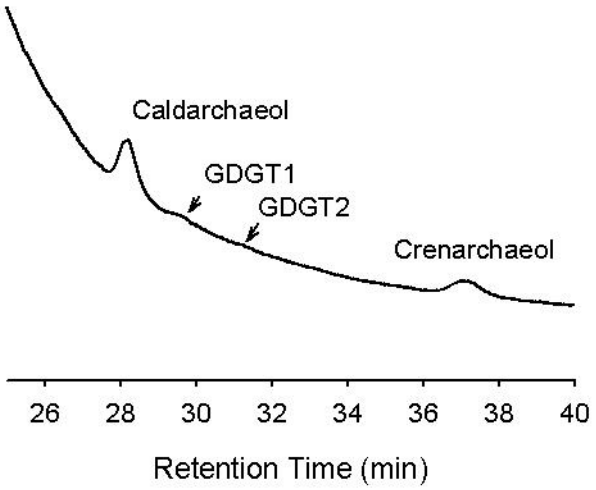


Fig. 4.12.3. Partial gas chromatogram showing ether lipids from a Dover farm experimental site.

The presence of archaeol (Figure 4.12.2) could not be confirmed, as the peak attributable to this compound was always obscured by broad peaks eluting at the same time. Archaeol was undoubtedly present in all samples, but the concentrations could not be determined.

4.12.1 Microbial biomass estimates

PLFA concentration data were converted into microbial biomass using the following factors: average bacteria contain 100 µmol PLFA g^{-1} (dry weight); 1 g of bacteria is equivalent to 5.9 x 10^{12} cells (dry weight); and the average molecular weight of phospholipid derived fatty acids was assumed to be 270 Daltons. This conversion assumes that bacteria contain a constant proportion of their biomass as phospholipids under natural conditions. Use of this quantitative approach to determine microbial biomass has been verified by various studies comparing this method with direct bacterial counts (e.g., Baulkwill et al. 1988).

4.12.2 Fatty acid nomenclature

Fatty acids are designated, number of carbon atoms:number of double bonds. The number (n) of carbon atoms of the closest double bond from the methyl end (□) of the molecule is given by □n. For example, eicosapentaenoic acid (EPA) is designated 20:5□3. All subsequent double bonds in PUFA are methylene interrupted unless otherwise noted by the suffix NMI (non-methylene interrupted). The prefixes i and a indicate iso and anteiso branching, and the suffixes c and t indicate cis and trans geometry respectively.

4.13 Statistical analysis

4.13.1 Univariate analysis of variance

Univariate data were analysed by Analysis of Variance (ANOVA). Various transformations were applied and these are noted in the results sections for the individual datasets. For the benthic infaunal data a nested two-way fixed effects model ANOVA with factors site (treatment) and time was used at Creeses Mistake to assess within treatment variations. Based on these results, sites were either pooled as treatments or analysed individually. At Stringers Cove, a two-way fixed effects model ANOVA with factors treatment and time was used to assess between treatment variations. For analyses of redox potential and sulphide concentration, where measurements were recorded at several depths within the sediment, a three-way fixed effects model ANOVA with factors site (or treatment), time and depth was used to assess between treatment variations. At both Stringers Cove and Creeses Mistake, initial sampling results (T0 –0 months) were not included for analysis of redox potential and sulphide concentration due to probe problems, whilst 1cm recordings were also excluded due to irregularities in the data.

A two-way fixed effects model ANOVA with factors treatment (or site) and time were used to assess variations in organic matter content (loss on ignition), redox potential, sulphide concentration, sedimentation rates, video data, visual characterisation of benthic sample data, sediment metals, macroinvertebrate abundance, number of species, major faunal groups (Phyla) and diversity (Shannon Index (Shannon & Weaver, 1963). Tukey's posthoc test was undertaken for subsequent pairwise comparisons to determine which treatments / groups were significantly different. In addition, photo and video scores were compared to Shannon index values for macrofaunal data using Regression analysis.

For analysis of microbiological samples, ANOVA was used to test for the effect of farm (2 levels), time (3 or 4 levels) and sediment depth (3 levels) on microbial numbers, microbial biomass and cellular morphology. Homogeneity of variances was checked visually by examining residual plots. Data that did not meet this assumption of ANOVA were log transformed. Significant factors were then compared using Tukeys HSD. When non-orthogonol comparisons were made a Bonferroni correction was used to adjust α. All statistical tests were tested at $\alpha = 0.05$. The statistical software SPSS v10 was used to perform tests.

4.13.2 Multivariate analysis of ecological structure

Patterns in the species community data were identified by means of agglomerative hierarchical cluster analysis and these patterns were then displayed both as dendrograms and ordination plots using multi-dimensional scaling (MDS). The relative contribution of each species to the average similarities of the groups (identified using cluster analysis) and average dissimilarities between groups was calculated and the results expressed as percentages (SIMPER). Planned comparisons of cages (one-way multivariate analysis of similarities – ANOSIM, Bonferroni corrected) were used to assess differences between cages and references in the species data and to determine if these were significant.

Cluster analysis and ordination was also used to determine patterns in the quantitative video assessment data. Groups identified in cluster analysis were assessed using SIMPER analysis to determine the relative contribution of each video parameter to the average similarity within groups and dissimilarity between groups. The video and biotic datasets were compared to determine how well they correlated (RELATE analysis). All the multivariate analyses were conducted using the Plymouth Routines in Multivariate Ecological Research (PRIMER) software package.

5. RESULTS & DISCUSSION - CREESES MISTAKE

5.1 General Site Information

Water flow at Creeses Mistake was generally quite slow between 2 and 4 cm/s, although it was consistently slightly higher at the sea surface (Fig. 5.1.1). However, relatively frequent storm events increased velocities throughout the water column (particularly during the summer) (Fig. 5.1.2). The predominant direction of current varied. Direction of flow was particularly variable during winter, probably in response to increased tidal effects. In summer, current flow was principally in an East / South-East direction with occasional westerly pulses. These pulses affected the entire water column and appeared to correspond to storm events (periods of markedly increased velocity) (Fig. 5.1.2). These storm events are an important feature at Creeses Mistake, as on occasions they may be strong enough to resuspend sediments which would have a significant effect on sediment recovery processes.

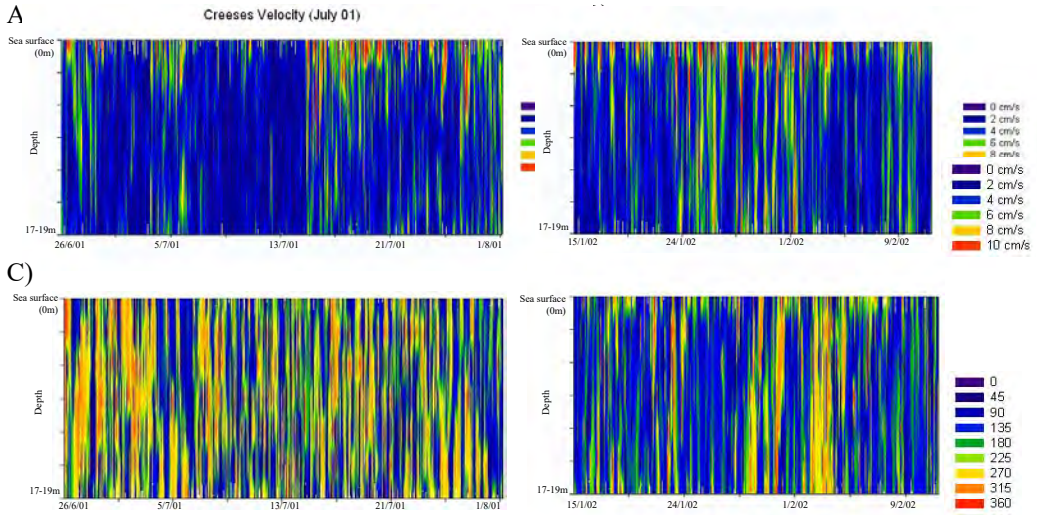


Fig.5.1.1. A) Current velocity at Creeses Mistake during winter (June / July 2001) and B) during summer (January / February 2002). C) Current direction during winter (June / July 2001) and D) during summer (Jan / Feb 2002).

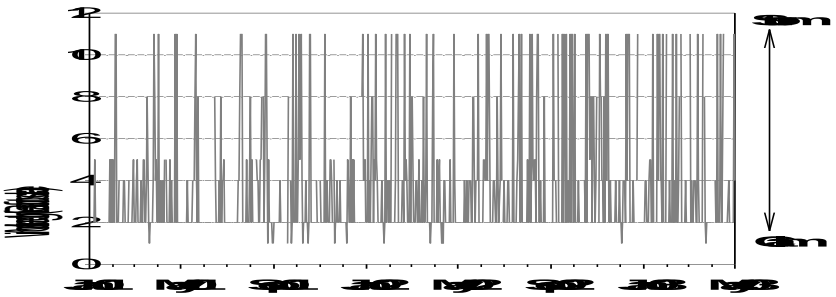


Fig.5.1.2. Daily weather conditions as recorded using the Beaufort scale by farm operators at Creeses Mistake.

Temperature and salinity varied seasonally and was highest in summer and lowest in winter (Fig.5.1.3.a,b), except on the few occasions when an appreciable increase in rainfall caused salinity depletion in surface waters. There was little change in either variable with water depth. Water temperatures were very similar to that recorded by

farm personnel (Fig.5.1.3c). Temperature and dissolved oxygen (DO) results collected daily by farm personnel from 5m depth at fixed positions within the lease area suggest that there is a strong relationship between temperature and DO. These data also displayed a seasonal (sine wave) response. There was no evidence of any generalised oxygen depletion within the farm. Temperatures ranged from 10-19°C and were highest between January - April. Average seasonal (spring, summer, autumn, winter) temperatures did not vary significantly over time within each season (Fig.5.1.3c).

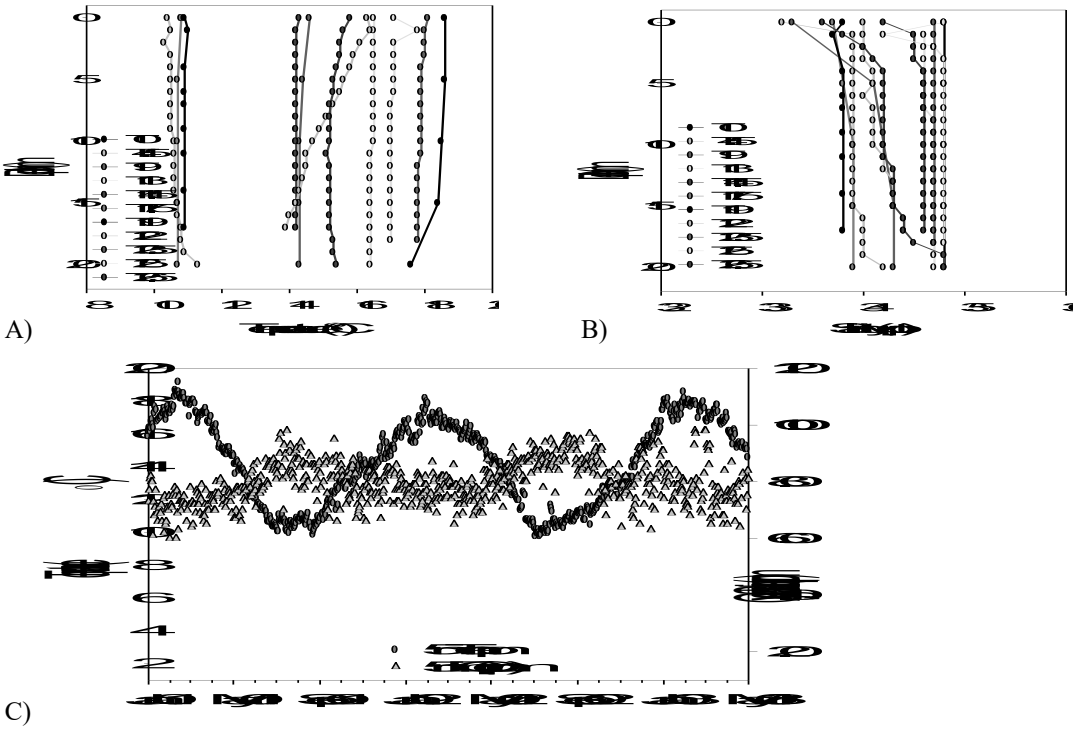


Fig.5.1.3. A) Temperature and B) Salinity at Creeses Mistake at each sampling time during the study period. C) Temperature and Dissolved Oxygen collected daily within the lease by farm operators at a depth of 5m ($F_{1,740}$=337.36, p=0.000, R^2=0.31).

Although the water around the cages was generally more turbid when the cages were stocked, the level of turbidity was not sufficiently different from the reference site to be useful in evaluating environmental impact (Fig. 4.1.4). In fact levels at the reference sites at 0 and 17.5 months were relatively high suggesting that some factor external to the direct farm inputs (e.g. phytoplankton bloom, freshwater run-off) may be influencing turbidity.

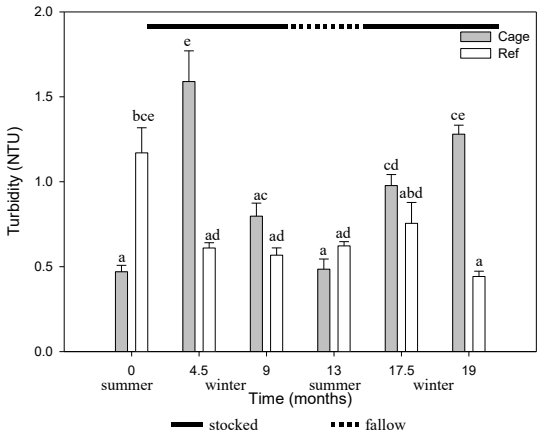


Fig.5.1.4. Turbidity at Creeses Mistake. Two-way ANOVA (Treatment*Time Interaction ($F_{5,36}$=25.855, p=0.000)). Tukeys posthoc pairwise comparison results indicated by letters.

5.2 Farm production information

Creeses Mistake operates a rotational fallowing policy, which means that over the course of the year all cage positions would be fallowed for a period, but at no time during the study was the entire lease vacant.

The average weight of the fish stocked on this site remained the same for both production cycles. However, biomass and feed input per cage was substantially reduced in the second production cycle (Fig.5.2.1). In the second cycle, biomass was nearly half that of the first cycle. In both production cycles the daily feed input was highly variable (Fig.5.2.2). This was principally a result of weather conditions, strong winds often making it difficult to access the site, resulting in periods where no feeding occurred, often followed by periods of compensatory high feed input when weather conditions improved.

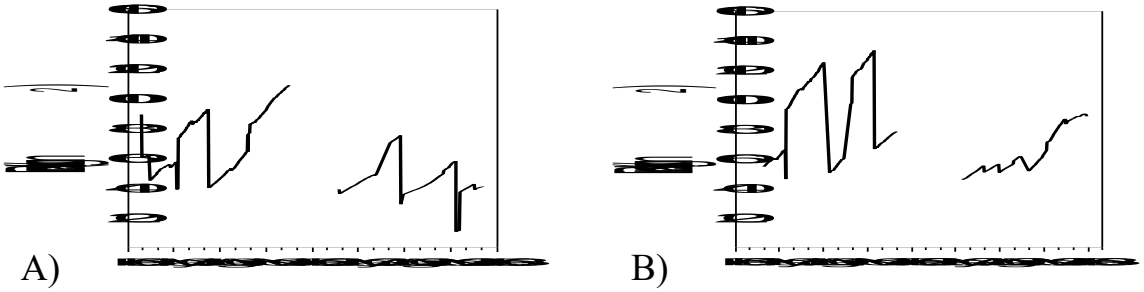


Fig.5.2.1. Cage biomass for study cages at Creeses Mistake during both production cycles. A) Cage 5 and B) Cage 8.

Due to farm management difficulties the two study positions were fallowed for different lengths of time between the two production cycles. In the first production cycle position 5 was emptied for the proposed 3 months, whilst position 8 received an additional 6 weeks fallowing. As a result, the two positions could not be treated as replicates in the second production cycle. However, both cages were stocked for the proposed nine months and were sampled during this stocked period and over the three month fallow period. In order to include the final sampling at position 8 (3 month fallow) the overall sampling programme had to be extended. It was decided to also sample position 5 at this time, as this would provide data after 4.5 months fallow in the second cycle and would allow an additional point of comparison between the first and second production cycles.

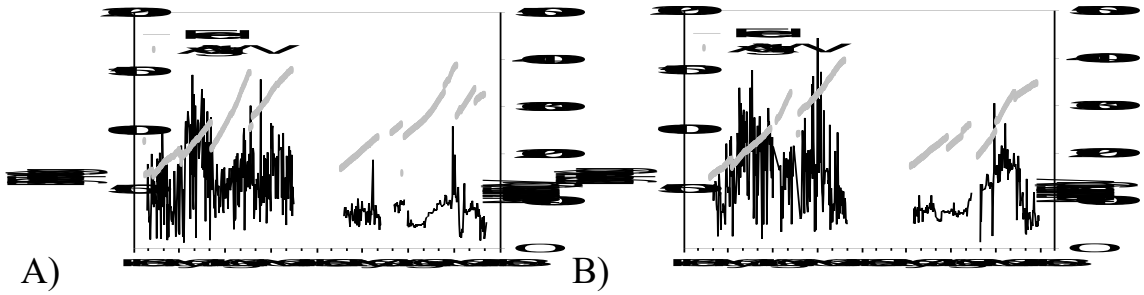


Fig.5.2.2. Farm production information for Creeses Mistake including mean individual fish weight and daily feed input at study cages over both production cycles. A) Cage 5 and B) Cage 8.

5.3 Benthic ecology

5.3.1 Characterisation of community change

The Multivariate analysis of the benthic community structure at Creeses Mistake showed several interesting patterns and relationships. Ordination of the Creeses community data indicated several distinct groups. Samples from the reference positions clustered together on the left hand side of the plot whilst the samples collected immediately after the cages were stocked tended towards the right hand side (Fig. 5.3.1.1). Samples from the cages pre-stocking lie between these two extremes. The vertical separation of the reference samples suggests that there may be an additional separation gradient, possibly a temporal effect.

Cluster analysis identified the first major dichotomy (groups 1 & 2) at a relatively low between group similarity level (23%). Group 1 contains all the samples during and immediately after the onset of farming, whilst group 2 contains all the reference samples and the samples collected pre-farming and from late in the fallowing cycle. Group 2 is separated into three further sub-groups, each with less than 30% overall similarity. These groups reflect differing levels of impact. Group 2A contained the samples collected prior to farming and position 8 at 14.5 months (after 4.5 months fallow), group 2B contained all of the reference samples and group 2C comprised the samples from the end of the recovery period in the second cycle.

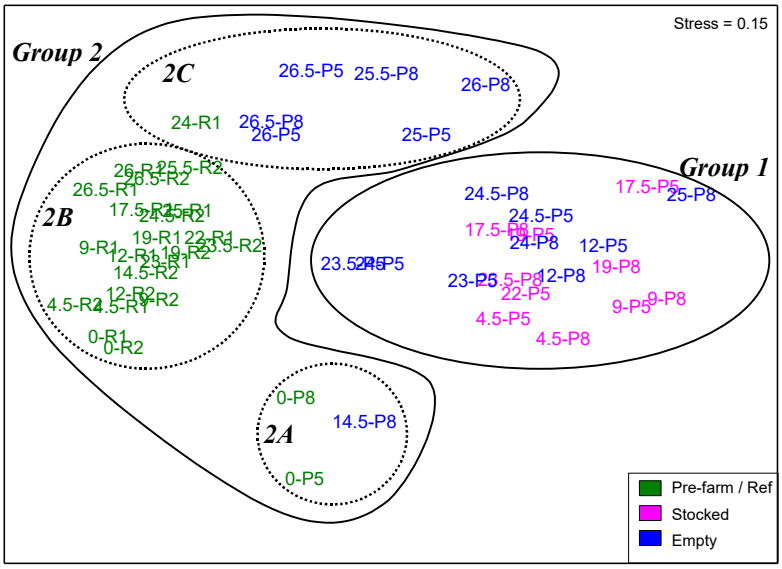


Fig. 5.3.1.1. MDS of all sites (replicates combined) at Creeses Mistake over both production cycles.

The species which characterise these groups tend to support the assumption that the groups reflect differing impact levels (Table 5.3.1.1). Group 1 was dominated by *Capitella capitata (complex),* which alone accounted for 97% of the overall group similarity. *C.capitata* is an opportunistic species well recognised as an indicator of organic enrichment (Pearson and Rosenberg, 1978, Iwama, 1991), and its abundant presence suggests that these samples are highly impacted. The five main species characterising group 2B (reference samples) were all crustaceans. Several studies have reported crustaceans as being sensitive to organic enrichment (Hall,1994).

Consequently, a faunal community dominated by crustacean ns could be considered indicative of relatively unspoiled environmental conditions. In this instance the amphipod, *Ampelisca* sp., a tanaid, *Apseudes* sp.2, and an ostracod, *Euphilomedes* sp.2, made up almost 50% of the overall similarity within this group. Ampeliscid amphipods have been reported as being particularly sensitive to organic enrichment.

Table 5.3.1.1. SIMPER output for the full community assessment indicating a) average abundance, ratio (average similarity / st.dev. similarity), % similarity and cumulative % similarity of the 5 most important species in each of the four main MDS cluster groups and b) average abundance, ratio (average similarity / standard deviation similarity) and cumulative % similarity of the three species which most clearly distinguish the main groups identified by cluster analysis

Species Name	Average abundance (No./m2)	Ratio	Percent Similarity	Cumulative % Similarity
a) GROUP 1				
Capitella capitata	15000	2.31	96.91	96.91
GROUP 2A				
Capitella capitata	232	1.82	22.81	22.81
Tethygenia sp.(MoV1304)	232	2.02	10.28	33.09
Paradexamine moorehousi	92	0.58	9.29	42.38
Polychaetea sp.1	122	0.58	5.55	47.94
Lyssianassidae sp.1	176	0.85	5.37	53.30
GROUP 2B				
Ampelisca sp.	449	1.97	18.54	18.54
Apseudes sp.2	713	0.74	15.41	33.96
Euphilomedes sp (MoV18)	217	2.03	13.78	47.74
Phoxocephalidae sp.	179	1.93	10.70	58.43
Corophium ascherusicum	146	0.81	4.84	63.28
GROUP 2C				
Spionidae sp.1	341	1.19	29.62	29.62
Euphilomedes sp (MoV18)	149	2.05	15.56	45.18
Nephtys australiensis	91	2.01	9.46	54.64
Capitella capitata	268	0.36	7.81	62.45
Solemya australis	55	1.61	6.17	68.62

Species Name	Group average abundance (no/m2)	Group average abundance (no/m2)	Ratio	Cumulative % Dissimilarity
b) BETWEEN GRPS				
Groups 1 & 2A	Group 1	Group 2A		(93.51)
Capitella capitata	15000	232	4.31	73.99
Apseudes sp.	24	530	0.61	77.71
Phoxocephalidae spp.	88	474	0.63	81.13
Groups 1 & 2B	Group 1	Group 2B		(95.00)
Capitella capitata	15000	78	4.36	74.07
Apseudes sp.	24	713	0.70	78.84
Ampelisca sp.	31	449	0.97	82.00
Groups 1 & 2C	Group 1	Group 2C		(91.37)
Capitella capitata	15000	268	5.85	84.34
Spionidae sp.	33	342	0.91	87.50
Euphilomedes sp (MoV18)	38	149	0.93	88.72
Groups 2A & 2B	Group 2A	Group 2B		(83.18)
Apseudes sp.2	530	713	1.12	14.51
Phoxocephalidae spp.	474	180	1.08	23.50
Ampelisca sp.	2	449	1.46	31.54
Groups 2A & 2C	Group 2A	Group 2C		(84.22)
Apseudes sp.2	530	21	0.72	10.62
Phoxocephalidae spp.	474	46	0.77	20.36
Spionidae sp.	0	342	1.37	29.48
Groups 2B & 2C	Group 2B	Group 2C		(80.75)
Apseudes sp.2	713	21	0.90	14.43
Ampelisca sp.	449	11	1.53	25.00
Spionidae sp.	0	342	1.28	34.88

Groups 2A and 2C contained a mixture of species from each of the unimpacted and impacted groups. Group 2A represents the recovered samples in the first cycle whilst group 2C represents the recovered samples in cycle 2. The difference between these groups (2A & 2C) and group 2B (83.18% and 80.75% respectively) was less than between groups 2B and group 1 (95%). Both *Apseudes* sp.2 and Phoxocephalid spp.were more prevalent at 2A than at 2C, whilst the Spionid, *Polydora* cf *socialis*, was more abundant at 2C. As *Apseudes* sp.2 and Phoxocephalid spp. are strongly indicative of the reference conditions, their proportionally greater abundance within group 2A would suggest a lesser impact there than in the post-stocking samples in group 2C. Comparison of the species lists for these two groups (2A and 2B) suggests that the temporal differences are mainly due to species being removed from the communities rather than the appearance of new species.

5.3.2 Classification of impact/ recovery stages (spatial & temporal)

The production regime was markedly altered between the two production cycles (stocking levels were reduced and the fallow period was extended at position 8 in the second cycle). Therefore it is not possible to compare the two cycles nor can the two pens be considered equivalent in the second cycle. Similarly, ANOSIM comparisons could not be made for the second cycle. However, planned comparisons of the cages in the first cycle (one-way ANOSIM, Bonferroni corrected (p=0.002)) indicated that there were significant differences between the cages and references at each given time and between the cages over time (Global Rho= 0.858) (Table 5.3.2.1). The cage positions were significantly different to the references at the start of the study, suggesting that the pre-farming communities were influenced by other operational cages within the lease. It is also important to note that the reference community differed over time and this seasonal /temporal influence must be taken into account when evaluating recovery.

Table 5.3.2.1. Sample statistic (Rho) values from the One-Way ANOSIM comparison of benthic data for selected sample positions (Cage (C) and Reference (R)) and times (Bonferroni corrected n=15, p<0.002). Categories with significant differences are shown in bold. Global Test, R=0.858, p<0.001.

Comparison	R Statistic	Significance Level	Comparison	R Statistic	Significance Level
0-C, 0-R	**0.652**	**0.0014**	13-C, 13-R	1.000	0.0022
0-C, 4.5-C	**0.776**	**0.0017**	**0-R, 4.5-R**	**0.724**	**0.0016**
0-C, 9-C	0.985	0.0021	0-R, 9-R	0.911	0.0020
0-C, 13-C	0.909	0.0020	0-R, 13-R	0.922	0.0020
4.5-C, 4.5-R	1.000	0.0022	**4.5-R, 9-R**	**0.824**	**0.0018**
4.5-C, 9-C	**0.706**	**0.0015**	**4.5-R, 13-R**	**0.774**	**0.0017**
4.5-C, 13-C	0.980	0.0021	**9-R, 13-R**	**0.430**	**0.0009**
9-C, 9-R	1.000	0.0022			
9-C, 13-C	**0.730**	**0.0016**			

The benthic community analyses (Fig. 5.3.1.1 and Table 5.3.1.1) indicate that whilst the cages were stocked the community was very clearly impacted. In the first cycle the cage communities were still clearly impacted after three months of fallow, only after the recovery period was extended for a further 6 weeks at pen position 2 did the community begin to show recovery. In the second cycle the cages were not stocked as heavily and in this instance both positions recovered to at least pre-farming conditions within 9 weeks after farming ceased. This is a considerable improvement on the first

cycle and clearly indicates that significant improvements can be achieved in sediment condition and recovery rate by adjusting farm stocking levels.

5.3.3 Temporal variability in rate of recovery

It is not possible to compare between the two production cycles at Creeses Mistake because the production strategies differed. The results for the full community assessment show that after 3 months fallow in the first cycle the infaunal community had not recovered to reference or pre-farming conditions. However, a further 6 weeks fallow resulted in a community similar to that pre-farming. In the second production cycle, the stocking level and feed input was markedly reduced and in this instance the community assessment suggests that recovery to pre-farming condition was achieved after 9 weeks fallow at position 5 and after only 7 weeks at position 8. These results also suggest that the negative effects of heavily impacting sediments in one cycle can be to some extent mitigated by modifying the production level in the next cycle. The marked reduction in the recovery time indicates that this change in the stocking level and concomitant reduction in feed input have had significant benefits for the sediment. This indicates that if farm managers had an understanding of the sediment conditions they could plan their production strategy accordingly and maximise use of their lease area.

5.3.4 Ecological significance of community changes

The predominant faunal group at the reference positions was Crustacea (Table 5.3.4.1). When environmental conditions deteriorate crustaceans are often amongst the first members of the infauna to be affected (Nilsson and Rosenberg, 1994). They are generally relatively sensitive to increased organic matter, sedimentation and low oxygen conditions, and would be quickly replaced by a faunal community more tolerant of these conditions. The ecology of the Tasmanian benthic infauna is very poorly known. For many of the species in this study functional identity has been inferred from taxonomic features of the species and information pertaining to similar species elsewhere. Consequently, it is not possible to say with certainty the main function of these sediment systems. However, the community mix at the reference positions (Table 5.3.4.1a) clearly suggests a broad range of functional types; suspension feeders and surface deposit feeders were amongst the most common groups. Filter feeders, a group which would be highly sensitive to the increased organic inputs from fish farming, were also found on occasion. This combination of species types suggests that little if any effect of farming is occurring at the reference sites.

Some species were particularly abundant. The tanaid crustacean, *Apseudes* sp.2, was present in especially high numbers at the reference and pre-farm positions, but was virtually eliminated when farming activities commenced, with little or no evidence of recovery over the fallow periods. Similarly *Ampelisca* sp. was consistently abundant over time at the reference stations but was almost entirely absent at the farm positions throughout the study. These species are suspension/surface deposit feeders which would be very sensitive to increased organic loading. Therefore they may be useful indicators of unimpacted conditions.

Table 5.3.4.1. Ten most abundant species at a) Reference position, b) Pen position 5 and c) Pen position 8 at each sampling time.

a) References

Species	0mths	4.5mths	9mths	12mths	17.5mths	23.5mths	25.5mths	26.5mths
Polychaeta sp.1				104				
Capitella cf capitata (MoV 2558)			59			119		
Serpulidae sp.1	370	356						
Phyllamphicteis (cf foliata***) sp.(MoV 3094)			104	89	30	178		
Lyssianassidae sp.1	89							
Corophium ascherusicum		133	156	178	474	104	74	30
Ampelisca sp.	914	985	452	696	178	637	222	148
Protolembos sp.1	173			74		119		74
Lyssianassidae sp.4			104			222	104	141
Ampelisca euroa				533				
Tethygeneia sp.(MoV 1304)	158	262						
Photis sp.			119	178	59	237		
Ischyrocerus sp	109						44	
Amphipod sp		49						
Jassa sp.	69		133					
Amphipod sp				104	148		30	163
Amphipoda sp.							44	59
Amphipoda sp. (Corophoidea)		89						
Phoxocephalidae	336	202	67	111	74	267	178	222
Amakusanthura olearia		79						
Euphilomedes sp.(MoV 18)	116	262	141	215	133	444	400	222
Apseudes sp.2	2383	1212	1304	1126	104	770	237	126
Decapoda sp (Hermit crab)								193
Dimorphostylis cottoni		119						
Cumacea sp.11	84							
Nebalia longicornis	123	72						59
Nassarius nigellus			44					
Maoricolpus roseus			267					119
Fusinus novaehollandiae					89			
Nemocardium thetidis	59	119	59					
Solemya australis				74				
Bivalve sp			44		44			
Ophuroid sp.7	195							
Echinocardium cordatum			163	163				
Pyura sp.							104	
Echinocardium cordatum						104		
Nemertea sp.1			49					

b) Pen Position 5

Species	0mths	4.5mths	9mths	12mths	17.5mths	23.5mths	25.5mths	26.5mths
Nephtys australiensis						99	74	148
Neanthes cricognatha				158		207		
Simplisetia amphidonta						94		
Polychaeta sp.1	123	454	49	6035				
Capitella cf capitata (MoV 2558)	94	16983	20173		10395	34375	1022	
Eupolymnia koorangia	114						30	
Maldanidae sp.			49					
Polydora cf socialis						119	296	681
Lyssianassidae sp.1	425							
Liljeborgia dubia								44
Corophium ascherusicum					69	667	15	
Jassa marmorata					25	104		
Oedicerotidae sp.							44	
Mediomastus australiensis	99							
Ericthonius sp.(MoV 544)			10					
Ampelisca sp.		64			49			
Tethygeneia sp.(MoV 1304)		420						
Photis sp.				15				
Amphithoidae sp.				35		79		
Paradexamine moorehousi	138							
Ischyrocerus sp	104							
Amphipod sp					25			
Phoxocephalidae		217	15	10			30	44
Euphilomedes sp.(MoV 18)			15	10	40		133	281
Ostracoda sp.23			20	20				30
Apseudes sp.2		104						44
Halicarcinus ovatus	286							
Dittosa undecimspinosa						79		
Lophopagurus nanus	64							
Amarinus laevis							30	
Dimorphostylis cottoni		109			202			59
Cumacea sp.8		365		40				
Nebalia longicornis			10					
Nassarius nigellus		44			49			89
Opistobranchia sp.1					59			
Mysella donaciformis				20				
Corbula gibba					104			
Hiatella australis		49						
Solemya australis	69		10	15				74

c) Pen Position 8

Species	0mths	4.5mths	9mths	12mths	17.5mths	23.5mths	25.5mths	26.5mths
Nephtys australiensis						74	178	
Neanthes cricognatha	40	163111	89	222		444		
Harmothoinae sp.(MoV 2848)				30				
Polychaeta sp.1	242		15					
Malacoceros tripartitus						74		
Capitella cf capitata (MoV 2558)	351	39541	14844	3422	3970	16385	89	
Polydora cf socialis							593	178
Maldanidae sp.			44					
Lyssianassidae sp.1	94							
Corophium ascherusicum					44	119		
Oedicerotidae sp.				15			44	30
Ampelisca sp.		15						
Lyssianassidae sp.4					59			89
Tethygeneia sp.(MoV 1304)	109	44		59				
Amphithoidae sp.								74
Jassa sp.				30				
Phoxocephalidae	1388	696			44	163		104
Euphilomedes sp.(MoV 18)							104	148
Ostracoda sp.23	59		30	30			59	222
Leptochelia dubia					30			
Apseudes sp.2	1546		15	44		44		
Hexapus granuliferus							30	
Dittosa undecimspinosa						44		
Dimorphostylis cottoni		59			59		44	104
Cyclaspis caprella		15						
Cumacea sp.8	69	44		44				
Cumacea sp					74			
Nebalia longicornis		30		89		133		
Nassarius nigellus					30			44
Opistobranchia sp.1					30			
Mysella donaciformis			15					
Mytilus edulis planulatus			15				30	
Corbula gibba			15		30			
Hiatella australis		15						
Solemya australis	158					104	30	89
Nemertea sp.1			15					

At the reference sites there were changes in the occurrence and abundance of the most common species. *Apseudes* sp.2 abundance declined over the course of the study (Table 5.3.4.1a), this may be a natural cyclic phenomenon, but alternatively could indicate that farming activities are having a broader influence than anticipated. In contrast, *Capitella capitata* was present at the reference stations during the warmer months of the second production cycle, this may have been a natural summer phenomenon or once again it may indicate a broader effect from farm operations. It might therefore be prudent to monitor the reference conditions until the real cause is established.

The significant shift in the community structure associated with the onset of farming produced a major change in the system function. The structure changed from a diverse community with a large proportion of suspension feeders and surface deposit feeders including some omnivores, predators and even a few filter feeders to one where the community is dominated by only a few species of deposit feeders and most of the original community is inhibited.

Several components of the data suggest that the reference communities had changed over time. It is not clear from the data whether this effect is a result of cyclic or long term natural change or whether it might be the result of broader system–wide effects from the farming operations. Unfortunately the temporal and spatial scale of sampling was insufficient to determine this. The precautionary principle would suggest that it may be appropriate to continue monitoring these reference positions until the nature of this change is established.

The species mix at the pen positions reflected both the impacts of farming as well as recovery over the fallowed periods (Table 5.3.4.1 b and c). The most notable change was the marked reduction in crustacean species and the increase in the incidence of polychaetes, particularly *Capitella capitata* over the stocked period and in the period immediately after stocking. *Capitella capitata* is now recognised as a species complex only distinguishable with molecular techniques. However, the species which comprise this complex are so similar in their function, that for the purpose of this study, they can be treated as equivalent. *C.capitata* is in many ways the ideal organism for the conditions beneath fish farms. It is well adapted for rapid colonisation; it is highly mobile (Tsutsumi & Kikuchi, 1984) and has huge reproductive potential, spawning 2-5 times with an interval of as little as 30 days between spawnings (Grassle & Grassle 1974). Each female can produce between 400-4,000 eggs, with a hatching success rate of around 79% (Tsutsumi & Kikuchi, 1984). Reproductive success seems to be greater in younger females, facilitating the colonisation of new areas (Tsutsumi & Kikuchi, 1984). *Capitella capitata* is also extremely well adapted to utilise the increased organic material resulting from fish farming; it has been reported as eating approximately 19% of its body weight in faecal pellets in 24 hours (Frankenberg & Smith, 1967). It is also extremely tolerant of low oxygen levels as a result of specialised respiratory pigments and an ability to extend its tubes into overlying oxygenated sediments and actively ventilate its burrows (Hutchings, 2000). Where organic inputs do not overwhelm these communities, they will very effectively process the material.

5.3.5 Simplified community features

Assessment of collective groups or sub-sets of the community provides useful insight into broad changes in community structure which may in turn give a simple indication

of environmental conditions. For instance Levin (2000) showed that the most significant changes associated with organic enrichment can be described just using polychaetes. The full community assessment in this study has indicated that there are significant changes in the abundance of polychaetes (Annelids) which are associated with impacted conditions, consequently evaluation of total or Annelid abundance may be a simple way to determine sediment condition.

The full community assessment showed a clear change in species composition over time with changing impact level. When organic enrichment was greatest (towards the end of the stocking cycle) the communities were dominated by only a few opportunistic species. At Creeses Mistake the number of species at the reference positions was generally high, between 35-50 species (Fig. 5.3.5.1). Numbers were relatively consistent over the first production cycle, but seemed to decline towards the end of the second cycle. At the farmed sites there was a marked decline in number of species after farming commenced and this was maintained throughout the study. Over the stocked period the number of species declined by between 30-70%.

In the first cycle there was a significant interaction between time and position ($n=40,3$, $F=9.471$, $p<0.001$, Fig. 5.3.5.1a). There was no difference between the cages and the reference stations prior to farming, but after farming commenced the number of species at the cage positions declined significantly (Fig. 5.3.5.1a).

In the second cycle there was also a significant interaction between time and position at both cages (Cage5/Ref1 $n=34,8$, $F=3.552$, $p=0.004$, Cage 8/Ref2 $n=28,6$, $F=3.160$, $p=0.017$, Fig. 5.3.5.1c). At the start of the second cycle the number of species at position 5 was low and actually increased during the stocked phase before declining again towards the end of the production cycle. Numbers were only significantly different from the reference immediately prior to and in the early stages of the stocked period (Fig. 5.3.5.1b). At position 8 the number of species was low throughout the second cycle, differing from the reference stations both before and throughout the early part of the stocked period. A decline in number of species at the reference stations between 23 and 26.5 months meant that levels at the cage positions were equivalent to those of the references within only a few weeks of the cages being fallowed (Fig. 5.3.5.1).

The results from the first production cycle suggest that the conditions at the cages had not recovered to pre-farming conditions after fallowing. This is in accordance with the full community assessment. However, with the reduction in farming intensity in the second cycle the impact appears not to have been so great, and compared with the full community assessment, evaluation of number of species appears to considerably underestimate the time required for recovery. Number of species does not provide any information about the changing mix of species and therefore is only useful to differentiate fairly major impacts.

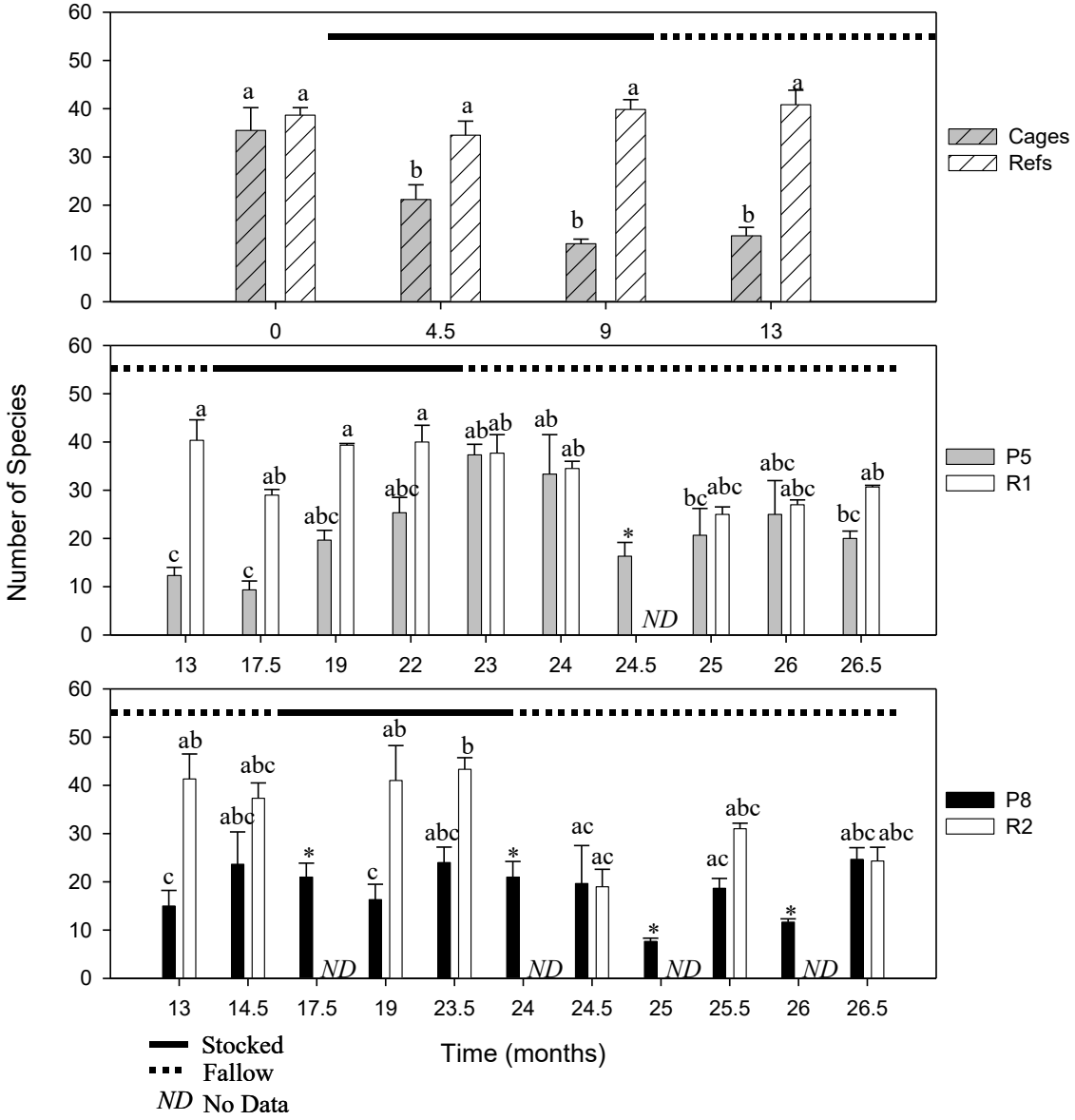


Fig.5.3.5.1 Number of species at Creeses Mistake in A) Production cycle one (treatments combined) B) Cage 5 / Ref 1 in Production cycle two and C) Cage 8 / Ref 2 in Production cycle two.

The number of individuals showed a similar inverse relationship to that suggested by the number of species (Fig. 5.3.5.2). Abundance at the reference positions was comparable over both production cycles. There was a significant interaction between time and position over both stocking cycles (Cages/Refs-cycle 1: n=40,3, F=14.570, $p<0.001$, Fig. 5.3.5.2a, Cage5/Ref1-cycle 2: n=34,8, F=4.937, $p<0.001$, Fig. 5.3.5.2b, Cage 8/Ref2-cycle 2: n=28,6, F=7.045, $p<0.001$, Fig. 5.3.5.2c). Over both production cycles total abundance was equivalent at the cages and reference positions prior to commencement of farming and at the end of the defined fallow period (3 months), suggesting complete recovery in both cycles. More frequent sampling over the fallow period in the second cycle indicates that this recovery occurred after 8 weeks at position 5 and after only a fortnight at position 8 (Fig. 5.3.5.2).

This suggests that in the first cycle abundance levels recovered much more rapidly than number of species but that over the second cycle the measures were more similar. However, when compared to the results for the full community assessment once again

abundance tended to overestimate the rate of recovery. The total number of individuals appears to be a good indicator of the decline of opportunistic species (i.e. *Capitella capitata*) but as with number of species this approach can not differentiate the differences in the species mix that occur in the recovery phase. Change in total abundance is a better measure of degradation than recovery.

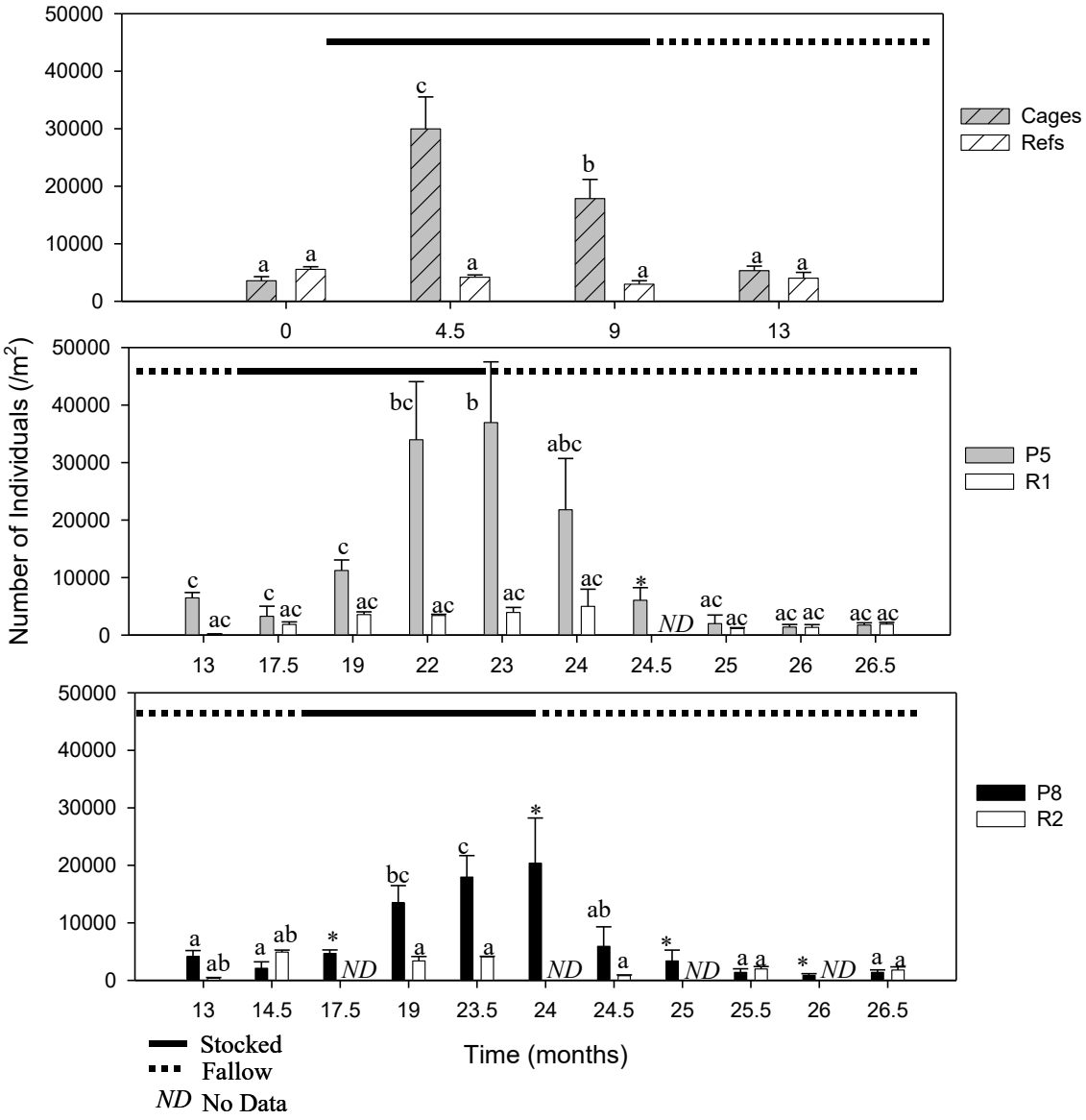


Fig.5.3.5.2. Number of Individuals at Creeses Mistake in A) Production cycle one (treatments combined) B) Cage 5 / Ref 1 in Production cycle two and C) Cage 8 / Ref 2 in Production cycle two.

Shannon diversity reflects both the number of species and the number of individuals, being a combination of both abundance and number of species it should be a more sensitive approach and better reflect community changes. This index is often cited as a measure of environmental condition. Shannon diversity was consistently high (approx. 3.0) at the reference sites (Fig. 5.3.5.3). This index varied both with time and position and consequently ANOVA indicated a significant interaction (Cages/Refs-cycle 1: n=40,3, F=42.356, p<0.001, Fig. 5.3.5.3a, Cage5/Ref1-cycle 2: n=34,8, F=6.600, p<0.001, Fig. 5.3.5.3b, Cage 8/Ref2-cycle 2: n=28,6, F=33.202, p<0.001, Fig. 5.3.5.3c). Before farming commenced in the first production cycle the Shannon

index at the cages and references was equivalent. It then declined rapidly and was significantly different throughout both the stocked and the fallow periods.

In the second production cycle the Shannon index was still very low at pen 5 (<1.0) prior to stocking. This corresponds to the community differences observed at the end of cycle 1 in the full faunal assessment. Index levels remained low throughout the farming cycle. The index levels suggest that conditions recovered fairly quickly after farming ceased (8 weeks) and indicate no difference between the reference and pen position for the rest of the fallow period (Fig. 5.3.5.3b). The Shannon index at pen position 8 was higher than at position 5 at the onset of the second farming cycle. This is probably a reflection of the extended fallow period at position 8 after the first production cycle. The index at both cages declined rapidly over the farmed phase and position 8 recovered more rapidly achieving reference levels after only 8 weeks (Fig. 5.3.5.3c).

When compared with the full community evaluation the Shannon diversity index also tended to overestimate the rate of recovery, particularly in the first cycle. However, use of this index appears to be a better approximation of the conditions than either of the previous measures. A similar level of effort is required in identification and enumeration of the fauna in calculating this index as would be required for the full community assessment. Consequently, it is hard to envisage why multivariate analysis of the data would not be preferable, as this would provide a more informative evaluation of the environmental conditions.

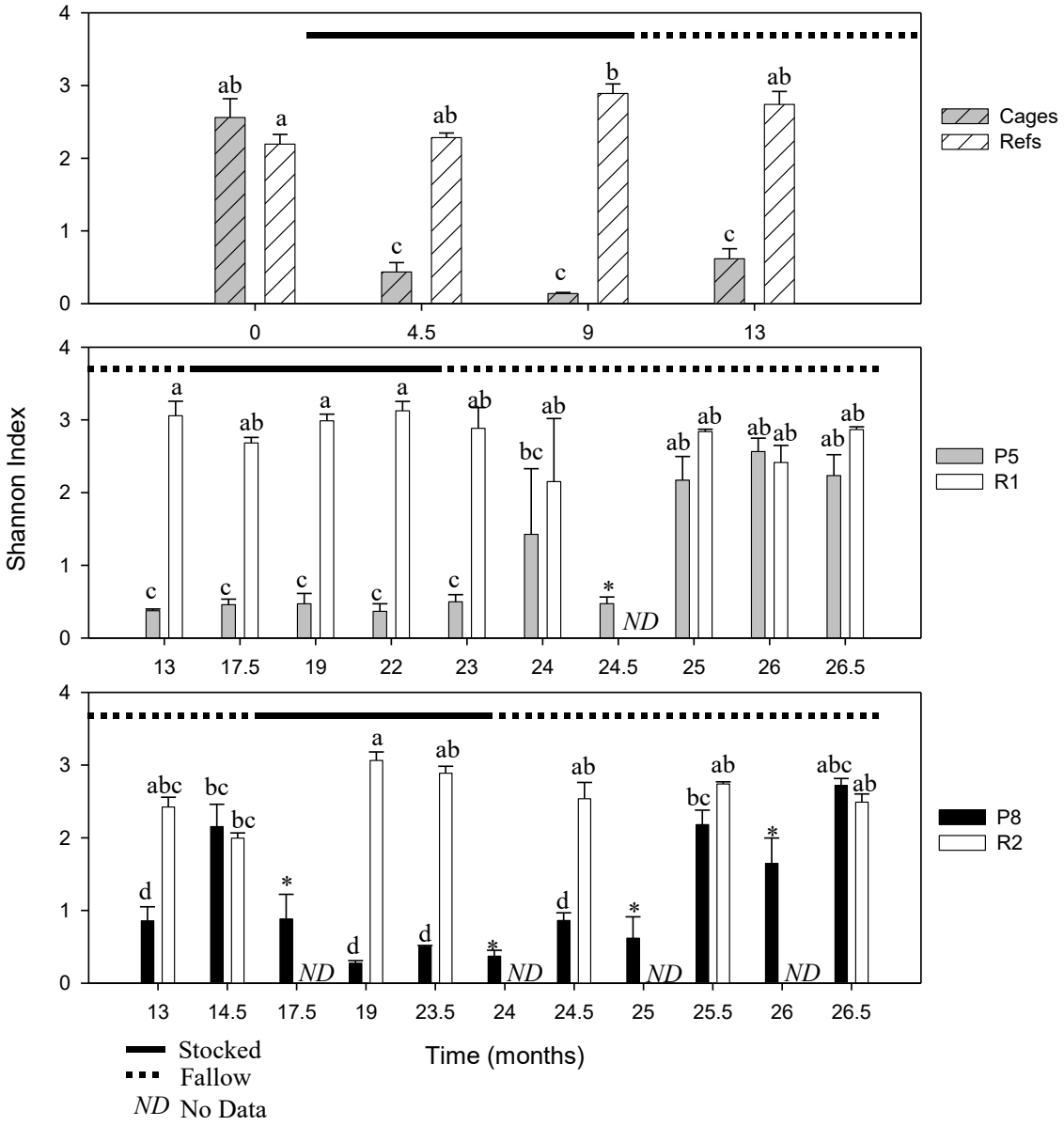


Fig.5.3.5.3. Shannon Diversity at Creeses Mistake in A) Production cycle one (treatments combined) B) Cage 5 / Ref 1 in Production cycle two and C) Cage 8 / Ref 2 in Production cycle two.

From the community analysis, the most significant change in the impacted community was the dominance of the polychaete *Capitella capitata* at the impacted communities. Consequently assessment of the change in the total abundance over time of this species might be expected to reflect the impact gradient.

The abundance of *Capitella capitata* (Fig. 5.3.5.4) shows a very similar pattern to that of total (Fig. 5.3.5.2) and annelid abundance. As with total numbers, *Capitella capitata* numbers were highest towards the end of, and immediately after, the stocked period. Abundance at the reference positions was similar over both production cycles and was much lower than at the impacted pen positions. In both cycles there was a significant interaction between time and position (Cages/Refs-cycle 1: n=40,3, $F=14.328$, $p<0.001$, Fig. 5.3.5.4a, Cage5/Ref1-cycle 2: n=34,8, $F=4.920$, $p<0.001$, Fig. 5.3.5.4b, Cage 8/Ref2-cycle 2: n=28,6, $F=11.072$, $p<0.001$, Fig. 5.3.5.4c). In both production cycles the cage and reference abundance was similar before farming

and at the end of the defined fallow period (3 months). As with total abundance, the increased sampling interval over the fallow period for the second cycle more clearly defined the point of recovery and indicated that position 5 had recovered after only 8 weeks and that position 8 had recovered within a month (Fig. 5.3.5.4). Variation in *Capitella capitata* abundance is clearly the main reason behind the overall abundance levels.

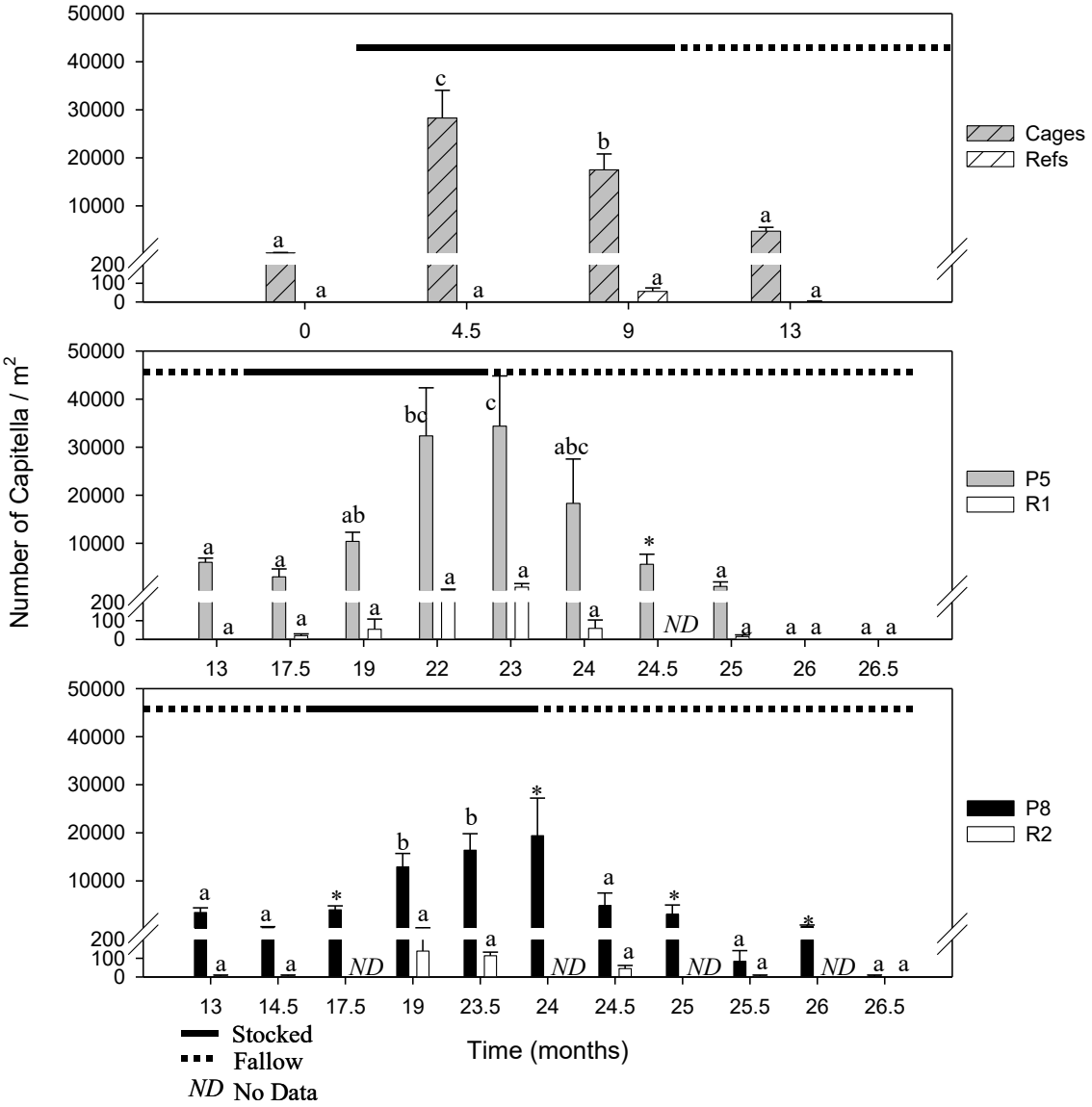


Fig. 5.3.5.4. Number of Capitella capitata at Creeses Mistake in A) Production cycle one (treatments combined) B) Cage 5 / Ref 1 in Production cycle two and C) Cage 8 / Ref 2 in Production cycle two.

5.3.6 Potential indicators and predictive capacity

Infaunal abundance clearly tracked the increase in *Capitella capitata* associated with increasing organic enrichment and the subsequent decline in this population over the fallow period. An increase in abundance greater than 100 fold over reference conditions appeared indicative of a relatively severe impact, whilst an increase greater than 10 fold seemed to indicate a lesser impact such as the residual effect of farm

operations or recovery. On its own absolute abundance overestimated the rate of recovery. It is suggested that a better understanding of recovery could be achieved if abundance was evaluated as a time series and in conjunction with either the number of species or key species information (Table 5.3.6.1). The number of species provided a useful suggestion of major environmental change; a reduction in species number relative to the reference conditions of 50% or greater indicating significant impact, conversely an equivalent improvement indicated recovering conditions. At Creeses Mistake the results show that more than 30 species in a sample might indicate a reasonably healthy sediment, less than 15 species might be deemed degraded, whilst sediments with between 15-30 species might be viewed as transitional. The presence / absence of key species in the infaunal communities can also be a useful indicator of environmental condition. At Creeses Mistake the presence of abundant *Capitella capitata* indicated deteriorated / impacted conditions whilst the presence of tanaid or ampeliscid amphipods consistently over several replicate samples and for consecutive samplings was a good indication of healthy/recovered sediments. Some simple information regarding these key species when used in conjunction with the broader biotic characterisation (i.e. total abundance and number of species) and with visual assessment techniques will give a better understanding of sediment conditions within farms and will enable more informed management decisions regarding stocking and fallowing.

Table 5.3.6.1. Summary of key indicators of impact level at Creeses Mistake.
(Species denoted by * are less significant individually for defining stage than they are in combination with others)

Impact Stage	Effect Category	Description	Generalised Benthic Categories	Key Indicator Spp (* use in combination with other species)	Shannon Index	Total Abundance	Redox Potential (mV)	Sulphide Conc. (uM)	Benthic Photo Score	Video Score	Video Features
I	No evidence of impact		Pristine indicator species present	Apseudes, Ampelisca	>2	<1,500/m2	>100mV	Below detection	Pos've	>5	Algae, Echiurans/Sipunculans
II	Minor effects (Degrading)	Small scale community change; Sediment chemistry unaffected or with only very minor effects	Larger, long lived species & pristine indicators absent. Diversity may be greater than pristine (zone of enhancement)	*Lyssianassidae, *Euphilomedes, *Polydora, *Phoxocephalidae	>2	<1,500/m2	0-100mV (or >50% ref)	Below detection	0 to -3	2.5-5	Prevalence of burrow/faunal track/tubes; Echiurans/Sipunculans
III	Moderate effects (Degrading)	Significant community change; Sediment chemistry affected	Rapid change in community mix; deposit feeding polychaetes/opportunists dominate. Filter/suspension feeders absent.	Capitella (dominant); Neanthes, *Corophium, *Polydora, *Tethygenia, *Cumacea, *Phoxocephalidae)	>1<2	>5,000/m2	0-100mV (or >50% ref)	>50uM	-4 to -3	<2.5	Sea slugs (Pleurobranchia)
IV	Major effects 1. (Degrading)	Major community change; Monospecific dominance; major sediment chemistry changes	Opportunists (esp. Capitellids) characterise community (abund >5000/m2)	Capitella (dominant); *Neanthes, *Phoxocephalidae, *Dimorphostylis	<1; No. spp. <50% of ref OR <10spp	>20,000/m2	<0mV	>100uM	<-4	Neg've	Any evidence of Beggiatoa, Gas bubbles, Black sediments;
V	Major effects 2. (Degrading)	As in Stage IV; Beggiatoa/outgassing on disturbance	Infaunal opportunists (esp Capitellids) dominate (abund >10,000/m2). Patchy beggiatoa/outgassing may be evident.	Capitella (greatly dominant); *Neanthes, *Phoxocephalidae							
VII	Major effects (Recovering)	Fauna returns to monospecific dominance; major sediment chemistry effects	Opportunists (Capitellids) still dominate but no.s dropping & other species colonising.	Capitella (dominant), *Neanthes, *Corophium, *Nebalia, *Phoxocephalidae	<1; No. spp. <50% of ref OR <10spp	>20,000/m2	<0mV	>100uM	<-4	Neg've	Any evidence of Beggiatoa, Gas bubbles, Black sediments;
VIII	Moderate effects (Recovering)	Fauna re-establishing (zone of enhancement); Sediment chemistry still affected	Transitional species prevalent - notable increase in epibenthic opportunists.	Capitella (lower no's), *Euphilomedes, *Polydora, *Euchone	>1<2	>5,000/m2	0-100mV (or >50% ref)	>50uM	-4 to -3	<2.5	Sea slugs (Pleurobranchia)
IX	Minor effects (Recovering)	Community largely recovered; Sediment chemistry recovered	Diversification of community but absence of climax/long lived species.	Mix of species with increasing crustacea and decreasing annelids. *Apseudes, *Polydora, *Euphilomedes, *Nephtys	>2	<1,500/m2	0-100mV (or >50% ref)	Below detection	0 to -3	2.5-5	Point at which sea slugs are displaced (temporal)

5.3.7 Visual assessment approaches

5.3.7.1 Benthic Community Visual Characterisation (benthic photos)

The results of the benthic community analyses showed significant changes in the abundances of several species which can be directly related to differing levels of impact and recovery. Many of these key indicator species or groups can be easily distinguished, either by virtue of their size, appearance or abundance. Consequently it might be possible to discern the main impact categories by visual comparison of sieved samples. A visual assessment technique was developed to evaluate the benthic faunal samples prior to sorting and identification.

A photograph of each sieved benthic sample was scored for a variety of features and the results analysed using multivariate techniques. Positive scores indicated good environmental conditions, with scores declining as conditions deteriorate, and highly negative scores reflecting severely degraded environments.

Analysis of photo scores from cage 5 and reference 1 showed a significant interaction between site (cage/ reference) and sampling time ($F_{7,32}$=4.360, p=0.002). Scores at the cage were significantly lower than at the reference towards the end of the stocking cycle and this persisted into the fallow period (Fig.5.3.7.1.1). After three months fallow (3 months and 25 months), although the photo score was still negative, conditions had improved and this improvement continued until the end of the fallow period in both production cycles (4.5 months, 26.5 months).

Cage 8 was empty for an additional 1.5 months at the end of the first production cycle. In this instance, although there was a significant difference between the cage and reference overall ($F_{1,18}$=135.864, p=0.000), there was no significant interaction over time ($F_{4,18}$=2.927, p=0.050), which suggests that conditions had not recovered after three months fallow. This contradicts the physical-chemical results (redox potential and sulphide concentration) but corresponds with the results of the full benthic community analysis, which indicated that although the community at cage 8 was recovering after 3 months fallow (26.5 months) it had not recovered to reference conditions. Photo scores showed a positive and significant ($F_{1,17}$=58.718, p=0.000) relationship to Shannon diversity (H') (Fig.5.3.7.1.1C). Accordingly, benthic photo analysis represents a more sensitive measure of change than the standard physical-chemical approaches for evaluation of sediment condition. Benthic photo scores represent a useful and cost-effective approach for analysing sediment condition that could easily be employed for farm-based assessment or as an early indicator of environmental degradation.

Positive video scores were always associated with unimpacted environments, values between 0 and –3 occurred in sediments which had only a minor impact, either were recently impacted or were well along in recovery. Scores between –4 and-3 suggest a moderate impact whilst scores greater than –4 would still be considered as highly impacted.The features which most strongly characterised the negative scores were capitellid worms, presence of *Beggiatoa* and gas emission (Table 5.3.6.1), whilst large invertebrtaes (eg. hermit crabs and heart urchins) best characterised unimpacted and improving conditions. With some training these features are relatively easy to distinguish and would give a quick evaluation of sediment infaunal condition that would complement both geochemical and epifau

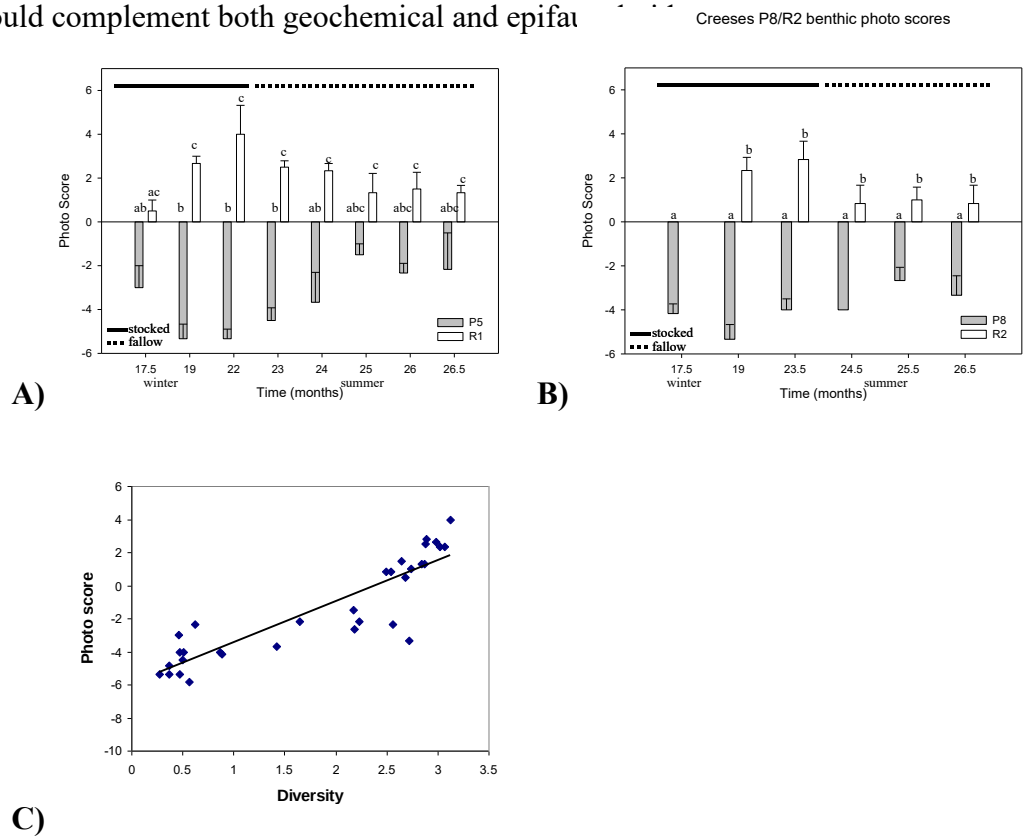


Fig.5.3.7.1.1. Photo scores at A) Cage 5 & Reference 1 and B) Cage 8 & Reference 2 at Creeses Mistake (second production cycle only). Letters represent ANOVA results. Scores are in the range of 10.5 to –14, with more positive scores representing good sediment conditions, and more negative scores representing impacted sediment conditions. C) Plot of Shannon Diversity and Benthic Photo Scores at Creeses Mistake during the second production cycle. Equation y = 2.4802x - 5.9525 and R^2 =0.7894.

5.3.7.2 Video Assessment

From the outset of this study it was anticipated that some form of video assessment would provide a simple method of rapidly assessing sediment condition. The video assessment technique is based on an approach proposed by Crawford *et al*. (2001). Refinement of the video assessment features with the inclusion of more site-specific criteria and weighting of these features has resulted in a considerable improvement in the sensitivity of the approach. Video footage was collected from spot dives conducted at each site. For each video key features indicative of varying effect levels were assessed and appropriate scores assigned. These scores were summed to give an overall ranking for each site and time and the scores for each feature were also used in multivariate analysis. The results were then compared between sites and over time and the outcomes compared with the findings of the full benthic community structure.

Like the benthic photo scores video scores reflect the level of impact at the site at that time; higher, positive scores representing good sediment conditions and lower, negative scores representing impacted sediment conditions. The results were analysed using both univariate and multivariate techniques. Since there is only a single piece of video footage from each site at each time, the cages have been treated as replicates in the univariate analyses. To allow for the differing lengths of fallow time at each cage prior to the second cycle, samples were combined relative to absolute time since stocking, rather than the sampling times in months.

In the first production cycle, there was a significant interaction between treatment (cage / reference) and time ($F_{3,8}$=11.977, p=0.003). At the start of the first production cycle the cage and reference positions were equivalent, at all other times (including after 3 months fallowing) the video scores at the cages were significantly lower than at the references (Fig.5.3.7.2.1a), showing a difference between the cages and references throughout the study. In the second production cycle, the two treatments (cage / reference) were significantly different throughout ($F_{1,11}$=67.053, p=0.000), with no interaction ($F_{5,11}$=0.414, p=0.830) (5.3.7.2.1b). This indicates that the cages did not recover after the fallow period in either cycle even with the reduced farming intensity in the second cycle. The total scores and the values for the individual features suggest that the sediments were not heavily impacted in the second cycle, the worst conditions in this cycle only scored –2, and at the onset of fallowing the total scores very quickly returned to positive values. Video assessment is comparatively sensitive, as it is able to distinguish between cages and references at relatively low impact levels.

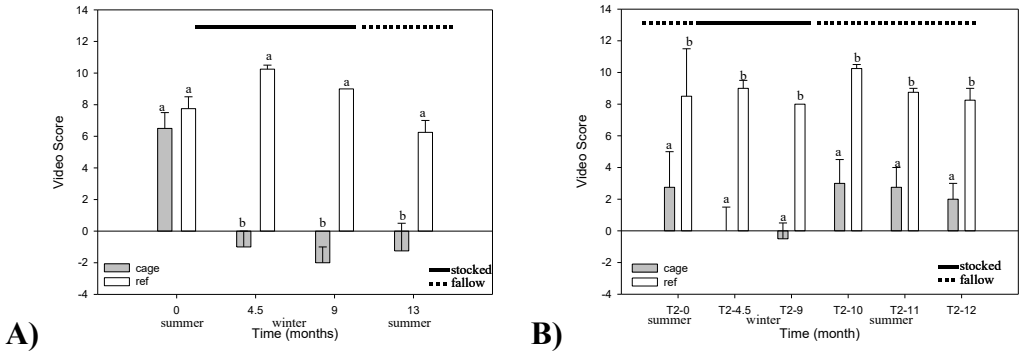


Fig. 5.3.7.2.1. Video scores at Creeses Mistake during A) the first production cycle and B) the second production cycle. Times shown indicate time in months post stocking not actual time. Where letters differ results were significantly different.

Multivariate analysis provides a better understanding of the features that distinguish the various categories of impact, with the samples separated into two principal groups (Fig.5.3.7.2.2). Group 1 comprised all reference sites as well as the two cages at the start of the study and was representative of unimpacted conditions. Group 2 consisted of the remaining cage sites and represents the impacted conditions. Group 2 was further separated into three sub-groups, 2A comprised the cages from the first production cycle, 2B comprised the stocked cages from the second production cycle and those from early in fallow period, whilst 2C contained the cages from the end of the 3-4.5 month fallow period from the second cycle. Group 2A was most dissimilar to the unimpacted conditions (group 1) and 2C was least dissimilar (Table 5.3.7.2.1).

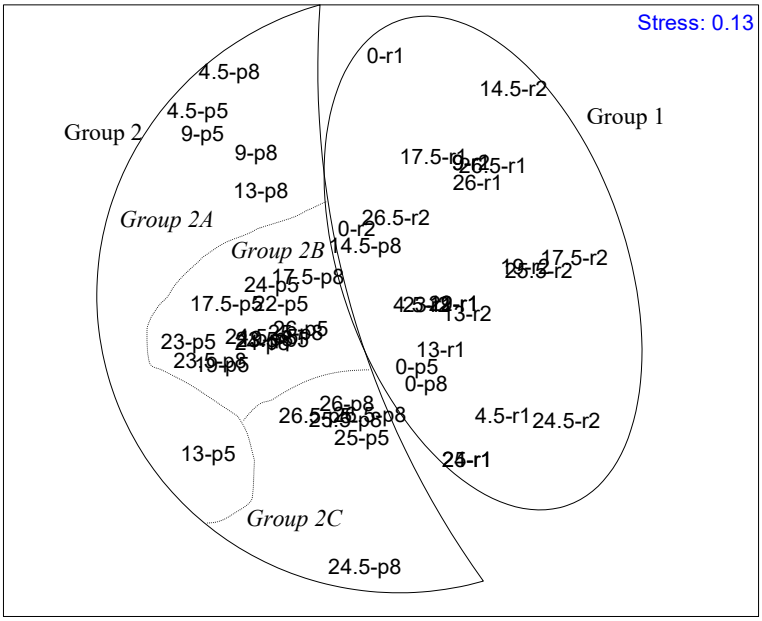


Fig. 5.3.7.2.2. Ordination analysis – 2 dimensional MDS plot of video assessment data from all sites and times at Site 1. Stress = 0.13.

The features discriminating the samples within the groups were very similar. Comparison between groups also showed differences in the features underpinning the group separations. The samples comprising group 1 generally had relatively high scores for burrow density and faunal tracks, and scores for % algal cover and presence of Echiurans and Annelids were consistently higher than in other groups. The echiuran *Ikeda* sp. was commonly found at the references and was never observed at the cage sites. Similarly, alga was commonly recorded at reference locations but was rarely seen at the cages after stocking. Decline in algae near tuna cages in South Australia was attributed to higher sedimentation rates and shading from the cages (Cheshire *et al*., 1994).

Bacterial mats (*Beggiatoa spp.*) and pellets/faeces were more prevalent and there was a greater incidence of burrows and worm tubes/casts under the cages stocked in the first production cycle (Group 2A) than in the second production cycle. The side-gilled sea slug (*Pleurobranchia maculata*) was frequently observed at cages in the second production cycle, whilst it was completely absent in the first production cycle. Comparison of groups 2B (cages stocked in the second cycle) and 2C (cages during the 2nd fallow period) suggests that some recovery is occurring at the cages in group 2C (Table 5.3.7.2.1). Burrows, faunal tracks and sea slugs were less obvious at group 2B than 2C sites, and at group 2C sites the sediment colour also improved. At group 2C sites more debris (e.g. fouling material such as mussels and mussel shells) was observed than at other sites, possibly as a result of the movement and cleaning of cages following removal of the fish. The increase in burrow density at group 2C sites may reflect increased faunal colonisation during recovery.

Table 5.3.7.2.1. SIMPER output for the video assessment indicating A) average scores, ratio (average similarity / st.dev. similarity), % similarity and cumulative % similarity of the 8 most important video parameters in each of the four main MDS cluster groups and B) average score, ratio (average similarity / standard deviation similarity) and cumulative % dissimilarity of the five parameters which most clearly distinguish the main groups identified by cluster analysis.

Video Parameter a) WITHIN GROUPS	Avg Score	Ratio	Percent Similarity	Cumulative % Similarity
Group 1				
Burrow Density	2.64	131.32	5.65	5.65
% Algal Cover	2.1	193.6	5.63	11.29
Faunal Tracks	0.96	394.03	5.59	16.87
Maoricolpus	0.92	220.07	5.58	22.45
Echiurans & Annelids	0.96	145.01	5.57	28.02
Sea Stars	0.36	319.99	5.54	33.56
Worms Tubes/Casts	0.32	345.83	5.54	39.11
Crustaceans	0.2	522.59	5.54	44.65
Group 2a				
Maoricolpus	0.5	241.67	5.58	5.58
Sea Stars	0.67	250.57	5.58	11.16
Faunal Tracks	0.33	378.74	5.57	16.73
Gas Bubbles	0	2010.2	5.57	22.3
% Algal Cover	0	2010.2	5.57	27.87
Burrow Density	0.25	2010.2	5.57	33.44
Worms Tubes/Casts	0	2010.2	5.57	39.01
Nassarid Gastropods	0	2010.2	5.57	44.58
Group 2b				
Burrow Density	1.5	2404.7	5.64	5.64
Sea Stars	0.77	208.39	5.59	11.23
Worms Tubes/Casts	0.69	203.33	5.58	16.82
Maoricolpus	0.31	360.21	5.56	22.38
Gas Bubbles	0	2404.7	5.56	27.94
Beggiatoa	0	2404.7	5.56	33.5
% Algal Cover	0	2404.7	5.56	39.06
Faunal Tracks	0.08	2404.7	5.56	44.62
Group 2c				
Burrow Density	3.25	1501.6	5.72	5.72
Faunal Tracks	0.67	209.47	5.57	11.29
Sea Stars	0.67	218.14	5.57	16.86
Worms Tubes/Casts	0.33	416.22	5.55	22.41
Maoricolpus	0.33	423.58	5.55	27.97
Gas Bubbles	0	1501.6	5.55	33.51
Beggiatoa	0	1501.6	5.55	39.06
% Algal Cover	0	1501.6	5.55	44.61

b) BETWEEN GROUPS	Group Avg Score	Group Avg Score	Ratio	Cumulative % Dissimilarity
Groups 1 & 2a	*Group 1*	*Group 2a*		
Burrow Density	2.64	0.25	1.98	20.34
% Algal Cover	2.1	0	2.85	38.21
Pellets/Faeces	0	-1.17	1.3	48.14
Echiurans & Annelids	0.96	0	1.15	56.31
Beggiatoa	-0.06	-0.83	1.22	63.4
Groups 1 & 2b	*Group 1*	*Group 2b*		
% Algal Cover	2.1	0	2.85	20.39
Burrow Density	2.64	1.5	1.07	31.46
Sea slugs	0	-1.08	1.47	41.92
Echiurans & Annelids	0.96	0	1.15	51.24
Faunal Tracks	0.96	0.08	2.83	59.87
Groups 1 & 2c	*Group 1*	*Group 2c*		
% Algal Cover	2.1	0	2.85	26.48
Burrow Density	2.64	3.25	1.13	39.22
Echiurans & Annelids	0.96	0	1.15	51.32
Debris	-0.12	-0.83	1.74	60.82
Maoricolpus	0.92	0.33	1.22	69.56
Groups 2a & 2b	*Group 2a*	*Group 2b*		
Burrow Density	0.25	1.5	2.22	16.43
Pellets/Faeces	-1.17	-0.23	1.32	30.75
Sea slugs	0	-1.08	1.47	44.91
Beggiatoa	-0.83	0	1.21	55.86
Worms Tubes/Casts	0	0.69	1.49	64.96
Groups 2a & 2c	*Group 2a*	*Group 2c*		
Burrow Density	0.25	3.25	3.74	35.3
Pellets/Faeces	-1.17	0	1.28	49.02
Beggiatoa	-0.83	0	1.2	58.83
Sea Stars	0.67	0.67	1.14	66.67
Sediment Colour	-0.67	-0.33	1.1	73.2
Groups 2b & 2c	*Group 2b*	*Group 2c*		
Burrow Density	1.5	3.25	3.11	28.47
Sea slugs	-1.08	-0.17	1.35	44.53
Faunal Tracks	0.08	0.67	1.33	54.95
Worms Tubes/Casts	0.69	0.33	1.13	64.13
Sediment Colour	-0.69	-0.33	1.13	73.3

The similarity matrix underpinning the video data was strongly correlated with the Bray-Curtis similarity matrix for the benthic infaunal data (RELATE analysis - sample statistic Rho= 0.606, $p<0.001$). Video scores were also compared with the Shannon diversity, a univariate measure of community diversity and change, and they correlated well (Fig.5.3.7.2.3), showing a significant relationship ($F_{1,47}=96.488$, $p=0.000$). Comparison with benthic infaunal assessment of impact level suggests that scores greater than +5 indicated extremely good environmental condition, values between 0 and +5 suggested a moderate impact or transitional conditions, whilst negative values indicated poor environmental conditions and values less than –5 suggested that the sediments were considerably degraded.

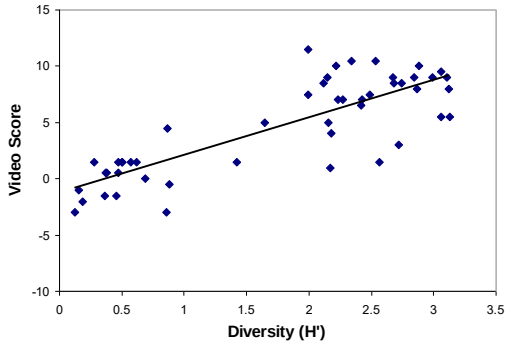


Fig.5.3.7.2.3. Plot of Shannon Diversity and Video Score at Creeses Mistake during the second production cycle. Equation y = 3.3179x - 1.216and R2 = 0.6724.

Assessment of video footage was clearly able to identify differing levels of impact both temporally and spatially. The visual indicators could also identify the transition period in recovery, and consequently this would be a very useful approach for farm-based evaluation of sediment condition.

5.3.8 Summary

The cages sampled at Creeses Mistake site were all from areas which had previously been subjected to farming and therefore the pre-farming samples could not be considered equivalent to those from the references.

Creeses Mistake was the more exposed site in this study and the sediments reflected these environmental conditions being predominantly fine sands. Sediment type is one of the most important features in determining the nature of infaunal communities (Hall, 1994). The infaunal community structure at Creeses Mistake changed markedly in response to cage farming, with several distinct stages of degradation and recovery evident (Fig. 5.3.8.1). These stages could be characterised by particular changes in the infaunal composition at both species and at higher levels. Some of these changes are relatively easy to distinguish and could be employed by farms to assess sediment condition.

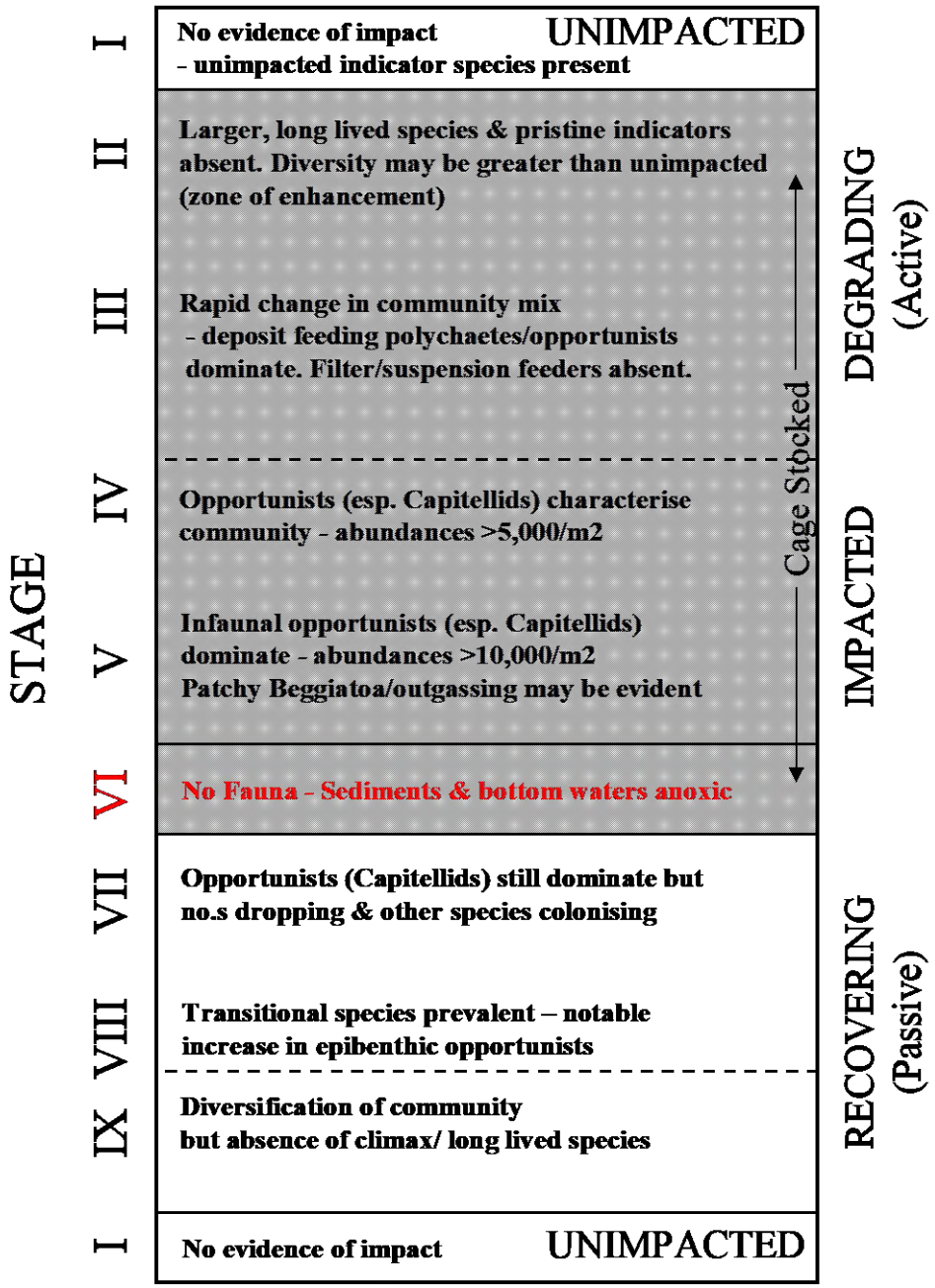


Fig.5.3.8.1. General characterisation of impact/recovery stages based on main community changes.

Environmental impacts affect the infaunal community at various levels depending on the severity of effect; minor effects result in species level changes whist more significant impacts will affect the community at a higher taxonomic level (Somerfield and Clarke, 1995). At Creeses Mistake the background faunal community was characterised by crustaceans. With the onset of farming this changed markedly and polychaetes became the dominant community group. This represents a community shift at the phylum level and indicates a severe change. Consequently discerning impacted from non-impacted community is relatively easy. Subtle changes reflecting degrees of impact and recovery occur at lower taxonomic level and are harder to distinguish and are perhaps beyond the capability of farm personnel. Nonetheless such changes could be used by skilled technical staff and researchers to discern the differing levels of impact (Fig. 5.3.8.2). These changes in the faunal composition will result in changes in the system function (Fig. 5.3.8.2), which in turn will affect the ability of the sediments to cope with continued enrichment.

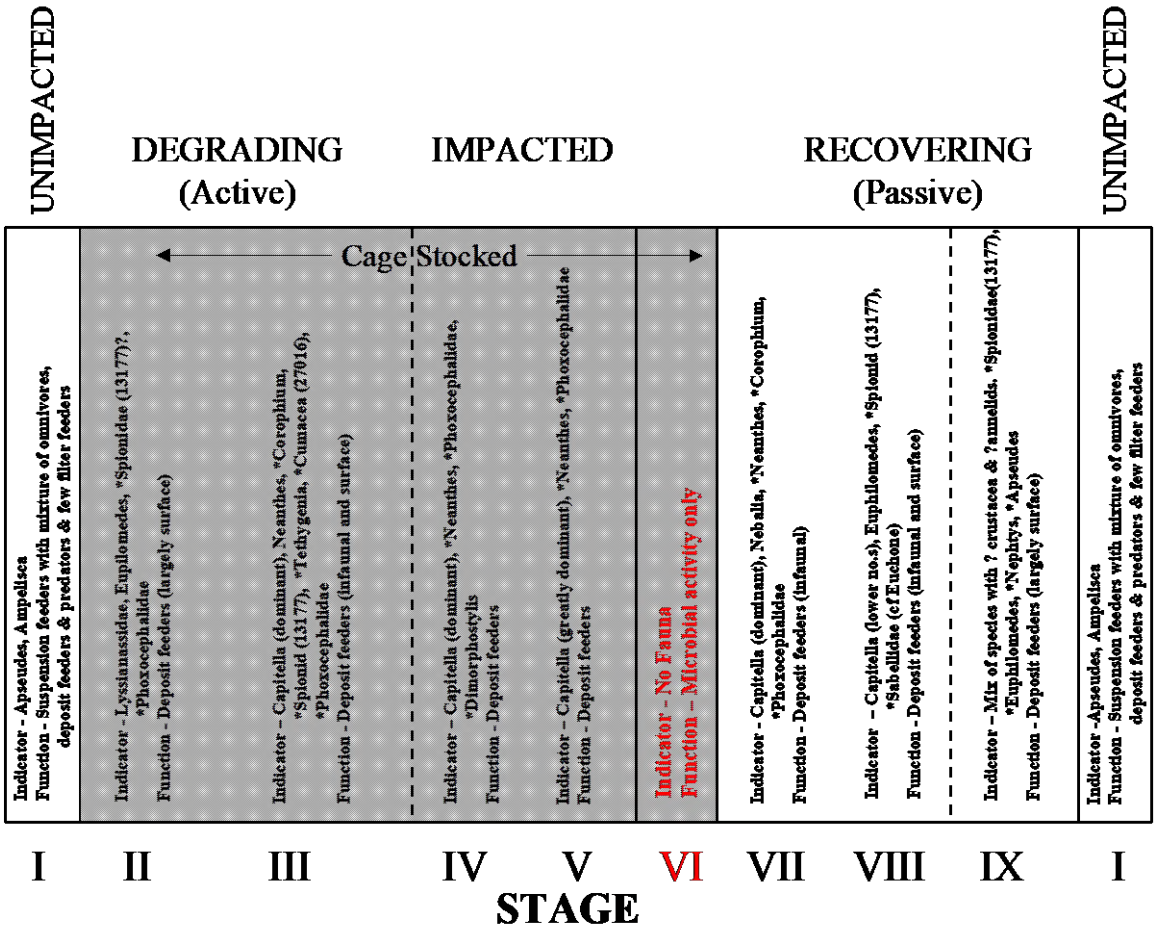


Fig.5.3.8.2. Indicator genera (or higher taxonomic groups) characterising impact/recovery stages and main functional identity of stages at Creeses Mistake.

Although farm-personnel may not be able to identify all of the faunal changes associated with the varying levels of impact, there were many changes in the community structure and abundance levels of particular key species that could be characterised relatively easily and which would provide an adequate understanding of the overall sediment condition (Fig. 5.3.8.3).

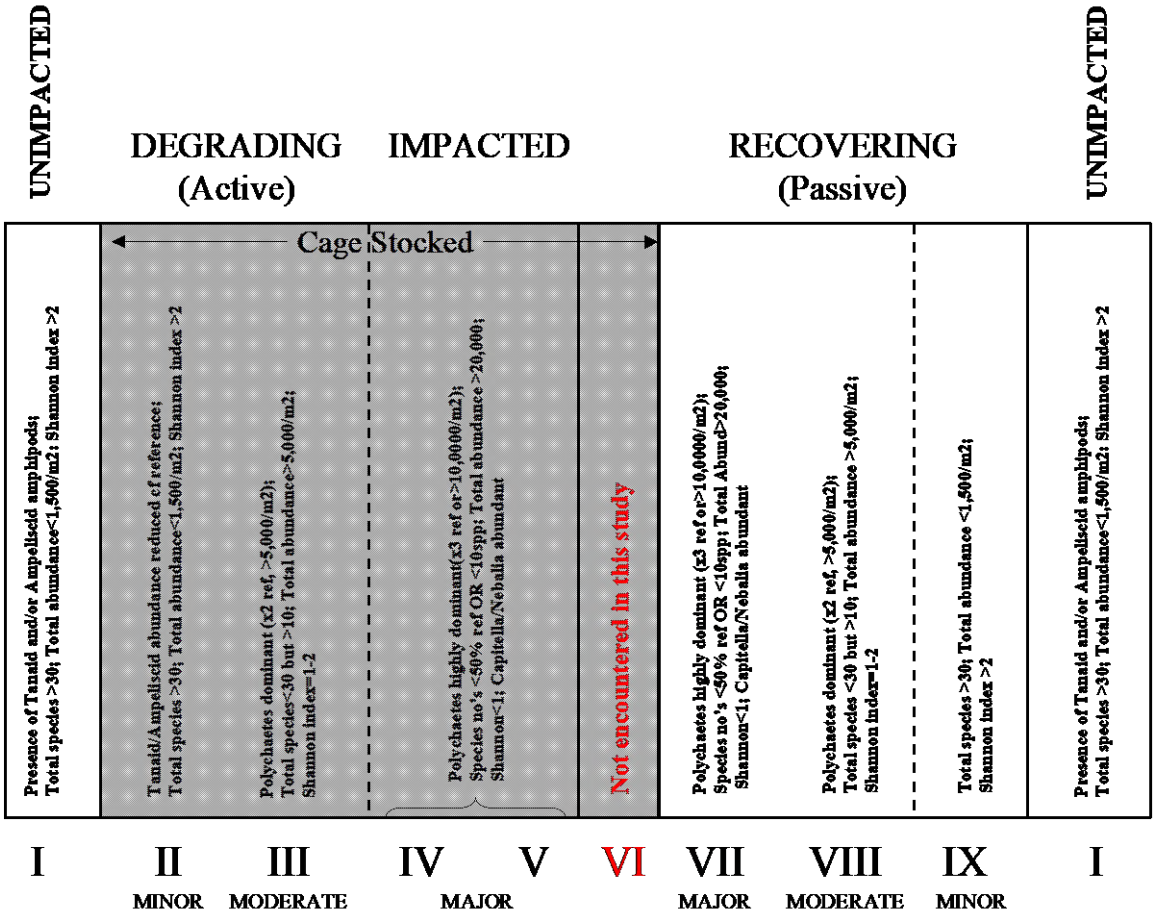


Fig. 5.3.8.3. Community features characterising impact/recovery stages at Creeses Mistake.

Two species were particularly significant in the unimpacted communities, *Ampelisca* and *Apseudes.* These species were present at recovered farm sites, and there were marked differences in abundance between recovered and reference samples. Unimpacted sediments generally contained more than 450 and 700 individuals /m2 of *Ampelisca* and *Apseudes* respectively. When sediment was impacted *Ampelisca* was entirely absent and only 1-2 individuals of *Apseudes* were noted. Consequently, the presence of between 150-300 individuals /m2 of *Apseudes* is considered indicative of recovery to at least a farm level. The presence of more than just one or two *Ampelisca* would indicate a high level of recovery. These species are quite large and distinctive and would be easy to distinguish even by a non-skilled ecologist, particularly when present in abundance. Phoxocephalids, another crustacean group, were also notably more abundant in the samples from recovered positions than at either the impacted or reference positions.

The reference sites at Creeses recorded between 35-50 species, where there was a major impact this was reduced to 10-15 species. Consequently where <10 species are identified it could be concluded that a marked impact had occurred (stage IV,V or VII) (Fig. 4.3.8.3). Total abundance was also slightly greater at Creeses at the reference positions. The impacted sites did not show as marked an increase in numbers as at Stringers Cove. Reference sites generally had less than < 5,000/m2 whereas impacted sites had >20,000 individuals/m2. Consequently at Creeses Mistake total abundance less than 1,500/m2 suggests that conditions are acceptable. Abundance greater than 5,000/m2 would suggest a moderate impact and more than 20,000 individuals /m2 would indicate a major impact (Fig. 5.3.8.3).

Total abundance and number of species can be combined to calculate the Shannon diversity index (Shannon and Weaver, 1963). This index is often applied to evaluate environmental condition. Low index values indicate poor environmental quality and high values represent relatively healthy sediments. In the present study Shannon index values greater than 2 were consistent with unimpacted sediments or conditions with very minor impacts, values between 1-2 indicated a moderate impact whilst Shannon values less than 1 were generally associated with significantly impacted sediments (Fig. 5.3.8.3). Although calculation of the Shannon index is reliant on determination of total number of individuals and species in a sample, it is not necessary to be absolutely exact in evaluating these parameters. This index and the definition of impact stage are quite robust to variation in both total abundance and number of species, the magnitude of the change involved is such that an estimate of abundance for large samples will not adversely affect the outcome.

Both benthic photo scores and video assessment show particular promise as farm based assessment options. The key features underpinning the scores reflect the faunal differences indicated above and therefore can be used to characterise the impact/recovery stages (Fig. 5.3.8.4). Although many of the key features were consistent between the two study sites there were some differences particular to Creeses Mistake. Bacterial mats (*Beggiatoa spp.*) are considered a very significant indicator of deteriorated environmental conditions (GESAMP, 1996), but even although the community information suggested that conditions were appreciably degraded there was little evidence of bacterial mats at Creeses Mistake. In contrast algae was a feature at the references/recovered sites and appeared to be a good indicator of recovery. Other features unique to Creeses Mistake included the presence of echiurans and large annelids which appeared to be good indicators of unimpacted/recovered conditions. Sea slugs were only present when impact was moderate, they were not observed in unimpacted conditions or when a major impact had occurred. Consequently in temporal monitoring of recovery the point at which sea slugs are displaced may indicate an appreciable improvement in sediment condition.

Most of these changes in community structure and the video/photo features are easy to discern and farm personnel could be trained to perform this.

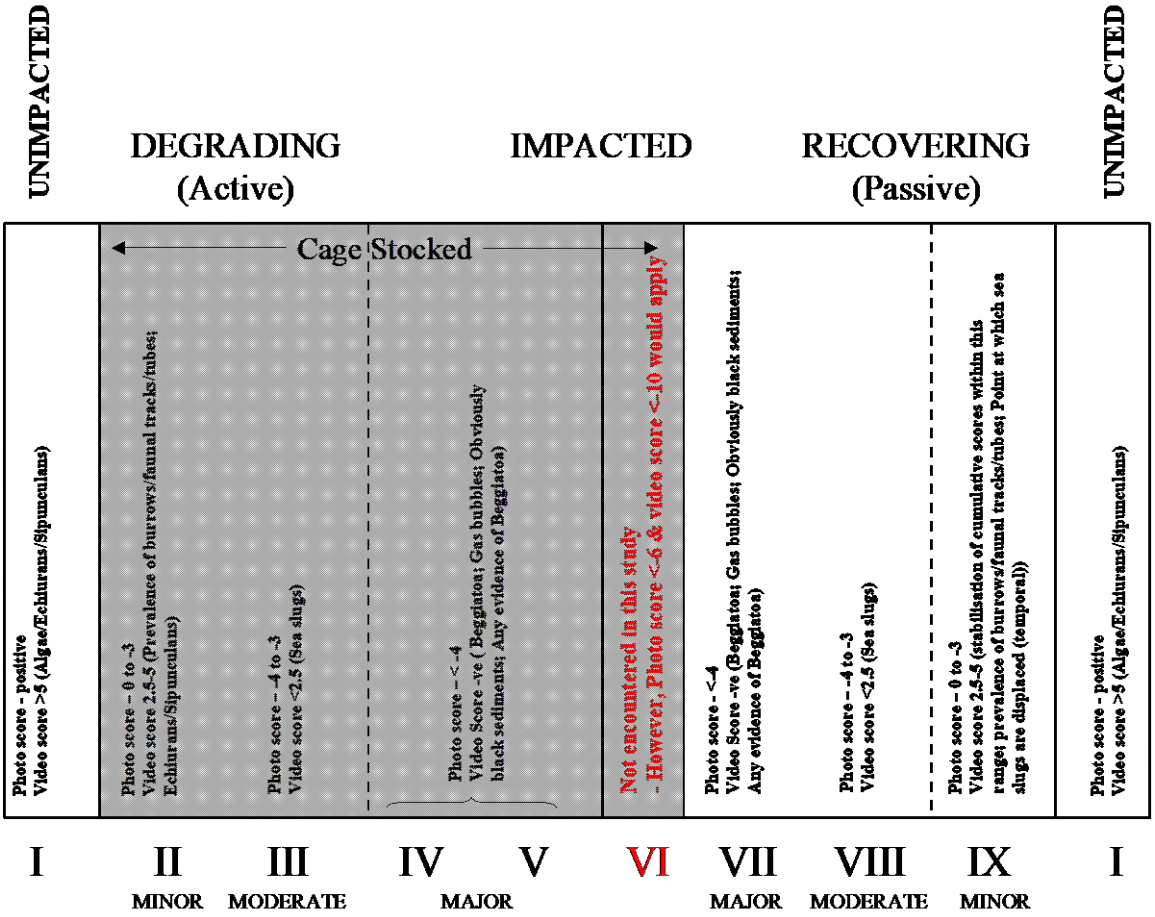


Fig. 5.3.8.4. Visual assessment features characterising impact/recovery stages at Creeses Mistake.

Several aspects of the reference data indicated a pattern of long-term temporal change. Community structure, individual species abundances and total number of species all exhibited changes that could be either natural variability or an indication of broader farming influence. Continued monitoring at these sites would ascertain the specific cause of these changes.

5.4 Sediment Biogeochemistry

5.4.1 Granulometry & Organic Matter Determination

5.4.1.1 Granulometry

The sediment was predominantly fine sand, with the silt/clay fraction constituting only a very minor component (Fig.5.4.1.1.1). The results indicated a significant interaction between treatment (cage/reference) and time in production cycle 1 ($F_{3,8}$=4.779, p=0.034) and a significant difference between cage and reference treatments in the second production cycle ($F_{1,12}$=5.331, p=0.040). This was the result of a small decline in the fine sand component at only one of the references at 13 months (end of first fallow cycle and start of second production cycle, therefore included in analysis of both cycles). As only one replicate was collected from each reference, this may be the result of small-scale patchiness within the environment.

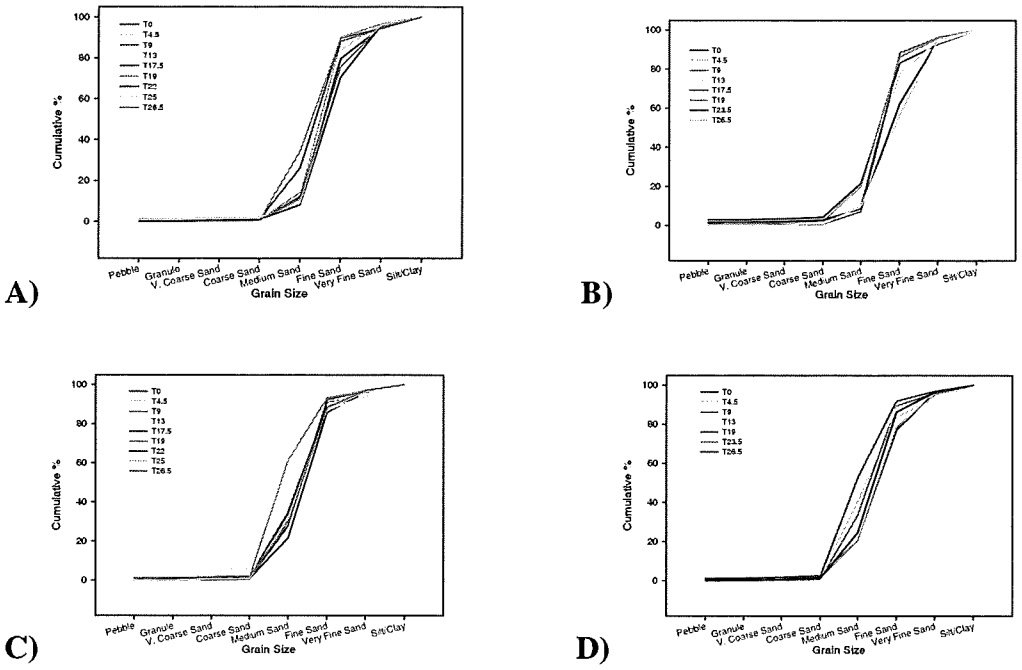


Fig.5.4.1.1.1. Grain size distribution at A) Cage 5 B) Cage 8 C) Reference 1 and D) Reference 2 at all major sampling times in both production cycles at Creeses Mistake.

There does not appear to be a shift towards finer sediments, as might be expected with increased farm deposition, as the silt/clay fraction did not show any significant changes with treatment or time (Cycle 1 treatment*time: $F_{3,8}$=3.531, p=0.068, treatment: $F_{1,8}$=0.504, p=0.498; Cycle 2 treatment*time: $F_{5,12}$=1.275, p=0.336, treatment: $F_{1,12}$=3.662, p=0.080).

5.4.1.2 Organic Matter Content

Total organic matter (TOM) was determined (using percentage loss on ignition) over the first production cycle. Levels did not differ between cage and reference at any time ($F_{1,56}$=0.278, p=0.600). However, there was a significant change over time, the TOM levels were higher at the start of the study than at any other time ($F_{3,56}$=70.708, p=0.000) (Fig. 5.4.1.2.1). This may be a sampling anomally, as the initial samples were collected and processed by a different individual than all subsequent samples.

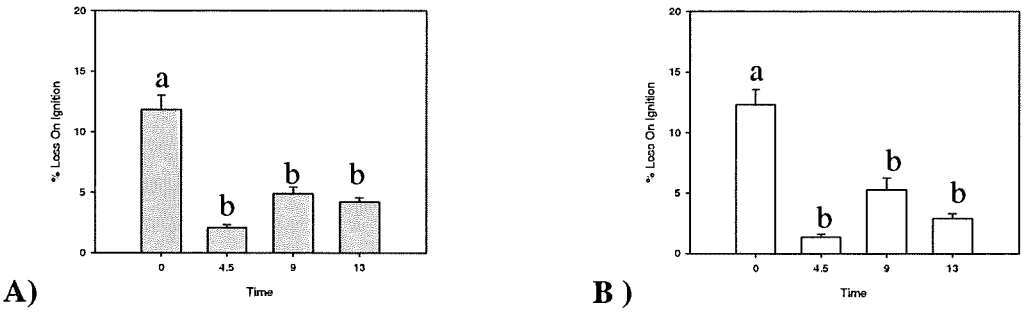


Fig.5.4.1.2.1. Total Organic Matter (Loss on Ignition) results (mean with standard error) at A) Cages and B) References during the first production cycle at Creeses Mistake. Letters indicate ANOVA results (applicable to both plots).

However, when the analysis was repeated excluding the initial sampling data there is still a significant temporal relationship ($F_{2,42}$=20.411, p=0.000), suggesting a seasonal or temporally cyclic pattern. There is no evidence that this pattern is related to farm activities. As a result of the variability in the results and the technique's inability to reflect the sedimentary changes at

stocked sites observed using other techniques, analysis of TOM was not continued for the second production cycle.

5.4.2 Redox potential & sulphide concentration

5.4.2.1 Redox Results

In the first production cycle at Creeses Mistake, both cages were stocked at similar levels and for the same duration. As a result there was no significant variation associated with site as a nested term within treatment (two-way nested ANOVA of time, depth, treatment (site)). However, due to changes to the production schedule at cage 8 in the second production cycle (see section 4.2), the two cages could no longer be treated as replicates (there was significant variation associated with the nested term ($F_{2,396}$=6.596, p=0.002)). Accordingly, for all analyses at Creeses Mistake, the cages were treated as a single treatment in the first production cycle, and separate treatments in the second production cycle.

In the first production cycle, there was a significant interaction between treatment (cage and reference) and depth ($F_{3,120}$=2.993, p=0.034). In addition, there was a significant difference between cage and reference treatments ($F_{1,120}$=529.384, p=0.000) and between sampling times ($F_{2,120}$=22.196, p=0.000), 4.5 months (half way through stocking) significantly differed from both 9 months (end of stocking) and 13 months (end of 3 months fallow) (Fig.5.4.2.1.1a). Posthoc comparison of treatment and depth showed that at the cages redox measurements at 2cm sediment depth were significantly different from those at 5cm. In addition, cage redox measurements at all depths were significantly different from all reference redox measurements but within the reference treatment there was no significant difference with depth. At the cages the reduced conditions at the surface appear to be limiting the amount of mixing in deeper layers, and therefore redox potential levels deeper in the sediment were significantly lower than those at the surface (Fig.5.4.2.1.1b).

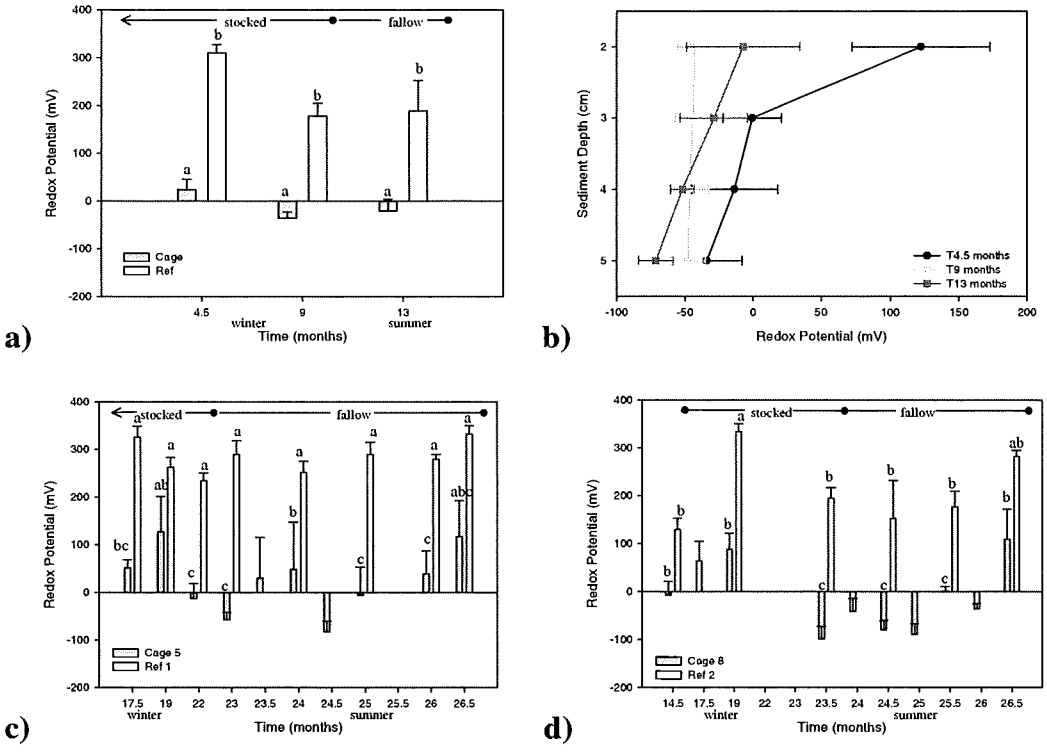


Fig.5.4.2.1.1 a) Redox potential (mean and standard error) at cage and references (combined) during production cycle 1. b) Redox potential at Creeses Mistake cage sediments at all measured depths (Cages 5 and 8 combined) in

the first production cycle only. c) Redox potential at cage 5 and ref 1 during production cycle 2. d) Redox potential at cage 8 and ref 2 during production cycle 2. Letters indicate ANOVA results (where letters are not present, data not included in statistical analysis due to unbalanced design).

In the second production cycle, the cages received differing treatments. Cage 5 was fallowed for three months between the two cycles (Scenario 1), whilst cage 8 was fallowed for 4.5 months (Scenario 2). As a result, the cages were treated separately, and analysed in relation to their respective references (ie. cage 5: ref.1; cage 8: ref.2).

In the first scenario, there was a significant interaction between site (cage 5 / ref.1) and time ($F_{7,127}$=3.479, p=0.002). Pairwise comparisons indicate that cage 5 was significantly different from reference 1 at all times in the second production cycle, including at the end of three and 4.5 months fallow (Fig. 5.4.2.1.1c). There appeared to be some level of 'recovery' in redox potential at cage 5 at 19 months (6 months into the second stocking cycle), possibly as a result of increased bioturbation activity by opportunistic species resulting in greater mixing and therefore increasing redox potential levels. In addition to the site/time interaction, depth was once again a significant factor ($F_{3,127}$=7.157, p=0.000). Posthoc testing showed that levels at 2cm differed significantly from those at 4 and 5cm.

In scenario two, cage 8 was fallowed for an additional 1.5 months between production cycles. This extra time appears to have resulted in greater recovery, (14.5 months, Fig. 5.4.2.1.1.d). However, there was a significant interaction between site (cage 8 / ref 2) and time ($F_{4,80}$=7.249, p=0.000) in the second production cycle. Pairwise comparison of the results showed that redox potential differed significantly between cage 8 and ref. 2 at all times during the second production cycle except at 14.5 months (end of extended fallow period / start of stocking in second cycle) and at 26.5 months (after three months fallow) (Fig. 5.4.2.1.1.d). This suggests that cage 8 did recover to reference redox conditions after 3 months fallowing in the second cycle. That cage 8 recovered after the second cycle whilst cage 5 did not suggests that the additional 1.5 months recovery at the end of the first production cycle was a significant factor in facilitating the subsequent recovery.

Once again, there were significant differences between measurement depths in scenario 2 ($F_{3,80}$=4.344, p=0.007), posthoc analysis showing that 2cm significantly differed from 4 and 5cm. These results suggest that 3cm would be the most appropriate depth for sampling redox to get an integrated evaluation of the whole core.

5.4.2.2 Sulphide Results

The two cages at Creeses Mistake effectively acted as replicates in the first production cycle (with no significant variation associated with sites nested within treatments). Due to the differences in fallow times at the two cages, there was significant variation associated with site within treatment in the second production cycle ($F_{2,361}$=4.056, p=0.018) and as a result the two cages were analysed separately in comparison to their respective reference (cage 5 – reference 1; cage 8 – reference 2).

In the first production cycle, there was a significant interaction between treatment (cage and reference) and time ($F_{2,115}$=16.349, p=0.000). Cage sulphide levels at 4.5 months (half way through stocking) differed significantly from those at the end of stocking (9 months) and at the end of the fallow period (13 months) (Fig. 5.4.2.2.1.a). Measurements mid-way through stocking (4.5 months) were not different from the references. Levels at the end of stocking (9 months) and after fallowing (13 months) were significantly different from the reference. The build up of organic material appears to have had a delayed effect on sulphide concentration, but once established the effect persisted through the fallow period.

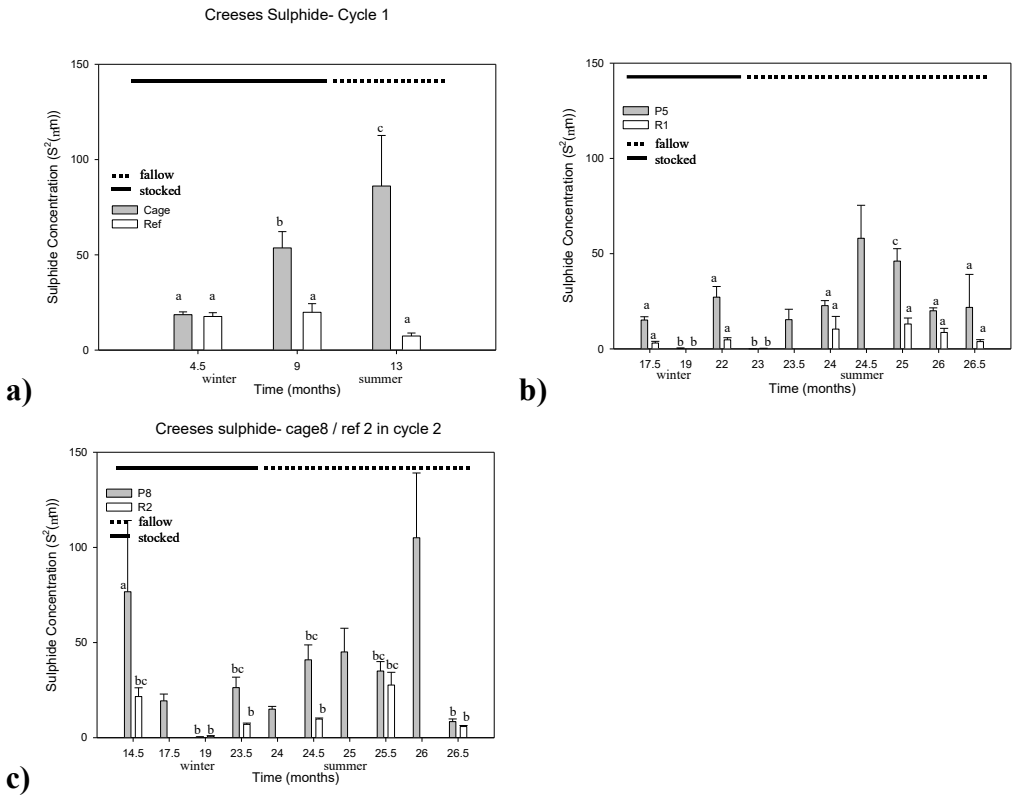


Fig.5.4.2.2.1. a) Sulphide concentration (mean and standard error) at cage and references (combined) during production cycle 1. b) Sulphide concentration at cage 5 and ref 1 during production cycle 2. c) Sulphide concentration at cage 8 and ref 2 during production cycle 2. Letters indicate ANOVA results (where letters are not present, measurements were recorded but data were not included in statistical analysis due to unbalanced design).

In the second production cycle, there was a significant interaction between sites and times in both scenario one (cage 5 / reference 1)($F_{7,122}$=6.077, p=0.000) and scenario two (cage 8 / reference 2)($F_{5,93}$=8.542, p=0.000). Sulphide concentration at cage 5 was extremely variable during both the stocking and fallowing periods. The effect of increased infaunal activity and the associated increase in bioturbation 6 months into the stocking cycle (19 months) may account for the reduction in sulphide concentration at this time (Fig. 5.4.2.2.1.b). After fallowing for 3 and 4.5 months (25 and 26.5 months respectively), sulphide concentration at cage 5 was still significantly higher than at reference 1. Sulphide concentrations at cage 8 were also highly variable, but was generally significantly higher than the reference except after three months fallow (26.5 months), suggesting that cage 8 did recover to reference conditions at the end of the second cycle.

The sulphide results are comparable with the results for redox potential. However, unlike redox potential, the depth at which sulphide was measured did not affect the outcome. Sulphide is extremely refractory and highly soluble and therefore will equilibrate throughout the sediments more readily than redox.

There was no evidence in the sulphide measurements of progressive deterioration at cage 8, which returned to reference conditions at the end of the second cycle. However, this was not as clear for cage 5, and either longer term temporal data or a more sensitive technique may be required to assess the likelihood of progressive deterioration in this instance.

5.4.2.3 Sulphide – Redox Relationship

Hargrave et al. (1997) and Wildish et al. (1999) described a strong direct linear correlation between redox and sulphide. Although there was a significant correlation (R^2=0.204,

$F_{1,535}$=137.212, p=0.000) between sulphide and redox in the present study (Fig. 5.4.2.3), the relationship was not as strong as that shown by Hargrave et al. (1997) and Wildish et al. (1999). However, the sulphide concentrations observed in this study were orders of magnitude lower than those reported by both Hargrave et al. and Wildish et al. and this may account for the poor relationship observed between redox and sulphide in this study (i.e. the low end of the impact scale doesn't show such a strong correlation).

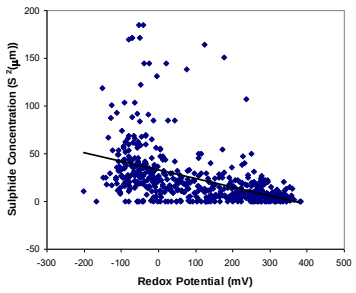


Fig.5.4.2.3.1. Redox potential and sulphide concentration at Creeses Mistake at all sites and times. Linear trendline has been fitted to the whole dataset, with the equation (y = -0.0907x + 32.937) and R^2=0.204.

5.4.3 Porewater Nutrients

5.4.3.1 Depth of Oxic Zone

Measurement of the depth of the oxic zone with microelectrodes clearly demonstrated differences between reference and cage sites. Reference sites commonly demonstrated a potential for photosynthesis (as indicated by an increase in oxygen concentration under illumination) whereas cage sites typically did not.

Because the oxic zone depth data lacked homogeneity of variance they were transformed as Log(depth + 1) and the 95% confidence limits calculated and back transformed for graphical presentation. As can be seen from Table 5.4.3.1, there was a significant interaction between site and time for both production cycles. Thus, a second ANOVA was carried out on the combined variable Site*Time for each year. This showed a significant effect of the variable in the first (F = 22.14, P=0.000, n=4) and second years (F=17.98, P=0.000, n=2). Tukey's Post Hoc test was used to determine homogenous subsets, as shown in Figures 5.4.3..1 and 5.4.3.2.

Table 5.4.3.1. ANOVA of the log transformed data for the mean oxic depth (mm) for both years of production assessed at Creeses Mistake.

Dependent Variable: LOG(Oxic depth+1)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.817(a)	19	.096	21.284	.000
Intercept	10.093	1	10.093	2246.795	.000
SITE	1.292	1	1.292	287.598	.000
TIME	.183	9	.020	4.521	.000
SITE * TIME	.248	9	.028	6.128	.000
Error	.171	38	.004		
Total	12.443	58			
Corrected Total	1.987	57			

a R Squared = .914 (Adjusted R Squared = .871)

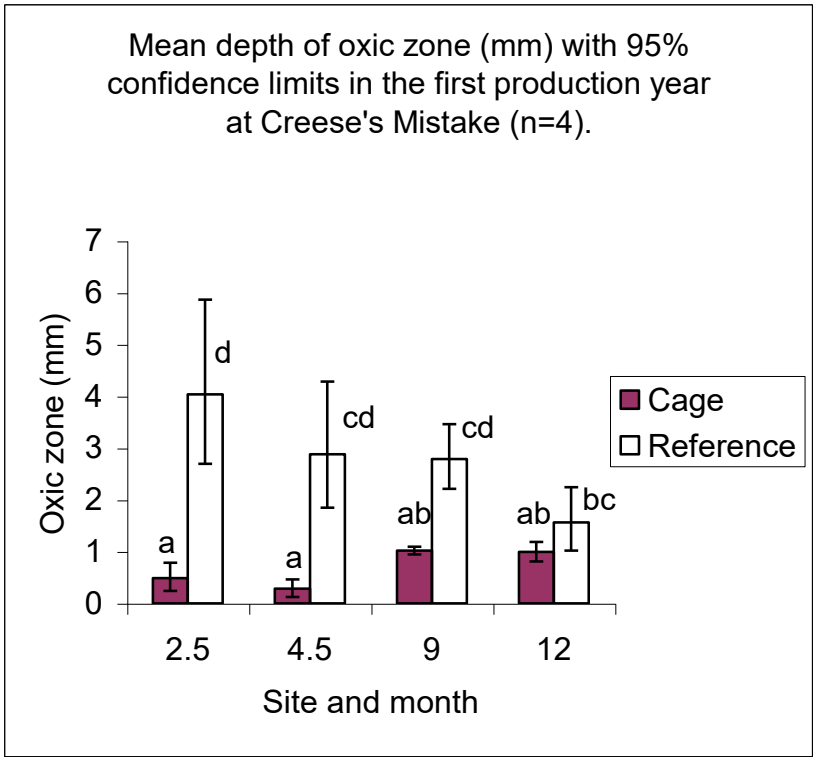


Fig. 5.4.3.1. Mean depth of oxygen penetration (mm) with 95% confidence limits for the first production cycle at Creeses Mistake. Different superscripts indicate significantly different depths of oxygen penetration.

In the second year of production (Fig. 5.4.3.2a and b) there was again no significant difference in depth of the oxic zone at the cage sites – the mean depth was never more than 1.65 mm. There was, however, a significant deepening of the depth of the oxic zone at the reference sites throughout the second year. Unfortunately, the statistical analysis was lacking in power (sample size, (n=2) because the cages could no longer be considered replicates as cage management was altered) reducing the ability to detect significant differences between sites and times. Consequently, at the end of the second year there was no significant difference in depth of the oxic zone at cage 8 and at reference site 2, despite the reference site mean oxic depth being nearly twice that at the cage site.

The depth of the oxic zone was compared over 2 production cycles using the data from 2.5, 12 and 25.5 months. A significant interaction between site and time was found ($F=16.62$, $P=0.000$, $n=4$) and so a second ANOVA was performed with Site-Time as the variable (Table 5.4.3.2). and Tukey's HSD used to determine homogeneous subsets (Fig. 5.4.3.3). In this case $n=4$ and the depth of the oxic zone at cage sites was significantly less than that at the reference sites. Cages were considered replicates in this analysis because they were sampled at the same time and had had the same length of stocking and fallowing on each occasion.

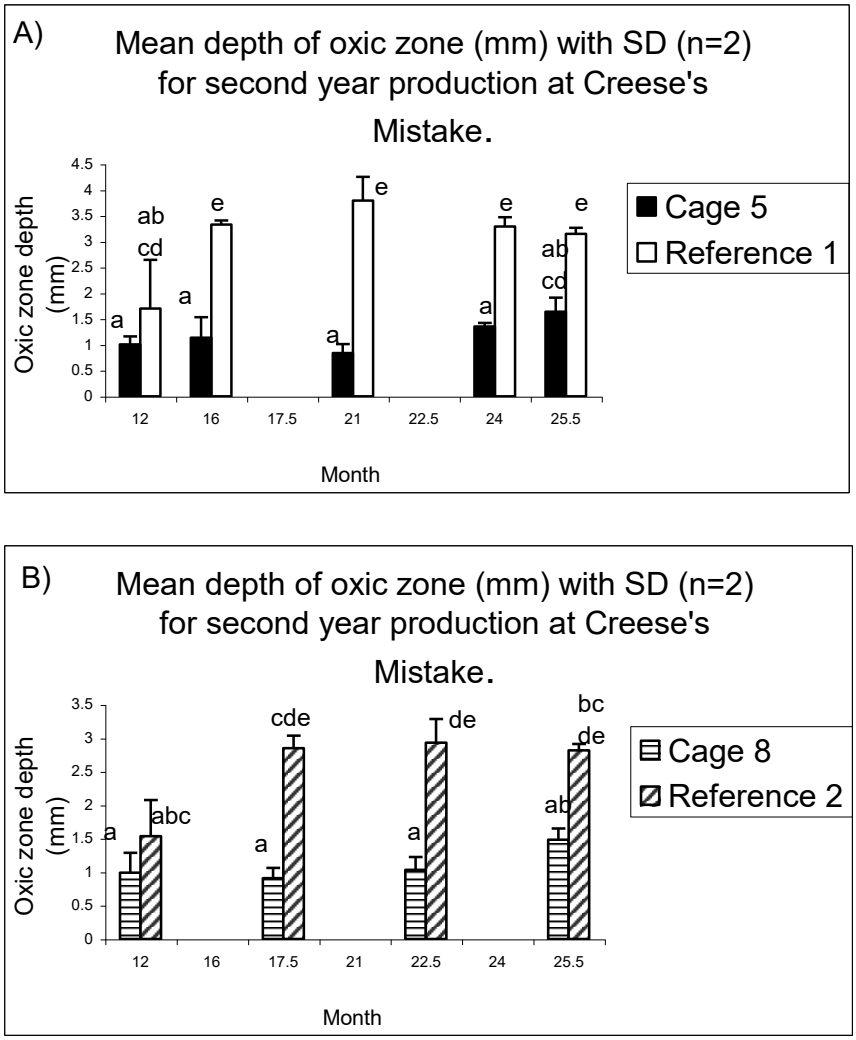


Fig. 5.4.3.2. Mean depth of oxygen penetration (mm) with standard deviation at Creeses Mistake farm. Different superscripts indicate significantly different means, (P=0.05, n=2) comparable across both figures.

Table 5.4.3.2. ANOVA of the mean depth of the oxic zone at Creeses Mistake over 2 production cycles. To achieve homogeneity of variance the data were transformed as Log(x+1). The variable is Site-Time and Tukey's HSD was used to determine homogeneous subsets.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.37(a)	5	0.074	22.49	0.001
Intercept	2.29	1	2.291	696.86	0.000
SITE-TIME	0.37	5	0.074	22.49	0.001
Error	0.02	6	0.003		
Total	2.68	12			
Corrected Total	0.39	11			

a R Squared = 0.949 (Adjusted R Squared = 0.907)

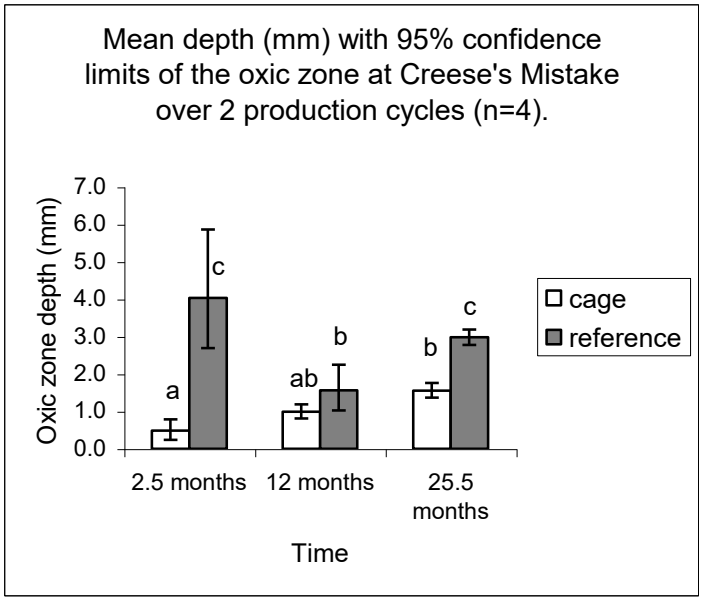


Fig. 5.4.3.3. Mean depth of oxygen penetration (mm) at Creeses Mistake over 2 production cycles. Different superscripts indicate significantly different mean depths of the oxic zone (P<0.05). Note that at 2.5 months for the reference n = 2.

At Creeses Mistake there were significant changes in the reference sites with time, which suggests that the whole environment there is dynamic. Although, the cage sites had a shallower oxic zone than the reference sites throughout time, at the end of the first year, the difference was not significant. This was primarily due to a decrease in the depth of the oxic zone at the reference sites, rather than a recovery of the cage sites during fallowing. Notably, the 2.5-month data demonstrated a marked deterioration in the depth of the oxic zone at the cage sites.

The removal rate for oxygen was calculated from 12 profiles (3 from each of 2 cores from cage sites and 3 from each of 2 cores from references) randomly chosen from the last sample period at 25.5 months. There was no effect of core nested within site, so data were analysed for site only, which demonstrated a significant difference in the mean removal rate for oxygen ($F=12.815$, $P<0.05$, $n=12$, Fig. 5.4.3.4). The rate of removal of oxygen from the cage sites was significantly higher than for the reference sites. We suggest that this means that the cage sediments still had more labile organic carbon than the reference sites after the fallowing period and that might be why the cage sites subsequently deteriorated so rapidly.

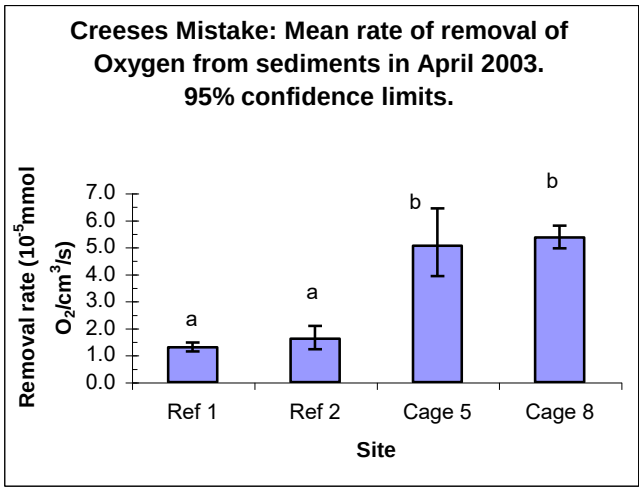


Fig. 5.4.3.4. Rate of removal of oxygen (10^{-5} mmol O_2 cm^{-3} s^{-1}) with 95% confidence limits from sediments at Creeses Mistake after 2 years of production cycles. Different superscripts indicate significantly different mean values (P<0.05, n=12).

5.4.3.2 Ammonia Measurements

Porewater measurements of ammonia from the top 6 to 8 cm of sediment cores indicated that ammonia rapidly increased at cage sites during the stocking period (Fig. 5.4.4.1). There was some variation at the reference sites. In March 2003 the concentration of ammonia at reference site 1 was considerably greater than the references at any other time, especially at depth. The concentration of ammonia was in fact higher than in sediments below the cage sites. By April 2003, the concentration at the reference sites was back to low values, in the range of < 50 µMol NH_3-N.

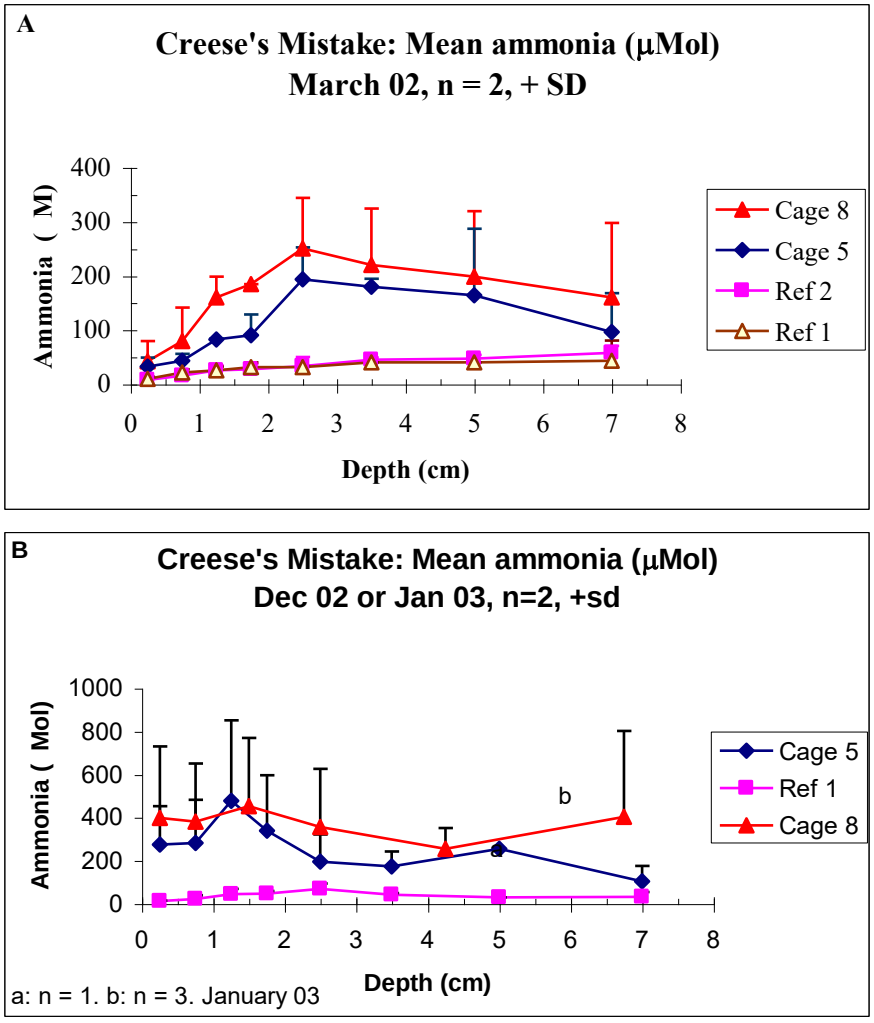


Fig. 5.4.4.1. Profiles of concentration of ammonia (µM) in porewaters extracted from sediments at Creeses Mistake. Note different scales for concentration. A) 15 months after commencement. B) 21 and 22.5 months after commencement of project.

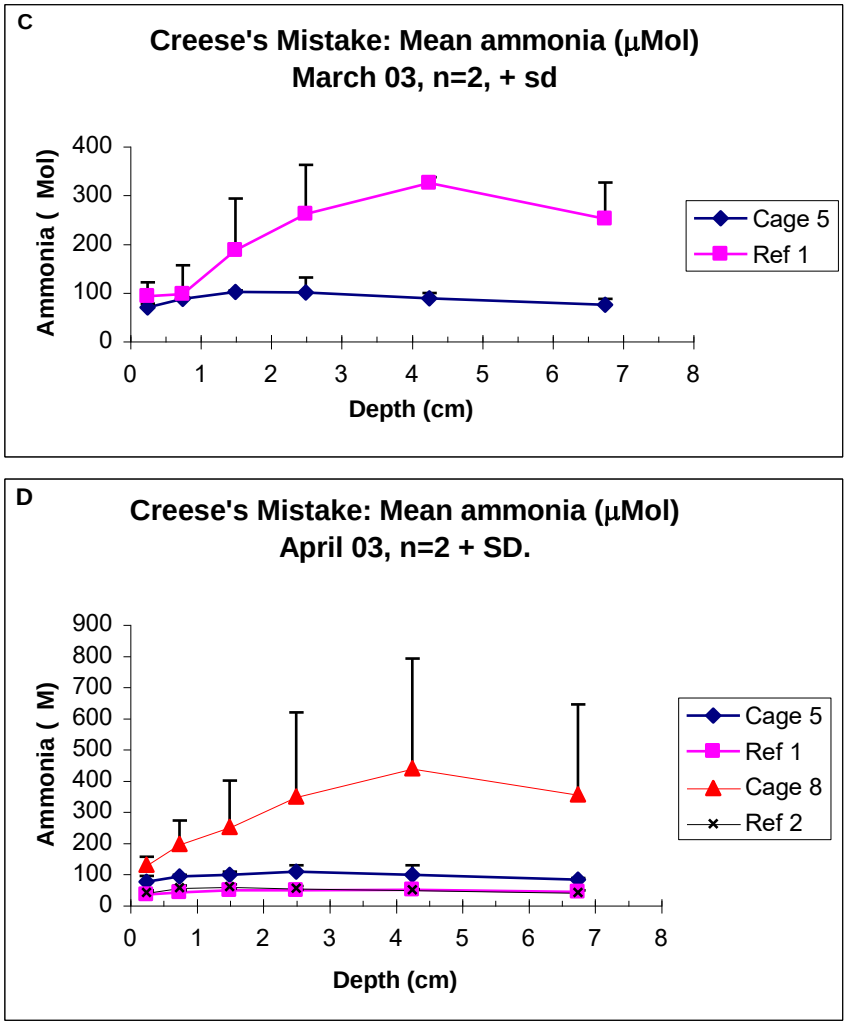


Fig. 5.4.4.2. Profiles of concentration of ammonia (mM) in porewaters extracted from sediments at Creeses Mistake. Note different scales for concentration. C) 24 months after commencement. D) 25.5 months after commencement.

There was a clear increase in ammonia in the sediments below cages after 3 months of fallowing after the first year of stocking (Fig. 5.4.4.2a). Thus, although the surface concentration was similar to the reference sites, the concentration at 2.5 cm depth at the cage sites was approximately 5 – 10x higher than the reference sites ate 2.5 cm. This suggests reworking of the sediments either physically or via bioturbation buries some of the organic carbon. Subsequent metabolism of this carbon released ammonia for some time after input of organic carbon had stopped. Further addition of organic carbon during the second year of production considerably increased the concentration at the surface of cage sediments by the end of the stocking period (Fig. 5.4.4.2b). A similar pattern was seen after the second fallowing period (Fig. 5.4.4.2.c and d), with decreased concentrations at the surfaces of cage sites, but high concentrations maintained at depth. The unusually high concentration observed at reference site 1 in April 2003 most likely occurred as a result of a random deposition of organic carbon from the overlying water. Metabolism of this carbon would have mineralized the organic carbon in a manner similar to that occurring under the cages.

5.4.3.3 Summary of oxic zone and porewater ammonia.

The data indicate that Creeses Mistake is a dynamic environment. Significant changes in the depth of the oxic zone were observed at reference sites. On the whole the oxic zone depth at cage sites was significantly shallower than at reference sites. Only after the completion of the

first year's production and fallowing was the difference in depth insignificant, but this appears to have been due to a decrease in oxygen penetration at the reference sites, rather than recovery at the cage sites. At the end of two years of production, the removal rate of oxygen was significantly higher at cage sites than at references, indicating a greater demand for oxygen at the cage sites. This increased demand was most likely due to higher concentrations of labile organic carbon. As for the oxygenation of the sediments, ammonia data indicate that once stocking had started, cage sites were fundamentally different from reference sites.

5.4.4 Carbon and nitrogen contents in sediments from Creeses.

Background levels of carbon in sediments at the Creeses site were very low (0.2%), reflecting the sandy nature of the sediment at this site. However, organic matter content at cage site 5 showed a pattern correlated with the stocking cycle, with sedimentary carbon increasing up to 5 times background to ca. 1% at the end of stocking (Fig. 5.4.5.1). This pattern is much less pronounced in the second stocking cycle, possibly reflecting lower stocking densities. Importantly, carbon levels fell rapidly to background levels with fallowing.

Background levels of nitrogen were extremely low (0.01 - 0.02%) making them very difficult to measure (Fig. 5.4.5.2). However, levels of nitrogen in the sediment did increase with stocking (but only reached 0.08%), but again fell rapidly to background levels during fallowing.

C:N values at reference sites were initially at 10 or greater (comparable to Stringers), but there was a trend over the study period to lower values, indicating an influx of more marine material. This trend was also apparent on the lease. However, given the errors associated with measurement of low C and N contents this must be treated with caution due to the low levels of nitrogen in the sediment.

Analysis of the stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) showed no significant change over the study period. This was mainly the result of the input from the farm being from marine derived organic matter (feed and faeces) onto a predominantly marine-derived background. $\delta^{15}N$ values remained fairly constant throughout the study period, with values around +6 to +8‰ . This indicates that the nitrogen was sourced primarily from "marine" derived inputs. There was a high degree of variability in some of the samples, reflecting the low levels of nitrogen present.

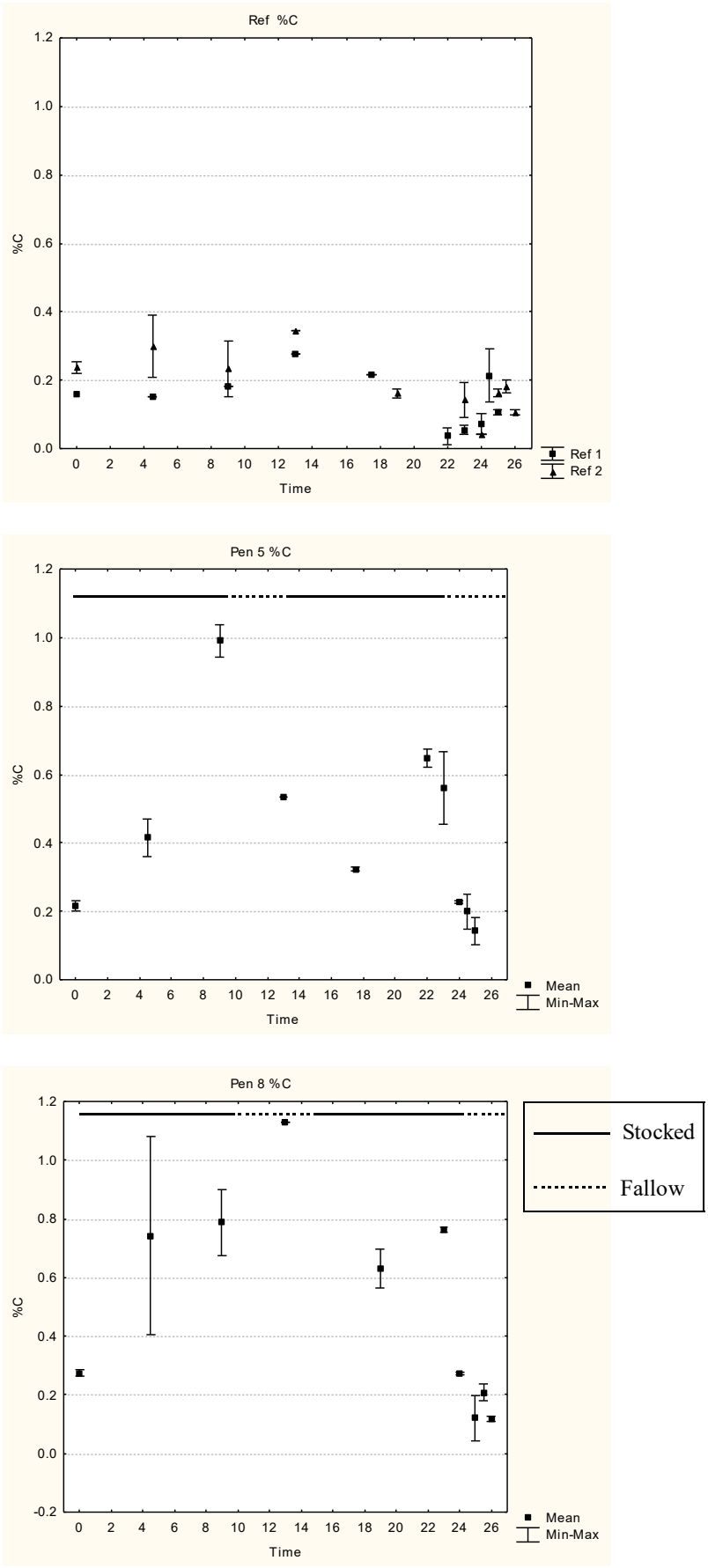


Fig. 5.4.5.1. Carbon contents (as % dry weight of sediment) at reference sites 1 and 2 and at cage 5.

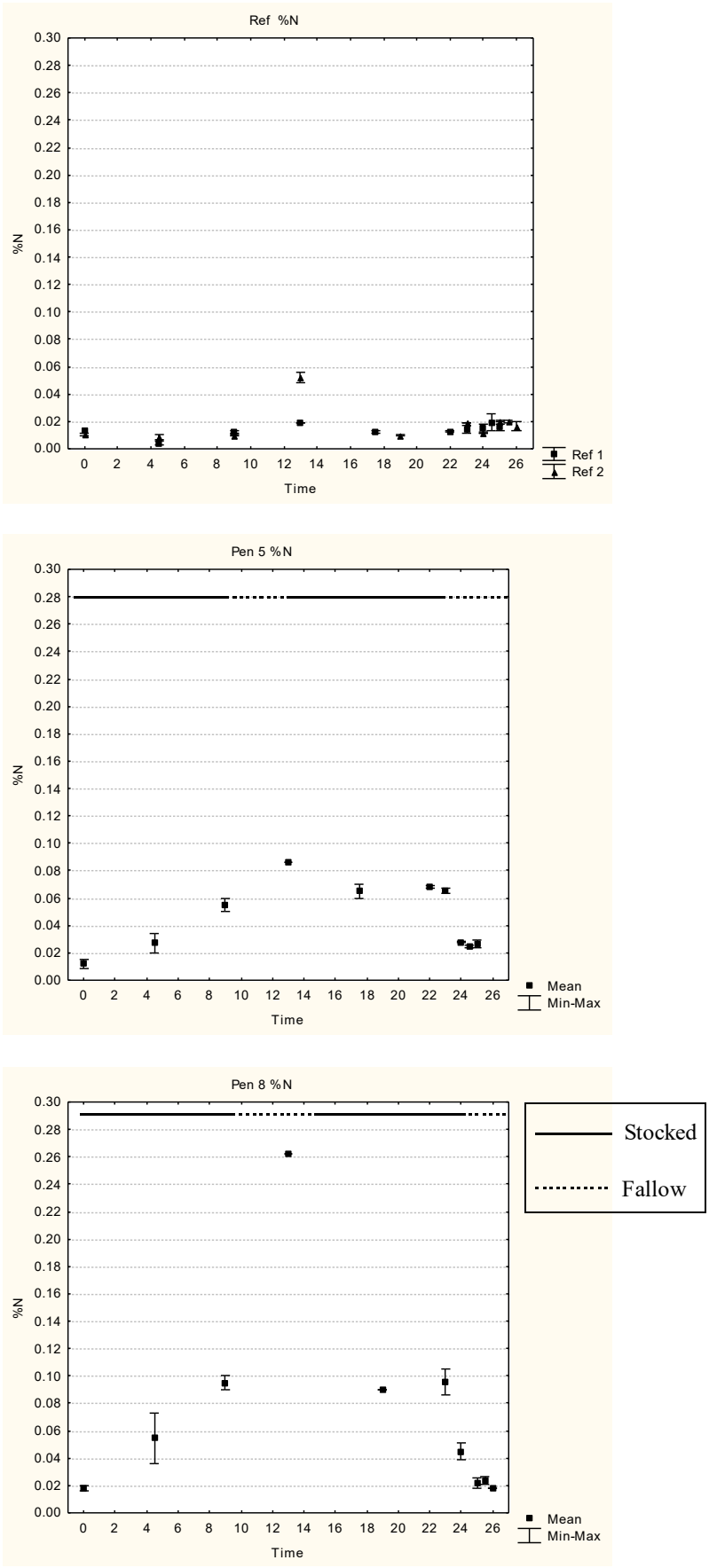


Fig. 5.4.5.2. Nitrogen contents (as % dry weight of sediment) at reference sites 1 and 2 and at cage site 5.

5.4.5 Stable Isotopes

Background concentrations of carbon at Creeses are very low (0.2%), reflecting the sandy nature of this site. However, values on the lease show a pattern correlated with the stocking cycle, with sedimentary carbon increasing up to 5 times background. This pattern is much less pronounced in the second stocking cycle, possibly reflecting lower stocking densities; i.e. carbon levels fall rapidly to background levels after fallowing.

Background levels of nitrogen are extremely low (0.01 - 0.02%) making them very difficult to measure. However, levels of nitrogen in the sediment increased with stocking and again fell rapidly to background levels during fallowing.

C:N values are similar to Stringers in that reference sites are initially at 10 or greater but there is a trend over the study period to lower values, indicating an influx of marine material. This trend is also apparent on the lease. However, this must be treated with caution due to the low levels of nitrogen in the sediment.

$\delta^{15}N$ values again remain fairly constant throughout the study period, with values around +6 to +8 ‰ . This indicates that the nitrogen is sourced primarily from "marine" derived inputs. There is a high degree of variability in some of the samples and this reflects the low levels of nitrogen present.

5.4.6 Sediment lipid biomarkers

5.4.6.1 Use of biomarkers to assess sources of organic matter

Marine sediments contain a great variety of lipids, many of which can be attributed to specific sources. These are often termed biological markers or simply biomarkers. Common examples include distributions of fatty acids, sterols, hydrocarbons and unusual lipids such as long-chain alkenones and alkyl diols (e.g. Volkman et al., 1998). Fatty acids have received most attention since within the one distribution one can find markers for bacteria (e.g. branched-chain fatty acids, cis-vaccenic acid), higher plants (e.g. C_{24}-C_{34} even-chain saturated fatty acids), microalgae (16:1n–7 and certain polyunsaturated fatty acids (PUFA)) and animals (e.g. PUFA such as 22:6(n–3)). Phytoplankton are the major source of sterols and these can display a wide range of structures with different side-chains and positions of double bonds (e.g. Volkman, 1986). A significant contribution from marine fauna is often indicated by high contents of cholesterol, while terrestrial plant inputs are often reflected in high contents of 24-ethylcholesterol and certain triterpenoid alcohols such as α- and β-amyrin (Volkman, 1986; Volkman et al., 1983; 1998). Macrofauna can play a significant role in sediment biogeochemistry both as a source of organic matter (e.g. Boon and Haverkamp, 1979; Leifkins et al., 1979), and as agents in its recycling with consequent effects on nutrient release (Hansen and Kristensen 1997; Banta et al., 1999; Heilskov and Holmer, 2001).

5.4.6.2 Previous applications to fish farming

A previous study of the potential of organic markers and other parameters to assess sediment conditions under a fish farm in the Huon estuary was carried out by McGhie et al. (2000). Sediment samples from two near-adjacent sites, but with different sediment types and depths, were analyzed for total organic matter, fatty acids, sterols, %C, $\delta^{13}C$, %N, $\delta^{15}N$, and redox potential during a 12-month fallowing period. Most of the organic matter accumulation was confined to an area directly underneath the fish cages, but at 30 m from the center of the cage, indicators of fish cage waste (faeces and feed) such as fatty acids were still elevated compared with reference sites. After 12 months fallowing, organic matter contents in surface sediment at

the center of the cage remained higher than at 30 m distance, even though redox potentials indicated that normal oxic conditions had returned. An earlier study by Ye et al. (1991) had used stable carbon isotopes to estimate organic matter inputs from a fish cage on marine sediments. The proportion of cage-derived organic C to total organic C in the sediment decreased with increasing distance from a fish cage. A highly organically enriched zone in sediments under the cage was characterized by >75% fish cage-derived organic C, a semi-enriched zone (> 10 m from the cage) was characterized by 60% and a lightly enriched zone (> 60 m) by 40%. Note that feeding efficiencies have improved considerably since this early work and thus the amounts found today should be much less.

The paper by Henderson et al. (1997) appears to be the only other study that has used organic lipid biomarkers to assess organic matter sources under salmon cages. Their data clearly showed that the lipid composition of sediments underlying marine fish cages in a Scottish sealoch was influenced by the waste material from the cages. Sediments were taken at regular intervals along a transect line perpendicular to the line of cages and extending 50 m on either side. Triacylglycerols, the main lipid class in the feed pellets, were present in sediments in highest concentration (0.44 mg g^{-1} sediment), while free fatty acids, sterols and polar lipids were also detected. The lipid content of the surface layer of sediment (0–5 mm) directly under the cages was very high (2 mg g^{-1} sediment) and substantially higher than that found in the deeper layers of sediment. The lipid content of surface sediments declined markedly with the distance from the cages; at 50 m from the cage the lipid content was only 0.4 mg g^{-1} sediment. The concentration of individual lipid classes in sediments decreased with distance from the cages and generally mirrored the changes in the total lipid content.

A more recent study of sediments beneath a well established fish farm in the Ligurian Sea (Western Mediterranean) examined the “biopolymeric carbon (BPC) fraction” of organic matter and phytopigment concentrations which both displayed very high values beneath the fish cages (Vezzulli et al., 2002). Lipid, carbohydrate and chlorophyll-a concentrations were higher in the farm sediment than at a control, whereas protein concentrations did not show significant change between farm sediment and control. These authors suggested that that the biochemical composition of sedimentary organic matter as well as selected microbial variables could provide useful tools for evaluating the effects of organic enrichment in sediments due to fish farming.

5.4.7 Non-saponifiable lipids from reference sites, in-between cage sites and under cages

Some 50 fatty acids and 24 sterols were identified and quantified for approximately 85 sediment samples. Amounts are expressed in μg g^{-1} of dry sediment or as percentages of the total lipids in that fraction. Sediments were collected at various intervals over a 26 month period covering two stocking and fallowing cycles. Illustrative capillary gas chromatograms showing the distributions of non-saponifiable (neutral) lipids at a reference site and at 0, 9 and 13 months for cage site 5 are shown in Fig. 5.4.8.1. and 5.4.8.2. Comparable data for the total fatty acids at the cage site are shown in Fig 5.4.8.3 and Fig. 5.4.8.4.

The chromatograms for the neutral lipids (Figs. 5.4.8.1 and 5.4.8.2) show a diversity of lipid constituents including phytol (side-chain of chlorophyll a), a complex mixture of sterols indicative of mixed algal and faunal inputs, plus smaller amounts of other compounds. General features of the distributions are discussed here, and a more detailed examination of amounts of selected biomarkers (phytol, cholesterol, α-tocopherol, desmosterol, sitosterol) are discussed separately in following sections.

Biomarker contents in reference sediments were quite low and more variable than at the Stringers site. All sediments contained similar amounts of phytol indicating comparable inputs of phytoplankton-derived chlorophyll a. Indicators of terrestrial organic matter of higher plant origin such as long-chain even-carbon-number *n*-alkanols and the C_{29} sterol 24-ethylcholesterol (sitosterol) were relatively minor constituents in marked contrast to the Stringers site. The sterol distributions were dominated by cholesterol of mainly animal origin plus sterols derived from a diversity of algal groups with significant inputs from diatoms (as shown by the high abundance of 24-methylcholesta-5,22E-dien-3β-ol) and dinoflagellates (high abundance of dinosterol: 4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol). These distributions can thus be used as a baseline for comparison with sediments that receive additional inputs of organic matter from farming activities. Biomarker contents in the sediments at the Creeses cages were about 50% less than those found at Stringers. For example, cholesterol in cage sediments at Stringers reached levels of 150 μg g^{-1} or more at both cages P1 and P2, but at the Creeses cage sites the total content of cholesterol never exceeded 60 μg g^{-1}.

Fatty acids at the beginning of stocking at cage 5 were very similar to those at the reference (Fig. 5.4.8.3), but were dominated by fatty acids from the feed at 9 months (Fig. 5.4.8.4.). Total fatty acid amounts had diminished by the end of 4 months fallowing, but the distribution still resembled that found in the feed. Fatty acids are more labile that sterols and thus their distributions should more quickly approach those of the reference sediments on fallowing.

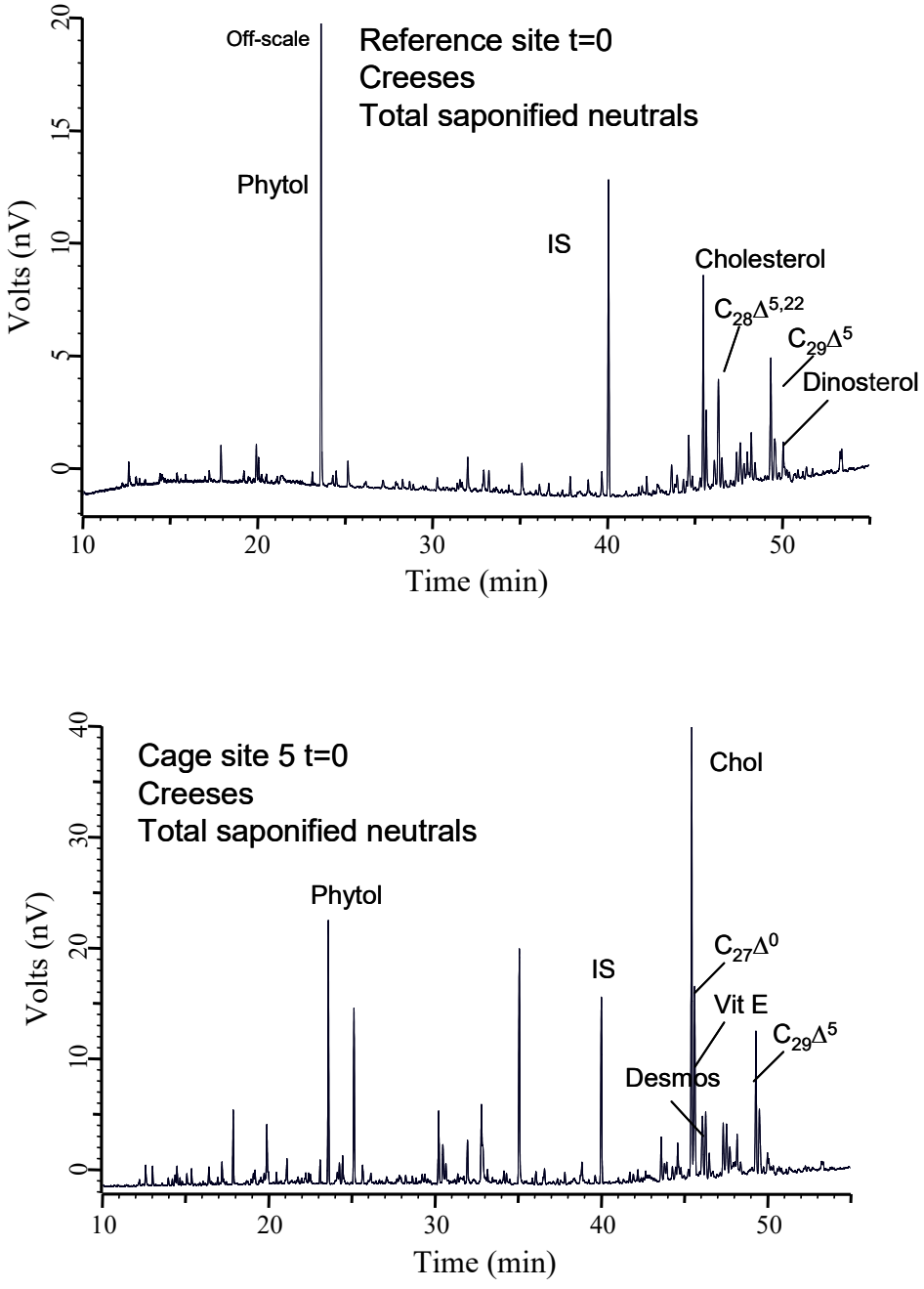


Fig. 5.4.8.1. Capillary gas chromatograms for total neutral lipids in sediments at a) reference site and b) cage 5 at the beginning of stocking (0 months). Biomarker contents are higher at the cage site and compounds indicative of past farming (vitamin E, desmosterol) are also present.

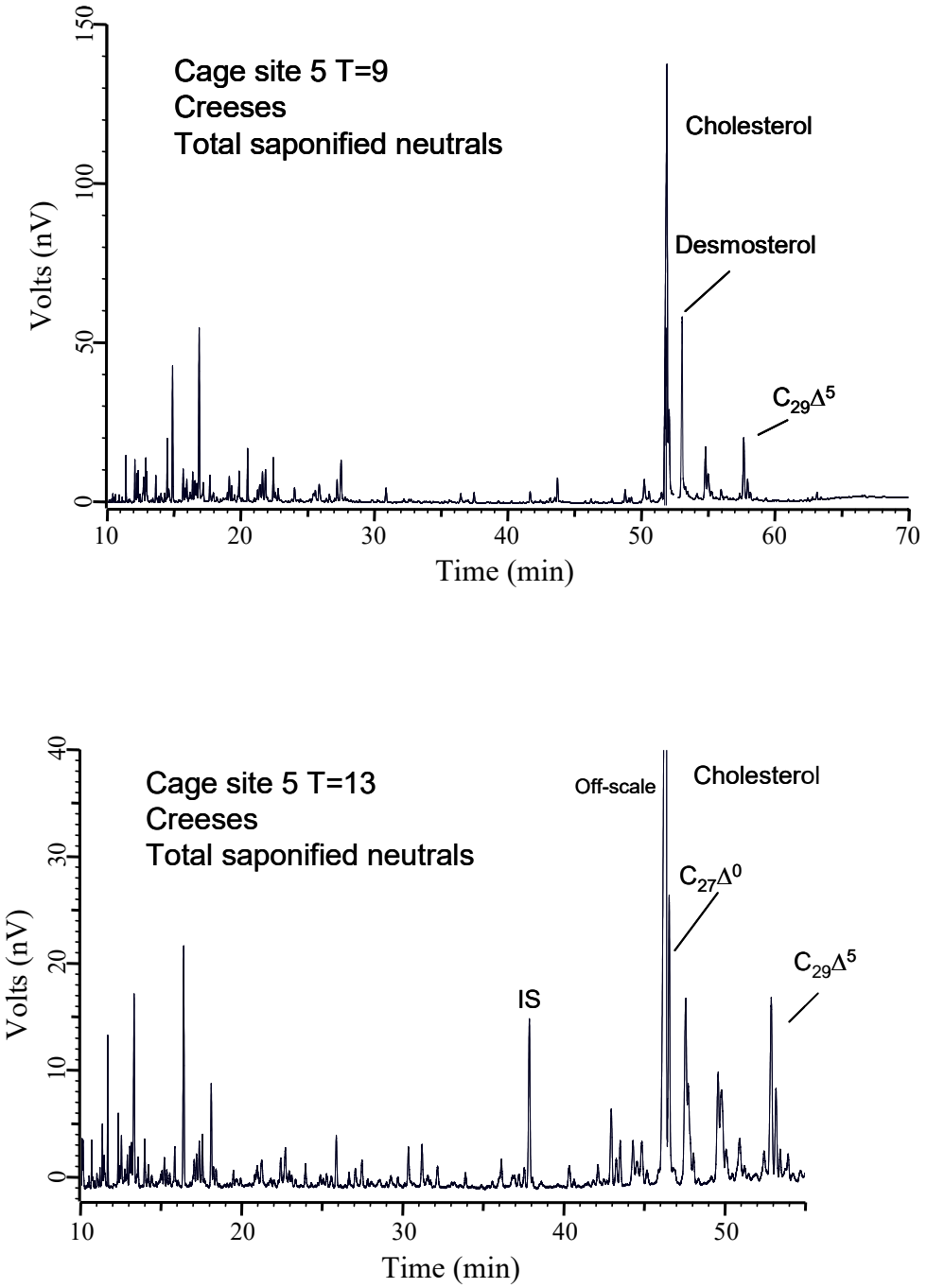


Fig. 5.4.8.2. Capillary gas chromatograms for total neutral lipids in sediments at cage 5 a) at the end of stocking (9 months) and b) after 3 months fallowing (12 months). Compounds indicative of past farming (vitamin E, desmosterol) are still present at 13 months.

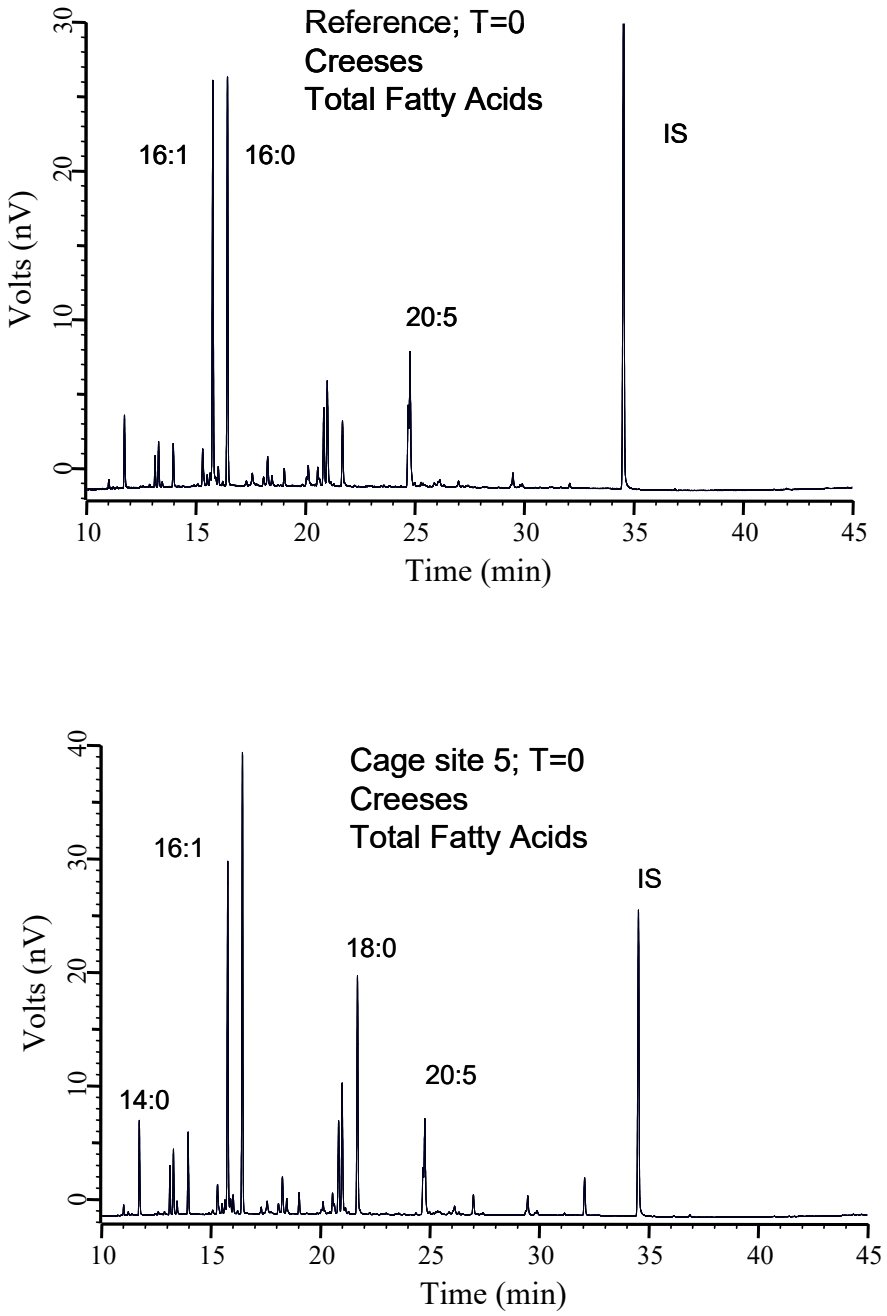


Fig. 5.4.8.3. Capillary gas chromatograms for total fatty acids after saponification (analysed as methyl esters) in sediments at a) reference site and at b) cage 5 at the beginning of stocking (0 months).

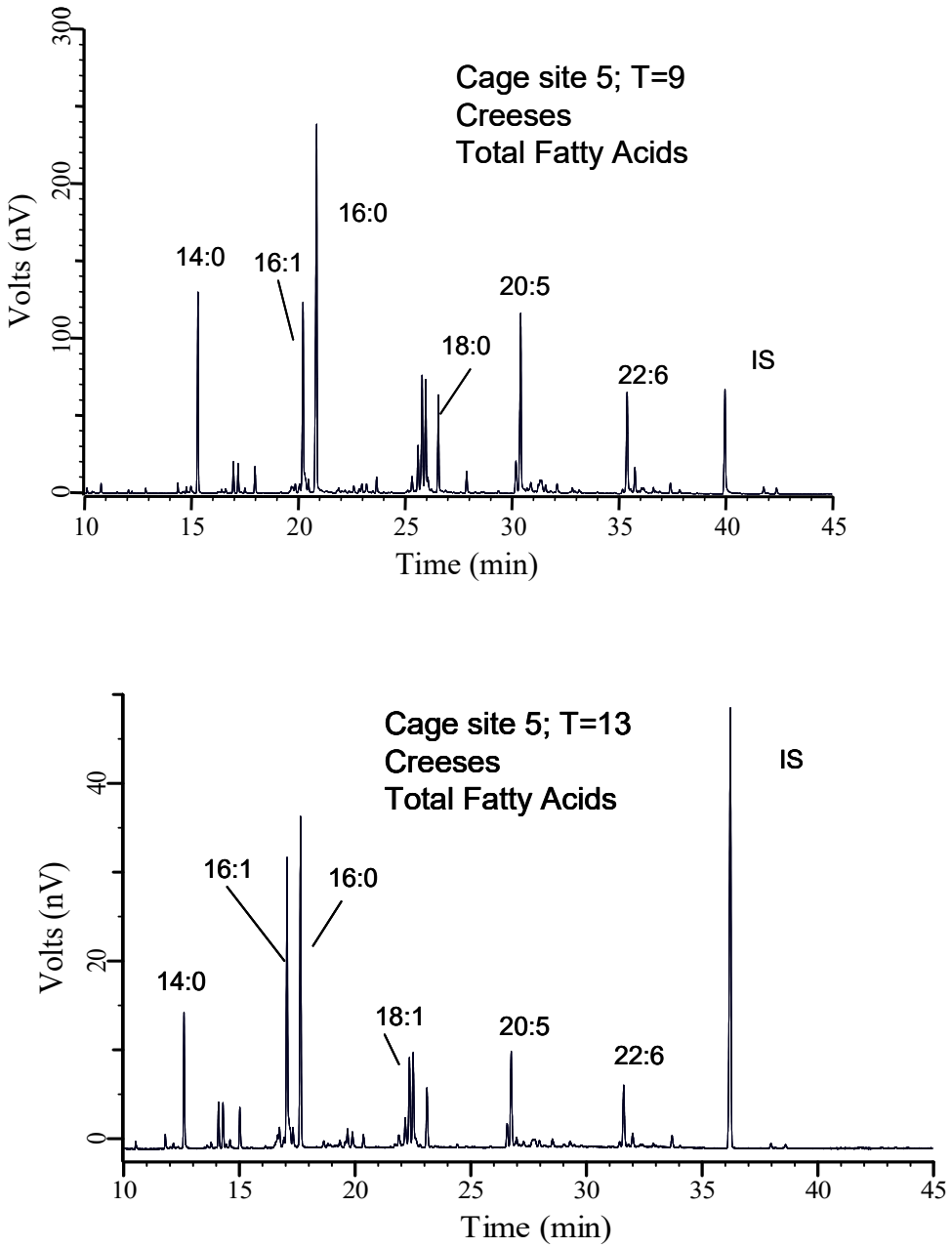


Fig. 5.4.8.4. Capillary gas chromatograms for total fatty acids after saponification (analysed as methyl esters) in sediments at cage 5 at the end of stocking (9 months) and after fallowing (13 months). Fatty acids typical of feed dominate the distribution, superimposed on fatty acids of natural bacterial and microalgal origin.

A key difference was found in the lipid distributions in sediments from cages 5 and 8 sampled at the same time. In particular, peak concentrations for most biomarkers occur at the end of stocking at cage 5, but at 4.5 months for cage 8. This almost certainly reflects the different stocking histories of the two cages. Fish were removed from cage 8 for two weeks prior to the scheduled end of stocking and then replaced. This two-week hiatus led to a dramatic reduction in the contents of most lipids in the sediments testifying to the value of fallowing and to the sensitivity of lipid contents to changes in stocking and sediment condition. Given this unfortunate break in our study, we have chosen to concentrate on data from cage 5 which follows a more expected cycle of concentration changes.

5.4.7.1 Temporal changes in specific biomarkers

5.4.7.1.1 Phytol Abundances

Phytol is the side-chain of chlorophyll a and thus its presence in sediments indicates a contribution from phytoplankton-derived organic matter to the sediments. It is not persistent in sediments and is broken down to a range of degradation products (Rontani and Volkman, 2003). Phytol was present in both reference sites at all sampling times. The amounts were generally low, with most falling in the range 1–3.5 µg g^{-1} (Fig. 5.4.8.1.1.1). Phytol contents were comparable at both reference sites and showed similar variability over time suggesting inputs from phytoplankton blooms in the warmer months. Contents at the cage sites were within the same ranges and also varied throughout the year implying a response to system-wide events rather than any particular influence of the farm operations.

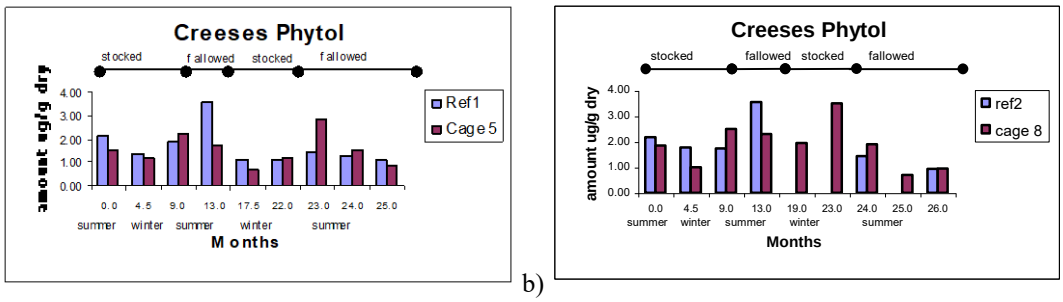
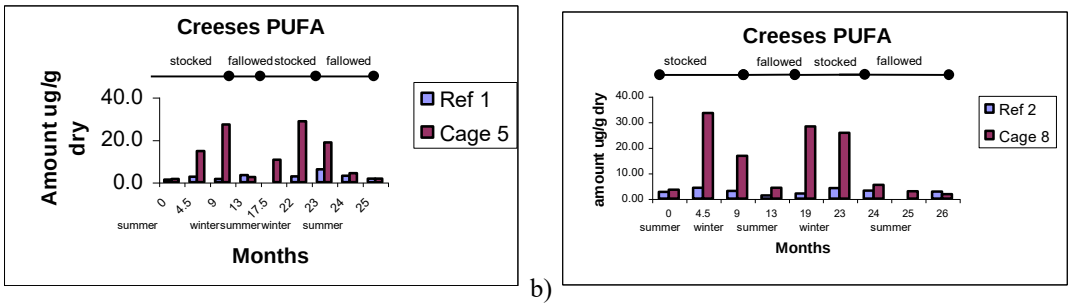


Fig. 5.4.8.1.1.1. Changes in the abundance of total phytol (after saponification) in sediments at the Creeses site. Phytol is an indirect measure of chlorophyll from microalgae.

5.4.7.1.2 Fatty acids in sediments

At reference sites, the distribution of fatty acids was dominated by 16:0, 16:1, 20:4(*n*-6) and 20:5(*n*-3) which is typical of distributions dominated by diatom inputs. Bacterial fatty acids were not particularly abundant (Fig. 5.4.8.1.2.1). The PUFA 22:6(*n*-3) was not abundant at reference sites reflecting the smaller contribution from animal lipids. In contrast, polyunsaturated fatty acids (PUFA) including 22:6(*n*-3) were particularly abundant in sediments at the cage-sites due to contributions from animals, uneaten feed and faecal matter (Fig. 5.4.8.1.2.1). Total amounts increased during stocking to 30–35 µg g^{-1} and then declined to near-background levels after 4 months fallowing (Fig. 5.4.8.1.2.1). PUFA are very labile compounds and thus the changes in their amounts gives an indication of build-up and degradation of labile organic matter in the sediments. These data suggest a faster recovery of sediments at Creeses than at the Stringers site.

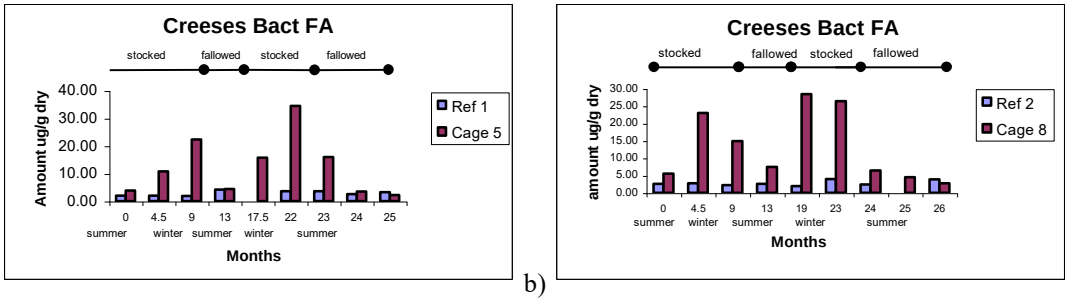


a) b)

Fig. 5.4.8.1.2.1. Changes in the abundance of total polyunsaturated fatty acids (PUFA) (after saponification) in sediments at the Creeses site.

5.4.7.1.3 Bacterial fatty acids

The content of bacterial fatty acids (*iso*-and *anteiso*-branched fatty acids plus 15:0 and 17:0 saturated fatty acids and 18:1(*n*-7) monounsaturated fatty acid) increased in sediments at the cage sites during the stocking cycle. However, on fallowing these amounts quickly fell to values typical of the reference sediments (Fig. 5.4.8.1.3.1).

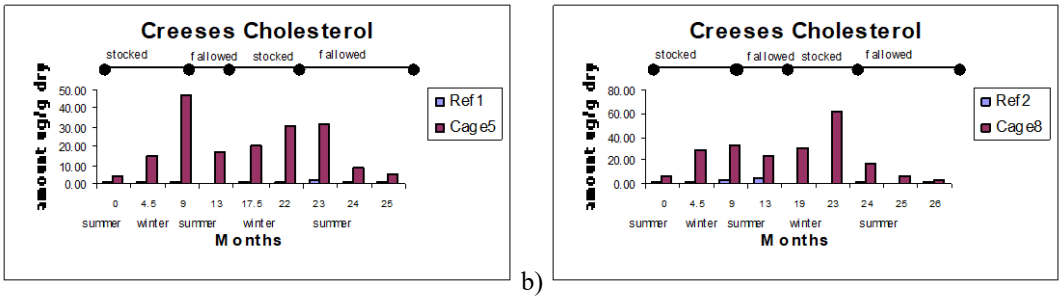


a) b)

Fig. 5.4.8.1.3.1. Changes in the abundance of bacterial fatty acids (after saponification) in sediments at the Creeses site.

5.4.7.1.4 Cholesterol in sediments

Cholesterol is the major sterol in most marine animals (including fish) and it is also the major sterol in salmon feed and faeces. Cholesterol contents were low in sediments at both reference sites, but showed major increases during the stocking period followed by a gradual fall as sediments were fallowed (Fig. 5.4.8.1.4.1). At no point did the sediment cholesterol contents at either site fall to values typical of the reference sites. Indeed, even at 0 months, cholesterol levels were elevated reflecting a residual effect due to previous stocking at this site.



a) b)

Fig. 5.4.8.1.4.1. Changes in content of cholesterol in sediments at the Creeses site.
Note the strong build-up during stocking and slow reduction during fallowing.

5.4.7.1.5 Desmosterol

Desmosterol is an intermediate in cholesterol biosynthesis but it is rare to find significant amounts in sediments or in marine animals. Indeed, only trace amounts were detected at the two reference sites. There was a clear build-up of desmosterol contents in sediments at the cage-sites during stocking and a gradual reduction to near-background levels with fallowing

(Fig. 5.4.8.1.5.1). Our work at the Stringers site showed that desmosterol is contributed by capitellid worms and a good correlation can be found between capitellid numbers and desmosterol content in the sediment (data from both sites combined). The maximum desmosterol levels are lower than those at Stringers (10–16 µg g^{-1} at Creeses compared with 20–50 µg g^{-1} at Stringers during the first cycle) reflecting the lower densities of *Capitella* at Creeses.

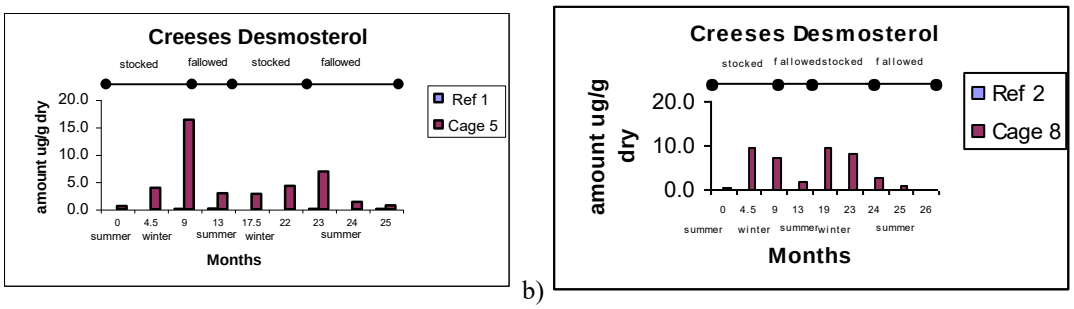


Fig. 5.4.8.1.5.1. Changes in the abundance of the sterol desmosterol in sediments at the Creeses site. In this environment, desmosterol is a marker for capitellid worms.

5.4.7.1.6 Vitamin E (α-tocopherol).

Vitamin E is added to the fish feed and small amounts are found in the salmon faecal material. It is also produced naturally by phytoplankton and so minor amounts are found at the reference sites. The vitamin E contents in cage-site sediments at Creeses are much greater than at the reference sites (Fig. 5.4.8.1.6.1), but nonetheless are much lower than those observed at the cage-sites at Stringers: the maximum content seen at Creeses is 5 µg g^{-1}, but at Stringers levels remained at around 35 µg g^{-1}. Although contents at the Creeses cage-sites were up to 5 times those at the reference sites during stocking, there was a recovery of Vitamin E levels to values approximating those at the reference sites after fallowing. This behaviour contrasts with the Stringers site where vitamin E levels remained high even after many months of fallowing.

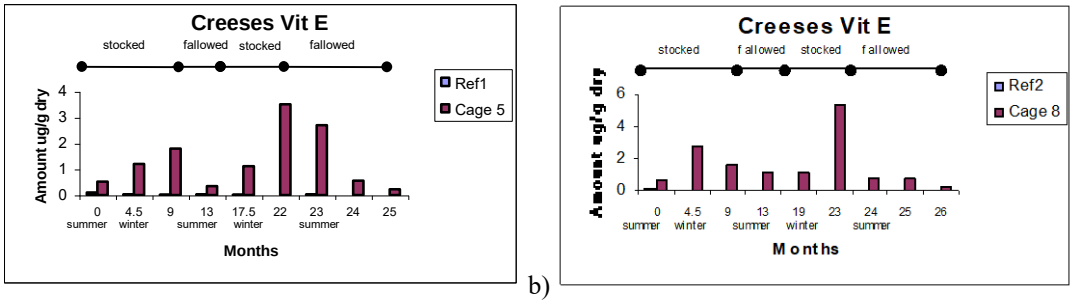
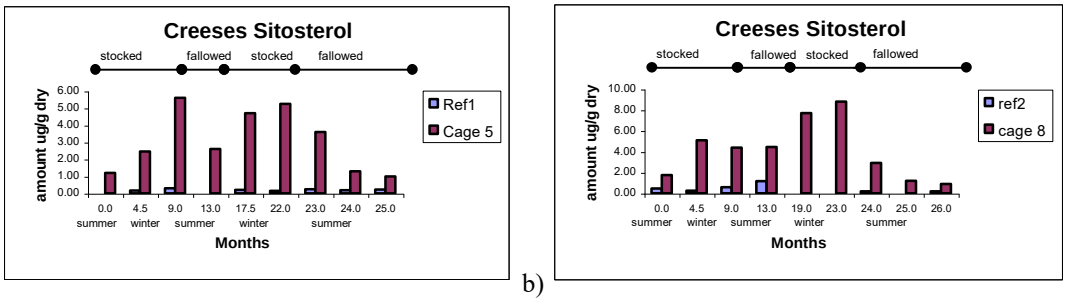


Fig. 5.4.8.1.6.1. Changes in the abundance of vitamin E (α-tocopherol) in sediments at the Creeses site. Vitamin E is added to the feed and thus is a marker for fish feed and faeces in this environment.

5.4.7.1.7 Sitosterol (24-ethylcholesterol)

The C_{29} sterol sitosterol is the major sterol in most higher plants and thus it is often used as a biomarker for plant input to coastal sediments. Amounts at the reference sites were quite low consistent with the predominance of marine-derived organic matter at this site. However at the cage sites the contents of sitosterol showed a significant increase with stocking followed by a reduction on fallowing (Fig. 5.4.8.1.7.1). Sitosterol is 6 % of the sterols in the feed and 13.6 % of the sterols in fish faeces. In this environment, sitosterol thus appears to be a good indicator of feed and/or faeces from farming operations. Indeed, there is a strong correlation with cholesterol when comparing the two plots: the profile of both biomarkers over the 2-year cycle

is almost identical. Sitosterol content decreased significantly during fallowing suggesting that there is limited long-term accumulation of waste feed at this site.



a) b)

Fig. 5.4.8.1.7.1. Content of the C_{29} sterol sitosterol (24-ethylcholesterol) in sediments at the Creeses site. Sitosterol is found in higher plants and in some microalgae. Here it is mostly contributed from uneaten feed.

5.4.7.1.8 Comparison of sediments directly under and adjacent to cages.

An opportunity was presented at 13 months to sample sediments at the cage 8 site from both the centre of the cage and at the edge. Contents were 2-3 times higher in the centre than at the edge of the cage. Even after fallowing for 3 months, biomarker contents in sediments from the centre site were 2 to 3 times those obtained from the edge of cage (Fig. 5.4.8.1.8.1). In fact cholesterol levels were 50 % higher at the centre cage position after fallowing (13 months) than at the edge of the cage at 9 months after stocking. Thus the impacts measured at the edge of the cage will be less that those in sediments directly beneath the cages.

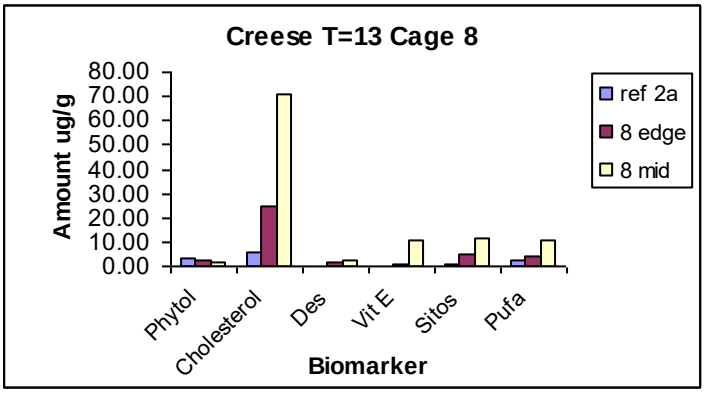


Fig. 5.4.8.1.8.1. Comparison of lipid contents in sediments collected from the original centre point of cage 8 with values found in sediments from the regular sampling position. The site had been fallow for 3.5 months by this stage.

5.4.8 Sedimentation

In production cycle 1, sedimentation rates were generally higher at the cage than at the reference positions. There was a significant interaction between site (cage / reference) and time ($F_{5,22}$=20.584; p=0.000). Pairwise comparisons showed that the cage sedimentation rate was significantly higher than the reference while the cage was stocked (up to and including 9 months) but not when the cage was empty (10, 11 months) (Fig.5.4.9.1a). The sedimentation rate at the cage again increased at the end of three months fallow (13 months). This may have been caused by preparation of the cage positions for restocking, i.e. replacing predator and cage nets.

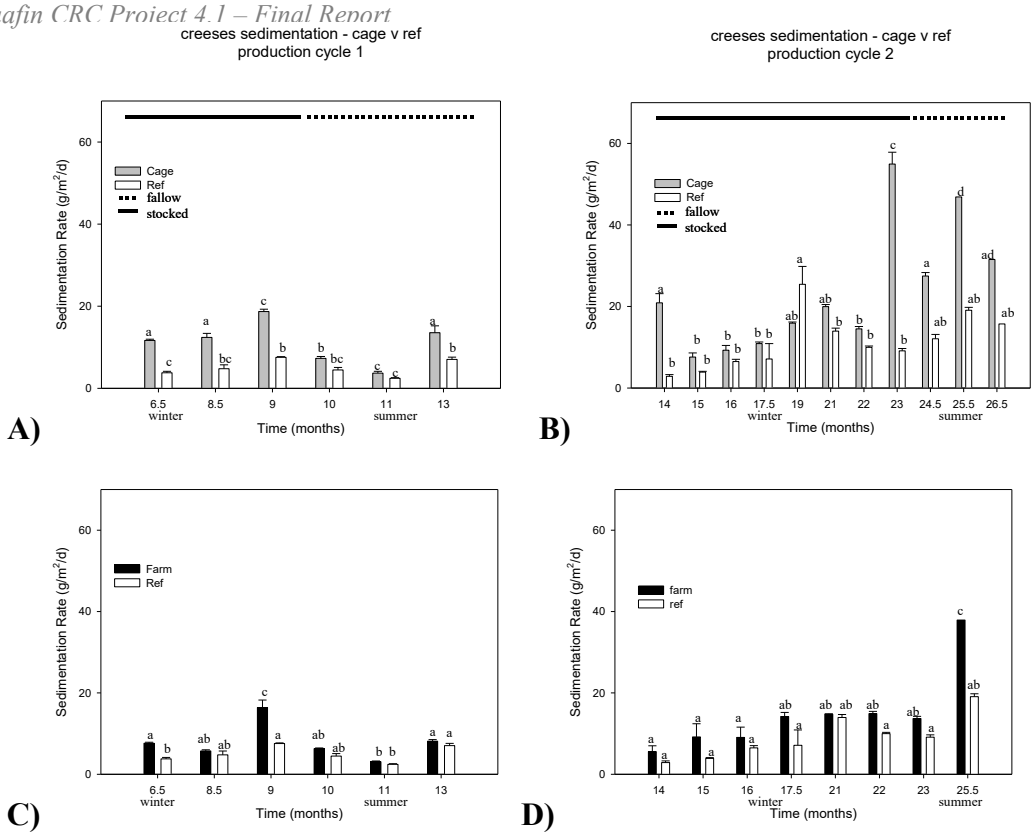


Fig.5.4.9.1. Sedimentation rates recorded at A) Cage and Reference in production cycle 1, B) Cage and Reference in production cycle 2, C) Farm and Reference in production cycle 1 and D) Farm and Reference in production cycle 2.

In production cycle 2, sedimentation rates showed a seasonal trend, with little difference between the cage and reference rates at most times. There was, however, a significant interaction between site and time ($F_{11,21}$=62.379; p=0.000). The cage sedimentation rate was significantly higher than the reference at both the start (14 months) and end of stocking (23 months) (Fig.5.4.9.1b). Sedimentation rates remained significantly higher at the cage than the reference during the fallow period (23 to 26.5 months), this may be a result of farming activities such as cleaning of cage fouling, or feed/faecal material from adjacent operational cages.

The effect of adjacent cages was assessed by placing a sediment trap in between two adjacent cages, at a "farm site". In the first production cycle there was a significant interaction between site (farm and reference) and time ($F_{5,22}$=10.064; p=0.000). Sedimentation rate at the farm site was variable, only occasionally were rates higher than at the reference and this was probably dependent on the level of feeding activity, stocking density and biomass of the adjacent cages (Fig.5.4.9.1c). There was no significant difference in sedimentation rates at farm and reference during the fallow period.

In the second production cycle, farm sedimentation rates were again variable, but often similar to the reference (Fig.5.4.9.1d). There was a significant interaction between site and time ($F_{8,16}$=13.502; p=0.000). As was the case with the cage sedimentation data, the farm sedimentation rate was significantly higher than the reference during the fallow period, suggesting that in the second cycle there was a broader farm-wide increase in sedimentation.

5.4.9 Sediment Metals

During the second production cycle at Creeses Mistake, copper treated antifouling nets were deployed at the study cages. As a result, copper concentration was measured in a selection of

Creeses Mistake sediments (cage and reference), and a feed sample, to determine if the treated nets were affecting copper levels in the sediments.

The data show that Cu has been transported to the sediments following the introduction of the antifouled nets. The copper concentration in sediments at the cages increased markedly after the deployment of the treated nets (approximately 13-14 months) and was significantly higher than the reference positions (2-Factor ANOVA, $F_{2,18}$=8.586, p=0.009) (Fig. 5.4.10.1).

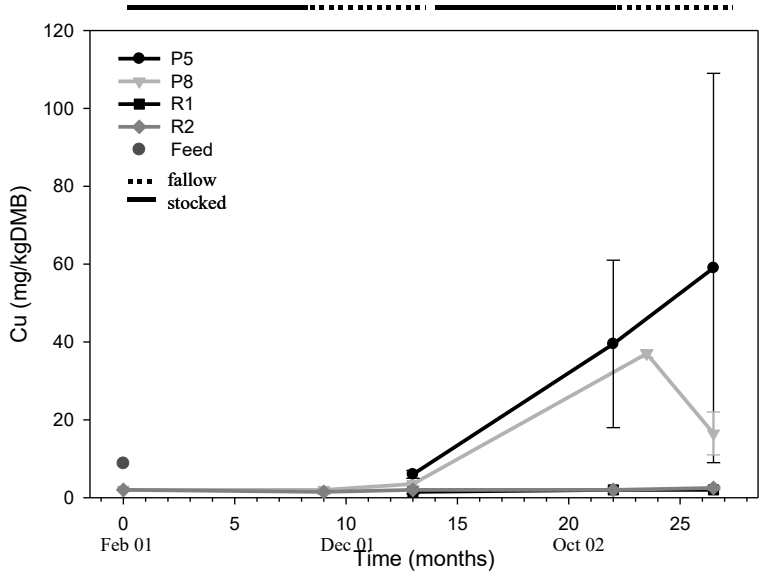


Fig.5.4.10.1. Copper concentration in sediment at Creeses Mistake between February 2001 and April 2003. Concentration in feed sample from November 2001 is included for comparison.

At 22 months (cage 5) and 23.5 months (cage 8) the fish were harvested from the study cages. The Cu level declined at cage 8 between 23.5 and 26.5 months, probably in response to the cage/net being removed. There was no decline in Cu level between 22 and 26.5 months at cage 5. We know that the fish were removed at 22 and 23.5 months but we have no information as to whether the net was also removed at this time.

The prime cause of the increase in the sediments is likely to be particulate matter, either as pieces of the antifoulant coating coming off the nets or as a result of fouling material absorbing Cu before being sloughed off the nets, rather than leaching of Cu into the water column. The Cu levels around the cages were highly variable suggesting a patchy effect, which supports the hypothesis that the increase is a result of localized deposition of material. Although sediment Cu levels were variable, increased sediment concentrations were evident from the time that the treated nets were deployed. Since the samples for analysis were obtained using remote sampling techniques from study sites in depths between 20-25 m and using individual cores collected adjacent to (not directly under) operational cages, the detection of a significant increase using this sampling regime, and at the replication level employed, indicates that the effect is widespread.

ANZECC interim sediment quality guidelines (ISQG) for copper in aquatic ecosystems identify levels up to 65 mg/kg as low and levels above 270 mg/kg as high. Although the mean Cu levels from this study generally fell within the lower range for these guidelines, on occasion higher levels were recorded (one replicate from pen 5 contained a level of 109 mg/kg) and in relation to toxicity the maximum level encountered can be far more important than the mean. Table 5.4.10.1 shows several recommended standards for sedimentary copper. The guidelines in Florida and for SEPA suggest that at the highest levels recorded in the present study there might be an adverse environmental effect.

Metals are generally not biodegradable: the only way to reduce levels in specific areas or avoid accumulation is by removal/dispersal by natural environmental mechanisms. These mechanisms can be either biological (i.e. bioturbation/ resuspension) or hydrological (resuspension). If Cu is accumulating in the sediments, the levels could rapidly approach high levels (ISQG) and this would have biological and ecological consequences.

Most of the literature on the effects of Cu on the biota has focused on levels in the ambient seawater with little information on the effects on sediment organisms. Brooks and Mahnken (2003) reviewed much of the information regarding the effects of Cu antifoulants in relation to salmon farming. Lu and Chen (1977) found that the release of Cu was significantly more pronounced in oxidizing than reducing environments and was slightly more pronounced in coarser sediments. Di Toro et al., (1992) showed that the reactive pool of acid volatile sulphide in sediment can bind metals rendering them biologically unavailable. Consequently Cu accumulated in fine, anaerobic sediments is less likely to be biologically available/toxic than equivalent levels in coarser aerobic, sediments (such as those at Creeses Mistake).

The toxicity of Cu is a function of its speciation (EPA, 1984). The data presented here are for total Cu, not EDTA-extractable Cu. In organically rich environments Cu may bind with organic ligands and therefore be biologically unavailable (Harrison et al., 1987). However, there is a lack of information on the relative toxicity of different Cu species/complexes. Brooks and Mahnken (2003) in a case study on the effects of Cu based antifouling paint in British Columbia showed no significant difference between treatments; the mean at unfarmed reference sites was 12.0 mg/kg (n=10, 95% confidence limit =4.3), at farm sites not using Cu antifoulant was 26.3 mg/kg (n=39, 95% confidence limit = 2.8), whilst at farm sites using Cu antifoulant the mean was 48.2 mg/kg (n=117, 95% confidence limit = 27.0). However, there was substantial variability in the results where Cu antifoulants were used and several sites exceeded the local government screening levels. Those sites which exceeded the standards were where the waste water from netwashing was commonly discharged into the environment (Brooks and Mahnken, 2003). Similar findings were reported in a study of Scottish farms by Solberg et al. (2002).

Table 5.4.10.1. Recommended standards for sedimentary copper concentration. Abbreviations indicate Threshold Effects Level (TEL), Probable Effects Level (PEL), Effects Range Low (ER-L) and Effects Range Median (ER-M).

Sediment Quality Criteria	Standard	Copper (mg/kg)
Washington	Apparent Effects Threshold	390
Florida	(TEL+PEL)/2	63.35
	(ER-L+ER-M)/2	152
Scottish Environmental Protection Agency	Background Concentration	16
	"Possible" Adverse Effects Level	35
	"Probable" Adverse Effects Level	390

Compared with similar farming sites elsewhere in Tasmania, Creeses Mistake is in a relatively dynamic location with at least 10 m of water between the cage bottom and the sediments. Consequently, the magnitude of the increase in sediment Cu is quite surprising. If anti-foulant material has been flaking off the cages then low level increases directly at the cages might be expected, and perhaps broader scale increases in the local system would be apparent over the

longer term. The results indicate that the levels in some sediments at the cages have increased rapidly, but so far the reference positions (150 m from the cages) have shown no evidence of Cu accumulation.

5.4.10 Summary

Deposition of faeces and excess feed to the sediments will increase the organic content (Hall et al., 1990, Holmer, 1991) and therefore evaluation of organic content should reflect the degree of impact. There are numerous ways in which organic matter can be measured. Several studies have shown that input of organic matter results in changes in the sediment particle size (Hall et al,. 1990, Holmer, 1991). However, this was not found to be the case at either location in the current study. Measurement of total organic matter (TOM) has also been used to identify different levels of organic enrichment but in this study TOM results did not correlate with either the farm production information or any of the other measures of environmental impact. It has been reported that this method is significantly influenced by the presence of shell and other refractory carbonates, although efforts were made to compensate for this by acidification of the samples, it is possible that the basic nature of the sediments compromised these results. However, it is clear that TOM is not sufficiently reliable to be considered as a useful approach for farm-based monitoring.

Measurement of physico-chemical parameters, such as redox and sulphide, has been applied as an indicator of sediment condition in many monitoring programmes worldwide. Redox has been included in the regulatory standards required for fish farm monitoring in Scotland, Canada, the U.S. and locally in Tasmania. Both sulphide and redox concentration showed a measurable response to farming activities, indicating a deterioration in environmental conditions throughout the farm production phase followed by an improvement in conditions when production ceased. The present study found that both redox and sulphide were more useful as measures of sediment degradation than recovery. Sulphide on its own was a particularly poor indicator of recovery. This is presumably because degrading conditions are associated with the continuous active input of organic material whereas recovery is a passive process. In New Brunswick, Canada it has been recommended that redox and sulphide be evaluated in combination (Wildish et al. 1999). Our findings indicate that sulphide is extremely unstable and degrades rapidly in recovering systems, such that the relationship identified in the study by Hargrave et al. (1997) does not persist.

Several levels of effect could be discerned which compare reasonably well with the levels characterised by the benthic infaunal evaluation (Fig's 5.3.8.2, 5.3.8.3 and 5.4.11.1). However, the boundaries were not as distinct, suggesting that redox potential and sulphide concentration were less sensitive than measures of community change.

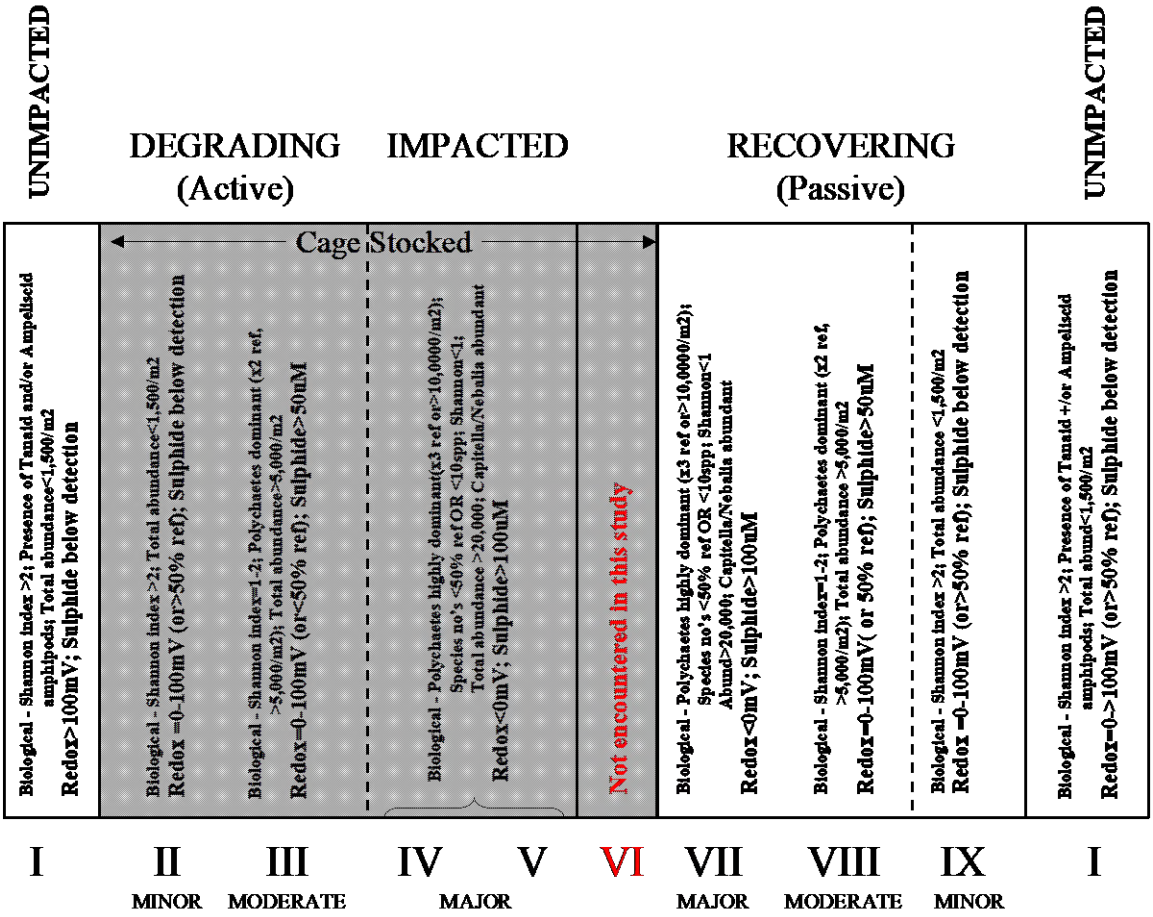


Fig. 5.4.11.1. Categories and criteria for redox and sulphide values based on effect stages defined by benthic community structure.

Both parameters varied with depth in the core, although measurement at 3cm adequately reflected the integrated core. In all cases a negative redox was considered undesirable and indicated impacted conditions, redox lower than 50% that at the reference indicated moderately impacted conditions. Redox levels above 100mV at 3cm appeared quite healthy. Redox levels higher than 50% of the reference but below 100mV may be considered acceptable but should be judged in conjunction with other parameters (eg. video or fauna). Sulphide levels above 50uM were generally associated with a moderate impact and levels greater than 100uM represented a significant impact. However, levels lower than 50uM do not necessarily mean that the sediment is recovered and under these circumstances the results should definitely be validated with other measures. Sulphide and redox measurement would be most useful when applied in combination with other measures of sediment condition.

Neither measure clearly identified progressive deterioration but comparison of the redox recovery rates over the two stocking cycles suggest that there is potential for progressive deterioration. This can be seen in the results for the first stocking cycle where the redox potential did not recover to pre-stocking levels nor did it reach the level achieved in the second cycle.

Since waste feed and faeces pass through the cages and drop to the sediments through the water column an increase in the amount of suspended material in the water column adjacent to the cages might be expected. However, turbidity and sedimentation rate were poor indicators of sediment condition, neither approach correlating with either farm production information or impact as reflected by the benthic infaunal assessment. In addition, sedimentation rate often appeared to be confounded by the effects of cage fouling and general farm operations. Sedimentation rate often appeared more closely related to natural changes than to the organic inputs from the farm. Given that these measurements were made adjacent to cages rather han directly beneath them, it may be that lateral dispersion of feed and faeces is limited and therefore the effect was reduced. If this is the case then for such measurements to be relevant they need to be made directly under cages. However, all of the other measurements of benthic impact were evaluated adjacent to the cages and did reflect the varying impact levels. Consequently, neither turbidity nor sedimentation rate could be recommended for farm monitoring.

Sediment metal levels were not evaluated as a means of monitoring the environmental conditions within salmon farm leases but were included in this study when it was identified that copper-based antifoulants were being used on nets and that this could represent a significant environmental risk. The results indicate that metal levels increased rapidly and significantly under the cages. It is suggested that further research is undertaken to determine the biological significance of this increase.

The significant inter-annual variation in concentration of both oxygen and ammonia as the reference sites suggests that Creeses Mistake is reasonably dynamic. The cage sites showed little or no recovery in terms of sediment oxygen penetration as a result of fallowing. Where there was no significant difference in the depth of the oxic zone between the reference and cage sites, this was a result of a decrease in oxygen penetration into the sediments of the reference site. The reason for the decrease in oxygen penetration at the reference sites after 12 months may have been a result of organic carbon loading or a change in porosity of the sediment grains or from both.

5.5 Microbial ecology

5.5.1 Community ecology

5.5.1.1 Bacterial Counts Over Production Cycle One

In the first production cycle, cages were combined for analysis resulting in two treatments, cage and reference. The production cycles at the cage sites were not synchronised in the second year, consequently cage 5, reference 1 and cage 8 reference 2 were treated separately. Mean microbial direct counts ranged from $1.56 \times 10^8 \pm 6.1 \times 10^7$ to $4.78 \pm 1.1 \times 10^8$ at reference sites and from $5.39 \pm 2.7 \times 10^8$ to $3.2 \pm 1.8 \times 10^9$ at cage sites.

Table 5.5.1.1.1 ANOVA for mean counts at Creeses Mistake during the first 12 month stocking period.

Tests of Between-Subjects Effects

Dependent Variable: LOGCOUNT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.633[a]	17	1.155	45.112	.000
Intercept	7054.923	1	7054.923	275573.4	.000
TREATMEN	11.377	1	11.377	444.400	.000
TIME	5.220	2	2.610	101.954	.000
DEPTH	.336	2	.168	6.559	.002
TREATMEN * TIME	.851	2	.425	16.620	.000
TREATMEN * DEPTH	8.186E-02	2	4.093E-02	1.599	.209
TIME * DEPTH	8.053E-02	4	2.013E-02	.786	.537
TREATMEN * TIME * DEPTH	9.006E-02	4	2.251E-02	.879	.480
Error	1.997	78	2.560E-02		
Total	7390.694	96			
Corrected Total	21.630	95			

a. R Squared = .908 (Adjusted R Squared = .888)

Microbial numbers were always significantly higher at the cage sites than at reference sites ($P<0.05$) (Table 5.5.1.1.1). Microbial numbers increased with organic loading at farm sites over the nine month stocking period and decreased during the three month fallowing period (Fig. 5.5.1.1.1). Although bacterial numbers continued to rise between 4.5 and nine months stocking, no significant difference was observed between these times. The large standard error observed at nine months for the cage site may have been caused by the different stocking and feeding rates each site received. Fish at one replicate cage were harvested part-way through the stocking period and were not restocked for several weeks. As a result organic loading for [illegible] er replicate cage.

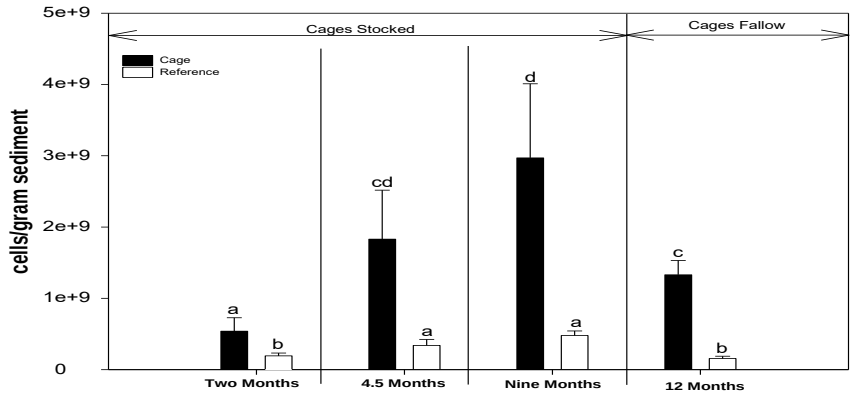


Fig. 5.5.1.1.1. Bacterial counts for cages and reference sites over the first 12 month farm cycle. Bars sharing a superscript are not significantly different ($p<0.05$). Error bars represent the standard error (n=4 for two-month samples, n=6 for all other samples).

ANOVA showed a significant effect of the sediment depth on bacterial numbers. Bacterial counts were higher in the surface layer than they were in the deepest layer (Table 5.5.1.1.2).

Table 5.5.1.1.2. Mean bacterial counts (cells/gram sediment) and standard errors at each sediment depth over the two year trial. Means sharing a common superscript within each sampling time/cycle are not significantly different.

DEPTH	First cycle		Cage 5, Ref 1, second cycle		Cage 8, Ref 2, second cycle	
	Mean	SE (n=44)	Mean	SE (n=32)	Mean	SE (n=24)
0-2 mm	1.25×10^9 *a*	2.64×10^8	8.87×10^8 *a*	1.86×10^8	6.2×10^8 *a*	1.4×10^8
2-5 mm	1.11×10^9 *b*	2.9×10^8	6.62×10^8 *ab*	1.28×10^8	5.0×10^8 *ab*	1.2×10^8
5-10 mm	8.25×10^9 *b*	1.59×10^8	6.16×10^8 *b*	1.31×10^8	4.2×10^8 *b*	8.4×10^7

5.5.1.2 Bacterial Counts Over Production Cycle Two

During the second 12 month production cycle mean bacterial numbers ranged from 8.6 ±1.4 x 10^8 to 1.9 x 10^9 ±3.1 x 10^8 at cage 5 and from 2.3 x 10^8 ±1.5 x 10^7 and 1.0 x 10^8 ±7.5 x 10^6 at reference 1. Bacterial numbers were higher at the cage site at the beginning of the stocking period, and remained higher for the entire twelve-month period (Table 4.5.1.2). Bacterial numbers at the cage site increased over the nine-month stocking period and declined over the fallow period (Fig. 5.5.1.2.1).

Table 5.5.1.2.1. ANOVA results for mean cell counts at Creeses Mistake, cage 5 and reference 1 over second production cycle.

Tests of Between-Subjects Effects

Dependent Variable: LOGCOUNT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14.306[a]	23	.622	24.790	.000
Intercept	5371.929	1	5371.929	214096.1	.000
TREATMEN	12.517	1	12.517	498.858	.000
TIME	1.032	3	.344	13.706	.000
DEPTH	.201	2	.101	4.013	.024
TREATMEN * TIME	.171	3	5.686E-02	2.266	.093
TREATMEN * DEPTH	2.480E-02	2	1.240E-02	.494	.613
TIME * DEPTH	3.902E-02	6	6.504E-03	.259	.953
TREATMEN * TIME * DEPTH	.322	6	5.360E-02	2.136	.066
Error	1.204	48	2.509E-02		
Total	5387.439	72			
Corrected Total	15.510	71			

a. R Squared = .922 (Adjusted R Squared = .885)

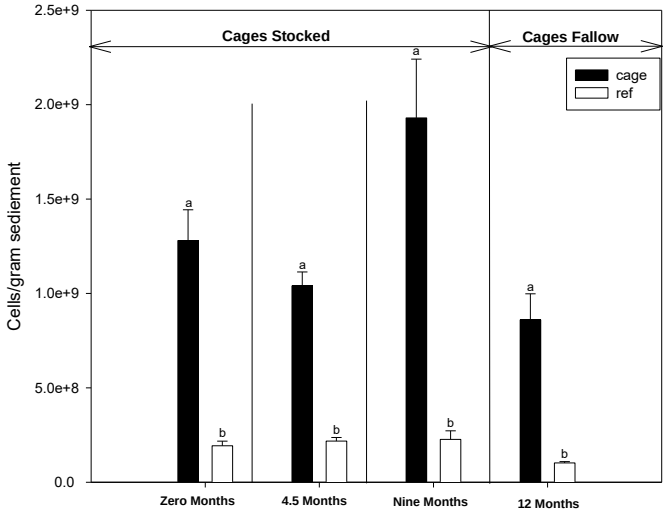


Fig. 5.5.1.2.1. Mean bacterial counts for cage 5 and reference site 1 over the second 12-month farm cycle. Bars sharing a superscript are not significantly different (p<0.05). Error bars represent the standard error (n=3).

During the second cycle cage 8 was fallowed for 4.5 months, instead of three months. ANOVA results are presented in Table 5.5.1.2.2. This extra fallow period allowed bacterial numbers to return to reference levels at the start of the new stocking period (Fig 5.5.1.2.3). Microbial numbers increased rapidly during the second stocking period, and declined during the fallow period. Although they did not return to the same levels as the reference site, they were not significantly different from the pre-stocking level. Again bacterial numbers were higher in the surface sediments (Table 5.5.1.1.2).

Table 5.5.12.2. ANOVA for mean counts at sites cage 8 and reference 2 over the second 12 month stocking/fallow cycle

Tests of Between-Subjects Effects

Dependent Variable: LOGCOUNT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Eta Squared
Corrected Model	13.926[a]	23	.605	33.866	.000	.942
Intercept	5168.496	1	5168.496	289092.2	.000	1.000
TREATMEN	7.348	1	7.348	411.000	.000	.895
TIME	1.937	3	.646	36.119	.000	.693
DEPTH	.264	2	.132	7.376	.002	.235
TREATMEN * TIME	4.207	3	1.402	78.435	.000	.831
TREATMEN * DEPTH	1.366E-02	2	6.828E-03	.382	.685	.016
TIME * DEPTH	9.050E-02	6	1.508E-02	.844	.543	.095
TREATMEN * TIME * DEPTH	6.570E-02	6	1.095E-02	.612	.719	.071
Error	.858	48	1.788E-02			
Total	5183.279	72				
Corrected Total	14.784	71				

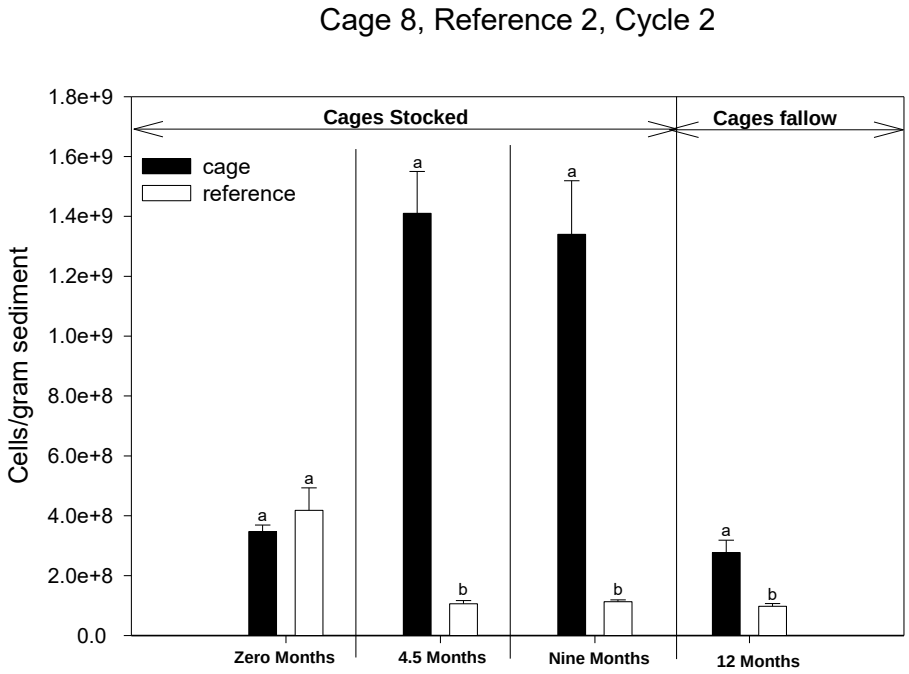


Fig. 5.5.1.2.3. Mean bacterial counts for cage 8 and reference site 2 over the second twelve-month farm cycle. Bars sharing a superscript are not significantly different ($p<0.05$). Error bars represent the standard error (n=3).

5.5.1.3 Accumulation Effects of Organic Load on Bacterial Numbers

To determine the accumulative effects of farm input on bacterial numbers, cage sites were compared at the beginning of the study (2 months), at the end of the first fallowing period (12 months) and at the completion of the study (end of the second fallowing period, 24 months). Because cages were not treated in the same manner during the first fallowing period and the second cycle the comparison was made for both cage sites separately.

At cage 5 there was a significant effect of time on the bacterial numbers ($F_{2,21}= 4.483$, $P<0.05$). After the first twelve-month cycle bacterial numbers were significantly higher than at the beginning of the study (2 months). However, at the end of the study (24 months) there was no significant difference between the bacterial numbers either at the beginning of the study (2 months) or after 12 months (Fig. 5.5.1.3.1).

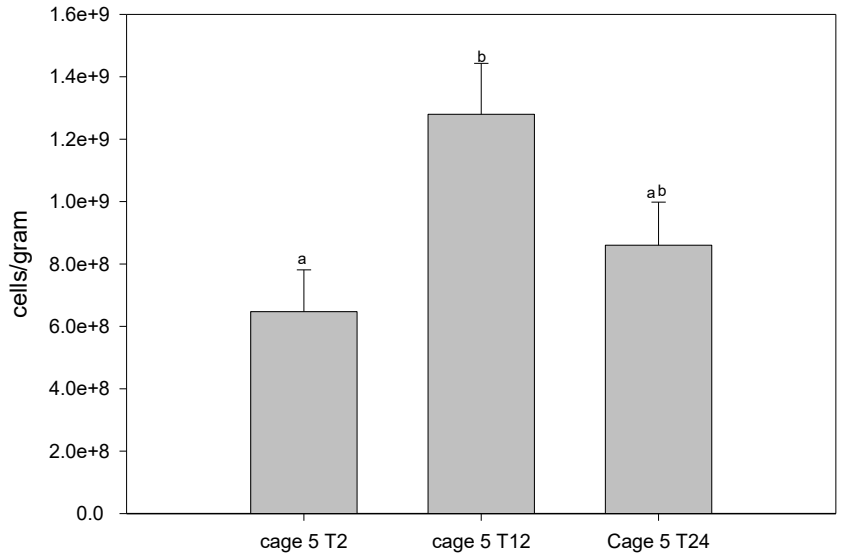


Fig. 5.5.1.3.1. Mean bacterial numbers at cage 5 at two months, 12 months and 24 months. Bars represent standard errors of the mean (n=3). Bars sharing a common superscript are not significantly different.

A similar pattern was evident for cage 8 ($F_{3,29}$=49.81, P<0.001), but the additional fallowing time given cage 8 resulted in a further decline in microbial numbers. Bacterial numbers were still significantly elevated after 12 months, (the completion of the initial three month fallowing period), but declined during the 1.5 month additional fallowing period to levels not significantly different from those at the beginning of the trial. After the second cycle bacterial numbers actually declined still further, to below those detected at the beginning of the trial (Fig. 5.5.1.3.2).

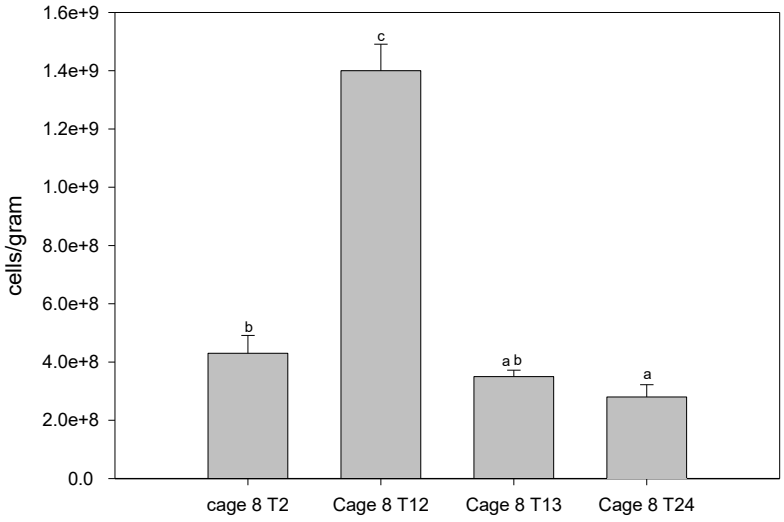


Fig. 5.5.1.3.2. Mean bacterial numbers at Cage 8 at two months, 12 months and 24 months. Bars represent standard errors of the mean (n=3). Bars sharing a common superscript are not significantly different.

5.5.1.4 Bacterial Biomass

Bacterial biomass increased during the stocking period and decreased during the fallowing period (Table 5.5.1.4.1). Biomass followed the same trends as total bacterial counts.

Table 5.5.1.4.1. Bacterial biomass (µgC/gram sediment) over both farm cycles

FARM CYCLE 1	TWO MONTHS	NINE MONTHS	12 MONTHS
Cage	40.11±16.9	305.53 ±46.9	167.86±13.4
Reference	14.81±1.8	36.41±2.1	15.43±1.2

FARM CYCLE 2	ZERO MONTHS	NINE MONTHS	12 MONTHS
Cage 5	76.82±5.5	124.17±18.4	62.38±11.17
Reference 1	14.37±1.7	12.6±1.9	7.9±0.53
Cage 8	22.53±1.0	88.59±11.04	18.71±2.5
Reference 2	25.59±3.2	7.15±0.38	6.60±0.56

5.5.1.5 Beggiatoa Counts

There was high variability in cage *Beggiatoa* filament counts (Fig. 5.5.1.5.1). The reference sites and fallowed cages both showed low numbers of filaments. Stocked cages showed an increase through stocking cycle. Photographs of *Beggiatoa* suggest the possibility that some cells were vacuolated. *Beggiatoa* filaments are clearly indicative of high organic loadings combined with significant concentrations of sulphide produced from sulphate reduction because they have a requirement for both organic carbon and reduced sulphur.

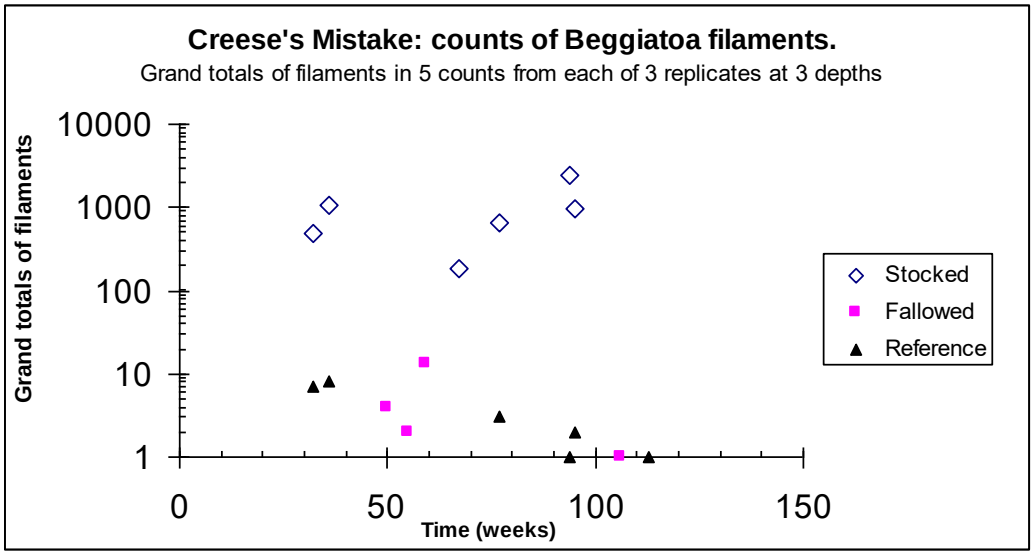


Fig. 5.5.1.5.1. Counts of *Beggiatoa* filaments in sediments from the Creeses Mistake farm.

5.5.1.6 Summary of Bacterial Community Ecology

Bacterial numbers and biomass generally increased with organic loading and then declined during fallowing. This trend was clearest over the first twelve-month cycle, but persisted throughout the study. During the first cycle a large amount of the variance seen at 9 months may be attributed to the different levels of loading experienced by replicate cages. Bacterial numbers at cage 5 reached higher levels than those at cage 8, and farm records show that cage 5 was stocked and fed more consistently over the 9 month period than cage 8. Despite this the impact of organic loading on microbial numbers was still detected. It has previously been reported (La Rosa et al., 2001) that microbiological parameters are very sensitive to fish-farm deposition. Although microbial numbers in this study did not decline to the same extent as reported by La Rosa et al. (2001) there was an initial decline in numbers, presumably as the highly labile organic matter was utilised. This rapid decline would occur any time organic

loading was decreased (e.g. during harvesting events or if feeding was stopped), not only if loading was halted for extended periods, as in fallowing. Such a response highlights the importance of sampling quickly after the cessation of loading if a reliable assessment of environmental impact is to be made.

Bacterial numbers were always higher in the surface sediments (0-2mm) than they were in the deeper sediment (5-10mm), regardless of treatment. This result suggests that bacterial growth rates are determined primarily by the availability of nutrients (Bastviken et al. 2001; Blume, et al. 2002) and not the pathways available for growth. It has been previously reported that waste from fish farms contains highly labile organic matter (McGhie, et al. 2000). Organic matter is first available to microbes at the sediment surface, which utilise what they can and less labile matter is passed into the underlying sediments. If metabolic pathway was of primary importance, then as the sediment layers become more homogeneously anoxic, numbers should be evenly distributed throughout the core. This was not observed.

A similar pattern was evident during the second cycle. However, because the cages were treated separately (due to the different timing of fallowing periods) an interesting result emerged. The extra 1.5 months fallowing afforded to cage 8 resulted in a significant decline in bacterial numbers over that period. This finding suggests that, with the level of loading experienced at this site, 3 months was not long enough for the highly labile organic matter to be utilised. At both sites bacterial numbers were elevated compared to the reference sites after the initial 3 months fallowing, but at cage 8 numbers declined further in the extra 1.5 months fallowing. Cage 8 also showed a more complete recovery after the second fallowing period. This suggests that the extra fallowing time given to cage 8 may have been long enough for the utilization of the highly labile organic matter, allowing the sediment microbial community to further "catch-up" before the second stocking cycle.

Consequently, the effect of farming on bacterial numbers is not necessarily cumulative. Numbers were elevated after the first cycle, but fell by the end of the second cycle. The high variability and the fact that the initial samples were taken two months after farming commenced suggests that the first sample is probably elevated above the true pre-stocking level. Whilst bacterial numbers did increase and decrease with organic load, they did not continue to increase and/or remain at maximum levels after farming ceased. Microbial numbers in the second cycle did not reach those exhibited in the first cycle. This is probably the result of these sites not being farmed as intensively in the second cycle.

5.5.2 Bacteriological Indicators

This study has made substantial progress in developing and assessing a bacteriological tool for monitoring available organic carbon in the sediments. Seven bacteria have been isolated from reference and cage site sediments at Creeses Mistake. They have been partially identified (Table 5.5.2.1). The genotypic affiliation is from the 16S rRNA gene similarity and does not exactly identify the isolate. Rather, it shows similarity to known strains. So far phenotypic identification largely agrees with the genotypic affiliation. Three of the isolates grew well in standard media and thus are potential candidates for bacteriological monitoring. The two *Vibrio* isolates have been used in dilution trials and have been successful with both diluted acetate and glucose as well as with sediment extracts (Table 5.5.2.2).

Table 5.5.2.1.Characterisation of selected heterotrophic bacteria from Creeses Mistake sediments.

Code	C8B1	C8B2	C8B3	CR1B1	CR2A1	CR2A2	C5B1
Gram	GNR	GNR	GNR	GNR	GNR	GNR	GPR
Aerobic	+++	+	+++	+++	+++	++	++
Anaerobic	-	-	+	+	+	+	-

Catalase	-	-	-	+	+	+	+
Oxidase	+	+	+	+	+	+	-
Motility	+	+	+	+	+	-	+
Oxid/ Ferment	NR	NR	NR	F	F	NR	NR
Growth TCBS	-	-	-	Y	G	-	-
Growth 37C	+	-	+++	-	-	+	+
Growth 0% SW	-	-	-	-	-	-	+
Growth 1% SW	+	-	+	+	+	+	+
Growth 3% SW	+	+	+	+	+	+	+
Sens. O129							
10 or 150 µg	-	-	-	+	+	-	-
ONPG	-	+	-	+	+	-	+
Glucose	-	-	-	-	+	-	-
Arabinose	-	-	-	-	-	-	-
Lysine decarb	-	-	-	-	-	-	-
Ornith decarb	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-
H2S	-	-	-	-	-	-	-
Urea	-	-	-	-	-	+	-
TDA	-	-	-	-	-	-	-
Indole	-	-	-	+	+	-	-
Methyl Red	-	-	-	+	+	-	-
Voges-Prosk.	-	-	-	-	-	-	-
Phenotype	*Shewanella* sp.	*Shewanella* sp.	*Shewanella* sp.	*V. splendidus* 2	*V. splendidus* 2	Not *Roseo-bacter*	Not *Altero-monas*
Genotypic affiliation	*Pseudoalt-eromonas*	*Pseudo-monas*	*Alteromonas*	*Vibrio* sp.	*Vibrio* sp.	*Roseobacter*	*Alteromonas*

Notes: GNR = Gram negative rod. GPR = Gram positive rod. NR = No reaction. F = Fermentative. O = Oxidative.

Table 5.5.2.2. Growth after 6 days incubation of selected heterotrophic bacteria at 20C in minimal medium with different concentrations of acetate.

Isolate	Code	0.5%	0.25%	0.125%	0.065%	0.032%	0.016%
Shewanella	C8B1	+	+	+	+	+	-
Shewanella	C8B2	-	-	-	-	-	-
Shewanella	C8B3	+	+	+	+	+	-
Vibrio	CR1B1	+	+	+	+	+	+
Vibrio	CR2A1	+	+	+	+	+	+
???	CR2A2	+	+	+	+	+	+

5.5.3 Summary

Microbial numbers increased and decreased in parallel with organic loading. With one exception (cage 8 after the extra 1.5 months fallowing at the end of the first year of production) there were always significantly more bacteria at the cage sites than at the reference sites. Numbers of microbes responded rapidly to organic loading – both increasing as loading commenced and decreasing after it was stopped. However, it is likely that 3 months of fallowing is not sufficient for the community to return to prestocking levels. There is potential for the development of a simple bacteriological assay for the concentration of biologically available organic carbon in sediment pore waters.

6. RESULTS & DISCUSSION - STRINGERS COVE

6.1 General Site Information

There was no difference in the water column parameters (temperature and salinity) measured from the various sample sites within the farm on each sampling occasion over the first twelve months. Consequently in the second production cycle only a single measurement was taken as representative of the entire farm at each of the sampling times (Fig.6.1.1a & b). Farm personnel recorded temperature and dissolved oxygen daily from 5m depth at fixed positions within the lease (Fig.6.1.1c). Water temperatures over the study period showed a clear seasonal response, with temperatures ranging from 10-19°C and the highest temperatures recorded over the summer period, between January and April. Average summer temperatures (January/February) were higher in 2001 than in 2002 ($F_{2,3}$=11.984, p=0.037). However, the temperature range both in summer and over the remainder of the years was similar (Fig.6.1.1d). Although there was no significant difference between the cage and reference positions in bottom water oxygen levels, there was a seasonal reduction in oxygen levels over the summer period (Fig. 6.1.1.c). Salinity and temperature both declined over the winter period, which corresponds with an increase in rainfall and river flow at this time (unpublished data). Turbidity measurements were also variable over this period, and there was no significant difference between cage, farm and reference turbidity levels throughout the study period.

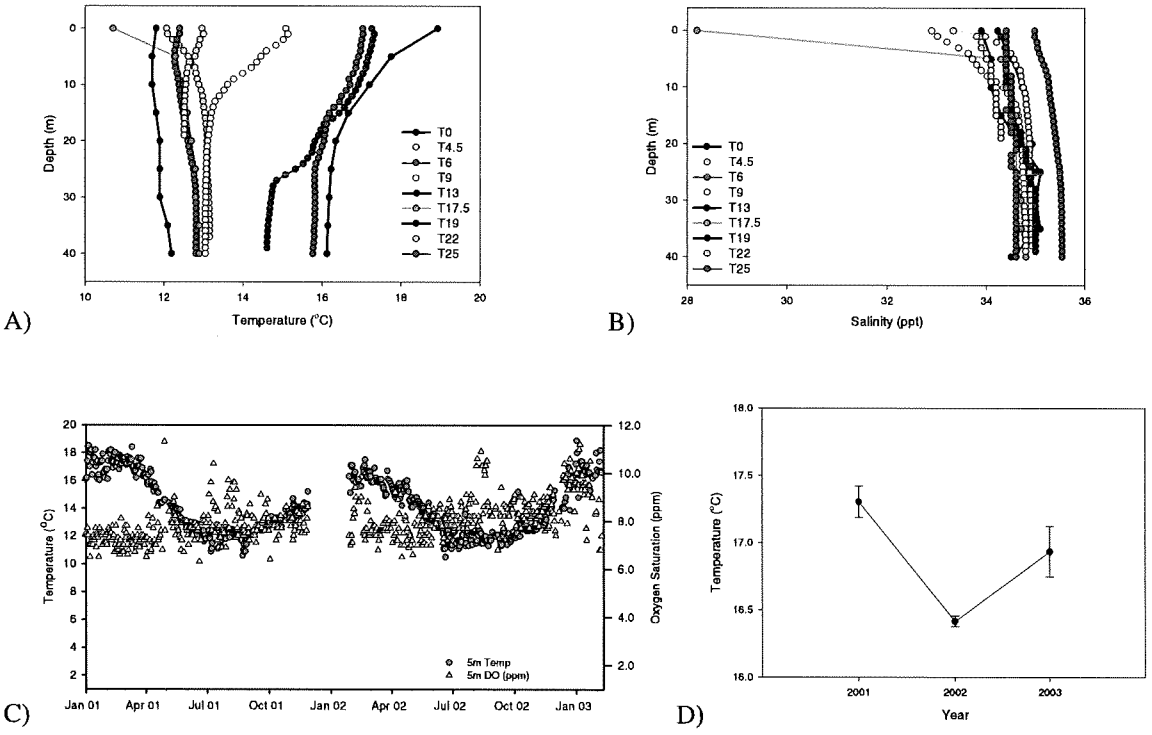


Fig.6.1.1. A) Temperature and B) Salinity at Stringers Cove at each sampling time during the study period. C) Temperature and Dissolved Oxygen collected daily within the lease by farm operators at a depth of 5m. D) Average summer (January, February) temperatures between 2001-2003.

Current velocity at Stringers Cove was generally in the range of 2-4cm/s and did not vary greatly, although there were several occasions where velocity increased markedly throughout the water column. Direction measurements show a strong tidal influence with direction oscillating between South-East predominantly, and North-West (Fig.6.1.2). Measurements of the Beaufort scale by farm operators show fewer storm events at Stringers Cove than Creeses

Mistake with an increase in the frequency of gale / storm activity in the spring period (September – November) of 2002 (Fig.6.1.3).

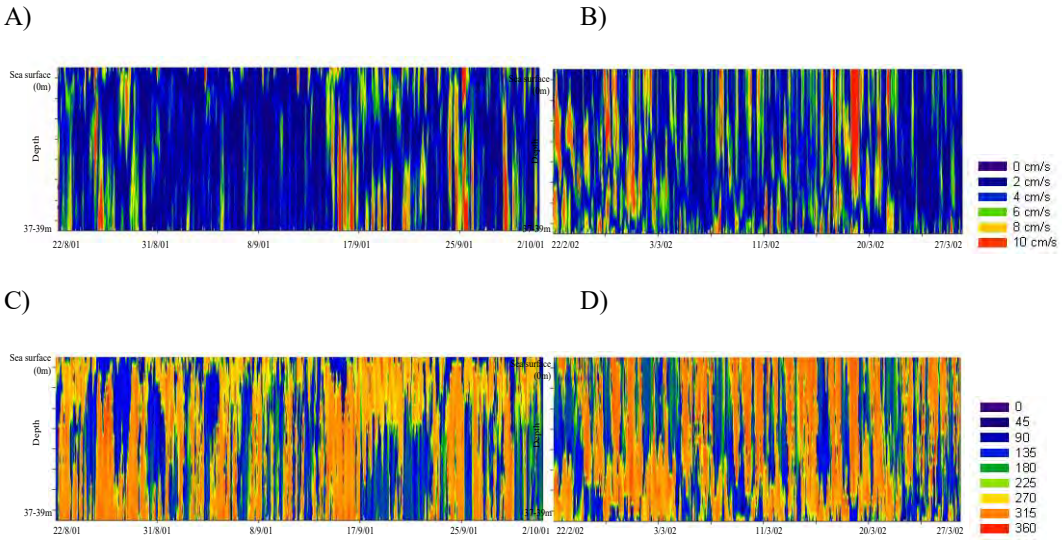


Fig.6.1.2. A) Current velocity at Stringers Cove during winter (September 2001) and B) during summer (March 2002). C) Current direction during winter (September 2001) and D) during summer (March 2002).

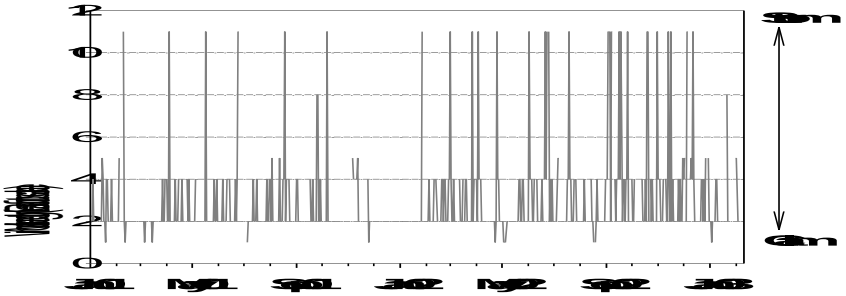


Fig.6.1.3. Daily weather conditions as recorded using the Beaufort scale by farm operators at Stringers Cove.

6.2 Farm production information

Biomass and mean individual fish weight were similar in all cages at Stringers Cove over both production cycles (Figure 6.2.1) and were broadly equivalent to the results at Creeses Mistake in the second production cycle. In the second production cycle at Stringers Cove, less feed was input to study cages than in the first cycle (Figure 6.2.2.). The effects of adjacent stocked cages was also investigated. The number of stocked cages surrounding each of the study cages may affect the impact level at those cages, particularly over the fallow period. The cages adjacent to the study cages were heavily utilised after the fallow period and in particular prior to the second production cycle (Figure 6.2.3). The entire site was only vacated for relatively short periods in either cycle; after production cycle 1 the site was fallowed from 28/11/01 to 24/1/02, whilst after the second production cycle the site was only fallowed from 17/12/02 to 22/1/03.

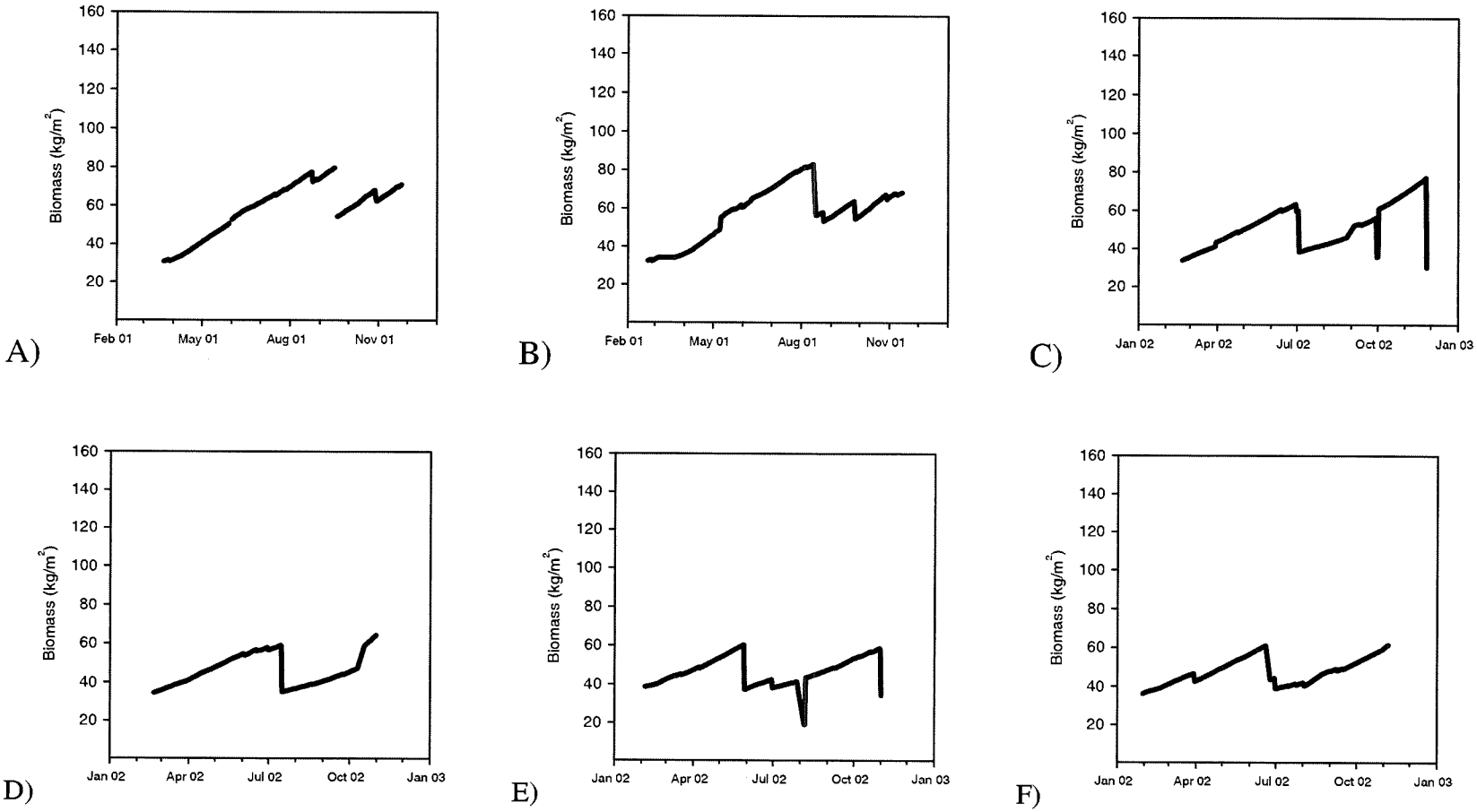


Fig.6.2.1. Cage biomass for study cages at Stringers Cove during production cycle 1 - (A) Position 1 *and* (B) Position 2; and production cycle 2 - (C) Position 1A, (D) Position 2A, (E) Position 3A *and* (F) Position 4A.

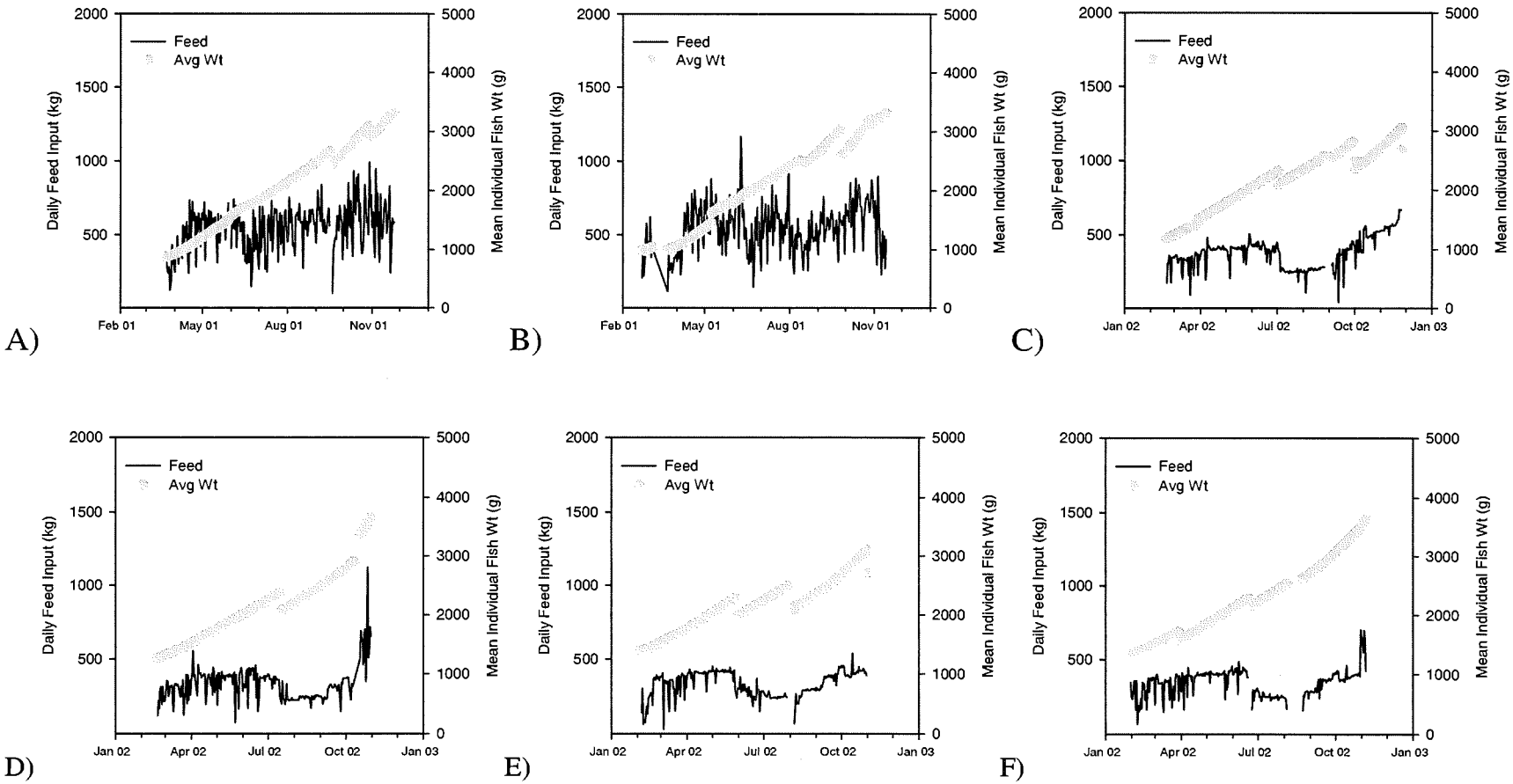


Fig.6.2.2. Farm production information for Stringers Cove including mean individual fish weight and daily feed input at study cages during production cycle 1 - (A) Position 1 *and* (B) Position 2; and production cycle 2 - (C) Position 1A, (D) Position 2A, (E) Position 3A *and* (F) Position 4A.

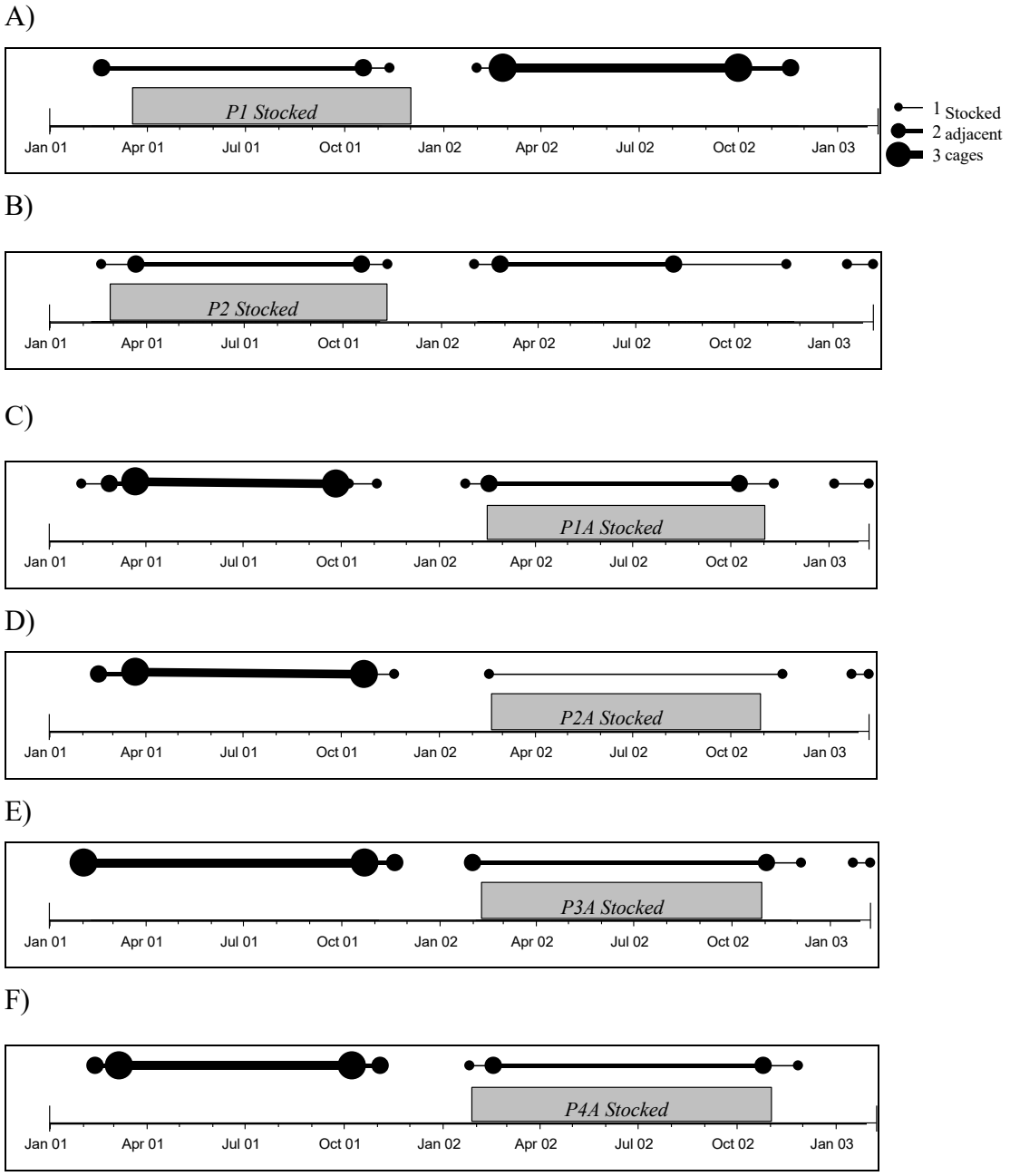


Figure 6.2.3. The number of stocked cages adjacent to each of the study cages at Stringers Cove during both production cycles. A) Position 1, B) Position 2, C) Position 1A, D) Position 2A, E) Position 3A, and F) Position 4A. Number of cages is indicated by thickness of line.

6.3 Benthic ecology

6.3.1 Characterisation of Community Change

There was a significant difference between "a priori" defined groups (reference, stocked cage, unstocked cage) (ANOSIM – Rho=0.717, $p<0.001$). Rho values (a multivariate correlation coefficient) from group pairwise comparisons indicate greater similarity between the unstocked and stocked cage groups (Rho= 0.515) than between the unstocked cage and reference communities (Rho=0.958) or the stocked cage and reference communities (Rho=1.000), which were all extremely different.

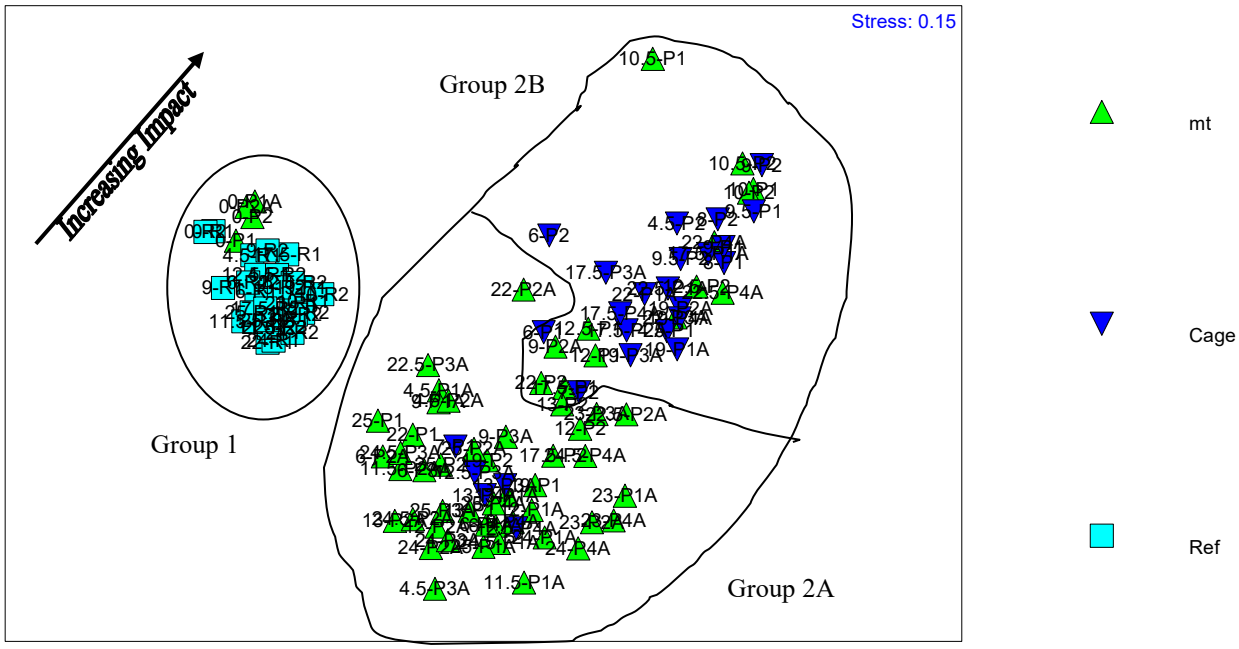


Fig. 6.3.1.1. Ordination analysis. 2-dimensional MDS plot of species abundance data. Stress=0.15. Label prefixes indicate the time of sampling in months, suffixes indicate the sampling position and symbols designate the impact category; stocked cage ( ), empty cage ( ) and reference ( ).

Multivariate analysis of the benthic infaunal abundance data indicates a clear separation of the farmed cage and reference locations (Fig. 6.3.1.1). The samples from the cage positions pre-farming associate more closely with the reference sites and unimpacted community than with the cage communities at any time after farming commenced. The fallowed sites do not associate with pre-farming or reference sites even after 14.5 months without stocked cages (P1/2). The impacted group is broadly spread, indicating a continuum of gradual change but generally the stocked cages are furthest from the reference group, and the cages which have been fallowed longest are nearest to the reference group. This suggests that the separation of the sites reflects an impact gradient running from left to right with the most impacted conditions towards the RHS of plot.

Cluster analysis enabled further separation of the impact group into two sub-groups. Although the communities which characterise these groups had some similarities they were sufficiently different at their extremes to be clearly distinguishable. Five stocked cage sites (7-P1, 12.5-P3A, 13-P4A, 13-P3A, 12.5-P4A) were represented in group 2A, the less impacted group. These sites had only recently been stocked with the exception of 7-P1, which was in the middle of the stocking period.

The communities which characterised the most impacted group (2B) were dominated by two species, *Capitella capitata* and *Nebalia longicornis* (Table 6.3.1.1). Together these species accounted for 95% of the overall similarity within this group (62% and 33% respectively). A broader range of species made up group 2A; *Nassarius nigellus* (31% of the within group similarity), *Corbula gibba* (21%) and Phoxocephalidae spp.(19%) with *Echinocardium cordatum* also making a notable contribution at 9.5% of the within group similarity. Together, these four species accounted for 80% of the overall within group similarity. The communities at the unimpacted sites (group 1) displayed a much greater range of species and there was no evidence of any particular species dominance. Nevertheless, *Mediomastus australiensis* and *Amphiura elandiformis* were significant members of the communities at the unimpacted sites, accounting for 19% and 15% of the within group similarity respectively.

Table 6.3.1.1. SIMPER output for the full community assessment indicating a) average abundance, ratio (average similarity / st.dev. similarity), % similarity and cumulative % similarity of the 5 most important species in each of the three main MDS cluster groups and b) average abundance, ratio (average similarity / standard deviation similarity) and cumulative % similarity of the three species which most clearly distinguish the main groups identified by cluster analysis.

Species Name	Average abundance (No./m2)	Ratio	Percent Similarity	Cumulative % Similarity
b) GROUP 1				
Mediomastus australiensis	202	10.91	19.34	19.34
Amphiura elandiformis	137	8.50	15.06	34.40
Nucula pusilla	98	4.44	7.87	42.26
Lysilla jennacubinae	73	3.96	7.02	49.29
Thyasira adelaideana	53	3.01	5.33	54.62
GROUP 2A				
Nassarius nigelus	391	9.92	30.85	30.85
Corbula gibba	663	6.74	20.97	51.82
Phoxocephalidae spp.	261	6.06	18.85	70.67
Echinocardium cordatum	109	3.06	9.51	80.18
Theora lubrica	279	1.97	6.12	86.30
GROUP 2B				
Capitella capitata	24053	25.21	61.86	61.86
Nebalia longicornis	7983	13.70	33.61	95.47

Species Name	Group Average Abundance	Group Average Abundance	Ratio	Cumulative % Similarity
b) BETWEEN GROUPS				
Groups 1 & 2A	Group 1	Group 2A		
Capitella capitata	13	24053	2.01	56.09
Nebalia longicornis	1	7983	1.42	83.65
Corbula gibba	35	359	0.59	85.66
Groups 1 & 2B	Group 1	Group 2B		
Corbula gibba	35	663	0.88	14.80
Nassarius nigellus	63	391	1.27	24.17
Phoxocephalidae spp.	20	262	0.93	31.93
Groups 1 & 2A	Group 2A	Group 2B		
Capitella capitata	24053	2440	1.94	57.51
Nebalia longicornis	7983	108	1.37	84.64
Corbula gibba	359	663	0.63	88.94

6.3.2 Classification of Impact / Recovery Stages

To more clearly evaluate the temporal and spatial differences in recovery at the Stringers Cove site it is useful to separate the three different production strategies that were employed at this site.

Strategy 1 - pens 1 and 2 had not previously been farmed and were stocked for 9 months in the first production cycle and thereafter were fallowed.

Strategy 2 - pen positions P1A and 2A were not stocked in this study until the start of the second production cycle and the associated sediments had never been exposed to farming activities.

Strategy 3 - pen positions P3A and 4A were also not stocked until the second production cycle but had previously been farmed.

Strategy 1 (*Pens 1 & 2 – stocked in first cycle)*

Ordination again revealed the differentiation between the reference and cage communities, with the more impacted conditions to top right hand side of the plot and the unimpacted/reference conditions to the lower left hand side (Fig. 6.3.2.1). As was the case with the MDS for the full dataset the distinction between the stocked and fallowed conditions was not clearly defined and was reflected by a larger, more disparate group where the samples indicated a progressive improvement in conditions over time. The stocked cages occupied the right hand side of this group, with those samples collected at the end of the stocked period (9 and 10 months) clustered to the far right. Samples collected at the beginning of the stocking cycle were located towards the centre of the main group (group 2). Samples taken over the recovery period (designated as MT) were mixed throughout group 2. Samples collected immediately after the commencement of fallowing (10-12 months) had communities which associated more closely with the stocked cages. However, environmental conditions changed rapidly and after 3 months conditions had markedly improved. Over the next twelve months there was further improvement in conditions, but the community structure never returned to that observed pre-farming. SIMPER analysis again indicated that *Capitella capitata* and *Nebalia lonicornis* were the main species characterising impacted locations (together accounting for 98% of the group similarity) whereas unimpacted conditions were characterised by a broader range of species (10 species making up 74% of similarity). The community at the recovering sites contained more species indicative of transitional conditions and was characterised by *Nassarius nigellus*, *Corbula gibba* and reduced levels of *Capitella capitata*, which together accounted for 70% of the within group similarity. The progressive temporal change in the benthic community was highlighted by the changes in the top ten species at each

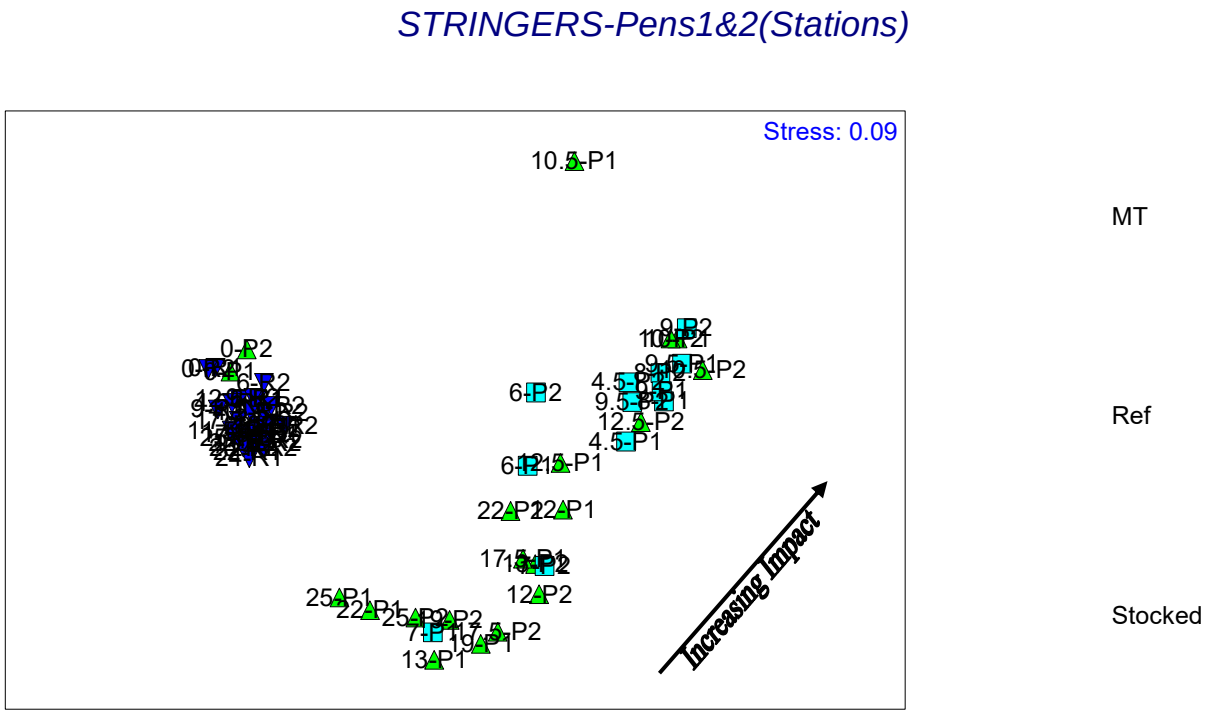


Fig. 6.3.2.1.Ordination analysis. 2-dimensional MDS plot of species abundance data for pen positions 1/2. Stress=0.09. Label prefixes indicate the time of sampling in months, suffixes indicate the sampling position and symbols designate the impact category; stocked cage ( ), empty cage ( ) and reference ( ).

Some interesting departures from the patterns were observed. Sample 7-P1 was located among the recovered sites, although 7 months was well into the stocked phase at P1. The production information does not indicate any reason why the environmental conditions should have improved at this time as the cage was consistently stocked and there was no reduction in feed.

The relative position of sample 10-P1 also appeared anomalous, the community at 10-P1 indicated impacted conditions but this position should have been recovering. Its position in the ordination was strongly affected by the presence of extremely high numbers of *Capitella capitata* and *Nebalia longicornis* in one replicate from this site. As this pen had only been emptied relatively recently it may be that there were still small areas where the impact persisted. The spatial pattern of recovery can be highly irregular (Zajac et al., 1998, Ellis et al., 2000).

Strategy 2 *(Pens 1A & 2A – stocked in second cycle, not previously farmed)*

Pens 1A/2A were fallowed over the first production cycle and therefore were not stocked until the second cycle. Samples from the start of the study associated more closely with the reference conditions (Fig. 6.3.2.2). There was a very clear distinction between samples from stocked and empty cages. All samples from the stocked cages grouped to the far right of the plot. The samples from the impact group but closest to the reference group were generally those obtained before the cages were stocked. The samples from empty cage stations immediately after stocking and in the early fallow period were closest to the impact group. The distribution of the stocked cages suggests progressive deterioration, moving to the right of the plot over time. Just before stocking (11.5 months) P1A appeared impacted. In this instance *Nebalia longicornis,* a species commonly associated with organic enrichment, was particularly prevalent at one of the replicates. Consequently the significance of the impact at P1A at this time should be viewed with caution. *Nebalia longicornis* is a swarming epibenthic species, located predominantly around the sediment-water interface, its distribution can be very patchy and may be affected by highly localised differences in conditions (e.g. presence of driftweed or small feed spills/patches) (Rainer & Unsworth, 1991, Edgar, 1997). The emptied cage positions most closely associated with the major impact group were those sampled immediately after the cages were emptied (22.5/23 months). Cage 2A at 9 months also clustered with the impacted groups, probably because the cages around this position were heavily stocked at that time. Again the data indicates that communities moved into recovery quickly. Recovered communities were similar to pre-farming after only 4-6 weeks. Samples collected from sites pre-stocking showed clear signs of impact, suggesting that there was a latent/residual farm effect. The species characterising the major groups were similar to those reported for Pens 1 &

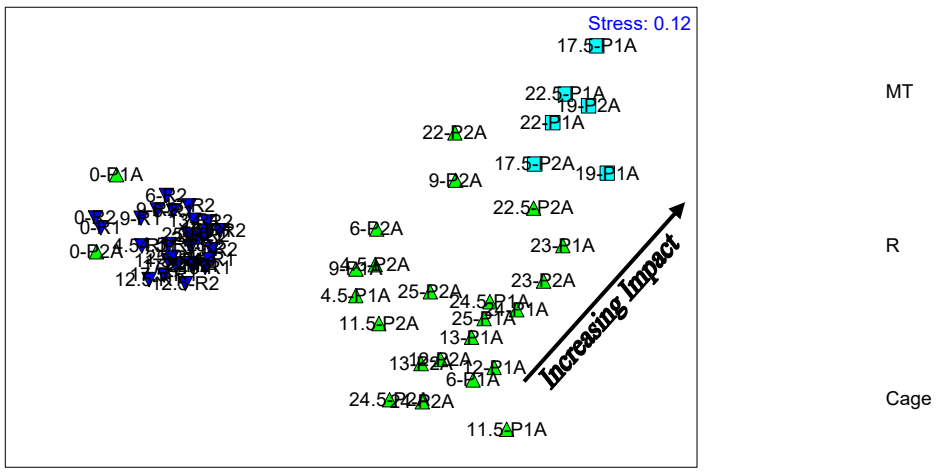


Figure 6.3.2.2.Ordination analysis. 2-dimensional MDS plot of species abundance data for pen positions 1A/2A. Stress=0.12. Label prefixes indicate the time of sampling in months, suffixes indicate the sampling position and symbols designate the impact category; stocked cage ( ), empty cage ( ) and reference ( ).

Strategy 3 *(Pens 3A & 4A – stocked in second cycle, previously farmed)*

The ordination of stations P3A/4A also demonstrated the differentiation between the impacted and unimpacted communities (Fig. 6.3.2.3). However, in this instance there appeared to be some distinction between the cages at the early and later phases of the stocking cycle. The impact did not appear to be as severe in the first 3-4 weeks of the stocking cycle (12.5 & 13 months) at which time the samples associated with reference and/or pre stocked conditions. Here, recovery again appeared quite rapid. Only 4 weeks after cage removal the community structure at position 3A (22.5 months) was comparable to that pre-farming (NB allowing for residual farm effects) and at position 4A the cage community was representative of generalised farm effects only 6 weeks after removal of cages (23 months). After this initial recovery

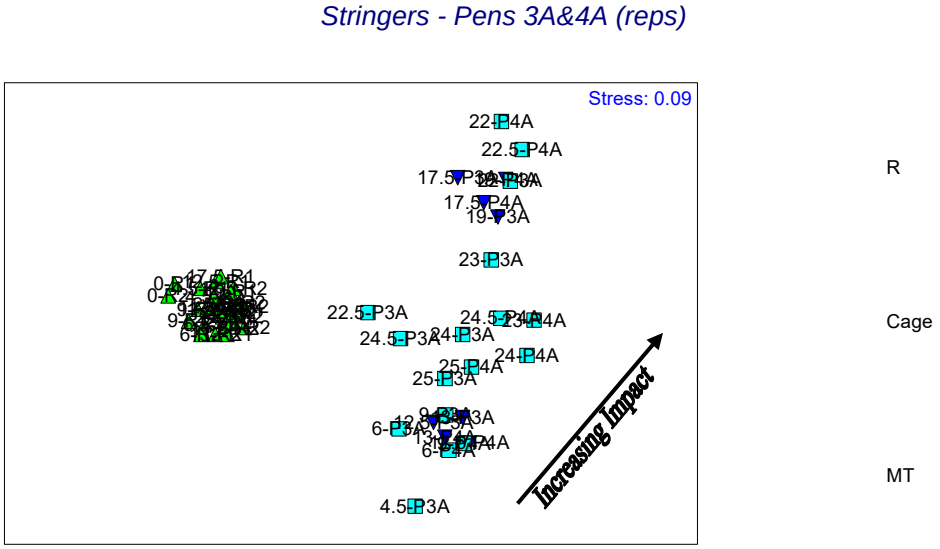


Figure 5.3.2.2. Ordination analysis. 2-dimensional MDS plot of species abundance data for pen positions 3A/4A. Stress=0.09. Label prefixes indicate the time of sampling in months, suffixes indicate the sampling position and symbols designate the impact category; stocked cage (▽), empty cage (□) and reference (▲).

6.3.3 Temporal Variability in Rate of Recovery

The ordination (Fig.6.3.3.1) shows three main groups and is the classic “horseshoe shape” often associated with environmental impact gradients (Clarke and Warwick, 2001). The first group correlates with zero or negligible impact and contains the samples collected before farming commenced anywhere on the site. A second group, which comprised all the stocked positions as well as those immediately after stocking, was indicative of impacted conditions, and a third group which contained the recovering positions and the samples from positions within the site, but prior to the occurrence of direct farm impacts, represented “intermediate” conditions.

Stringers - Cage

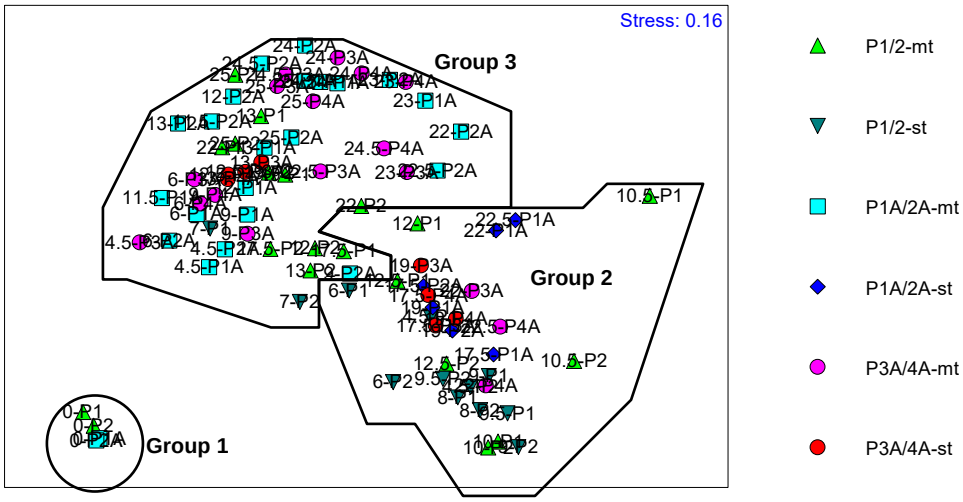


Fig. 6.3.3.1. Ordination analysis. 2-dimensional MDS plot of species abundance data for cage positions. Stress=0.16. Label prefixes indicate the time of sampling in months, suffixes indicate the sampling position and symbols designate the production strategy and impact status of the cage.

From the preceding analyses it is apparent that the temporal progression post-stocking represents a recovery gradient. If only those samples collected over the recovery phase are plotted (Fig. 6.3.3.2) some interesting patterns become evident. The rate of recovery at all of the study sites suggests differences in the level of impact. Generally the sites immediately after removal of the cages were the most impacted. The impact appears to be most pronounced at the positions stocked in the first cycle (P1/P2). These two pen positions responded slightly differently over the recovery phase, P1 recovered very rapidly and after only 3 months of fallowing had a community structure which stayed much the same over the next 12 months. P2 was still impacted after 3 months but thereafter recovered very quickly. The effect at P1A /2A seemed to be less than that at P1/2, within only a few weeks of the cages being emptied and feed input ceasing these positions recovered such that the infaunal community was equivalent to that present before stocking. At positions P3A/4A the impact pre-stocking once again appeared less severe. Overall the impact seems to have been less in the second cycle than in the first cycle.

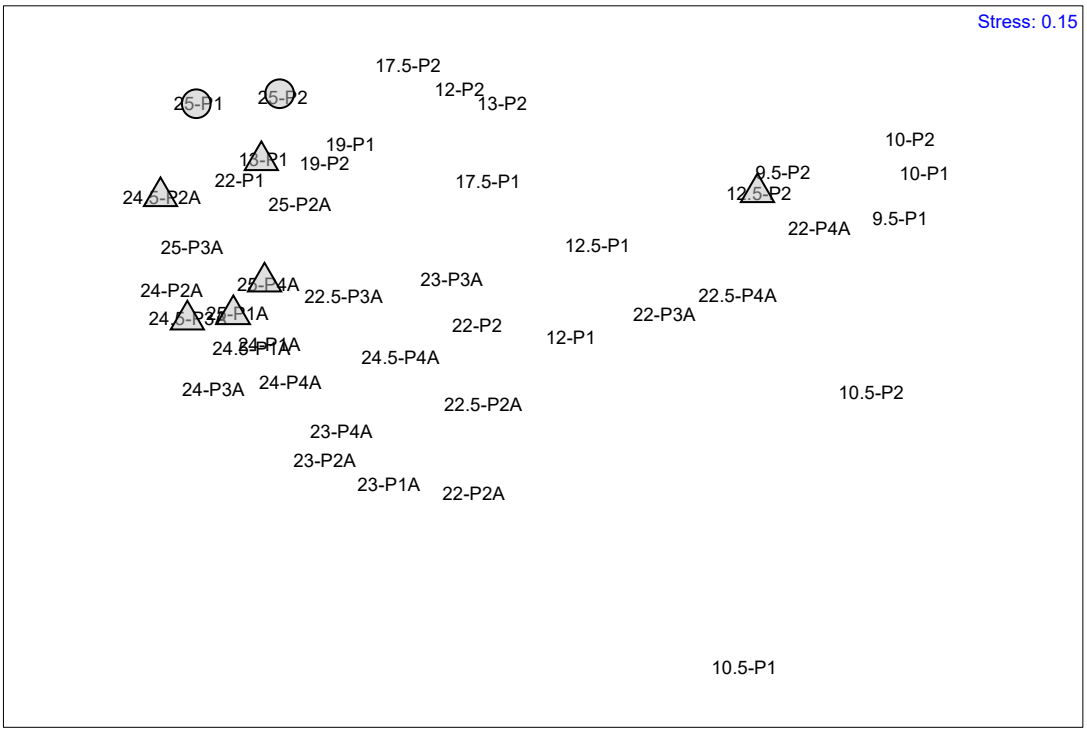


Fig. 6.3.3.2. Cage positions post-stocking i.e. in recovery phase. Label prefixes indicate the time of sampling in months and suffixes indicate the sampling position. Symbols designate fallow period of 3 months (△) and 15 months (○).

In the second cycle the overall feed input at the site was reduced and it is likely that this was a major factor in both the decrease in the overall impact and the decrease in the time required for recovery. After only 2 weeks fallow (23 months) in the second cycle the community at pen position P1A was similar to that at P1 after 12 weeks fallow (13 months) (Fig. 6.3.3.2) and after 10 weeks fallow the community at P1A had recovered to the extent that it was more similar to the unimpacted community than to P1 after 15 months fallow (Fig. 6.3.3.2).

Despite the fact that some recovery was apparent there was no evidence of return to pre-farming impact conditions at any Stringers position even after relatively long fallow periods (15 months). This suggests that fallowing of individual cage positions within an operational farm has limited capacity to achieve sediment recovery (i.e. conditions will never get better than transitional community because of generalised farm effects/impact). However, a transitional community may be the optimal state to achieve as this not only contains a broad range of species but may also provide the best reservoir of fauna to assimilate organic material in the next cycle. This may also be acceptable in the broader environmental context so long as the generalised farm effect doesn't extend beyond the lease boundaries and start to more widely affect longer-lived climax species in the environment.

6.3.4 Ecological significance of community changes

The different levels of spatial and temporal impact tended to be characterised by a number of key species. The variation in the primary function of those species reflects the change in ecology at the different stages.

Table 6.3.4.1. Ten most abundant species at a) Reference, b) P1/2, c)P1A/2A and d) P3A/4A positions at each sampling time.

a) Reference Sites

ID/Time	0mth	4.5mth	6mth	9mth	13mth	17.5mth	22mth	25mth
Lumbrinereis sp.(MoV 322)	293	183	329	549	366			
Spiophanes kroeyeri						366	329	
Scalibregma sp.(MoV 638)								549
Euchone limnicola				293		256		
Aricidea sp.1	695							
Asychis sp.2 (13079)	1061							
Capitella cf capitata (MoV 2558)		219		658				
Mediomastus australiensis	3402	2305	3256	1866	4719	1975	1939	3475
Aricidea sp.6		256	1792		1939	549	951	1024
Lysilla jennacubinae	1024	805	1975	1171	1536		658	951
Asychis sp.2			878	768	1975	439	1646	1280
Aedicira sp.2	293							
Paraonella sp.1					658			
Phoxocephalidae			256					
Euphilomedes sp.(MoV 18)	1097	988	476				293	512
Euphilomedes sp.(MoV 1021)	329	0			549	841	0	658
Nassarius nigellus		329		293	2231	1427	1939	951
Maoricolpus roseus						219		
Nucula pusilla	293	2158	1244	1171	3182	1280	1024	585
Nemocardium thetidis		402				366		402
Thyasira adelaideana	402	768	476	622	1097	841	732	622
Corbula gibba		293	988	256				
Theora fragilis								1390
Amphiura elandiformis	2048	1866	1646	2195	2231	2305	1536	2451
Nemertea sp.1	1024	1207	1171	878	732	476	1061	695

b) Pen position P1/2

ID Code/Time	0mth	4.5mth	6mth	9mth	13mth	17.5mth	22mth	25mth
Glycera sp.1					22			
Neanthes cricognatha						158		
Simplisetia amphidonta		10		2				
Schistomeringos loveni					32			
Euchone limnicola				2				
Paraprionospio coora				2	27	20		27
Aricidea sp.1	79							
Asychis sp.2	86							
Asychis sp.1 (MoV 907)	27	12	64	17				
Capitella cf capitata (MoV 2558)		21089	7049	58380	1002	2020		
Mediomastus australiensis	249		89			22	22	15
Paraonides sp.(MoV 1358)	42							
Aricidea sp.6			79				32	15
Lysilla jennacubinae	30							
Paraonella sp.1	35							
Oligochaeta								12
Jassa marmorata		12						
Phoxocephalidae		59	25	0	101	689	343	27
Euphilomedes sp.(MoV 18)	104	10					7	
Archasterope sp.1					15	32	15	
Callianassidae sp.4		22						
Caprella sp.1				7				
Nebalia longicornis		7138	3306	11264			951	
Nassarius nigellus		131	96	47	368	874	459	143
Nucula pusilla	30							
Mysella donaciformis		12					25	10
Mytilus edulis planulatus				2				
Corbula gibba	27	523	975	240	175	1519	254	356
Theora lubrica		10	452		12	146	207	69
Hiatella australis		17		12				
Tellinidae sp. (cf T. deltoidalis)		42		20	40			
Mysella sp.							15	
Amphiura elandiformis	143		27					7
Echinocardium cordatum		15	17	12	30		30	25
Plathelminthes sp.1							17	0
Nemertea sp.1	109	0	57	49	146	20	17	7
Nemertea sp.2						67		15
Sipuncula sp.1						35		

c) Pen position P1A/2A

ID Code/Time	0mth	4.5mth	6mth	9mth	13mth	17.5mth	22mth	25mth
Neanthes cricognatha						49	202	
Simplisetia amphidonta			12		17	20		10
Harmothoinae sp.(MoV 2848)						10		
Prionospio kulin			35	10				
Paraprionospio coora		15	12	17	25			
Aricidea sp.1	309							
Asychis sp.2 (13079)	119							
Asychis sp.1 (MoV 907)	44							
Capitella cf capitata (MoV 2558)		79		514	2	12593	52	
Mediomastus australiensis	244	67	12	94				
Paraonides sp.(MoV 1358)	116							
Aricidea sp.6		54	47	40	12			
Lysilla jennacubinae	22							
Paraonella sp.1	72		15					
Lyssianassidae sp.1								5
Jassa marmorata							7	
Phoxocephalidae		195	284	227	49	2	67	148
Euphilomedes sp.(MoV 18)	185	47	17		7			
Euphilomedes sp.(MoV 1021)	35							
Cypridinidae sp.1								7
Hexapus granuliferus					10		12	7
Halicarcinus rostratus						10		
Nebalia longicornis				612		5069	4607	
Nassarius nigellus		1395	612	143	165	190	25	131
Polinices didymus					5			
Mysella donaciformis							37	15
Corbula gibba	57	2291	904	600	373	141	54	156
Theora lubrica		1822	1188	509	15	47		35
Amphiura elandiformis	175							
Echinocardium cordatum		146	207	35	111	40	64	96
Platyhelminthes sp.1							17	
Platyhelminthes sp.2							27	
Nemertea sp.1	193	79	35	57	22	12	12	12
Nemertea sp.2						17	20	25

d) Pen position P3A/4A

ID Code/Time	0mth	4.5mth	6mth	9mth	13mth	17.5mth	22mth	25mth
Glycera sp.1		10		10	5			
Neanthes cricognatha						373	252	5
Harmothoinae sp.(MoV 2848)						15		
Prionospio kulin		15						
Paraprionospio coora			22	37	42			5
Capitella cf capitata (MoV 2558)						9946	34699	0
Mediomastus australiensis			25		10	30		5
Aricidea sp.6		15	25	27	17			
Paraonella sp.1			12					
Oligochaeta			10					
Lyssianassidae sp.1							15	
Oedicerotidae sp.		10	10					
Tiron sp.1							15	
Phoxocephalidae		106	380	257	69	89	393	91
Euphilomedes sp.(MoV 18)		15						7
Cypridinidae sp.1								20
Archasterope sp.1					5			
Ostracoda sp.23					7			
Halicarcinus rostratus						42		
Nebalia longicornis				96		3380	9652	15
Munida haswelli				35				
Nassarius nigellus			899	701	281	459	44	175
Polinices didymus			15	10	27		22	
Mysella donaciformis							20	15
Corbula gibba		1519	1040	1106	1119	64	156	32
Theora lubrica		617	830	911	20	49		32
Raeta pulchella				5				
Echinocardium cordatum		94	202	210	225	22	77	138
Platyhelminthes sp.2							64	
Nemertea sp.1			17	35		35		15
Nemertea sp.2							49	
Sipuncula sp.2			59					

Overall there was a greater diversity of species at the reference positions and although a few species were conspicuous in terms of their consistency and abundance there were no clearly dominant species. The species consistently found at the reference sites included the capitellid polychaete, *Mediomastus australiensis*. This species has a familial relationship to the pollution indicator species *Capitella capitata* but has a much lower tolerance for organic enrichment and hypoxia. Nonetheless, *Mediomastus* species are often found in areas where organic content has been slightly enhanced. Dauvin (2000) found *Mediomastus australiensis* increased in abundance just after an oil spill in the Bay of Morlaix. In this instance the effects of the spill were weak and the normal fauna of the bay adapted to the higher organic load. In a review of polychaetes as environmental indicators in N America (Levin, 2000) *Mediomastus* was cited as one of the genera indicative of enrichment in shallow waters. In Australia *Mediomastus* was identified as a major species in the assemblage characterising fine mud/sand in Western Port Bay, Victoria (Coleman et al. 1978).

Lysilla jennacubinae was also a common inhabitant of the reference sites. *L.jennacubinae* is a sedentary terrebellid polychaete which lives naked in sediments (Hutchings, P.A., 2000) making it very susceptible to organic pollution (particularly localised hypoxia and increased sedimentation). Although relatively immobile, this species can have an effect on relatively large areas of sediments as it has a large tentacle spread. It acts as a selective deposit feeder, eating diatoms and sediment particles within a specific size range (Hutchings, P.A., 2000). *L.jennacubinae* as with other terebellids is restricted to non-polluted, fully marine environments (Hutchings, P.A., 2000). Terebellids lack the ecological capacity to tolerate pollution events, and if the population is compromised they have limited capacity to re-establish themselves. Terebellids are broadcast spawners which generally produce large numbers of gametes over a relatively defined spawning season of days/weeks (Hutchings, P.A., 2000). Therefore, unfavourable environmental conditions over the reproductive or settlement period can have serious implications for survival of the population.

According to Pearson and Rosenberg (1978) stable communities are typically dominated by larger, long lived species such as *Nucula, Amphiura, Terebellides, Rhodine, Echinocardium* and *Nephrops* which require more stable/benign environmental conditions. The local bivalve *Nucula pusilla* was relatively common at the reference sites, as was the large bivalve *Thyasira adelaideana*. Pearson and Rosenberg (1978) suggested that the genus *Thyasira* might be found in communities indicative of "transitory" conditions such as might arise when organic enrichment levels are enhanced. However, *Thyasira* species are also suspension feeders and as such are susceptible to any activities which significantly increase the suspended matter loads.

One of the most important species characterising the reference communities was the brittlestar *Amphiura elandiformis*. In previous local studies of the impacts of aquaculture *A.elandiformis* was a key species in unimpacted sediments (Macleod, 2000, Crawford et al, 2002). It has been recorded in relatively large numbers (700/m2 (Macleod, 2000, 160/m2 (Crawford et al, 2002)), often dominating the soft sediment communities, suggesting that it plays a major role in community and sediment processes. Ophiuroids, or brittlestars, are often significant, even keystone, species in soft sediment communities (Heip et al, 1992, Rosenberg et al., 1997, Rumohr et al., 2001). *Amphiura elandiformis* is relatively common around Tasmania and is found on the North, South and East coasts (Dartnall, 1980, Edgar et al., 1999). In spite of its prevalence there is very little known about the biology and ecology of this species. Video footage from the current and previous studies of soft-sediment communities in Southern-Tasmania (Crawford et al., 2002) have shown that although it is principally a surface deposit feeder, burying its basal disc in the sediment and using its arms to scavenge for food on the surface, it can also use its arms to remove particles from the water column. These observations suggest that this species may serve a very similar functional role in the local soft sediment

ecosystem to the boreal *Amphiura* communities defined by *A. chiajei* and *A. filiformis*. *A. elandiformis* appears relatively intolerant of organic enrichment and low oxygen conditions (Crawford et al, 2002) and comparison of this and other field observations with available biological and ecological information on *A.chiajei* and *A.filiformis* (Nillson, 1999) suggest that functionally *A.elandiformis* may more closely resemble *A.filiformis*. This species is likely to be important both in physically structuring the sediments and in nutrient cycling. Where present, brittle stars more or less continuously rework the sediments as a result of vertical and horizontal migration (Rosenberg et al., 1997). This bioturbation can also have important implications for the fate of contaminants **(**Mazik & Elliott, 2000, Gunnarsson et al., 2000). Selective feeding by brittlestars on labile organic matter may represent a major exposure route for organic contaminants (Gunnarsson et al., 1997). Bioturbation by *Amphiura* spp.may also stimulate both nitrification and dissimilatory nitrate reduction (Enoksson & Samuelsson, 1987). Faunal mediated oxidation of sediments can increase removal of nitrogen through increased nitrification and denitrification and enhanced binding of phosphorus, thereby reducing nutrient fluxes to the water column (Heilskov & Holmer**,** 2001).

The faunal composition of reference sites gives a fairly clear picture of environmental condition and function. That *Mediomastus australiensis* is a significant component of the reference community at Port Esperance suggests that the background conditions are naturally subject to a relatively high level of organic material. However, the presence of brittle stars and terrebellids, in particular *Lysilla jenacubinae,* and suspension feeders such as *Thyasira adelaideana* suggests that the environmental condition is good, the sediments well oxygenated and that the overall sedimentation rate is relatively low. The faunal mix also suggests that there is a range of ecological function occurring within these sediments, including suspension and surface and infaunal deposit feeding.

Pen positions 1 and 2 were stocked in the first cycle and fallowed for 15 months. Species characteristic of unimpacted conditions were only present immediately before the cages were stocked and either did not reappear or were present only sporadically and in low numbers over the fallow period (Table 6.3.4.1b). Impacted conditions were characterised by two main features; an absence of species commonly associated with unimpacted conditions and the outstanding abundance of two species, *Capitella capitata and Nebalia longicornis* (Table 6.3.4.1b). *Capitella capitata* is highly tolerant of increased sedimentation rates and extremely high levels of organic carbon and is often regarded as an indicator of organic enrichment (Grassle & Grassle 1974, Pearson & Rosenberg, 1978, Levin, 2000). *Capitella capitata* can also tolerate extremely low oxygen levels as a result of specialised respiratory pigments and by extending their tubes into overlying oxygenated sediments and actively ventilating their burrows (Hutchings, 2000). This species is highly mobile (Tsutsumi & Kikuchi, 1984) and can rapidly reproduce, spawning 2-5 times in its life with an interval of as little as 30 days between spawnings. It is extremely fecund, each female can produce between 400-4,000 eggs, with a hatching success rate of around 79%, although reproductive success seems to be greater in younger females, which may facilitate the colonisation of new areas. In the current study, at its most abundant, more than 75,000 individuals/m2 were sampled from cage positions, assuming a 1:1 sex ratio (Tsutsumi & Kikuchi, 1984) and little or no mortality this would equate to 37,500 females/m2 which in turn suggests the reproductive potential for a further 120 million individuals/m2 at the next cycle. It is clear that this species is extremely well adapted for the rapid colonisation of disturbed environments. *C.capitata* is also extremely well adapted to utilise the organic material within these sediments. Frankenberg & Smith (1967) reported that *C.capitata* could eat 19% of its body weight in faecal pellets in 24 hours. Assuming that a large proportion of the sediments under the cages comprise faecal material and an average worm weight of 0.0118g (Macleod, unpublished data), the population densities encountered at the

cages towards the end of the first stocking period (average 40,000 per m^2) suggest that around 90g per m^2 of organic material could be processed by these worms daily. Clearly *C.capitata* is extremely efficient at breaking down and assimilating the excess organic material resulting from aquaculture operations.

The other main species associated with impacted conditions were the leptostracan crustacean, *Nebalia longicornis*, and the introduced bivalve, *Corbula gibba. Nebalia sp* are generally epibenthic scavengers which are often associated with areas of increased organic enrichment (Rainer & Unsworth, 1991). *Nebalia longicornis* is highly mobile and is often found as dense swarms around areas of decaying detrital material (Edgar, 1997). However, there is little information on the ecology of this species locally.

Corbula gibba was introduced to Australia from the Mediterannean and has become a significant component of many temperate Australian soft-sediment communities (Wilson et al., 1998). *C.gibba* is well adapted to live in unstable mixed muddy bottoms, dominating where physical-chemical and sedimentary parameters show large variations and where the sediments are unstable (Crema et al., 1991). It generally resides in surface sediments and is a suspension-feeding bivalve, however, it can exhibit a high degree of particle selection (Kioerboe & Moehlenberg, 1981). It is extremely tolerant of low oxygen condition even periods of partial anoxia (Crema et al., 1991) and can survive levels of turbidity which would be prohibitive to many other suspension feeding species. It is widely distributed in the estuaries of Northern Europe and the Mediterranean, and is often abundant in eutrophic areas at the edge of anoxic/azoic zones (Jensen, 1990). Early studies suggested that it was relatively long-lived (>5yrs) and slow growing (Jones, 1956) although more recent studies suggest a life span of only 1-2yrs (Jensen, 1990). Once established *C.gibba* can become extremely abundant; densities of up to 2,600m^{-2} have been reported from Port Phillip Bay (Curry & Parry, 1999) whilst Jensen (1990) in a Danish study estimated densities of 67,000 m^{-2} were possible. This bivalve was present at very low levels before farming, increased in abundance over the stocked period but was also present in relatively high numbers over the recovery period. This species was only absent immediately after the cages were emptied, perhaps indicating the period of greatest impact and the respective tolerance level of this species. It was present at levels comparable to those reported from Port Phillip Bay at pen positions 1A/2A (Table 6.3.4.1c).

Pen positions 1A and 2A were stocked in the second cycle, but as a consequence of adjacent cages being operational throughout the first cycle there was also an effect on the faunal community throughout the first stocking cycle. The sediments associated with the stocked cages were again dominated by *Capitella capitata* and *Nebalia longicornis* (Table 6.3.4.1c). Species characteristic of unimpacted conditions were only encountered at the initial sampling times. *Amphiura elandiformis* and *Lysilla jennacubinae* were only recorded at the very first sampling, before any major farm activity commenced. Several transitional species, notably the introduced bivalves, *Corbula gibba* and *Theora lubrica,* were significant components of the benthic communities throughout the pre-farming and fallow periods, although there was some reduction in these species over the periods of greatest impact.

Theora lubrica originated from Japan and around the Korean peninsula. It has been widely reported around Australia and New Zealand (Parry et al, 1997, Hayward et al., 1997, Wilson et al., 1998) and is often amongst the dominant species in soft sediment communities (Hayward et al., 1997, Wilson et al, 1998). In Tasmania it has only officially been reported from Georges Bay, the Derwent and the Tamar although it is believed to be considerably more widespread. In its native range it is a selective deposit-feeder commonly found in soft sediments (Talman, 1998) and has several features making it particularly suitable as a key transitional species. It is known to be tolerant of organic pollution and is extremely tolerant of both hypoxic conditions (Tamai, 1996) and high levels of organic matter sedimentation (Saito et al., 1998). Like

Corbula gibba it can also recover rapidly from temporary pollution episodes, however, it is susceptible to freshwater input and reduced salinity will decrease both its density and life expectancy (Poore & Kudenov, 1978) making it less robust than *Corbula gibba*.

Pen positions 3A and 4A were only stocked in the second cycle and as a result showed community relationships very similar to those at positions 1A and 2A (Table 6.3.4.1d). Since positions 3A and 4A had previously been farmed, the disappearance of species characteristic of unimpacted conditions after farming commenced was even more rapid than at positions 1A and 2A. This suggests that after the initial impact sediments may be more vulnerable to impact, reverting more quickly to a degraded community, which in turn suggests that the community, and potentially the chemistry, of the system is less stable. In fact *Amphiura elandiformis, Lysilla jennacubinae Nucula pusilla* and *Thyasira adelaideana* were not recorded from these positions at the start of the study. Stocked conditions were again dominated by *Capitella capitata* and *Nebalia longicornis,* and transitional species were prevalent throughout the pre-farming and fallow periods.

The structure of the sediment communities associated with the impacted and recovering conditions is important in determining the capacity of the sediment to tolerate organic enrichment and to bring about recovery. The species associated with impacted conditions exist by virtue of their tolerance to extreme/ inclement conditions and are specifically adapted to survive and thrive under these conditions. In doing so they utilise and breakdown accumulated organic material, they do this very well so long as the cage operations continue (i.e. there is a food source) and so long as the amount of organic material being supplied does not overwhelm the community. Accordingly, we suggest that there is an optimal balance between the rate of organic material input to the system and the systems ability to assimilate this material; when this balance is achieved the system will function most effectively. However, if the sediments ability to assimilate organic material is exceeded, then the fauna will be unable to cope, environmental conditions will deteriorate and the fauna will ultimately be eliminated. This would be a very undesirable situation as without the fauna to re-work and oxygenate the sediment it would quickly become anoxic, larval settlement and species recruitment would be inhibited and once the macrofauna had been eliminated it would be much more difficult to re-establish and therefore recovery would slow/cease. Consequently it is in the interests of good farm management to have an understanding of the sediment status and to maintain the infaunal community at a level appropriate to the farm requirements.

6.3.5 Simplified Evaluation Options

Broad changes in community structure may be assessed by relatively simple means (e.g. characterisation of major groups and evaluation of general diversity). Some of these approaches indicated very clearly the response of the infaunal community to the introduction of the fish cage, the deterioration throughout the stocking period and the subsequent recovery of the sediments. However, these measures generally suggested that recovery was relatively rapid after the cages were removed and showed little or no evidence of an ongoing difference/change in the community/ environmental status. Consequently, their value for regular management would be limited. Nonetheless, they are generally simple to apply and may give an indication of sediment condition.

The number of species recorded from the reference sites generally varied between 20-30 species. There was some variation at the references over time ($n=55,13$, $F=3.265$, $p=0.001$) however, there was not a significant interaction between the two reference sites and time ($n=55,13$, $F=1.460$, $p=0.163$) (Fig. 6.3.5.1). At the pen positions the number of species generally declined after commencement of farming operations. At positions 1 and 2 the number

of species declined in the period immediately after the pen positions were stocked, remained low over the stocked period and for some time thereafter (Fig. 6.3.5.1). This effect was also evident, although not as pronounced, at positions 1A/2A and 3A/4A in the second cycle. ANOVA for the number of species showed a significant interaction between site and time for all positions (P1/2- n=109,10, F=106.4, $p<0.001$; P1A/2A- n=118,11, F=106.6, $p<0.001$; P3A/4A- n=95,9, F=3.9 $p<0.001$). After 3 months fallow the number of species did not return to a level equivalent to that at the reference stations or to that pre-farming at any of the study positions. Although the number of species did increase over the fallow period, there were still significantly less species at positions 1 and 2 after 15 months fallowing than at the reference or pre-farming. The number of species also did not increase significantly at the pen positions from impacted and post-impact levels over the recovery period in the second cycle. This lack of differentiation between impact levels suggests that number of species would not by itself be a useful criterion for assessment of recovery. However, the significant decline in species numbers associated with farming impact may represent a useful definition of impact. Reference and pre-farming positions generally contained between 20-30 species, whilst at the most impacted points in the farming cycle this was reduced to around 10 species, suggesting that a decline in species numbers greater than 50% would be indicative of a relatively severe impact.

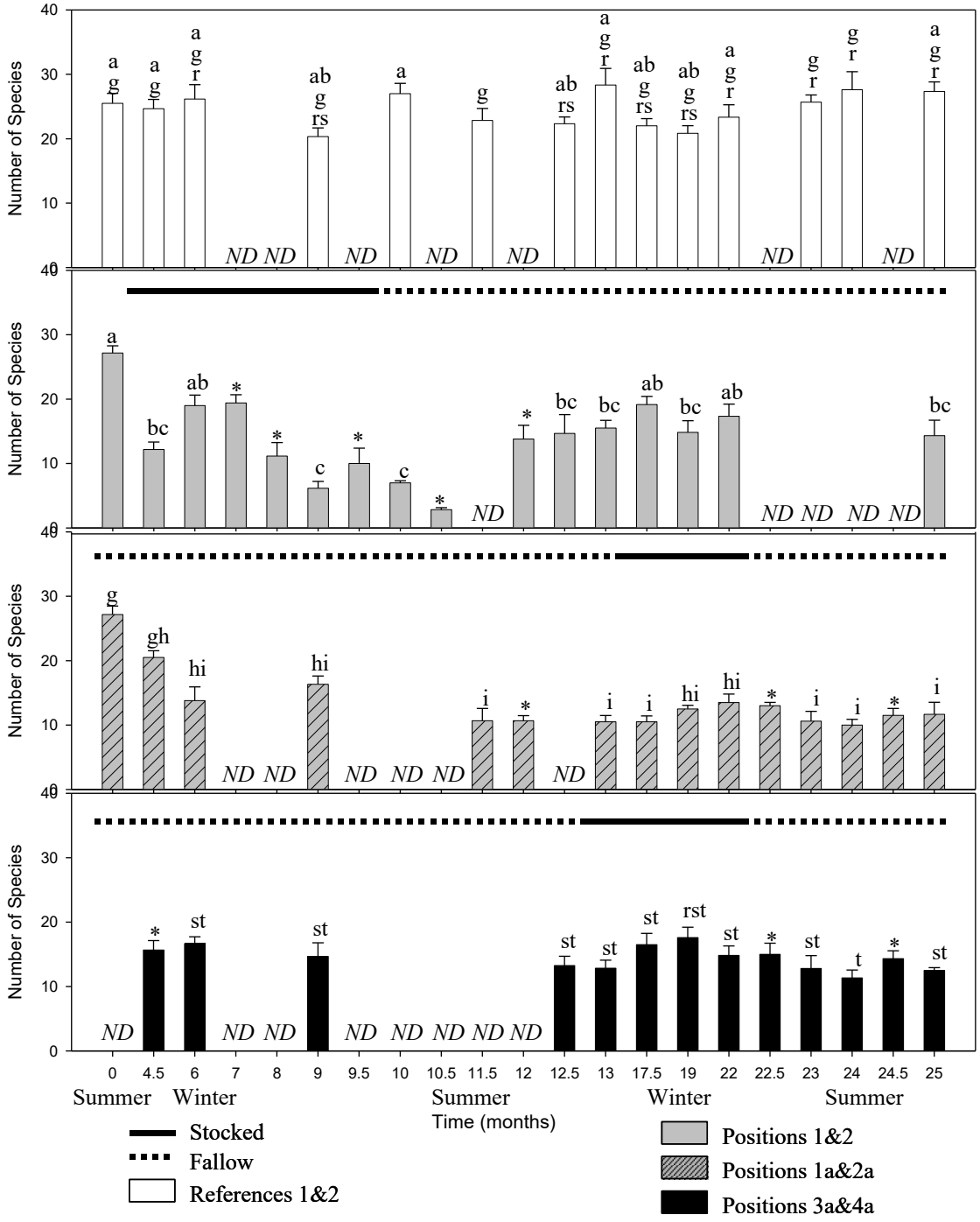


Fig.6.3.5.1. Number of Species at Stringers Cove. ND indicates no data available. * indicates data not included in the ANOVA analysis.

Abundance at the reference sites did not vary over time. When the cage positions were stocked abundance increased significantly for the best part of the stocked period, declining fairly rapidly after the cages were fallowed (Fig. 6.3.5.2). The number of individuals showed a significant interaction between site and time for all positions (P1/2- n=109,10 F=11.044, p<0.001; P1A/2A- n=118,11, F=10.029, p<0.001; P3A/4A- n=95,9 F=7.964, p<0.001). Abundance at the reference sites and prior to farming was generally fairly low (<3,000 individuals/m^2). Farming impact resulted in a significant increase in abundance. In the second cycle abundance more than doubled, whilst under the more intensive farming in the first

production cycle numbers increased to between 10,000-100,000 individuals/m^2. Accordingly, a doubling of the number of individuals could be considered indicative of a moderate change in environmental conditions whilst an increase of three fold or greater than the pre-farming/reference levels would suggest a major impact. The number of individuals declined rapidly after farming ceased, recovering to pre-farm conditions well within 3 months in the first cycle and even before the end of the stocking period in the second cycle. This suggests a much more rapid recovery than indicated with the full community assessment (recovery to pre-stocking levels within 3 months for the first cycle and 4-6 weeks in the second cycle), and consequently it is suggested that total abundance may not be as useful a measure for evaluation of recovery.

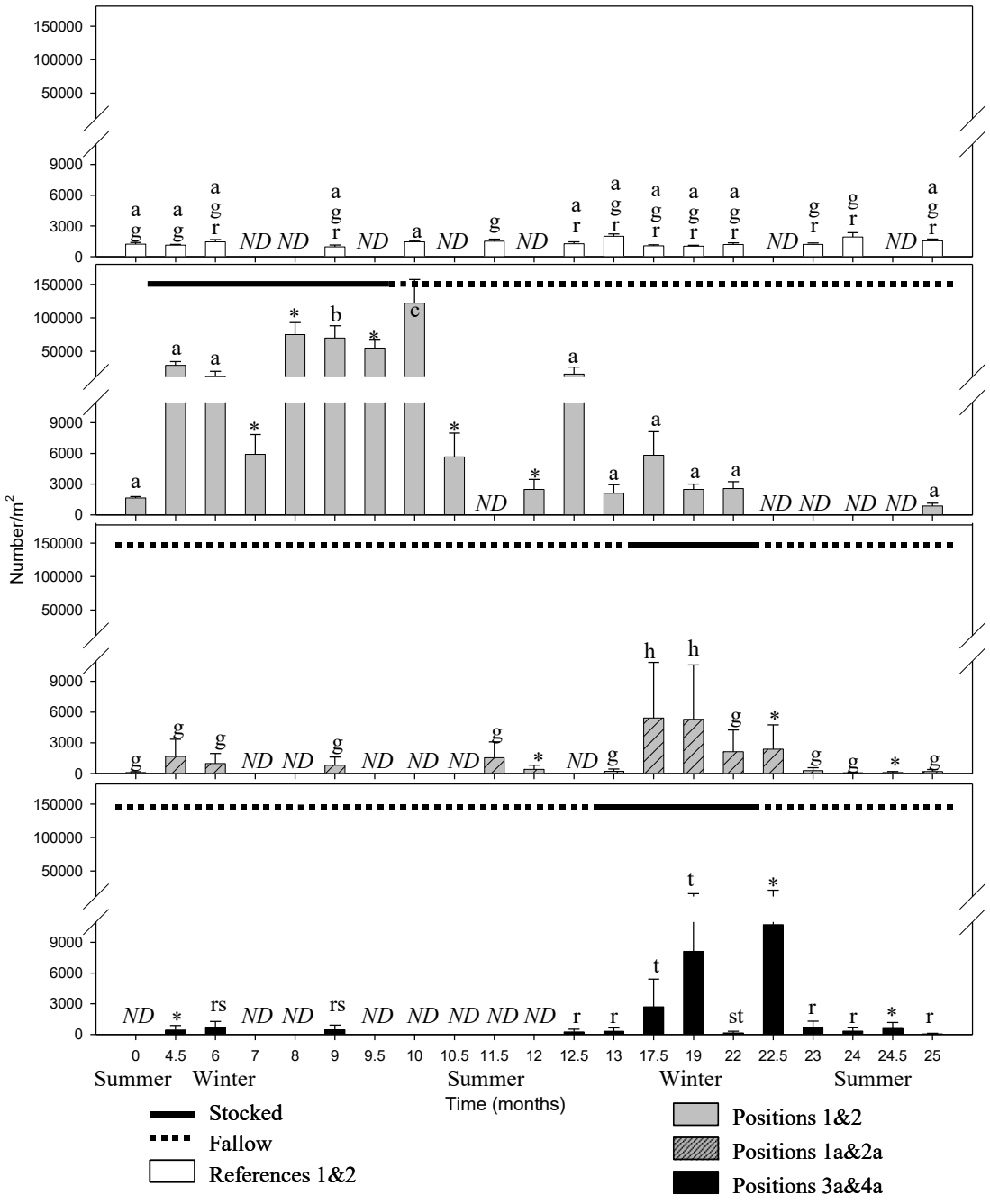


Fig. 6.3.5.2. Number of Individuals at Stringers Cove. ND indicates no data available. * indicates data not included in the ANOVA analysis.

Annelid abundance is the main factor affecting total abundance and therefore the two measures are synonymous. The impact criteria defined for total abundance would equally apply to Annelid abundance. The increases in total faunal abundance at the cage positions were primarily as a result of changes in abundance of *C.capitata* (Fig. 6.3.5.3). Analysis of the variations in abundance of this species indicated the same interaction pattern as was evident in both total and annelid abundance (*Capitella capitata* abundance P1/2- n=109,10 F=9.064, $p<0.001$; P1A/2A- n=118,11 F=12.727, $p<0.001$; P3A/4A- n=92,9 F=6.998, $p<0.001$). As evaluation of the number of *C.capitata,* number of Annelida and the total number of individuals are all essentially measuring the same thing (i.e. number of *C.capitata)* the easiest approach for the farms may be to assess the total number of individuals as this would not require any specific identification skills and the samples can be collected and analysed without any further separation being required.

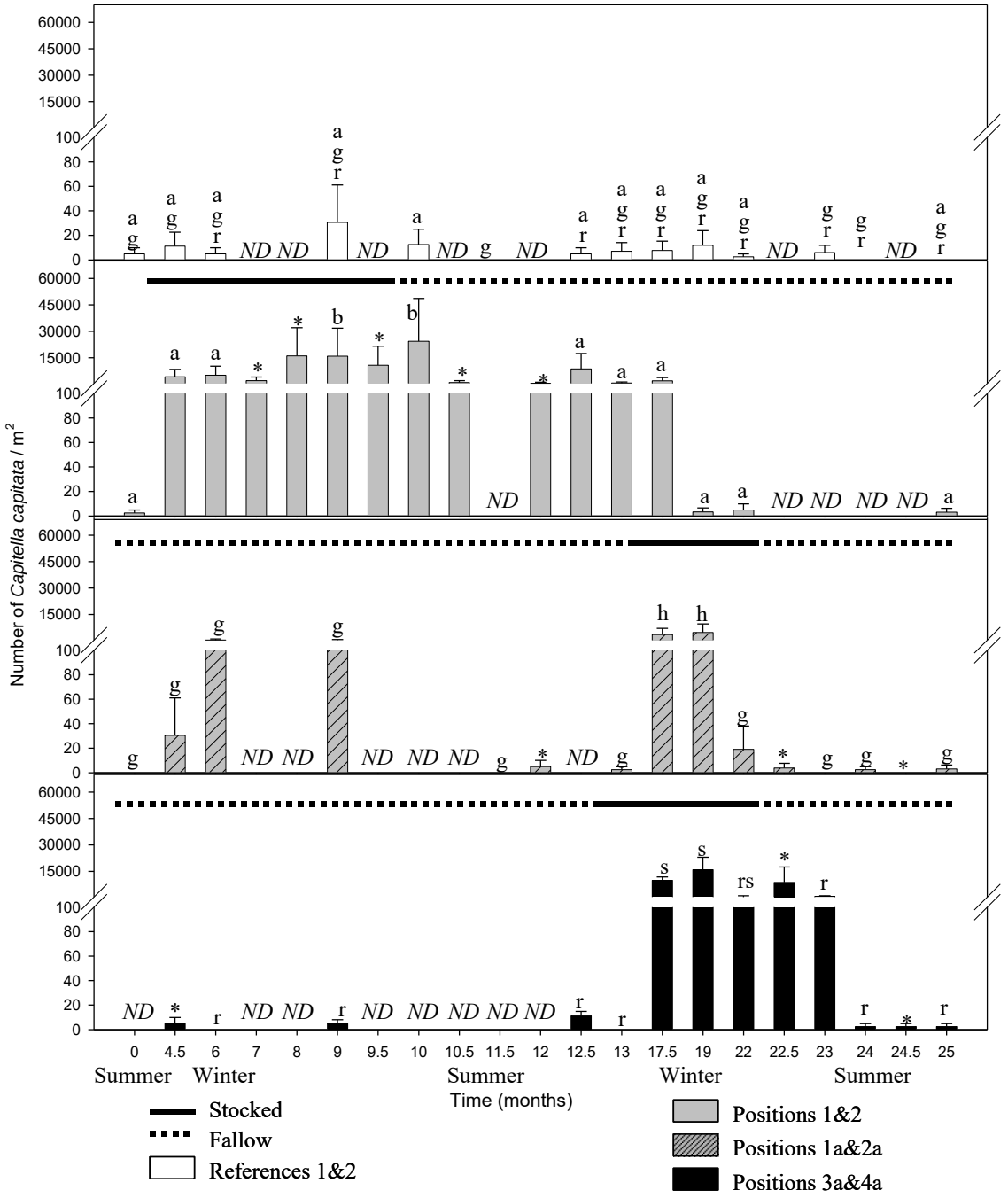


Fig. 6.3.5.3. Number of Capitella at Stringers Cove. ND indicates no data available. * indicates data not included in the ANOVA analysis.

The Shannon diversity index is a univariate measure based on the number of species and the number of individuals in each sample. ANOVA revealed a strong interaction between site and time at all pen positions (P1/2- n=109,10 F=9.864, p<0.001; P1A/2A- n=118,11 F=6.289, p<0.001; P3A/4A- n=95,9 F=5.844, p<0.001). The Shannon index at the references remained consistently high (average 2.80) throughout the study (Fig. 6.3.5.4). Levels generally declined throughout the farm after the onset of farming and were significantly lower than the references and the pre-farming conditions for the duration of this study. After the cages were stocked levels declined further and remained low over the stocked period. Once the cages were emptied the Shannon index showed some recovery, but only to the general farm levels and never to reference or pre-farming levels. An index between 1.5-2.0 is indicative of general farm

operation, a value greater than 2.0 was generally associated with good environmental conditions, values less than 1.5 indicated impacted conditions and values less than 1.0 suggested a major impact. The Shannon index indicated recovery to generalised farm levels within 10 weeks of fallowing in the first cycle and 4 weeks in the second cycle, which compares quite favourably with the recovery estimates from the full community assessment (3 months for cycle 1 and 4-6 weeks for cycle 2). However, it is important to recognise that calculation of the Shannon index requires identification and enumeration of the fauna to species level. If this level of taxonomic identification is to be done, then it is suggested that a more reliable estimate of recovery could be extracted from the data if they were analysed using multivariate techniques.

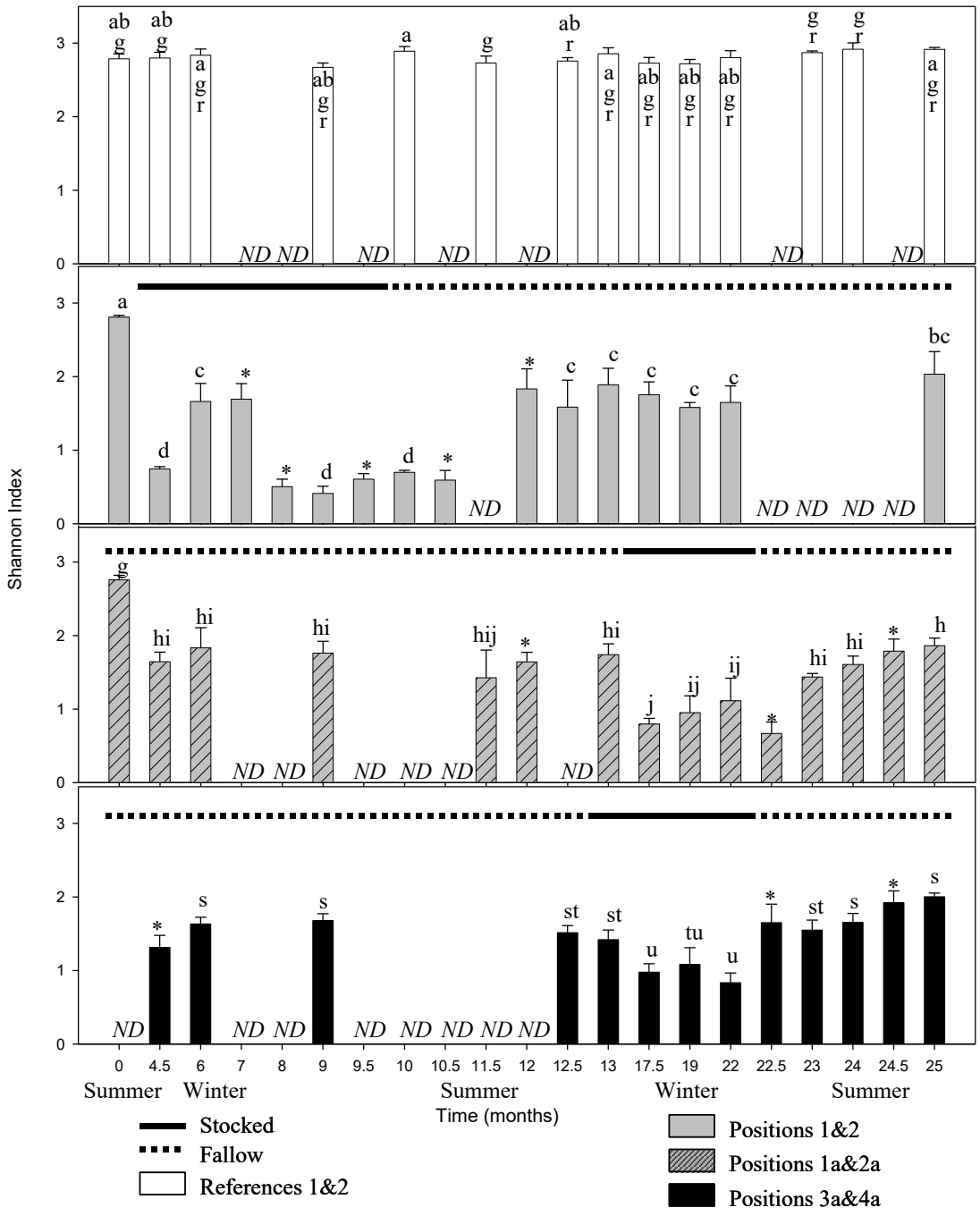


Fig.6.3.5.4. Shannon Index at Stringers Cove. *ND* = no data. ND indicates no data available. * indicates data not included in the ANOVA analysis.

Simple measures of community abundance and diversity can provide a strong indication of the sediment condition. However, changes in specific community groups such as crustaceans, molluscs and echinoderms may also be used either independently or in combination to obtain a better understanding of environmental changes.

At Stringers Cove crustacean abundances responsed to cage farming and in most instances abundance increased over the stocked period. Once again ANOVA indicated a strong interaction between site and time at all pen positions (P1/2- n=109,10 $F=12.759$, $p<0.001$; P1A/2A- n=118,11 $F=3.688$, $p<0.001$; P3A/4A- n=95,9 $F=7.715$, $p<0.001$). However, the correlation was not as clear or consistent as observed for the annelid numbers. Increases were principally related to the opportunistic leptostracan *Nebalia longicornis*. *N. longicornis* is a free swimming, swarming crustacean which is principally found at the sediment/ water interface associated with areas of high organic content, such as accumulations of detrital or algal material. It is also a highly mobile epibenthic species and its presence and abundance is difficult to accurately estimate. It is particularly susceptible to some types of sampling equipment and therefore may be an unreliable indicator.

There were changes in the total abundances in the mollusc community over time, although the relationship between these changes and the impact gradient was less clear than for either the annelids or crustaceans. There was a significant interaction between time and position at all of the pen positions (P1/2- n=109,10 $F=6.497$, $p<0.001$; P1A/2A- n=118,11 $F=4.739$, $p<0.001$; P3A/4A- n=95,9 $F=15.559$, $p<0.001$). The changes were reflected in a variety of different species and were in response to several different environmental triggers. As outlined in the community analysis, several bivalve species (e.g. *Theora lubrica* and *Corbula gibba*) increased in abundance when environmental impact was moderate, whereas more sensitive molluscan species (e.g. *Nucula pusilla* and *Thyasira adelaideana)* declined when environmental conditions deteriorated. Overall mollusc abundance was significantly greater at the farmed sites compared with the references but the composition of the molluscan community varied both in relation to the level of organic enrichment and to natural seasonal influences. Consequently, evaluation of total mollusc abundance does not, in isolation, appear to be a very useful measure of sediment condition.

Conversely, echinoderms generally declined under farming conditions. There was a significant interaction between time and position for pen positions 1&2 and 3A&4A (P1/2- n=109,10 $F=2.589$, $p=0.007$; P1A/2A- n=118,11 $F=1.516$, $p=0.134$; P3A/4A- n=95,9 $F=2.975$, $p=0.004$). Once again it was difficult to explain the changes in relation to the farming activities. One species of heart urchin, *Echinocardium cordatum,* appeared to be enhanced at low levels of organic enrichment. The brittle star (*Amphiura elandiformis*), as has already been mentioned in the community analysis, appeared to be a keystone species in the unimpacted communities and was all but eliminated from the farm communities. This species potentially represents a significant indicator of recovered/unimpacted conditions. Since these two species represent a large proportion of the echinoderm community and they respond in opposite ways to impact, it would be difficult to use evaluation of total echinoderm abundance as a meaningful indicator of changing sediment condition.

There were other species which showed significant changes in response to farming activities, most notably two species of Nemertean worm. However, when collectively grouped as other fauna there was little or no evidence of their usefulness as a measure of environmental condition. Although ANOVA indicated an interaction between time and position for all pen positions (P1/2- n=109,10 $F=2.322$, $p=0.016$; P1A/2A- n=118,11 $F=3.733$, $p<0.001$; P3A/4A- n=95,9 $F=3.258$, $p=0.002$) there was little biological evidence to indicate that the changes related specifically to the impact levels.

Evaluation of major groups is clearly most useful when reviewed in relation to known farm information and as a time series. Of all these simple approaches total abundance appears to be the most reliable and provides an indication of sediment condition, particularly in relation to major impacts. However, if some evaluation of the key species (e.g. presence/absence) is also included, then a slightly better understanding of the sediment condition can be obtained.

6.3.6 Potential Indicators & Predictive Capacity

This study has identified several single species and community categories which have the potential to be useful indicators of impact and recovery. Impacted sites were clearly characterised by two species, the polychaete *Capitella capitata* and the leptostracan crustacean *Nebalia longicornis* (Table 6.3.4.1). These species are distinctive and would require little training to identify in benthic samples. *C.capitata* is slightly more reliable than *N.longicornis* as it is an infaunal species which it is not as likely to be affected by sampling technique; the epibenthic and fairly mobile *N.longicornis* may be missed or its abundance misjudged by some sampling approaches. The magnitude of the change in abundance of *C.capitata* is such that it is not necessary to obtain an accurate count of the number of individuals in a sample, a relative measure such as highly abundant, i.e. greater than 1,000, or even a simple volumetric assessment would be sufficient to characterise the environmental conditions. Since it is the overwhelming abundance of *C.capitata* that distinguishes the impacted conditions, it is actually not even necessary to identify or separate this species, simply quantifying the general abundance/mass of worms will have the same outcome.

The brittle star *Ampiura elandiformis* appears to be a very good indicator of unimpacted conditions (Table 6.3.4.1). With limited training this species would also be relatively easy to identify. It was consistently present at the reference positions at Stringers Cove, and was not found anywhere where there was significant organic enrichment. Consequently the presence of brittle stars in samples would indicate that the environmental conditions are relatively good.

From the farm management perspective it is most important to be able to distinguish transitional conditions (i.e. deteriorating/recovering) within the sediments, particularly sediment recovery. Unfortunately this is the most difficult period to characterise. Nonetheless, two introduced bivalves *Theora lubrica* and *Corbula gibba* appear to be fairly consistently associated with transitional conditions (Table 6.3.4.1). These species were never present when conditions were highly impacted and were rarely encountered at the reference/unimpacted conditions. When present at the reference/unimpacted sites they were in relatively low numbers, whilst in contrast, they were abundant in transitional communities. Both species are easy to identify and are large enough to count with the naked eye or a simple magnifying light. Although the appearance of these species in time series data could not be considered conclusive evidence of recovery, in conjunction with other indicators (e.g. video or sediment chemistry) this information would enable a relatively reliable assessment of sediment condition to be made.

6.3.7 Visual Assessment Approaches

6.3.7.1 Benthic Community Visual Characterisation (Benthic Photos)

Photo scores were only obtained from the second production cycle. As at Creeses Mistake, scores for a variety of features were calculated from photographs of the pre-sorted benthic infauna samples and subsequently analysed using univariate and multivariate techniques.

Photo scores were consistently and significantly lower at the cage positions than at the reference positions (Fig.6.3.7.1.1) (Position 1 &2 -$F_{1,39}$=65.350,p=0.000, position 1A & 2A - $F_{1,57}$=514.236,p=0.000, positions 3A & 4A - $F_{5,58}$=4.327, p=0.002) suggesting that this approach is a reliable determinant of impacted from unimpacted conditions.

Photo scores for positions 1 and 2 (not stocked in the second cycle) differed significantly over time ($F_{3,39}$=3.674, p=0.020). However, although there was some improvement in the photo scores for these pens positions over time the final score was still lower than the reference (Fig.6.3.7.1.1a). This is consistent with the findings of the full benthic macrofaunal analysis which also showed an ongoing effect at these 'fallowed' cages, such that they did not return to pre-farming or reference conditions. A similar response was also found at positions 1A and 2A (stocked in the second cycle) ($F_{5,57}$=3.188, p=0.013) (Fig.6.3.7.1.1b).In this instance conditions appeared to recover with the cessation of farming and were markedly improved after only one month of fallowing (23 months). Scores then declined again with the result that at the end of the three months fallow (25 months) there was no significant difference between the fallowed and stocked scores. Positions 3A and 4A (also stocked in the second cycle) showed a significant interaction between treatment and time ($F_{5,58}$=4.327, p=0.002). These positions showed some recovery over the fallow period, but did not recover to reference conditions by the end of the 3 months fallow period (25 months) (Fig.6.3.7.1.1c).

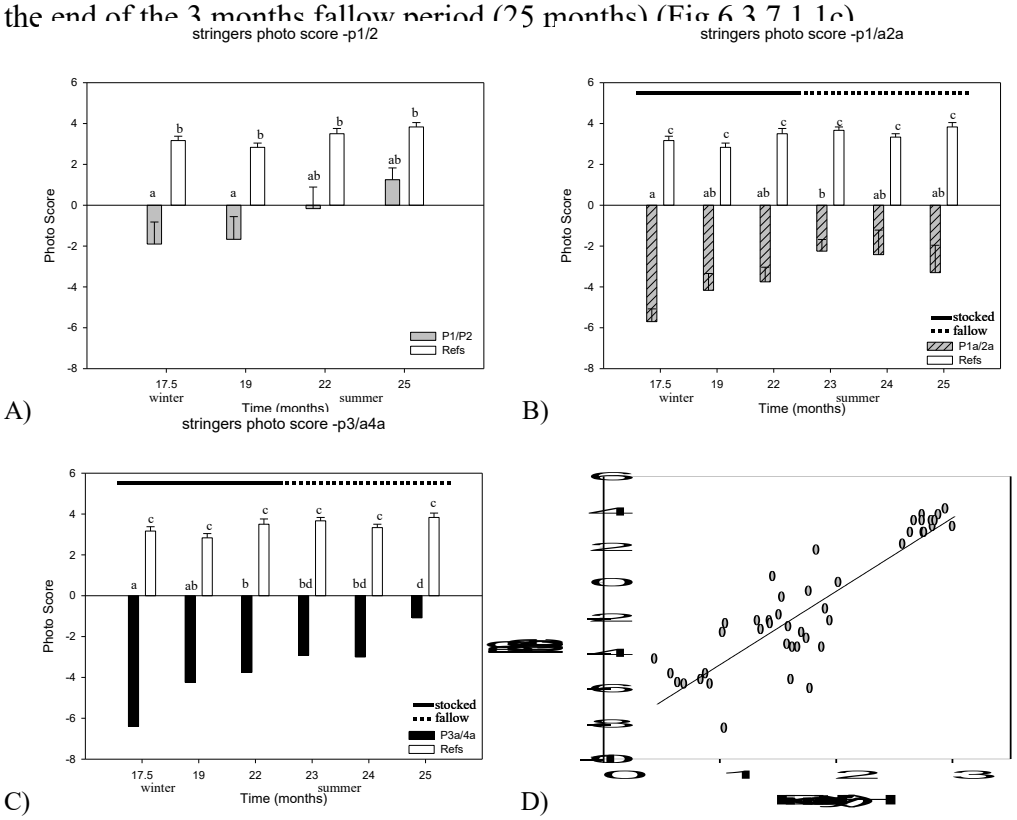


Fig.6.3.7.1.1. Benthic photo scores (mean and standard error) at Stringers Cove sites A) Positions 1&2 and References, B) Positions 1A & 2A and References and C) Positions 3A & 4A and References, during the second production cycle. ANOVA results are indicated by letters. D) Plot of Shannon Diversity and Benthic Photo Scores at Stringers Cove during the second production cycle. Equation y = 4.1575x - 8.8261 and R^2 = 0.7834.

Comparison of the benthic photo scores with Shannon Diversity, as a measure of the benthic community status, indicated a significant positive relationship ($F_{1,42}$=152.007, p=0.000) (Fig.5.3.7.1d). This suggests that benthic photo scores can distinguish the level of impact to the same extent as can the Shannon index. It has already been established that Shannon index values can be used to separate not only impacted from unimpacted conditions. When viewed as a continuous scale, the index can also differentiate intermediate or transitional impacts. This

suggests that benthic photo scores can be used in a similar manner to indicate degrading or recovering sediments.

6.3.7.2 Video Assessment

Video footage from each site at each time was evaluated and the same scoring system as described for Creeses Mistake was applied. This resulted in a matrix of video scores for each of the features assessed. This data was then analysed using both univariate and multivariate techniques.

The results are shown separately for each of the three different production strategies (see Section 6.3.2).

Strategy 1

Overall the video scores for positions 1 and 2 differed significantly from the references ($F_{1,22}$=76.953, p=0.000) and over time ($F_{10,22}$=3.393, p=0.008) but there was no interaction between treatment and time ($F_{10,22}$=2.021, p=0.081) (Fig. 6.3.7.2.1a). At the end of the first stocking cycle (10 months) gas bubbles were observed at position P2, gas bubbles are a very serious indicator of environmental degradation and as such generate an extremely low video score. Gas bubbles were not observed at the replicate site (position P1) and therefore there was a very high standard error for this data point (se=7.25). This extremely high variance would have affected the overall calculation of between and within treatment variance in the entire analysis. When the analysis is repeated excluding this score, there is a significant interaction between treatment and time ($F_{9,20}$=3.305, p=0.012) (Fig. 6.3.7.21.b).

The cage video scores were significantly lower than the reference scores on several occasions both in the first production cycle and during the fallow period and during the second production cycle, despite these positions not being stocked in this cycle (Fig. 6.3.7.2.1b). This may indicate that the sediments were affected by adjacent stocked cages, which is consistent with the findings of the benthic infaunal assessment. It was surprising that the video scores for the cage and reference were not different at the end of the stocked period (9 months). Review of the individual features indicates that there were very few visual features (either good or bad) in either the reference or cage footage at this time and therefore it was not possible to differentiate conditions. This particular situation was quite unusual and did not occur at any other site or time. However, it is a problem that needs to be addressed. Reviewing the video assessment as time series data will help to identify such problems if/when they occur and validation of findings with other alternative approaches will also help to avoid misinterpretation of the data.

At the end of the three month fallow period (13 months), and at the end of the study (25 months), the cage scores were not significantly different to either the reference or pre-stocking levels suggesting that with respect to the visual assessment features the conditions had recovered.

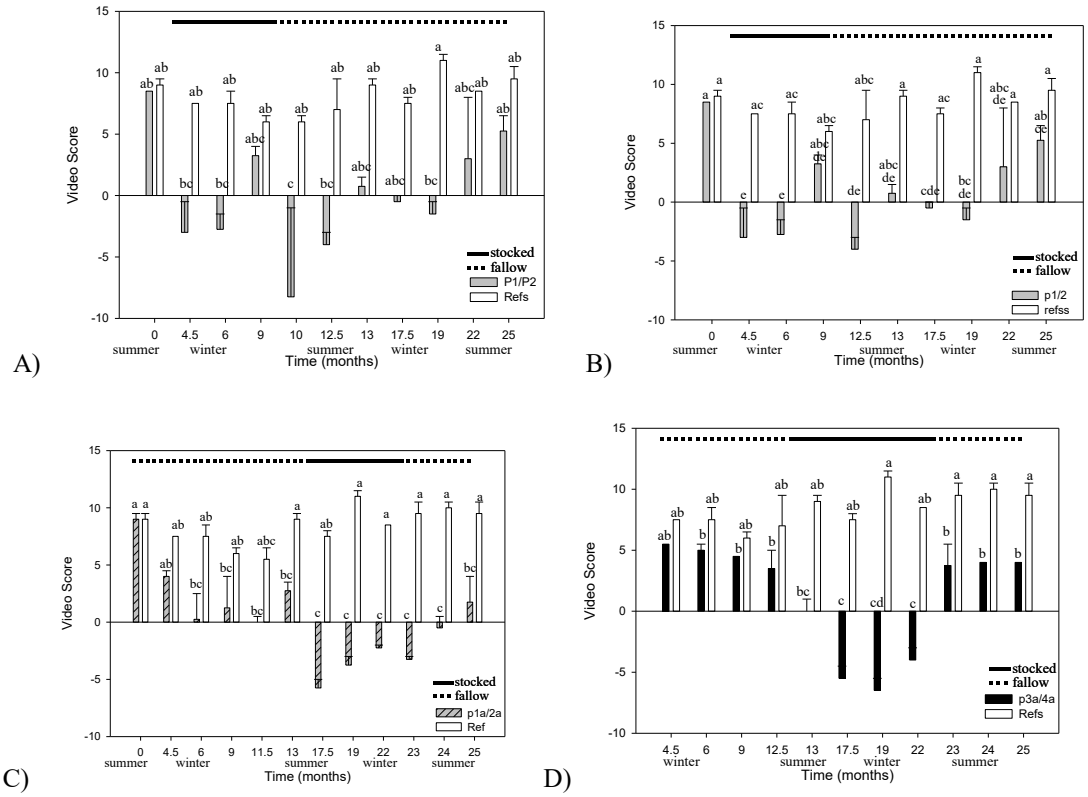


Fig. 6.3.7.2.1. Video scores (mean and standard error) at A) Positions 1&2 and References, B) Positions 1&2 (revised) and References, C) Positions 1A & 2A and References and D) Positions 3A & 4A and References; at Stringers Cove during both production cycles. Letters indicate ANOVA results.

Strategy 2

There was a significant interaction between treatment (positions 1A & 2A and references) and time ($F_{11,24}$=8.734, p=0.000). Video scores at positions 1A & 2A were variable over the first cycle (when these positions were empty) and were significantly lower than the reference scores on several occasions (Figure 6.3.7.2.1c). This is again consistent with findings of the full infaunal analysis and probably reflects the effect of adjacent stocked cages. Throughout the second production cycle video scores at positions 1A & 2A were significantly lower than at the references, both when the positions were stocked and when empty. Scores did not return to reference or pre-farm (0 months) levels at the end of the fallow period (25 months), but they did return to a level equivalent to that before the cages were stocked (12 months).

Strategy 3

There was once again a significant interaction between treatment (Positions 3A & 4A and references) and sampling time ($F_{10,21}$=13.798, p=0.000). The cage and reference video scores were similar during the first production cycle when these cages were empty (Fig. 6.3.7.2.1d). Unlike positions 1A & 2A, positions 3A & 4A had previously been subject to farming and this result suggests that the video score did not detect an influence of that previous farming on the sediment condition. That the cage scores remained high and equivalent to the references throughout the first farming cycle suggests that in this instance the adjacent cages had a negligible effect on the sediment condition. After the cages were stocked at 12.5 months, the video scores declined rapidly. Video scores declined further over the stocking period, and were significantly different from the references. Over the fallow period the video scores improved markedly, fairly quickly returning to a level equivalent to that pre-stocking (12.5 months) but still remaining significantly lower than the references.

6.3.7.3 Multivariate Analysis

Cluster analysis and ordination of the Stringers Cove video data matrix (from major sampling times only) indicated two distinct groups (Euclidean distance 4.5) (Fig.6.3.7.3.1). Group 1 was representative of unimpacted conditions as it included all the reference sites and the cages prior to the start of the first production cycle (0 months) (Fig.6.3.7.3.1). Whilst group 2 included the cages during the two stocking cycles, and could be viewed as representing impacted conditions. Within these two groups there was a further gradation of impact separating the sites. In group 1 the samples show vertical separation, from the top to bottom of the plot, which appears to reflect a temporal gradient. Although there is a similar gradation in group 2 there is a stronger separation laterally, reflecting the degree of impact. Those samples with the greatest impact tending towards the far right of the group and the less impacted samples positioned more to the left of the plot. Group 2a includes the cages during the stocked periods of both production cycles, whilst group 2b contains the cage sites during the fallow periods. Group 2b lies between groups 1 and 2a suggesting that it is intermediate to these two groups.

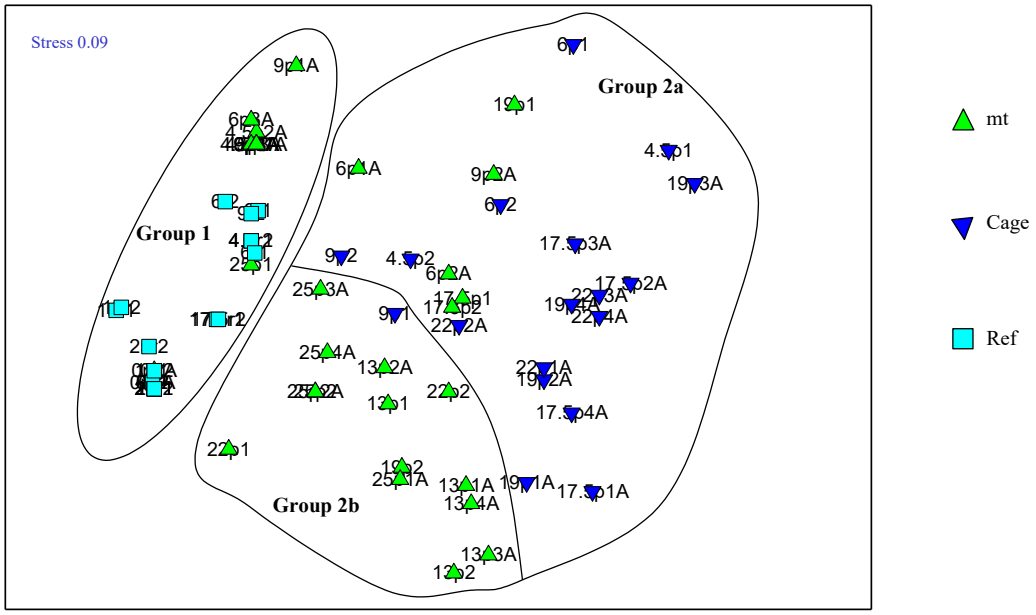


Figure 6.3.7.3.1. Ordination analysis – 2 dimensional MDS plot of video assessment data from all sites at major sampling times. Stress = 0.09.

SIMPER analysis indicated the specific features that define these groups (Table 6.3.7.3.1). Burrow density, presence of brittle stars and *Beggiatoa* accounted for 56% of the dissimilarity between these two groups. The presence of brittle stars and a prevalence of infaunal burrows was strongly indicative of unimpacted conditions. Conversely the absence of these features in conjunction with the presence of *Beggiatoa* and a blackening of the sediments indicated degraded conditions. Previous studies in southern Tasmania (Crawford *et al*, 2001, Macleod *et al*, 2002) showed that brittle stars and burrows were indicative of unimpacted conditions in silt/clay sediments (Crawford *et al*, 2001, Macleod *et al*, 2002), whilst *Beggiatoa* is a good indicator of disturbed environments (GESAMP, 1996).

Table 6.3.7.3.1. SIMPER output for the video assessment indicating a) average scores, ratio (average similarity / st.dev. similarity), % similarity and cumulative % similarity of the 8 most important video parameters in each of the four main MDS cluster groups and b) average score, ratio (average similarity / standard deviation similarity) and cumulative % dissimilarity of the five parameters which most clearly distinguish the two main groups identified by cluster analysis.

Video Parameter a) WITHIN GROUPS	Avg Score	Ratio	Percent Similarity	Cumulative % Similarity
Group 1				
Burrow density	4.37	192.03	6.11	6.11
Faunal tracks	0.88	225.82	5.91	12.02
Brittlestars	1.41	89.64	5.9	17.93
Fish	0.74	199.52	5.9	23.82
Planktonic Crustaceans	0.44	281.82	5.87	29.7
Seastars	0.06	966.4	5.87	35.56
Gas Bubbles	0	1100.63	5.87	41.43
Pellets/Faeces	0	1100.63	5.87	47.29
Group 2a				
Burrow density	0.64	192.53	5.92	5.92
Seastars	0.43	255.25	5.92	11.84
Fish	0.29	398.72	5.91	17.75
Planktonic Crustaceans	0.14	704.14	5.91	23.65
Faunal tracks	0.14	709.16	5.91	29.56
Gas Bubbles	0	1178.02	5.91	35.47
Nassarids	0	1178.02	5.91	41.37
Brittlestars	0	1178.02	5.91	47.28
Group 2b				
Faunal tracks	0.93	315.19	5.94	5.94
Burrow density	1.5	112.81	5.93	11.87
Fish	0.79	228.37	5.92	17.79
Planktonic Crustaceans	0.29	434.06	5.89	23.68
Gas Bubbles	0	1212.33	5.89	29.57
SeaSlugs	0	1212.33	5.89	35.45
Brittlestars	0	1212.33	5.89	41.34
Heart Urchins	0	1212.33	5.89	47.22

b) BETWEEN GROUPS	Group Avg Score	Group Avg Score	Ratio	Cumulative % Dissimilarity
Groups 1 & 2a	*Group 1*	*Group 2a*		
Burrow density	4.37	0.64	4.34	29.38
Beggiatoa	-0.09	-2.21	1.65	46.28
Brittlestars	1.41	0	0.97	57.4
Sediment Colour	-0.03	-0.95	3.53	64.71
Squat Lobsters	-0.62	-0.76	1.08	71.68
Groups 1 & 2b	*Group 1*	*Group 2b*		
Burrow density	4.37	1.5	2.36	35.59
Brittlestars	1.41	0	0.97	53.09
Nassarids	-0.03	-1.07	1.21	66.29
Squat Lobsters	-0.62	-0.14	0.8	74.48
Planktonic Crustaceans	0.44	0.29	0.86	80.99
Groups 2a & 2b	*Group 2a*	*Group 2b*		
Beggiatoa	-2.21	-0.11	1.63	22.21
Burrow density	0.64	1.5	1.18	34.99
Nassarids	0	-1.07	1.21	46.17
Faunal tracks	0.14	0.93	2.04	54.59
Sediment Colour	-0.95	-0.21	1.77	62.5

The visual features defining groups 2a and 2b support the proposition that these groups represent a gradient of impact (Table 6.3.7.1). The main features distinguishing group 2b from group 1 were burrow density, brittle stars and Nassarid gastropods. Increased burrow density and the presence of brittle stars have already been identified as being indicative of good environmental conditions and were important features in group 1 samples. In contrast Nassarid gastropods (dog whelks) are opportunistic epibenthic scavengers that take advantage of any additional organic matter and were prevalent in group 2b samples but rare at group 1. The variables distinguishing the impacted groups (2b & 2a) were *Beggiatoa* cover, burrow density, Nassarid gastropod and faunal tracks. *Beggiatoa* was more common in group 2a sites (the more impacted group), whilst burrow density and Nassarid gastropods increased in the group 2b sites. *Beggiatoa* was commonly seen underneath and in close proximity to cages, and reduced rapidly in both occurrence and thickness with distance from the cages. Although previously described as opportunistic scavengers, Nassarid gastropods were rarely found directly beneath cages; this is probably because they are unable to tolerate the low oxygen environment associated with more degraded conditions.

6.3.7.4 Comparison with benthic community structure analysis

Comparison of the video scores with Shannon Diversity indictaes a significant positive relationship ($F_{1,104}$=171.813, p=0.000) (Fig. 6.3.7.4.1). This quantitative video assessment technique is as sensitive to differing levels of impact as the Shannon index, and can distinguish impacted and unimpacted conditions, and also intermediate and transitional impacts.

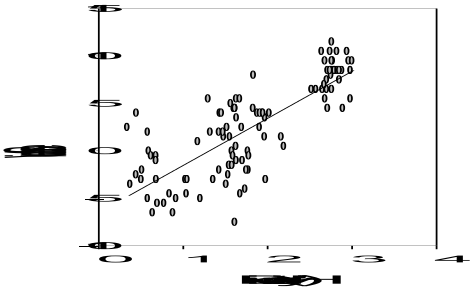


Fig. 6.3.7.4.1. Plot of Shannon Diversity and Video Scores at Stringers Cove during the second production cycle. Equation y = 4.9587x - 6.4931 and R^2 = 0.6229.

The correlation between the similarity matrices for the video (Euclidean distance) and benthic (Bray-Curtis similarity co-efficient) data was strong (Sample statistic (Rho): 0.512, significance of sample statistic =0.1%), indicating that the two data sets were achieving a similar level of site differentiation.

6.3.8 Discussion

The Stringers Cove data indicates very clear differences between the samples collected at the different farm positions over time which could be related both to the faunal composition and to changes in farm production levels.

The community data clearly distinguished impacted and unimpacted sediment conditions. The results showed that the pre-farming community was very similar to the reference community (P1/2/1A/2A samples at 0 months), suggesting that the references selected were appropriate for the study (Fig. 5.3.1.1). That no farm sites were associated with the reference group after the onset of farming suggests that conditions never returned to a level equivalent to that at the reference/pre-farming, despite up to 14.5 months fallow (P1/2). Previous work in Tasmania by Ritz et al. (1989) had suggested that sediments recovered in only 7 weeks. However, this study

was conducted under different environmental conditions (ie. sandy sediments around the Tasman peninsula) and under less intensive farming conditions. More recently Black et al. (2001) and Karakassis et al. (1999) studied Sea Bream culture in the Mediterranean and indicated that recovery could take much longer, 21 and 23 months respectively. However, these studies were looking at the complete removal of the cage operations not rotation of cages within an operational site as in the current study. It would be unrealistic to expect full recovery to reference/pre-farming conditions where fallowing periods are short and where the rest of the farm/lease is still being utilised. All of the study sites showed a level of recovery, and from the perspective of farm sustainability, the sediments in this study could be considered recovered if they attained an equivalent community structure to that existing pre-stocking.

There were a broad range of conditions represented by the changing community structure, which reflected the increasing organic enrichment gradient, with communities indicative of low levels of impact at one extreme and more marked impacts at the other. Although the community structure changed continuously along this gradient it was possible to define general characteristics, such as communities or faunal indicators, typical of differing levels of impact and even to distinguish deteriorating and recovering conditions (Fig. 6.3.8.1). It was difficult to separate conditions/communities which fell near the boundaries of these categories. However, such precision would not be necessary for farmers, they would only have to determine approximately where a particular site might be placed along the general impact/ recovery gradient. The present data suggest that this would be possible.

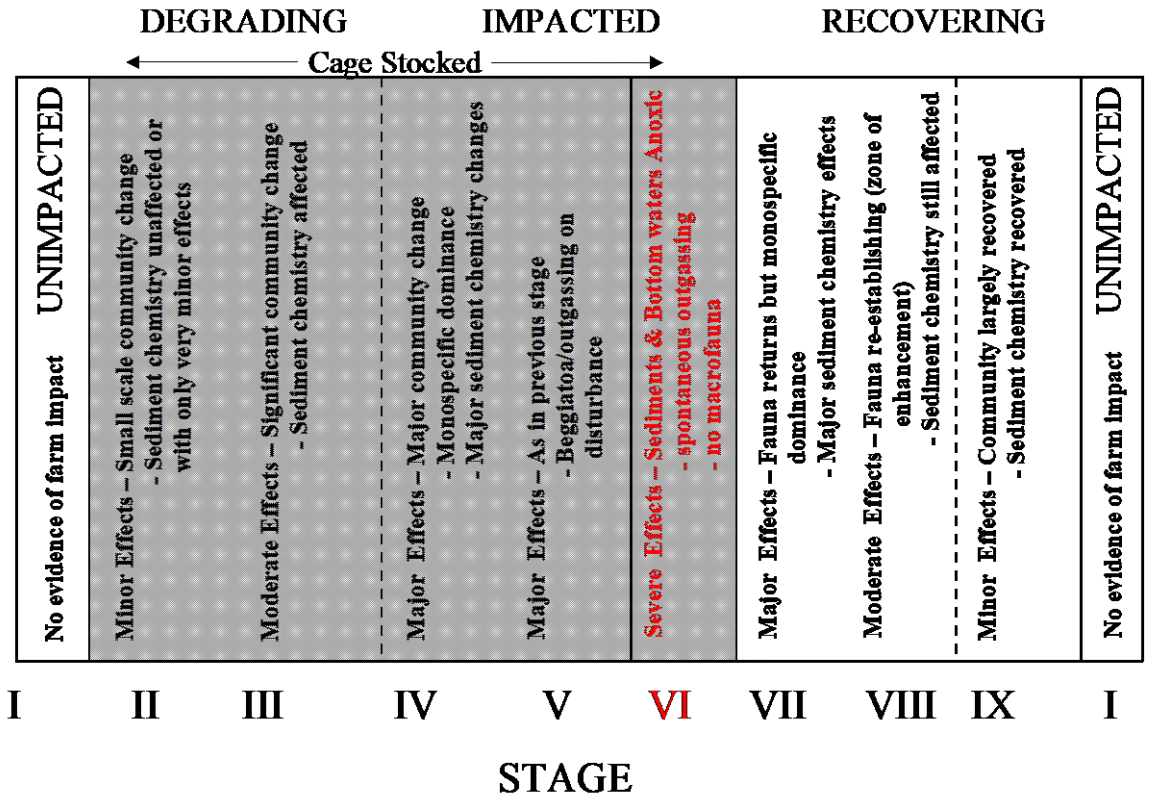


Fig.6.3.8.1. General characterisation of impact/recovery stages based on main community changes.

There was considerable temporal variability in the degree of sediment impact over the two years of the study. This was principally a function of the production strategy employed. Consequently the characterisation of impact level shown above was developed without reference to any specific temporal scale. Changes in intensity of farming and attendant changes in degree of impact would be reflected in both the interval between impact stages and the maximum impact stage occurring.

The study identified a number of key indicator species which characterised particular conditions (Fig. 6.3.8.2). *Capitella capitata* and *Nebalia longicornis*, both of which are well recognised opportunists, typified the impacted conditions. Intermediate levels of impact were characterised by several species, including several representative of taxonomic or functional groups which have previously been considered characteristic of transitional conditions (Pearson & Rosenberg, 1978). These included, *Nassarius nigellus*, a native dog whelk and a commonly occurring epibenthic scavenger, *Corbula gibba,* an introduced bivalve mollusc tolerant of low oxygen and organically enriched environments, and Phoxocephalidae species. Unfortunately Phoxocephalidae is an amphipod family which is notoriously difficult to identify and separate at species level; accordingly, within the present study they were simply grouped as Phoxocephalidae spp. However, several species of Phoxocephalidae spp. are known to be tolerant of low oxygen and slightly organically enriched environments and it is probably these, or functionally similar species which are involved in this instance. *Echinocardium cordatum* was also a notable contributor to the intermediate impact communities. This heart urchin has a cosmopolitan distribution and is not often thought of as an indicator of organic enrichment. However, locally it appeared to thrive in areas where there were low levels of organic enrichment, so long as the oxygen levels remain adequate.

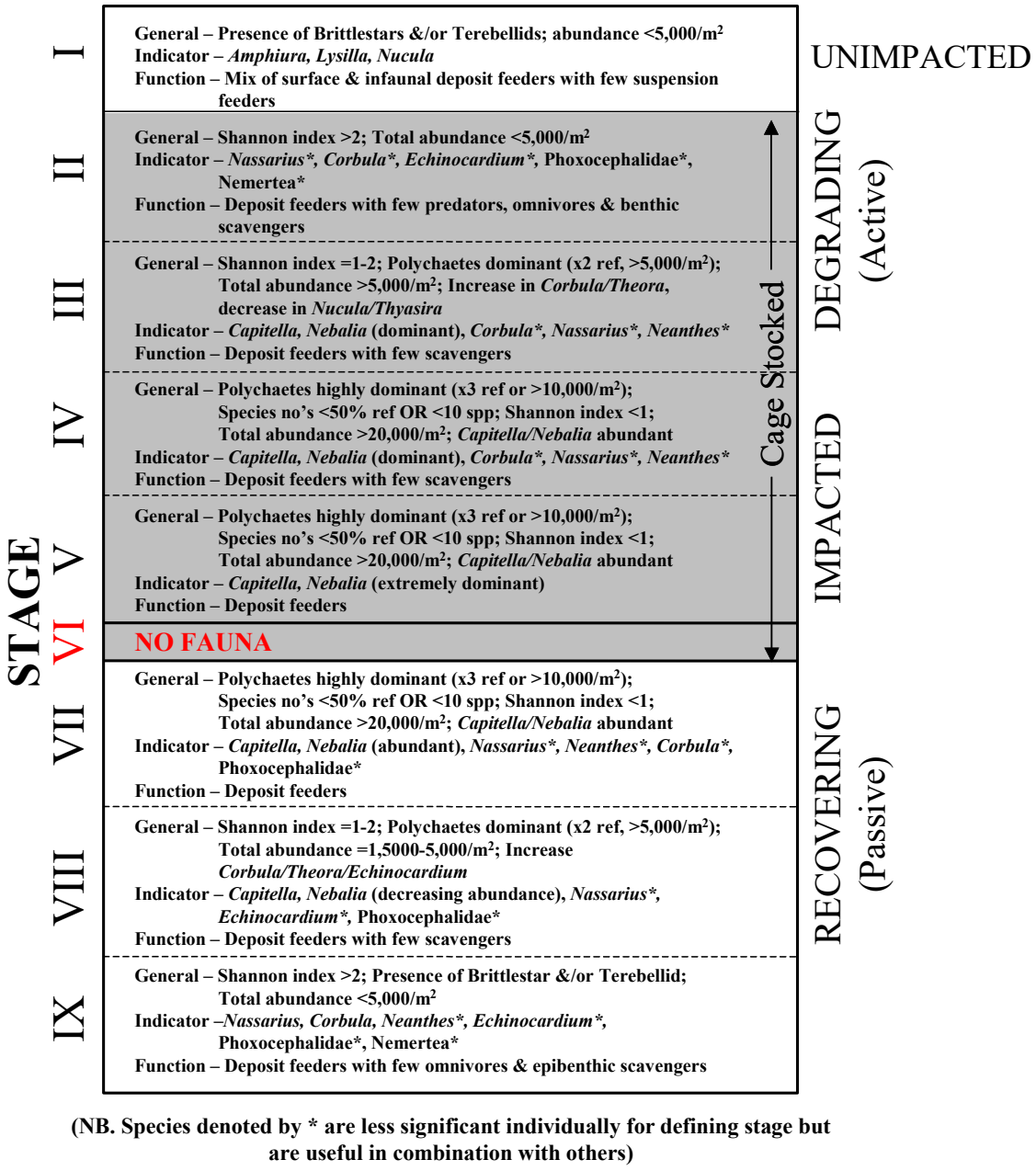


Fig.6.3.8.2. Characterisation of impact/recovery stages based on general community features, key indicator species and ecological function.

At the unimpacted sites the communities were considerably more diverse, with no obvious dominants. Nonetheless, a couple of species appeared to be specifically associated with these conditions. The brittle star *Amphiura elandiformis* appeared to be relatively intolerant of impacted environmental conditions. It was never found where environmental conditions were degraded or where sediment oxygen levels were depleted. Therefore when present it appears to be a very reliable indicator of unimpacted conditions. The presence of *Mediomastus australiensis* in place of *Capitella capitata* may also be a possible indication of improved environmental conditions.

Sediment recovery began almost as soon as the pens were removed and was initially quite rapid. Marked changes in the first few months of fallowing resulted in a transitional/minor impact community (stage II/IX). The initial change from impacted to transitional community was initiated by the cessation of enrichment and a concomitant reduction in nutrients which had supported proliferation of the main opportunistic species. Once the organic input ceased, any remaining material was very quickly utilised and the opportunistic impact species died off. Re-establishment of the normal community then began. This started with the arrival of the more mobile species (epifauna and scavengers), which can quickly colonise the vacant environmental niches. Other infaunal species with a relatively high tolerance to poorly oxygenated, organically enriched environments also began recruiting to the recovering sediments from nearby populations (e.g. *Corbula gibba* & *Theora lubrica*). These species either have a rapid reproductive phase or an appropriate larval settlement /survival strategy. Once the initial influx of new species was complete the recovery rate slowed markedly. Further stabilisation of the community was reliant on the establishment of slow growing, less adaptable species whose reproductive strategies are more limited and for which population growth is a slower process. Change from this transitional community to a more stable system would be slower. Recovery to a full climax community (stage I) would take a long time, and was not observed in this study.

The recovery process was reflected in the changes in species, and also in ecological function over time. Once the input of organic material ceased the initial change in the community structure was rapid, with numbers of *Capitella capitata* declining precipitously. However, during this phase the bioturbation of the sediments would still be significant, actually improving conditions and helping to promote the next stage of colonisation. The epifaunal scavengers and some of the more hypoxia tolerant opportunists (*Nassarius, Corbula* and Phoxocephalids) were the first to relocate. These species dominated when competition was limited. As conditions continued to improve their dominance of the system diminished and the community developed a broader range of species better adapted to well oxygenated and lower organic content sediments (e.g. *Mediomastus australiensis*, *Aricidea* sp.6, *Archasterope* sp.1, *Mysella donaciformis*). However, several stable community species (eg. *Amphiura elandiformis*, *Lysilla jennacubinae, Nemocardium thetidis, Nucula pusilla,* Nemertean sp.1) were consistently found at the reference stations, and did not re-establish at the farm sites even after the longest fallow period (15 months). Either the reproductive strategies of these species were incompatible with the colonisation conditions (i.e. long development stages and/or larval/juvenile sensitivity), or they may have had feeding modes/life strategies which preclude them from even slightly impacted environments. Since in this instance there was a local reservoir for colonisation (i.e. they were present at the reference sites), it is more likely that it was the condition of the sediments within the farm that inhibited their establishment.

The recovered community comprised a mix of opportunistic, transitional and stable species. This community would still be relatively tolerant of a broad range of environmental conditions and might be considered the optimal recovered state for a fish-farm sediment community. It could provide a ready supply of opportunistic species to the farm sediments when farming

resumed. The results from the current study indicate that deposit feeding and burrowing capitellids are extremely efficient at breaking down and assimilating organic material and oxygenating the sediments. Consequently, from a farm perspective, this community (stage IX) may be preferable to a more stable community (stage I). A stage I community would take a very long time to achieve and would probably not be as effective in recycling the excess nutrients within the system.

Although it may be possible to maintain an impacted fauna on an ongoing basis, this would probably not be the best outcome in the longer term. It is important that the sediments be allowed to recover and that more diverse communities are established between impacts. Diverse communities have specific advantages, larger bivalves and burrowing/tube building crustaceans associated with the transitional/recovering fauna will oxygenate the sediments to a much deeper level than the capitellids, ensuring that an anoxic inversion layer does not become established. If the sediments are continuously degraded then it is possible that the capitellids may only oxygenate the upper sediments, isolating the deeper sediments. Over time these sediments could become depleted in oxygen and this anoxic layer might then slowly rise to the surface, eliminating all interstitial fauna (i.e. slowly souring the system). Allowing a recovered community (stage IX) to establish will ensure that this does not happen. The farm production regimes considered in this study enabled stage IX recovery to occur.

In the current study there was a difference in the extent of impact/recovery between the two production cycles. The impact at the end of the second stocked phase appeared to be less than after the first cycle, and after the second production cycle the sediments appeared to recover more quickly. The production data indicated that although the biomass of fish cultured remained consistent the amount of feed input was markedly reduced in the second cycle. Adjustments in farm management practices have a considerable influence on the level of impact on the sediments.

Visual assessment techniques were evaluated in relation to the 9 distinct impact/recovery stages identified by the infanal community assessment, and were found to relate extremely well (Fig. 6.3.8.3). Benthic photo evaluation is a very simple approach, which uses images of sieved infaunal samples to identify the main community features and characterise the sediment condition accordingly. There was less data available to assess this technique than for other approaches evaluated. The results show that it effectively characterised the main impact stages (Fig. 6.3.8.3), and that it could be a very useful technique for farm based assessment. The advantage that this approach has over video assessment is that it represents conditions within the sediments not just at the sediment surface.

Video assessment is a relatively easy technique that can readily be undertaken on farms. In many instances farms already use video footage to obtain a broad and subjective evaluation of sediment condition. However, the approach outlined in this study would provide an objective evaluation of the sediment condition and a quantitative categorisation of sediment condition that can be compared between sites and over time.

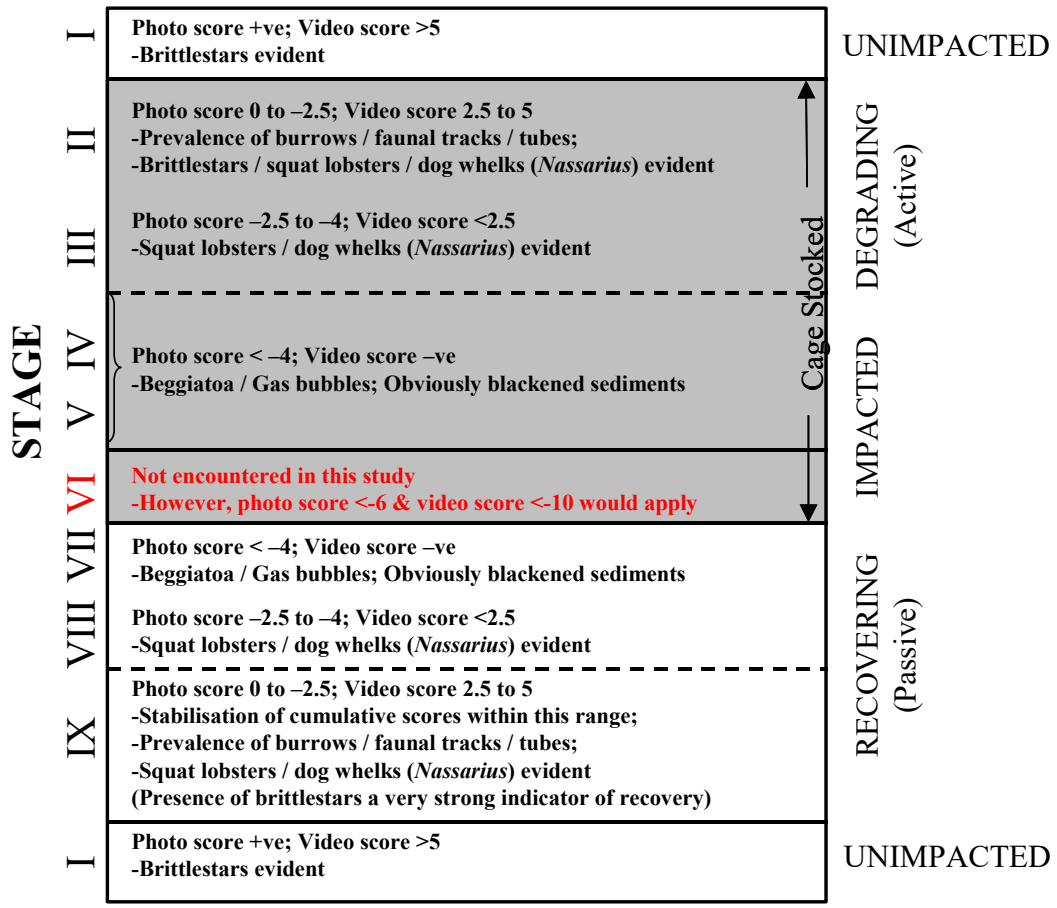


Fig. 6.3.8.3. Characterisation of impact/recovery stages based on visual assessment features.

Both the infaunal and visual assessments identified approaches which could be used by farmers to more effectively monitor the sediment condition within their leases. Incorporating this information into their regular management protocols will enable farmers to either manage production levels within their lease to facilitate particular environmental outcomes or at least better understand the environmental implications of particular stocking/fallowing regimes.

6.4 Sediment Biogeochemistry

6.4.1 Granulometry and Organic Matter Determination

6.4.1.1 Granulometry

Sediment particle size distribution remained similar throughout the study. In particular, there was no increase in the silt/clay fraction at the cage sites, as might be expected with the increased input of organic material (Fig.6.4.1.1.1). Silt/clays dominated the sediments and the breakdown of this fraction showed that the size composition was extremely variable. There was no specific pattern that could be associated with stocking or fallowing. The natural temporal and spatial variability of this fraction overwhelmed any changes that might be associated with organic enrichment.

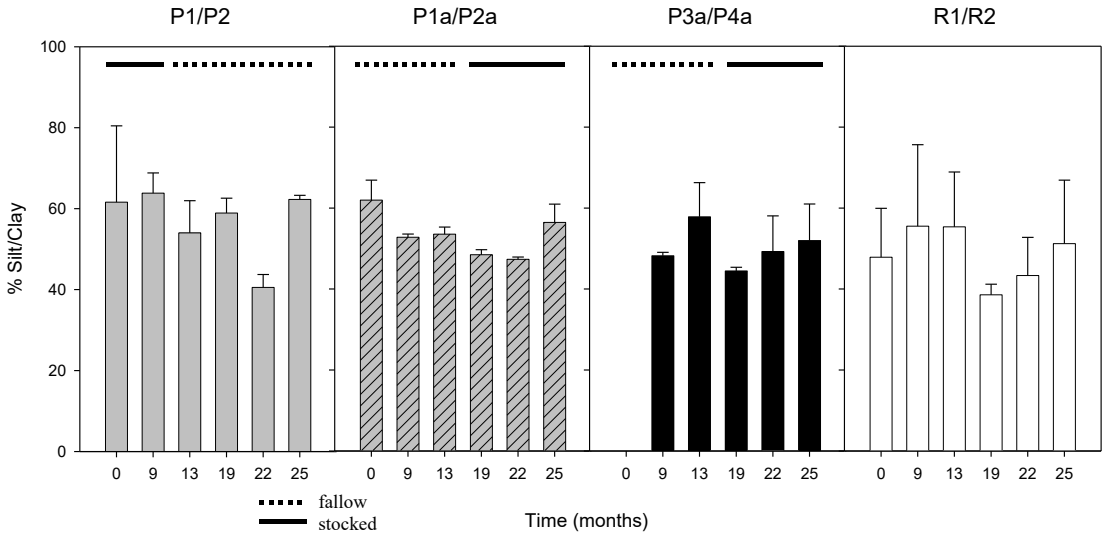


Fig. 6.4.1.1.1 Percentage of Silt/Clay in sediments at Stringers Cove sites on major sampling occasions. Stocked and fallow periods at farm sites are indicated by dotted (fallow) and solid (stocked) lines. (Positions 1 & 2 , positions 1a & 2a, positions 3a & 4a and references 1 & 2 have been combined for this analysis). There were no significant differences.

6.4.1.2 Organic Matter Content

Total organic matter (TOM) was assessed (by loss on ignition) for the first production cycle at Stringers Cove and as a result, only the stocked cages (P1 and P2) and references (R1 and R2) were analysed. There was significant interaction between treatment (cage / reference) and sampling time ($F_{5,84}$=2.611, p=0.030), with posthoc analysis suggesting that, as at Creeses Mistake, there was more TOM present in the initial sample set than in later samples. This finding is unusual and counter-intuitive as these samples were collected before farming commenced and would suggest that farming resulted in lower organic matter levels. Since the result is consistent for both Creeses Mistake and Stringers Cove samples, it is possible that there may have been a problem in processing these initial samples. Consequently, the results for the initial samples were removed from the dataset and the analysis was repeated. Once again, there was a significant interaction between treatment and sampling time ($F_{4,70}$=4.348, p=0.003). In this instance post hoc comparison indicated a significant difference in TOM between the cage and reference treatments after the cages were stocked; midway through the stocking cycle (4.5 months), at the end of stocking period (10 months) and during the fallow period (12.5 months) (Fig. 6.4.1.2.1). However, there was no significant difference between cage and reference at either 9 or 13 months and in both these instances the organic matter level at the cages was lower than on the previous sample occasion. Although a reduced level at 13 months might be a valid indication of recovery, the reduction in level for both the cage and reference at 9 months does not seem likely. It is possible that the microbial and macrofaunal communities in the sediments at this time were successfully breaking down the organic material. The Creeses Mistake results suggested several problems with this technique and the Stringers Cove data also seems to support those assertions. As a result of these problems and the fact that organic matter measurement did not reflect the sedimentary changes at stocked sites observed with other approaches, particularly the benthic infauna, assessment was not continued for the second production cycle.

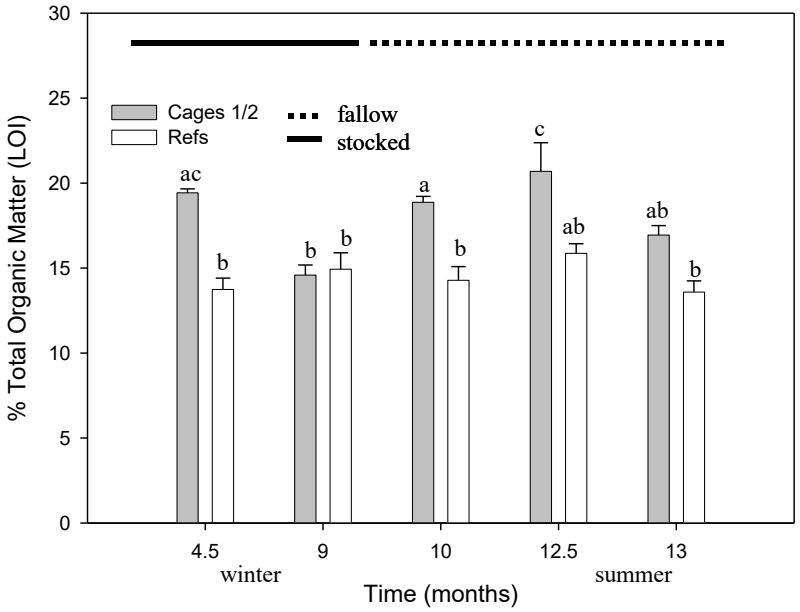


Fig. 6.4.1.2.1. Total Organic Matter (% loss on ignition) (mean and standard error) at positions 1 & 2 and references 1 & 2 during the first production cycle at Stringers Cove. Letters indicate ANOVA results.

6.4.2 Redox Potential & Sulphide Concentration

For the analysis of redox and sulphide data, sites were combined according to the different production strategies/ treatments described in section 6.3.2.

6.4.2.1 Redox Potential

Strategy 1:

There was a significant interaction between treatment (positions 1/2 & references) and sampling time ($F_{9,400}$=5.675, p=0.000). Post hoc comparison indicated that except at 6 months redox potential was significantly lower at the cages than at the references over the stocking period; this occurred up to and including the end of the three month fallow period (13 months) (Fig. 6.4.2.1.1a). The apparent recovery of the redox potential after 6 months corresponds with the period where there was a significant increase in faunal abundance (Fig. 6.3.5.2.1) and might therefore reflect the effects of increased bioturbation associated with such large numbers of opportunistic species. At the end of the stocked period there was negative redox potential at the cages. Over the fallow period there was a steady improvement until 17.5 months, after which time redox levels stabilised, but remained lower than the references. Even after 15 months fallow, redox potential at the cages was still significantly lower than at the reference. However, redox levels were significantly higher at the end of the study (25 months), than at any time after 4.5 months in the stocked phase (6, 9, 10 months), suggesting that redox level can readily distinguish deteriorating conditions. After three months fallow redox at these positions was similar to that pre-farm and did not change markedly over the next twelve months. That redox levels did not continue to recover likely suggests that adjacent stocked cages are having an influence on these empty/recovering sites.

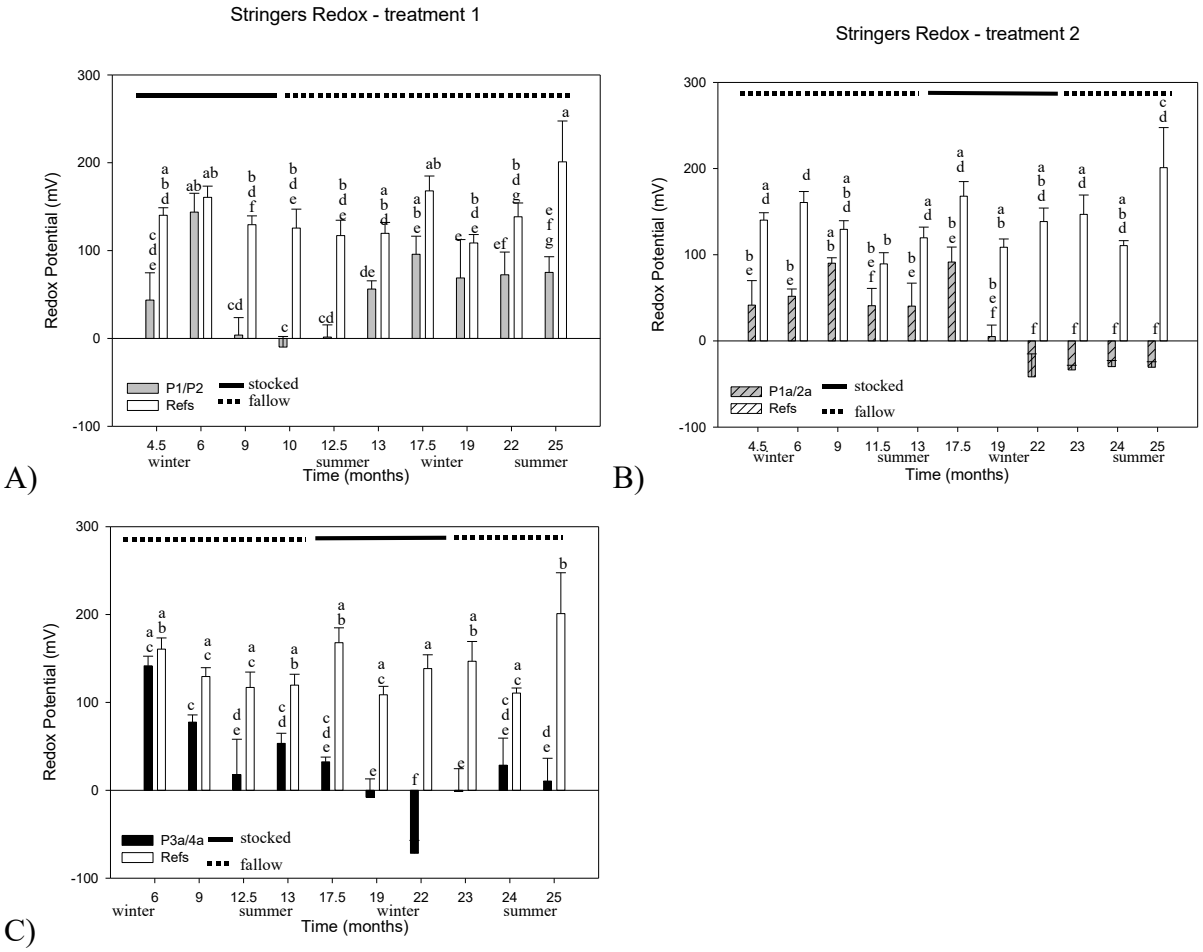


Fig. 6.4.2.1.1. Redox potential at 3cm sediment depth (mean and standard error) for A) Strategy 1 (positions 1/2 and references), B) Strategy 2 (positions 1A/2A and references) and C) Strategy 3 (positions 3A/ 4A and references) at Stringers Cove over both production cycles. Letters indicate ANOVA results (nb. letters between plots are not related).

Strategy 2:

As with strategy 1, there was a significant interaction between treatment (positions 1A & 2A / references) and sampling time ($F_{10,440}$=18.55, p=0.000). Tukeys posthoc pairwise comparison indicated that for the most part redox at the cages was significantly lower than at the references but that levels at the cages varied over time (Fig.6.4.2.1.1b). Over the first production cycle cage redox was consistently lower than the references, despite the cages being empty over this period. This once again indicated that adjacent stocked cages have an influence on sediment conditions. Although lower than the references, the level at the cages remained relatively stable over this period, with no major differences over time. Redox potential at the cages was significantly lower than at the references at all times after the cages were stocked in the second production cycle, including at the end of three months fallow (25 months). After stocking redox potential at the cages dropped markedly to negative levels and remained very low, even after three months fallow. The cage positions did not recover to pre-stocking levels indicating that there is the potential for progressive deterioration at these positions.

Strategy 3:

Once again there was a significant interaction between treatment (positions 3A&4A / references) and sampling time ($F_{9,400}$=13.567,p=0.000), reflecting the changing impact (stocking) level at the cage positions over time. However, in contrast to positions 1A/2A redox potential at positions 3A/4A was not significantly different from the references over the first production cycle (Fig. 6.4.2.1.1c). This indictaes that the effect of the adjacent stocked cages

was not as great at these positions as it was at positions 1A/2A. After stocking and on all subsequent sampling occasions, redox potential at positions 3A/4A was significantly lower than at the references. Redox level dropped below zero towards the end of the stocked period (19, 22 & 23 months) but thereafter improved rapidly and was positive throughout the fallow period. Although redox potential was still significantly lower at the cage positions at the end of the three month fallow period (25 month) than at either the references or at the start of the study (4.5 months) ($F_{2,60}$=44.833, p=0.000), there was evidence of recovery, and at the end of the study redox levels at the cages were not significantly different to pre-stocking levels (12.5 months). However, since these positions had previously been farmed, the fact that they didn't recover to pre-farming conditions suggests there is the potential for progressive deterioration.

Comparsions with the findings of the full benthic infanal analysis indicates that although redox potential appears to be very effective at detecting the difference between impacted and unimpacted conditions it is not a very sensitive technique for assessing recovery. The results are inconclusive regarding progressive deterioration. It is unlikely that within the relatively short time-frame of this study, it would be possible to detect progressive degradation. In spite of this, the results from both positions 1A/2A and 3A/4A suggest that there may be the potential for progressive deterioration.

6.4.2.1.1 Depth of Redox Measurement

This study also examined the variation in redox with depth to determine whether any particular depth was most suitable for measurement. The results showed that redox varied significantly with depth (Strategy 1:. $F_{3,400}$=6.144, p=0.000; Strategy 2: $F_{3,440}$=7.777, p=0.000; Strategy 3: $F_{3,400}$=5.944, p=0.000). Posthoc tests showed that redox levels at 2cm depth were significantly higher than those at 4cm and 5cm and suggest stratification within the sediments. However, these changes in depth did not appear to affect either the differentiation between cage and reference or the temporal pattern of response as there was no significant interaction with treatment or time (Depth*Treatment - Strategy 1:.$F_{3,400}$=0.183, p=0.908; Strategy 2: $F_{3,440}$=0.572, p=0.634; Strategy 3: $F_{3,400}$=0.208, p=0.891; Depth*Time - Strategy 1:.$F_{27,400}$=0.161, p=1.000; Strategy 2: $F_{30,440}$=0.749, p=0.831; Strategy 3: $F_{27,400}$=0.311, p=1.000). This does not diminish its importance as a factor to consider when employing redox potential as a monitoring approach. In all analyses, 3cm depth was not significantly different to 2cm or 4 and 5cm, which suggests that this would be the most appropriate depth from which to obtain measurements. All redox comparisons in this study have been presented using the measurements from this depth.

6.4.2.2 Sulphide Concentration

Strategy 1:

Under strategy 1 there was a significant interaction between treatment (positions 1&2 / references) and sampling time ($F_{8,354}$=20.329, p=0.000) for sulphide concentration. Pairwise comparison showed that during the stocked period of the first production cycle sulphide concentration was significantly higher at positions 1/2 than at the references (Fig.6.4.2.2.1a). At the onset of fallowing sulphide concentrations initially decreased (10 months) then rose markedly (12.5 months). However, the values for the replicates at 12.5 months were very variable, suggesting that there was considerable small scale patchiness in sulphide concentration (possibly as a function of the variability in micro and macrofaunal activities in recovery). At the end of three months fallow sulphide concentration was still higher at positions 1/2 than at the references. Over the second production cycle, when positions 1/2 were empty, the sulphide concentration was consistently higher than at the references although the levels were very low and the differences were not significant. Wildish et al. (2001) ascribed

sulphide concentrations less than 300µM as indicative of normal conditions. In contrast to the findings for redox potential and assessment of the benthic macrofauna, these results suggest that there was no effect of adjacent stocked cages, which in turn implies that sulphide concentration is a less sensitive measure of intermediate and low levels of impact from organic enrichment.

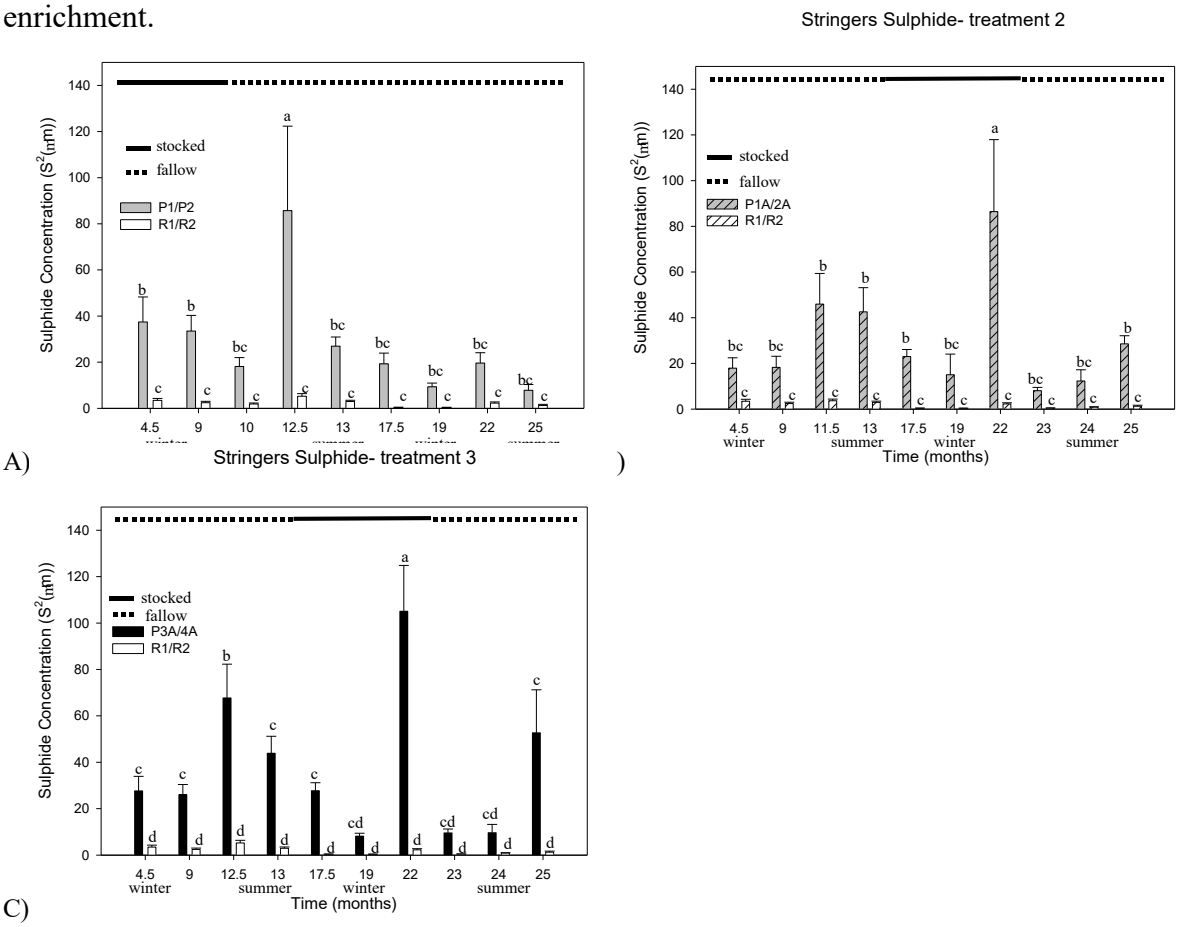


Fig. 6.4.2.2.1. Sulphide concentration at 3cm sediment depth (mean and standard error) at A) Strategy 1 (positions 1&2) and References, B) Strategy 2 (positions 1a&2a) and References and C) Strategy 3 (positions 3a&4a) and References at Stringers Cove over both production cycles. Letters indicate ANOVA results for each different production strategy and do not compare between plots.

Strategy 2:

Once again there was a significant interaction between treatment (positions 1A & 2A / references) and sampling time ($F_{9,400}$=14.175, p=0.000). Sulphide concentration did not vary significantly between treatments over the first production cycle, when the cages were not stocked (Fig. 6.4.2.2.1b). Only towards the end of the second stocking cycle (22 months) did the sulphide concentration increase sufficiently to differ from the references. Sulphide concentration responded rapidly to the reduction in organic loading at the end of the stocking cycle, with levels at positions 1A/2A returning to both pre-farming and reference conditions within one month of the onset of fallowing. This was maintained for the remainder of the three month fallow period. This contradicts the redox potential results (Fig. 6.4.2.1.1b), which showed a more prolonged difference between the cages and references and suggested the potential for progressive deterioration occurring at these sites. Consequently it is proposed that sulphide concentration is not a sensitive measure of recovery or a good definition of the transitional stages of fallowing, but that it may still be a useful indicator of degradation.

Strategy 3:

There was again a significant interaction between treatment (positions 3A&4A / references) and sampling time ($F_{9,387}$=34.806, p=0.000). Sulphide concentration at the references was very low and did not differ significantly over time. However, over the first production cycle sulphide concentration at positions 3A/4A was significantly higher than at the references (Fig.6.4.2.2.1c). Since at this time these cages were not stocked, this might suggest that adjacent stocked cages were having a marked effect. Sulphide concentration at the cages declined slightly after the onset of stocking (19 months) which corresponds to the redox potential and infaunal findings and indicates that at this time the fauna were assimilating the extra organic material associated with farming. Thereafter the sulphide concentration increased markedly (22 months), indicating more degraded conditions, before dropping off once again with the start of the fallow period. Sulphide concentrations were more variable at positions 3A/4A (strategy 3) than at positions 1A/2A (strategy 2), which indicates that factors other than the farm inputs were affecting the sulphide concentration (although there was no change observed in reference sulphide levels throughout the study).

In contrast to the redox potential findings there was no significant difference between the depths at which sulphide concentration was measured (2 – 5cm). Consequently, it is proposed that 3cm would be the most appropriate depth for sampling, as this would provide a result that is directly comparable to redox measurements. However, the results above indicate that sulphide concentration is not a sensitive measure of environmental condition except in relatively impacted condition, and is particulary poor in differentiating intermediate and transitional impact.

6.4.2.3 Redox – Sulphide Relationship

Wildish et al. (2000) suggest that defining the relationship between sulphide and redox gives a better understanding of the sediment conditions than that possible with either technique alone. However, in this study the relationship between redox potential and sulphide concentration at Stringers Cove was poor, although it was significant ($F_{1,1204}$=324.76, p=0.000) (Fig.6.4.2.3.1). This is probably because the levels encountered in this study were much lower than those in the study by Wildish et al. (2000). The relationship was best described using a quadratic equation ($y = 16.469e^{-0.0125x}$, R2=0.392), and suggests that there is an inverse correlation between redox and sulphide at the impacted sites (i.e. high sulphide concentration corresponding to low redox potential). The poor relationship in the current study may be the result of the numerous reference sites, with very low/ no sulphide, included in the analysis. However, the relationship was not improved when only stocked cage sites were examined (F1,286=88.346, p=0.000, R2=0.236).

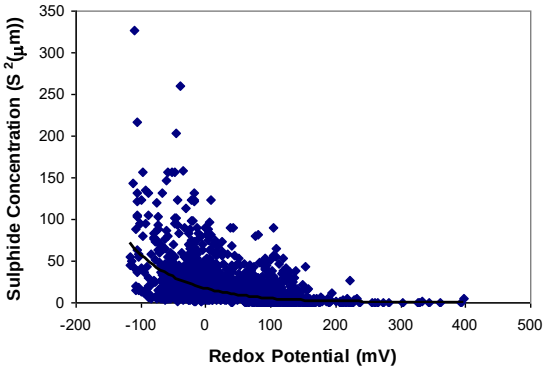


Fig.6.4.2.3.1. Redox potential and sulphide concentration at Stringers Cove at all sites and times. Quadratic trendline has been fitted to the whole dataset, with the equation ($y = 16.469e^{-0.0125x}$) and R^2=0.392.

6.4.3 Porewater Nutrients

6.4.3.1 Depth of the Oxic Zone

Production Cycle 1:

A univariate ANOVA with 2 cages, 2 fallowed cages and 2 reference sites demonstrated that only site differed significantly in the first production cycle (2001-2). There was no effect of standardised elapsed time (time of sampling was equated to length of time from when cages were initially stocked) in the production cycle, nor any interaction between time and site (Table 6.4.3.1.1). The standardised elapsed time is used as times of sampling replicates differed and an intermediate time is used. The mean depth of oxic zone (mm) with the standard error of the mean for stocked and fallowed cages and the references for 1.5, 8.2 and 12.2 months of standardised elapsed time is shown in figure 6.4.3.1.1.

Table 6.4.3.1.1 ANOVA of depth of oxic zone (mm) at farm and reference sites in the first year.

Tests of Between-Subjects Effects

Dependent Variable: Mean depth of oxic zone (mm)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	Source	1	93.715	106.791	.010
	Error	1.707	1.945	.878[a]		
SITENAME	Hypothesis	24.713	2	12.357	46.743	.005
	Error	.831	3.142	.264[b]		
STANDTIM	Hypothesis	1.704	2	.852	3.238	.178
	Error	.793	3.014	.263[c]		
SITENAME * STANDTIM	Hypothesis	.789	3	.263	.767	.544
	Error	2.742	8	.343[d]		

a. 1.045 MS(STANDTIM) - 3.631E-02 MS(SITENAME * STANDTIM) - 8.196E-03 MS(Error)

b. .982 MS(SITENAME * STANDTIM) + 1.767E-02 MS(Error)

c. .998 MS(SITENAME * STANDTIM) + 1.825E-03 MS(Error)

d. MS(Error)

The farm sites consistently had shallower oxygen penetration into the sediments compared to the reference sites (Fig. 6.4.3.1.1). On average, the depth of the oxic zone at farm sites was 50% lower than that at the reference sites. This is despite the fact that the stocked cages had not previously been farmed and that the fallowed cages had been destocked for 15 months at the time of sampling at 12.2 months. This indicates that the oxygen dynamics at the farm sites was fundamentally different from the reference sites. As oxygen dynamics are largely driven by organic carbon respiration, then either labile organic carbon was retained in the sediments for a long time, or there was a more general influence of farm practices throughout the farm area. In this instance the latter is more likely and means that recovery of cage sites was not only a function of how long the site was stocked and fallowed, but was also affected by organic carbon from surrounding cages.

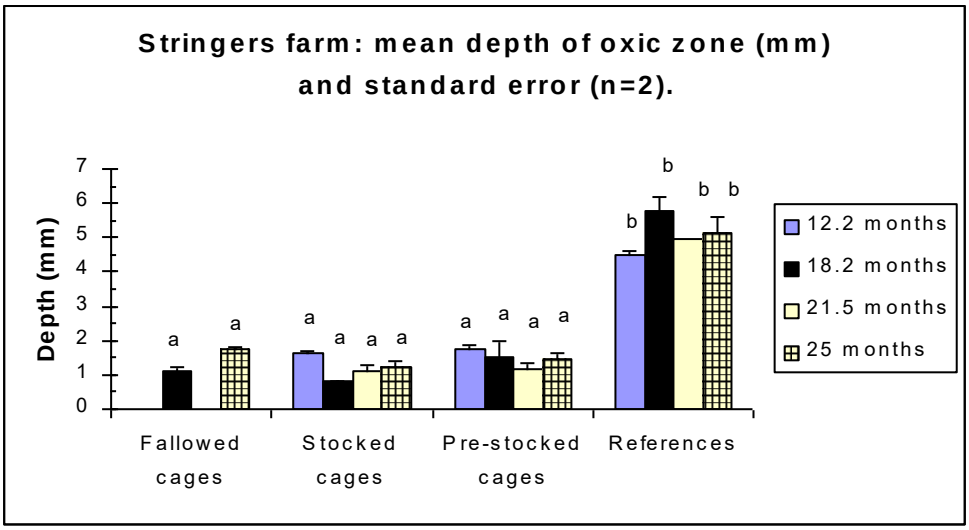


Fig. 6.4.3.1.1. The mean depth (mm) of the oxic zone with standard error of the mean at Stringers farm for the first year of production examined (Feb 01 to Feb. 02). Different superscripts indicate significantly different mean depths of oxygen penetration into the sediments (P<0.05, n=2).

Production Cycle 2:

The potential for cumulative effects on the oxic zone was evaluated by comparing the depth of the oxic zones near the beginning of the first cycle (1.5 months), after the first cycle (12.2 months) and after the second cycle of production (25 months). Table 6.4.3.1.3 compares all cage sites, both stocked and fallowed, and Table 6.4.3.1.4 compares both of the reference sites for these times. It can be seen that there was no significant difference in the depth of the oxic zone at either the cage or the reference sites for these times. Therefore, neither stocking for 9 months nor fallowing for up to 15 months significantly altered the depth of the oxic zone over time. Similarly, because there was no significant difference in oxygen penetration at the reference sites, then there was no obvious effect of inter-annual variation affecting the wider ecosystem. The important issue is that farm sites were always significantly different from reference site, most likely indicating farm-wide effects on organic loading and not just the impact of the cage over the particular site.

Table 6.4.3.1.2. ANOVA of depth of oxic zone (mm) at farm and reference sites in the second year.

Tests of Between-Subjects Effects

Mean depth of oxic zone (mm)

Source		Type III Sum of S	df	Mean Square	F	Sig.
Intercept	Hypothesis	112.9734	1	112.973356	439.9226	0.000265
	Error	0.757239	2.9487187	0.25680279		
SITENAME	Hypothesis	71.80093	3	23.9336426	100.8778	6.82E-05
	Error	1.18627	5	0.23725392		
STANDTIM	Hypothesis	0.769865	3	0.25662175	1.081633	0.43654
	Error	1.18627	5	0.23725392		
SITENAME * STANDTIM	Hypothesis	1.18627	5	0.23725392	1.72871	0.202573
	Error	1.64692	12	0.1372433		
a	1.009 MS(STANDTIM) - 9.347E-03 MS(SITENAME * STANDTIM)					
b	MS(SITENAME * STANDTIM)					
c	MS(Error)					

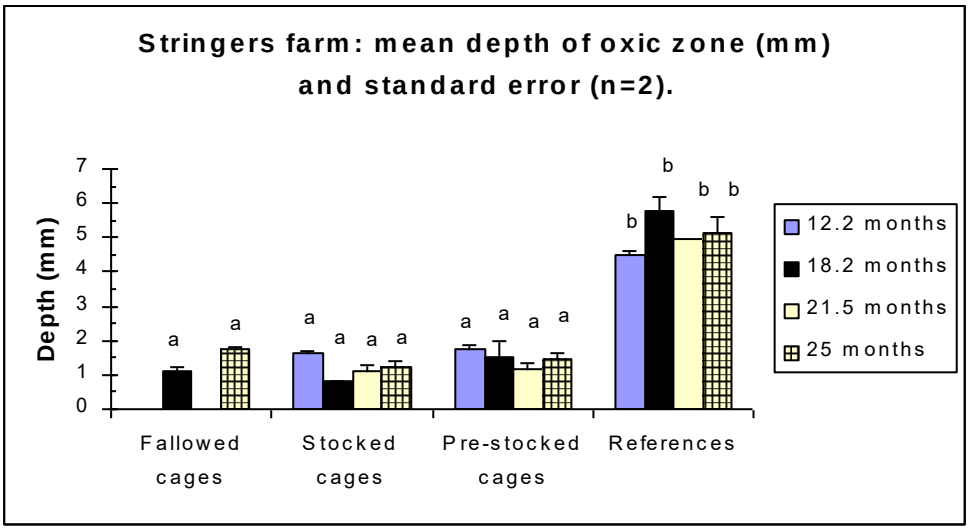


Fig. 6.4.3.1.2. Mean depth of the oxic zone (mm) and standard error of the mean for the second year of production. Different superscripts indicate significantly different depths of penetration.

Table 6.4.3.1.3. One-way ANOVA of the mean depth of the oxic zone at cage sites for Stringers farm at 1.5, 12.2 and 25 months.

Tests of Between-Subjects Effects

Mean depth of oxic zone (mm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.444	2	0.222	6.601	0.080
Intercept	18.965	1	18.965	563.415	0.000
STANDTIM	0.444	2	0.222	6.601	0.080
Error	0.101	3	0.034		
Total	19.511	6			
Corrected Total	0.545	5			

a R Squared = .815 (Adjusted R Squared = .691)

Table 6.4.3.1.4. One-way ANOVA of the mean depth of the oxic zone (mm) of the reference sites over two years of production at the Stringers farm.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.502	2	.251	1.086	.442
Within Groups	.693	3	.231		
Total	1.195	5			

6.4.3.2 Ammonia Measurements

Ammonia concentration was measured in the top 6 to 8 cm of sediment cores before and after production cycles. In all cases, the concentration of ammonia at cage sites (whether in production or being fallowed) was greater than in sediments from the reference sites. After only 2.5 months of production, there was an increase in ammonia in the surface sediments of one cage compared to the reference site (Fig. 6.4.3.2.1 A). Maximum ammonia concentrations commonly occurred at 2 – 4 cm depth at farm sites (Fig. 6.4.3.2.1).

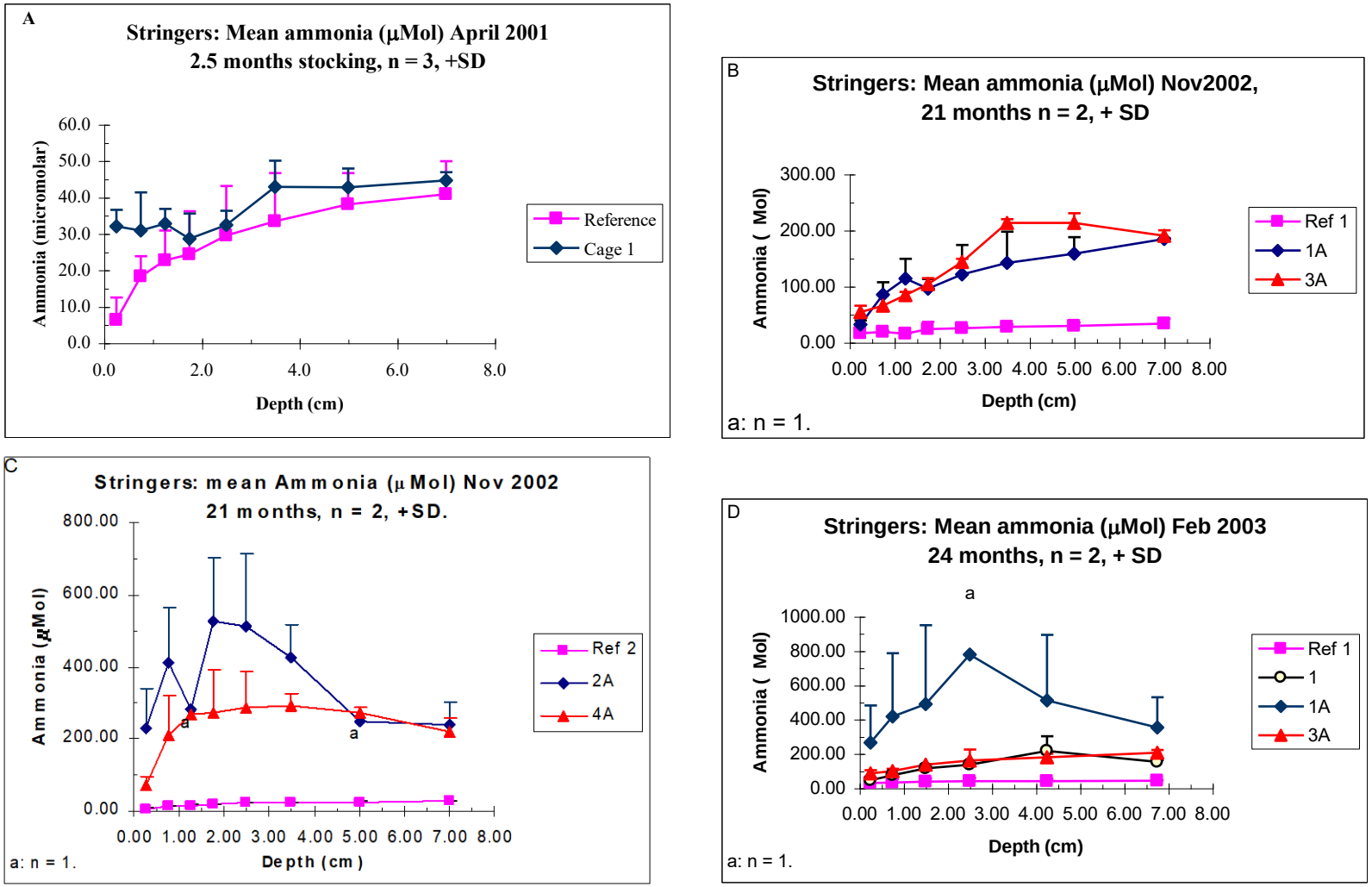


Fig. 6.4.3.2.1. Profiles of ammonia concentration (µM) in porewaters of sediments from cage, fallowed and reference sites at Stringers Cove over 2 years of production.

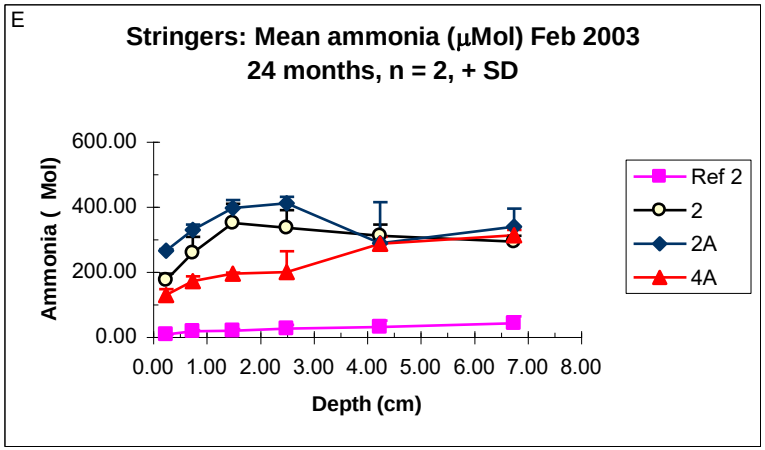


Fig. 6.4.3.2.1(Continued). Profiles of ammonia concentration (µM) in porewaters of sediments from cage, fallowed and reference sites at Stringers Cove over 2 years of production.

Table 6.4.3.2.1 shows the concentration of ammonia in the surface sediments at the end of the first stocking cycle for cages 1 and 2. Cages 3A and 4A had been fallowed for 12 months at this time. It can be seen that an extraordinarily high value was obtained from cage 2. The sediments at cage 2 were outgassing hydrogen sulphide as well as other gases derived from anaerobic metabolism at this time. The impact of this can possibly be seen in figure 6.4.3.2.1 D and E in which the concentration of ammonia present in porewater from cage 2 (fallowed for 15 months by then) was similar to the cages that had been just stocked and fallowed for 3 months.

Table 6.4.3.2.1. Concentration of ammonia (μM) in porewaters extracted from surface sediments at Stringers on October 30, 2001. Detection limit = 0.4 μM.

Site	Ammonia (μM)
1	161
2	10,253
3A	52
4A	102
R1	24
R2	3

All cage sites showed increasing concentration of ammonia with depth, indicating burial of organic carbon, which would act as the source of ammonia through mineralisation. As Stringers is relatively sheltered, at least compared to Creeses Mistake, then the movement of organic carbon into the sediments is most likely mediated by bioturbation.

6.4.3.3 Denitrification

Cores taken from Stringers Cove in November 2002 (21 months) showed no accumulation of N_2O in response to acetylene inhibition. This could be either because:

1. there was no denitrification occurring in the sediments. Reference sites may have been limited in organic carbon or nitrate and cages may have been limited in nitrate. However, the latter is unlikely as nitrate was added to sediment samples.
2. there was no denitrification occurring in the sediments because of sulphide inhibition of denitrification (this is a possibility for the cage sites).
3. sulphide was antagonistic to the effect of acetylene inhibition of denitrification, thereby preventing the accumulation of N_2O. (Again a possibility for the cage sites).

It is unlikely that failure to detect N_2O resulted from malfunctioning of the electrode, because it responded in calibration and in an initial test exercise with glucose and nitrate added to a sediment sample.

Ammonia concentrations were elevated at cage sites at all times even after 15 months fallowing. As cage sites had oxic zones less than 2 mm deep, then having the peak of ammonia concentration at 2 – 4 cm suggests that ammonia is being removed anaerobically, or that high rates of nitrification are occurring in the oxic zone. Possible mechanisms for anaerobic consumption of ammonia include: 1) heterotrophic uptake, which would leave the nitrogen available to the biota, or 2) anaerobic ammonia oxidation (ANAMMOX) which ultimately converts the ammonia to nitrogen gas, leaving it unavailable to the biosphere. If strong nitrification is occurring in the oxic zone, then the resulting nitrate may be consumed by denitrification (but little of this process was detected), or by dissimilatory reduction to ammonia by organisms such as *Beggiatoa* spp. In the latter case the ammonia would still be available to the biosphere. Thus, there are several possible mechanisms that could result in eutrophication of the overlying water.

6.4.3.4 Summary of pore water nutrient results

The oxic zone at cage sites was always significantly shallower than at the reference sites for all cages and times of sampling. Therefore, even 15 months fallowing was unable to increase oxygen penetration of the sediments compared to cages currently stocked. This suggests that the surrounding cages were affecting the cage sites assessed in this project. Similarly, ammonia concentrations were greater in pore waters extracted from cage sites than pore waters from references. Furthermore, the concentration of ammonia increased with depth. Combined with the shallow oxic zones observed, this suggests that it is possible that the sediments around cages can supply ammonia to the overlying water.

6.4.4 Carbon and nitrogen contents in sediments.

Carbon contents in the reference sediments are consistently high throughout the study period with a mean of ca. 3 % (Fig. 6.4.4.1). Samples collected at 13 months (both on and off-farm), showed elevated carbon contents suggesting an influx of carbon at all sites. Thebackground levels of carbon were quite high for a marine site, reflecting both the high proportion of silt and mud-sized particles and the high proportion of terrestrial higher-plant organic matter in the sediments. Benthic biota will thus be pre-adapted to high carbon loadings. At the cage sites P1 and P2, the carbon content is somewhat higher (4-5%), and shows little change during the stocking phase (despite very high carbon deposition), nor does it decrease during fallowing. This is in marked contrast to the Creeses site where sediments had naturally low levels of carbon and showed significant changes in carbon content during the stocking and fallowing cycles. Carbon contents at the in-between sites P1A and P2A were also higher than at the references, but showed little change through the stocking-fallowing cycle.

Sedimentary N was consistently low at the reference sites at about 0.4% (Fig. 6.4.4.2). Sediment contents were much higher at the Stringers cage sites, but they also show considerable variation over time, and on occasions dropped to background levels. There is some indication of an increase through the stocking cycle, but this was not statistically robust. The form of N is not known at this stage, although sediment flux measurements did show a large increase in NH_4 efflux through the stocking cycle. N contents were also high at the in-between sites, with some values approaching those found at the cage sites.

Background C:N values tended to be 8 or greater, indicating a mixture of marine and terrestrially sourced material. Interestingly, at 13 months, the background C:N value maximised at 14, which corresponds to a maximum in the %C values and would appear to indicate an input of material with a significant terrestrial component. C:N values on the lease showed a trend from background levels to values around 6, possibly indicating a shift to more labile organic matter and/or an accumulation of nitrogen species c.f. carbon.

As with the site at Creeses Mistake, analysis of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) showed no significant change through the study period. This was again due to the background and inputs being too similar in their isotopic signatures.

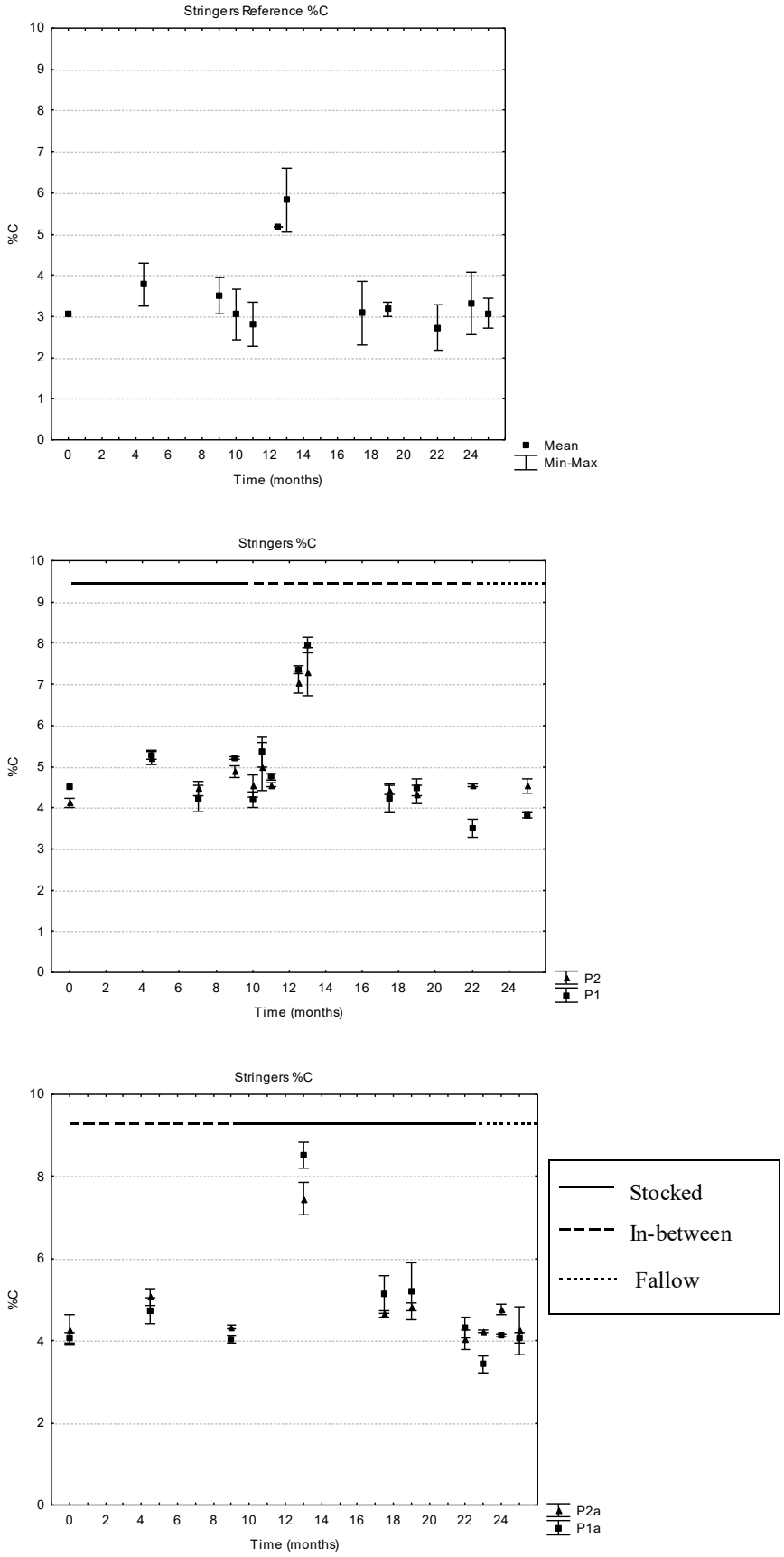


Fig. 6.4.4.1. Carbon contents (as % dry weight of sediment) at a) reference sites and at b) cage sites P1 and P2, and c) in-between sites P1a and P2a.

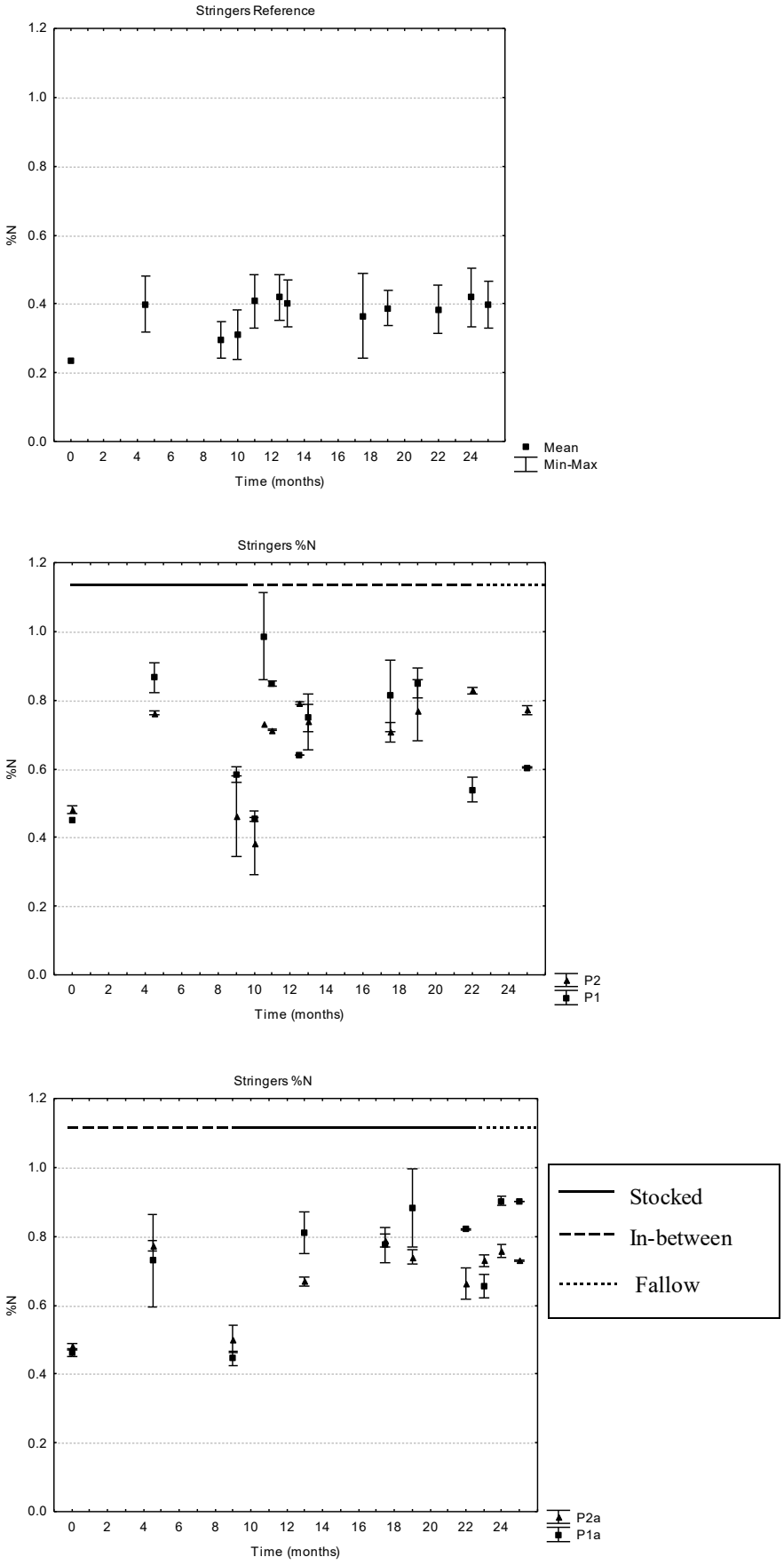


Fig. 6.4.4.2. Nitrogen contents (as % dry weight of sediment) at a) reference sites and at b) cage sites P1 and P2, and c) in-between sites P1a and P2a.

6.4.5 Sedimentary lipid biomarkers

6.4.5.1 Non-saponifiable lipids from reference sites, in-between cage sites and under cages

Non-saponifiable (neutral) lipids were measured at two reference sites, in-between cage sites (P1a and P2a) and at the edge of two cages (P1 and P2) at 0, 4.5, 9, 10, 13, 17.5, 19, 22, 23, 24 and 25 months. Illustrative capillary gas chromatograms showing changes in the distributions from 0, 9.5 (end of stocking) and 13 months (3 months of fallowing) are shown in Figures 6.4.5.1.1 and 6.4.5.1.2. These chromatograms show a diversity of lipid constituents including phytol (side-chain of chlorophyll a), long-chain alcohols, a complex mixture of sterols, hopanoids (bacterial markers; Ourisson et al., 1979) plus smaller amounts of other compounds. General features of the distributions are discussed here, and a more detailed examination of amounts of selected biomarkers (phytol, cholesterol, α-tocopherol, desmosterol and sitosterol) are discussed separately in following sections.

At the beginning of stocking, the lipid distributions in sediments at the reference sites and under the cages were remarkably similar (Fig. 6.4.5.1.1). All sediments contained similar amounts of phytol indicating comparable inputs of phytoplankton-derived chlorophyll a. Indicators of terrestrial organic matter of higher plant origin such as long-chain even-carbon-number *n*-alkanols and the C_{29} sterol 24-ethylcholesterol (sitosterol) were particularly abundant, as might be expected for a location sited close to land and the Esperance river. The relative proportions of the long-chain alkanols were remarkably similar in all samples, all show a predominance at 24:0, and the ratio of alkanols to sitosterol was also almost identical indicating the same source of terrestrial organic matter.

The sterol distributions suggest a diversity of algal groups contributing to the organic matter with significant inputs from diatoms (as shown by the high abundance of 24-methylcholesta-5,22E-dien-3β-ol) and dinoflagellates (high abundance of dinosterol: 4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol). Extensive reworking of the organic matter is shown by the presence of stanols, which are produced from the sterols by microbial reduction, and by various hopanoids of bacterial origin. In each case, the major sterol was cholesterol which is often observed in marine sediments and usually attributed to inputs from marine animals. These data in combination imply that the organic matter in all the sediments analysed was mostly derived from a mixture of marine and terrestrial inputs of natural origin. These distributions can thus be used as a baseline for comparison with sediments that receive additional inputs of organic matter from farming activities.

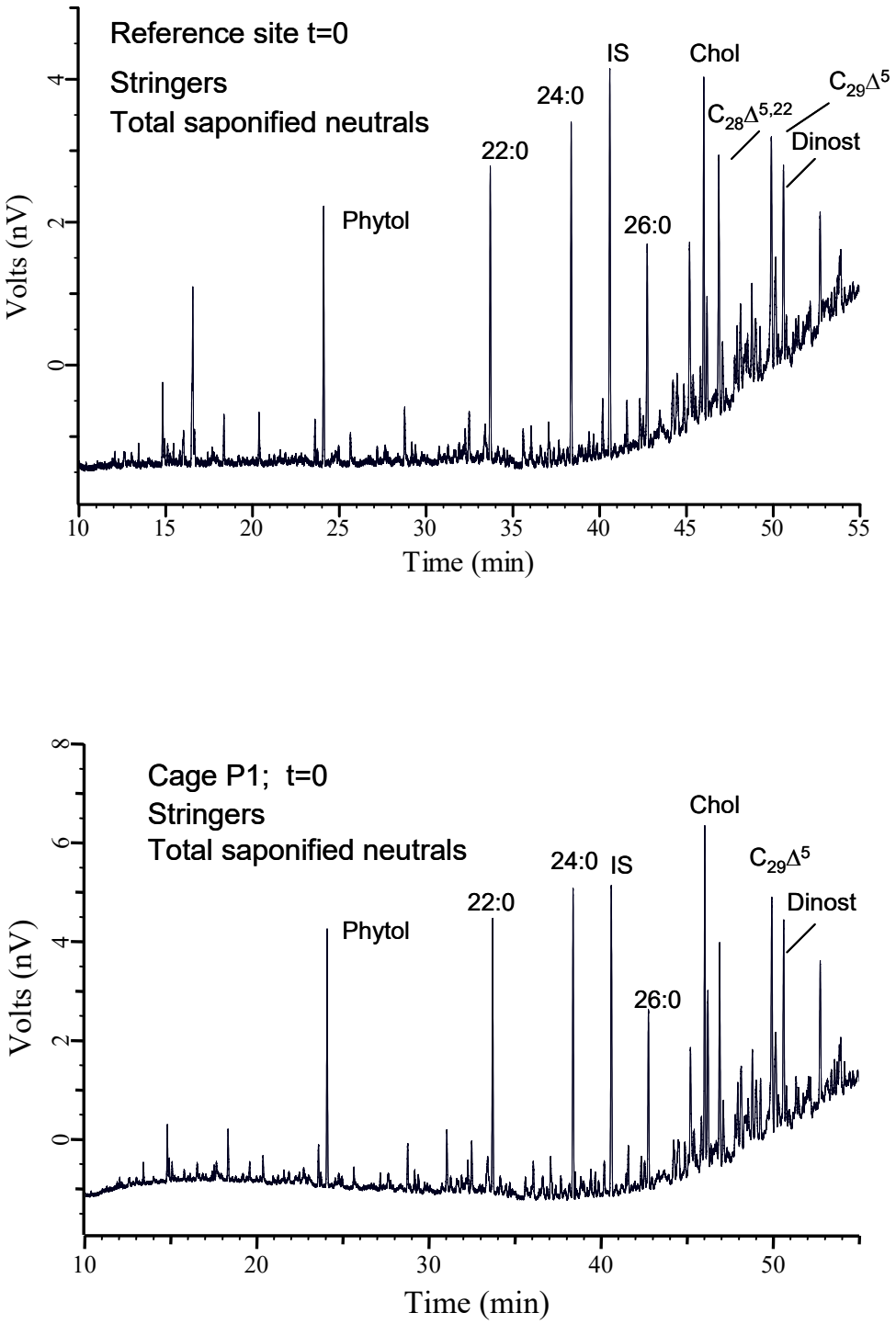


Fig. 6.4.5.1.1. Capillary gas chromatograms (time for 10 to 55 minutes shown) of total neutrals after saponification (as TMSi-ethers) in sediments from a) reference site and b) cage P1 at the initiation of fish stocking. Note the similarity in the distributions of alcohols (shown as carbon number: number of double bonds) and sterol distributions, apart from higher cholesterol amounts in the cage site. IS is the internal standard. Baseline rise is due to column bleed at high temperatures.

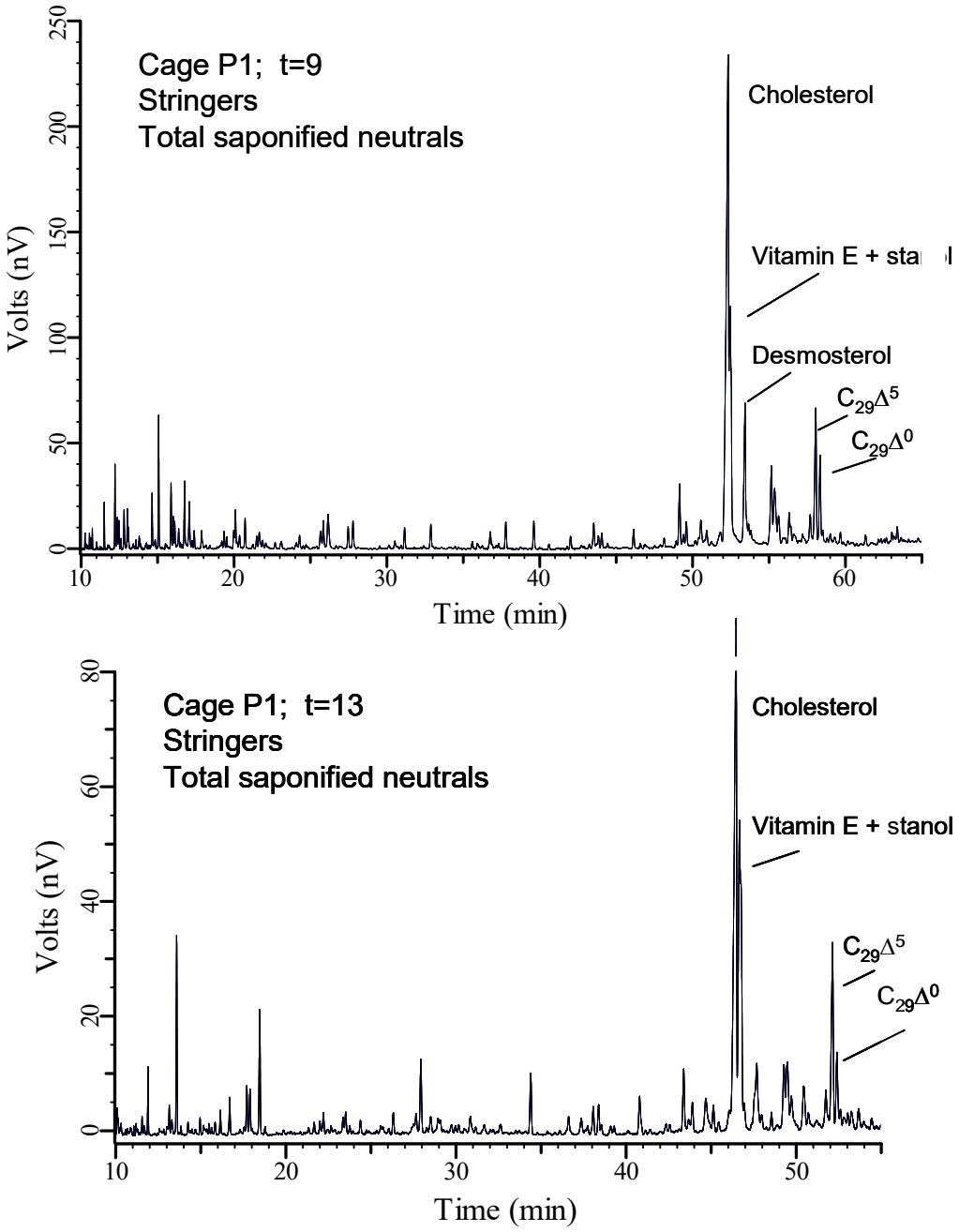


Fig. 6.4.5.1.2. Capillary gas chromatograms (time for 10 to 55 minutes shown) of total neutrals after saponification (as TMSi-ethers) in sediments from cage P1 at a) 9 months (end of stocking) and at b) 13 months (after 4 months fallowing). IS is the internal standard.

However, we did notice a few distinctive differences between the chromatograms for the reference sites and the cage sites at 0 months. The most obvious is the high proportion of vitamin E (α-tocopherol) in the sediments from the cage sites and its low abundance in the reference sites. This compound elutes just after the cholesterol/5α-cholestanol pair in the gas chromatogram (Figs 6.4.5.1.1 and 6.4.5.1.2). Although α-tocopherol naturally occurs in some marine organisms, particularly microalgae (Brown et al., 1999), we show below that this is most likely a residue from previous farming activities at this site. Cholesterol contents were also slightly elevated in the cage site sediments, which is also consistent with a small residue of organic matter from previous fish stocking.

After 9 months of fish being stocked at this site, a dramatic change in the distribution and total quantity of lipids can be seen in the chromatograms for the two cage sites (Fig. 6.4.5.1.2), and elevated levels at in-between cages sites, in contrast to the very minor changes observed at the reference site (Fig. 6.4.5.1.1). The distributions at the cage site sediments are now dominated by cholesterol, its 5α-stanol, α-tocopherol, sitosterol and its 5α-sterol plus several additional sterols including desmosterol. Also present in enhanced concentration are the 5β-stanols such as coprostanol indicative of extensive microbial reworking of the organic matter. Phytol is now a minor component, but its absolute abundance and ratio to the phytoplankton sterols is hardly changed so there is little variation in the natural marine organic matter component. Similarly, the higher-plant derived alkanols show only minor variations in distribution and their relative abundance is hardly changed so we can rule out changes due to variations in terrestrial inputs of organic matter. It is clear that the dramatic changes seen are the result of fish farming activities. Factors likely to be responsible for the enhanced cholesterol, desmosterol and α-tocopherol abundances are discussed in more detail below.

At 13 months, the distribution of non-saponifiable lipids at the reference site is hardly changed and the total abundances are also very similar to those at 0 and 4.5 months. This implies that there is not a pronounced seasonal variation in either the natural marine or terrestrial inputs. However, if we examine the chromatograms for the two cage sites at 13 months there is still a marked enhancement of the relative abundances of cholesterol, its microbial reduction products 5α-cholesterol and 5β-cholestanol (coprostanol) and α-tocopherol, although absolute amounts are considerably less than at the end of the stocking period (9 months) (Fig. 6.4.5.1.2). Contents at the in-between sites P1a and P2a were also still elevated at approximately 50% of the values seen at the cage sites (data not shown). It is apparent that organic residues from farming activities have been reduced by 3 months fallowing, but still dominate the extractable lipids present in the farm sediments.

6.4.5.2 Fatty acids in sediments at reference, in-between cages and under cages.

Total fatty acids were measured in surface sediments collected at two reference sites, and at the edge of cage sites P1a and P2a, and P1 and P2 at 0, 4.5, 9,10, 13, 17.5, 19, 22, 23, 24 and 25 months. Illustrative capillary gas chromatograms of the fatty acids as methyl esters for references and cages P1/P2 showing changes in the distributions from 0, 9 (end of stocking) and 13 months (4 months of fallowing) are shown in Figs 6.4.5.2.1 and 6.4.5.2.2 These chromatograms show a diversity of fatty acids reflecting the mixed marine and terrestrial sources also observed in the non-saponifiable lipids. General features of the distributions and a more detailed examination of the amounts of PUFA are discussed separately below.

Monounsaturated fatty acids 20:1, 22:1 and 24:1 were present in high contents in the fish faeces and feed. Elevated amounts were seen at the cage sites (Fig. 6.4.5.2.2) but not the reference sites (Fig. 6.4.5.2.1). Henderson et al. (1997) also reported that the principal monounsaturated fatty acid of their fish diet was 22:1(*n*–11). This comprised 14.7% total fatty acids in feed compared with 9% of the fatty acids in the surface sediment layer directly under the cages, but less than 5% of those at 50 m. The proportions of the other characteristic fatty acids of the diet, 20:1(*n*–9) and the PUFA 20:5(*n*–3) and 22:6(*n*–3), showed a similar decrease with distance. It may be that these monounsaturated fatty acids have potential for discriminating between fish feed and other marine sources of organic matter, but we have not explored this further since these long-chain components are also major constituents of zooplankton species such as copepods.

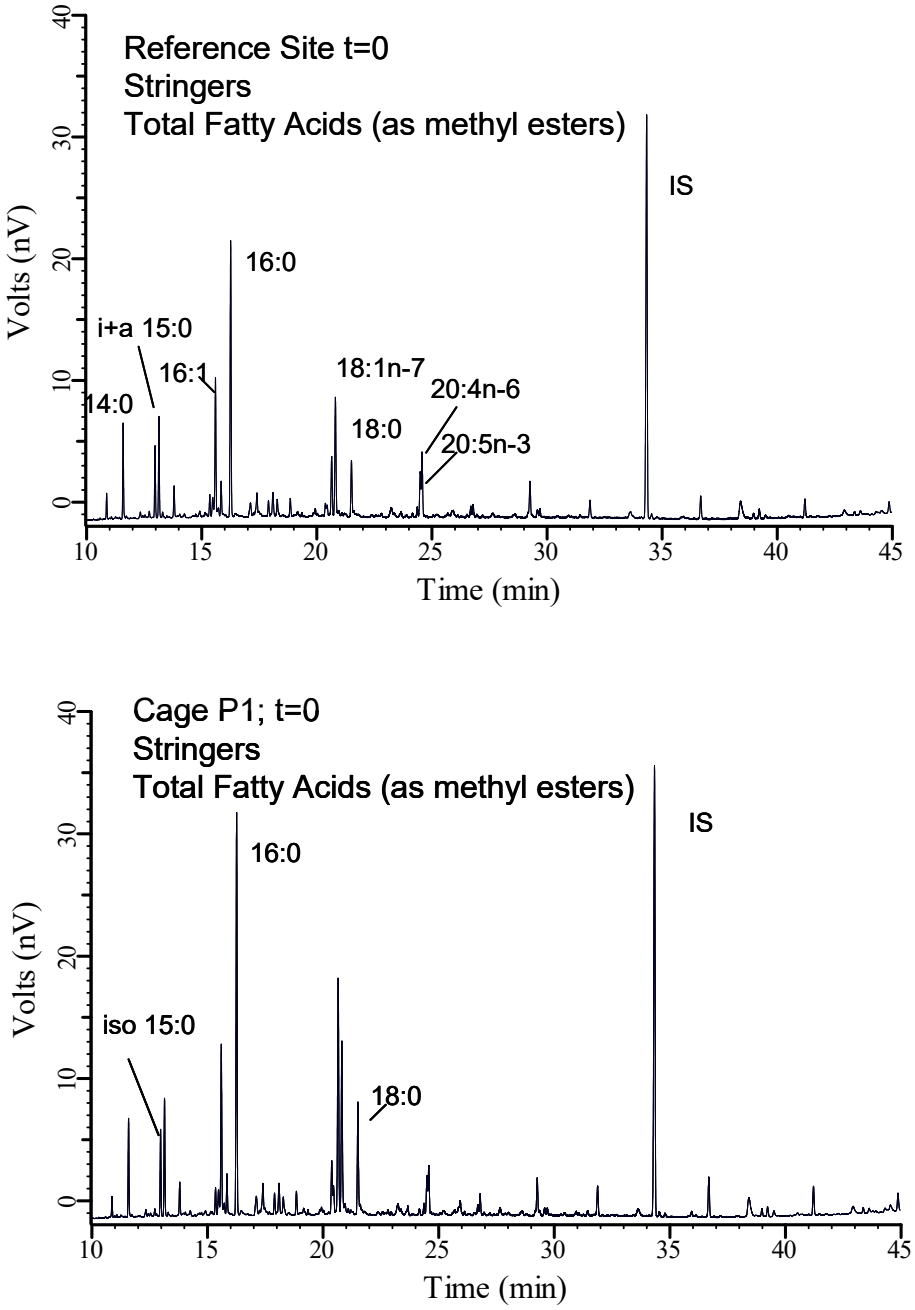


Fig. 6.4.5.2.1. Total fatty acids (after saponification) analysed as methyl esters in sediments at a) the reference site and b) at site P1 at 0 months. Fatty acids are given as number of carbon atoms: number of double bonds. The position of the double bond relative to the methyl end of the molecule is given as *n*-x.

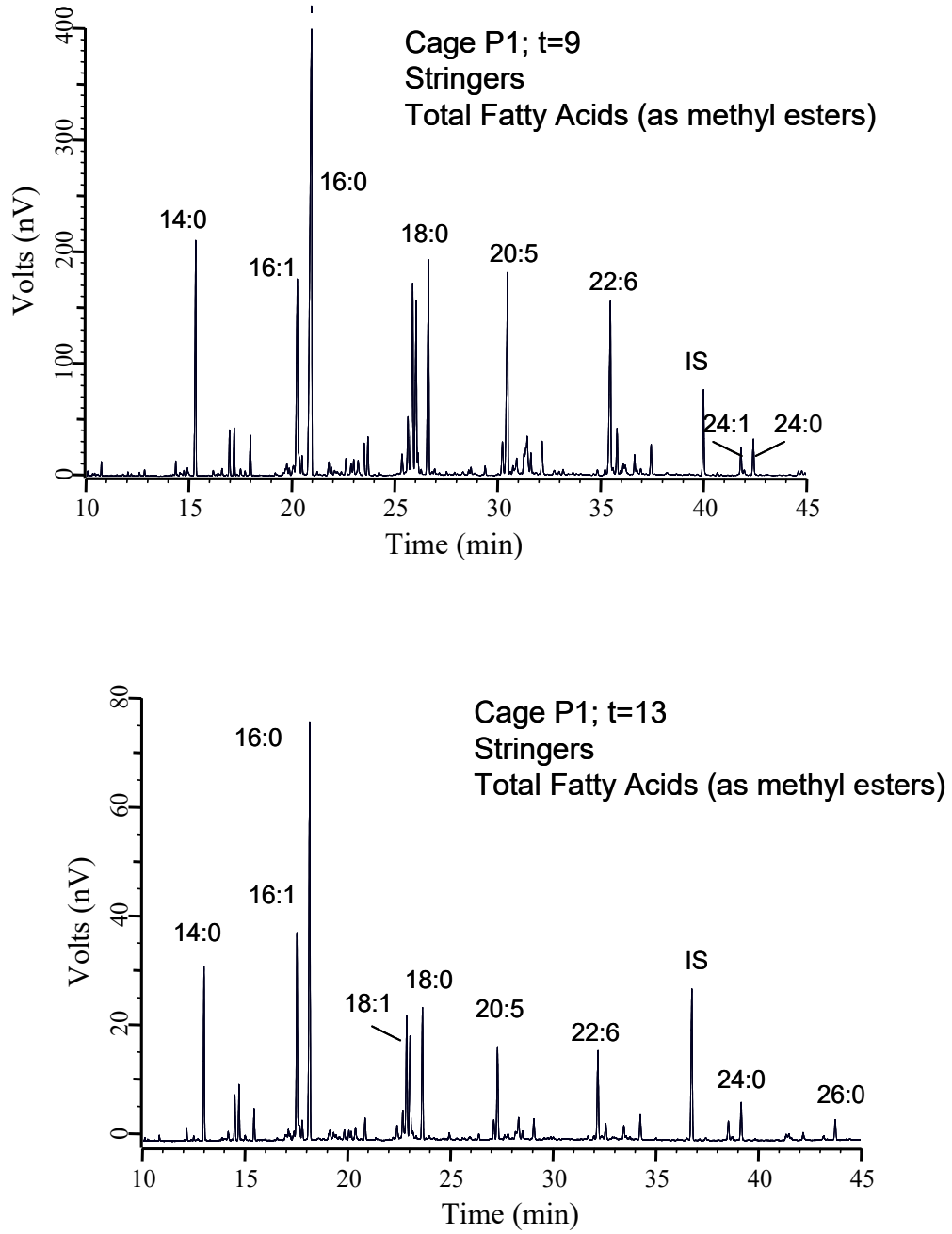


Fig. 6.4.5.2.2. Total fatty acids (after saponification) analysed as methyl esters in sediments at the cage site P1 at a) 9 months (end of stocking) and b) 13 months (4 months of fallowing). Fatty acids are given as number of carbon atoms: number of double bonds. The position of the double bond relative to the methyl end of the molecule is given as *n*-x. The chromatographic conditions used are different for the two samples and so the chromatograms are offset to facilitate comparison.

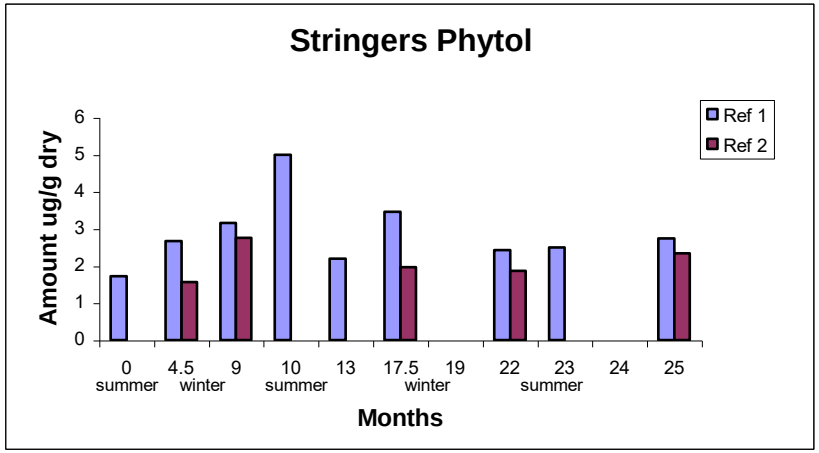
6.4.5.3 Temporal changes in specific biomarkers

6.4.5.3.1 Phytol Abundances

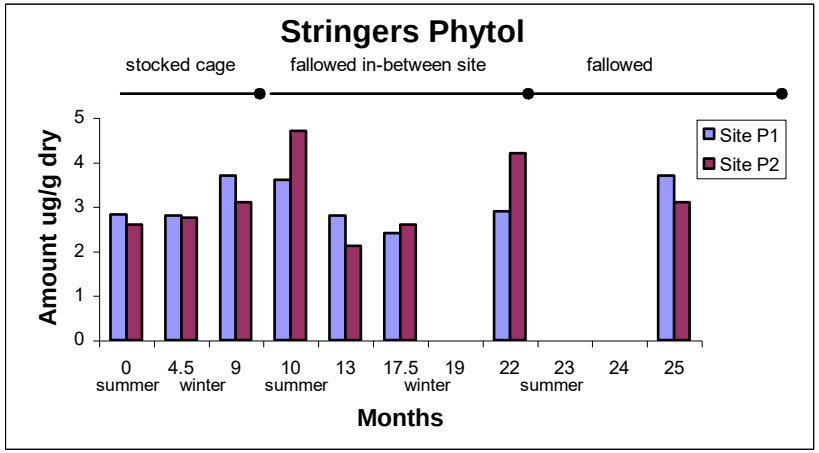
Phytol is the side-chain of chlorophyll a and thus its presence in sediments indicates a contribution from phytoplankton-derived organic matter to the sediments. It is not persistent in sediments and is broken down to a range of degradation products (Rontani and Volkman, 2003). Phytol was present in both of the reference sites at all sampling times. The amounts were generally low, with most falling in the range 2–3 μg g^{-1} (Fig. 6.4.5.3.1.1). Contents at reference site 2 were always slightly less than those at reference site 1. The highest value

recorded was about 5 μg g^{-1} in summer at reference site 1, but there were no clear seasonal trends despite expected higher phytoplankton abundances in the warmer months. Contents at the cage sites were within the same ranges and showed little variation throughout the year. This suggests that there is little or even no enhancement of the flux of phytoplankton-derived organic matter to the sediments within the farm lease either at the cage sites or in-between the cages.

a)



b)



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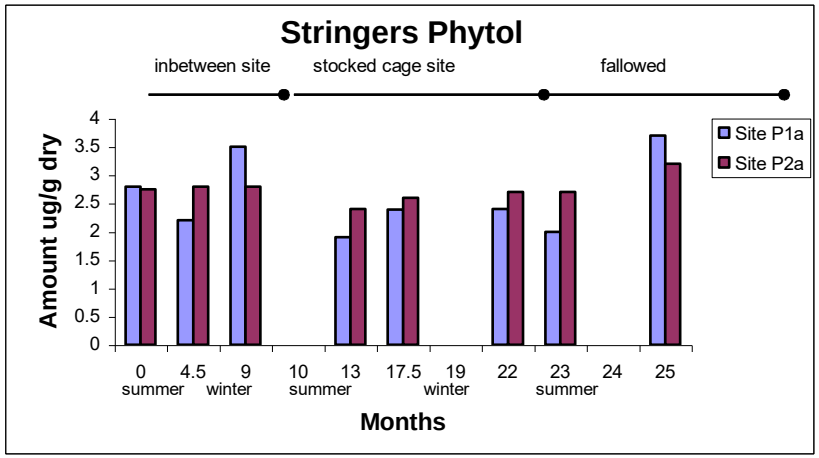
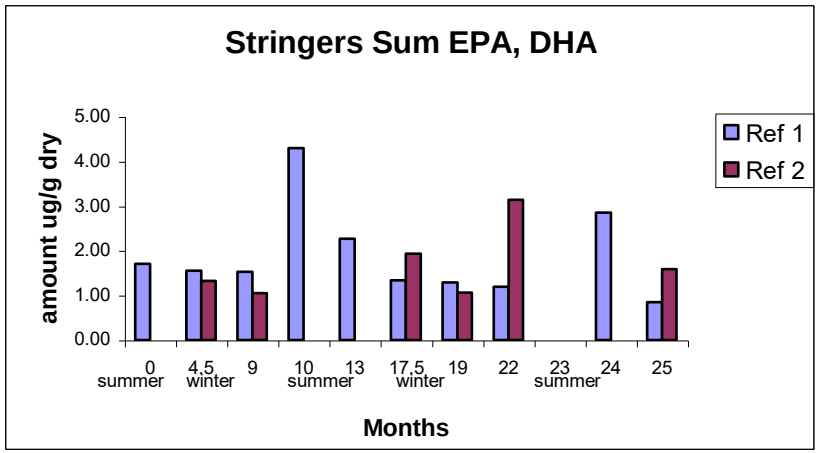


Fig. 6.4.5.3.1.1. Changes in the abundance of total phytol (after saponification) in sediments at the Stringers site; a) references, b) site P1/P2 and c) site P1A/P2A. Phytol is the side-chain of chlorophyll and is an approximate measure of chlorophyll abundance.

6.4.5.3.2 PUFA

The presence of polyunsaturated fatty acids (PUFA) in sediments is indicative of the presence of labile organic matter and /or living organisms. Small amounts would be expected in reference sediments and indeed this was found. The sum of the two main PUFA, 20:5n-3 and 22:6n-3, was generally less than 2 μg g^{-1} (Fig. 5.4.5.6). The highest value was recorded at reference site 1 at 9 months and this also had the highest content of cholesterol and phytol. Contents of these PUFA increased dramatically during stocking at both site P1 and P2, with amounts at the latter being approximately twice as high, reaching almost 200 μg g^{-1}. A rapid diminution occurred on fallowing, but values still remained elevated relative to the reference sites (Fig. 6.4.5.3.2.1) even after 13 months fallowing (22 months). A similar trend was observed for sites P1a and P2a, although the maximum reached was only 50 μg g^{-1} at P1a and 20 μg g^{-1} for P2a. Contents of these PUFA at the in-between sites also built up as stocking progressed reaching a value of 10 μg g^{-1} (Fig. 6.4.5.3.2.1).

a)



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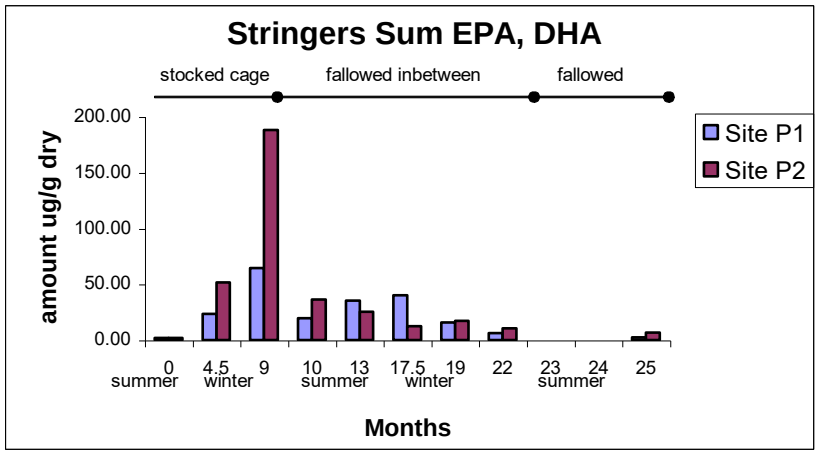

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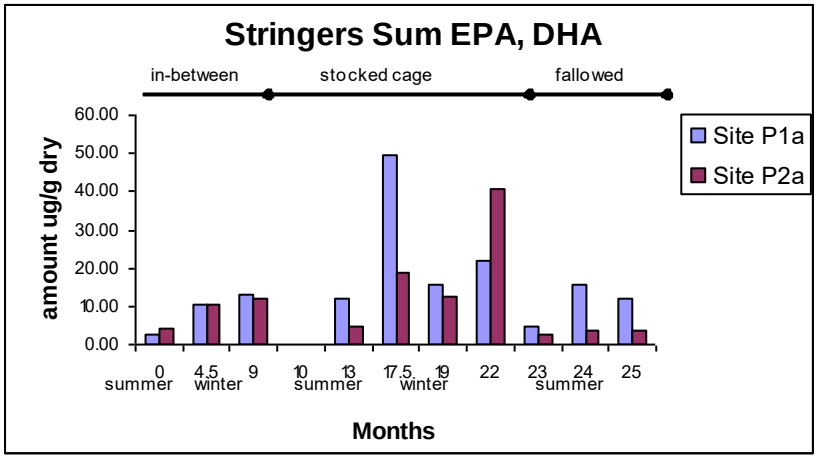


Fig. 6.4.5.3.2.1. Changes in the abundance of polyunsaturated fatty acids (PUFA) 20:5n-3 (EPA) and 22:6n-3 (DHA) (total amounts after saponification) in sediments at the Stringers site; a) references, b) site P1/2 and c) site P1A/2A.

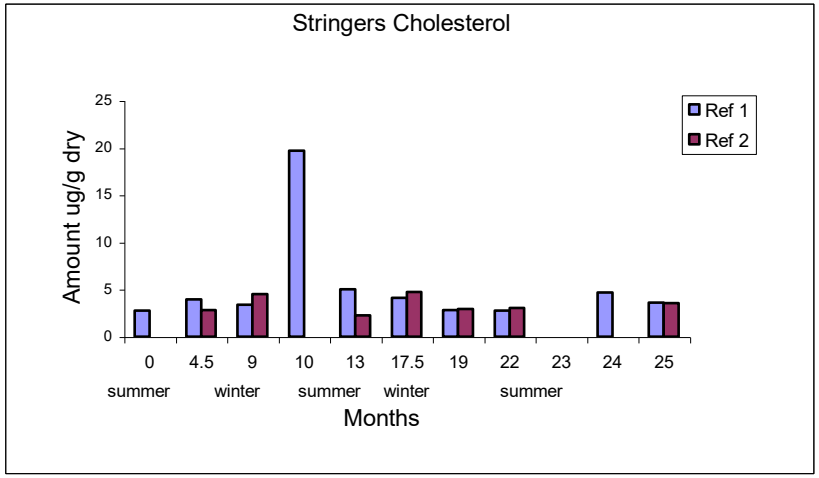
These PUFA can be derived directly from the fish feed, fish faecal pellets, phytoplankton and benthic fauna. The lipid component of the fish feed contained a higher level of polyunsaturated fatty acids, particularly 20:5n-3 and 22:6n-3, than lipid extracted from the sediments clearly pointing to additional sources. The build up of PUFA follows the trend in cholesterol (see below) (Fig. 6.4.5.3.3.1) and biomass of benthic fauna suggesting that animals are also a contributor of these PUFA. Given the very high abundances of these PUFA compared with the reference sites we can exclude phytoplankton as a significant source of these PUFA and this is corroborated by the low phytol abundances.

The presence of long-chain polyunsaturated fatty acids (PUFA; ca. 9% of total fatty acids) including the unusual PUFA 24:6n-3 at the reference sites prompted a search for their likely source. Analysis of a number of different species of benthic fauna isolated from these sediments revealed that the brittle star *Amphiura elandiformis* contained abundant PUFA including high contents of 24:6n-3, but much smaller amounts of the more common animal PUFA 22:6n-3. This is the first report of the lipid composition of this animal and a paper is being prepared for publication. Small amounts of 24:6(n-3) were found in *Nassarius nigellus* and *Nebalia longicornis,* but none was detected in *Capitella capitata* or *Neanthes cricognatha*. DMDS adducts were used to identify the positions of double bonds in the monounsaturated fatty acids. The major 20:1 isomer in the brittle star was identified as the rarely reported 20:1*n*-13 fatty acid which may be another useful marker for contributions of organic matter from this benthic animal to marine sediments.

6.4.5.3.3 Cholesterol

Cholesterol was present at the reference sites through the study period (Fig. 6.4.5.3.3.1) and was usually the major sterol present. Contents were generally less than 5 μg g^{-1} and similar at both reference sites except for an exceptionally high value of about 20 μg g^{-1} at site 1 in summer (9 months). This sample also had the highest content of phytol and so the elevated content of cholesterol likely reflects a larger population of animals responding to increase phytoplankton-derived food.

a)



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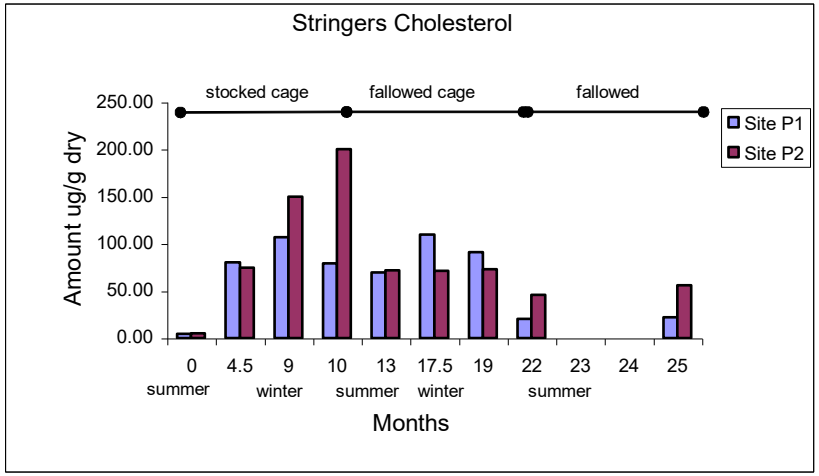

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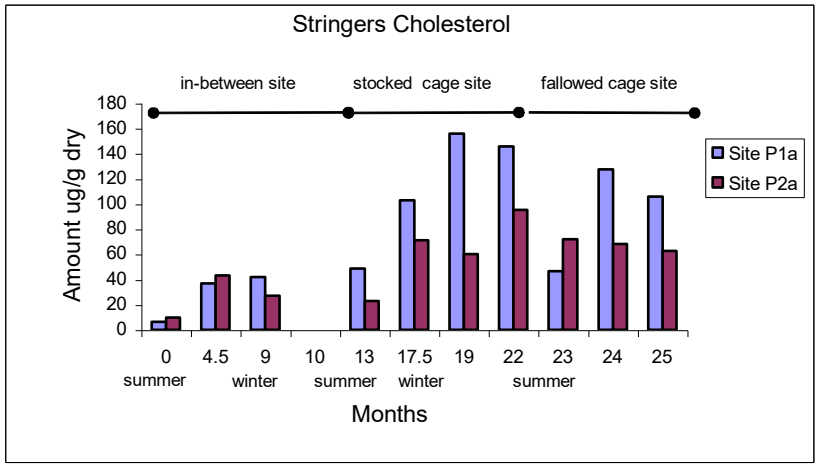


Fig. 6.4.5.3.3.1. Changes in abundance of cholesterol in sediments at the Stringers site; a) references, b) site P1/P2 and c)site P1A/P2A.

In marked contrast, the content of cholesterol in sediments at sites P1 and P2 increased dramatically during the stocking period reaching about 200 μg g^{-1} at site P2 at 10 months (i.e. more than 40 times the average background level). Contents at site P1 showed the same trend, but the values were lower only reaching 100 μg g^{-1} at 9 months and a decline at 10 months. Cholesterol contents dropped quickly after removal of the fish, but were still elevated (ca. 75 μg g^{-1}) at 13 months and remained at these levels throughout the fallowing period to 19 months. Even after 13 months fallowing (22 months), the contents were still much above the background levels at both sites, particularly at P2.

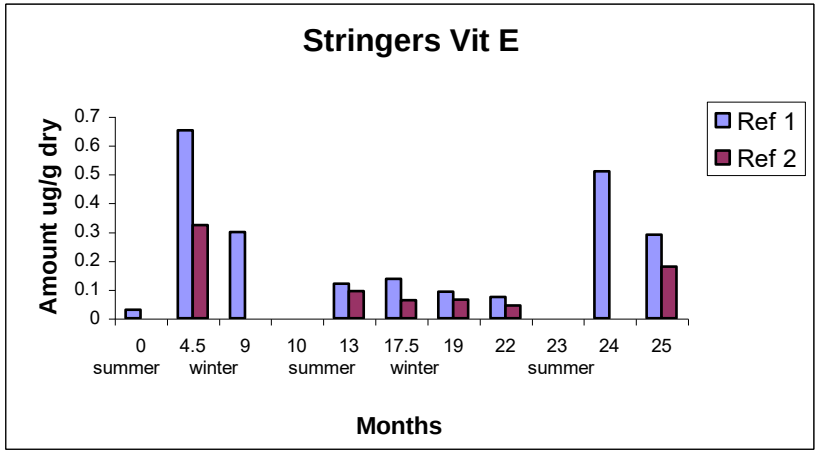
Cholesterol contents also increased at sites P1a and P2a during stocking of the adjacent cages consistent with transport of organic material from the cages to surrounding areas. During stocking, the P1a and P2a sites showed a similar trend of increasing cholesterol contents peaking at about 150 μg g^{-1} (i.e. somewhat less that at P2). However, cholesterol levels did not drop markedly during fallowing (Fig. 6.4.5.3.3.1) and remained above 50 μg g^{-1}.

Cholesterol is contributed to sediments from inputs from the water column (generally minor) and benthic fauna. Under the cages there are additional and major inputs from uneaten feed and fish faeces. It is not possible to discriminate between these various sources without reference to other biomarker data. The general trends, however, are consistent with the build-up of animal biomass under the cages as stocking progresses.

6.4.5.3.4 Vitamin E

High contents of vitamin E were found in sediments at the cage sites, up to 25 μg g^{-1} during the first cycle and 40 μg g^{-1} in the second (Fig. 6.4.5.3.4.1). These values are up to 20 times that observed at the reference sites. Vitamin E levels were very high in sieved sediment sample from which fauna had been removed indicating that (unlike desmosterol) it is not from fauna present under the cage. Vitamin E is added to the fish feed and small amounts are found in the faecal material. The fact that the content of vitamin E builds up in the sediment over the stocking cycle and is maintained during fallowing suggests that this compound is quite stable to biological degradation in this environment and hence can accumulate in sediments. This contrast with the Creeses site, where vitamin E was slowly degraded over time during fallowing.

a)



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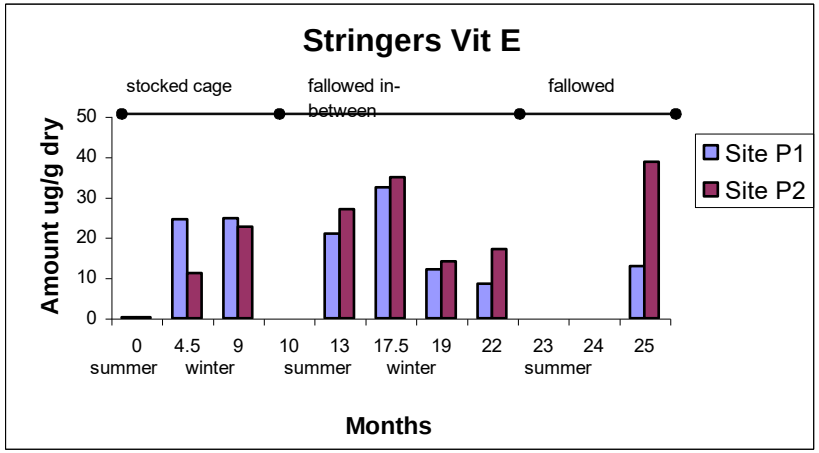

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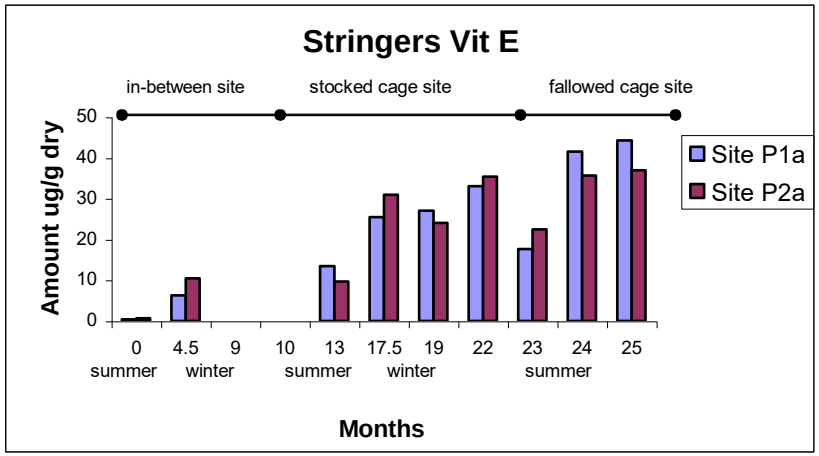
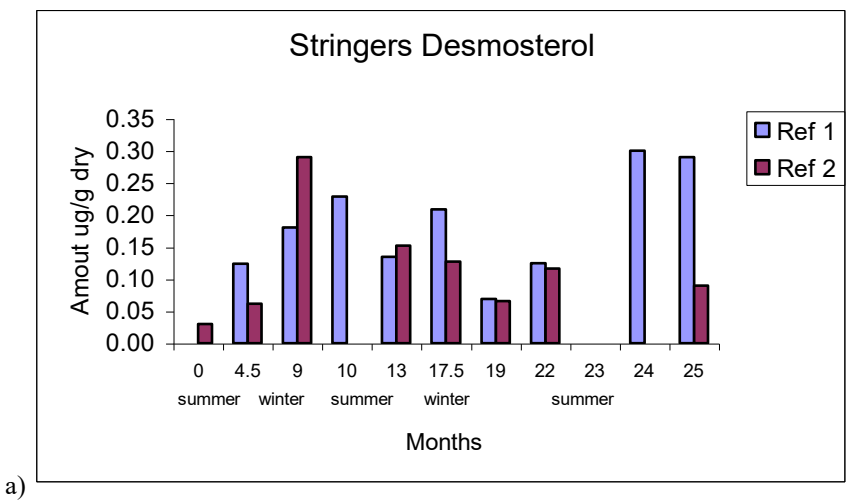


Fig. 6.4.5.3.4.1. Changes in the abundance of vitamin E (α-tocopherol) in sediments at the Stringers site; a) references, b) site P1/2 and c)site P1A/2A.

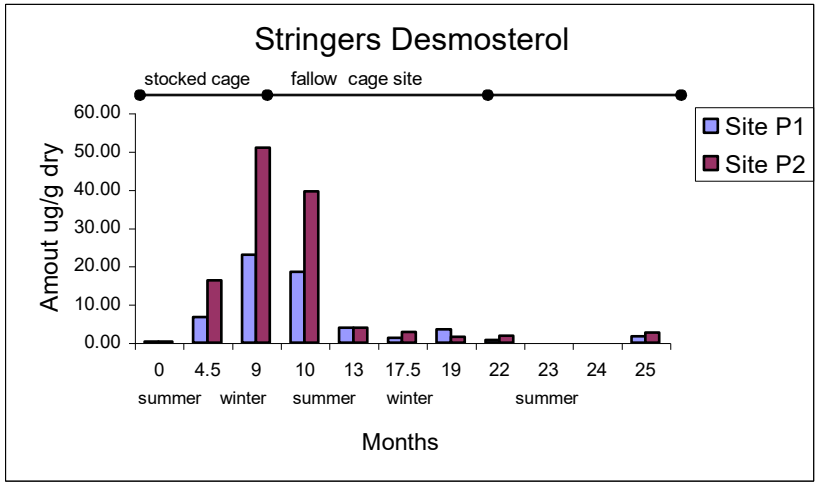
Tocopherols occur in a wide variety of marine microalgae (e.g. Brown et al., 1999) and in sediments (Brassell and Eglinton, 1983). It has previously been suggested that tocopherols can be preserved in sediments by binding to macromolecular organic matter and then become a significant source of C20 isoprenoids on thermal breakdown (Goossens et al., 1984).

Concerns have been raised that discharges of vitamins and therapeutics from aquaculture could have deleterious effects on aquatic populations (e.g. Wu et al. 1995). No vitamin E was detected in animal populations under the cages and this compound is considered to have extremely low toxicity (e.g. Thakur et al., 1996), so the levels of vitamin E seem unlikely to have any significant effect on the sediment biota.

6.4.5.3.5 Desmosterol



a)



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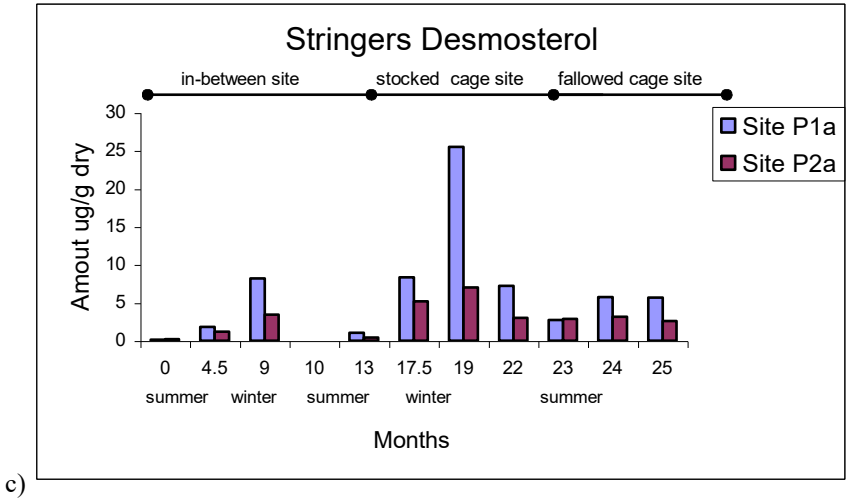


c)

Fig. 6.4.5.3.5.1. Changes in the abundance of the sterol desmosterol in sediments at the Stringers site; a) references, b) site P1/2 and c)site P1A/2A. Desmosterol is mainly derived from capitellids.

Desmosterol is an intermediate in cholesterol biosynthesis and is commonly found in sediments but usually at very low levels. Initial desmosterol contents in the cage sediments were very similar to the reference sites (0.1 to 0.3 µg g^{-1} at reference, and 0.1 to 1.0 at the 0 months cage sediments). Levels increased to 20 to 50 µg g^{-1} at 9 and 10 months during the first cycle and up to 25 µg g^{-1} at 6 months in the second cycle with levels dropping away after fallowing (Fig. 6.4.5.3.5.1).

A strong correlation was noted between the abundance of capitellids and the content of desmosterol (Fig. 6.4.5.3.5.2). The r^2 value after removal of two outliers was 0.891 with a standard error of estimate of 2.991. This correlation is a novel finding and provides strong support for the concept that measurement of particular biomarkers may provide an alternative to time-consuming enumeration of specific animals. However, in this case it is probably easier to directly measure the volume of capitellids (rather than counts) in highly impacted sediments rather than resort to more expensive chemical analysis. Note that the relationship does not have a zero-zero intercept, which indicates that there is a small contribution of desmosterol from other animal sources.

To test this correlation, we analysed the sterols in a sample of capitellids isolated from the sediment collected from cage site P2a at 17.5 and 19 months. Desmosterol and cholesterol were the two major sterols observed. In theory, by knowing the relationship between cholesterol and desmosterol in the capitellids, it should be possible to work out how much of the cholesterol is coming from these organisms in the sediments at Stringers.

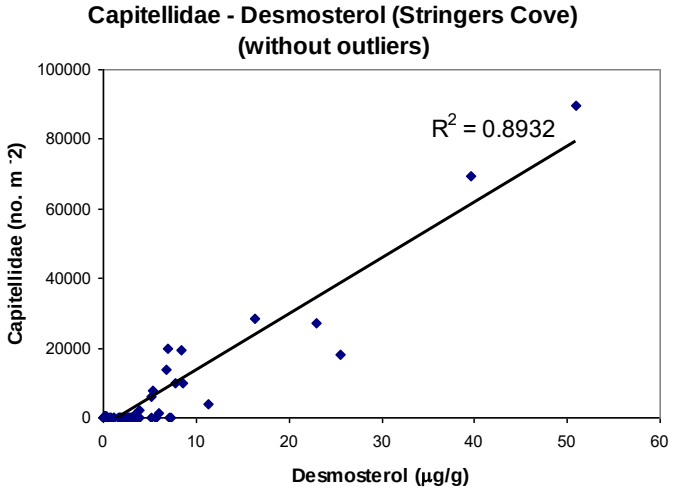


Fig. 6.4.5.3.5.2. Correlation of the numbers of Capitellidae with content of desmosterol.

The dominance of capitellids in highly organic-enriched sediments is commonly observed. For example, Karakassis et al. (2000) observed that *Capitella* cf. *capitata* dominated the macrofauna up to 10 m from the cages in two fish farms in the Mediterranean, whereas the third was dominated by the functionally similar *Protodorvillea kefersteini*. Seasonal variability in geochemistry and macrofauna was higher in proximity of the cages, and their work clearly demonstrated that the impacts of fish farming on benthos in the Mediterranean varies considerably depending on the characteristics of the farming site. Although desmosterol would clearly be a good indicator of capitellids and hence organic enrichment at Stringers Cove, we do not have biochemical information from other functionally equivalent species such as *Protodorvillea* and so this marker may not be appropriate in all environments.

6.4.5.3.6 Sitosterol as feed marker

Sitosterol (24-ethylcholesterol) is a marker for organic matter derived from terrestrial higher plants. Amounts at the Stringers reference sediments indicate a fairly constant background of terrestrial organic matter, except for an unusually high value at 10 months. As at the Creeses Mistake site, we noted a marked build-up of sitosterol at the cage-sites which we ascribe to accumulation of organic matter from feed and faeces. Sitosterol amounts decline slowly with fallowing suggesting that sitosterol might be a surrogate for the more refractory components of organic matter in this environment.

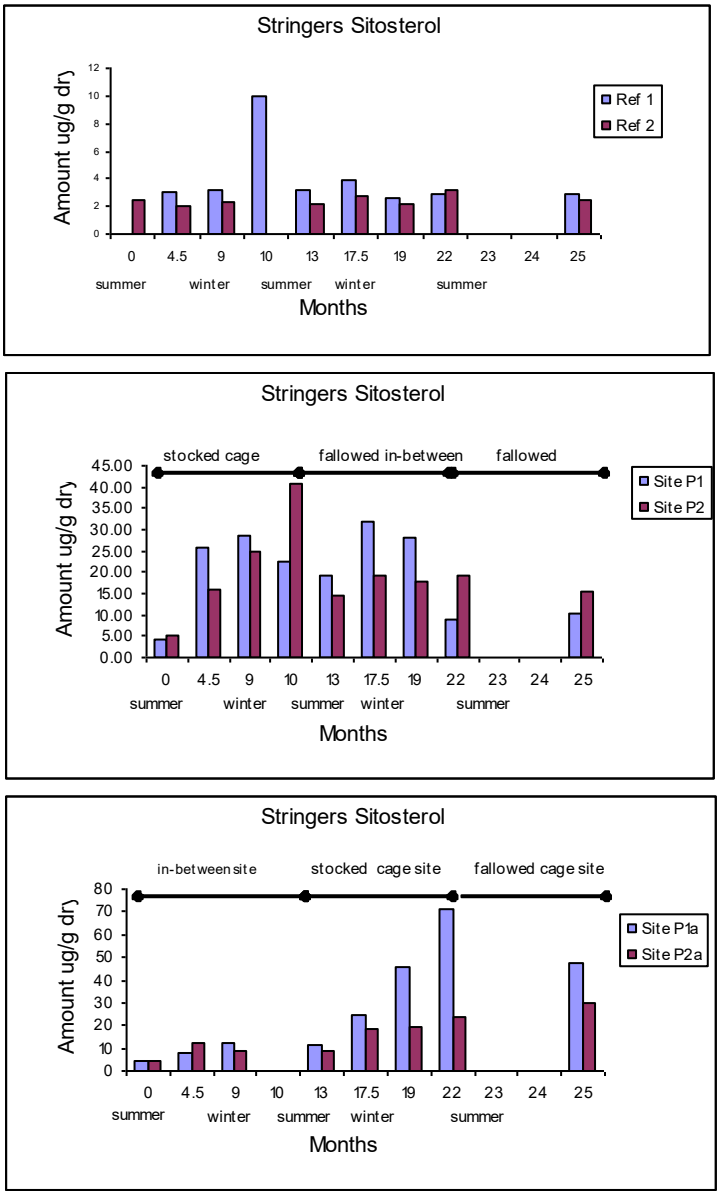


Fig. 6.4.5.3.6.1. Changes in the abundance of the sterol sitosterol (24-ethylcholesterol) in sediments at the Stringers site; a) references, b) site P1/2 and c) site P1A/2A. Sitosterol is found in higher plants, but here is it mainly derived from the feed pellets.

6.4.6 Sedimentation

Sedimentation rates were recorded at cage and reference locations, as well as a farm site (an empty position between operational cages) at Stringers Cove (Fig.6.4.6.1a). Background (reference) sedimentation rates were high (20 – 40 $gm^{-2}d^{-1}$) compared to studies overseas.

Sutherland *et al.* (2001) recorded sedimentation rates of approximately 18 $gm^{-2}d^{-1}$ adjacent to salmonid cages whilst Black (2001) reported rates of 11-33 $gm^{-2}d^{-1}$ directly beneath salmonid cages. Background sedimentation rates appear to follow a seasonal temporal cycle similar to that shown in the temperature data (Fig.6.4.6.1b).

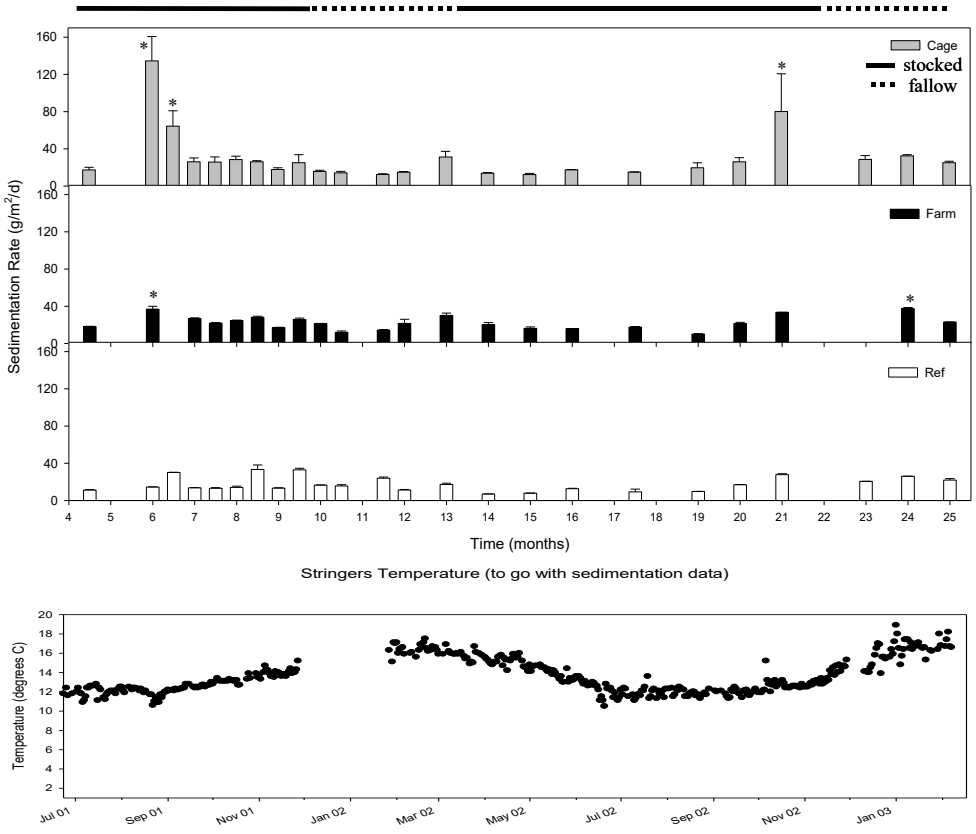


Fig. 6.4.6.1. a) Sedimentation rates at cage, farm and reference at Stringers Cove over both production cycles. Rates identified through Tukeys posthoc pairwise comparison as being significantly different from the reference are indicated by asterisk*. b) Temperature at 5m water depth over the same period of time.

There was no correlation between feed input and sedimentation rates. Sedimentation rates at the cages were variable but did not differ significantly from that at the reference for the majority of the study. Sedimentation rates were significantly higher at the study cage (position 2) than the reference on only three occasions (6, 6.5 and 21 months) during the first production cycle (time*site interaction; $F_{18,75}$=12.187, p=0.000) (Fig. 6.4.6.1a). Since these increases do not correspond to feed input and, with the exception of 6 months, were not observed in the farm data then it must be assumed that these high sedimentation rates are a function of some other farming activity, possibly deposition of fouling material either naturally or as a result of net cleaning. Farm sedimentation rates were only significantly higher than the references on two occasions (6 and 24 months) (time*site interaction; $F_{17, 71}$=13.079, p=0.000) and there is no clear explanation for why this should be. At both the cage and farm positions there appears to be quite a strongly seasonal response in sedimentation rate.

6.4.7 Sediment Respiration

Changes in benthic respiration (total CO_2 fluxes) were measured by Perran Cook (Uinversity of Tasmania/CSIRO) using sediment reactors incubated at *in-situ* temperatures in the laboratory. Sediment respiration increased dramatically towards the end of the stocking period in concert with the increase in labile organic matter (e.g. as reflected in PUFA contents) and biomass of

benthic infauna such as capitellids which colonised the sediment at this time (Fig. 6.4.7.1). The highest measured respiration rate exceeds most rates reported in coastal sediments and is likely enhanced by bioirrigation driven by the high numbers of capitellids.

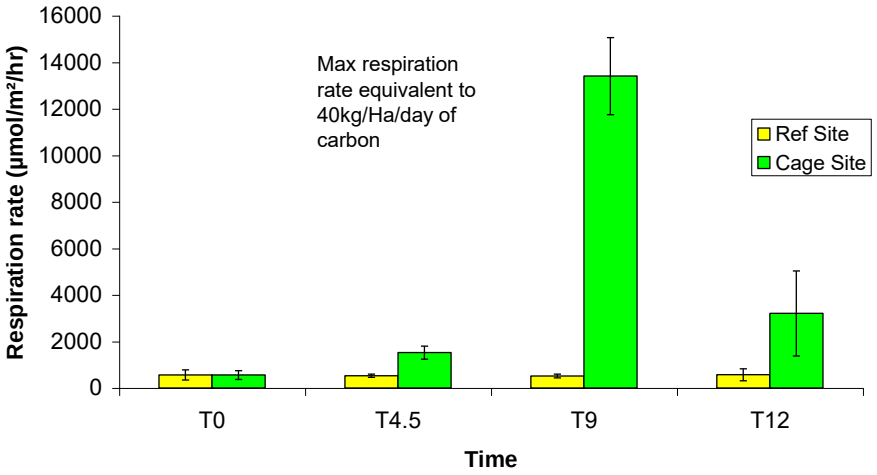


Fig. 6.4.7.1. Sediment respiration rates at Stringers site from the beginning of cage stocking (T0 months) until after 3 months fallowing (T12 months) (Perran Cook; unpublished data).

The measured respiration rates indicate removal of up to 40 kgC $Ha^{-1}day^{-1}$ at the end of the stocked period. Measurement of sedimentation rates at a cage site indicated that at the same point in the stocking cycle, 12 g of organic matter (as determined by loss on ignition) were being deposited per m^2 per day. Using a published relationship between LOI and organic carbon content of approximately 3:1 (Leong and Tanner, 1999), this corresponds to 4 gC $m^{-2}day^{-1}$ which equates to a deposition of 40 kgC $Ha^{-1}day^{-1}$. Thus, the rate of respiration matches the rate of deposition. This supports our suggestion that the sediment fauna is "pre-adapted" to deal with relatively high levels of organic carbon and is in agreement with the fact that no significant carbon accumulation in sediments could be detected at this site. This also has implications for sites where an increase in organic carbon can be measured as it indicates lower rates of re-mineralisation through sedimentary fauna.

Prior to 9 months there was a net efflux of nitrogen from the sediments under the cage, as NO_3 and N_2 (Fig. 6.4.7.2). This indicates that nitrogen deposited as part of the organic matter is successfully re-mineralised through nitrification and denitrification, as at the reference sites. However, towards the end of the stocking cycle, nitrogen is predominantly lost from the sediments as more biologically available ammonia and the sediments become a net sink for nitrate (Fig. 6.4.7.3). Denitrification rates also increase, to a point where this process is removing approximately ten times the amount of nitrogen entering the sediment as nitrate (Fig. 6.4.7.4). This suggests that there is a small proportion of the sedimentary ammonia undergoing nitrification and subsequent denitrification. The total amount of nitrogen lost via this route is still only 5% that lost directly as ammonia, which is the converse of that seen in normal "healthy" sediments and observations at the reference site, presumably due to a reduction in oxygen associated with the high respiration rates.

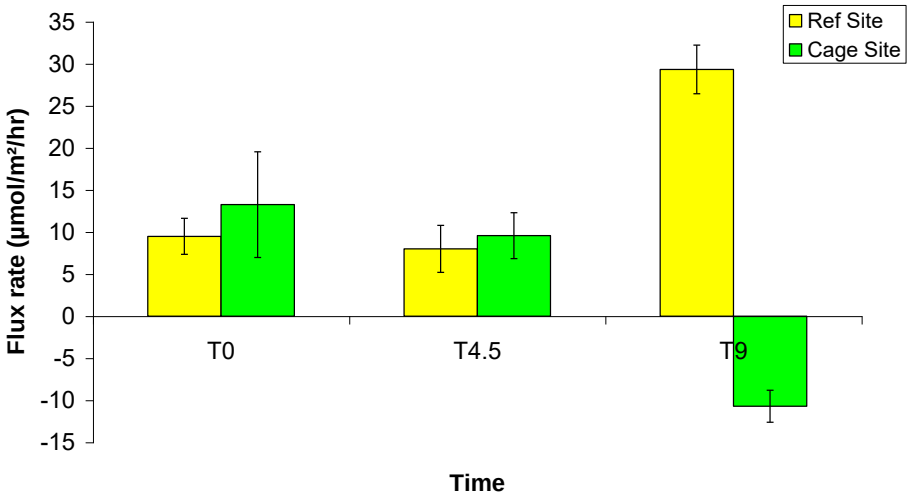


Fig. 6.4.7.2. Sediment nitrate fluxes at Stringers over the period from cage stocking (T0 months) until destocking (T9 months). (Perran Cook; unpublished data).

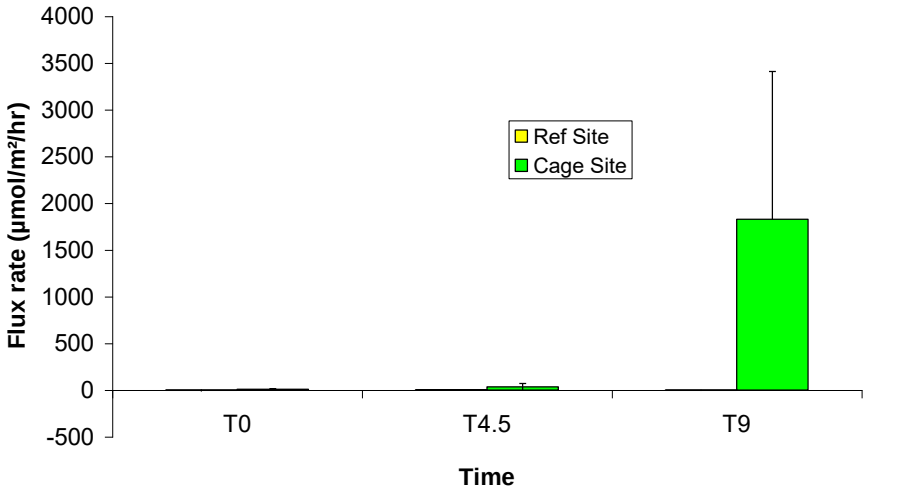


Fig. 6.4.7.3. Sediment ammonia fluxes at Stringers site over the period from cage stocking (T0 months) until destocking (T9 months). (Perran Cook; unpublished data).

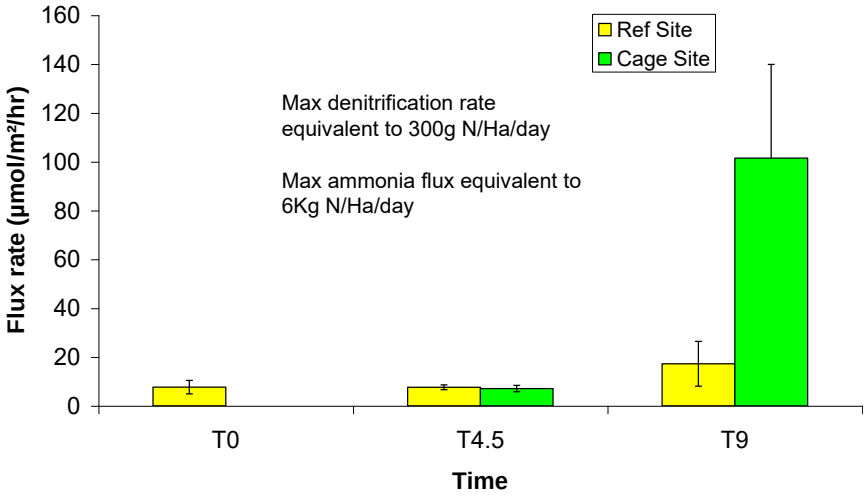


Fig. 6.4.7.4. Sediment denitrification rates at Stringers over the period from cage stocking (0 months) until destocking (9 months). (Perran Cook; unpublished data).

6.4.8 Discussion

The farm operations did not affect either the organic matter levels in the sediments or the grain size distribution at Stringers Cove. Measurement of organic content and evaluation of change in the sediment grain size, particularly the silt/clay fraction, have previously been shown to be strongly related to level of organic enrichment (Hall et al,. 1990, Holmer, 1991). In this instance there was no correlation with either the farm production information or with the findings of the benthic infaunal community assessment. Sedimentation rate would be expected to increase markedly in association with farm operations but in the present study this too was a very poor measure of environmental condition, which did not correlate with farm inputs and instead seemed to reflect seasonal variability.

Physico-chemical parameters, such as redox and sulphide, are regularly employed in monitoring programmes to determine sediment condition. Regular measurement of redox potential is currently a requirement in both the baseline and ongoing environmental monitoring programmes for salmonid farms in Tasmania. As was the case at Creeses Mistake both redox potential and sulphide concentration were good indicators of deteriorating sediment conditions, particularly those associated with major impacts, but were very poor indicators of recovering sediment stages (Fig. 6.4.8.1). The correlation between redox potential and sulphide concentration described by Wildish et al (2000) was supported by the present data when environmental conditions were deteriorating, although the absolute levels were considerably reduced. However, this relationship broke down completely under recovery conditions and it is suggested that the chemistry underpinning the degradation process is quite different to that in recovery. It was determined that 3cm was the optimal depth at which to measure redox potential and sulphide concentration to ensure an accurate representation of the overall sediment condition.

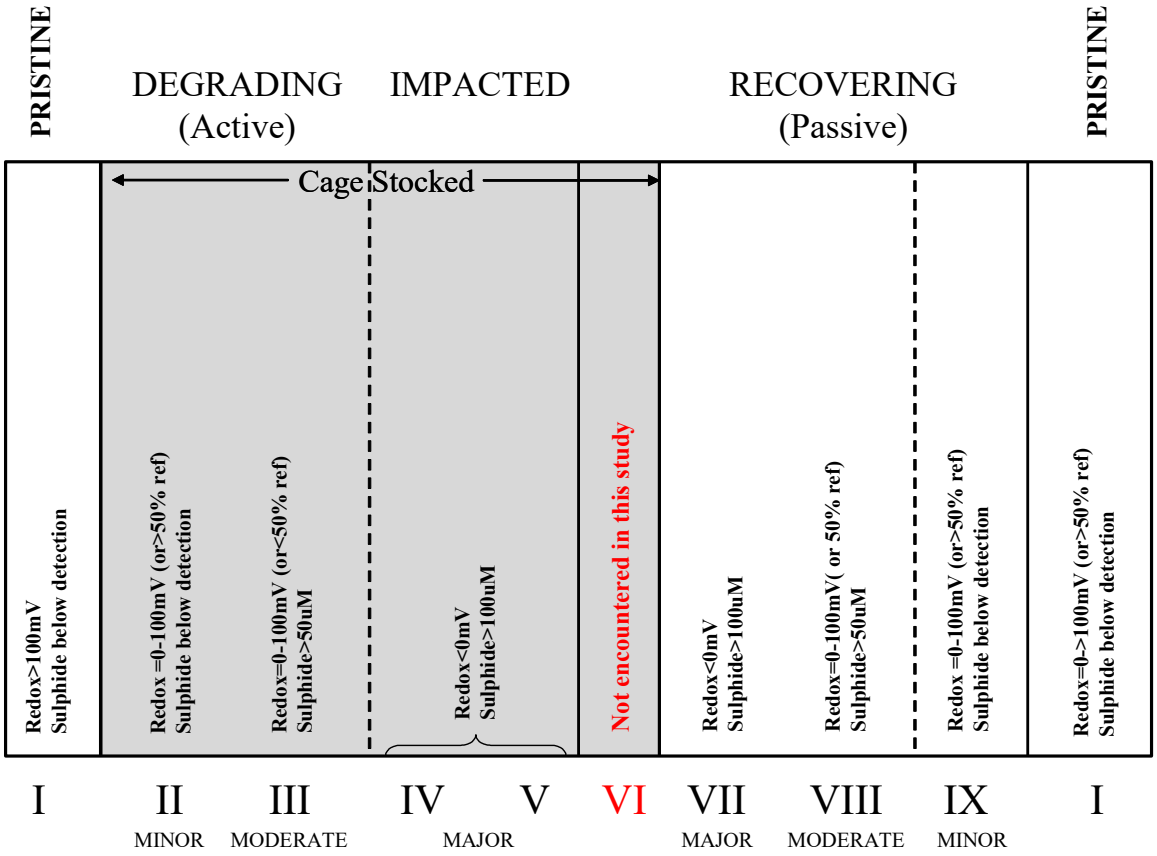


Fig. 6.4.8.1. Characterisation of impact/recovery stages based on sediment redox potential and sulphide concentration.

Oxygen penetration was always deeper at the reference sites than at the farm sites, irrespective of whether the farm site was stocked or being fallowed. This shows that farming changes the dynamics of oxygen cycling in the sediments associated with mariculture. There appears to be a strong influence of surrounding cages on what happens to oxygen at a particular cage site, irrespective of whether that cage was being farmed or not. This suggests either that the dispersion of labile organic carbon from stocked cages is greater than had been initially predicted, or that benthic invertebrates are able to rapidly bury organic carbon, but their continual bioturbation of the sediments enables a slow feeding of labile organic carbon to microbes at the sediment surface. Thus, aerobic respiration continued to deplete oxygen. It is not possible with the current dataset to conclude which of these hypotheses is correct.

Analysis of organic components correlates with the level of impact. In both production cycles, levels of bacterial fatty acids, cholesterol and polyunsaturated fatty acids (PUFA) increased to a maximum at the end of the stocked period, but decreased again after 3 months fallowing. However, levels after fallowing were still elevated relative to the reference conditions. Cages stocked in the first production cycle, and fallowed over the second cycle, also showed an increase in fatty acids, cholesterol and PUFA levels, suggesting an effect from the surrounding operational cages. The C_{27} sterol desmosterol (cholesta-5,24-dien-3β-ol) is shown to be a good indicator of the biomass of capitellids.

The two cages studied for biomarkers behaved differently depending on their position within the farm. Cage site P2 was stocked in the first cycle 2001–2002 and is situated on the southern end of the farm. All biomarkers showed large increases in concentrations during the stocking cycle up to 9.5 months. The cage site was then fallowed and became an in-between site for 2002–2003. Concentrations of most biomarkers then dropped by more than 50% within 2 months but not to the levels at 0 months or at levels seen at the reference sites. Amounts rose

slightly at the end of the second stocking cycle (22 months) when this site was an in-between site.

Site P2A was an in-between site in 2001 and then a stocked cage site in 2002–2003. Low levels of biomarker were seen at 0 months but amounts increased during 2001 while an in-between site, especially cholesterol (possible washing of excess feed into this area adjacent to the cages or an increase in benthic fauna). The amounts of all biomarkers increased substantially (typically doubling) during the stocking cycle in 2002

Cage site P1 was stocked in the first cycle of 2001-2002 but behaved very differently from cage site P2, most likely due to its position within the farm between two other cages. All biomarker concentrations increased dramatically during the first stocking up to T=9 months. Biomarker levels decreased during the first two months of fallowing, and then amounts returned to high levels during 2002 when this was an in-between site, suggesting a large influence from the adjacent cages.

Site P1A was an in-between site in 2001 and then stocked in 2002-2003. Low levels of biomarkers were seen at 0 months, and there were small increases in amounts during 2001 while it was an in-between site especially with cholesterol. The amounts of all biomarkers increased during the stocking cycle 2002 from in-between site levels of 40 $\mu g\ g^{-1}$ cholesterol to 140 $\mu g\ g^{-1}$ at the end of stocking.

Both reference sites at Stringers showed consistent behaviour over the two stocking cycles, despite their different sediment type, with very small increases and decreases due seasonal variation. Biomarker concentations were typically 40-50 times less than at the cage sites and 10 times less than at in-between sites during stocking.

6.5 Microbial ecology

6.5.1 Community ecology

6.5.1.1 Bacterial Counts

Mean microbial direct counts ranged from $2.2 \times 10^8 \pm 2.1 \times 10^7$ to $2.6 \times 10^8 \pm 1.5 \times 10^7$ at reference sites and from $2.42 \times 10^8 \pm 1.7 \times 10^7$ to $5.37 \times 10^9 \pm 4.2 \times 10^8$ at cage sites. Results of the ANOVA performed are presented in Table 6.5.1.1.1 and indicate a significant interaction between time and treatment.

Table 6.5.1.1.1. ANOVA table for mean counts at Stringers Cove, first 12 month cycle.

Tests of Between-Subjects Effects

Dependent Variable: LOGCOUNT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42.038[a]	23	1.828	117.584	.000
Intercept	8516.748	1	8516.748	547906.6	.000
TREATMEN	9.343	1	9.343	601.058	.000
TIME2	17.611	3	5.870	377.647	.000
DEPTH	.352	2	.176	11.329	.000
TREATMEN * TIME2	10.442	3	3.481	223.929	.000
TREATMEN * DEPTH	1.284E-04	2	6.421E-05	.004	.996
TIME2 * DEPTH	3.414E-02	6	5.690E-03	.366	.899
TREATMEN * TIME2 * DEPTH	3.100E-02	6	5.167E-03	.332	.919
Error	1.679	108	1.554E-02		
Total	9857.490	132			
Corrected Total	43.717	131			

a. R Squared = .962 (Adjusted R Squared = .953)

Bacterial numbers increased as farming progressed during the first production cycle and decreased during the fallowing period (Fig. 6.5.1.1.1.). Bacterial numbers did not, however, return to pre-stocking or reference levels by the end of the 3-month fallowing period. Bacterial numbers did return to reference levels over the following 12 months, during which time no cages were over these sites. Depth at which samples were taken within the sediment had a significant effect on bacterial numbers. Numbers at the surface were significantly higher (1.9 10^8) ($p<0.05$).

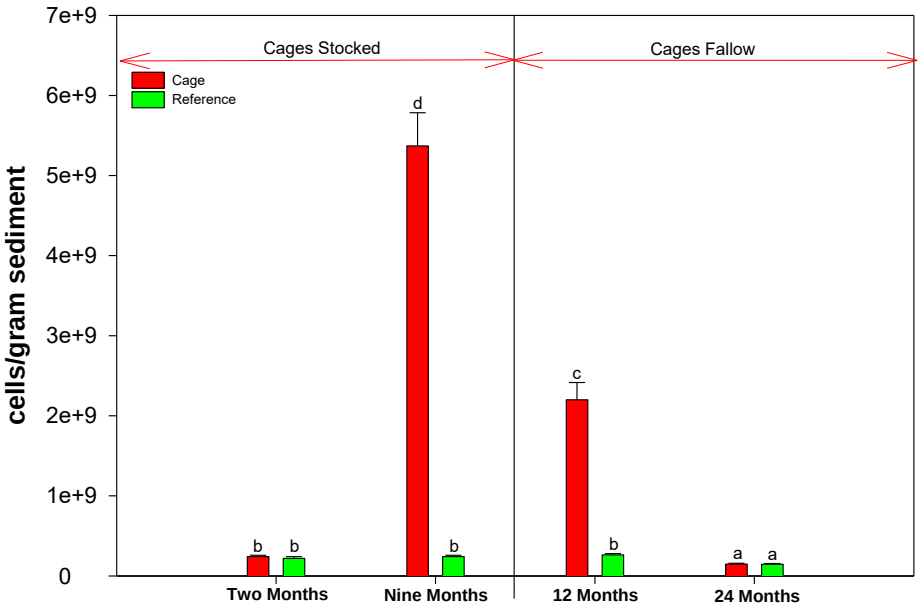


Fig. 6.5.1.1.1. Mean bacterial numbers (cells/gram) at cage and reference sites during the first production cycle. Treatments sharing a common superscript are not significantly different.

During the second 12 months the original sites were fallowed and two more farmed cages were sampled. ANOVA results from these sites are presented in Table 6.5.1.1.2. Bacterial numbers increased with farming and decreased with fallowing (Fig. 6.5.1.1.2), but numbers at the cage sites were not significantly different from those at reference sites at the end of the fallow period. Again, bacterial numbers were higher in the surface sediments($1.6 \times 10^9 \pm 3.6 \times 10^8$, 0-2mm; $9.7 \pm 2.4 \times 10^8$, 5-10mm) of the core ($p<0.05$).

The lack of differentiation between the layers may be an artefact of our imprecise means of sectioning the cores, the surfaces of which were often uneven. The middle (2-5mm) layer acted more as a buffer to separate the other two layers than as a layer in its own right. This result suggests that bacterial growth rates are determined primarily by the availability of nutrients (Bastviken et al., 2001; Blume et al., 2002) and not the pathways available for growth. It has been previously reported that waste from fish farms contains highly labile organic matter (McGhie et al., 2000). This organic matter is first available to microbes at the sediment surface, which utilise what they can and pass on less labile matter to the underlying sediments. If metabolic pathway was of primary importance, then as the sediment layers become more homgeneously anoxic, numbers should be more evenly distributed throughout the core. This was not the case.

Table 6.5.1.1.2. ANOVA table for mean counts at Stringers Cove, during the second 12 month production cycle.

Tests of Between-Subjects Effects

Dependent Variable: LOGCOUNT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20.438[a]	17	1.202	50.869	.000
Intercept	7563.465	1	7563.465	320031.5	.000
TREATMEN	5.074	1	5.074	214.682	.000
TIME2	4.841	2	2.420	102.409	.000
DEPTH	.422	2	.211	8.926	.000
TREATMEN * TIME2	9.829	2	4.914	207.939	.000
TREATMEN * DEPTH	9.868E-03	2	4.934E-03	.209	.812
TIME2 * DEPTH	4.303E-02	4	1.076E-02	.455	.768
TREATMEN * TIME2 * DEPTH	.123	4	3.067E-02	1.298	.277
Error	2.103	89	2.363E-02		

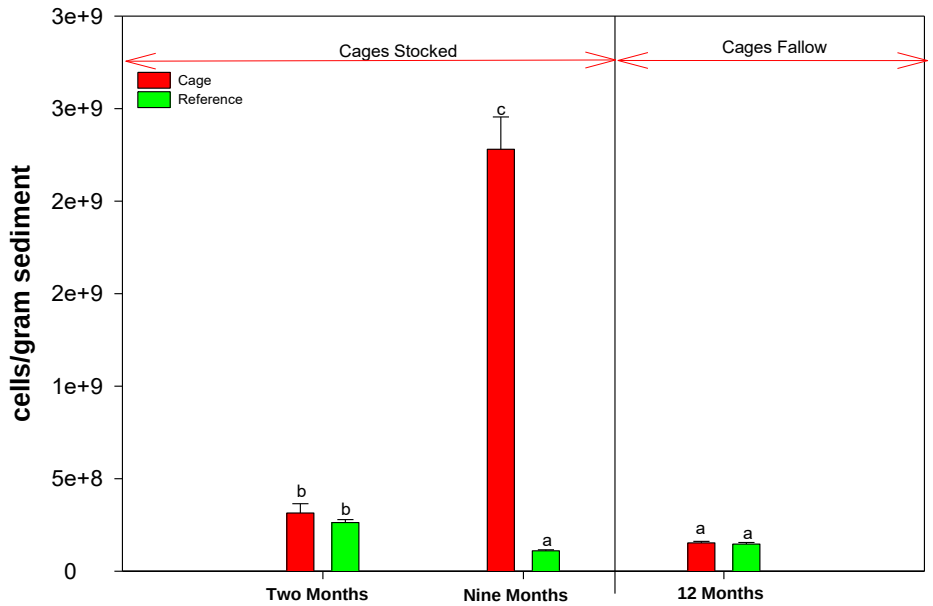


Fig. 6.5.1.1.2 Mean bacterial numbers (cells/gram) at cage and reference sites during the second production cycle. Treatments sharing a common superscript are not significantly different. Bars represent standard errors of the mean (n = 2.)

6.5.1.2 Bacterial Biomass

As at Creeses Mistake bacterial biomass increased significantly during the stocking period and decreased during the fallow period (Table 6.5.1.2.1). Biomass at cage sites was not as high during the second twelve-month production period.

Table 6.5.1.2.1. Bacterial biomass (µgC/gram sediment) at Stringers Cove over both production cycles

CYCLE 1	2 MONTHS	9 MONTHS	12 MONTHS	24 MONTHS
Cage	20.82±1.9	476.37±36.4	234.78±25.77	11.22±0.85
Reference	11.26±1.15	24.38±1.9	27.76±2.7	10.60±0.77

CYCLE 2	0 MONTHS	9 MONTHS	12 MONTHS
Cage	28.09±3.2	166.53±21.7	13.57±0.92
Reference	27.76±2.7	9.45±0.78	10.60±0.77

6.5.1.3 Beggiatoa counts

Stringers Cove farm had high *Beggiatoa* numbers associated with stocked cages at 0 months, which suggests that these positions were influenced by neighbouring cages (Fig. 6.5.1.3.1). This agrees with the findings on the depth of the oxic zone under fallowed cages. Counts early in the fallow period remained high, suggesting that the effects of stocking persisted. The zero count at 9 months in stocked cages probably occurred because of outgassing of anaerobic gases such as H_2S. Vacuolate *Beggiatoa* have a capacity to store nitrate and anaerobically respire sulphide, producing ammonia in the course of this metabolism, rather than dinitrogen gas. The result is, like the lack of denitrification, that nitrogen remains available to the biota and may contribute to eutrophication.

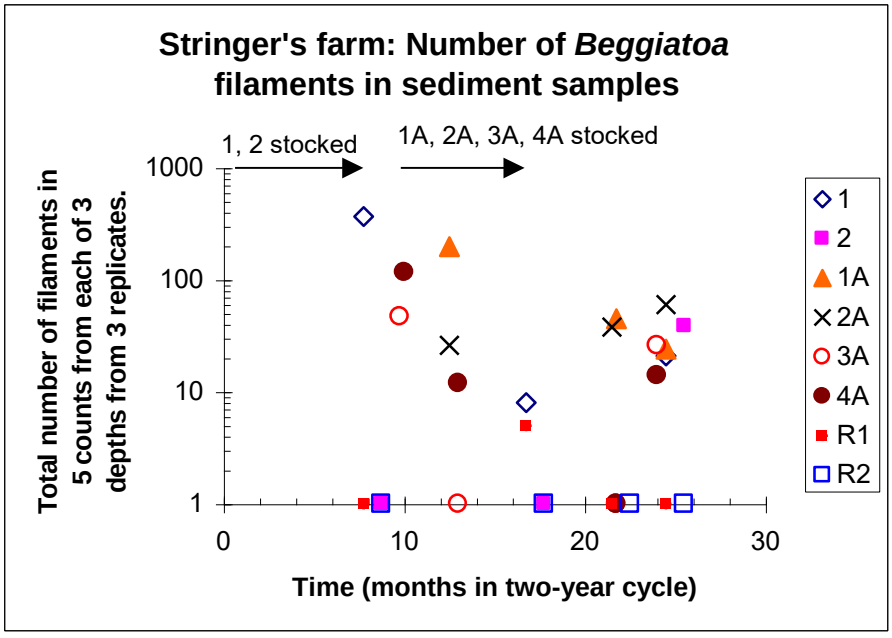


Fig. 6.5.1.3.1. *Beggiatoa* filament counts at Stringers Cove over a two years of production.

6.5.2 Polar Lipid Fatty Acids and Ether Lipids

6.5.2.1 Polar Lipid Fatty Acids (PLFA)

PLFA compositional (percent and absolute basis) data are presented in Tables 1 and 2 respectively (Appendix 4). Seventy-four fatty acids were identified in one or more of the samples (Fig. 6.5.2.1.1, Table 1 - Appendix 4). The results reported below will be separated into specific classes of fatty acids, with selected components also discussed according to microbial groupings.

Total PLFA content generally increased approximately twenty fold over the 9 month stocking period (samples T0, T4.5 and T9) (see also Fig. 6.4.5.2.1). The PLFA content at 13 months was lower than 4.5 months. Total PLFA content at the reference sites and in the two cage samples at 0 months was similar to values observed for Port Phillip Bay (PPB) sediments during an environmental study in the mid 1990s (O'Leary et al. 1994). The 4.5 and 9 months PLFA concentrations were markedly higher than observed in the PPB-wide survey, and for sediments from other pristine coastal environments.

Using signature PLFA, microbial biomass can be expressed in two forms (Table 3 (Appendix 4) - mg g^{-1} and cells g^{-1}). Biomass was similar for the cage at 0 months and all the reference site samples when expressed in either format. In comparison, cell abundances determined by microscopic methods were in the 10^9 range (Bissett et al., 2002), similar to values estimated from signature PLFA results for the 0 months cage site and reference site samples (Table 3 - Appendix 4). As noted for PLFA content (μg g^{-1} basis), biomass also increased markedly at 4.5 and 9 months in the cage samples reaching 2.4-3.7 x 10^{10} (Table 3 - Appendix 4). These biomass estimates presently include input from faunal sources (see PUFA section below), as the sediments were not sieved.

Faunal input can be estimated, and therefore corrected for, assuming that most of the PUFA is derived from benthic marine fauna. This approach may be worth testing in future analyses, and or treatment of the current data set.

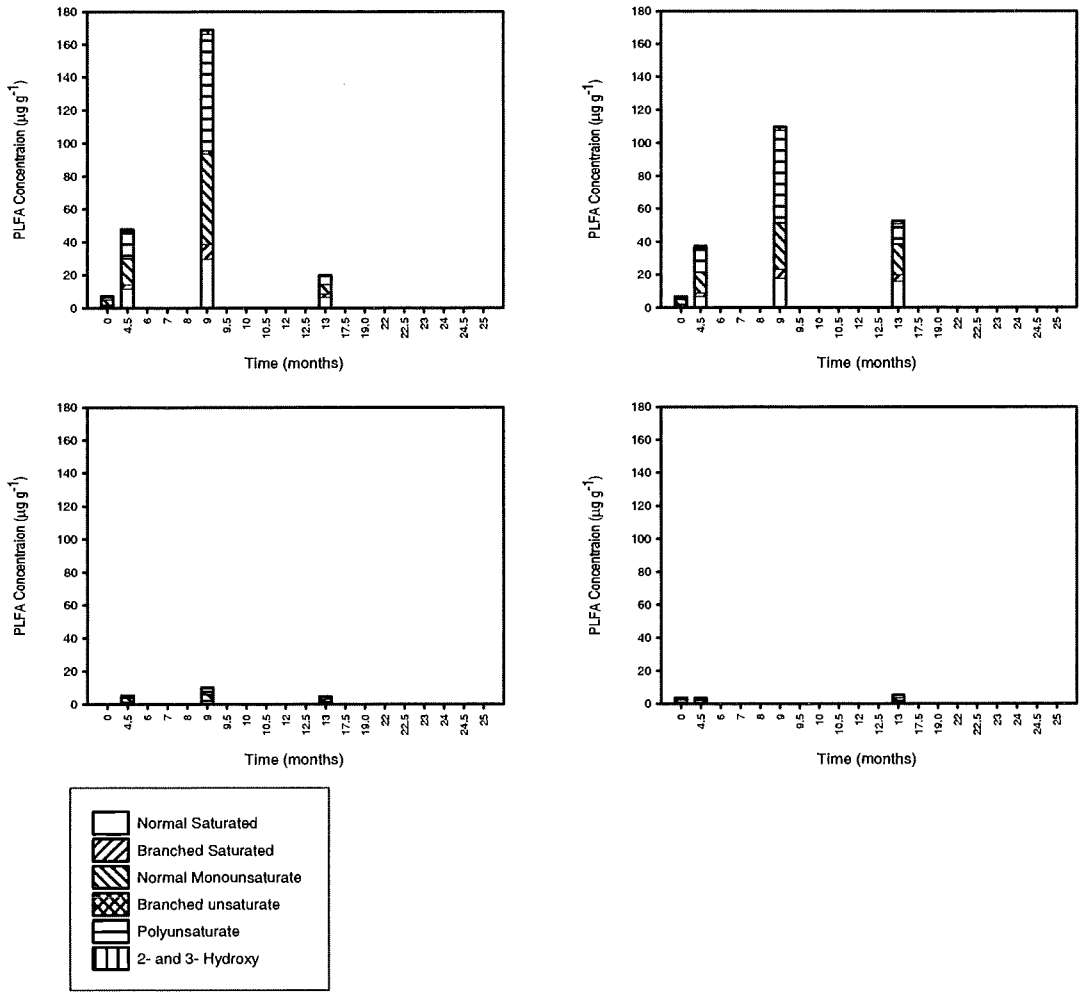


Figure 6.5.2.1.1. Concentration of the PLFA classes in the Dover samples and the reference sites. Upper Figures, cage site; lower Figures reference sites.

6.5.2.2 Saturated fatty acids (SFA)

Saturated, normal-chain fatty acids (SFA) accounted for 20-25% of the total PLFA in the reference and 0 month samples (Fig. 6.5.2.1.1). The highest concentration were 16:0 (50-60% of the total SFA) and 18:0 (20-30%). The percentage that SFA made up of the total PLFA decreased during the period that the fish cage was in place, dropping to approximately 15% at T9. Amongst the SFA, the relative percentages of 18:0 dropped markedly, while 16:0 increased slightly. The relative concentration of 14:0 increased ten fold, to around 3%.

In absolute terms, the concentrations of all SFA increased markedly during the period that the fish cages were in place. The increase was greatest (100-200 fold) for the shorter chain acids (14:0), and decreased with increasing chain length (Table 2 - Appendix 4).

6.5.2.3 Branched, saturated fatty acids

Branched, saturated fatty acids (BSFA) are often used as indicators of bacterial activity. In particular, odd-chain iso- and anteiso-acids are particularly abundant in many bacteria, but are largely absent from higher organisms. The relative levels of BSFA in cage site sediment at 0 months and in the reference samples was between 8.9 and 16.7% (Fig. 6.5.2.1.1), with the major acids being a15:0, 10Me16:0, a17:0 and i16:0. At 4.5 months, the percentage that BSFA made up of the total PLFA decreased to circa 5.5%, and dropped slightly further at 9 months. At 13 months the proportion remained above that at 0 months. The relative proportion of i15:0

and a15:0 increased from 0 to 9 months, while the other initially abundant acids decreased in relative proportion.

In absolute terms, the concentration of the BSFA increased sharply, but, as reflected in the decrease in the percentage these acids made up of the total, less markedly than some other acid groups. The concentrations of i15:0 and a15:0 increased ten to twenty five fold, though there was a marked difference between the two cage samples. The concentrations increased less in sample Pen 1-2 compared to Pen 1-1. The increases in the concentrations of i16:0, i17:0 and a17:0 were less marked (five to nine fold), while the concentration of 10Me16:0 at most doubled. A consistent slight increase in total BSFA concentration was observed at the reference sites, with a15:0 and 10Me16:0 remaining the most important acids.

It is evident from these data that a new suite of bacteria developed in the sediments under the fish cage. The shifts in the relative proportions of the BSFA indicated that species containing i15:0 and a15:0 became relatively less important. The presence of 10Me16:0-containing organisms also clearly decreased relatively compared to the original community. The similar relative level of this acid at the reference sites over the sampling period suggests that the increase in BSFA concentration at these sites reflected natural, possibly seasonal, change. The BSFA i15:0 and a15:0 are present in many species of bacteria, and it is not possible at this stage to suggest which species or genera may have become more prevalent.

6.5.2.4 Monounsaturated fatty acids (MUFA)

MUFA were major components on a relative and absolute basis in all samples (25-44%, Fig. 6.5.2.1.1, Table 1 - Appendix 4). Major MUFA were: 16:1ω7c, 18:1ω9c and 18:1ω7c. For cage sediments, MUFA showed a general decrease on a relative basis at 4.5 and 9 months compared to 0 months. At an individual component level, trends were less consistent on a relative basis, including between the two cage samples, e.g., 16:1ω7c increased in one sample, and increased then decreased for the other cage sample. 18:1ω7c decreased at both the cage and reference sites from 0 to 9 months, and the ratio 18:1ω7c to 18:1ω9c also generally decreased. On an absolute basis, all of three of the above-mentioned MUFA markedly increased. The increase in 16:1ω7c, including relative to 16:0 and accompanied by an increase in 20:5ω3 (EPA), is consistent with a greater contribution from diatoms and/or fauna (see below).

Higher proportion of specific *trans* MUFA relative to their *cis* counterpart can be indicative of certain stress conditions in bacterial cultures and environmental samples (Guckert et al. 1986). An increase in 16:1ω7t and 18:1ω7t was not observed in either the cage, fallowed or reference site samples over time, consistent with a lack of such environmental influences.

6.5.2.5 Polyunsaturated fatty acids (PUFA)

At the start of the stocking period, PUFA made up 15-19% of the total PLFA (Fig. 6.5.2.1.1). This proportion increased markedly at the cage site after 4.5 and 9 months, reaching 40-50%, but then dropped sharply during fallowing to less than the initial percentages (12-14%) in the final sample (13 months).

The true increase in total PUFA concentration was more marked. The total concentration at 0 months was on average 1.0 μg g^{-1} dry weight. For the two cage samples, the average at 4.5 months was 15.5 μg g^{-1} dry weight, and after 9 months 63.4 μg g^{-1} dry weight. The concentrations at the reference sites remained in the range 0.6-3.6 μg g^{-1} dry weight over this period, without any apparent systematic variation. The average total PUFA concentration for the two cage samples at 13 months, 3.5 mg g^{-1} dry weight, was far lower than at 4.5 and 9 months during the stocking period, but still above the reference site at the same time. Of the

individual PUFA, the two dominant acids were 20:5ω3 (EPA) and 22:6ω3 (DHA), which accounted for approximately 50% and 20% of the total PUFA respectively at 4.5 and 9 months. Both EPA and DHA increased dramatically in concentration between 0 and 9 months; other less abundant PUFA that increased by similar relative amounts included 18:4ω3, 18:2ω6, 20:3ω6, 20:4ω3, 22:5ω3 and non-methylene interrupted 22:2 (22:2NMI). As mentioned above, the concentrations of PUFA at the reference sites remained much lower, and even though EPA and DHA were important acids, others, including 20:4ω6 and 22:4ω6, were present at greater relative abundance than at the cage site.

It is clear that the presence of the fish cage resulted in a dramatic increase in the concentration of PUFA. The relative proportions of the acids indicated that the increase did not reflect 'natural' input at the cage site (as reflected in the PUFA ratios at the reference sites), but rather was related to the nature of the food fed to the fish which would either reach the sediments by direct sedimentation, via faeces, or by input from recycling including from fauna. It is also possible that bacteria and other organisms, such as diatoms as noted above, and other flora and fauna that could either synthesis or incorporate these PUFA grew in the sediments under the fish cages, and that the observed changes are due to such a species shift. A related novel example of one of these possible sources is EPA-producing bacteria, with isolates having recently been isolated from the Huon Estuary (Skerratt 2001). Although further research is required to determine the precise source(s) of polar lipid-derived PUFA in these sedimentary environments, other data, including the recognized high fauna contribution (Section 5.3), point to benthic fauna as the major source.

6.5.2.6 Hydroxy fatty acids

Hydroxy fatty acids (HFA) contributed up to 3 - 5 % of the total PLFA in the reference and at 0 months. The most prevalent HFA prior to the beginning of the experiment were 16:0 3OH, which accounted for approximately 35% of the HFA. Other important HFA at 0 months were a17:0 2OH and a17:0 3OH, which were not separated in the GC analysis, and the corresponding i17:0 hydroxy acids. Members of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) cluster are one possible source of HFA within the PLFA (Skerratt et al. 1991); such bacteria have been recently shown to be major components of Huon estuary water column samples (Skerratt 2001).

The percentage that HFA made up of the total PLFA decreased for the cage site (4.5 and 9 months) to 1.3 – 1.4% of the total. Most acids maintained their approximate percentage contributions to total HFA. One exception were the 14:0 HFA, which accounted for about 10% of the HFA at the start of the experiment, but less than 1% at 9 months.

The total concentrations of HFA increased during the stocking period, but less so than other components of the PLFA, as reflected in the lower percentage. The HFA that increased most in concentration were 15:0 2OH and the a17:0 HFA, while the concentration of the 14:0 hydroxy acids actually decreased.

These results indicate that HFA-containing bacteria increased in number under the fish cage, but not to the extent where species high in HFA dominated the consortia. It is clear that some species (including those for which the 14:0 HFA are markers) decreased in abundance. The relatively unusual i17:0 and a17:0 HFA may well be indicators for particular bacterial strains in the sediments.

6.5.2.7 Markers for Sulphate-reducing bacteria (SRB)

In addition to the preceding discussion on FA groups, it is also possible to observe trends in signature FA specific to key microbial groups such as sulphate-reducing bacteria (SRB).

10Me16:0 is regarded as a biomarker for acetate-utilizing SRB, specifically members of the genus *Desulfobacter* (e.g. Nichols et al. 1987). The results for this fatty acid suggest that the sediments remained oxygenated, and that significant populations of this genus did not develop (Tables 1 and 2 - Appendix 4, Fig. 6.5.2.7.1). These findings for *Desulfobacter* can be compared with other SRB biomarkers. The acid i17:1ω7c (see below), which increased on a concentration basis by a factor of 5-10, is a biomarker for another genus of SRB, the lactate-utilizing *Desulfovibrio* (Nichols et al. 1987), and it appears this genus was relatively more successful than *Desulfobacter* (Fig. 6.5.2.7.1). Similarly, the lactate-utilizing genus *Desulfobulbus* is characterized by the presence of 17:1ω6 and 16:1ω5 as predominant fatty acids (Taylor et al. 1985), although these acids may be present in other microbial taxa. Both fatty acids increased approximately twenty fold at the cage sites by 9 months on an absolute basis (Fig. 6.5.2.7.1). On a relative basis, the proportion of 17:1ω6 and 16:1ω5 decreased at 4.5 and 9 months, and then increased at 13 months during fallowing to similar levels observed for the 0 month samples (Table 2 - Appendix 4). The relative levels at 13 and 0 months at the cage site were similar to values for the two reference sites. The relative levels of all SRB biomarkers at 13 months were overall similar to those observed at the reference sites. Based on PLFA and EL compositional data, the cage site sediments are not considered highly anoxic either at 13 months, or during the farming cycle (4.5 and 9 months).

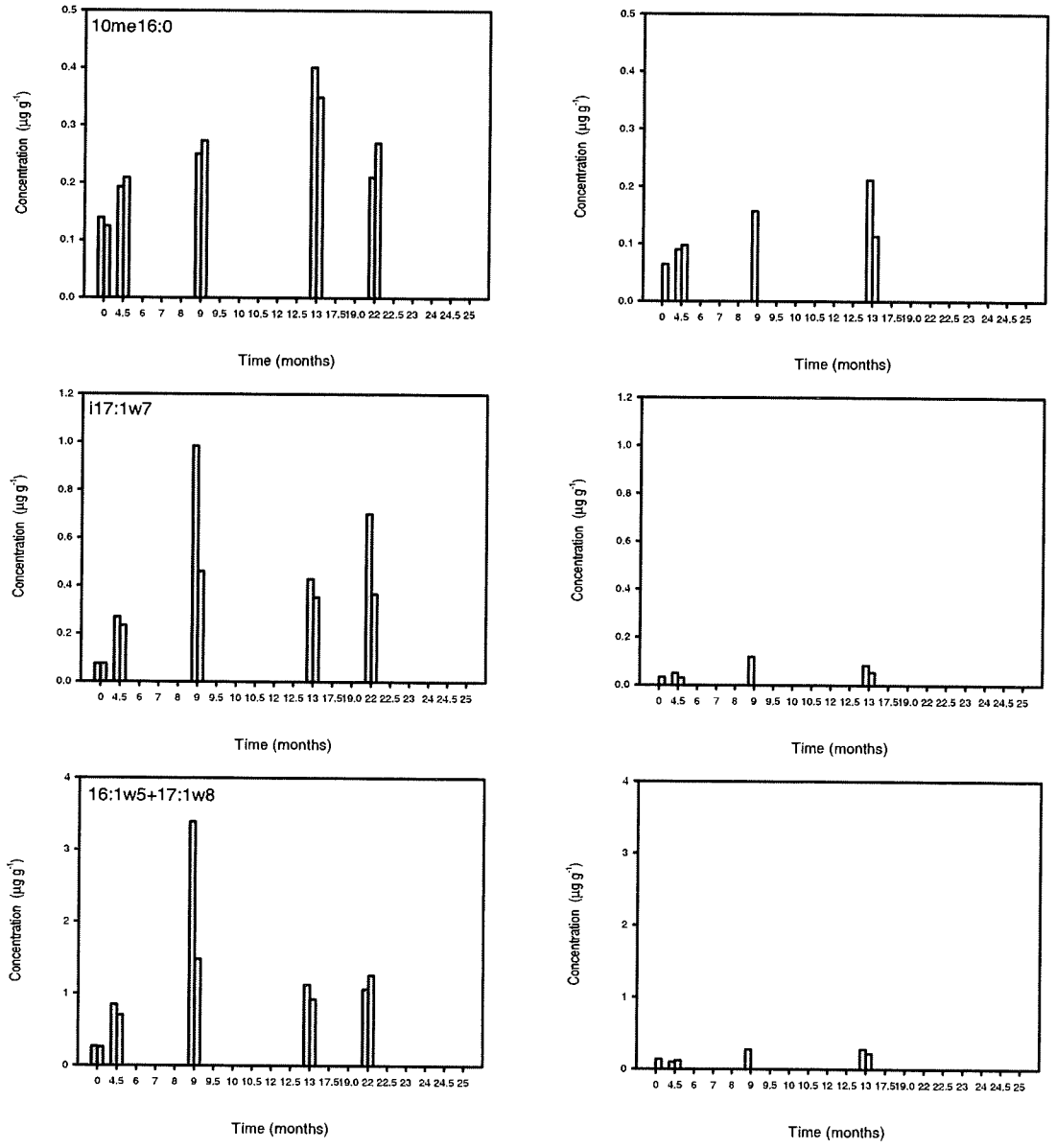


Fig. 6.5.2.7.1. Concentration of SRB markers: 1. a), 10Me16:0, this acid is present in the genus *Desulfobacter*; 2. b), i17:1ω7c, this acid is present in the genus *Desulfovibrio*; 3. c), 16:1ω5c and 17:1ω6c, these acids are present in the genus *Desulfobulbus*. For each SRB biomarker, left hand panels represent cage site, and right hand panels represent reference sites.

On an absolute basis, 13 months values for SRB biomarkers at the cage site were all higher (up to five fold) than measured at 0 months. Two further samples of 22 months cage sediments (17-1 and 17-2, Tables 1 and 2 - Appendix 4) were also analysed. The samples were collected at the end of the second 9 month stocking cycle, and were specifically examined for PLFA due to the suspected presence of H_2S odour (Susan Forbes, personal communication). No appreciable increases in the three SRB signature PLFA were observed for the 22 months samples; these findings are consistent with the earlier observation for the 9 months month samples that the cage site sediments are overall not considered highly anoxic.

6.5.2.8 Ether lipids

GDGT were observed in all sediment samples at concentrations ranging from 71 to 423 ng g^{-1} dry weight (average 156 ng g^{-1} dry weight, standard deviation 89 ng g^{-1} dry weight). Caldarchaeol was the most abundant GDGT in all samples, accounting for on average 65% of

the total GDGT (standard deviation 3%) (Fig. 6.5.2.8.1). GDGT1 and GDGT2 were present at 2 to 6% and 0 to 3% respectively, with the remainder (24-33%) being crenarchaeol. This distribution of GDGTs is typical of marine sediments (Schouten et al. 2000; Bowman et al. 2003; J. Gibson et al., unpublished results).

There were no consistent trends in the GDGT data. The concentrations at the two reference sites showed as much variation as those from the two cage sediment samples.

From the GDGT results, it appears that the concentration of ether lipids is generally not affected by the presence of fish cages. It is possible that most of the ether lipid present in the sediment is derived from dead cell material, and is therefore present as free ether lipid as distinct from phospholipid ether lipid. If a community of methanogenic Archaea had developed beneath the cage (ie. at 4.5 and 9 months) due to the increased input of organic material, and the subsequent exhaustion of sulphate as an electron acceptor in the oxidation of organic matter, it would be expected that the concentration of ether lipid would increase. As noted above, no such marked increase in SRB biomass was observed, nor did EL increase, supporting the interpretation that the development of an active methanogenic community did not occur under the fish cage.

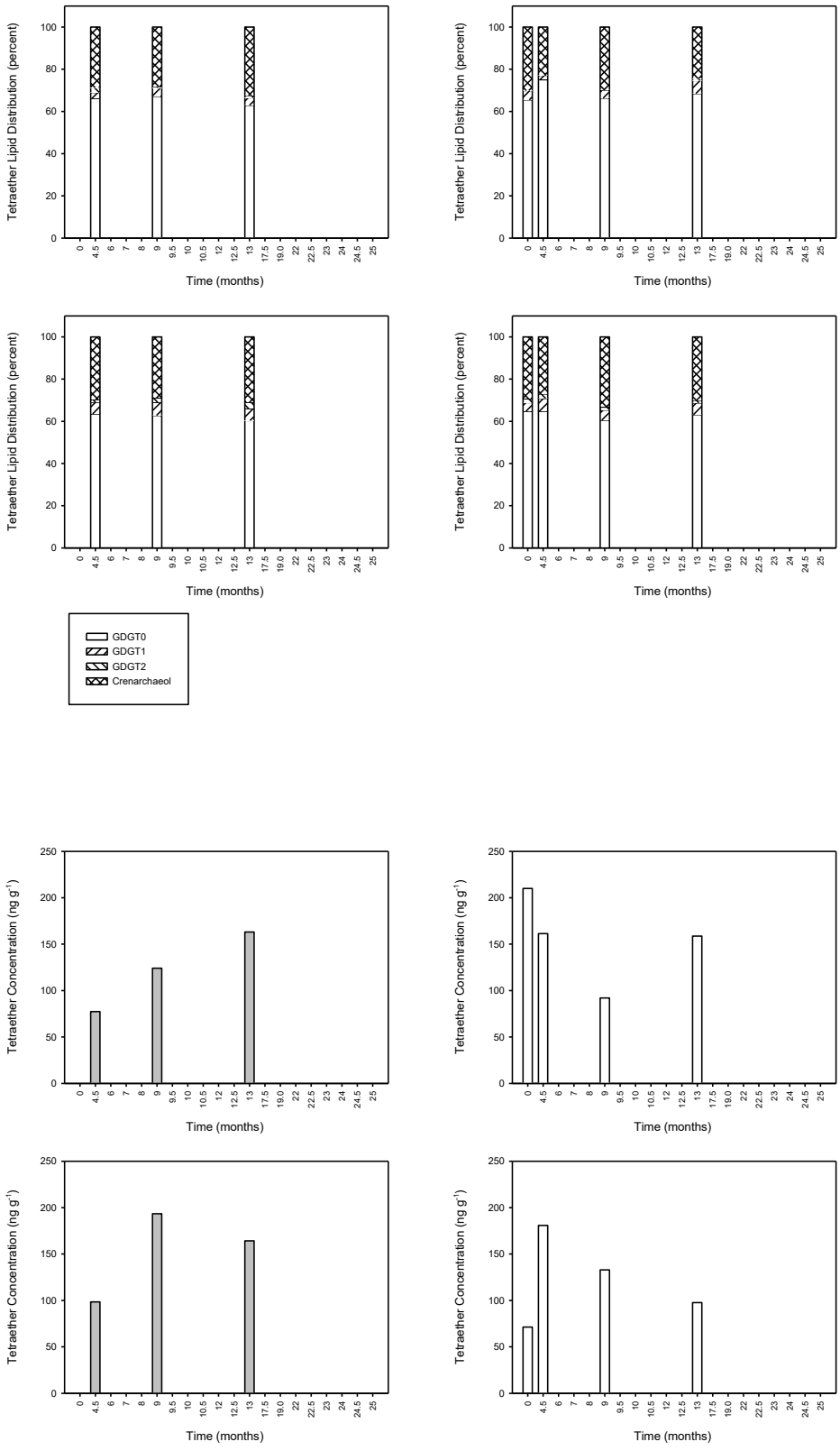


Fig. 6.5.2.8.1. Relative proportion (upper plots) and absolute concentration (lower plots) of GDGTs in over farm site (left hand panels) and reference (right hand panels) sediments.

6.5.3 Summary/ Discussion

At Stringers Cove the response of bacterial numbers to farm loading was similar to that at Creeses Mistake. Numbers increased throughout the nine-month stocking period and declined over the three-month fallow period. Over the first farm cycle bacterial numbers did not return to reference site levels, and continued to decline over the following 12 months. As was the case at Creeses Mistake, the farming intensity was higher during the first twelve-month cycle. Over the second production cycle bacterial numbers at cage sites were not different from those at reference sites at the beginning or end of the cycle, and were still elevated at the end of the stocking period. This result further supports the conclusion that microbial numbers respond rapidly and directly to labile organic input. During the second production cycle, farming intensity was less than during the first cycle. As a result, bacterial numbers were not as high at the end of the second stocking period, there was less labile carbon in the sediments and bacterial numbers consequently declined more rapidly.

Bacterial numbers were always higher in the surface sediments (0-2mm) than they were in the deeper layers (5-10mm), regardless of treatment, although there was no statistically detectable difference between the middle layer and the other two.

Changes were apparent with a number of microbial signature lipid and biological parameters. However, it appears from examination of specific sensitive environmental signature lipid indicators of the methanogens and SRB that the fish farming conditions employed has not markedly enhanced growth of these potentially deleterious sedimentary microbial groups.

Quantitatively, bacterial numbers increase with stocking and decrease during fallowing. They did not return to the original levels in the first year, but did so in the second, probably as a result of reduced stocking intensity. Also, there was an indication of a qualitative change in taxa present as there were more *Beggiatoa* filaments present in stocked or fallowed cage sediments compared to references.

7. GENERAL CONCLUSIONS

This study aimed to identify monitoring techniques which could be used by farm managers to simply and reliably evaluate sediment condition under and adjacent to their cages and to relate this information to farm production data in such a way that managers could more effectively monitor and manage environmental conditions. To this end a variety of different approaches for farm-based monitoring of enevironmental condition were assessed. The relative performance (sensitivity and reliability) of the techniques was judged against the findings of the benthic infaunal community assessment, which was deemed to be the most sensitive approach for determining sediment condition. Some techniques were unable to adequately detect change or were deemed too complex to be applied for farm-based monitoring. However, several simple and reliable methods were identified.

7.1 Benthic Infaunal Characterisation

Analysis of the combined benthic infaunal data from the two study sites (Creeses Mistake and Stringers Cove) indicated significant differences between the benthic infaunal communities characterising unimpacted and impacted (farmed) sediments (Fig. 7.1.1). These differences were consistent with successional community patterns previously described in association with organic enrichment from fish farms (Brown et al., 1987, Weston, 1990, Wildish & Cranston, 1997, Black, 2001). Although many of the species involved were clearly specific to the Tasmanian environment, the functional changes were largely equivalent and will be outlined in the following sections.

There were significant differences between the unimpacted conditions at each of the study sites. i.e. the baseline communities at the two study sites were very different. This is important as an understanding of the community composition prior to farming is essential for determining recovery and may provide an indication of the potential resilience of the system.

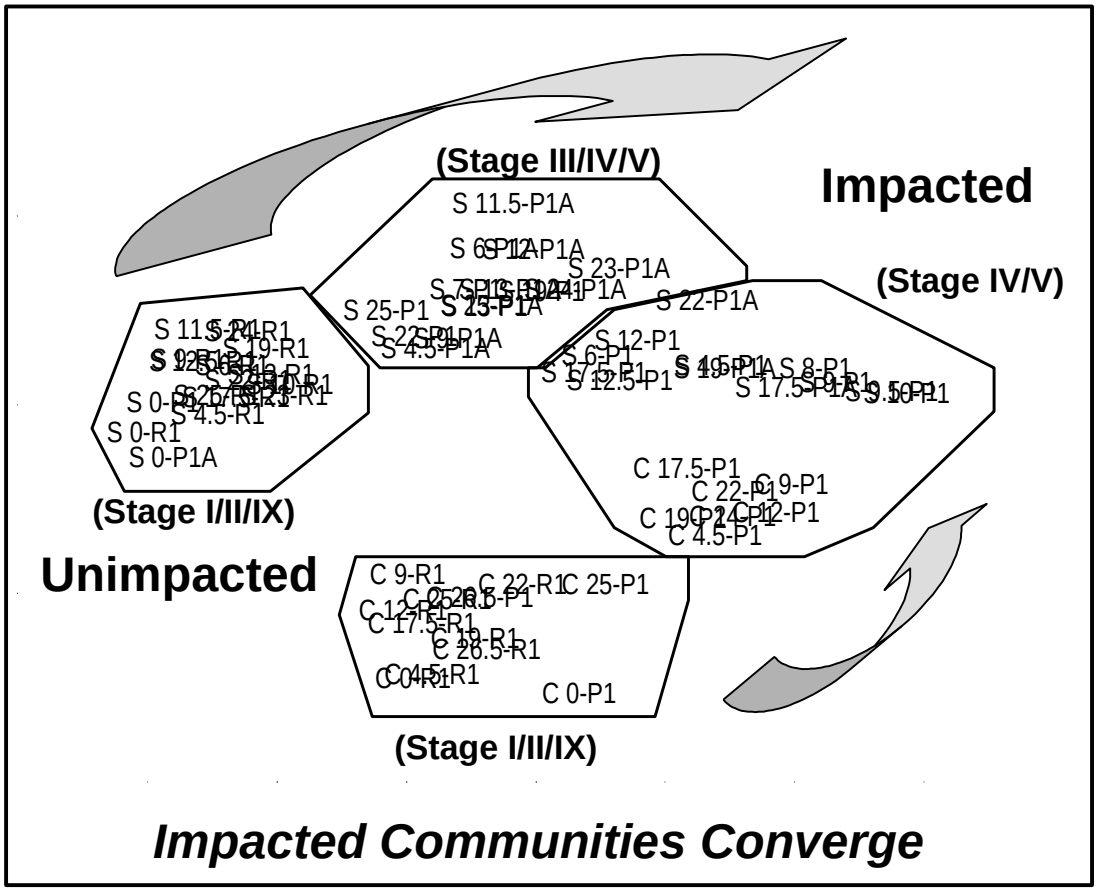
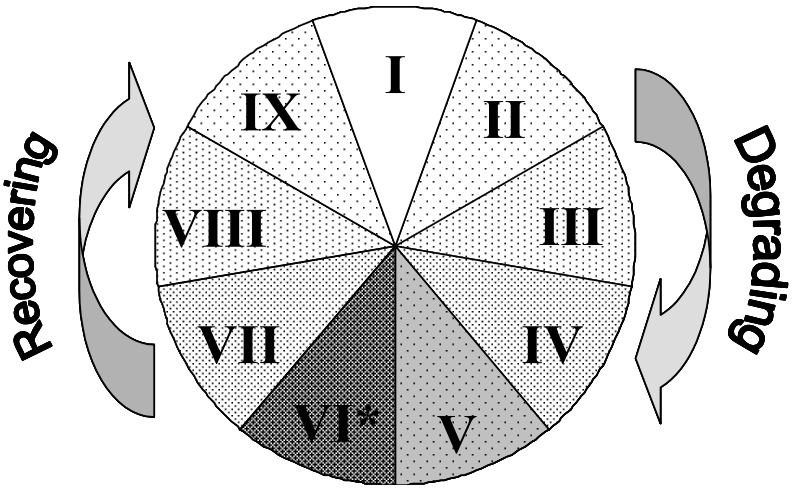


Fig.7.1.1. Ordination of the community data for the community structure data for both Creeses Mistake (references and cages 5(P1) & 8(P2)) and Stringers Cove (references and pen positions 1,2,1A,). Stress= 0.16. Prefix indicates site and time in months and suffix indicates position.

7.1.1 Areas of Equivalence between Sites

Although the unimpacted communities at the two study sites were quite different, the infaunal community structure became more similar as the level of organic enrichment increased (Fig. 7.1.1.1). The impacted sediments were also similar in many other sediment characteristics and even in the early impact stages there were broad similarities in the general characterisation of the two sites. Consequently, this study defined 9 different stages of impact, which effectively distinguished degrading and recovering sediments, based on these generalised changes in community features and sediment criteria (Fig.7.1.1.1).



* Indicates conditions not observed in this study
Suggest stage IX is sufficiently recovered for restocking

STAGE – Category		STAGE – Description	
I	- Unimpacted	I	- No evidence of impact
II	- Minor Effects	II	- Slight infaunal & community change observed
III	- Moderate Effects	III	- Clear change in infauna & chemistry
IV	- Major Effects (1)	IV	- Major change in infauna & chemistry
V	- Major Effects (2)	V	- Bacterial mats evident, outgassing on disturbance
VI*	- Severe Effects	VI*	- Anoxic/ abiotic, spontaneous outgassing
VII	- Major Effects	VII	- Monospecific fauna, major chemistry effects
VIII	- Moderate Effects	VIII	- Fauna recovering, chemistry still clearly effected
IX	- Minor Effects	IX	- Largely recovered, although slight faunal/ chemical effects still apparent

Fig. 7.1.1.1. Impact and recovery stages.

Changes in the level of organic enrichment resulted in a variety of changes in the type and abundance of species comprising the infaunal community. Several simple approaches for evaluation of the faunal community, such as total number of species, total abundance and the presence of key indicator species, all provided useful information on the sediment condition, even distinguishing particular impact stages (Fig. 7.1.1.2). Some of these features were equally applicable at both study sites, were easy to define and were clearly linked to particular impact stages, whilst others characterised a broader change or were site specific.

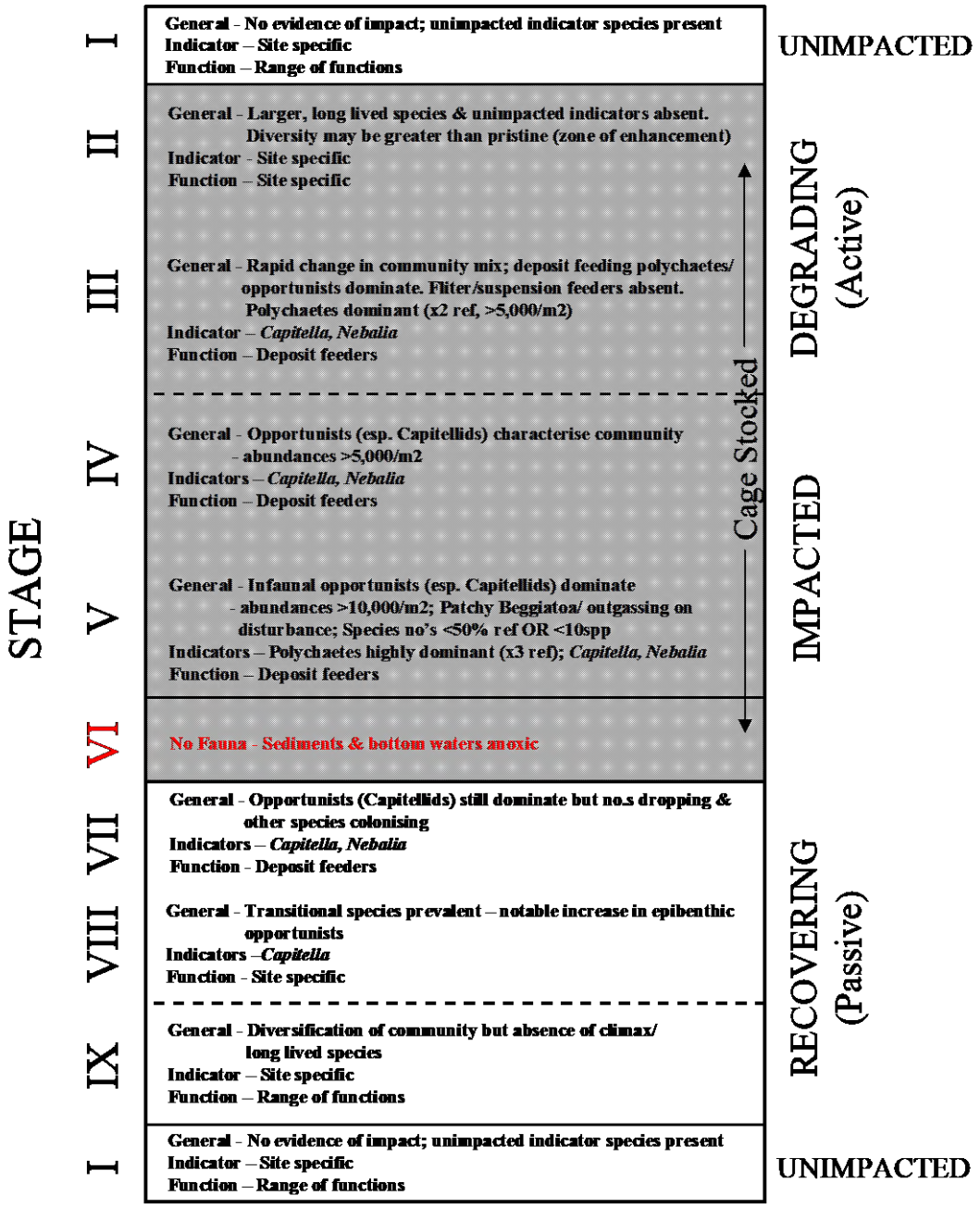


Fig.7.1.1.2. General characterisation of impact/recovery stages based on main infaunal community, key faunal indicators and functional changes.

This study assessed the benthic community information from both sites to determine whether any simplification of the data was possible or whether any generalisations could be made in regard to the infaunal results and the level of impact. The results show that when sediment conditions were degraded polychaete worms were the dominant faunal group, both in terms of abundance and diversity. In organically enriched sediments capitellid polychaetes (distinctive, red, opportunistic worms) can occur in huge numbers, and in this study these worms were strongly indicative of the moderate and major impact stages, III-VIII (Fig. 7.1.1.2). In impacted sediments the worms represent the greatest proportion of the fauna, and evaluation of total abundance effectively reflected these changes. Consequently a doubling in the number of organisms per sample compared with reference samples indicated a moderate impact (stage III), whilst an increase of threefold or greater was indicative of a major impact (stage V).

Where there was a major impact species numbers were also generally reduced; a 50% or greater reduction in the number of species compared to the reference site or less than 10 species present (based on a $0.2m^2$ sample) suggested a significant impact (stage V). However, number of species by itself was a poor indicator of impact. For example if a farm sediment sample contained the same number or more species than an equivalent reference it did not necessarily mean that the sediment had recovered, since the mix of species might be quite different. In the initial stages of impact (or in recovery) diversity can increase, as the conditions have not yet deteriorated sufficiently to eliminate the stable species, and there are sufficient resources to encourage the establishment of opportunistic species (this is sometimes referred to as a zone of enhancement).

There were also changes in some general faunal groups which could be related to the level of apparent impact. Most of these were species and site specific and are discussed in the next section. The results suggest that where crustacea are a dominant component of the reference community a significant drop in numbers should be considered carefully, particularly if also associated with an increase in abundance of worms. Change in the abundance of molluscs as a group was on the whole a fairly poor indicator of environmental condition. However, echinoderms appeared to be relatively sensitive to organic enrichment, particularly when this was associated with a decline in the oxic status of the sediments.

Of the geochemical measures evaluated estimation of redox potential and sulphide concentration were the most useful for distinguishing between impacted and unimpacted sediments (Fig. 7.1.1.3). Redox has been included in the regulatory standards required for fish farm monitoring in Scotland, Canada, the U.S. and locally in Tasmania. However, assessment of both redox and sulphide were poor indicators of recovery, generally greatly overestimating the extent of recovery. Wildish et al. (2001) produced a model that defined four stages of impact in relation to redox potential and sulphide concentration. Comparison of the present data with the organic enrichment model proposed by Wildish et al. (2001) suggests that although redox levels defining the impact stages in the current study were comparable to those of the Canadian study, the local sulphide concentrations were greatly reduced. In the current study a major impact was suggested by sulphide levels more than 10 times lower than that indicated by Wildish et al. (2001). In New Brunswick, Canada the requirements for environmental monitoring of salmon farms require that redox and sulphide be evaluated in combination (Wildish et al., 1999) and certainly the results from this study indicate that measurement of both redox and sulphide concurrently would

increase the reliability of the result. Redox appeared to be influenced by seasonal changes and both redox potential and sulphide concentration were variable in recovering sediments. Consequently it is recommended that redox and sulphide only be considered either as part of a reliable and frequent time series data set or in conjunction with other measures of sediment condition (e.g. faunal information or video assessment).

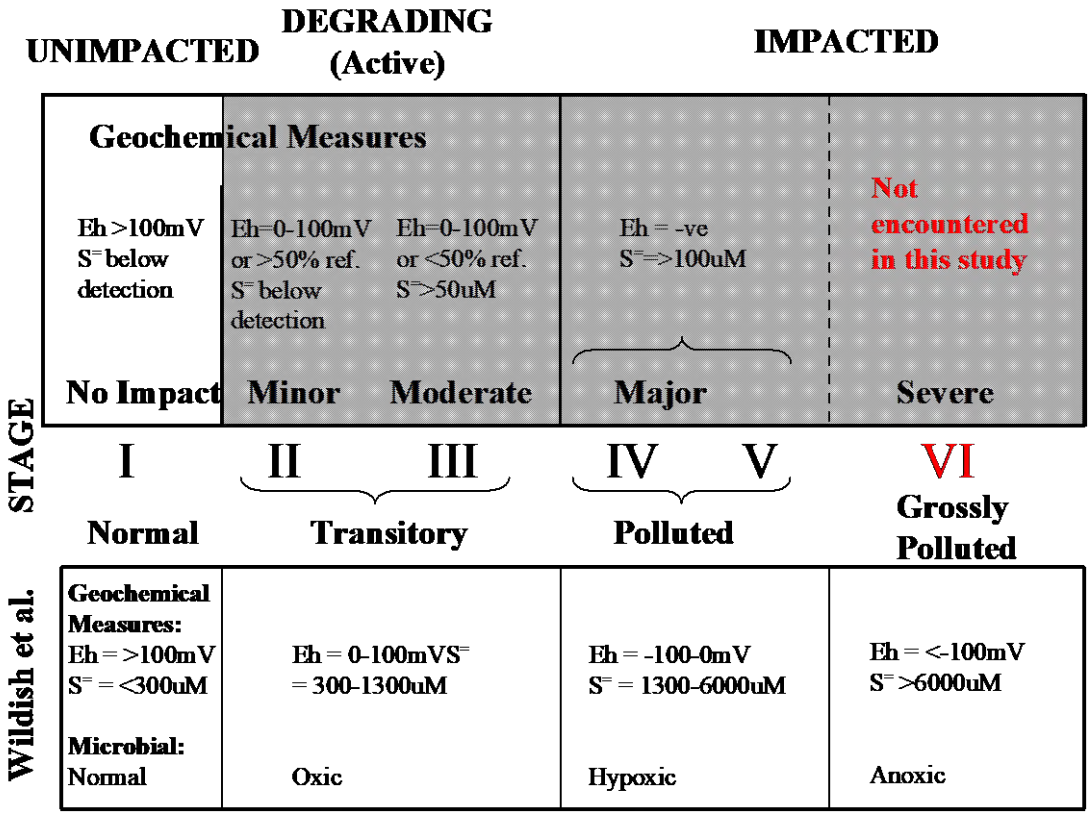


Fig. 7.1.1.3. Redox potential and sulphide concentration associated with impact stage and compared with organic enrichment model as defined by Wildish et al. (2001).

Visual assessment techniques were found to be very reliable in defining the level of impact, and were applicable to both degrading and recovering sediments (Fig. 7.1.1.4). However, the differentiation between degrading and recovering sediments was most easily accomplished when the information was reviewed as a time series.

Analysis of video footage was a very good indicator of benthic condition. Negative scores indicated degraded conditions, scores greater than 5 suggested a relatively healthy environment, whilst values between 2.5-5 would be considered acceptable within farms. Where video scores were less than 2.5 conditions were still transitional and further time would be needed for recovery. In determining the video scores several key visual features were apparent which have significance in their own right, several of these were site specific but some were more generally applicable. The presence of *Beggiatoa* mats and emission of gas bubbles were definite signs of major impact. In contrast prevalence of burrows, faunal tracks and tubes was a positive sign, indicating that sediments were recovering well. Stabilisation of the cumulative scores for these features could be used to indicate recovery.

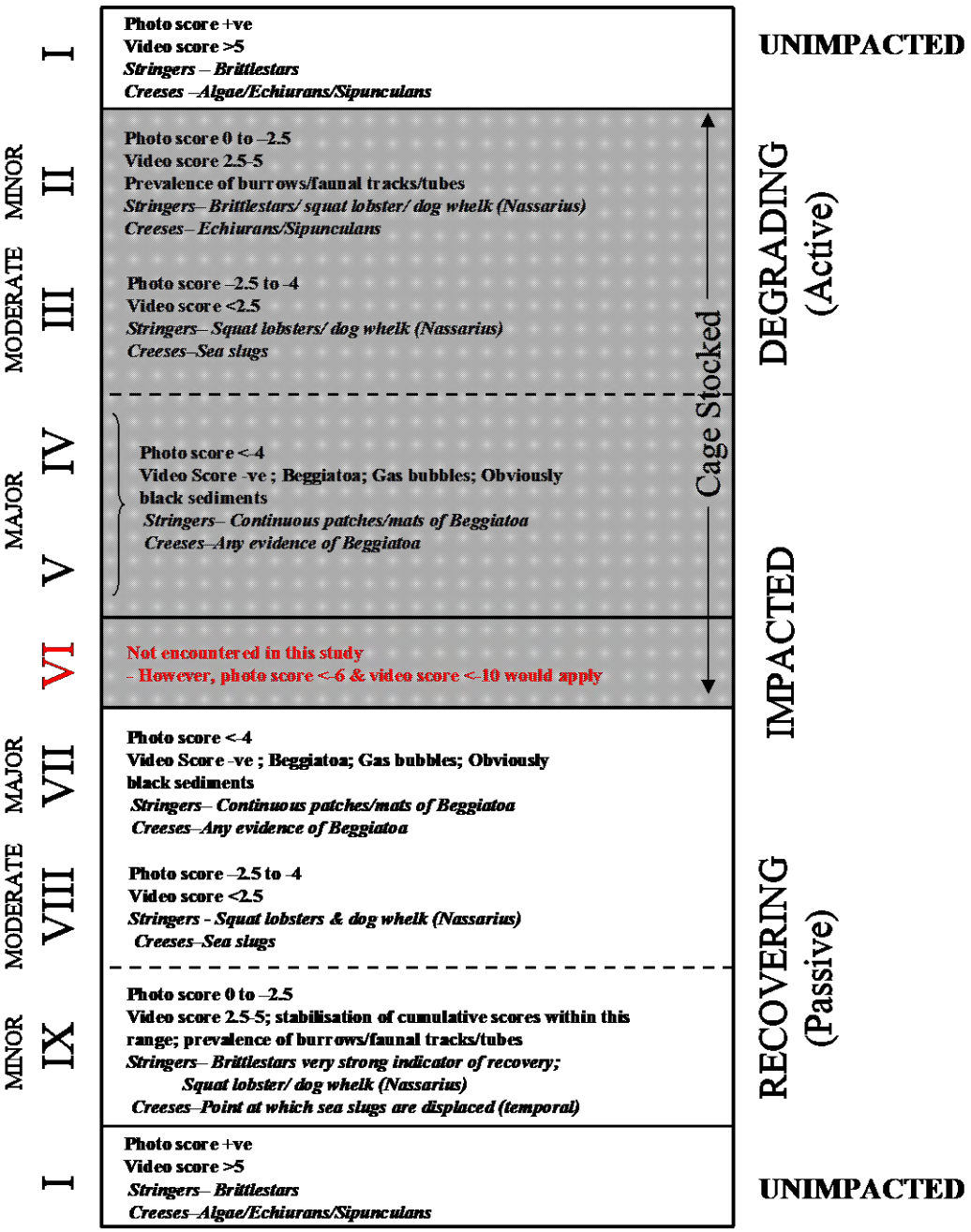


Fig.7.1.1.4. Characterisation of impact/recovery stages based on visual assessment scores and main indicator features at both Stringers Cove and Creeses Mistake.

The benthic photo evaluation also identified generally applicable criteria indicative of levels of impact. As with the video scores a positive score indicated reasonably healthy sediments, a photo score greater than 2 could be considered very good, whilst a score above 0 could, to all intents and purposes, be considered recovered. Negative scores suggested that the system was degraded, although between –2.5 and 0 would be acceptable for farm conditions. Sediments with a photo score below –4 should be considered severely degraded.

7.1.2 Site Specific Conclusions (Regional variability)

Substrate type is a critical determinant of infaunal community structure (Hall, 1994, Snelgrove & Butman, 1994). The unimpacted communities at each of the two sites were very different. Creeses Mistake had many features typical of a sandy sediment community, and although functionally relatively diverse there was a high proportion of suspension feeding crustaceans. In contrast the Stringers Cove community was characteristic of soft mud sediments which are polychaete dominated with few suspension feeders and many surface and infaunal deposit feeders. With the onset of farming the communities at the two sites changed and over time became more similar. Each impact stage can be characterised by specific genera or community changes (Fig. 7.1.2.1). At Stringers Cove the key genera defining the various stages were very similar to those proposed by Pearson & Rosenberg (1978). There were some notable differences in the sandier environment at Creeses Mistake, particularly in the early stages of impact.

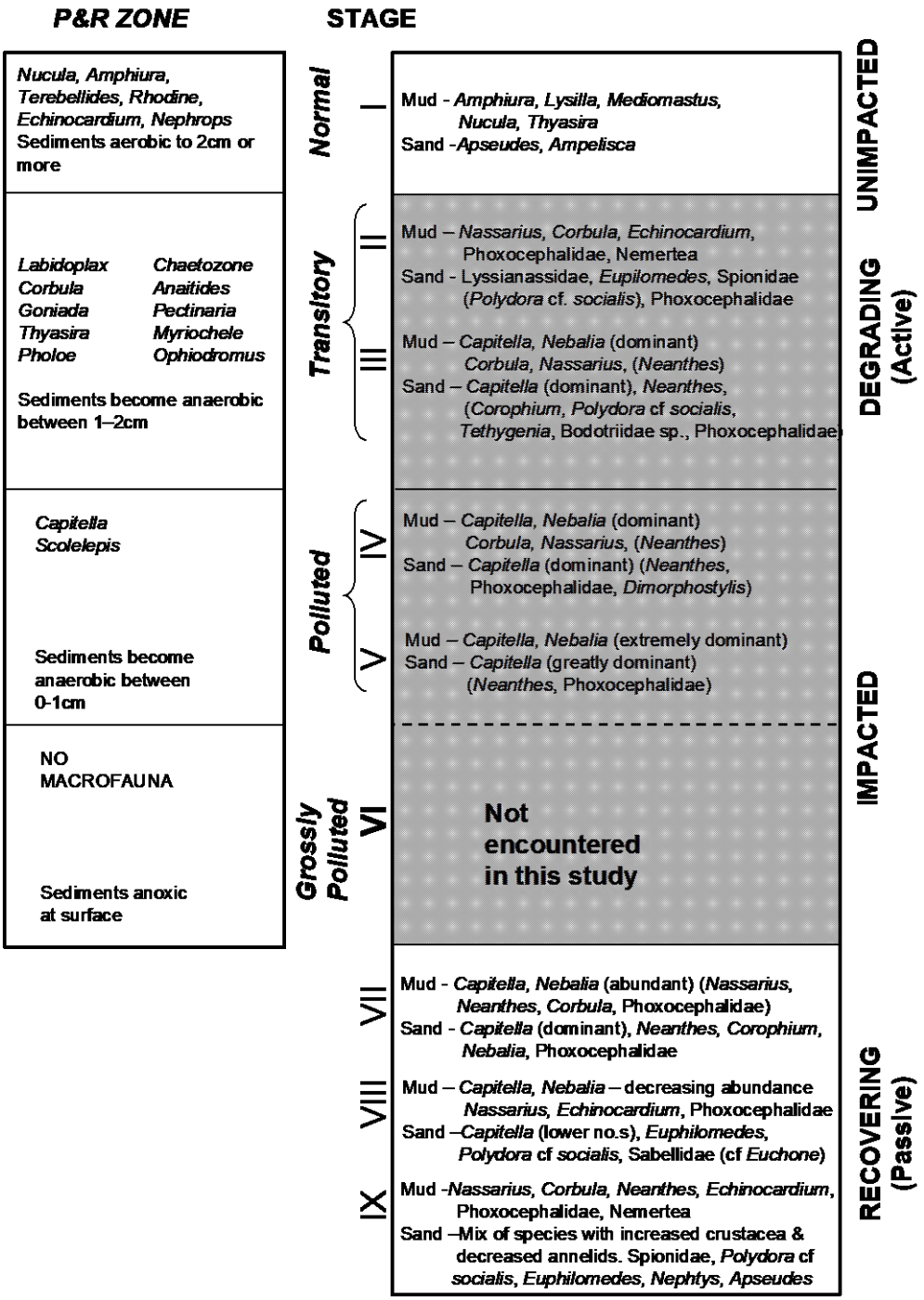


Fig.7.1.2.1. Comparison of main indicator species with P&R model (1978). Shaded area indicates period when cage was stocked.

All of the monitoring approaches examined identified differences between the two study locations. For the most part these differences refined the sensitivity of the more generally applicable criteria. To facilitate comparison between the two sites these differences are listed in detail below.

CREESES MISTAKE:

In contrast to Stringers Cove the cages sampled at Creeses Mistake site were all from areas which had previously been subjected to farming and therefore the pre-farming samples could not be considered equivalent to those from the references.

The background faunal community at Creeses Mistake was characterised by crustaceans, two species in particular *Ampelisca sp* and *Apseudes sp*. These species were present at recovered/farm sites and there were marked differences in abundance between recovered and reference samples (Table 7.1.2.1). Unimpacted sediments generally contained more than 450/m^2 and 700/m^2 individuals per sample (0.01m^2) of *Ampelisca sp.* and *Apseudes sp.* respectively. When the sediment was impacted *Ampelisca sp* was entirely absent and only about 15-30 individuals m^{-2} of *Apseudes sp.* were noted. Consequently, the presence of between 150-300 individuals m^{-2} of *Apseudes sp* might be considered indicative of recovery to at least a farm level. The presence of more than just one or two *Ampelisca sp.* would be a good indicator of a high level of recovery. These species are comparatively large and distinctive and would be relatively easy to distinguish even by a non-skilled ecologist, particularly when present in abundance. Phoxocephalids, another crustacean group, were also notably more abundant in the samples from recovered positions than at either the impacted or reference positions.

Table 7.1.2.1. Characterisation of impact/recovery stages at Creeses Mistake (a sandy site) based on key features for each of the techniques deemed suitable for farm based assessment.

Impact Stage	Effect Category	Description	Generalised Benthic Categories	Key Indicator Spp (* use in combination with other species)	Shannon Index	Total Abundance	Redox Potential (mV)	Sulphide Conc. (uM)	Benthic Photo Score	Video Score	Video Features
I	**No evidence of impact**		Pristine indicator species present	Apseudes, Ampelisca	>2	<1,500/m2	>100mV	Below detection	Pos've	>5	Algae, Echiurans/Sipunculans
II	**Minor effects (Degrading)**	Small scale community change; Sediment chemistry unaffected or with only very minor effects	Larger, long lived species & pristine indicators absent. Diversity may be greater than pristine (zone of enhancement)	*Lyssianassidae, *Euphilomedes, *Polydora, *Phoxocephalidae	>2	<1,500/m2	0-100mV (or >50% ref)	Below detection	0 to -3	2.5-5	Prevalence of burrow/faunal track/tubes; Echiurans/Sipunculans
III	**Moderate effects (Degrading)**	Significant community change; Sediment chemistry affected	Rapid change in community mix; deposit feeding polychaetes/opportunists dominate. Filter/suspension feeders absent.	Capitella (dominant); Neanthes, *Corophium, *Polydora, *Tethygenia, *Cumacea, *Phoxocephalidae)	>1<2	>5,000/m2	0-100mV (or >50% ref)	>50uM	-4 to -3	<2.5	Sea slugs (Pleurobranchia)
IV	**Major effects 1. (Degrading)**	Major community change; Monospecific dominance; major sediment chemistry changes	Opportunists (esp. Capitellids) characterise community (abund >5000/m2)	Capitella (dominant); *Neanthes, *Phoxocephalidae, *Dimorphostylis	<1; No. spp. <50% of ref OR <10spp	>20,000/m2	<0mV	>100uM	<-4	Neg've	Any evidence of Beggiatoa, Gas bubbles, Black sediments;
V	**Major effects 2. (Degrading)**	As in Stage IV; Beggiatoa/outgassing on disturbance	Infaunal opportunists (esp Capitellids) dominate (abund >10,000/m2). Patchy beggiatoa/outgassing may be evident.	Capitella (greatly dominant); *Neanthes, *Phoxocephalidae							
VII	**Major effects (Recovering)**	Fauna returns to monospecific dominance; major sediment chemistry effects	Opportunists (Capitellids) still dominate but no.s dropping & other species colonising.	Capitella (dominant), *Neanthes, *Corophium, *Nebalia, *Phoxocephalidae	<1; No. spp. <50% of ref OR <10spp	>20,000/m2	<0mV	>100uM	<-4	Neg've	Any evidence of Beggiatoa, Gas bubbles, Black sediments;
VIII	**Moderate effects (Recovering)**	Fauna re-establishing (zone of enhancement); Sediment chemistry still affected	Transitional species prevalent - notable increase in epibenthic opportunists.	Capitella (lower no's), *Euphilomedes, *Polydora, *Euchone	>1<2	>5,000/m2	0-100mV (or >50% ref)	>50uM	-4 to -3	<2.5	Sea slugs (Pleurobranchia)
IX	**Minor effects (Recovering)**	Community largely recovered; Sediment chemistry recovered	Diversification of community but absence of climax/long lived species.	Mix of species with increasing crustacea and decreasing annelids. *Apseudes, *Polydora, *Euphilomedes, *Nephtys	>2	<1,500/m2	0-100mV (or >50% ref)	Below detection	0 to -3	2.5-5	Point at which sea slugs are displaced (temporal)

On the whole slightly more species were recorded from Creeses Mistake than Stringers Cove. The reference sites at Creeses recorded between 35-50 species and where there was a major impact this was reduced to 10-15 species (Table 7.1.2.1). At Stringers only 20-30 species were recorded from the reference sites and as with Creeses Mistake this declined to approximately 10 species at impacted sites. Consequently where <10 species are identified it could be concluded that a marked impact had occurred. Total abundance was also slightly greater at Creeses at the reference positions but the impacted sites did not show as marked an increase in numbers as at Stringers Cove. Reference sites generally had less than < 5,000/m^2 whereas impacted sites had >20,000 individuals/m^2 and as at Stringers Cove this increase was mostly due to capitellid worms. Consequently at Creeses Mistake total abundance less than 1,500/m^2 suggests that conditions are acceptable, although an abundance greater than 5,000/m^2 would suggest a moderate impact and more than 20,000 individuals m^{-2} would indicate a major impact.

The findings and scoring for the visual assessment techniques was similar between the two sites. The main differences were in the key fauna underpinning the scores, which for the most part reflected the species differences indicated above. At Creeses Mistake there was less evidence of bacterial (*Beggiatoa*) mats at impacted sites compared to Stringers Cove (Table 7.1.2.1). Alga was not a feature at Stringers Cove but was observed at the references/recovered sites at Creeses Mistake and was therefore a good indicator of recovery at this site. Similarly echiurans and large annelids appeared to be good indicators of unimpacted/recovered conditions at Creeses Mistake but did not occur at Stringers Cove. Sea slugs were only present when impact was moderate, they were not observed in unimpacted conditions or when a major impact had occurred. Consequently in temporal monitoring of recovery the point at which sea slugs are displaced may indicate an appreciable improvement in sediment condition.

STRINGERS COVE:

In contrast to the crustacean dominated community at Creeses Mistake, the unimpacted community at Stringers Cove was characterised by a diverse range of polychaete worms including several surface deposit feeders (Table 7.1.2.2). Impacted conditions were characterised by a significant increase in abundance of opportunistic capitellid worms and a scavenging epibenthic crustacean (*Nebalia longicornis*). *N. longicornis* is very distinctive and in combination with increased Capitellid abundance would be a very good indicator that sediments were markedly impacted (stages III-VIII). Any significant variations in crustacean abundance at Stringers tended to be associated with *N. longicornis*. However, this species is a swarming epibenthic deposit feeder and is both highly clumped in its distribution and very susceptible to sampling technique so it can be easily missed. Consequently its absence from samples is not as significant as its presence.

Table 7.1.2.2. Characterisation of impact/recovery stages at Stringers Cove based on key features of each of the techniques deemed to be suitable for farm based assessment.

Impact Stage	Effect Category	Description	Generalised Benthic Categories	Key Indicator Spp (* use in combination with other species)	Shannon Index	Total Abundance	Redox Potential (mV)	Sulphide Conc. (uM)	Photo Score	Video Score	Video Features
I	No evidence of impact		Pristine indicator species present	Amphiura, Lysilla, *Mediomastus, *Nucula, *Thyasira	>2	<5,000/m2	>100mV	Below detection	Pos've	>5	Brittlestars
II	Minor effects	Small scale community change; Sediment chemistry unaffected or with only very minor effects	Larger, long lived species & pristine indicators absent. Diversity may be greater than pristine (zone of enhancement)	*Nassarius, *Corbula, *Echinocardium, *Phoxocephalidae, *Nemertea	>2	<5,000/m2	0-100mV (or >50% ref)	Below detection	0 to -2.5	2.5-5	Prevalence of burrow/faunal track/tubes; Brittlestars, squat lobsters, dog whelk
III	Moderate effects	Significant community change; Sediment chemistry affected	Rapid change in community mix; deposit feeding polychaetes/opportunists dominate. Filter/suspension feeders absent.	Capitella, Nebalia (dominant); *Corbula, *Nassarius,*Neanthes	>1<2	>5,000/m2	0-100mV (or >50% ref)	>50uM	-2.5 to -4	<2.5	Squat lobsters, dog whelk
IV	Major effects	Major community change; Monospecific dominance; major sediment chemistry changes	Opportunists (esp. Capitellids) characterise community (abund >5000/m2)	Capitella, Nebalia (dominant); *Corbula, *Nassarius, *Neanthes	<1; No. spp. <50% of ref OR <10spp	>20,000/m2	<0mV	>100uM	<-4	Neg've	Continuous patches/mats of Beggiatoa, Gas bubbles, Black sediments;
V	Major effects	As in Stage IV; Beggiatoa/outgassing on disturbance	Infaunal opportunists (esp Capitellids) dominate (abund >10,000/m2). Patchy beggiatoa/outgassing may be evident.	Capitella, Nebalia (extremely dominant)							
VII	Major effects	Fauna returns to monospecific dominance; major sediment chemistry effects	Opportunists (Capitellids) still dominate but no.s dropping & other species colonising.	Capitella, Nebalia (abundant); *Nassarius, *Neanthes, *Corbula, *Phoxocephalidae	<1; No. spp. <50% of ref OR <10spp	>20,000/m2	<0mV	>100uM	-2.5 to -4	Neg've	Continuous patches/mats of Beggiatoa, Gas bubbles, Black sediments;
VIII	Moderate effects	Fauna re-establishing (zone of enhancement); Sediment chemistry still affected	Transitional species prevalent - notable increase in epibenthic opportunists.	Capitella, Nebalia (decreasing abundance); *Nassarius, *Echinocardium, *Phoxocephalidae	>1<2	1,500 - 5,000/m2	0-100mV (or >50% ref)	>50uM	0 to -2.5	<2.5	Squat lobsters, dog whelk
IX	Minor effects	Community largely recovered; Sediment chemistry still slightly affected	Diversification of community but absence of climax/long lived species.	Nassarius, Corbula, *Neanthes, *Echinocardium, *Phoxocephalidae, *Nemertea	>2	<5,000/m2	0-100mV (or >50% ref)	Below detection	Pos've	2.5-5	Prevalence of burrow/faunal track/tubes; Brittlestars, squat lobsters, dog whelk

The samples from Stringers Cove indicated some interesting changes in the local molluscan communities which appear to be associated with farming operations and level of impact (Table 7.1.2.2). Changes are species specific and best evaluated as time series rather than one off assessment. Two introduced bivalves (*Corbula gibba* & *Theora lubrica*) may be useful indicators of minor/moderate impact (stage II-III, VII-VIII) as over time and with increasing enrichment of the sediments they appeared to become more abundant, although numbers rapidly diminished after a certain level of impact. In contrast the abundance of two native bivalves (*Nucula pusilla* & *Thyasira adelaideana*) declined rapidly with impact and therefore their re-establishment in sediments would suggest conditions had much improved.

There were several significant echinoderm species at Stringers Cove. The heart urchin *Echinocardium cordatum* was occasionally found at reference positions but was absent where there was any significant impact, although at low levels of enrichment it appeared to thrive. In contrast the local brittlestar (*Amphiura elandiformis*) was never found where there was any organic enrichment and consequently its presence in infaunal or video samples represents a very reliable indicator of good sediment conditions.

There were two very important site specific differences in the video assessment features at Stringers. Squat lobsters (*Munida haswelli*) and the little local dog whelk (*Nassarius* sp.) were extremely good indicators of intermediate impact. Consequently when monitoring the progress of fallowing, the presence of these two species would indicate that the sediments are well on the way to recovery. Both species are very easy to identify in video footage.

7.2 Integrated Management Model

The results demonstrate that at both sites there was a very close relationship between environmental condition and farm production levels (Figures 7.2.1 and 7.2.2). At both sites changes in management practices were associated with quite significant alterations in environmental condition. At Creeses Mistake the reduction in both biomass and feed input over the second production cycle resulted in a marked improvement in overall environmental condition; diversity, redox /sulphide and visual assessment scores all indicated improved conditions at the completion of second cycle than at the end of the first stocked phase (Fig. 7.2.1). As a result the degree to which the sediments were impacted was also reduced in the second cycle, and it seems as though this lower impact level has enabled the sediments to recover more quickly.

In the first cycle at Creeses Mistake one cage was fallowed for 6 weeks longer than planned; this resulted in a greater improvement in the condition of that particular position, and this extra level of recovery also had continued benefits in the subsequent production cycle. The sediments at this position were less impacted at the end of the second cycle than at the other cage position, which had not had this extra fallow period.

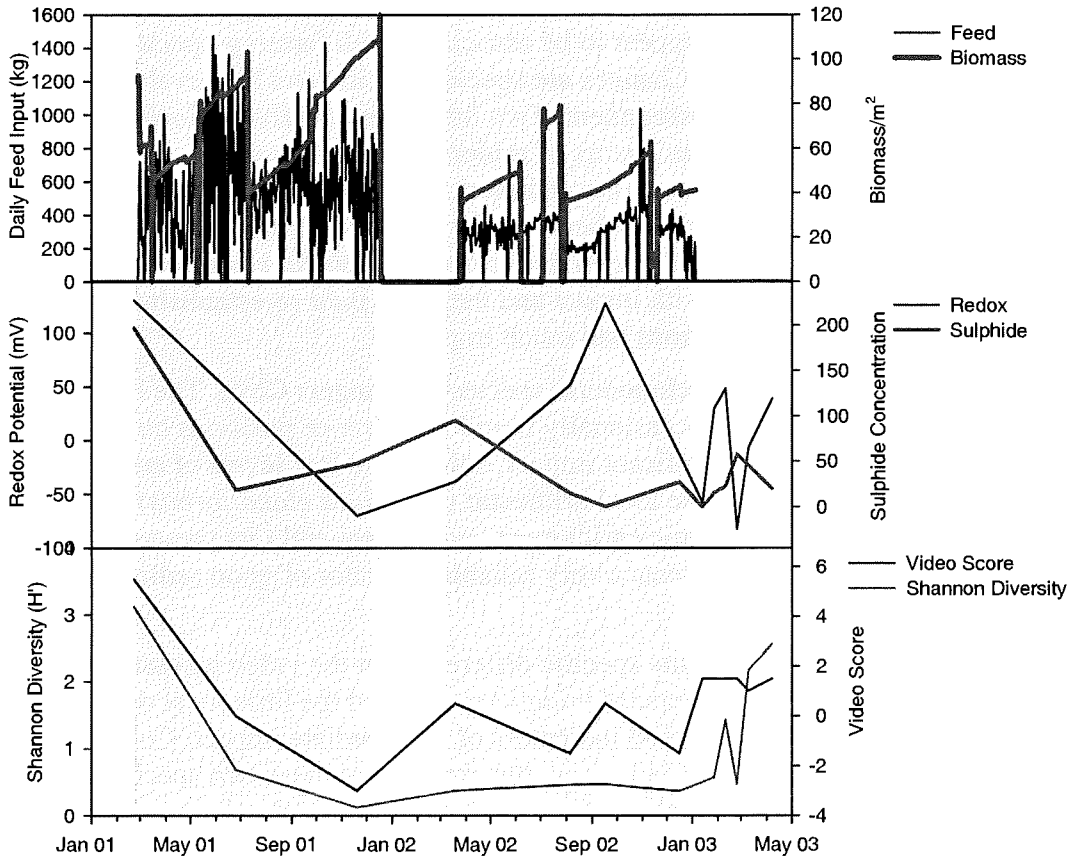


Fig. 7.2.1. Creeses Mistake – Representative data from pen position 5 showing the relationship between farm production (feed input and fish biomass), biogeochemical characterisation (redox and sulphide), Shannon diversity index and visual assessment of sediment condition. Shaded area indicates period where cages were stocked.

At Stringers Cove, the biomass of fish stocked was relatively consistent and the feed input dropped appreciably in the second production cycle (Fig. 6.2.2). Once again environmental conditions appeared to respond and the degree of impact was substantially reduced over the second production cycle.

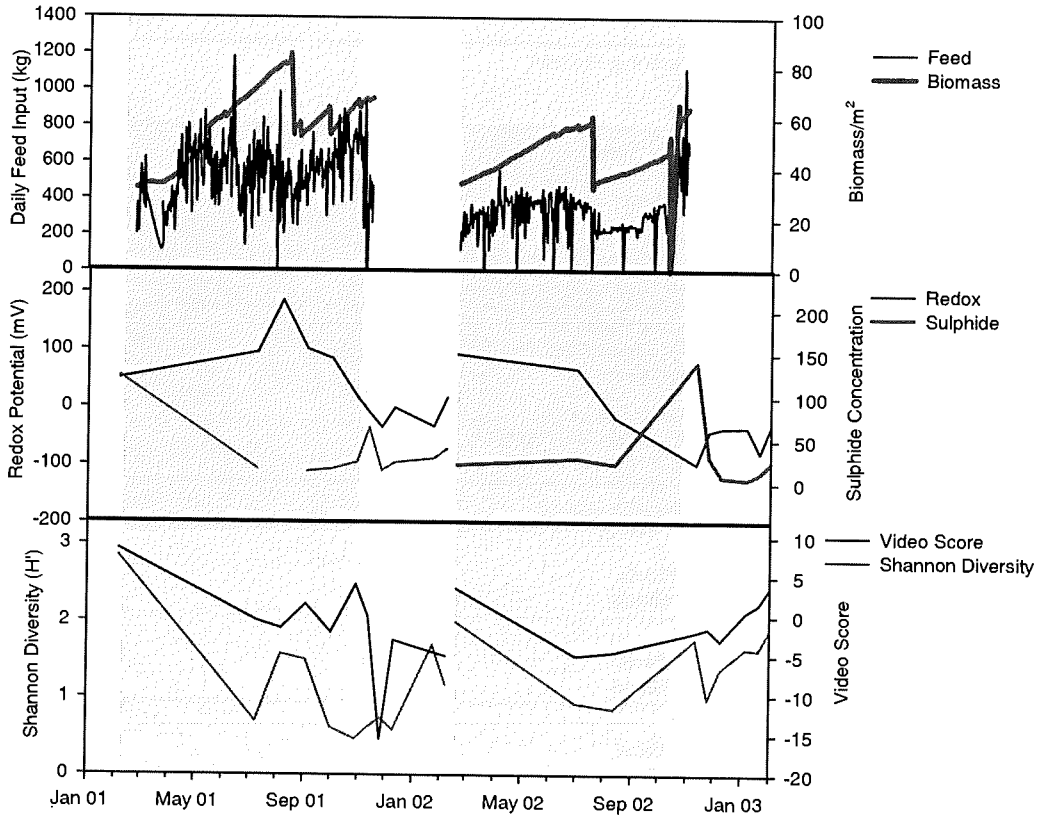


Fig. 7.2.2. Stringers Cove – Representative data for cages from the first and second production cycles combined showing the relationship between farm production (feed input and fish biomass), biogeochemical characterisation (redox and sulphide), Shannon diversity index and visual assessment of sediment condition. Shaded area indicates period where cages were stocked.

The environmental condition reflects farm production level and accordingly farm production can be managed to produce desirable environmental outcomes. These findings suggest that farmers can compensate for environmental impacts sustained in any given production year, and that it would be possible to manage their production strategies to forward plan and adjust for likely episodes of increased or prolonged stocking in particular areas.

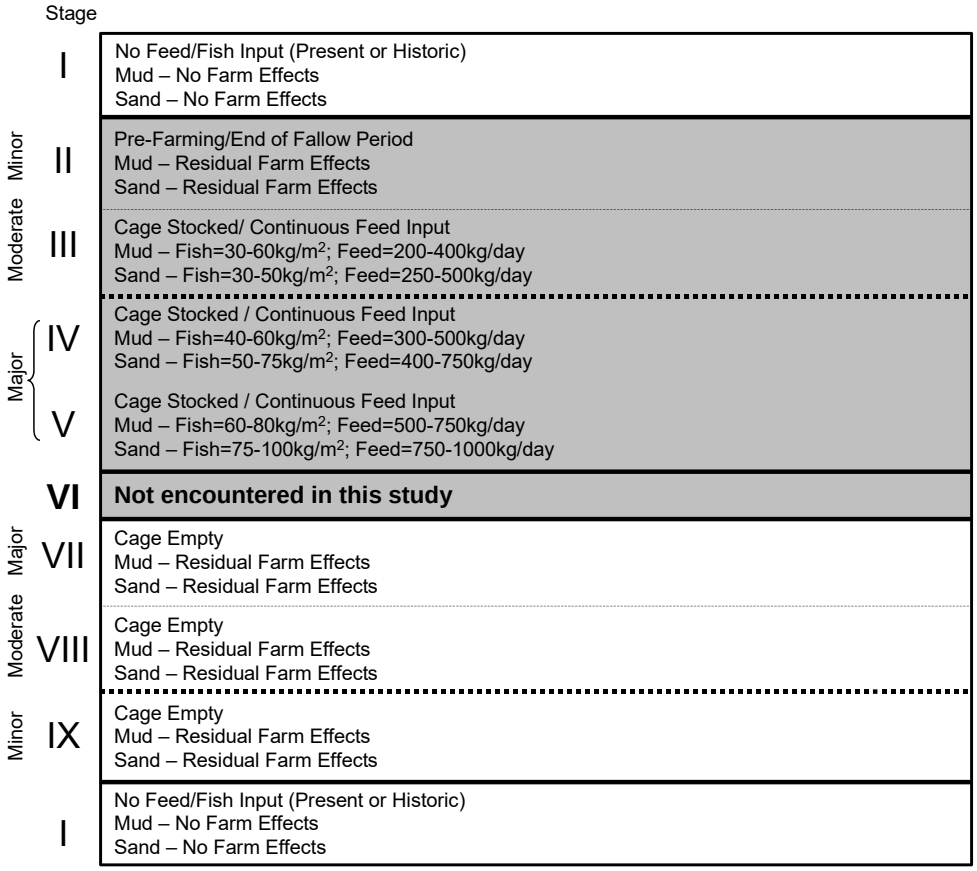


Fig.7.2.3. Comparison of impact/recovery stages with generalised farm production information at both Stringers Cove (mud) and Creeses Mistake (sand).

Relating the production information to the impact stages defined earlier shows that the levels of impact defined occurred at lower levels of stocking and feed input at Creeses Mistake (sand sediments) than at Stringers Cove (mud sediments) (Fig. 7.2.3). Which in turn supports the suggestion that the benthic community at Stringers Cove was better adapted to cope with organic impacts.

However, it should be noted that there is no adjustment for the duration of impact in this model, and although in this particular study the stocking cycles were equivalent this would not always be the case, and differing farming circumstances would have to be taken into account in any other comparisons.

7.3 Scientific Outcomes

This study has generated a substantial dataset. Some of these results have produced scientific outcomes which lie outside the scope of the original project objectives. The following section provides a brief outline of these various research areas. Further details can be obtained by contacting the principal investigator.

Prior to this study being undertaken it was recognised that farm inputs (feed & faeces) would have a significant effect on the sediment condition. However, it was not appreciated just how closely the infaunal (macro & micro) community dynamics were related to farm practices. Our results show a strong association between the macrofaunal and microbial ecology. It seems that the macrofauna may be a more significant determinant of sediment condition than originally anticipated and may in fact be influencing many of the major geochemical changes in the sediments.

It was expected that at the more exposed site (Creeses Mistake), organic material would be widely dispersed and therefore that a less severe impact would result. The impact at this site was in fact greater than we might have anticipated and was just as great as at the more depositional site. The overall change in the community structure was much greater at the more exposed site and may be harder to reverse. This appears to be largely as a result of the composition of the background infaunal community, which was not well adapted to organic enrichment. In contrast the background fauna at the more sheltered site (Stringers Cove) appeared to be functionally pre-disposed to organic material. This has broader implications for the resilience of communities to environmental impacts generally.

At Creeses Mistake there was some suggestion of a more general temporal/long term decline in the background crustacean community. The significance of these changes needs to be established in order to ascertain whether they reflect natural variability or are symptomatic of broader scale farming effects.

Sediment metal levels under cages (particularly Cu) are also particularly noteworthy. The results suggest that Cu concentrations can reach relatively high levels, potentially even resulting in toxic effects for some of the fauna within the sediments. This could significantly affect the ecological balance and compromise the ability of sediments to regulate organic matter inputs.

This study has developed a novel technique for determining sedimentation rate. Unfortunately this approach was not suitable for farm-based assessment of sediment condition resulting from fish-farming as it was not possible to isolate the cage sedimentation from other external sources. However, the method may have application in other contexts.

The large numbers of *Beggiatoa* spp present in the cage sediments at the end of the stocking cycle, together with the shallowness of the oxic zone and the apparent removal of ammonia under anaerobic conditions deeper into the sediment, suggests that anaerobic forms of metabolism are dominant in nitrogen cycling at this time. It is likely that the sediments serve as a reservoir for biologically available nitrogen for the overlying water potentially leading to eutrophication.

Heterotrophic bacteria isolated from Creeses Mistake reference sediments showed potential for development of a simple technique for estimating the amount of biologically available organic carbon in pore waters. The method needs to be refined and compared with other methods for assessment of organic carbon environmental impact.

8. BENEFITS

This project was expressly designed to support the Tasmanian aquaculture industry, and the environmental information and techniques developed apply specifically to these conditions. However, there has been widespread recognition of the potential for the findings, and particularly the extension products, to be applied in other areas. There has been considerable interest in the outputs from the Tasmanian shellfish industry, other finfish aquaculture sectors throughout Australia, from environmental regulators in Tasmania and elsewhere and from several aquaculture training/ educational institutions who would like to use the field guide as a teaching resource.

There are several specific benefits for industry associated with this research. These include an increased understanding of the impacts of farming practices within individual lease areas and the ability to define levels of impact such that sediment condition can be included as a factor in farm management and planning. The findings of the project generally support the assertion that the aquaculture industry is environmentally responsible and this along with adoption of the recommendations by industry will help to increase public confidence in the sustainability of the aquaculture industry. It is extremely hard to define specific financial gains in relation to environmental research but the willingness with which the local industry has adopted the recommendations of this study suggests that there must be a significant benefit.

In the original proposal the benefits and beneficiaries of this research were identified as:

	Commercial		Recreational	Traditional
SA	25	0	0	
Tas	65	0	0	
WA	5	0	0	
Vic/ Qld/ NSW		5	0	0

This would still be a fairly accurate representation of the likely flow of benefits within Australia.

A final benefit of this research is the potential for transfer and commercialisation of the outputs, in particular the field guide and database package. This is currently under review by the CRC technology transfer committee.

9. FURTHER DEVELOPMENT

There are four main areas in which further research/development is needed.

Development of a farm field guide.
This is currently underway and an interactive field guide and database is expected to be available by mid-2004.

Evaluation of impact of copper.
Industry is currently undertaking an in-house evaluation of copper levels in farm sediments as part of their licence to use copper based antifoulant products. However, this does not include an evaluation of the ecological impacts; this information is essential to obtain a realistic understanding of the overall effects of copper in the environment.

Ongoing assessment/refinement of video and benthic photo techniques.
It would be extremely useful to follow-up the workshops with an evaluation of the visual assessment techniques at other farm sites, under different environmental conditions and even for other forms of environmental impact.

Further investigation of the specific role of benthic infauna and its relationship with microbial degradation in the remineralisation of organic carbon in sediments as factors determining the overall capacity of sediments to assimilate deposited organic matter.

Microbiota and macrofauna are both critical for effective processing of organic material in sediments. This study has clearly shown that in organically enriched sediments the benthic macrofauna can adapt to accommodate quite significant changes in organic matter input. What is not clear is how the differing benthic communities process organic material and how these communities interact with the microbiota. Understanding these interactions and the fundamental processes by which organic carbon is degraded would improve our ability to predict the response of different sediment types to organic loading and would help to characterize the overall carrying capacity of the sedimentary system of each farm.

10. PLANNED OUTCOMES

The project's outputs have contributed considerably to the following planned outcomes:

Assessment of the regenerative capacity of sediments associated with intensive cage aquaculture.

A comprehensive assessment of the recovery of sediments at two finfish farms in South-East Tasmania has been completed. This multidisciplinary study assessed the capacity of sediments to recover using a range of techniques. A series of impact and recovery stages were identified based on all the techniques examined, with recovery to a stage IX recommended before restocking of cages. Recovery of sediments at both farms was closely related to farm management and production schedules and it was clear from the results that adjustment of stocking density and feed input can influence the level to which sediments are impacted and subsequently recover.

Evaluation of the suitability of techniques for industry-based management of sediment condition.

All techniques used in this project were assessed for their suitability for industry-based management of sediment condition. The following criteria were examined for each technique: level of sensitivity, reliability, ease of use, cost effectiveness and ability to deliver rapid results. Several techniques are recommended, including the semi-quantitative video assessment, benthic photo assessment, simplified faunal assessment and redox potential and sulphide concentration. The video assessment in particular provides a very cost effective and sensitive technique for industry-based management of sediment condition. Further details on the recommended techniques, methods and equipment specifications are presented in the Field Manual.

Development of techniques which may be readily applied to other sources of organic enrichment.

The techniques recommended for industry-based assessment may also be readily applied to assess other sources of organic enrichment. Interest has already been shown by various researchers, and the field manual and database produced as part of the extension to this project provide an easy and effective means for transferring the methodologies for these techniques to interested parties.

Development of guidelines for management of under-cage sediments which will promote maximum sustainable production.

The key findings and recommendations, as well as the recommended techniques, have all been made available to the Department of Primary Industries, Water and Environment, Marine Farming Branch, and the project researchers have had a lot of contact with the Marine Farming Branch in order to assist in the use of the results in the redevelopment of their monitoring program.

Increased understanding of sediment processes - particularly in relation to microbial communities (may be applicable to other microbial problems eg. Gill amoeba, sediments as disease reservoirs)

The scientific outcomes listed in Section 7.3 outline the major advancements in our understanding of sediment processes that are a direct result of this project. This includes advancements in our understanding of microbial communities, with results suggesting these communities respond to organic enrichment in a very similar fashion to the benthic infaunal community, particularly in terms of total abundance. In addition, the project has contributed to related studies, including the assessment of gill amoeba in sediments (project leader: Barbara Nowak), with results from this project currently being processed.

11. KEY FINDINGS AND RECOMMENDATIONS

This study had three principal objectives.

1. To assess the potential for progressive degeneration of sediments in association with cage aquaculture operations.

The results from the first cycle suggested that at the end of the initial fallow period conditions at both farms, and particularly at Stringers Cove, had deteriorated compared to those pre-farming. Whilst this did not conclusively indicate that progressive deterioration had occurred, it was of some concern as it demonstrated that there was the potential for degeneration. We were unable to evaluate whether progressive deterioration would have occurred under the defined production scenario, as the stocking and feed regimes at both sites were markedly reduced in the second cycle, so that it was no longer appropriate to compare the two cycles.

These changes to the stocking/feed input in the second cycle did provide some useful information, suggesting that reduction in the farming intensity could result in marked improvement in both the rate and degree of recovery. This in turn suggests that relatively minor farm management adjustments can produce substantial environmental improvements.

It is important to note that in order to effectively evaluate whether progressive deterioration is occuring it is necessary to determine whether the conditions post-farming differ from what existed pre-farming. In this study the two farm sites had very different pre-farming community structures. Consequently, to determine the potential for progressive deterioration it is essential to establish baseline environmental conditions.

2. To adapt and develop novel combinations of monitoring techniques (identified by TAFI and CSIRO) to facilitate evaluation of sediment degradation associated with ongoing marine cage aquaculture operations.

Many approaches for farm-based monitoring have been assessed as part of this project. Benthic infaunal assessment was used as the standard by which the sensitivity, reliability and suitability of techniques was evaluated. Our findings suggest that visual assessment techniques are the most useful approach for farm-based monitoring.

We have made the visual assessment more objective by defining indices, based on easily identifiable visual criteria, which relate to specific stages of impact (Fig. 7.1.1.4). The proposed visual approaches can determine different levels of impact and therefore can be used to monitor both degradation and recovery. Discussions with industry stakeholders, environmental consultants and government regulators indicate that these indices would apply equally well in environments other than those included in the study. Consequently we are confident that with only minor and relatively simple modifications they would be applicable to the broader farming community.

Key faunal indicators have also been identified, and these complement the visual techniques. The indicator species are easy to distinguish and provide additional reliable ecological information on the sediment condition. In conjunction with the quantitative visual analysis information, this ecological information will enable farmers to obtain an

understanding of their sediment condition that they could only previously have achieved with the assistance of highly trained scientific professionals.

3. To incorporate these techniques into farm management protocols as tools for the evaluation and management of sediment condition in order to maximise sustainable aquaculture production.

The methods described in this study could be used for a variety of monitoring purposes. The proposed protocol was developed specifically in relation to on-farm monitoring, and was not intended for regulatory or compliance purposes. The purpose of the proposed monitoring programme is to provide farmers with sufficient information to enable them to incorporate an environmental condition factor into their current farm management strategies. To this end this study has defined a range of impact stages (Fig. 7.1.1.1) which categorise the sediment condition, which are applicable to a range of environments, and which can be easily established by farmers using the proposed techniques.

Accordingly, it is recommended that video assessment be adopted as the main approach for farm-based monitoring. Video footage should be obtained relatively frequently (at least monthly but preferably fortnightly) from cages within the farm, towards the end of the stocking cycle and over the fallow period, and this should be compared with footage from reference positions taken at the same times. Only a short (1-2 minute) video drop is necessary. Assessment can be done in the field or post-processed. If there is any uncertainty as to the classification resulting from the visual assessment, the findings could be validated with infaunal grabs and subsequent evaluation of key species. Other approaches (e.g. redox/sulphide, signature lipid analysis, microbial status) can be undertaken if a greater sensitivity or understanding of the system processes is required.

To ensure that the characterising features for the video assessment are relevant to a particular site it is essential to have baseline information on the benthic community structure and sediment conditions for that site. It is also recommended that infaunal grab samples be collected from representative reference and farm locations at regular intervals (i.e. every 2-3 years) to calibrate key indicators and identify any significant community changes. These samples would be quick to obtain, would not require any complex processing, their principal function being to identify and validate the key/dominant species. Photographic records should be taken of these samples to establish a baseline environmental archive, providing a pictorial record of the community structure. This can then be compared with subsequent evaluations (i.e. to identify any major community shifts, to validate indicator identifications and to validate categorisation of impact levels).

The application of all these recommended techniques is more specifically described in the associated interactive farm field guide (Macleod et al., 2004). This guide was developed in response to an extension of the original project, which was approved in January 2004 and included two additional objectives:

i. To develop a training package (field guide and multimedia cd) for the aquaculture industry to simply explain the techniques proposed in CRC project 4.1.

ii. To conduct a series of workshops in Tasmania to instruct farm personnel in the field sampling requirements, analysis and interpretation of the techniques recommended in CRC project 4.1 and in the field guide.

The field guide fully explains the sampling procedures and analytical techniques underpinning the visual assessments. Workshops have been conducted around Tasmania to train farm personnel in all of the recommended techniques so that these approaches can be rapidly adopted by industry.

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13. APPENDICES

13.1 Appendix 1 – Intellectual Property

13.2 Appendix 2 – Project Staff

Ms Catriona Macleod	Project Leader/ TAFI – University of Tasmania
Mrs Susan Forbes	TAFI – University of Tasmania
Mr Bob Connell/ Mr Sam Foster	TAFI – University of Tasmania
Dr John Volkman	CSIRO Marine Research
Dr Peter Nichols	CSIRO Marine Research
Dr Andy Revill	CSIRO Marine Research
Mr Danny Holdsworth	CSIRO Marine Research
Dr Chris Burke	TAFI – University of Tasmania
Mr Andrew Bissett	TAFI – University of Tasmania

13.3 Appendix 3 - Project Publications

13.3.1 Articles and Reports:

- Gibson, J., Armand, S., Rayner, M. and Nichols, P. (2003) Environmental Impact of Salmon Sea Cages on Marine Sediments at Dover, Tasmania: Signature Phospholipid Fatty Acids and Archaeally-derived Ether Lipids. Report 2003-CMR2R.

13.3.2 Conference Presentations:

2001

- Bissett (May 2001). Australian Society for Microbiology (Tasmanian Branch) – One day seminar on water microbiology.
- FRDC Atlantic Salmon Aquaculture Sub-Program conference July 18th – 19th 2001

2002

ASAS Conference (8th July 2002)

- C. Macleod (July 2002) Development of Novel Methods for the assessment of sedimetn condition and determination of management protocols for sustainable finfish cage aquaculture operations – Progress Report. ASAS Conference Handbook pp 43 – 47.
- S.Forbes, C.Macleod, I.Mitchell (Poster) (July 2002) Design, development and field trial of a sediment trap for use in evaluating organic loading associated with cage finfish culture. ASAS Conference Handbook pp 35-37.

AMSA Conference (Fremantle, Western Australia 10-12th July 2002)

- Revill, A. Holdsworth, D., Cook, P., Volkman, J., Nichols, P. and Macleod,C. (July 2002) Organic carbon and nitrogen cycling under salmon aquaculture cages in Tasmania.
- S. Forbes, C. Macleod, I. Mitchell (July 2002) What can sediment traps tell us about the environmental impact of marine finfish culture in Tasmania, Australia?
- S.Forbes, C.Macleod, I.Mitchell (Poster) (July 2002) Design, development and field trial of a sediment trap for use in evaluating organic loading associated with cage finfish culture.

Aquafin CRC Conference (22-24th September 2002)

- C. Macleod (September 2002) One Thing Leads to Another – The Relationship Between Biology and Chemistry in Sediment Recovery. Aquafin CRC Conference Abstracts p 29.
- Bisset (September 2002) Microbial Ecology of Fish Farm Sediments. Aquafin CRC Conference Abstracts p 30.

National Scientific Conference of the Australian Society for Microbiology (September 2002)

- Bissett, A.P., C.M. Burke and J.P. Bowman (September 2002) Microbial Ecology of Fish Farm Sediments.

CRC Workshop/Review (2002)

- Information on CRC project 4.1 presented by Dr John Volkman.

ASLO 2002 Conference, Victoria, Canada (10-14th June 2002)

- Macleod, C., Forbes, S., Volkman, J., Revill, A., Holdsworth, D., Burke, C. and Bissett, A. (June 2002). A multidisciplinary approach for evaluation and management of recovery processes in sediments associated with marine finfish culture in Tasmania, Australia.

2003

ASAS Conference (21st May 2003)

- C. Macleod (May 2003) Development of Novel Methods for the assessment of sediment condition and determination of management protocols for sustainable finfish cage aquaculture operations – An update on research progress. ASAS Conference Handbook pp 42-44.

Second Year CRC Review (14th August 2003)

- Information on CRC project 4.1 presented by Dr John Volkman.

The Institute of Biology, Aarhus University, Aarhus, Denmark (September 2003)

- The following paper was presented by Dr Chris Burke:
  Macleod, C., Bissett, A., Burke, C., Connell, R., Holdsworth, D., Forbes, S , Nichols, P., Revill, A and J. Volkman. 2003. Environmental Management of Sediments Underlying Salmon Seacage Farms in Tasmania, Australia. Preliminary findings.

34th Australian Entomological Society / 6th Invertebrate Biodiversity and Conservation Combined Conference in Hobart (October, 2003)

- Forbes, S. and Macleod, C. 2003. Development of Quantitative Visual Assessment Techniques for the Assessment of Sediment Condition at Marine Finfish Farms

Scientific Conference of the Australian/NZ Society for Microbiology (October 2003)

- Bissett, A., Bowman, J. and Burke, C. (2003) Ammonia-oxidiser communities in fish farm sediments.

Aquafin CRC conference (28th-30th October 2003)

- Forbes, S. and Macleod, C. (Poster) (October 2003). Development of Quantitative Visual Assessment Techniques for the Assessment of Sediment Condition at Marine Finfish Farms
- Macleod, C., Forbes, S., Bissett, A., Burke, C., Gibson, J., Holdsworth, D., Nichols, P., Revill, A and J. Volkman. (Poster) (October 2003). "Links Between Physical/Chemical and Ecological Techniques for the Assessment of Sediment Condition".

The Max Planck Institute for Marine Microbiology, Bremen, Germany (December 2003)

- The following paper was presented by Dr Chris Burke:
  Macleod, C., Bissett, A., Burke, C., Connell, R., Holdsworth, D., Forbes, S , Nichols, P., Revill, A, and J. Volkman. 2003. Environmental Management of Sediments Underlying Salmon Seacage Farms in Tasmania, Australia. Preliminary findings.

13.3.3 Media Presentations:

- Television interview with ABC News (C.Macleod) – Sunday 10th June 2001.
- ASAS Newsletter (Salmon Snippets, Newsflash, 27 March 2002) – Article detailing the recent collaboration between current project and AGD researchers in conducting a pilot study testing for the presence of gill amoeba in sediments.

13.4 Appendix 4 - Polar Lipid Fatty Acids and Ether Lipids

Table 1. PLFA composition (%) of Stringers Cove farm and reference sediments. 'tr', <0.05%; '-', not detected.

	Site															
Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
Normal Saturated Acids																
12:0	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
13:0	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
14:0	0.3	1.2	2.7	1.3	0.4	0.7	2.7	1.4	-	0.3	0.7	-	-	1.4	2.9	2.7
15:0	0.6	0.7	0.8	1.2	0.7	0.5	0.6	0.8	0.6	0.5	0.6	0.5	0.4	1.2	0.3	1.2
16:0	14.5	15.9	11.5	21.4	15.0	12.7	9.6	19.1	13.1	12.8	16.7	15.2	12.8	16.2	18.9	18.0
17:0	1.5	1.1	0.8	1.7	1.9	1.1	0.8	1.5	2.3	1.2	2.2	2.1	1.9	1.9	1.6	1.3
18:0	6.5	5.4	1.8	7.3	8.0	3.7	2.6	6.6	6.8	5.3	8.7	8.7	8.9	7.8	5.3	4.8
20:0	0.6	0.4	0.1	1.5	0.9	0.2	0.2	1.0	1.5	0.4	2.4	1.0	1.3	1.9	1.3	1.0
21:0	0.4	0.3	tr	0.1	0.7	0.2	0.1	0.1	0.9	-	0.2	-	0.8	0.2	tr	0.2
22:0	-	tr	tr	0.5	-	-	-	0.3	-	-	1.9	-	-	1.4	0.5	0.3
Total	24.4	24.9	17.8	35.0	27.6	19.2	16.6	30.9	25.1	20.5	33.5	27.5	25.9	32.1	30.9	29.5
Branched Saturated Acids																
i13:0	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
a13:0	-	-	0.1	-	-	-	tr	-	-	-	-	-	-	-	-	-
i14:0	-	0.1	0.3	0.1	-	0.1	0.2	0.1	-	-	0.5	-	-	0.2	0.6	0.6
i15:0	1.2	1.0	1.3	2.7	1.3	0.9	1.0	2.2	0.7	0.9	1.7	0.9	0.8	2.8	3.7	3.3
a15:0	2.6	1.5	1.9	3.3	2.9	1.4	1.6	2.5	2.1	2.0	2.4	1.4	2.1	4.4	4.9	4.2
br16:0	-	0.1	0.1	-	-	0.1	0.1	-	-	-	-	0.1	-	-	-	-
i16:0	1.3	0.6	0.5	1.0	1.3	0.6	0.4	0.6	1.2	0.9	1.4	1.3	1.2	1.6	1.1	0.9
10Me16:0	2.0	0.4	0.1	0.8	1.9	0.6	0.2	0.4	1.7	1.6	1.4	1.9	2.8	1.3	0.7	0.6
i17:0	1.2	0.7	0.5	1.0	1.3	0.8	0.5	0.8	1.4	0.8	1.6	1.3	1.4	1.3	0.9	0.7
Table 1 (cont)	Site															

Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
a17:0	1.9	0.9	0.5	1.4	2.0	1.1	0.6	0.9	2.3	1.2	1.7	2.0	1.9	2.0	1.4	1.2
Total	10.1	5.4	5.4	10.2	10.7	5.6	4.7	7.5	9.6	7.5	10.7	8.9	10.0	13.6	13.2	11.5
Normal Monounsaturated Acids																
13:1w7	-	-	-	-	-	-	tr	-	-	-	-	-	-	-	-	-
13:1w5	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
14:1w9	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
14:1w7	-	tr	0.1	-	-	-	0.1	0.2	-	-	-	-	-	-	0.1	0.2
14:1w5	-	-	0.1	-	-	-	0.1	-	-	-	-	-	-	-	-	-
15:1w6	-	0.2	0.4	0.1	-	0.1	0.2	0.1	-	-	-	-	-	-	-	0.3
16:1w9c	1.2	0.6	0.1	0.7	1.3	0.6	0.5	0.4	1.3	1.1	0.5	1.3	1.2	0.6	1.0	0.9
16:1w7c	6.9	9.3	10.3	-	6.9	7.7	6.0	11.5	6.2	6.3	5.1	5.6	4.9	6.0	13.3	12.4
16:1w7t	1.0	0.7	1.0	1.4	0.8	0.7	0.6	1.2	2.0	0.6	0.2	0.6	0.5	0.3	1.3	1.2
16:1w5c	2.4	1.1	1.1	1.9	2.5	1.2	0.8	1.5	0.3	1.7	1.9	2.3	2.0	2.3	2.4	2.0
17:1w8	1.3	0.6	0.9	0.9	1.5	0.7	0.6	0.6	1.6	1.0	0.6	1.6	1.4	0.8	0.9	0.8
17:1w6	1.9	1.0	1.1	1.3	2.3	1.1	0.7	0.9	2.7	1.5	1.2	2.2	2.2	1.4	1.4	1.2
18:1w9c	9.0	7.0	7.9	9.0	8.2	8.9	6.7	7.7	9.2	8.7	6.5	8.6	8.3	5.6	6.4	8.8
18:1w7c	13.4	8.7	7.2	10.9	14.6	9.7	5.6	9.1	16.4	10.2	11.9	15.3	15.2	10.5	9.6	10.3
18:1w7t	1.1	0.5	0.6	0.9	0.8	0.8	0.7	0.8	1.1	0.7	0.3	1.1	0.9	0.2	0.6	0.6
18:1w5c	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.4	0.2	0.2	0.5	0.4	0.2	0.2	0.2
20:1w9	1.8	1.8	0.5	0.8	1.1	0.8	1.7	1.0	2.7	0.6	2.3	2.0	-	3.0	1.3	0.7
20:1w7	0.3	0.6	0.4	0.3	0.3	0.7	0.7	0.1	-	0.4	0.5	-	1.0	0.2	0.2	0.1
22:1w11	-	0.1	0.1	-	-	0.1	0.1	-	-	-	-	-	-	-	-	-
22:1w9	-	0.2	0.1	-	-	0.2	0.2	-	-	-	-	-	-	-	-	-
22:1w7	-	0.1	tr	-	-	tr	0.1	-	-	-	-	-	-	-	-	-
Total	40.7	32.9	32.3	28.2	40.6	33.5	25.6	35.2	43.7	33.0	31.4	41.0	37.9	31.3	0.0	0.0
Table 1 (cont)	Site															

Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
Branched Monounsaturated Acids																
i13:1w5	-	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	-
i15:1w6	0.2	0.1	0.3	-	-	tr	0.1	-	-	0.3	-	-	-	-	-	-
i16:1w5	0.3	0.1	0.1	-	0.3	0.1	0.1	-	-	0.2	-	0.3	-	-	-	-
i17:1w7	1.0	0.6	0.6	1.0	1.2	0.6	0.4	0.7	0.9	1.2	0.3	0.9	0.9	0.2	2.2	0.8
Total	1.5	0.8	1.0	1.0	1.5	0.8	0.6	0.7	0.9	1.7	0.3	1.2	0.9	0.2	2.2	0.8
Polyunsaturated Acids																
18:3w6	-	0.1	0.2	0.1	-	0.1	0.2	0.1	-	-	-	-	-	-	-	0.1
18:2	0.3	0.1	-	-	0.6	-	-	-	-	0.2	-	0.3	0.3	-	-	-
18:4w3	0.3	0.4	0.8	0.4	-	0.6	0.7	0.5	-	0.4	0.3	0.2	0.1	0.4	0.7	1.0
18:2w9?	-	0.3	-	0.3	-	0.3	0.1	0.3	-	-	0.3	0.5	0.5	0.4	0.3	0.5
18:2w6	1.8	2.3	2.9	3.7	1.5	2.6	2.7	3.7	1.3	2.3	1.2	0.9	0.9	1.1	2.3	2.8
20:4w6	3.9	3.0	2.8	1.7	3.7	3.5	3.6	1.5	5.2	5.1	3.1	5.6	6.0	4.0	1.2	1.4
20:5w3	5.0	13.6	20.1	3.9	2.4	15.9	23.4	5.0	3.6	15.6	4.2	4.1	4.1	5.4	2.2	4.8
20:3w6	0.1	0.5	0.4	0.1	0.1	0.5	0.6	0.1	0.3	0.3	0.2	-	-	-	0.1	tr
20:4w3 + 20:2 NMI	0.4	0.9	1.1	0.9	-	0.9	1.6	0.5	-	0.8	2.9	-	-	1.8	0.8	0.1
20:2w6	2.0	0.7	0.7	0.8	0.8	1.2	1.1	0.5	-	0.6	3.4	-	2.9	2.4	0.6	0.2
C21 PUFA	-	0.2	0.1	0.3	0.9	0.2	0.2	0.4	-	0.4	0.7	-	1.1	1.9	0.3	0.3
22:5w6	-	0.3	0.2	0.3	0.3	0.3	0.3	0.2	-	0.2	0.3	0.3	0.4	0.2	0.2	0.3
22:6w3	2.3	7.1	8.3	9.2	2.3	7.9	8.9	9.0	2.2	5.2	2.9	3.2	2.9	1.9	2.9	4.4
22:4w6	1.7	0.7	0.4	0.3	1.7	0.8	0.7	0.2	2.3	1.2	0.6	2.6	2.1	0.2	0.1	0.1
22:5w3	0.9	2.4	2.3	1.1	0.6	2.8	3.9	1.4	0.7	2.3	0.7	-	1.1	0.5	0.4	0.6
22:3w6	-	0.3	0.3	-	-	0.3	0.8	-	-	0.3	-	-	-	-	-	-
22:2 NMI	-	1.0	0.4	-	-	0.6	1.0	-	-	0.4	-	-	-	-	-	-

Table 1 (cont)	Site

Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
22:2 NMI	-	0.1	0.5	-	0.5	0.3	0.9	-	-	0.3	-	-	-	-	-	-
22:2w6	-	0.1	0.1	-	-	0.1	0.2	-	-	-	-	-	-	-	-	-
Total	18.8	34.2	41.7	23.3	15.6	38.9	51.0	23.4	15.6	35.6	21.1	17.7	22.3	20.4	12.1	16.7
2- and 3-Hydroxy Acids																
12:0 2OH	-	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	-
13:0 2OH	-	0.1	0.1	-	-	0.1	0.1	-	-	-	-	-	-	-	1.4	-
14:0 3OH + 14:0 2OH	0.5	tr	tr	0.1	0.4	0.1	-	0.1	-	0.1	0.3	-	-	0.3	0.1	tr
15:0 2OH	0.2	0.2	0.1	0.7	0.2	0.2	0.1	-	-	-	-	0.2	-	-	-	-
16:0 3OH	1.5	0.4	0.3	0.4	1.1	0.4	0.3	0.3	1.1	0.5	1.2	2.1	1.2	0.9	0.5	0.4
16:0 2OH	-	-	0.1	-	-	-	0.1	0.3	1.1	-	-	-	-	-	0.5	0.4
i17:0 3OH	0.5	0.5	0.2	0.6	0.6	0.3	0.1	0.2	0.7	0.3	-	0.5	0.6	1.0	0.1	0.3
i17:0 2OH	0.5	-	0.1	-	0.5	0.2	0.2	0.3	0.6	0.2	1.2	-	0.6	-	0.4	0.7
a17:0 3OH + a17:0 2OH	0.8	0.4	0.3	0.4	0.7	0.5	0.4	1.1	1.6	0.5	-	0.9	0.6	-	-	-
18:0 2OH	0.5	0.2	0.1	-	0.5	0.2	0.2	-	-	-	-	-	-	-	-	-
Total	4.4	1.8	1.3	2.3	4.0	2.1	1.4	2.3	5.0	1.7	2.7	3.7	3.0	2.2	2.9	1.9

Table 2. PLFA composition (µg g^{-1} dry weight) of Stringers Cove farm and reference sediments. 'tr', <0.05 µg g^{-1} dry weight; '-', not detected.

	Site															
Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
Normal Saturated Acids																
12:0	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
13:0	-	-	0.1	-	-	-	tr	-	-	-	-	-	-	-	-	-
14:0	tr	0.6	4.6	0.3	tr	0.3	3.0	0.7	-	tr	tr	-	-	0.1	0.9	1.2
15:0	tr	0.3	1.3	0.2	tr	0.2	0.6	0.4	tr	tr	tr	tr	tr	0.1	0.1	0.6
16:0	1.0	7.6	19.5	4.2	1.0	4.8	10.5	10.0	0.7	1.3	0.8	0.5	0.4	0.8	6.0	8.2
17:0	0.1	0.5	1.3	0.3	0.1	0.4	0.9	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0.5	0.6
18:0	0.5	2.6	3.0	1.4	0.5	1.4	2.8	3.4	0.4	0.5	0.4	0.3	0.3	0.4	1.7	2.2
20:0	tr	0.2	0.2	0.3	0.1	0.1	0.2	0.5	0.1	tr	0.1	tr	tr	0.1	0.4	0.4
21:0	tr	0.1	0.1	tr	tr	0.1	0.1	0.1	tr	-	tr	-	tr	tr	tr	0.1
22:0	-	tr	tr	0.1	-	-	-	0.2	-	-	0.1	-	-	0.1	0.2	0.1
Total	1.6	11.9	30.0	6.9	1.6	7.2	18.1	16.2	1.2	1.9	1.5	0.9	0.8	1.7	9.7	13.5
Branched Saturated Acids																
i13:0	-	-	tr	-	-	-	tr	-	-	-	-	-	-	-	-	-
a13:0	-	-	0.1	-	-	-	tr	-	-	-	-	-	-	-	-	-
i14:0	-	0.1	0.6	tr	-	tr	0.3	0.1	-	-	tr	-	-	tr	0.2	0.3
i15:0	0.1	0.5	2.1	0.5	0.1	0.3	1.1	1.2	tr	0.1	0.1	tr	tr	0.1	1.2	1.5
a15:0	0.2	0.7	3.3	0.7	0.2	0.5	1.7	1.3	0.1	0.2	0.1	tr	0.1	0.2	1.5	1.9
br16:0	-	tr	0.2	-	-	tr	0.1	-	-	-	-	tr	-	-	-	-
i16:0	0.1	0.3	0.9	0.2	0.1	0.2	0.4	0.3	0.1	0.1	0.1	tr	tr	0.1	0.3	0.4
10Me16:0	0.1	0.2	0.2	0.2	0.1	0.2	0.3	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.3
i17:0	0.1	0.3	0.8	0.2	0.1	0.3	0.6	0.4	0.1	0.1	0.1	tr	tr	0.1	0.3	0.3

Table 2 (cont)	Site

Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
a17:0	0.1	0.5	0.8	0.3	0.1	0.4	0.7	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.5	0.5
Total	0.7	2.5	9.0	2.0	0.7	2.0	5.1	3.9	0.5	0.8	0.5	0.1	0.2	0.7	4.2	5.3
Normal Monounsaturated Acids																
13:1w7	-	-	-	-	-	-	tr	-	-	-	-	-	-	-	-	-
13:1w5	-	-	0.1	-	-	-	tr	-	-	-	-	-	-	-	-	-
14:1w9	-	-	0.1	-	-	-	tr	-	-	-	-	-	-	-	-	-
14:1w7	-	tr	0.2	-	-	-	0.1	0.1	-	-	-	-	-	-	tr	0.1
14:1w5	-	-	0.2	-	-	-	0.1	-	-	-	-	-	-	-	-	-
15:1w6	-	0.1	0.7	tr	-	0.1	0.2	0.1	-	-	-	-	-	-	-	0.1
16:1w9c	0.1	0.3	0.2	0.1	0.1	0.2	0.5	0.2	0.1	0.1	tr	tr	tr	tr	0.3	0.4
16:1w7c	0.5	4.5	17.4	-	0.4	2.9	6.5	6.0	0.3	0.6	0.2	0.2	0.2	0.3	4.2	5.7
16:1w7t	0.1	0.3	1.7	0.3	0.1	0.3	0.6	0.7	0.1	0.1	tr	tr	tr	tr	0.4	0.5
16:1w5c	0.2	0.5	1.9	0.4	0.2	0.4	0.9	0.8	tr	0.2	0.1	0.1	0.1	0.1	0.8	0.9
17:1w8	0.1	0.3	1.5	0.2	0.1	0.3	0.6	0.3	0.1	0.1	tr	0.1	tr	tr	0.3	0.4
17:1w6	0.1	0.5	1.9	0.3	0.1	0.4	0.8	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.5	0.6
18:1w9c	0.6	3.4	13.3	1.8	0.5	3.3	7.4	4.1	0.5	0.9	0.3	0.3	0.3	0.3	2.0	4.0
18:1w7c	0.9	4.2	12.1	2.2	0.9	3.6	6.1	4.8	0.9	1.0	0.5	0.5	0.5	0.5	3.0	4.7
18:1w7t	0.1	0.2	1.1	0.2	0.1	0.3	0.8	0.4	0.1	0.1	tr	tr	tr	tr	0.2	0.3
18:1w5c	tr	0.1	0.3	tr	tr	0.1	0.2	0.1	tr	tr	tr	tr	tr	tr	0.1	0.1
20:1w9	0.1	0.9	0.9	0.2	0.1	0.3	1.9	0.5	0.1	0.1	0.1	0.1	-	0.2	0.4	0.3
20:1w7	tr	0.3	0.8	0.1	tr	0.2	0.8	0.1	-	tr	tr	-	tr	tr	0.1	0.1
22:1w11	-	0.1	0.1	-	-	tr	0.2	-	-	-	-	-	-	-	-	-
22:1w9	-	0.1	0.2	-	-	0.1	0.2	-	-	-	-	-	-	-	-	-
22:1w7	-	tr	tr	-	-	tr	0.1	-	-	-	-	-	-	-	-	-
Total	2.8	15.7	54.6	5.5	2.6	12.5	27.9	18.5	2.3	3.3	1.3	1.3	1.1	1.5	12.2	18.1

Table 2 (cont)	Site

Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
Branched Monounsaturated Acids																
i13:1w5	-	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	-
i15:1w6	tr	tr	0.5	-	-	tr	0.1	-	-	tr	-	-	-	-	-	-
i16:1w5	tr	0.1	0.2	-	tr	tr	0.1	-	-	tr	-	tr	-	-	-	-
i17:1w7	0.1	0.3	1.0	0.2	0.1	0.2	0.5	0.4	tr	0.1	tr	tr	tr	tr	0.7	0.4
Total	0.1	0.3	1.7	0.2	0.1	0.2	0.7	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.7	0.4
Polyunsaturated Acids																
18:3w6	-	0.1	0.3	tr	-	tr	0.2	tr	-	-	-	-	-	-	-	tr
18:2	tr	0.1	-	-	tr	-	-	-	-	tr	-	tr	tr	-	-	-
18:4w3	tr	0.2	1.4	0.1	-	0.2	0.8	0.2	-	tr	tr	tr	tr	tr	0.2	0.5
18:2w9?	-	0.1	-	0.1	-	0.1	0.1	0.2	-	-	tr	tr	tr	tr	0.1	0.2
18:2w6	0.1	1.1	4.8	0.7	0.1	1.0	3.0	2.0	0.1	0.2	0.1	tr	tr	0.1	0.7	1.3
20:4w6	0.3	1.5	4.7	0.3	0.2	1.3	3.9	0.8	0.3	0.5	0.1	0.2	0.2	0.2	0.4	0.6
20:5w3	0.4	6.5	34.0	0.8	0.2	5.9	25.6	2.6	0.2	1.6	0.2	0.1	0.1	0.3	0.7	2.2
20:3w6	tr	0.2	0.6	tr	tr	0.2	0.7	tr	tr	tr	tr	-	-	-	tr	tr
20:4w3 + 20:2 NMI	tr	0.4	1.8	0.2	-	0.4	1.7	0.2	-	0.1	0.1	-	-	0.1	0.2	0.1
20:2w6	0.1	0.3	1.2	0.2	0.1	0.4	1.3	0.3	-	0.1	0.2	-	0.1	0.1	0.2	0.1
C21 PUFA	-	0.1	0.2	0.1	0.1	0.1	0.2	0.2	-	tr	tr	-	tr	0.1	0.1	0.1
22:5w6	-	0.1	0.4	0.1	tr	0.1	0.3	0.1	-	tr	tr	tr	tr	tr	0.1	0.1
22:6w3	0.2	3.4	14.0	1.8	0.1	3.0	9.7	4.7	0.1	0.5	0.1	0.1	0.1	0.1	0.9	2.0
22:4w6	0.1	0.3	0.7	0.1	0.1	0.3	0.8	0.1	0.1	0.1	tr	0.1	0.1	tr	tr	tr
22:5w3	0.1	1.1	4.0	0.2	tr	1.1	4.3	0.7	tr	0.2	tr	-	tr	tr	0.1	0.3
22:3w6	-	0.2	0.6	-	-	0.1	0.8	-	-	tr	-	-	-	-	-	-
22:2 NMI	-	0.5	0.7	-	-	0.2	1.1	-	-	tr	-	-	-	-	-	-

Table 2 (cont)	Site

Fatty Acid	17-1				17-2				Ref 1			Ref 2			17-1	17-2
	T 0	T 4.5	T 9	T 13	T 0	T 4.5	T 9	T 13	T 4.5	T 9	T 13	T 0	T 4.5	T 13	T 22	T 22
22:2 NMI	-	0.1	0.8	-	tr	0.1	1.0	-	-	tr	-	-	-	-	-	-
22:2w6	-	tr	0.2	-	-	tr	0.2	-	-	-	-	-	-	-	-	-
Total	1.2	16.3	70.4	4.6	0.9	14.5	55.8	12.2	0.8	3.3	0.8	0.5	0.6	1.0	3.8	7.5
2- and 3-Hydroxy Acids			42.4													
12:0 2OH	-	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	-
13:0 2OH	-	tr	0.1	-	-	tr	0.1	-	-	-	-	-	-	-	0.4	-
14:0 3OH + 14:0 2OH	0.5	tr	tr	0.1	0.4	0.1	-	0.1	-	0.1	0.3	-	-	0.3	0.1	tr
15:0 2OH	0.2	0.2	0.1	0.7	0.2	0.2	0.1	-	-	-	-	0.2	-	-	-	-
16:0 3OH	1.5	0.4	0.3	0.4	1.1	0.4	0.3	0.3	1.1	0.5	1.2	2.1	1.2	0.9	0.5	0.4
16:0 2OH	-	-	0.1	-	-	-	0.1	0.3	1.1	-	-	-	-	-	0.5	0.4
i17:0 3OH	0.5	0.5	0.2	0.6	0.6	0.3	0.1	0.2	0.7	0.3	-	0.5	0.6	1.0	0.1	0.3
i17:0 2OH	0.5	-	0.1	-	0.5	0.2	0.2	0.3	0.6	0.2	1.2	-	0.6	-	0.4	0.7
a17:0 3OH + a17:0 2OH	0.8	0.4	0.3	0.4	0.7	0.5	0.4	1.1	1.6	0.5	-	0.9	0.6	-	-	-
18:0 2OH	0.5	0.2	0.1	-	0.5	0.2	0.2	-	-	-	-	-	-	-	-	-
Total	4.4	1.8	1.3	2.3	4.0	2.1	1.4	2.3	5.0	1.7	2.7	3.7	3.0	2.2	2.9	1.9

Table 3. Total PLFA concentration, estimated microbial biomass and estimated microbial abundance of Stringers Cove farm site and reference sediments.

Sample	Total PLFA µg g^{-1} dry weight	Microbial biomass mg g^{-1} dry weight	Bacterial abundance cells g^{-1} dry weight
17-1 T0	7.1	0.26	1.5E+09
17-1 T4.5	47.9	1.77	1.0E+10
17-1 T9	168.9	6.26	3.7E+10
17-1 T13	19.8	0.73	4.3E+09
17-2 T0	6.4	0.24	1.4E+09
17-2 T4.5	37.4	1.39	8.2E+09
17-2 T9	109.5	4.06	2.4E+10
17-2 T13	52.5	1.94	1.2E+10
17A-1 T22	31.7	1.17	6.9E+09
17A-2 T22	45.7	1.69	9.9E+09
Ref 1 T4.5	5.2	0.19	1.1E+09
Ref 1 T9	10.0	0.37	2.2E+09
Ref 1 T13	4.6	0.17	1.0E+09
Ref 2 T0	3.5	0.13	7.6E+08
Ref 2 T4.5	3.5	0.13	7.6E+08
Ref 2 T13	5.2	0.19	1.1E+09