Final Report

AQUAFIN CRC - SOUTHERN BLUEFIN TUNA AQUACULTURE SUBPROGRAM: TUNA ENVIRONMENT SUBPROJECT EVALUATION OF WASTE COMPOSITION AND WASTE MITIGATION

Milena Fernandes, Peter Lauer, Anthony Cheshire, Ib Svane, Sapto Putro, Genevieve Mount, Michael Angove, Talya Sedawie, Jason Tanner, Peter Fairweather, Jeremy Barnett and Annette Doonan

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Objectives

1. To determine the type and quantity of waste produced by sea-cage operations across a range of management and environmental regimes.
2. To develop and validate a model or modify an existing model (e.g. DEPOMOD) of the waste dynamics of sea-cage operations incorporating information on stocking density, feed type and a number of environmental and management parameters to quantify the extent and intensity of localised impacts.
3. Obtain information on the composition of the fouling community on and adjacent to tuna cages with reference to potential polyculture species with an assessment of their potential biological and economic viability.
4. To develop a sampling program for benthic assemblages exposed to waste from tuna farming operations with reference to the efficacy of fallowing as a waste remediation process. This will form the basis for consideration of alternative waste mitigation strategies and for recommending improvements to environmental monitoring regimes.
5. To test the potential of integrated farming in mitigation studies. This involves the use of benthic filter feeders (e.g. blue mussels) benthic surface feeders (holothurids and crabs) and bioturbators (e.g. stingrays, fish or native oysters).
6. To evaluate potential applications of technological approaches (e.g. diaper systems) to waste mitigation.

Outcomes achieved to date

The project results and outputs have contributed to the following outcomes:

- Information to quantitatively assess the impact from SBT farming that will provide greater certainty in planning and thereby help to secure tenure and access to sites for aquaculture industries in marine environments.
- An ability to model changes in waste under different management strategies that will allow predictions about the ecological consequences (and through this the risks to stock) of the application of new technologies or mitigation solutions.
- A better understanding of environmental issues associated with SBT farming, necessary to refine monitoring programs, licensing and regulatory frameworks. Government bodies use this information to facilitate the development of a strategic approach to adaptive management, essential to warrant public support to coastal aquaculture developments.

This project investigated the dynamics of waste production within southern bluefin tuna (SBT) pens in waters off Port Lincoln in lower Spencer Gulf, South Australia, and the impacts associated with the release of these wastes in the environment. Whereas coastal aquaculture operations are known to be input sources of nutrients into the marine environment (e.g. uneaten feed, faecal matter, metabolic products), little was known about the composition or quantity of wastes released by SBT farms or the appropriate measures for managing or minimising environmental impacts. This information was necessary to promote environmental conditions that are optimum for both production and the health of the aquatic environment, ensuring access to sites and security of tenure for finfish farmers within a framework that is proven to be environmentally sustainable.

To determine the type and quantity of waste produced by sea-cages, we measured nutrient contents and leaching rates from feeds and faeces, sedimentation rates and benthic fluxes in the farms. Wastes are released mostly in dissolved form as a result of the extremely high metabolic rates of SBT and associated high discharges of urine and gill excretory products. Dissolved nutrients are also lost to the water column by leaching from solid wastes, and losses are 3-4 times higher when SBT are fed baitfish compared to pellets. Although sedimentation rates are up to 10 times the natural background, solid wastes are quickly metabolized and released back into the water column, resulting in low accumulation in the sediments. The high feed conversion ratios (FCR) associated with a diet of baitfish result in nitrogen loads to the environment in excess of 260 kg N $tonne^{-1}$ growth, more than double values for other aquaculture species fed manufactured pellets. Considering Australian current production of 4,380 tonnes per year, total annual loads to the environment can reach 1,137 tonnes N, including 983 tonnes N released as dissolved products. Actions to reduce these loads could include improving feeding strategies and associated technologies, and producing diets with an optimal protein/energy ratio so that energy requirements are met by non-protein sources.

To develop a model of waste-dynamics, we assessed the fate of nitrogen in the system based on environmental and farm management data, and estimates of fish metabolism. Only a small fraction (7-12%) of nitrogen in feed is retained for growth. Metabolic losses correspond to 59-64% of inputs, leaching to the water column to 10% and remineralization in the sediments to 7-11%. Overall, between 84 and 92% of nitrogen in feed is lost as dissolved wastes. Particulate wastes account for only a small fraction (8-12%) of environmental losses, occurring mostly through sediment accumulation (maximum 6 %) or export out of the system with current flows (maximum 12 %). The importance of dissolved wastes combined with the low settling velocity of SBT faeces and the high scavenging activity in the area lead to minimal impacts to the benthos at current stocking densities and holding periods. Effects are transitory rather than chronic and changes are reversible as a result of fast turnover periods. Although nutrients are rapidly recycled within the system, the benthic impact of pens is noticeable through a shift to finer sediments where infauna abundance is greater and diversity, number of taxa and evenness is lower.

The nature of the wastes indicates that these are not confined to the footprint of the pens and might spread over a large area. When compared to other coastal sites within Spencer Gulf, the offshore SBT farming zone has finer sediments, with occasional high levels of organic carbon and total nitrogen, and distinct macrobenthic assemblages, as well as higher water column phosphate concentrations. These variables suggest that the entire area might be subject to some disturbance, particularly during the farming season. Potential regional effects are now being considered through a follow up project of the Aquafin CRC.

To obtain information on the composition of the fouling community on and adjacent to sea-cages, an experiment was conducted to test the efficacy of the proprietary antifoulant Wattyl NetClear® on the nets. The dominating fouling organisms were the green algae *Entermorpha sp*. at shallow depths and sponges at deeper depths, with low settlement of blue mussels and paper oysters. The largest cover of fouling was observed in April-May. The use of effective and environmentally friendly anti-fouling alternatives could help minimize deposition to the benthos and therefore increase rates of recovery during fallowing. The use of Wattyl NetClear® coating in this study proved unsuccessful in significantly reducing biofouling on SBT nets as a strategy to minimize deposition.

To develop a sampling program to assess the efficacy of fallowing, we monitored infauna in 8 fallowed and 8 control sites over a full year. Fallowing is the only strategy currently employed to allow benthic recovery, with pen sites left to fallow for a period of two years. Lumbrinerids and spionids were identified as the best macrobenthic taxa for assessing the level of disturbance at fallowed sites. The examination of infauna assemblages suggests that the recovery of the benthos is slow and farmed sites remain moderately disturbed after 12 months. Some variation in rates of recovery between sites is expected as the area covers distinct sediment types.

To test the potential of integrated farming and evaluate potential applications of technological approaches, we completed a desktop study to evaluate (1) the species of economic interest occurring naturally in and around commercial farms and (2) engineering solutions to waste mitigation. Polyculture appears as a promising waste mitigation alternative for SBT farming, with the added benefit of increasing profitability. An integrated system combining SBT, abalone/sea urchins and red macroalgae would be beneficial on both economical and environmental terms. Abalone/sea urchins would intercept particulate wastes while macroalgae would reduce the loads of dissolved wastes. Other native benthic scavengers such as spider crabs and sea cucumbers could also be used to reduce benthic impacts. Organisms considered for polyculture should be tested for their potential to act as reservoirs of SBT parasites. On a regional level, production of mussels and/or oysters could be enhanced by correct positioning in relation to the movement of particulate wastes from SBT farms. At this stage, engineering solutions for waste mitigation in such open water systems would be prohibitive as a consequence of high cost and the need to test and validate available technologies in open ocean environments.

Although the major aspects of waste production have been covered by this work, we still have no clear idea of the spatial and temporal gradients of impact. This information is necessary to refine fallowing and site rotation regimes. More information is necessary to establish how effects on sediment composition, structure and fauna vary with distance from stocked pens according to sediment types and water circulation. To better understand how nutrient loads vary during the course of the season, there is a need to estimate how SBT feed intake, growth and body composition vary with size of fish and water temperature. This level of detail would allow calculation of loads on more suitable time scales (e.g. months) to pinpoint periods where inputs from SBT farms are more likely to have an effect on the natural functioning of the ecosystem.

Keywords

Spencer Gulf, Port Lincoln, southern bluefin tuna, finfish, aquaculture, polyculture, environmental impacts, environmental mitigation, fallowing, nutrients, organic carbon, nitrogen, phosphorus, oxygen uptake rates, nutrient fluxes, benthic infauna, fouling, sediments, geochemistry, mass balances, modelling.

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We also would like to thank all those who have been involved in the project. The primary contributors are listed in Appendix 2, although many volunteers and other SARDI and University staff have also contributed to various aspects of the project. The latter appear in the individual acknowledgement sections of the different data chapters. Special thanks to Professor Anthony Cheshire, who originated this project, and who acted as its Principal Investigator in the early stages.

Chapter 1: General Introduction

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1.1. Background

1.1.1. Fish aquaculture and the environment

The aquaculture sector currently produces over 36% of the world's fish supply, up from 7% in 1970 (FAO, 2003). Australian domestic demand for seafood is expected to reach 80,000 tonnes above current consumption by 2020 and the expectation is to meet the gap by increasing aquaculture production (FRDC, 2003). As a consequence, the past decade has seen considerable expansion of the industry in the country, with the value of production more than doubling from $331 million in 1992/1993 to $743 million in 2002/2003 (FRDC, 2004). Industry sustainability is directly linked to a healthy environment and its rapid growth has increased awareness of environmental effects. Finfish pens in particular are open systems that act as point sources of wastes in coastal areas (Figure 1.1). Nutrients supplied to the fish with feed are digested and metabolic wastes released directly into the water column as dissolved products. The material that is not digested is excreted as faeces. Soluble nutrients in faeces and uneaten feed will leach into water during transit to the seafloor (Phillips et al., 1993; Chen et al., 2003). Solid wastes reaching the sediments accumulate in the vicinity of the pens leading to an increase in microbial biomass (Karakassis et al., 2000), oxygen consumption by heterotrophic organisms and overall sediment metabolism (Holmer & Kristensen, 1992). These processes promote remineralization of organic matter at the sediment-water interface leading to an efflux of inorganic nutrients from the sediments (Christensen et al., 2000; Strain & Hargrave, 2005).

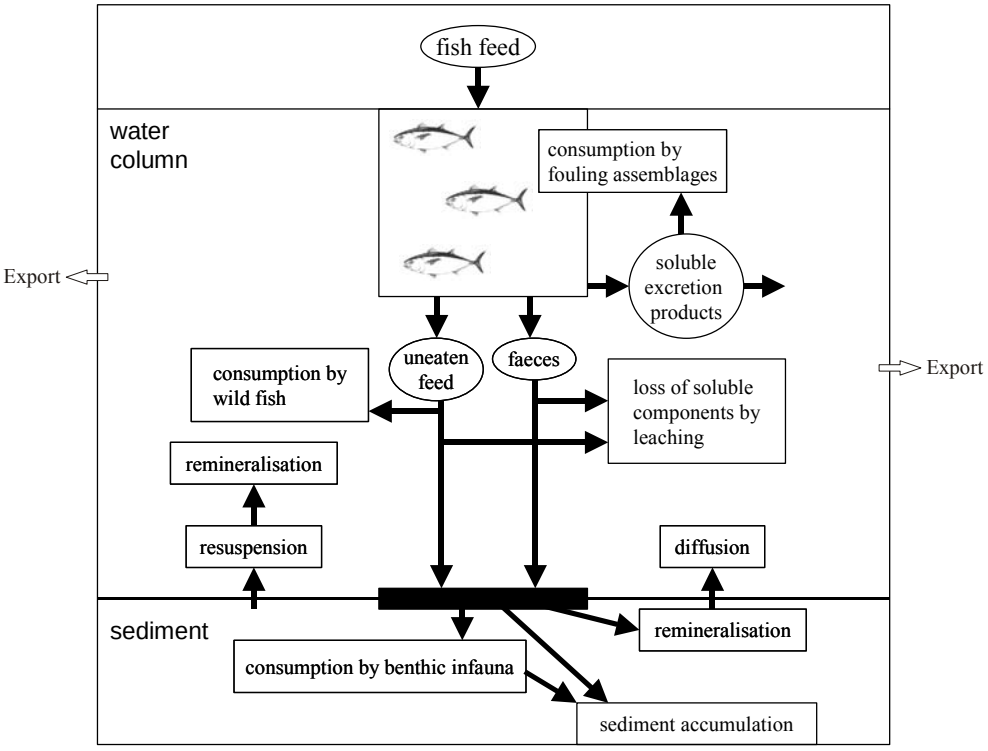

Figure 1.1. Conceptual model of waste flows in a marine fish farm.

The excretion of metabolic products, leaching of solid wastes and regeneration of nutrients in the sediments as a result of fish farming will act to increase the load of nitrogen and phosphorus available for primary productivity in the water column. Coastal marine systems are generally nutrient-limited and a surplus of nitrogen and phosphorus may affect ecosystem balance by promoting algal growth and potentially blooms (Paerl, 1997; Russell et al., 2005). This is an undesirable effect not only from an environmental point of view, but also for the farm operator as algal blooms can affect the health of the stock (Cembella et al., 2002; Treasurer et al., 2003). The increase in feed availability in the water column may also disturb other levels of the trophic chain, e.g. changing the composition and increasing densities of scavenger populations such as fish and birds. Although pelagic effects might be various and far reaching, with implications for ecosystem health that are currently not fully understood, these will depend on industry practices and environmental conditions and can potentially be avoided with adaptive management.

The large amount and continuous deposition of solid wastes underneath the pens act to change the physical structure and nutrient availability in the sediments and can affect the community structure of both benthic fauna and flora. Benthic assemblages characteristic of the area are replaced by opportunistic species that have an adaptive advantage and can use the change in conditions to establish an ecological niche (Brown et al., 1987; Weston, 1990; Karakassis et al., 2000). In areas of heavy deposition the demand for oxygen at the sediment-water interface may exceed supply from water circulation and sediments become anoxic. One of the consequences can be the formation of an azoic zone devoid of benthic infauna. Anaerobic conditions also favour the formation of toxic compounds such as ammonia and hydrogen sulphide, and other reduced compounds such as methane, which in extreme cases degas from the sediments and impact fish growing in the pens (Gowen & Bradbury, 1987). Other adverse effects on farmed stocks directly related to self-pollution include development of conditions favourable to the spread of parasitic and microbial infections (Braaten et al., 1983). The scale of benthic impacts will largely depend on the size of the operation and the assimilative characteristics of the surrounding environment.

Several reports on the environmental effects of finfish farming have appeared in the scientific literature as a consequence of long-term experiences with salmon and trout farms, primarily in the Northern Hemisphere. Variables affecting the amount of wastes released include management (e.g. feed wastage, stocking density, feeding regime), feed quality (e.g. stability and solubility in water, digestibility, nutrient contents) and fish metabolism (e.g. growth, excretion) (Islam, 2005). Feed management and engineering advances have significantly reduced waste over the last three decades (Enell, 1995). The amount of nitrogen in feed actually retained in fish growth is expected to be lower than 40% for most farmed fish species, whereas for phosphorus values are even lower, between 10 and 30% (Islam, 2005; Strain & Hargrave, 2005; Fernandes et al., submitted-b). The fraction not used for fish growth is lost to the environment as dissolved metabolic products or as faecal matter. More than 80% of nitrogen environmental losses are believed to occur in dissolved form (Islam, 2005). In contrast, much less phosphorus is released in dissolved form. Although values reported in the literature vary over a wide range (38-66%), most of the phosphorus released is expected to accumulate in the sediments (Phillips et al., 1985; Holby & Hall, 1991; Islam, 2005). The limit adopted by the UK for the nitrogen load from aquaculture production into coastal areas is 123 kg N $tonne^{-1}$ growth (Islam, 2005). In Scandinavia, typical loads are less than 55 kg N $tonne^{-1}$ growth and 5 kg P $tonne^{-1}$ growth (Enell, 1995; Fernandes et al., submitted-b). Although it is advisable to have a limit for nutrient release per tonne of production, any ecological effects will be ultimately driven by the total load released into the

environment according to the total production tonnage for a given farming area. These total loads need then to be considered in the context of local conditions so that they do not exceed the environmental carrying capacity of the area. Environmental characteristics, such as water circulation and benthic assimilative capacity, will ultimately determine the fate of wastes and its effect on the environment and farm operations.

In order to manage the impact of wastes released to the environment, the finfish aquaculture industry usually employs a number of preventative or remediation strategies. To combat the build up of organic matter beneath salmon pens and to limit the health risks to stock, operators are required to move pens on a regular basis with the resulting "fallow" period allowing recovery of the site before pens are reinstalled (Frid & Mercer, 1989; Bron et al., 1993). While fallowing is the most common approach to waste management in sea-cage aquaculture, rates of recovery vary substantially between locations from as little as 10 weeks up to 18 months or more (Ritz et al., 1989; Stewart, 1998; Carroll et al., 2003; Nash, 2003; Macleod et al., 2004b; Pereira et al., 2004; Lauer, 2005). Reasons for these differences are related to the variety of benthic systems involved and their assimilative capacity, species being farmed, stocking density, water movement and temperature. Other waste remediation strategies used by industry may never fully replace the need for fallowing, but may reduce the recovery time or extend the period that a pen may be left in operation. Most of the methods are technological (filtration systems), but various forms of biofilters and integrated farming methods (polyculture) have been attempted. Technological solutions for waste removal are likely to enhance the pace of recovery during fallowing but the cost of such operations may be prohibitive. Polyculture generally combines finfish with filter feeders of particulate matter (e.g. mussels) and macroalgae as nutrient sinks but further attention should be given to the use of cultured subsurface and surface deposit feeders that act to stir up sediment under cages and maintain oxygen levels (Brzeski & Newkirk, 1997). Integrated farming can be successful in utilising waste and has the added advantage of increasing both the commercial value and stability of the industry through diversification of its product base (such as edible macroalgae, mussels or pharmaceutical products extracted from tunicates). However, little information is available on the productivity of such operations at a commercial scale. The targeting of alternative mitigation strategies in finfish aquaculture is problematic because operators have little or no control over water movements, sediment nutrient loads and external ecological variables, hindering progress in this area. Fallowing appears to be the only mitigation process currently employed in Australia and the only research on impacts and recovery rates has been undertaken in Tasmania for salmon farming (Macleod et al., 2004a; Macleod et al., 2004b; Edgar et al., 2005).

1.1.2. The southern bluefin tuna aquaculture industry

The reduction of southern bluefin tuna (SBT) (*Thunnus maccoyii*) wild quotas from 14,500 tonnes in 1987/1988 to its current level of 5,265 tonnes prompted the move of the industry away from canning to value-adding through farming (Jeffriess, 2003). SBT were first farmed in Australia in 1991 with sea-cage technology adapted from aquaculture systems developed for other species (in particular salmonids). Only 17 tonnes were farmed in this initial year, but since 1999 more than 95% of the total wild quota has been farmed (Jeffriess, 2003; Love & Langenkamp, 2003). The sector is currently the leading aquaculture industry in South Australia, accounting for over 85 % of the gross value of production between 2000 and 2003 (EconSearch Pty Ltd, 2002; Knight et al., 2004). In economic terms, SBT farming is also the largest aquaculture industry in the country, contributing to more than 20% of the gross value

of production, followed by pearls and Atlantic salmon (Love & Langenkamp, 2003; FRDC, 2004; Newton et al., 2006). Approximately 99% of exports are sold to the Japanese sashimi market, with the rest of the production sold to the domestic or US markets (EconSearch Pty Ltd, 2002). Current threats to the industry include a decline in price and potential reductions in the total allowable catch as a response to concerns about the size of the wild stock. The increased supplies of farmed tuna from Mediterranean countries, an appreciation of the Australian dollar against the Japanese yen, and a significant decline in SBT farm output have forced a drop in the export value of SBT from a peak of 267 million in 2002/2003 to 140 million in 2004/2005 (EconSearch Pty Ltd, 2006).

Although there has been interest in developing SBT farms in other states, SBT aquaculture currently only occurs in waters off Port Lincoln, lower Spencer Gulf, South Australia. This reflects the suitability of the area for SBT grow-out and availability of juvenile wild stock in waters of the adjacent Great Australian Bight during their annual migration off the coast of western and southern Australia. Initially SBT farms were located in the protected waters of Boston Bay between the city of Port Lincoln and Boston Island. In April 1996, unusually severe weather affected the area leading to the loss of 75 % of all stock. Mortalities during that time were attributed to resuspension of fine sediments into the water column (Clarke, 1996). By 1997 most farms had re-located to the more exposed waters seaward of Boston Island (Figure 1.2), where current speeds are stronger and flushing times faster (Bierman, 2005). There are currently 21 operators and 32 lease sites in this offshore farming zone (PIRSA, 2006). Leases range from 10 to 215 ha, occupying a total area of 1,794 ha, with maximum stocking biomass of 6 tonnes per hectare (PIRSA Aquaculture, 2003; PIRSA, 2006). Farmers are issued with a lease area three times as large as the licence area they are allowed to farm inside the lease in any particular year. This management strategy allows for moving cages inside the lease every year, therefore allowing each farmed area to fallow for a period of at least 2 years after harvest.

Juveline SBT weighing between 15 and 25 kg are caught between December and March in waters off the South Australian coast and towed back to Port Lincoln for transfer into sea-cages. Licenses issued prior to 2006 had to ensure that maximum stocking densities did not exceed 4 kg m^{-3}. However, stocking densities are no longer included in licence conditions (Stephen Madigan, personal communication). SBT are fattened over the next 3 to 7 months in 40-50 m diameter "Polar Cirkel" and "Bridgestone" type pens (Clarke et al., 1999), and harvested between July and September. Feeding relies on baitfish feed sourced mostly locally (Australian sardines, *Sardinops neopilchardus*), but also from overseas, e.g. American (*Sardinops sagax*) and Californian sardines (*Sardinops caeruleus*) (Ellis & Rough, 2005). Feed conversion ratios are generally in the range 10-17 (wet weight feed/wet weight gain) (Fernandes et al., submitted-b). Harvest fish weigh between 25 and 40 kg, with the total annual production averaging 9,640 tonnes between 2000 and 2003 (Jeffriess, 2004). Until now, this production is achieved without the use of anti-fouling agents or drugs.

Limited data has been available on the waste produced by SBT farms and their environmental effects (for a review, see below). Comparatively more information has been published on salmon aquaculture in Tasmania, an industry that started in 1984, 7 years earlier than SBT farming in South Australia (Crawford et al., 2002; Crawford, 2003; Macleod et al., 2004a; Macleod et al., 2004b). The environmental issues associated with SBT aquaculture are likely to have unique characteristics compared to salmon and other finfish because of differences in food type, management practices, nutritional efficiency of the stock and the assimilative capacity of the local environment. In particular, the rates of nutrient retention and partition of

wastes between the dissolved and particulate phases have not previously been quantified, and we have little understanding of the regional effects of farming on the oligotrophic waters of lower Spencer Gulf, subject to strong flushing regimes and high levels of scavenger/predator activity. Localized impacts have not been detected in several monitoring campaigns undertaken since 1996.

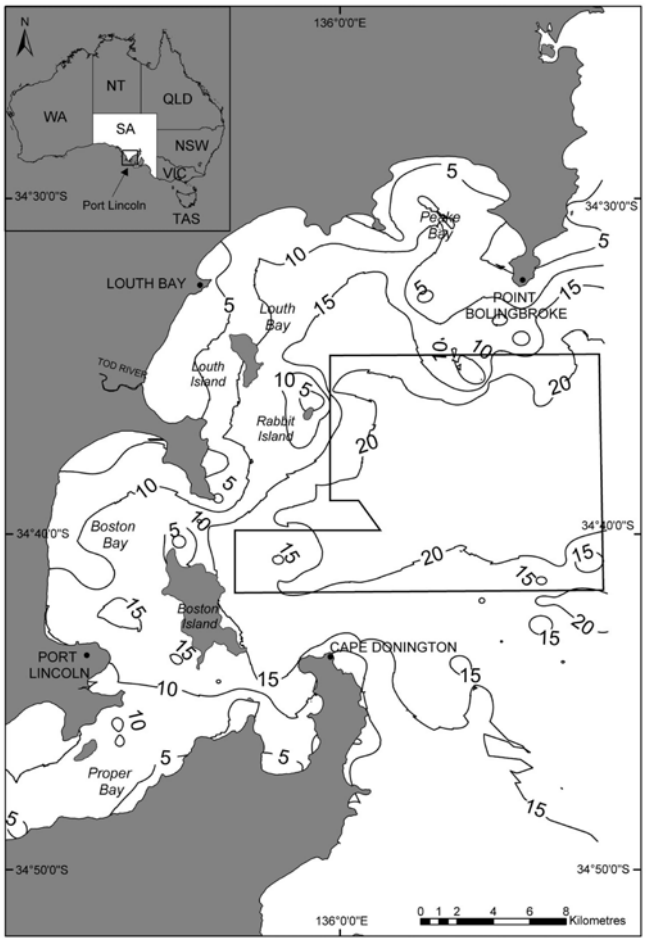

Figure 1.2. Map showing the location of the SBT offshore farming zone in lower Spencer Gulf.

Pelagic impacts

An initial study was carried out in the early 1990s, when SBT pens were still contained within Boston Bay (Bond, 1993). Results suggested an increase in nitrogen and phosphorus within SBT leases, up to 40 m from the pens. Total Kjeldahl nitrogen (TKN) and total phosphorus reached values approximately twice background within the pens. In contrast, cholorophyll-*a* levels (0.2-1.3 μg L^{-1}), dissolved oxygen concentrations, pH and turbidity showed no detectable impacts. SBT pens were also found to act as fish aggregating devices. Despite some obvious local disturbances to the natural pelagic system, the effects to the water column were considered minor.

This initial study was followed by an assessment in 1995 of the impact of SBT farming on dissolved oxygen levels in the water column (Cronin et al., 1999). For this purpose, the authors assessed the role of biofouling growing on the nets in the production and respiration of oxygen. The fouling community was found to be a net consumer of oxygen, particularly at depth where the abundance of autotrophs declined. The relative contribution of the fouling community to oxygen consumption was estimated as 3% of the total, with the remainder deriving from SBT respiration and sediment metabolism. This study suggested that fouling might have a greater effect on dissolved oxygen concentrations inside the pens by restricting water exchange. However, it was not clear what minimal levels are likely to be attained in the vicinity of the pens, and how the decline could disturb water column characteristics in the area.

After the April 1996 mortalities, most farms were relocated to the offshore farming zone seaward of Boston Island. Typical total algal counts (<1,000 cells ml^{-1}) and chlorophyll-*a* levels (<1.5 μg L^{-1}) recorded during environmental monitoring campaigns carried out between July 1996 and December 1998 were characteristic of an oligotrophic coastal system (Clarke et al., 1999). These levels were found to be only marginally higher in the vicinity of SBT pens. Abundance and composition differed significantly between locations and time of the year, with samples collected offshore of Boston Island and less so in the vicinity of Rabbit Island showing an imprint of a coastal or perhaps more disturbed environment compared with samples collected closer to the mouth of the Gulf near Taylor Island. Blooms of *Gymnodinium mikimotoi* and *Gymnodinium breve* and high concentrations of dinoflagellate cysts were observed in Boston Bay during the study. Nutrient levels were homogeneous throughout the area, showing seasonal changes. Concentrations of ammonia were particularly high, between 0.005 and 0.125 mg N L^{-1} with a mean value of 0.027 mg N L^{-1}, and surpassed the ANZECC guidelines for coastal waters in southern western Australia (0.005 mg N L^{-1})(ANZECC/ARMCANZ, 2000). South Australian guidelines are higher (0.050 mg N L^{-1}) but need to be considered with caution as they were developed from a restricted dataset for a single location (Greg Collings, personnal communication). Phosphate levels were also relatively high, with mean values <0.020 mg P L^{-1} although the ANZECC guideline is 0.005 mg P L^{-1} for southern western Australia and 0.010 mg P L^{-1} for South Australia. In contrast, concentrations of nitrate were low, typically <0.005 mg N L^{-1}, and concentrations of silica and nitrite were below detection limits (1 mg Si L^{-1} and 0.005 mg N L^{-1}, respectively).

The subsequent monitoring campaign undertaken in the summer of 1999/2000 was modified to look at small-scale impacts based on changes in gradients away from the pens (Clarke et al., 2000). That work highlighted lower but acceptable dissolved oxygen levels (> 6 mg L^{-1}, 88.6% saturation) in the vicinity of the pens, attributed to biofouling. Temperature, salinity, turbidity, pH, nutrients, chlorophyll-*a* and chlorophyll-*b* as well as plankton counts did not change significantly with distance from pens. Although changes with distance were not statistically significant, nutrients and chlorophyll-*a* showed a trend of decreasing values away from pens.

Recent work by Bierman (2005) confirmed earlier findings by Clarke et al. (1999) that chlorophyll-*a* concentrations are slightly higher in the vicinity of SBT pens. Bierman (2005) compared areas of greater and lower density of pens and found chlorophyll-*a* levels to be higher where density was greater. Interestingly, he found this to be true both when pens were stocked and unstocked. In this work, the area with greatest density of pens was also the area closer to the coastline. Whether the differences observed were a result of proximity to land or

a direct effect of the presence of SBT pens, is not clear. Phosphate concentrations in the water column were also found to increase in areas of greater density of stocked pens. The overall levels of chlorophyll-*a* and dissolved nutrients across the entire study area seaward of Boston Island did not increase during the SBT grow-out season, suggesting that although small localized impacts may occur (e.g. chlorophyll-*a* and phosphate concentrations), impacts to the whole area are not detectable.

A model has been constructed to predict dissolved nitrogen and phosphorus concentrations in the farming zone with respect to ambient levels as a function of SBT stocking rates (Collings et al., 2006). The model relies on a relatively simple mass balance requiring knowledge of the levels of input to the system with fish feed, amount assimilated in the growth of the fish and the flushing rate of the water body. By adding to the existing (pre-input) level, it is possible to predict the levels of dissolved nutrients in the aquaculture zone for specified levels of production. The model outputs are compared with Australian and New Zealand Environment and Conservation Council recommended concentrations (ANZECC/ARMCANZ, 2000) to establish environmentally acceptable production levels. Based on current farming practices, the model predicts that the environmental effects of SBT farming are driven by nitrogen loads rather than phosphorus. When guidelines for South Australia are taken into consideration, the system could support an increase of approximately 7,000 tonnes above current production before exceeding nitrate and ammonia critical levels (0.050 mg N L^{-1})(ANZECC/ARMCANZ, 2000).

The increase in food availability in the water column (uneaten feed, SBT stock, and fish attracted to the pens) also affects the structure of pelagic communities. This in turn might affect large predators such as dolphins, seals, sea lions and sharks, leading to behavioural changes, mortalities by entanglement in the nets, or entrapment in the pens (Bond, 1993; Theil et al., 2006). Birds have also been shown to be affected, with the local population of silver gulls doubling between 2000 and 2003 (Harrison, 2003). Other possible impacts to the pelagic ecosystem include the potential spread of diseases through imported baitfish (Biosecurity Australia, 2001).

Benthic impacts

The first investigations into seabed souring associated with SBT farming date back to the early 1990s (Bond, 1993), when the industry was primarily inside the protected and shallower waters of Boston Bay (current speeds typically less than 2 cm s^{-1}, water depths between 15 and 18 m) and the pens were fitted with an outer net that draped to the seafloor and acted as a barrier to predators such as seals. Results suggested that sedimentation rates increased to 2-7 times the natural background (10-20 g m^{-2} d^{-1}) under the pens, to decline quickly in the next 20-40 m from the edge. Increased sedimentation rates were associated with a decrease in oxygen availability, and an increase in total organic carbon, phosphate and TKN in the sediments below the pens, although the magnitude of these changes was not reported. The natural benthic communities in the area were dominated by the heavily epiphytised red algae *Botryocladia obovata*, which decreased in abundance after SBT pens were put in place, giving way to a number of opportunistic species. The area underneath the pens became devoid of macro flora and fauna (except for spider crabs) within 6 to 12 months of pen set up, indicating significant impacts limited to the footprint of the pens.

The preliminary study conducted by Bond (1993) was followed by a detailed assessment of benthic impacts undertaken in 1994/1995 (Cheshire et al., 1996a; Cheshire et al., 1996b).

This work failed to detect changes in sediment chemistry but highlighted severe disturbance of benthic communities in the footprint of the pens. The study also pointed to the lack of adequate environmental data before the introduction of the industry to the area and the difficulty in determining regional changes. Based on their results, a gradient of localized impacts was delineated, as shown in Table 1.1.

Table 1.1. Zones of benthic impact defined for SBT pens when these were located inside Boston Bay, from Cheshire et al. (1996a; 1996b).

Impact	Distance from pen (m)	Nature of wastes	Benthic taxa in high numbers	Benthic taxa in moderate numbers
High	0-5	Large and small particles	Sea urchins, polychaetes, nebalids, gastropods, brachyurans (crabs) and anthozoans	Ascidians, holothurians
Moderate	5-20	Small particles	Ascidians, holothurians	Sea urchins, polychaetes
Low	20-150	Not visible	Ascidians, holothurians[1]	----
Undisturbed	>150	Not visible	Background communities	----

[1]Maximum values were found between 50 and 150 m from the pens.

The gradient depicted in Table 1.1 was greatly influenced by the presence of predator nets, which act to reduce water exchange therefore minimizing the spread of impacts (Bruce, 1997). Infaunal communities alone did not provide enough information to differentiate the zones identified in this gradient, but were clearly in greater numbers in the footprint of the pens where lower diversity was observed. There was also clear evidence of changes in the epibenthic community structure up to 20 m from the pens. The biota inhabiting the zone of high impact under the pen and up to 5 m from the edges comprised opportunistic species benefiting from the continuous rain of large organic detritus, able to avoid smothering and tolerant to low light incidence caused by shading from the nets. High numbers of bivalves and green algae (*Ulva*) were attributed to biofouling dislodged from the nets. In a companion study, the bivalve *Electroma georgiana* was found to contribute the greatest proportion of the fouling biomass on SBT nets (Cronin et al., 1999). In contrast, shrimps, brown and red algae (*Phaeophyta and Rhodophyta*) showed significantly lower numbers in the footprint of the pens. Smaller particles (mostly faeces and uneaten feed) accumulated in the zone characterized by a moderate level of impact from 5 to 20 m from the pens, where numbers of sea urchins and polychaetes declined considerably. Lesser impacts were seen up to 150 m from the pens where the epibenthic community had higher taxa richness and there was little evidence of detrital accumulation. Although not visibly disturbed, these sediments supported the highest numbers of ascidians and holothurians recorded anywhere in the Bay, indicating that sedimentation rates were high enough to cause disturbance. It would seem that the area of highest sedimentation in the footprint of the pens would favour the settlement of deposit feeders such as polychaetes but hinder the establishment of suspension feeders such as ascidians, which would have problems keeping cilia and siphons of filtering mechanisms unclogged. This would explain the high numbers of these organisms in the area of lower sedimentation away from the pens but still under their influence. Background communities characterized by high diversity and low abundance were found in sediments more than 150 m from the pens. Mysid shrimps in particular, were only found in these undisturbed areas.

In 1996, Bruce (1997) investigated the deposition of wastes from tuna farms in Boston Bay. This work found that 4-23 % of feed inputs, and 0.1-0.4% of the fouling biomass on the nets, reached the seafloor daily. Given estimates of fouling biomass between 2 and 6 tonnes depending on cage dimensions, history and location (Bruce, 1997; Cronin et al., 1999), this represents a considerable amount of sinking matter, which can outweigh the amount of uneaten feed reaching the benthos when feeding rates are low. Deposition of uneaten feed trapped in the nets, and of fouling organisms, was suspected to considerably increase during storm events. Sedimentation rates below pens stocked with only 19 fish each reached values as high as 156 g m^{-2} d^{-1} and 13 g organic matter m^{-2} d^{-1}. These values were 30-400% higher than the natural background, but decreased exponentially with distance from the pens. The impact of particulate wastes was localized, with the maximum observed distances for waste dispersal varying from 55 to 217 m depending on weather and tide conditions.

After these initial studies, pens were mostly relocated to the area outside Boston Bay, east of Boston Island, and the use of predator nets was discontinued. Data collected during the environmental monitoring campaigns undertaken between 1996 and 1998 show that organic detritus, fauna and bivalves increased up to 50 m from the edge of stocked SBT pens (Clarke et al., 1999). Although pens were largely unstocked in the summer of 1999/2000, Capitellidae, Ampharetidae and Spionidae polychaetes were also in higher numbers in the footprint of the pens (and gastropods at distances between 75 and 200 m from the pens), whereas ascidians and scallops were more abundant at control transects (Clarke et al., 2000). Organic detritus found in the vicinity of the pens in these studies was mostly composed of biofouling from the nets, and occasionally waste feed was recorded up to 10-50 m from the edge of the pens. Other benthic variables monitored in these studies, such as bioturbation, substrate colour, algal and sponge coverage, organic matter content and mineral grain size, remained unchanged with distance from pens.

The subsequent environmental monitoring campaigns undertaken between 2001 and 2003 targeted benthic disturbance by the qualitative assessment of biota and sediment appearance on video transects and the quantitative assessment of infaunal abundance and diversity together with mineral grain size (Loo, 2006). These variables were measured 150 m outside and downcurrent from lease boundaries and at control sites at least 1 km away from any lease site. No localized impact was detected during the three years of the study. However, a regional increase in abundance and decrease in diversity was found in the infauna, as well as an increase in species more prone to benefit from organic enrichment, such as Spionidae. The data are inconclusive and it is not clear at this stage whether these results relate to natural interannual variability or a consequence of the effects of SBT farming.

More recently, a composite mass balance/advection model was constructed to estimate the increase in deposition of particulate carbon on the seafloor as a consequence of SBT farming (Collings et al., 2006). In the first instance, the model calculates the carbon load in terms of uneaten food and faecal material from the feed input and metabolic capabilities of the fish. These carbon loads are then separately utilised by the advection/diffusion module, which takes into account both the prevailing currents (tidal and residual) and the diffusion that could be expected in the absence of any water movement. The result is a map of carbon loading on the seafloor. Another module can be applied to simulate the metabolism of carbon by benthic feeders and within bacterial cycles. Many assumptions of the model require empirical validation and/or need to be refined and therefore it cannot provide a quantitative estimate of deposition rates at this stage. However, it does provide a qualitative two-dimensional plot of

the likely pattern of carbon deposition for any specified lease area and therefore can be used to direct monitoring programs and management issues in the future.

The only environmental management strategy to reduce benthic impacts of SBT farming currently in place is fallowing. The management regime stipulates that pens should be moved at a minimum of every two years (Bond, 1993), although the actual recovery time is not known. A requirement for a minimum distance of 1 km between farms has also been implemented to reduce the risk of carryover from one lease to the next and to avoid the spread of diseases. Overall, results from the previous studies reviewed above suggest that both pelagic and benthic impacts are minimal and restricted to the immediate vicinity of SBT pens. Some evidence of minor changes on a regional level does exist (e.g. nutrient concentrations, phytoplankton/infauna diversity and abundance), but these are inconclusive. More information on regional effects will become available as a result of the new Aquafin CRC environment project "Risk and Response", which will develop a complete biogeochemical model for the whole region.

1.2. Need

There is a need to minimise the impact of sea-cage aquaculture systems on the environment and thereby to ensure access to sites and security of tenure for finfish farmers, the productivity of the farming operation, the health of fish (and consequent reduced mortalities) and the ability of farmers to comply with environmental management requirements. Whereas sea-cage aquaculture operations are known to be sources of wastes into the marine environment (e.g. uneaten food, faecal matter, organic and inorganic nutrients), little is known about the nature or composition of these wastes for SBT or the appropriate measures for managing or minimising the environmental effects. This limits the degree to which waste mitigation and remedial strategies can be applied and may have important economic and ecological consequences. There is a need therefore, to quantify the dynamics of waste production from tuna cages in relation to differing management regimes. Given the knowledge of waste composition, alternative mitigation strategies can be evaluated. Fallowing is the only strategy currently employed to manage impacts. Cage sites are left to fallow for a period of two years to allow for local environmental recovery. There are, however, no data to indicate whether this period is too short (which may ultimately lead to long-term environmental degradation) or too long (which limits the effective use of available sites). There is no information on the efficacy of alternative waste mitigation approaches (e.g. stocking density, feeding systems, diaper systems, polyculture systems). There is thus a need to develop our understanding of the environmental recovery that occurs during the fallow period and to assess alternative waste mitigation strategies. This project provides information in relation to the nature of the wastes, its effects on a local scale, and then uses this knowledge to identify and evaluate alternative mitigation strategies.

1.3. Objectives

1. To determine the type and quantity of waste produced by sea-cage operations across a range of management and environmental regimes.

2. To develop and validate a model or modify an existing model (e.g. DEPOMOD) of the waste dynamics of sea-cage operations incorporating information on stocking

density, feed type and a number of environmental and management parameters to quantify the extent and intensity of localised impacts.

3. To obtain information on the composition of the fouling community on and adjacent to tuna cages with reference to potential polyculture species with an assessment of their potential biological and economic viability.

4. To develop a sampling program for benthic assemblages exposed to waste from tuna farming operations with reference to the efficacy of fallowing as a waste remediation process. This will form the basis for consideration of alternative waste mitigation strategies and for recommending improvements to environmental monitoring regimes.

5. To test the potential of integrated farming in mitigation studies. This involves the use of benthic filter feeders (e.g. blue mussels) benthic surface feeders (holothurids and crabs) and bioturbators (e.g. stingrays, fish or native oysters).

6. To evaluate potential applications of technological approaches (e.g. diaper systems) to waste mitigation.

1.4. Study scope and design

This project was a pro-active initiative by industry to understand environmental effects, assess mitigation solutions and improve environmental services. The project aimed at the development of a clearer understanding of the natural setting of the offshore SBT farming zone, the localized impacts of wastes from SBT pens to the area and an assessment of strategies aimed at reducing or mitigating these impacts. These have important implications for the better management to limit waste (and therefore costs) and improve the health of stock (and hence productivity) within a framework that is proven to be environmentally sustainable. As a consequence, the data chapters in this report are divided into three parts.

Part 1 consists of two chapters that describe the natural background characteristics of Spencer Gulf and the offshore tuna farming zone:

Chapter 2 provides a quantitative analysis of benthic oxygen uptake rates and nutrient fluxes, as well as sedimentary concentrations of surface organic carbon, total nitrogen and chlorophyll-*a* at sites in the northern, central and southern areas of the Gulf. Other parameters examined included redox potential and mineral grain size distributions of sediments and nutrient concentrations in the water column. Results are compared to values obtained in other subtidal temperate areas and in the SBT farming zone.

Chapter 3 investigates the natural sedimentary setting in the offshore SBT farming zone. Maps were produced for the distribution of mineral grain size, carbonate, organic carbon and total nitrogen and their stable isotopes. These parameters were used to explore the links between the natural distribution of sediments and potential environmental effects and risks to the industry. In particular, to address how different sediment types might affect benthic recovery and the likelihood of resuspension into the water column.

Part 2 investigates the nature, composition and environmental effects of farm wastes:

In **Chapter 4** we determined settling rates and the amount of nutrients in pellets and baitfish feed as well as faeces of farmed SBT, and estimated the fraction and forms available to leach into seawater. Results were used to calculate nitrogen and phosphorus environmental loads from leaching of solid wastes.

In **Chapter 5** we investigated whether SBT farming had significant effects on benthic metabolism. For this purpose, we measured sedimentation rates in the water column, oxygen uptake rates (OUR) and nutrient fluxes at the sediment-water interface, as well as nutrient concentrations in porewaters and standing stocks of organic carbon, total nitrogen and chlorophyll-*a* in the sediments. Other benthic variables measured included macroinfauna abundance and biomass, redox potential and mineral grain size. These parameters were compared between farmed and control sites to determine the magnitude of local changes, and between SBT and other fish aquaculture to obtain some insight into differences and similarities between the industries.

Chapter 6 uses meta-analysis to quantitatively assess the magnitude of impacts from fish farming upon benthic metabolism as compared to other anthropogenic activities such as mussel farms and sewage treatment plants. For this purpose, data on oxygen uptake rates (OUR) in subtidal marine environments from temperate latitudes were analysed using a series of statistical tools.

In **Chapter 7**, we investigated perturbations to natural processes determining benthic nitrogen cycling as a result of SBT farming. Isotope dilution techniques were used to characterise gross and net ammonium mineralisation rates at farm *versus* control sites over a 24-hour period during spring. The isotope dilution technique permitted partitioning of nitrogen available to be released back into the pelagic environment from that retained in the sediments by the microbenthos or in standing stocks. These data are complemented by measurements of ammonium standing stocks to calculate ammonium turnover rates.

Chapter 8 discusses the role of scavengers in consuming uneaten feed reaching the seafloor. An underwater video platform with baitfish was used to identify the most common benthic scavengers, estimate their occurrence and feeding rates.

In **Chapter 9**, we integrated data from previous chapters into a model of nitrogen retention and environmental flows in commercial SBT pens. Based on results from the model, we calculated nitrogen loads to the environment in both dissolved and particulate forms and compared these loads to other finfish industries.

In Part 3 we explore strategies available to mitigate impacts from SBT farming:

Chapter 10 investigates changes in macrobenthic assemblages over a 12-month period to describe the rates of recovery of the benthos as a result of fallowing of SBT sites. Abundance and diversity data are examined using several statistical and indicator tools (multi-dimensional scaling plots, taxon richness, evenness and diversity, abundance-biomass and multiple *k*-dominance curves).

As previous studies indicate that biofouling is a significant vector for sinking matter to the seafloor under SBT pens, the use of an antifouling coating on nets was investigated as a mitigation solution in **Chapter 11**. This work also provided information on fouling organisms with potential for polyculture.

Chapter 12 reviews waste management strategies available for the SBT industry, with emphasis on polyculture techniques and engineering solutions. The study involved a literature survey on current open ocean polyculture studies and a discussion on the major issues presented by polyculture. This information, combined with a literature investigation into the marine biodiversity of the Port Lincoln area, was used to suggest potential models that could be used by the SBT industry. We also reviewed engineering solutions currently available for use in waste mitigation.

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Chapter 2: Spencer Gulf natural sedimentary setting

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Abstract

This chapter presents sediment and water physicochemical data gathered during field trips undertaken in the spring of 2003 (October) and autumn of 2004 (April) in Spencer Gulf, South Australia. Results from six different locations showed that benthic metabolism and nutrient cycling were spatially and temporally variable within the Gulf. Despite low sedimentary organic loadings (organic carbon <0.8%, total nitrogen <0.1%), benthic recycling of nutrients appeared to be an important process to support primary productivity. Redox potential was positive up to 8 cm depth, suggesting that benthic metabolic processes were not oxygen limited. Oxygen uptake rates (429-1,477 μmol m^{-2} h^{-1}) increased with water temperatures. Benthic fluxes of ammonium (-9-23 μmol m^{-2} h^{-1}) and phosphate (2-5 μmol m^{-2} h^{-1}) were in the lower range of values published for other temperate subtidal areas, whereas values for nitrate+nitrite fluxes (28-120 μmol m^{-2} h^{-1}) were higher. These results suggest that a relatively high proportion of ammonium undergoes nitrification (and perhaps denitrification) within the Gulf. The ranges in oxygen uptake rates, nutrient fluxes and benthic chlorophyll-*a* concentrations (< 100 mg m^{-2}) were similar to values observed at control sites within the SBT farming zone. However, mean grain size was higher, and sedimentary organic carbon and total nitrogen contents, as well as water column phosphate concentrations, were lower than values measured in the SBT farming zone.

2.1. Introduction

Spencer Gulf occupies an area of 21,700 km^2 (Smith & Veeh, 1989) between Yorke and Eyre Peninsulas on the South Australian coast (Figure 2.1). Spencer Gulf extends almost 320 km inland (Noye, 1984) from approximately 35°00'S near Cape Spencer to 32°30'S several kilometres north of Port Augusta. The greatest width of the Gulf is 129 km (Noye, 1984) from near Port Lincoln on Eyre Peninsula to Point Turton on Yorke Peninsula. Spencer Gulf is a shallow semi-enclosed water body with a mean depth of 13 m (Nunes & Lennon, 1986), but a considerable north to south gradient from below 10 to about 60 m (Figure 2.2 a). The mouth of the Gulf is approximately 79 km in width, with the main connection to the Southern Ocean circulation consisting of a series of channels having an average depth of 40 m, which extend nearly 100 km north into the centre of the Gulf (Noye, 1984) (Figure 2.2 a).

Throughout the Gulf, salinity and temperature vary spatially and temporally (Green, 1984). Nunes & Lennon (1986) conducted a 30 month oceanographic survey of the Gulf, north of 34ºS (Wallaroo), which covered some 7,500 km^2. Seasonal temperature ranged from around 12 ºC in winter to about 24 ºC in summer. Water temperatures near the entrance to the Gulf range from 13 ºC in winter to 18 ºC in summer (Edyvane, 1999). High evaporation in the Gulf gives rise to maximum salinities in the northern reaches approaching 48 during summer, which decreases to about 43 in winter (Green, 1984; Nunes & Lennon, 1986). Across the entrance to the Gulf, salinity increases in a west to east direction from about 36 to 38 (Figure 2.2 b) (Bye & Whitehead, 1975). Interestingly, Nunes & Lennon (1986) reported that several years of observation showed no long-term drift in the magnitude of the salinity gradient in the northern reaches, which suggests equilibrium between annual salt production from evaporation and its removal from Gulf water circulation.

The Gulf is characterised by a net fluid loss due to evaporation, which results in an average northward water movement of 0.016 cm s^{-1} (Green, 1984), particularly in surface waters (Bye & Whitehead, 1975) (Figure 2.2 c, d). Average annual rainfalls for Port Augusta and Port Pirie are approximately 300 mm, while evaporation exceeds 2,200 mm throughout the region (Green, 1984; Noye, 1984). Spencer Gulf is referred to as an inverse estuary because salinity increases toward the head of the Gulf. This gradient is due to minimal freshwater inputs at the head of the Gulf and the excess evaporation. Southern Ocean waters from the Great Australian Bight flow north along the west of the Gulf and are deflected south around the Tippara Reef area, southeast of Cowell (Edyvane, 1999) (Figure 2.2 c, d). A southward current characterises the eastern side of the Gulf and this carries with it more saline water, which generates the aforementioned salinity gradient observed at the entrance to the Gulf (Edyvane, 1999) (Figure 2.2 b). Maximum current flows occur during spring tides and reach 0.5 to 0.75 m s^{-1} in the reaches north of Port Pirie and about 1 m s^{-1} in regions to the south (Noye, 1984). Dodge tides occur fortnightly when semi-diurnal lunar and solar constituents have nearly the same amplitude and tidal variation is approximately zero for 24 h (Green, 1984; Noye, 1984; Nunes & Lennon, 1986). Consequently, tidal currents may be expected to be less than 0.1 m s^{-1} during the dodge tide, increasing to the maximum velocity a week later (Noye, 1984).

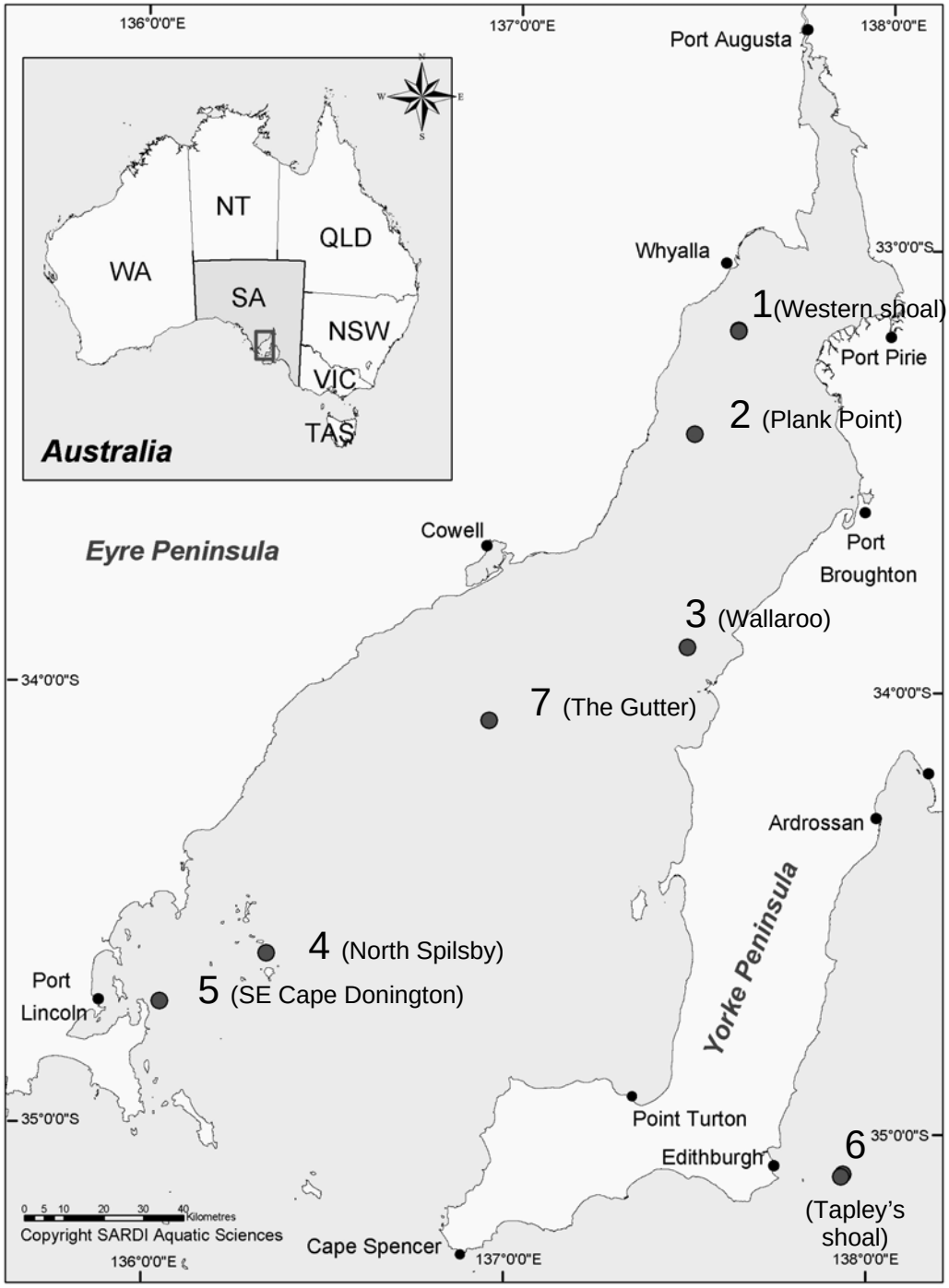

Figure 2.1. Map of Spencer Gulf, South Australia, showing sampling sites described in Table 2.1.

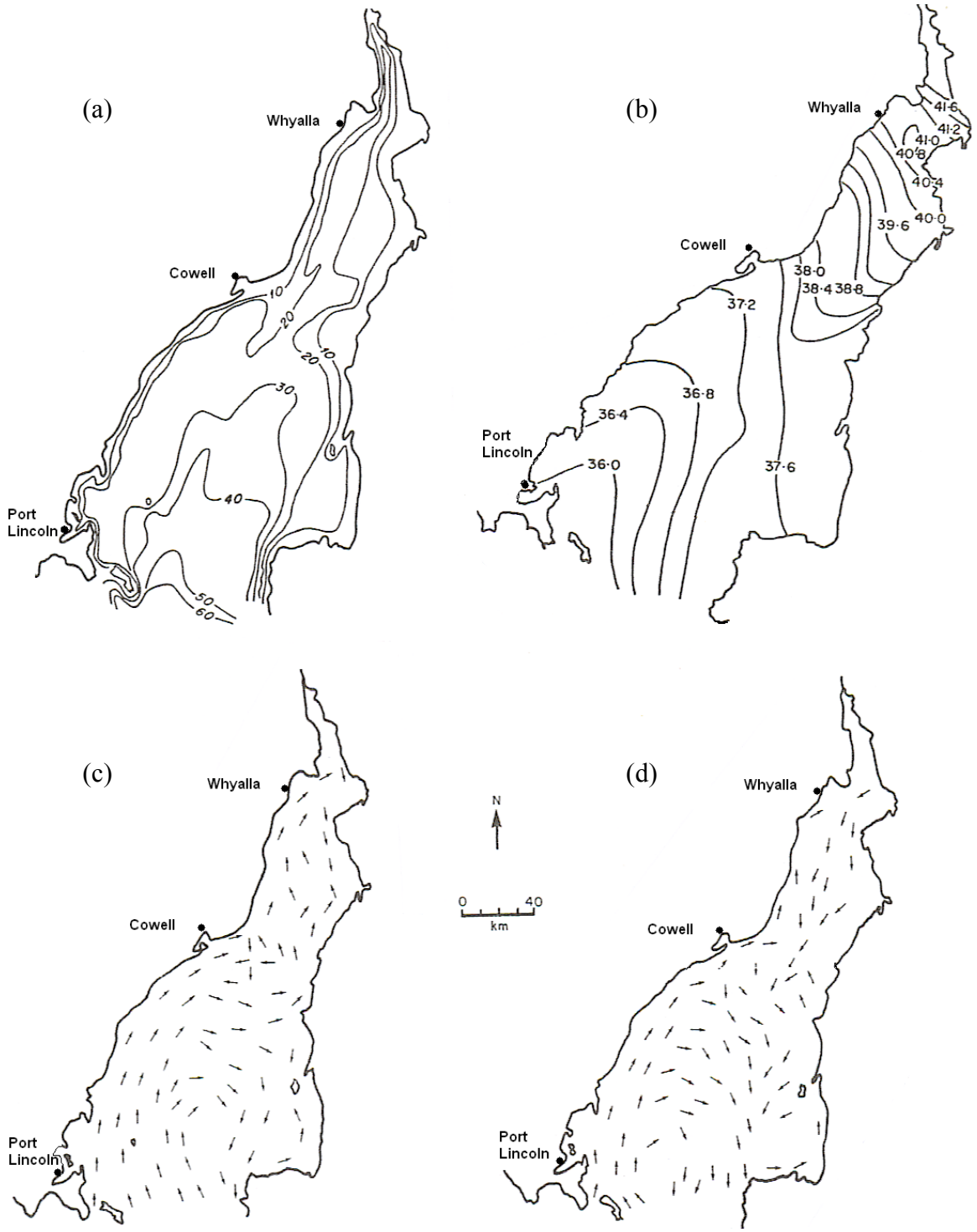

Figure 2.2. Spencer Gulf (a) bathymetry, (b) depth averaged salinity, (c) surface currents and (d) bottom currents. From Bye and Whitehead (1975).

An extensive review of the intertidal and subtidal marine ecology of the Spencer Gulf region was compiled by Edyvane (1999) and thus only the defining features of flora and fauna are introduced here. The substrate of the intertidal and subtidal areas of the Gulf vary considerably, from mud and sand areas in the north to sand patches and algal-dominated platform reefs in the central and southern extents. Consequently, there are a number of ecosystems and related biogeochemical processes that occur within Spencer Gulf. The northern reaches of the Gulf support approximately 57% (or 5,512 km^2) of the total recorded area of seagrasses for South Australia. These meadows occur mainly in waters less than 10 m deep and support various species of infauna and epiphytes which in turn contribute to the carbonate basis of the region's sediments. Several species of seagrass, including *Halophila australis*, exist at depths reaching 23 m throughout the central and southern Gulf (Womersley, 1984), which offers evidence of sufficient light penetration for photosynthesis in these regions, at this depth. The intertidal mangrove forests (*Avicennia marina*) of the central and northern regions are the most extensive in South Australia. Mud flats, samphires and seagrasses form part of the ecosystem surrounding the 46,000 ha of mangroves in the northern region. Mangrove communities also exist in southern regions of the Gulf (6,000 ha) and in both regions provides habitat for waterfowl and waders, as well as nursery, feeding and breeding areas for fish and crustaceans. Mammals are well represented, including the largest breeding colonies of New Zealand fur seals (*Arctocephalus fosteri*) in South Australian waters on the Neptune Islands (approximately 51% of the Australian population), just outside the mouth of the Gulf. Other mammalian species include the common (*Delphinus delphis*) and bottlenose (*Tursiops truncatus*) dolphins, Australian sea lions (*Neophoca cinerea*), little penguins (*Endyptula minor*) and a further 7 bird species protected under the South Australian National Parks and Wildlife Act (Edyvane, 1999).

Only one published report investigates the mass balance of carbon, nitrogen and phosphorus within Spencer Gulf (Smith & Veeh, 1989). This report was based on a single cruise during May 1987 that sampled 25 stations throughout the Gulf. The authors concluded that about 90% of primary production was sustained by internal recycling of nutrients. Furthermore, they concluded that the Gulf as a whole is phosphorus-limited and that phosphorus controls net system production as well as other carbon fluxes (organic, calcium carbonate and carbon dioxide) in the system. Finally, they found that most of the particulate organic matter produced is exported to the Southern Ocean, where its fate becomes unknown; possibly either going into food webs elsewhere along the coast, or as sedimentary material for the shelf or deep ocean. Notable omissions from this study included measurements of nutrient fluxes at the sediment-water interface, and inclusion of a temporal component to the sampling program for this temperate region. The models of the nutrient dynamics produced by Smith & Veeh (1989) also omitted remineralisation processes that return nutrient from the sediments to the overlying water. The exclusion of nutrient fluxes from the benthos may have led to inaccuracies in estimates of the sources for carbon, nitrogen and phosphorus within the Gulf. Smith & Veeh (1989) primarily attributed sources of carbon and nitrogen to the atmosphere, and phosphorus to water exchange with the Southern Ocean.

Although Smith & Veeh (1989) mentioned that the human population of around 100,000 people discharged sewage into the Spencer Gulf environment (population numbers are similar at present), no mention besides 'other pollutants' was made of the significant industrial activity in the northern reaches of the Gulf. There are significant industries occupying the northern region, namely Whyalla with iron and steel manufacturing, Port Pirie with lead and zinc smelters, Port Augusta with power stations, and Stony Point with a liquid hydrocarbon facility (Noye, 1984; Edyvane, 1999). However, while the BHP steel

manufacturing facility at Whyalla discharges 170 tonnes of oxidised nitrogen and 80 to 110 tonnes of ammonium per year into the marine environment (Edyvane, 1999), there is no available information on the fate of these discharges or their consequences for biogeochemical cycling. Furthermore, Edyvane (1999) reported that very few systematic subtidal studies have been conducted on the marine biology of the central and southern reaches of the Gulf.

There are currently a number of other anthropogenic activities that occur within the Spencer Gulf region. These include aquaculture, wild-catch fisheries and recreational activities. Aquaculture activities other than SBT, include yellowtail kingfish (*Seriola lalandi*), oysters (*Crassostrea gigas*), abalone (*Haliotis laevigata*) and mussels. Given the number and variety of inputs into this system, information regarding the cycling of organic compounds and regeneration of nutrients within Spencer Gulf is useful for proper resource management. With this information, a better spatial and temporal understanding of nutrient regeneration processes for various areas of Spencer Gulf may be achieved.

The aim of this chapter was to characterise the natural sedimentary setting in Spencer Gulf to provide a comparative framework for the SBT farming zone located in the lower Gulf. In the absence of a large body of knowledge about sedimentary characteristics of the area, the information in this chapter broadens the context of this project into a more regional scale. Specifically, this study assessed the sediment biogeochemistry of the Spencer Gulf region at water depths ranging from 18 to 25 m, with sites sampled in the northern, central and southern areas of the Gulf in the spring of 2003 (October) and autumn of 2004 (April). This water depth was chosen to match the studies carried out around the SBT farms off the coast of Port Lincoln discussed in chapters to follow. Measurements of oxygen uptake rates (OUR) and nutrient exchange at the sediment-water interface were made. These measurements were complemented with standing stock estimates of surface sediment organic carbon and total nitrogen content, chlorophyll-*a*, redox potential and grain size distribution. Ambient seawater samples were taken at 3 and 10 m water depth, which also paralleled investigations around the SBT farms. Water nutrient samples were analysed for dissolved organic carbon (DOC), ammonium, nitrate plus nitrite, total nitrogen and phosphate**.**

2.2. Methods

2.2.1. Sampling

Samples were collected onboard the R.V. Ngerin in the spring of 2003 (1^{st}-9^{th} and 17^{th}-18^{th} of October) and autumn of 2004 (21^{st}-26^{th} April). Sites were spread out, with Western Shoal (1) and Plank Point (2) in the north, The Gutter (7) and Wallaroo (3) in the central region and North Spilsby (4) and SE Cape Donington (5) in the southwest (see Figure 2.1 for site locations and Table 2.1 for details of the sites). An additional site, Tapley's Shoal (6), is in the southwest corner of Gulf St Vincent to the east, but has been included in this analysis to further broaden the context of the data. A series of measurements were made at each of the sites (except for the Gutter) in the spring of 2003 (Table 2.2). During autumn of 2004 extra measurements were made at fewer sites in the northern part of the study area (sites 1-3 and 7, Western Shoal, Plank Point, Wallaroo and The Gutter).

Sediments cores were collected using a HAPS Corer (KC Denmark). For the analyses of OUR, nutrient fluxes, chlorophyll-*a* and redox potential we used 105 mm (i.d.) opaque PVC

core barrels. These cores with overlying water were transferred into an incubation system upon collection for measurement of OUR and nutrient fluxes (see below). Immediately after the incubations were completed, redox potential was measured and sub-samples were collected for the analyses of chlorophyll-*a*. For chlorophyll-*a*, the top 1.5 cm of the sediment was sampled using a modified 50 mL syringe (29 mm i.d.) with the sample being placed in a sterile, preweighed 50 mL centrifuge tube. Samples were immediately frozen on ship at -20 ºC, prior to storage in the laboratory at -80 ºC. During storage and processing, samples were kept from exposure to sunlight by wrapping the tubes in aluminium foil. Profiles of redox potential were measured by inserting a platinum electrode with a calomel reference (ORP, Phoenix) through holes pre-drilled into the PVC barrels at different sediment depths. These holes were 12 mm in diameter, 20 mm apart (centre to centre) to a depth of 8 cm, and sealed with rubber grommets. Surface redox measurements were made by vertically inserting the 10 mm platinum tip into the top of the sediment core. The redox probe tip was rinsed clean in seawater before each insertion and readings were allowed to stabilize for 20 s before measurements were recorded. Voltage output (mV) measurements were adjusted relative to the normal hydrogen electrode (Eaton et al., 1995).

Table 2.1. Details of sites sampled in Spencer Gulf.

Season and Year	Date sampled	Site	Number	Water depth (m)
Spring 2003	3-10-03	Western Shoal	1	25
	4-10-03	Plank Point	2	26.5
	5-10-03	Wallaroo	3	24
	7-10-03	Nth Spilsby	4	17.5
	17-10-03	SE C. Donington	5	19
	18-10-03	Tapley's Shoal	6	22
Autumn 2004	21-4-04	The Gutter	7	24
	22-4-04	Plank Point	2	25.5
	24-4-04	Western Shoal	1	24
	26-4-04	Wallaroo	3	23

Table 2.2. Measurements made at sites sampled in the Spencer Gulf.

Season and Year	Sediment-water exchange	*n*	Standing stock	*n*	Water chemistry	*n*
Spring 2003	Oxygen	4 to 6	n/d	n/d	n/d	n/d
Autumn 2004	Oxygen, ammonium, nitrate + nitrite, phosphate	3 to 6	Organic carbon, total nitrogen, chlorophyll-*a*, redox potential	4 to 6	Dissolved organic carbon, ammonium, nitrate + nitrite, phosphate	2

n/d = no data.

To collect samples for the analysis of organic carbon, total nitrogen and mineral grain size we used stainless steel sediment core barrels 67 mm in diameter. Upon retrieval, the overlying water in the barrel was carefully discarded to minimise surface disturbance to the sediment. The sediment was then extruded onto a clean stainless steel table. For mineral grain size distribution, the top 4 cm of the sediment was sectioned from a core sample, placed in a pre-combusted (450 ºC for 12 hours) aluminium tray with lid and then into a zip-lock plastic bag. For organic carbon and total nitrogen, the top 1 cm of the sediment was sectioned from a core

sample and placed in a pre-combusted glass jar. Samples were stored on ship at -20 ºC and transferred to laboratory storage (-30 ºC) prior to analysis.

Ambient seawater was sampled from 3 and 10 m water depths using a modified messenger bottle. Samples were filtered using a pre-rinsed sterile syringe onto a 0.45 µm filter and stored shipboard (-20 ºC) before being transferred to laboratory storage (-30 ºC). Due to equipment losses, Wallaroo was not sampled for dissolved nutrients.

2.2.2. Oxygen uptake rate and nutrient fluxes

Shipboard measurements of OUR (n = 3 to 6 per site) and nutrient fluxes (n = 4 to 6 per site) were made on intact sediment cores collected in the PVC barrels with a manually operated incubation system. The PVC barrels were impervious to light and thus constituted dark incubations of sediment cores. These barrels contained sediment cores and a minimum volume of 800 mL of *in situ* bottom seawater. Sediment cores with a visibly undisturbed sediment surface, clear overlying seawater and containing at least a 10 cm depth of sediment were accepted for incubation. OUR measurements were made onboard immediately after sampling and at ambient temperatures**.** The incubation system consisted of six oxygen electrodes, two temperature probes, six seawater stirrers and a temperature-controlled water bath, thus allowing six sediment cores to be incubated at once. The oxygen and temperature sensors were connected to a data logger (DT 50, Datataker) which was programmed in the field with a laptop computer. The oxygen electrodes were microcathode oxygen electrodes (Vicki Cheshire, Adelaide). The stainless steel seawater stirrers had a single blade, 7 mm wide, 4 mm long, brass propeller. The rotation of the blade maintained complete circulation of the seawater overlying the sediment within the barrel but did not cause sediment resuspension. The water bath consisted of a 200 L PVC outer container in which a 60 L polystyrene box was placed. The PVC barrels were placed into the polystyrene box during the incubation period. The space between the outer container and the inner polystyrene box was filled with ice. The ice constantly chilled the water contained inside the polystyrene box. The polystyrene box was filled with freshwater and had a pump to circulate the water and an aquarium heater to heat the water. The aquarium heater was connected to a temperature controller that was set at ambient temperatures. This thermostat system maintained temperatures within the polystyrene box to ± 0.8 ºC of the temperature set.

The water bath was set at ambient bottom seawater temperature (measured with a mercury thermometer) from the time of sediment collection. The time taken to collect six sediment samples and get the incubation started was up to one and half hours. Samples of the overlying seawater in each core were taken in duplicate at the start and end of the incubation for nutrient analysis. Nutrient fluxes were determined from the change between initial and final concentrations in the overlying seawater. The short duration of the incubations in this work (i.e. 2 to 4 hours) was assumed to limit the chances of non-linear nutrient changes. The overlying seawater was sampled using a sterile plastic syringe, filtered (0.45 µm) and then stored at -20 ºC until analysis. Samples were analysed for nitrate plus nitrite (NO_x, mg L^{-1}), ammonium (NH_4^+, mg L^{-1}) and phosphate (PO_4^{3-}, mg L^{-1}), using flow injection analysis according to the same methods used for the analyses of water column dissolved nutrients described below. The duplicate samples were averaged and the change in nutrient concentration was then adjusted to account for the sediment surface area, duration of incubation and volume of overlying seawater to determine the rate of nutrient release or uptake. The resultant rate of change was expressed in units of µmol m^{-2} h^{-1}. For nutrient

fluxes, positive measurements represented a flux out of the sediments into the overlying water and negative measurements represented a flux into the sediments from the overlying water.

The oxygen electrodes coupled to the data logger generated a record of the oxygen concentration in the seawater overlying the sediment within the barrel with a resolution of 60 seconds. The magnetic-coupled stirrers maintained circulation of the overlying water to prevent stratification and maintain a constant flow of seawater over the membrane of the oxygen electrodes. To avoid artificial limitation of oxygen for sediment processes, yet gain a sufficient change in oxygen and nutrient concentration from which to calculate a meaningful rate of change, incubations lasted 2 to 4 hours. Simple linear regression between the 100% and 0% saturation mV readings from calibration were used to convert the mV readings recorded during the incubation into mg L^{-1} according to ambient seawater temperature (measured from the mercury thermometer) and salinity (measured using a HORIBA multistation instrument, Japan). To ensure oxygen electrodes operated correctly throughout an incubation period, calibration was carried out prior to incubation (maximum of 1 hour) and immediately after incubation (maximum of 1 hour). If a given oxygen electrode lost calibration during the incubation, the associated OUR data were not used. Consequently, there was often uneven replication of OUR among sites. The dissolved oxygen concentration in the water overlying the sediments within the barrels decreased throughout the incubation period. Oxygen concentrations were related to time using simple linear regression. The rate of change (mg L^{-1} h^{-1}) was then adjusted for the sediment area and volume of overlying water to determine the OUR. The resultant rate of change was expressed in the units of μmol O_2 m^{-2} h^{-1}. Contrary to nutrient fluxes, positive measurements of OUR represented a flux into the sediments from the overlying water.

2.2.3. Sedimentary chlorophyll-*a*

Sedimentary chlorophyll-*a* was measured on the cores used for OUR and nutrient incubations (n = 4 to 6 per site). Samples for the analysis of chlorophyll were processed within 3 weeks, when they were weighed and thawed. Subsequently, plant pigments were extracted from the wet sediment with 35 mL of 100% acetone. Samples were initially shaken and placed in a refrigerator (4 ºC) for 24 hours. Samples were shaken once more during the extraction period. Prior to analysis, samples were centrifuged at 1,500 rpm for 10 minutes. Spectrophotometric measurements were made on the supernatant before and after acidification according to Lorenzen (1967). Specifically, 2.7 mL of the extract was pipetted into a quartz cuvette and 0.3 mL of milli-Q water was added to make the final volume 3 mL (90% acetone). The water used for rinsing, dilution and preparation of solutions was organic-free water dispensed by a Milli-Q water system (Milli-Q Plus by Millipore). The blank was 2.7 mL of 100% acetone with 0.3 mL of Milli-Q water. Acidification of the sample was done by adding 0.09 mL of 0.1 N HCl to the sample in the quartz cuvette. To ensure complete acidification, samples were mixed using glass pipettes (without the introduction of air bubbles) and allowed to react for a further 90 seconds prior to measurement. The results are reported as chlorophyll-*a* concentrations per unit area (mg m^{-2}).

2.2.4. Mineral grain size distribution

Cores (n = 3 per site) were thawed, oven dried overnight at 105 ^{o}C and homogenised. A 50 g aliquot of each core was ashed for 12 h at 350 °C to oxidise organic matter and allowed to

cool. This sample was stirred with a dispersing agent (40 g L^{-1} sodium hexametaphosphate in Milli-Q water) for 15 minutes and left to soak overnight. Blank hydrometer (Calton Glass Marketing) readings were noted for the dispersing solution. The sample was stirred for 10 minutes and transferred into a 1 L measuring cylinder and the volume made up to 1 L using milli-Q water. The cylinder was inverted until the sediment was evenly suspended throughout the water column and then placed on a level surface. Hydrometer and temperature readings were taken exactly 2 hours after placement to determine clay content (< 4 μm). The content of the cylinder was then wet sieved through a 63 μm sieve and the retained fraction was dried at 100 °C. A stacked series of graded sieves comprising 2000, 1000, 500, 250, 125 and 63 μm mesh sizes were used to obtain sand fractions. The sample was dry sieved using an automatic sieve shaker (Endecotts EFL2000) set at 5 mins. The different size fractions were then weighed (g). The silt content (4 to 63 μm) was calculated as the difference between the initial weight of the sample and the sand and clay fractions. The resultant weight distributions for the different size fractions were analysed using GRADISTAT software (Blott & Pye, 2001). The mean grain size was computed with GRADISTAT as per the geometric method of Folk and Ward (1957) .

2.2.5. Organic carbon and total nitrogen contents

In the laboratory the top 1 cm of each core (n = 4 per site) was freeze-dried, sieved to 500 μm to remove large shell fragments, and homogenised with a mortar and pestle. Pre-treatment of samples included acidification with 1 N HCl to remove carbonates. Specifically, 2.5 g of the dry sample was weighed directly into a 35 mL glass centrifuge tube and wet with 10 mL of milli-Q water. Subsequently, 1 N HCl was added slowly and in small quantities (~1 mL at a time to a total of 5 mL). The acidified slurry was agitated in an ultrasonic bath at room temperature for 5 minutes. The tube was then filled with Milli-Q water and centrifuged at room temperature (10 minutes at 1,000 rpm) and the water discarded. The same treatment was repeated until no further effervescence was observed. The residue remaining after HCl treatment was rinsed 3 times with Milli-Q water and centrifuged for 10 minutes at 1,000 rpm between rinses to separate the water. The final residue was dried overnight at 50 ^{o}C, homogenised with a mortar and pestle and transferred to a storage vial. Samples were then sent to the Isotope Analysis Service, CSIRO Land and Water, Waite Institute (Adelaide). Organic carbon and total nitrogen were analysed by Continuous-Flow stable Isotope Ratio Mass Spectrometry (CF IRMS) using a Europa Scientific ANCA-SL elemental analyser coupled to a Geo 20-20 Mass Spectrometer. Weight percentages of organic carbon and total nitrogen were corrected for carbonate content and were reported as a fraction (%) of total sediment. The carbonate content was determined from the difference between carbonate free (from acidification) and carbonated samples.

2.2.6. Dissolved nutrients

Dissolved organic carbon (DOC, mg L^{-1}), ammonium (NH_4^+, mg L^{-1}), nitrate plus nitrite (NO_x, mg L^{-1}), total nitrogen (TN, mg L^{-1}) and phosphate (PO_4^{3-}, mg L^{-1}) were also measured in seawater samples (n = 2 per site and depth). Samples were analysed within 1 month of collection by the Water Studies Centre, Monash University, Victoria. The same laboratory analysed the nutrient samples from benthic incubations. Samples for the analysis of DOC were acidified with HCl and purged before being analysed in a Shimadzu TOC-5000 elemental analyzer. Determination of nitrates/nitrites involved reducing nitrates to nitrites in a column packed with copper coated cadmium granules and measuring total nitrites

colourimetrically by flow injection analysis (FIA) (QuickChem 8000 Automated Ion Analyser) (APHA-AWWA-WPCF, 1998b). Briefly, the sample was mixed with acidic sulphanilamide to form a diazo compound, which was then mixed with N-(1-Naphtyl)-ethylene diamine dihydrochloride to form a purple azo dye, the intensity of which is proportional to the sum of the nitrate and nitrite concentration measured spectrophotometrically at 520 nm. The automated phenate method was used for the determination of ammonia by FIA (APHA-AWWA-WPCF, 1998c). Ammonia reacts with hypochlorite to form monochloramine, which in the presence of phenol, nitroprusside and excess hypochlorite, gives indophenol blue. The intensity of indophenol blue is proportional to the ammonia concentration in the sample and is measured spectrophotometrically at 630 nm. For determination of total nitrogen (APHA-AWWA-WPCF, 2001), the sample was digested for 45 minutes in an autoclave with an alkaline persulphate solution to convert N-containing compounds to nitrates which were determined as described above. The digestion process was repeated twice. The persulphate solution was prepared by dissolving 4.5 g of NaOH in 200 mL of MilliQ-water, this solution was cooled to room temperature, 20 g of $K_2S_2O_8$ added and the volume adjusted to 500 mL. FIA was also used to determine phosphate (APHA-AWWA-WPCF, 1998a). The sample was mixed with ammonium molybdate and antimony potassium tartrate to form phosphomolybdic acid, which was reduced by the addition of ascorbic acid to form a blue complex, the intensity of the colour being proportional to the concentration of phosphate. The absorbance was measured at 880 nm.

2.2.7. Statistical analysis for 2003 data

To test the null hypotheses that OUR was not significantly different among sites in 2003, one-way ANOVAs were used (with Tukey's HSD *post-hoc* test). Univariate analyses were conducted using SPSS (version 11.5, SPSS Inc., Chicago). For OUR measurements, a $\log_{10}$ transformation was used to normalise the positively skewed data.

2.2.8. Statistical analysis for 2004 data

To test the null hypothesis that there was no significant difference among sites in OUR, ammonium, nitrate plus nitrite and phosphate fluxes, chlorophyll-*a* and redox potentials measured at 4 cm, one-way ANOVAs (with Tukey's HSD *post-hoc* test) were conducted. Nitrate plus nitrite and phosphate fluxes were $\log_{10}$ transformed prior to analysis to normalise positively skewed distributions.

To determine the potential for the ammonium, nitrate plus nitrite and phosphate fluxes, chlorophyll-*a* and redox potential to significantly predict OUR, a multiple regression was performed. These five variables were included as they were all measured from the same sediment sample and have previously been shown through other studies to influence OUR (Jorgensen, 1977; Valdovinos & Figueroa, 2000; Wenzhofer & Glud, 2004).

To test the null hypotheses that there were no significant differences in organic carbon or nitrogen among sites, one-way ANOVAs were used (with Tukey's HSD *post-hoc* test). Particle size distribution of the sediments was analysed using GRADISTAT software to determine the mean grain size (Blott & Pye, 2001). To test the null hypotheses that there was no significant difference in mean grain size or the percentage of silt and clay among sites one-way ANOVAs were used (with Tukey's HSD *post-hoc* test).

For those sites sampled in both spring of 2003 and autumn of 2004 (Plank Point, Western Shoal and Wallaroo), a two-way ANOVA (with Tukey's HSD *post-hoc* test) was used to assess the factors of the three sites, two seasons and the interaction of site and season on the OUR. The analysis assessed the null hypotheses that there were no significant differences in OUR among sites or between seasons, or as a result of the interaction between site and season.

To explicitly describe linear relationships among the dissolved nutrient concentrations (dissolved organic carbon, ammonium, phosphate and nitrate plus nitrite) principal component analysis (PCA) was used (PRIMER, Plymouth). PCA scores with eigenvalues greater than 1 were used to assess the null hypotheses that nutrient flux data and the standing stock measurements did not vary significantly with site using ANOVA (with Tukey's HSD *post-hoc*).

2.3. Results

2.3.1. OUR in 2003

The highest mean OUR values were recorded in the southeast at North Spilsby (1477 µmol m^{-2} h^{-1}) and SE Cape Donington (1,432 µmol m^{-2} h^{-1}) with the lowest recorded in the north at Plank Point (429 µmol m^{-2} h^{-1}) and Western Shoal (435 µmol m^{-2} h^{-1}) (Figure 2.3). Mean OUR was significantly different among sites (Table 2.3 a). North Spilsby had significantly greater OUR to all sites ($p < 0.045$) except SE Cape Donington, and SE Cape Donington was significantly greater than Plank Point and Western Shoal ($p < 0.03$) (Figure 2.3).

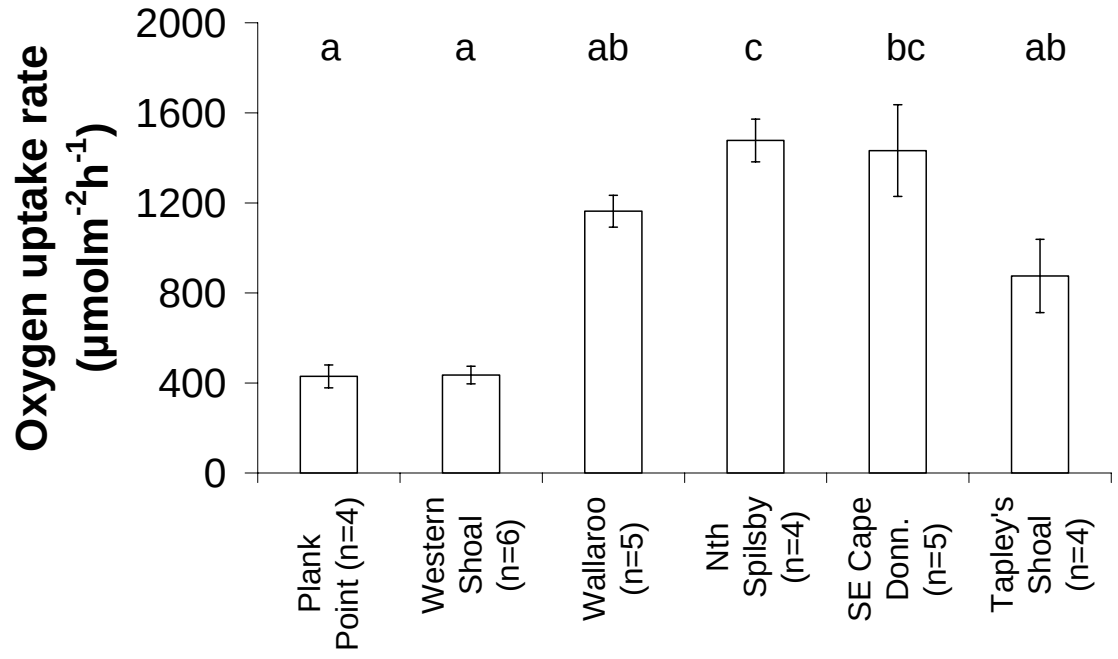

Figure 2.3. Benthic oxygen uptake rate in Spencer Gulf during 2003. Bars indicate means (± SE). Letters denote significantly different ($p < 0.05$) sites according to Tukey's *post-hoc* test.

Table 2.3. Influence of spatial variability in Spencer Gulf on OUR and nutrient fluxes in (a) spring 2003 and (b) autumn 2004.

(a)

Variable		df	Mean Square	F	Sig.
Log_{10} OUR	Site	5	0.399	19.408	<0.001
	Residual	28	0.021		

(b)

Variable		df	Mean Square	F	Sig.
OUR	Site	3	456237	3.051	0.064
	Residual	14	149542		
Ammonium flux	Site	3	1044	4.084	0.021
	Residual	19	256		
Log_{10} nitrate plus nitrite flux	Site	3	0.469	15.758	<0.001
	Residual	19	0.030		
Log_{10} phosphate flux	Site	3	0.315	9.734	<0.001
	Residual	19	0.032		
Chlorophyll-*a*	Site	3	848	1.584	0.226
	Residual	19	536		
Redox potential	Site	3	6201	10.972	<0.001
	Residual	16	565		

2.3.2. Benthic fluxes and standing stock measurements in 2004

The highest mean OUR was recorded in the centre of the Gulf at The Gutter (1,240 µmol m^{-2} h^{-1}) and the lowest in the north at Western Shoal (483 µmol m^{-2} h^{-1}), although the difference was not significant (Table 2.3 b, Figure 2.4 a). The highest mean ammonium flux was recorded at Wallaroo (22.8 µmol m^{-2} h^{-1}) with Western Shoal recording the lowest (-8.72 µmol m^{-2} h^{-1}) (Figure 2.4 b). The mean ammonium fluxes were significantly different among sites (Table 2.3 b), with Western Shoal being the only site to show a net uptake of ammonium into the sediment. For nitrate plus nitrite fluxes, the overall difference among sites was significant (Table 2.3 b), with higher rates recorded in the centre of the Gulf at The Gutter (119.5 µmol m^{-2} h^{-1}) than at any other site ($p < 0.011$) (Figure 2.4 c). For phosphate fluxes, The Gutter showed the greatest flux (5.29 µmol m^{-2} h^{-1}) and Western Shoal the lowest (1.56 µmol m^{-2} h^{-1}) (Figure 2.4 d). Again, as for the other two nutrient fluxes, the differences in phosphate fluxes between sites were significant (Table 2.3 b). The Gutter had the highest concentration of chlorophyll-*a* per unit area (89.6 mg m^{-2}) and Plank Point in the north the lowest (62.1 mg m^{-2}), but the difference among sites was not significant (Table 2.3 b, Figure 2.4 e). The redox potential at all sites remained positive above 8 cm of sediment and at 4 cm depth the difference among sites was significant (Table 2.3 b, Figure 2.4 f). Plank Point had significantly lower redox potential measured at 4 cm than the other 3 sites ($p < 0.025$).

Variation in OUR was explained by variation in the other core level variables (Multiple Regression, $r^2 = 0.579$, $n = 16$). The equation was OUR (µmol m^{-2} h^{-1}) = 660.342 + 1.741

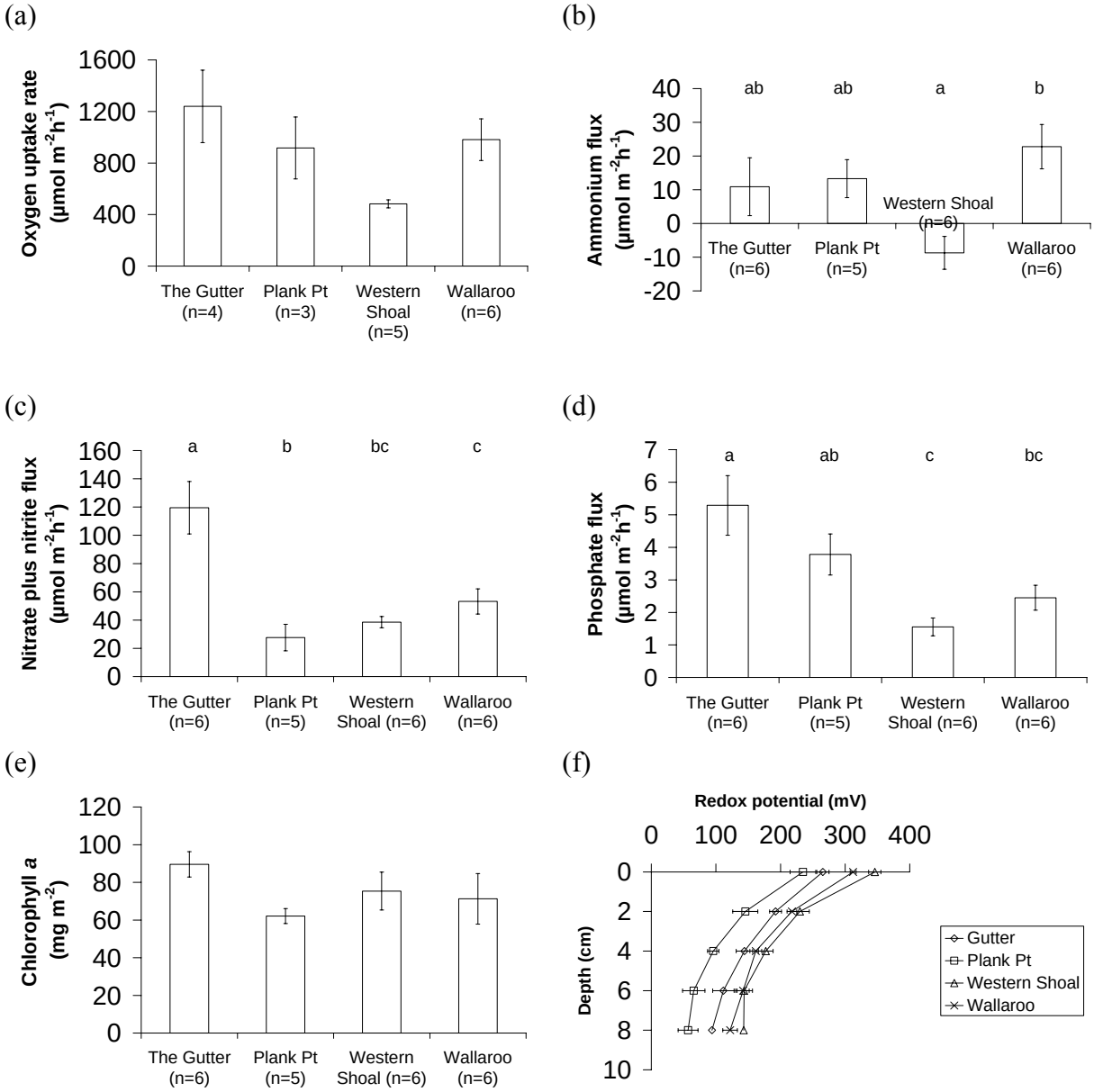

Figure 2.4. Benthic (a) OUR, (b) ammonium fluxes, (c) nitrate plus nitrite fluxes, (d) phosphate fluxes, (e) chlorophyll-*a* concentrations and (f) redox potential profiles in Spencer Gulf during 2004. Bars and points indicate means (± SE). Letters denote significantly different ($p < 0.05$) sites according to Tukey's *post-hoc* test.

ammonium flux (μmol m^{-2} h^{-1}) + 1.569 nitrate plus nitrite flux (μmol m^{-2} h^{-1}) + 148.465 phosphate flux (μmol m^{-2} h^{-1}) – 1.001 redox potential (mV) – 2.946 chlorophyll-*a* (mg m^{-2}).

The organic carbon content at Wallaroo was the highest (0.68%) and The Gutter the lowest (0.39%) (Figure 2.5 a). There was no significant difference among sites in the organic carbon content (Table 2.4). The total nitrogen content of the sediments did vary significantly among sites (Table 2.4), with this result mainly due to a significant difference between The Gutter in the center of the Gulf (0.06%) and Plank Point in the north (0.09%) (Figure 2.5 b). The average C:N ratio was 7.4 and ranged from 6.2 at The Gutter to 8.5 at Wallaroo (Figure 2.5 c).

Mean grain size ranged from 108 μm (very fine sand) at The Gutter to 180 μm (fine sand) at Wallaroo (Figure 2.6 a) and there was no significant difference between sites (Table 2.5). The percentage of silt and clay was the highest at The Gutter (27%) and lowest at Western Shoal (18%) (Figure 2.6 b), and as for mean grain size there was no significant difference among sites (Table 2.5).

The largest change in OUR between seasons was found at Plank Point, where the OUR in spring of 2003 (429 μmol m^{-2} h^{-1}) was less than half of the OUR in autumn of 2004 (917 μmol m^{-2} h^{-1}) (Figure 2.7). Wallaroo showed a small decrease in OUR between seasons from 1163 μmol m^{-2} h^{-1} in spring of 2003 to 981 μmol m^{-2} h^{-1} in autumn of 2004. Western Shoal showed consistently low OUR, being 435 and 483 μmol m^{-2} h^{-1} in spring 2003 and autumn 2004, respectively. A significant interaction effect of site and season on OUR was found (Table 2.6).

Table 2.4. Effects of spatial variability in Spencer Gulf on organic carbon and nitrogen content of sediments sampled in 2004.

Variable		df	Mean Square	F	Sig.
Organic carbon	Site	3	0.067	2.277	0.132
	Residual	12	0.030		
Total nitrogen	Site	3	0.001	4.682	0.022
	Residual	12	0.000		

Table 2.5. Effects of spatial variability in Spencer Gulf on mean grain size and the percentage of silt and clay of sediments sampled in 2004.

Variable		df	Mean Square	F	Sig.
Mean grain size	Site	3	3180	0.748	0.553
	Residual	8	4251		
% silt and clay	Site	3	50	1.980	0.196
	Residual	8	25		

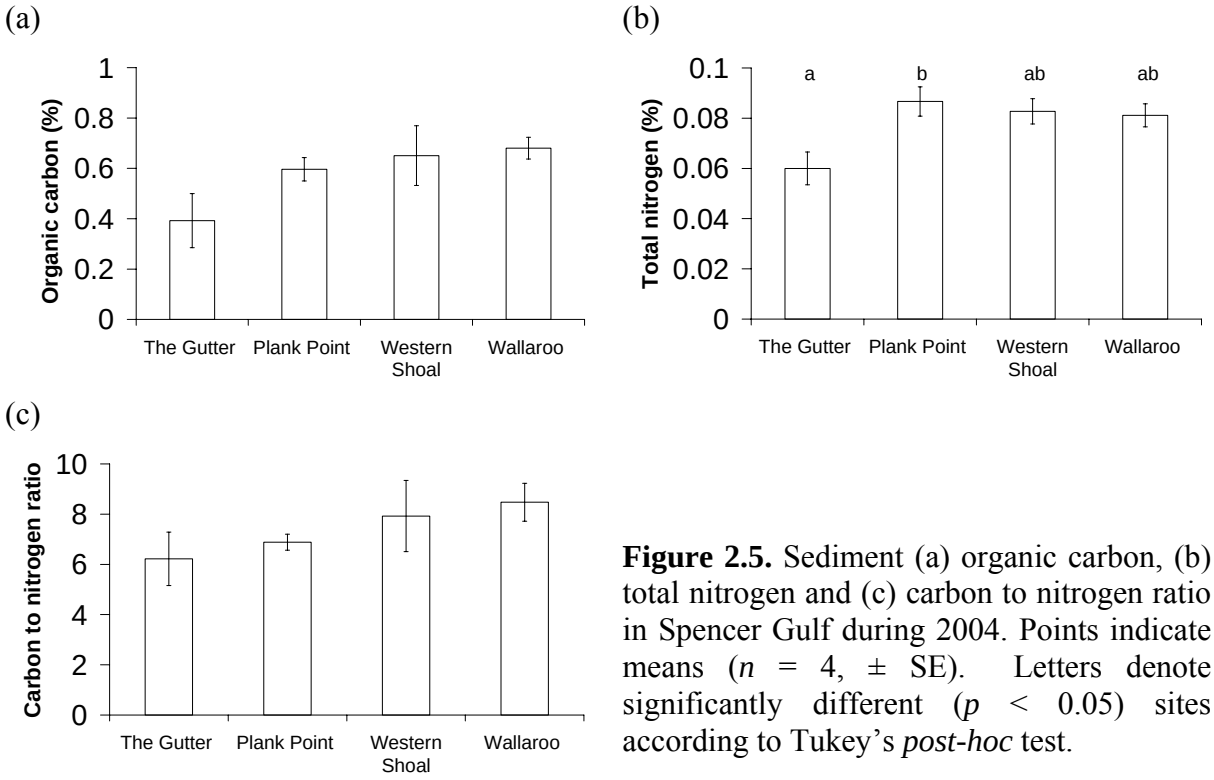

Figure 2.5. Sediment (a) organic carbon, (b) total nitrogen and (c) carbon to nitrogen ratio in Spencer Gulf during 2004. Points indicate means (n = 4, ± SE). Letters denote significantly different (p < 0.05) sites according to Tukey's *post-hoc* test.

Table 2.6. Effects of spatial and seasonal variability in Spencer Gulf on OUR measurements.

Source	df	Mean Square	F	Sig.
SITE	2	963493.526	16.258	.000
SEASON	1	94986.930	1.603	.218
SITE * SEASON	2	260402.238	4.394	.024
Error	23	59263.108		

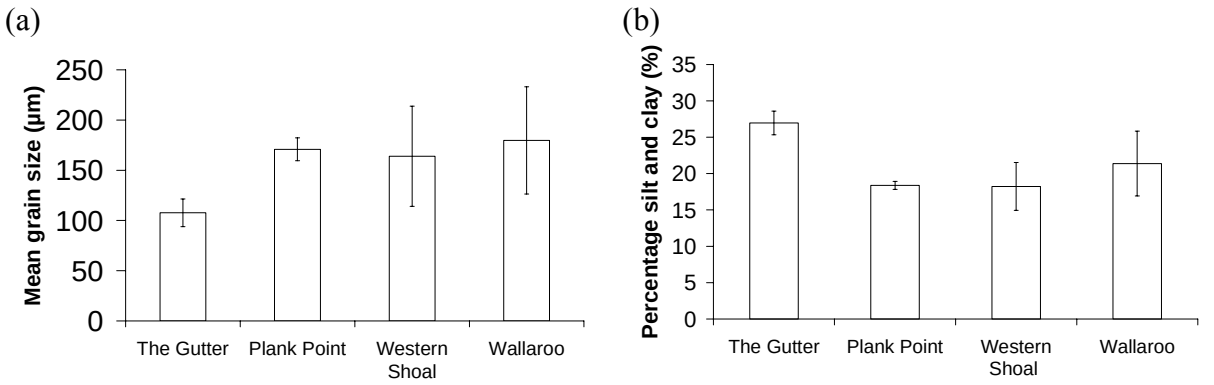

Figure 2.6. Grain size characteristics in Spencer Gulf in 2004: (a) mean grain size and (b) percentage of silt and clay. Bars indicate means (n = 3, ± SE).

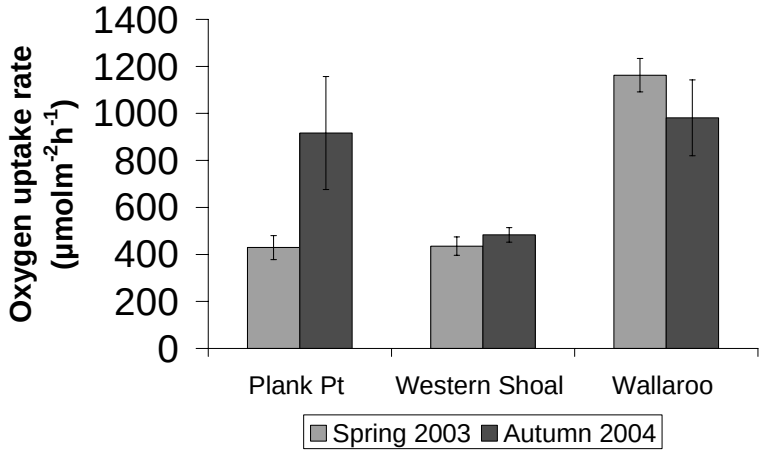

Figure 2.7. OUR in Spencer Gulf measured at the same sites during spring of 2003 and autumn of 2004. Bars indicate means (± SE).

2.3.3. Dissolved nutrient concentrations

For the seawater nutrients measured, there was no evidence of concentration differences with depth among sites (Figure 2.8). The DOC concentration at the three sites sampled did not show great variation, ranging between 1.5 to 2 mg L^{-1} (Figure 2.8 a). Total nitrogen levels were at their highest at Western Shoal (0.15 mg L^{-1}) and lowest at The Gutter (0.09 mg L^{-1}) (Figure 2.8 b). Ammonium levels at Western Shoal were an order of magnitude greater than at the other two sites (0.02 mg L^{-1} versus approximately 0.002 mg L^{-1} at The Gutter and Plank Point) (Figure 2.8 c). Phosphate concentrations were below detectable levels (<0.001 mg L^{-1}) at all sites. Nitrate plus nitrite concentrations also varied among sites, from below detection (<0.001 mg L^{-1}) at Plank Point to 0.001 mg L^{-1} at Western Shoal (Figure 2.8 d).

The seawater samples from Western Shoal grouped separately in ordination space from the samples taken at The Gutter and Plank Point, which grouped together on the PCA (Figure 2.9). The first PC explained 60.1% of the variation with another 23.1% explained by the second PC (Table 2.7 a). Positive scores on the first PC corresponded to decreasing ammonium concentrations and positive scores on the second PC corresponded to increasing nitrate plus nitrite concentrations and decreasing DOC concentrations (Table 2.7 b). Significant differences among sites were found upon analysis of PC1 scores (Table 2.8), where Western Shoal was significantly different to The Gutter and Plank Point ($p < 0.004$). From the PC1 loadings (Table 2.7 b) and inspection of the raw data (Figure 2.8 c), the significant difference among sites was driven by higher ammonium concentrations at Western Shoal.

Table 2.7. (a) Eigenvalues and (b) eigenvectors from the PCA of the dissolved nutrient concentrations measured at the 4 sites sampled in Spencer Gulf in 2004.

(a)

Principal Component	Eigenvalue	% Variation
1	2.4	60.1
2	0.93	23.1
3	0.46	11.4
4	0.21	5.4

(b)

Dissolved Nutrient	PC1	PC2
Ammonium	**-0.563**	0.351
Nitrate plus nitrite	-0.498	**0.550**
Total nitrogen	-0.507	-0.387
DOC	-0.422	**-0.651**

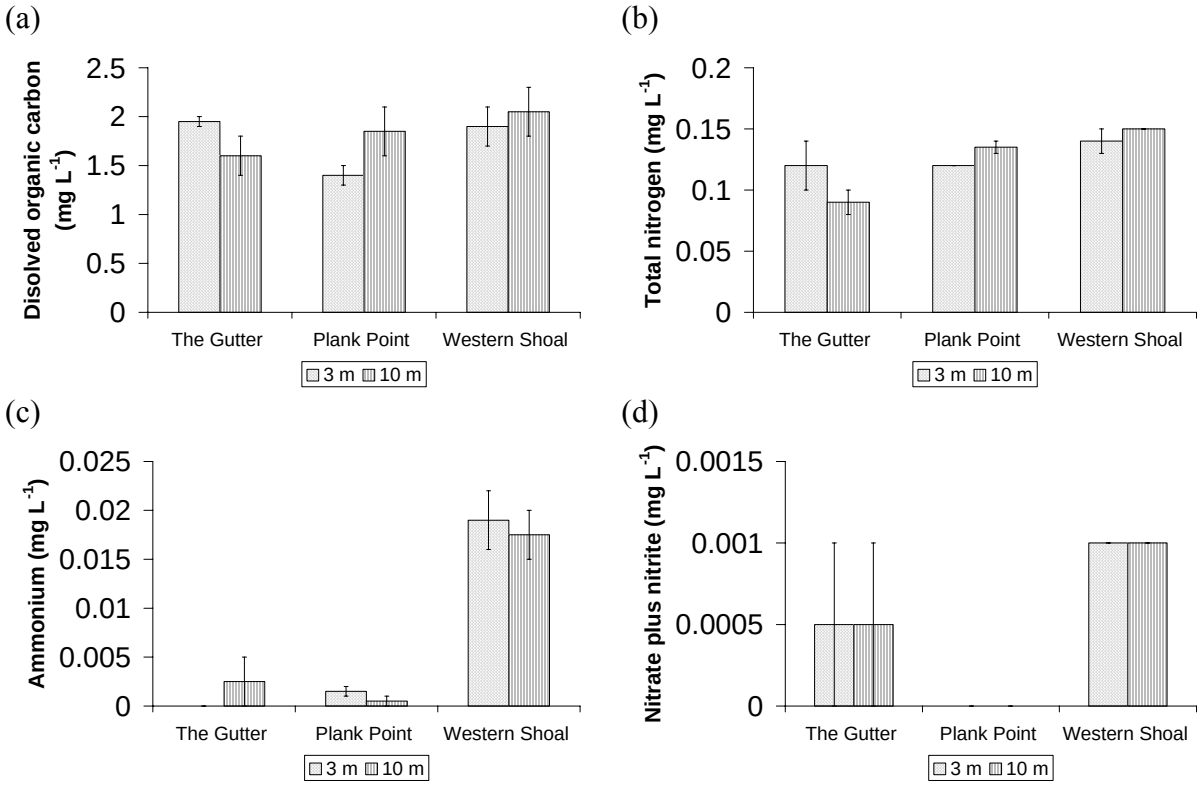

Figure 2.8. Water nutrient concentrations of (a) DOC, (b) total nitrogen, (c) ammonium and (d) nitrate plus nitrite in Spencer Gulf during 2004. Bars indicate means (± SE). Blank data indicate zero values.

Table 2.8. ANOVA table for the PC1 scores from principal component analysis of dissolved nutrients in Spencer Gulf in 2004.

Source	df	Mean Square	F	Sig.
SITE	2	10.804	22.051	.002
DEPTH	1	.018	.037	.853
SITE * DEPTH	2	.934	1.906	.229
Error	6	.490		

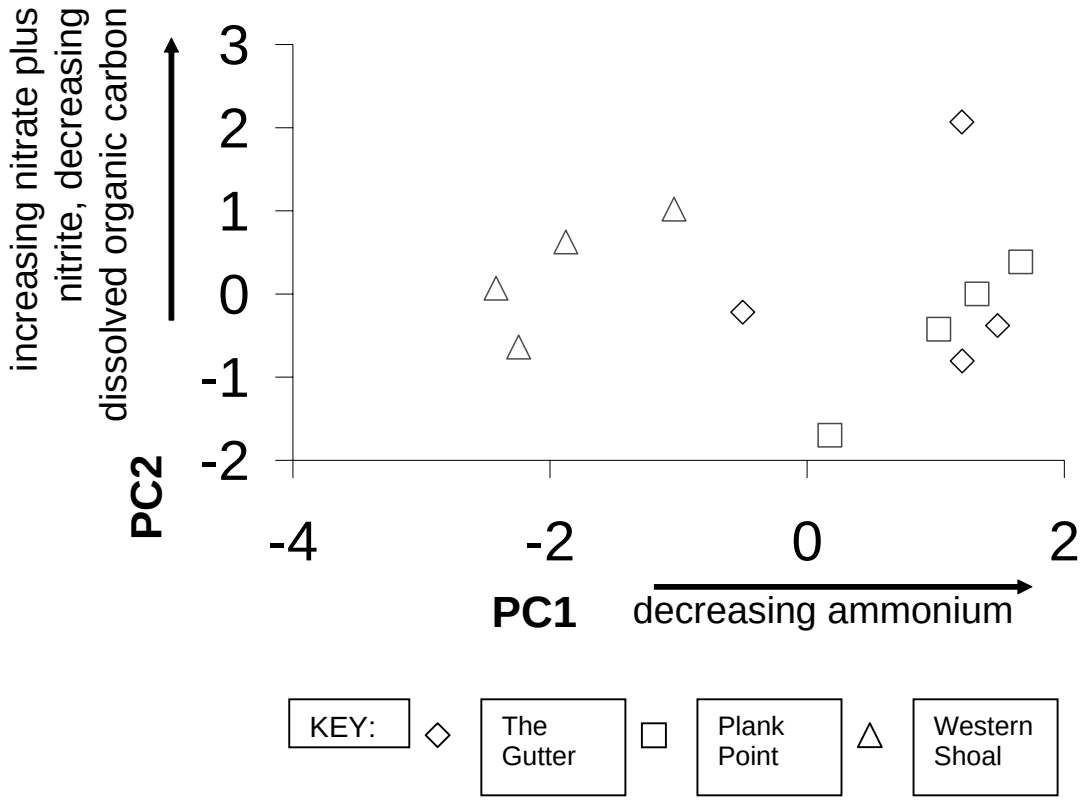

Figure 2.9. PCA plot for the dissolved nutrient concentrations in Spencer Gulf during 2004.

2.4. Discussion

2.4.1. Oxygen uptake rates

In 2003, OUR varied at large spatial scales (> 100 km) throughout Spencer Gulf. However, there was no significant difference among the four sites sampled in 2004. The range in OUR for these four sites was relatively low compared with the total variation observed among the 6 sites sampled in 2003. There was a significant interaction effect of site and season on the OUR measured at the three sites that were sampled in both years; stemming from a

significant increase in OUR at Plank Point from spring 2003 to autumn 2004. This interaction suggests that temporal as well as spatial variability may lead to significant variation among measured OUR. Indeed, an effect of seasonality on OUR at temperate latitudes has been previously documented (Jorgensen & Sorensen, 1985; Sampou & Oviatt, 1991; Kemp et al., 1992; Vidal et al., 1997). Consequently, the total variability in OUR observed at the sites throughout Spencer Gulf consists of significant temporal and spatial components and future sampling strategies should aim to encompass these aspects.

The OUR measured throughout Spencer Gulf fall within the range of published literature values for subtidal coastal marine sites (Table 2.9) (Pamatmat, 1971; Jorgensen, 1977; Hopkinson & Wetzel, 1982; Kaspar et al., 1985; Baudinet et al., 1990; Kemp et al., 1992; Aller et al., 1996; Burke, 1999; Nicholson et al., 1999; Grenz et al., 2003; Rabouille et al., 2003). The sites included in Table 2.9 do not include studies where anthropogenic activities were the focus of investigations and were re-calculated for comparison in common units of measurement (i.e. μmol m^{-2} h^{-1}). The variability in OUR among these sites, both within and between studies, may be attributed to various site specific differences. Among other study-wide differences such as the method of measurement of OUR, site specific differences include water depth, temperature range, sediment type and benthic infauna. The OUR measured throughout the Spencer Gulf are similar in magnitude to the measurements made within the SBT farming region at control sites and for farming sites when measured before or after stocking of the pontoons (Chapter 5).

Studies of OUR at temperate latitudes have shown decreased OUR during winter associated with lower seawater temperatures, when compared with summer periods (Mazouni et al., 1996; Hopkinson et al., 1999). At Plank Point and Western Shoal, higher OUR were recorded in 2004 when seawater temperatures were higher. More specific differences contributing to seasonal change in OUR have been highlighted by Epping and Helder (1997), who showed that benthic photosynthesis during summer months contributed to variation in OUR. The finding that OUR may be predicted from nutrient flux data and other measures of sediment biogeochemistry (e.g. redox potential) offers evidence that OUR is a sensible measure of total community metabolism for the sediments that were sampled within Spencer Gulf.

2.4.2. Nutrient fluxes

The ammonium and phosphate fluxes measured within the Gulf fall at the lower end of comparable published values, whereas nitrate plus nitrite fluxes were at the upper end (Table 2.10) (Kaspar et al., 1985; Baudinet et al., 1990; Kemp et al., 1992; Aller et al., 1996; Nicholson et al., 1999). The studies included for comparison are from other subtidal temperate marine sites where anthropogenic activities were not the main focus of investigation and the rates were re-calculated for comparison in common units of measurement (i.e. μmol m^{-2} h^{-1}). However, the work in Port Phillip Bay by Nicholson et al. (1999) undertaken to test the compatibility of two types of *in situ* benthic samplers did include sites that were proximal to outfall stations and a freshwater discharge point. Even so, the ammonium and phosphate fluxes measured adjacent to active SBT pontoons within the farming area off the coast of Port Lincoln greatly exceeded previously observed natural variability (Chapter 5). Overall, the nutrient fluxes follow the same trend as for OUR, where measurements made within the Gulf are comparable to those made at control sites and unstocked pontoon sites within the SBT farming region (Chapter 5).

Table 2.9. The range in OUR measured at various subtidal coastal marine sites worldwide.

Author	Site	Depth (m)	Sediment type	Temp. (ºC)	Range of OUR (µmol m^{-2} h^{-1})
Aller et al. 1996	Amazon Shelf, Brazil	10 to 40	Fine grained	20	291 to 1041
Burke 1999	Port Phillip Bay, Aust.	8 to 24	n/d	20	75 to 1291
Hopkinson and Wetzel 1982	Georgia Bight, U.S.A.	1 to 10	96% sand, 1.5% silt, 1.5% clay	Max 28	Mean of 3780
Jorgensen 1977	Limfjorden, Denmark	4 to 12	Silt/clay to fine sand	0 to 20	500 to 3041
Kemp et al. 1992	Chesapeake Bay, U.S.A.	9 to 18	n/d	8 to 27	651 to 2343
Nicholson et al. 1999	Port Phillip Bay, Aust.	9 to 24	soft	n/d	791 to 3500
Pamatmat 1971	Puget Sound, U.S.A.	22	n/d	7	268 to 281
Rabouille et al. 2003	Golfe de Fos, France	9	Muddy sands	n/d	200 to 1966
Grenz et al. 2003	New Caledonia, Sth Pacific	10 to 52	Mud to sand	n/d	450 to 2250
Present Chapter	Spencer Gulf	17.5 to 26.5	Very fine sand to fine sand	15 (2003) to 18 (2004)	429 to 1477
This study, Chapter 5	Port Lincoln farming area	18 to 22	Very coarse silt to fine sand	14.5 to 22	448 to 4329

n/d = no data.

Table 2.10. The range in nutrient fluxes measured at various subtidal coastal marine sites worldwide.

Author	Site	Ammonium flux (μmol m^{-2} h^{-1})	Nitrate plus nitrite flux (μmol m^{-2} h^{-1})	Phosphate flux (μmol m^{-2} h^{-1})
Aller et al. 1996	Amazon Shelf, Brazil	-354 to 63	-83 to 37	-7 to -0.8
Kemp et al. 1992	Chesapeake Bay, U.S.A.	209 to 943	n/d	n/d
Nicholson et al. 1999	Port Phillip Bay, Aust.	8 to 492	-2 to 26	4 to 79
Kaspar et al. 1985	South Island, New Zealand	17 to 21	8 to 25	n/d
Baudinet et al. 1990	Gulf of Fos, France	4 to 366	n/d	-3.2 to 116
Present Chapter	Spencer Gulf, Aust.	-9 to 23	28 to 120	2 to 5
This study, Chapter 5	Port Lincoln farming area, Aust.	32 to 9,963	2 to 122	4 to 2,177

n/d = no data.

From comparison of the ammonium fluxes obtained in the present study to those in Table 2.10, it may be suggested that either nitrogen mineralisation occurred at a slower rate within the sedimentary environment of the Gulf compared with other sites, or that a relatively larger proportion of mineralised ammonium undergoes nitrification and denitrification within the Gulf. Previous studies have shown that under increasing levels of organic loading to the sediments, the ammonium flux increases, while the proportion of nitrification increases slightly then decreases (Blackburn & Blackburn, 1992b). A similar result was reported by Heggie et al. (1999), where, under high loads of organic matter to the sediments, denitrification efficiencies were near zero and nitrogen was returned to the water column as ammonium. Indeed the organic carbon (0.4 to 0.7%) and total nitrogen (0.06 to 0.09%) levels in the sediments sampled within the Gulf were not particularly high, relative to levels associated with high loads of organic matter in similar sediment types (e.g. > 3% for sands). Therefore, as nitrification was measured via nitrate plus nitrite fluxes, it seems reasonable to conclude that the sedimentary environment of the sites sampled within the Gulf was not subject to high levels of organic loading. Furthermore, as the nitrate plus nitrite fluxes were measured at all sites sampled, nitrification was an active sink for mineralised ammonium.

There was a net uptake of ammonium by the sediments sampled at Western Shoal. Aller et al. (1996) also found uptake of ammonium by Amazon Shelf sediments sampled at 10 to 40 m depth. Although Aller et al. (1996) had more corroborative evidence from directly measured carbon dynamics than may necessarily be inferred from OUR measured in the present study, they concluded that the sediments had a capacity to nitrify and denitrify 100% of the mineralised ammonium. Indeed, it was found in the present study that nitrification occurred within the sediments at Western Shoal, as evidenced by positive nitrate plus nitrite fluxes. However, dentrification was not directly measured in the present study. The amount of organic material within the sediment system can affect the dynamics of ammonium remineralisation and nitrification (Blackburn & Blackburn, 1992b; Heggie et al., 1999) and may have explained the discrepancy in ammonium fluxes between sites within Spencer Gulf. As there were no significant differences in the organic carbon or total nitrogen content of the sediments at Western Shoal compared with the other sites, it seems unlikely that variations in organic loading can explain the ammonium fluxes, although we caution that the labile

component was not investigated. Further investigations of the nutrient fluxes at Western Shoal may focus on the nitrogen cycle more closely, specifically on the sinks and sources for mineralised ammonium.

2.4.3. Standing stock measurements

The greatest difference in chlorophyll-*a* concentrations was between The Gutter and Plank Point. The range of sedimentary chlorophyll-*a* concentrations measured throughout Spencer Gulf was comparable in magnitude to that measured around the SBT farming region, irrespective of site usage. Magni et al.(2000) stated that much research in the last decade has been focused upon the role of benthic microalgae as a carbon source for food webs and its implications for cycling of nutrients. Through the use of a carbon to chlorophyll-*a* ratio the relative contribution of benthic microalgal derived carbon within the sediments may be estimated. Using a C:chl a ratio of 50 (Garrigue, 1998; Light & Beardall, 1998) (and the concentrations of chlorophyll-*a* in $\mu g\ g^{-1}$ for the sediments sampled in the present study; data not presented here) chlorophyll-*a* constituted 2.7% of the organic carbon of the sediments across all sites (range from 1.8% at Plank Point to 4.9% at The Gutter). The range found in Spencer Gulf falls within the range of 0.1 to 12% reported by Light & Beardall (1998) for 64 sites sampled at 2 to 24 m depth in Port Phillip Bay, Victoria. The remainder of the carbon at the sites in Spencer Gulf is made up of varying amounts of microalgal degradation products, sedimentary phytoplankton, microbial origins (e.g. from bacteria), degraded root material of macrophytes, meiofauna, macrofauna and other labile or refractory material.

Interestingly, the relatively high percentage of benthic microalgal-derived carbon at The Gutter corresponded with the highest OUR, nitrate plus nitrite and phosphate fluxes. Phytoplankton derived material is considered to have a greater proportion of labile material than seagrass detritus (Miller et al., 1996; Herbert, 1999), and hence the difference in the source of organic material may have contributed to the generally higher benthic fluxes observed at The Gutter compared with Wallaroo. The organic carbon and total nitrogen contents of sediments at The Gutter were also the lowest among the four sites sampled in 2004, which suggested that there was less accumulation of organic material in the sediments there. Consequently, there may have been less organic inputs into the sedimentary environment of The Gutter or, given equal amounts of inputs, a greater labile component. Benthic microalgae are a food source for a range of animal species from several trophic groups, including surface deposit feeders (Garrigue, 1998) and therefore, the higher concentrations of chlorophyll-*a* at The Gutter may also be associated with greater macroinfaunal abundances. Further investigation into the benthic community structure of Spencer Gulf may provide valuable insight into the links between trophic levels and community metabolic processes.

The carbon to nitrogen ratios of the sediment sampled at the four sites in 2004 suggest the organic material is derived from marine sources. Thunnell et al. (2000) cited a report that marine organic matter has carbon to nitrogen ratios of 6 to 7, while terrestrially derived matter often has ratios exceeding 20. The carbon to nitrogen ratios measured at the four sites sampled in 2004 (6.2 to 8.5) suggest that terrestrial sources do not contribute any substantial amount of organic material into the sedimentary systems sampled and this is not surprising given the lack of terrestrially derived freshwater inputs. The marine nature of the sedimentary organic material does reinforce previous conclusions that internal recycling of

nutrients must be an ecologically important process supporting primary production within the Gulf (Smith & Veeh, 1989).

The organic carbon and total nitrogen contents, mean grain size and the percentage of silt and clay in the sediments sampled throughout the Gulf were comparable in magnitude to the northern sites sampled in the SBT farming region, although at the lower end of the variation observed among sites (Chapter 5). The sediments of the sites towards the south of the SBT farming had double the organic carbon and total nitrogen content, about half the mean grain size and 50% more silt and clay than those sites sampled in the Gulf. Consequently, the variation in sediment types observed within the SBT farming area was greater than the variation observed at the four sites sampled in 2004. Therefore, physical properties of the sedimentary environments within the Gulf may vary upon relatively small spatial scales. Indeed, patchy distribution of organic material within the marine environment has been well documented, at various spatial and temporal scales (Brooks, 2001).

The consequences of sediment characteristics for the redox state of the sediments and the redox potential may be used to determine whether sedimentary organic material is more likely to be oxidised or reduced (Zobell, 1946). Although significantly lower redox potentials were measured at 4 cm at Plank Point than at the other three sites sampled in 2004, all measurements remained positive above 8 cm depth. This offers evidence for the aerobic state of the sediments to this depth and suggests that the sediments were well oxygenated and benthic metabolic processes were not oxygen limited. Again, the redox potentials measured throughout the Gulf were comparable in magnitude to those recorded within the SBT farming region at control sites and for farming sites when measured before or after stocking of the pontoons (Chapter 5).

2.4.4. Dissolved nutrients

The dissolved nutrient levels found here are comparable to Smith & Veeh (1989) measurements within the Gulf. Phosphate was below detectable levels at the four sites sampled in 2004. This is in contrast to the measurements made within the SBT farming region, where measurable concentrations were found at all sites and at all sampling times (Chapter 5). Smith & Veeh (1989) suggested that the source of phosphate in the Gulf was from oceanic water and that virtually all dissolved inorganic phosphorus in the Gulf is taken up (presumably by photosynthetic organisms, mineral precipitation or adsorption by particulate material). Consequently, as the SBT farming region is positioned at the western side of the mouth of the Gulf, where the oceanic water enters the Gulf, it may seem reasonable that phosphate concentrations within the farming area would more closely reflect oceanic water concentrations rather than that of the central and northern reaches of the Gulf.

The observations made at Western Shoal, where ten times greater ammonium concentrations in the water column were sampled than at the other two sites, may have been a result of sediment resuspension from a recent storm event. It is noteworthy that DOC, total nitrogen, and nitrate plus nitrite concentrations in the water column were also the highest at Western Shoal. In fact, a two-day storm event in Spencer Gulf directly preceded sampling at Western Shoal. Tengberg et al. (2003) stated that sediment resuspension temporarily liberates porewater and mixes it with the overlying water, which generates a sudden increase in nitrogen in the water column. However, reports have stated that the BHP facility at Whyalla discharges 170 tonnes of oxidised nitrogen and 80 to 110 tonnes of ammonium per year into

the marine environment (Edyvane, 1999), which may impact on Western Shoal which is about 20 km south. Therefore repeated sampling of dissolved nutrient concentrations at Western Shoal during various sea-states may clarify the underlying causes of the observations made during the present study; whether they are natural or anthropogenic.

2.5. Conclusions

The information from this chapter questions part of the biogeochemical cycling model put forth by Smith & Veeh (1989). Specifically, the model suggested by Smith & Veeh (1989) had no nutrient inputs from the sediment into the water column but rather only a sink from the water column into the sediments. The results here clearly show that in general, inputs of nitrogen and phosphorus into the water column also emanate from the sediments, suggesting that the atmospheric and hydrographical inputs outlined by Smith & Veeh (1989) may be overestimates. Moreover, significant site specific differences in OUR and nutrient fluxes observed within the Gulf suggest that modelling system-wide biogeochemical processes based on limited averages from a few sites only is inherently flawed and can lead to inaccurate conclusions. In the global context, the benthic fluxes within Spencer Gulf fall within the range of the published values for other subtidal coastal marine waters. In the regional context, these fluxes and dissolved nutrient concentrations were similar to those recorded at control sites sampled within the SBT farming region (Chapter 5).

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Chapter 3: Sediment geochemistry in lower Spencer Gulf, South Australia: implications for southern bluefin tuna farming

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Abstract

Farming of southern bluefin tuna in South Australia currently contributes to more than 30% of the value of the aquaculture production in Australia. This study investigated the natural sedimentary setting of the area designated for this important industry in coastal waters off Port Lincoln, and explored the links between the natural distribution of sediments and potential environmental effects and risks to the industry. Sediments were mostly composed of poorly sorted silts and fine sands, predominantly skeletal remains of carbonate-secreting organisms. The contribution of plankton to the organic matter remaining in the sediments was calculated to be in excess of 80% using concentration-dependent stable isotope mixing models. An erosional area was identified south of Rabbit Island where sediments contained up to 50% siliciclastic material, grainsize distributions were better sorted and coarser, and organic carbon and total nitrogen contents were very low. In contrast, deeper waters north of Cape Donington were identified as a depocentre for fine sediments, which contained organic matter levels twice those elsewhere in the region despite the extremely high carbonate contents (>80%). The heavier stable isotopic signature of nitrogen suggested that this organic matter comprised a greater fraction of weathered components, probably advected to the area by suspended and bedload transport. This local variability of sediment characteristics in the farming zone suggests that the benthic assimilative capacity of farmed sites will depend on their location. Wastes from pens located south of Rabbit Island in particular are likely to be quickly winnowed out by wave and tidal action. These pens are also less likely to be affected by resuspension of fine sediments that might be associated with unusually severe storms.

3.1. Introduction

Farming of southern bluefin tuna is the most valuable aquaculture industry in Australia, contributing to more than 30% of the total value of production in 2002-2003 (FRDC, 2004). The industry has expanded considerably in the last decade, with stocks farmed off Port Lincoln in southern Spencer Gulf increasing from 138 tonnes in 1991/1992 to current levels of more than 5,000 tonnes (Jeffriess, 2004). While fish farms rely on an unpolluted marine environment to sustain production, they are a point source of wastes and invariably raise public concerns over potential environmental effects caused by the rapid expansion of the industry.

The ability of the environment to assimilate farm wastes and the resulting long-term effects on sedimentary textural, chemical and biological characteristics will depend on farm management but also on the natural setting, e.g. sediment composition, scavenger activity and dispersion patterns. Whereas most studies dealing with the environmental impacts of fish farms concentrate on the sedimentary accumulation of organic matter, the inorganic composition and textural characteristics of sediments will affect the rates of mineralisation and dispersal of farm-derived wastes and are therefore essential parameters in modelling farm effects.

While the effects of fish farming on the environment is a topic of debate, the reliance of the industry on a healthy environment needs to be acknowledged and discussed. Sudden environmental changes can have a devastating effect on production, as was demonstrated in Port Lincoln in April 1996 when unusual stormy weather resuspended sediments into the water column, leading to substantial southern bluefin tuna mortality, 60% of standing stock or more for some operators (Clarke, 1996; Grzechnik, 2000). That incident encouraged the industry to complete relocation of pens from the shallow protected waters of Boston Bay to the deeper and more exposed waters further offshore of Boston Island. However, while many studies have investigated sediments in northern Spencer Gulf (Belperio et al., 1984; Gostin et al., 1984; Gostin et al., 1988; Harris, 1994) little has been done on its southern end, and only limited information has been available on sediment characteristics in the offshore southern bluefin tuna farming zone. The most comprehensive work on sediments in the southern Spencer Gulf was published by Fuller et al. (1994), and suggested that fine molluscan sands accumulate on the western coast as a result of northward bottom currents flowing into the gulf from the southern Australian shelf.

This study aimed to evaluate the natural geochemical characteristics of sediments offshore of Boston Island with a view to assessing the suitability of the area for southern bluefin tuna farming. For this purpose, we determined sedimentary mineral grainsize distributions, carbonate, organic carbon and total nitrogen contents, and stable isotopes of carbon and nitrogen. Mineral grainsize affects the entrainment, transport and deposition of particulate matter, and therefore gives an indication of sediment provenance, dispersal pathways and depositional environments (Storlazzi & Field, 2000; Buynevich & FitzGerald, 2003). Carbonate content distinguishes the fraction of biogenic (i.e. calcareous skeletal remains of marine fauna) over siliciclastic (i.e. detrital mineral and rock fragments) sediments. Organic carbon and total nitrogen contents indicate changes in the rate of organic matter accumulation, while stable isotopes of carbon and nitrogen may be used for evaluation of the source and degree of decomposition of organic detritus (Ye et al., 1991; Velinsky & Vogel, 1999; Hedges et al., 2000; Karakassis et al., 2000). We used these variables to construct

prediction maps of sediment characteristics that allowed us to explore the links between the sedimentary environment, southern bluefin tuna aquaculture and associated risks to the industry.

3.2. Methods

3.2.1. Study area

Spencer Gulf extends 300 km northward from the continental margin of southern Australia. Sampling sites were located in 18-25 m water depth, between Point Bolingbroke and Cape Donington in the lower gulf (Figure 3.1). Salinity and water temperature ranges in the area are 35-37 and 12-22^{o}C, respectively. The coastal geometry affords some shelter from predominantly southwest ocean-generated swell (Fuller et al., 1994; Harris, 1994; Porter-Smith et al., 2004). This high-energy coastal region receives negligible terrigenous inputs as a consequence of a semiarid climate (annual rainfall <550 mm), low relief and small riverine discharge (Fuller et al., 1994; Schwarz, 2003). The Tod River represents the only riverine input into the study area, with flow rates barely reaching 10.5 m^3 s^{-1} in winter (Oceanique Perspectives, 1997). Current velocities are generally between 2.5 and 5 cm s^{-1}, with maximum values attaining 17-20 cm s^{-1} (Petrusevics, 1993). The system is microtidal (<2 m) and during neap tides (also known as dodge tides), tidal movement may cease for up to 3 days (Lennon et al., 1987).

3.2.2. Sampling

Eight control sites (Table 3.1; Figure 3.1), each located at least 1 km away from southern bluefin tuna leases, were sampled in October 2002 after the conclusion of the farming season (February-August). Two stations were sampled from each sampling site. These two stations were 25 m apart in an area where no farming had previously been conducted. Sediments were collected with 6.7 cm (i.d.) tubes using a HAPS Corer (KC Denmark). Ten replicate cores (10-15 cm in length) were collected at each sampling site (five from each sampling station), to obtain data in relation to mineral grainsize and sediment morphology (two cores) and chemistry (eight cores). Each core was transferred into a pre-combusted aluminium tray and stored frozen (-30^{o}C).

Table 3.1. Sampling locations and water depth.

Sampling Sites	Latitude	Longitude	Water depth (m)
RC1	-34^{o} 38.5290'	135^{o} 59.2824'	20
RC3	-34^{o} 37.6848'	136^{o} 03.6426'	23
RC5	-34^{o} 35.7276'	135^{o} 59.8206'	21
RC7	-34^{o} 35.5038'	136^{o} 04.4472'	21
BC4	-34^{o} 41.7564'	136^{o} 01.1256'	19
BC5	-34^{o} 41.3106'	135^{o} 58.9842'	21
BC7	-34^{o} 39.4224'	135^{o} 58.2522'	21
BC8	-34^{o} 39.6520'	136^{o} 00.8580'	23

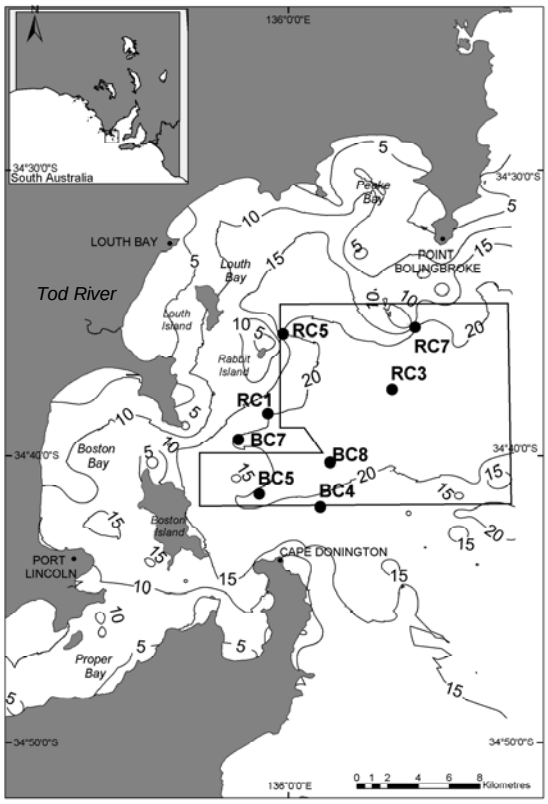


Figure 3.1. Map of the study area in lower Spencer Gulf showing sampling sites. Also indicated are depth contours (m) and the southern bluefin tuna farming zone.

3.2.3. Mineral grainsize

Individual cores were thawed, oven-dried overnight at 105 ^{o}C, and homogenised. A 50 g aliquot of each core was muffled for 12 h at 350°C to remove organic matter and allowed to cool. This sample was stirred with a dispersing agent (40 g L^{-1} sodium hexametaphosphate in MilliQ water) for 15 min and left to soak overnight. Blank hydrometer (Calton Glass Marketing) readings were noted for the dispersing solution. The sample was stirred for 10 minutes, transferred into a 1 L measuring cylinder, and the volume made up to 1 L using MilliQ water. The cylinder was then inverted until the sediment was evenly suspended throughout the water column and placed on a level surface. A hydrometer and temperature reading was taken exactly 2 h after this placement to determine clay content (<4 μm). The content of the cylinder was then wet sieved through a 63 μm sieve and the retained fraction was dried at 100°C. A stacked series of graded sieves comprising 2,000, 1,000, 500, 250, 125, and 63 μm mesh size was used to obtain sand fractions. The sample was dry sieved using an automatic sieve shaker (Endecotts EFL2000) set at 5 min. The silt content (4-63 μm) was calculated as the difference between the muffled weight of the sample and the sand and clay fractions.

3.2.4. Sediment morphology

Aliquots of selected oven-dried cores used for mineral grainsize analyses were sieved to 500 μm to remove large shell fragments. These samples were placed on a 12.6 mm diameter mount with a double sided adhesive tab and then coated with carbon (15 nm) and gold (15 nm) in a Denton Evaporative Vacuum Coater before being examined with a Philips XL30 Field Emission Gun Scanning Electron Microscope (SEM). Secondary micrographs were taken at 10 kV with a working distance in the range 10-30 mm.

3.2.5. Sediment chemistry

In the laboratory, the top layers (0-1 cm) of sediment cores were sliced, transferred into pre-combusted glass jars, and stored frozen. Each sample was freeze-dried, sieved to 500 μm to remove large shell fragments, and homogenised with a mortar and pestle.

Carbonate content

Carbonate content was determined with a pressure transducer (RS Components, part 348-8065, Iso-Tech voltameter IDM 91) by measuring the increase in pressure generated by the CO_2 released after acidification of core aliquots with 5.5 N HCl at room temperature.

Organic matter content and stable isotopes

Total nitrogen (TN), organic carbon (OC), and their stable isotopes ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) were analysed by Continuous-Flow stable Isotope Ratio Mass Spectrometry (CF IRMS) using a Europa Scientific ANCA-SL elemental analyser coupled to a Geo 20-20 mass spectrometer. Pre-treatment of samples included acidification with 1 N HCl to remove carbonates. Weight percentages of TN and OC were corrected for carbonate content and are therefore reported as a fraction of total sediment.

Natural isotopic abundances for carbon and nitrogen are reported as $\delta^{13}C$ and $\delta^{15}N$, which correspond to the deviation (in ‰) of the isotopic composition of a sample from an internationally accepted standard (std) (Pee Dee Belemnite limestone for $\delta^{13}C$ and nitrogen in air for $\delta^{15}N$):

$$\delta^{13}C \text{ or } \delta^{15}N = \left(\frac{R_{sample}}{R_{std}} - 1 \right) x\, 10^3 \qquad [1]$$

where

$$R = \frac{^{13}C}{^{12}C} \text{ or } \frac{^{15}N}{^{14}N} \qquad [2]$$

3.2.6. Statistical analysis

Mineral grainsize distributions were analysed with the software package GRADISTAT (Blott & Pye, 2001). The Folk and Ward graphical method was used to calculate grainsize parameters. This method is relatively insensitive to samples with a large particle range in the tails of the distribution and provides a robust tool to compare compositionally variable samples. Parameters used to describe grainsize distributions included the mean grainsize, the spread of sizes around the mean (sorting), the symmetry or preferential spread of the distribution to one side of the mean (skewness), and the degree of concentration of the grains relative to the average (kurtosis). Although the clay fraction was measured as sediments <4 μm, it was considered to vary in the range 0.25-4 μm for calculation of statistical parameters. Unpublished sediment trap data showed that <1.6% of material sinking through the water column is <0.25 μm.

The contribution of each grainsize fraction is expressed as a percentage of the total and therefore is not free to vary as an independent variable, leading to the problem of the constant sum. To avoid this problem, grainsize fraction percentages were arcsine-transformed. Statistical analyses were performed with the software package STATISTICA. Principal component analysis and cluster analysis were used as exploratory techniques to extract relationships between variables and sample groupings. Analysis of variance was used to determine the significance of observed differences.

3.2.7. Geostatistical analysis

Mineral grainsize, carbonate, organic matter contents and stable isotope data were used to construct prediction maps (surface grids) of background sediment properties by applying the kriging interpolation method in ArcMap GIS software. Kriging predicts values at unsampled locations, based on knowing the value at measured locations and taking into account distance and clustering. In kriging, each point is given a weight. The weights are based on how far apart each point is to another point that is near it. It looks at the distance between the measured points and the prediction location as well as the overall spatial arrangement (spatial autocorrelation) among the measured points and their values. The initial analysis showed spatial trends in the form of second order polynomials. The data points were used to obtain empirical semivariograms, and cross-validation enabled the selection of the best model that fitted the data. The cross-validation process used all of the data to estimate the autocorrelation model. In this study, cross-validation omitted a point (from the 16 stations) and calculated the value of this location using the remaining 15 points. It went through this process for all 16 points. The models showed a scatter plot of predicted *versus* measured values. The closer the two lines were, the better the model fitted. All data fitted either an exponential model or a Gaussian model with anisotropy and second order trend.

3.3. Results

3.3.1. Mineral grainsize and sediment morphology

Mean grainsize varied in the range 53-307 μm, as silts and fine sands were the dominant fractions. Most samples had a unimodal size-frequency distribution peaking in the fine sand range at 187.5 μm (ϕ=2.5) characteristic of the silty sand textural group (Figure 3.2). Sorting

values (σ_G) varied between 3.83 and 7.29, indicating very poorly sorted sediments (σ_G = 4-16) with a large range of grainsizes. Negative skewness values (Sk_G from -0.46 to -0.16) highlighted an excess of fine particles from very fine (-1 to -0.3) to fine (-0.3 to -0.1) skewed grainsize distributions. Kurtosis values (K_G) from 0.99 to 1.67 indicated that the central portion of these distributions were better sorted than the tails, leading to strongly peaked mesokurtic (0.90-1.11) to mostly leptokurtic (1.11-1.50) and very leptokurtic (1.50-3.00) curves.

Grainsize distributions significantly changed with geographical location ($p < 0.001$). Figure 3.3 depicts the occurrence of three main clusters: very coarse sediments in the vicinity of Rabbit Island at site RC1, fine sediments north of Cape Donington at sites BC5 and BC8, and intermediate grainsize distributions in the other sites. Mineral grainsize distributions for each of these areas are summarised in Table 3.2. Sediments in the vicinity of Rabbit Island had lower percentages of silts and very fine sands, with distribution curves showing a tail of coarse sediments reflected in higher skewness values, mean grainsize and better sorted sediments (Figure 3.4). These sediments consisted of skeletal remains of carbonate-secreting organisms (e.g. foraminifers, bryozoans and molluscs) and some rock and mineral fragments (Figure 3.5a). In contrast, the finest and most poorly sorted sediments in the study area occurred north of Cape Donington (Figure 3.4) where silts and very fine sands constituted more than half of the total (Table 3.2). These sediments were dominated by highly weathered biogenic fragments covered with a coating of fine particles (Figure 3.5b,c).

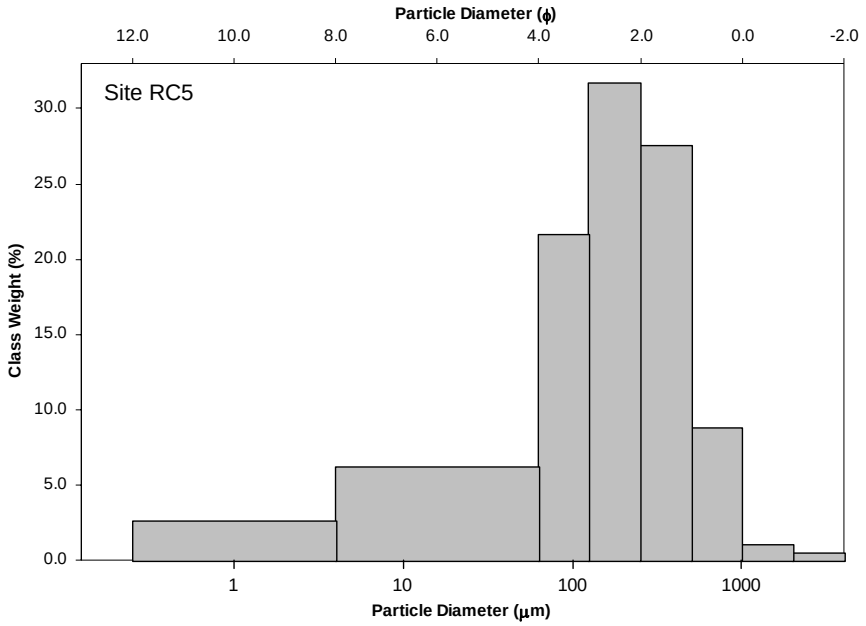


Figure 3.2. Typical grainsize-frequency distribution of sediments in the offshore southern bluefin tuna farming zone. Values reported are for site RC5.

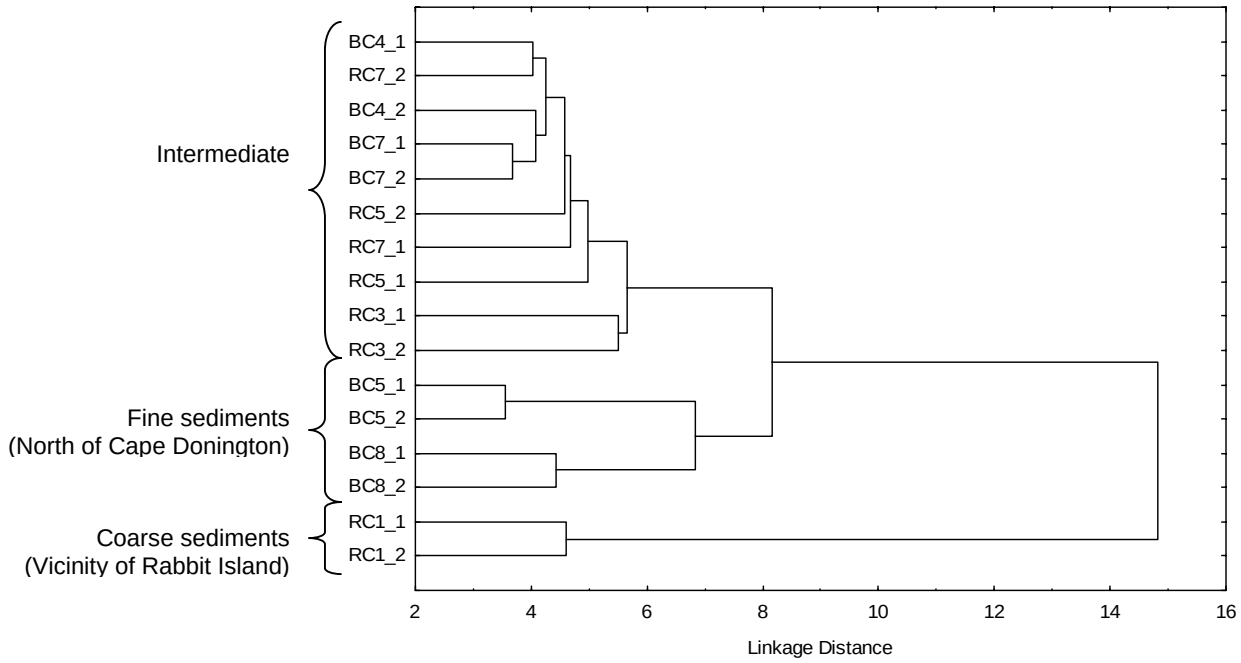


Figure 3.3. Dendrogram using single linkage (Euclidean distances) highlighting the spatial separation of sampling sites (two stations per site) based on mineral grainsize fractions as defined in Table 3.2.

Table 3.2. Mineral grainsize fractions and distribution parameters with geographical location.

Grainsize distribution[a]	**Vicinity of Rabbit Island**[b] (***n*=2**)	**North of Cape Donington**[c] (***n*=4**)	**Other** (***n*=10**)
Grainsize fractions (%)			
Clay (<4 μm)	4.07-4.08 (4.08)	6.22-10.36 (8.28)	4.11-8.29 (6.00)
Silt (4-63 μm)	4.84-5.46 (5.15)	25.92-36.42 (31.49)	13.01-21.87 (17.93)
Very fine sand (63-125 μm)	9.52-10.59 (10.06)	20.77-25.65 (23.15)	15.65-21.42 (17.92)
Fine sand (125-250 μm)	22.91-27.34 (25.12)	16.64-19.00(17.85)	24.01-28.71 (25.81)
Medium sand (250-500 μm)	24.63-25.22 (24.92)	9.78-13.28 (11.63)	16.15-23.04 (19.96)
Coarse sand (500-1000 μm)	17.65-19.91 (18.78)	3.46-5.48 (4.34)	6.99-10.70 (8.39)
Very coarse sand (1-2 mm)	8.45-11.26 (9.86)	1.44-1.99 (1.69)	0.87-4.73 (2.68)
Gravel (>2 mm)	1.83-2.24 (2.03)	1.10-2.15 (1.56)	0.13-3.87 (1.32)
Distribution parameters			
Mean (μm)	278.89-306.68 (292.79)	52.94-66.11(58.69)	84.60-137.44 (111.42)
Sorting (σ_G) (μm)	3.83-4.05 (3.94)	5.66-7.29 (6.44)	4.41-6.92 (5.41)
Skewness (Sk_G)	-0.21/-0.16 (-0.19)	-0.34/-0.26 (-0.31)	-0.46/-0.31 (-0.36)
Kurtosis (K_G)	1.52-1.63 (1.58)	0.99-1.19 (1.06)	1.22-1.67 (1.46)

Sample grouping is according to results of the cluster analysis depicted in Figure 3.3.
[a]values reported as range (mean).
[b]Coarse sediments at site RC1.
[c]Fine sediments at sites BC5 and BC8.

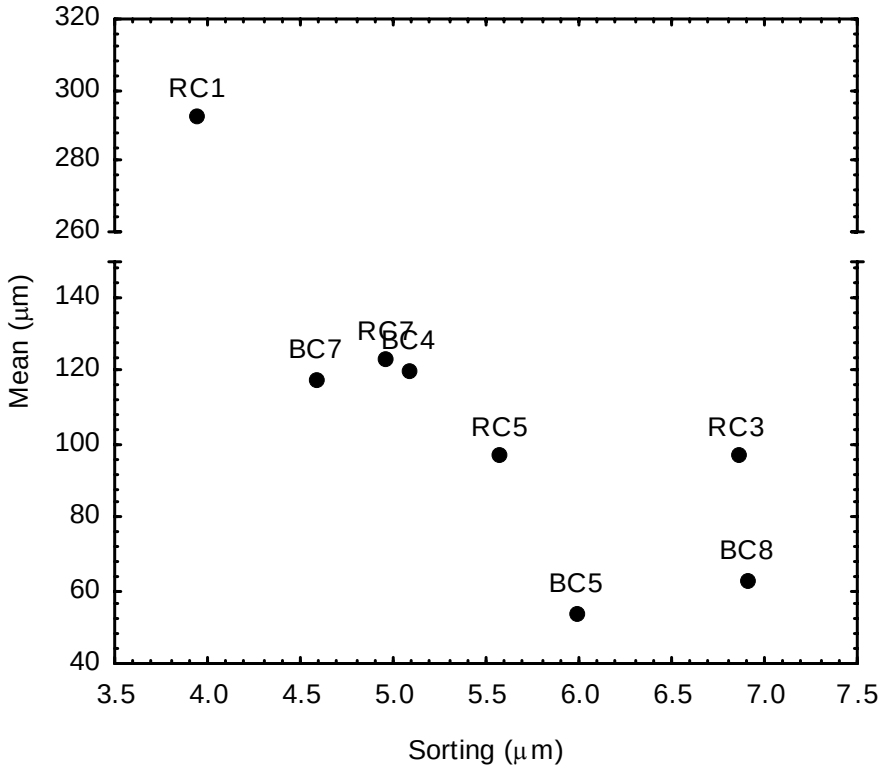


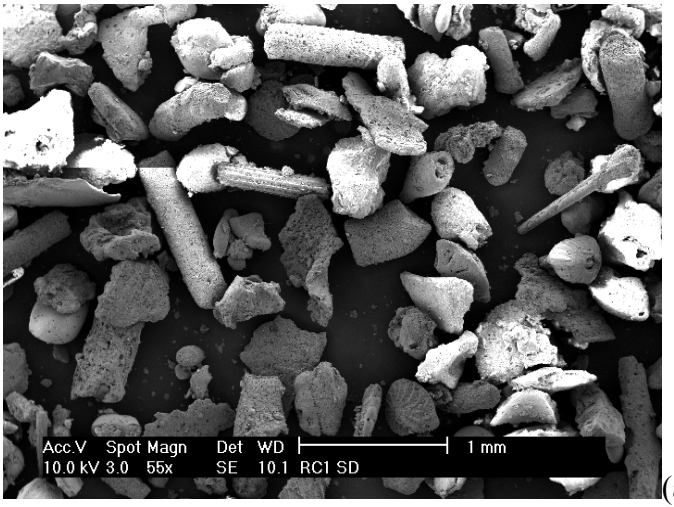
Figure 3.4. Mean grainsize *vs* sorting values for sediments offshore of Boston Island.

3.3.2. Carbonate content

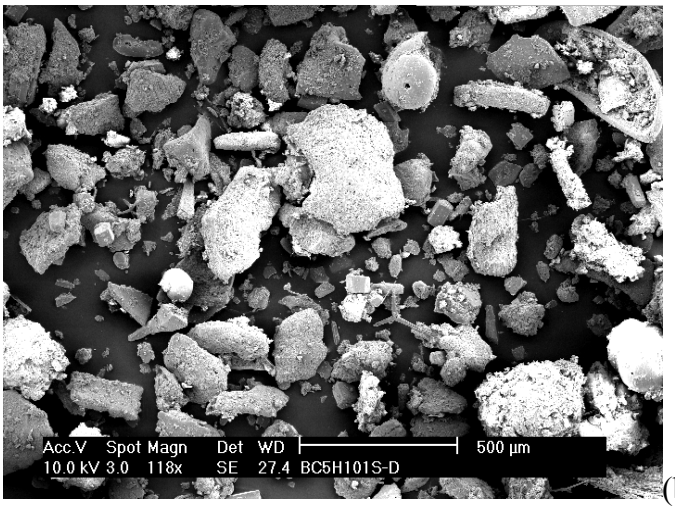
Sediments were calcareous with carbonate content in the range 47-95%. Sediments in the vicinity of Rabbit Island had the lowest carbonate values and consequently the highest siliciclastic contribution (Figure 3.6). The area extending from Cape Donington up to Point Bolingbroke, hereafter referred to as north of Cape Donington, typically consisted of more than 80% carbonate. The coarsest sediments in the study area also had the lowest carbonate values, while fine sediments north of Cape Donington were made up almost exclusively of biogenic calcareous grains.

3.3.3. Organic matter content

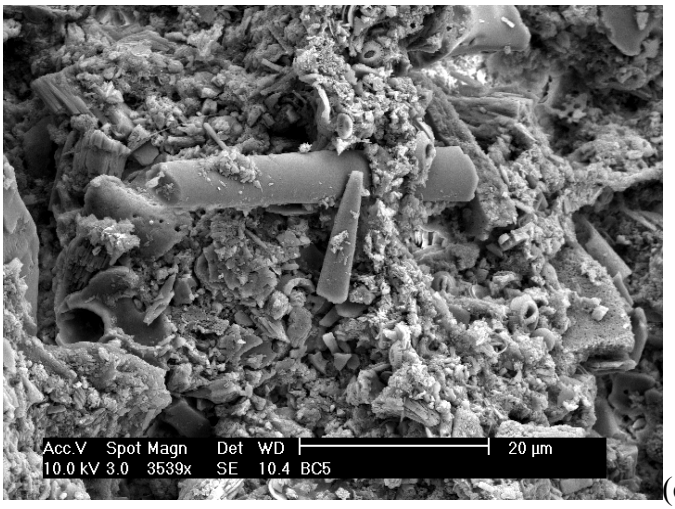
Organic carbon (OC) and total nitrogen (TN) contents in these calcareous sediments were very low, comparable with other areas of the coast of South Australia (Harbison, 1984; Blom & Alsop, 1988; Veeh et al., 1999). Similarly to the pattern observed for carbonate, OC and TN contents were higher in the area of fine sediments north of Cape Donington (Figure 3.7). Principal component analysis showed that OC, TN and carbonate values were closely related to fine mineral fractions (<125 μm), clays in particular, with the first principal component or factor accounting for 61% of the variance in the dataset (Figure 3.8).



(a)



(b)



(c)

Figure 3.5. Scanning electron micrographs of sediments (a) south of Rabbit Island at site RC1 and (b,c) north of Cape Donington at site BC5.

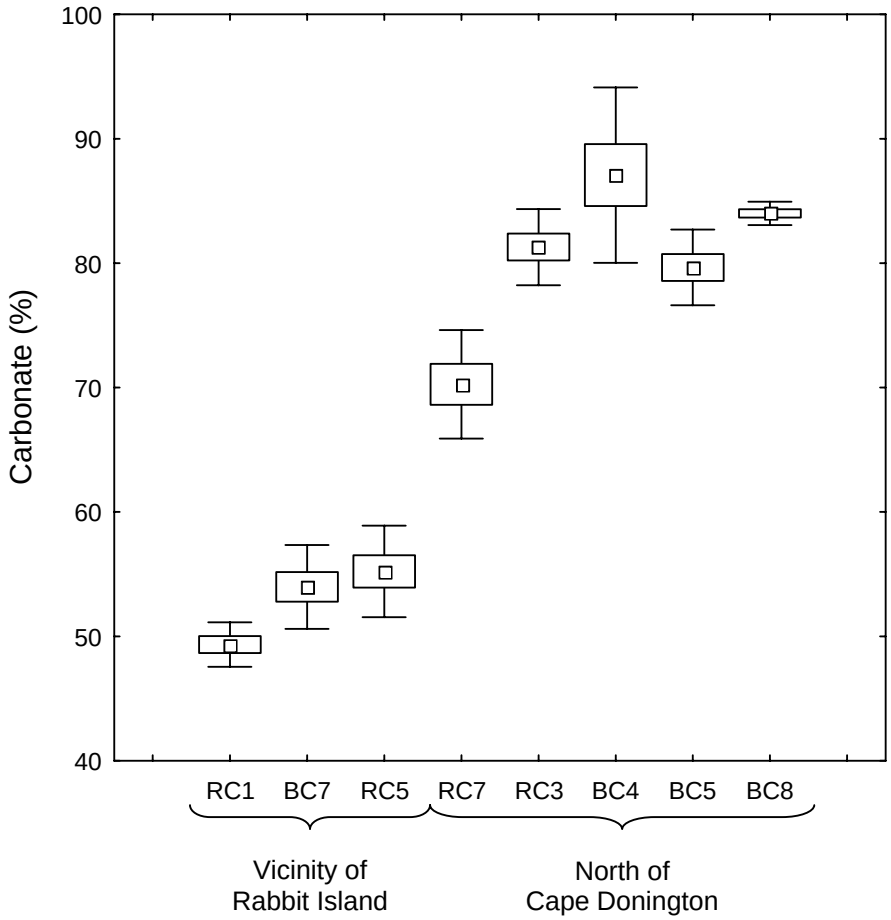


Figure 3.6. Carbonate content in sediments offshore of Boston Island. Values are reported as the mean; the inner spread corresponds to the standard error, and the outer spread to 95% confidence intervals.

3.3.4. Organic matter composition

The ratio of OC to TN (C:N) was consistently between 6.2 and 9.6, indicative of marine origin. The variation between sites was small, with slightly lower values in deeper waters north of Cape Donington, particularly evident at site RC3. The stable isotope signature of sedimentary organic matter was more variable with geographical location (Figure 3.9). Sites located in the vicinity of Rabbit Island and in the north of the study area (site RC7) had the most depleted $\delta^{15}N$ values, typically between 3 and 3.5‰, while sites north of Cape Donington were ^{15}N-enriched in comparison, with $\delta^{15}N$ values generally between 4 and 4.5‰. The $\delta^{13}C$ signature of surface sediments was heavier at the northernmost sites of the study area (RC5, RC7) located closer to the protected embayments of Louth and Peake Bay. At sites north of Cape Donington, those located closer to shore (BC4, BC5) also had higher $\delta^{13}C$ values compared with those located in deeper waters.

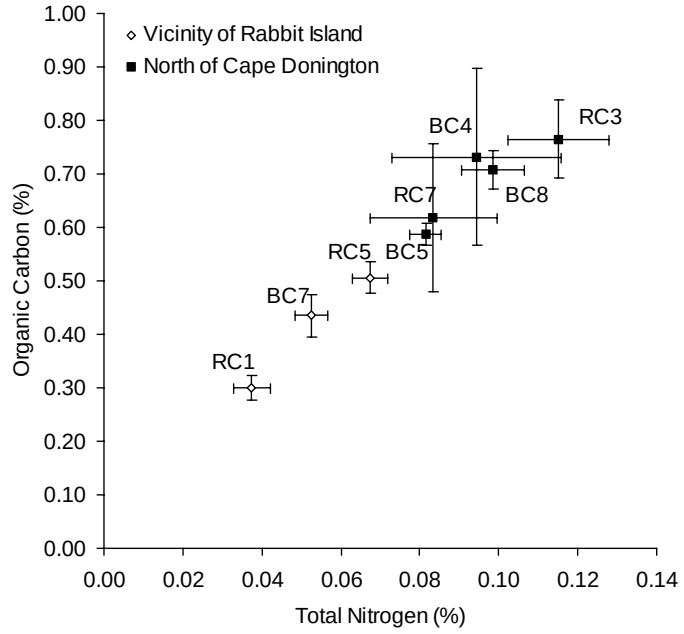


Figure 3.7. Organic carbon *vs* total nitrogen in sediments offshore of Boston Island. Values are reported as the mean, and the spread corresponds to the standard error.

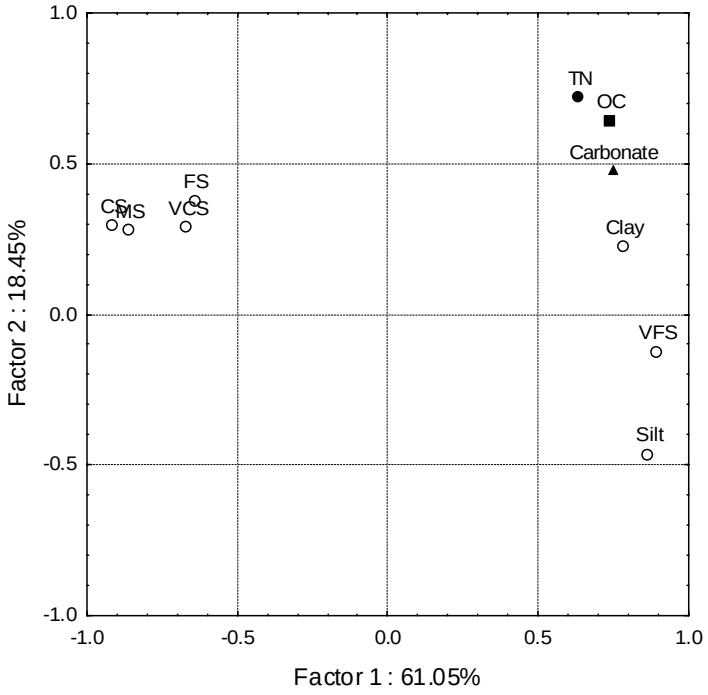


Figure 3.8. Principal component analysis loading plot showing the projection of organic carbon (OC), total nitrogen (TN), carbonate and mineral grainsize fractions in sediments offshore of Boston Island. Gravel was excluded from this analysis; all data were standardised. VFS, very fine sand; FS, fine sand; MS, medium sand; CS, coarse sand; VCS, very coarse sand.

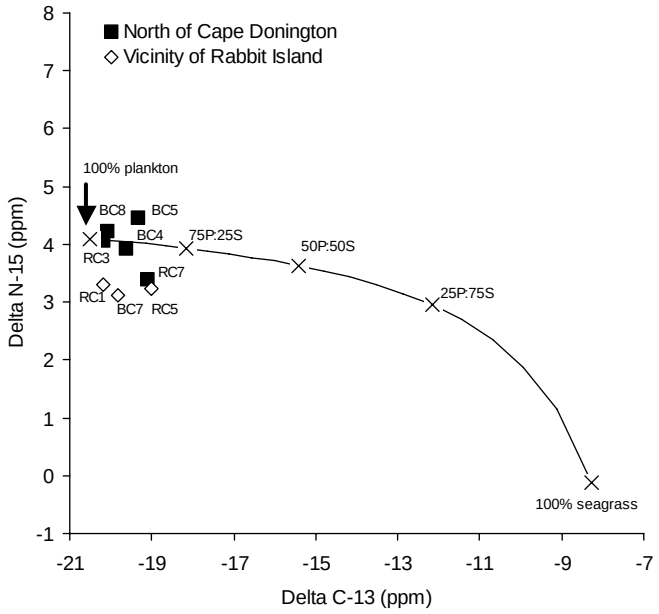


Figure 3.9. $\delta^{15}N$ *vs* $\delta^{13}C$ values of the organic matter in sediments offshore of Boston Island. Also indicated is the binary mixing curve showing predicted isotopic values obtained from mixing different proportions of plankton (P) and seagrass-derived (S) organic matter.

3.4. Discussion

3.4.1. Natural setting

The southern Australian margins are known as one of the largest cool-water carbonate facies on the planet with unconsolidated sediments populated by recent and relict skeletons of molluscs, bryozoans and benthic foraminifers (Gostin et al., 1988; Fuller et al., 1994; Li et al., 1996). Sediments in the southern bluefin tuna farming zone off Port Lincoln are no exception with calcareous sediment fractions <500 μm comprising between 67 and 94% of the total. These poorly sorted fine sediments depleted in organic matter are similar to the molluscan sand biofacies described by Fuller et al. (1994) for the lower part of western Spencer Gulf.

Although some fine sediments in the area could derive from southern bluefin tuna farming wastes, the low organic matter and high carbonate contents suggest that extensive seagrass meadows common in protected coastal embayments in the region are major players in the production of biogenic sediments found offshore of Boston Island. This is broadly consistent with observations from other subtidal zones of Spencer Gulf (Gostin et al., 1984; Gostin et al., 1988) where the fauna associated with seagrass meadows acts as 'carbonate factories' (Gostin et al., 1984; De Falco et al., 2003). The close relationship between carbonate content and sediments <125 μm, suggests that the natural distribution of biogenic detritus is controlled by the movement of fine sediment fractions. These are most likely generated by mechanical and biological fragmentation of soft carbonate grains during suspended and bedload transport (Blom & Alsop, 1988; Harris, 1994; Porter-Smith et al., 2004).

Two distinct sedimentary environments were identified: sediments in the vicinity of Rabbit Island were coarse, better sorted and organic-poor, with a high siliciclastic fraction, whereas sediments north of Cape Donington were finer, poorly sorted, mostly calcareous, with significantly higher OC and TN contents (Figure 3.10). Sediments in the vicinity of Rabbit Island are located away from the majority of southern bluefin tuna leases and likely to be exposed to an erosional regime where fine fractions are winnowed out by wave action and tidal currents that accelerate as water flows into Boston Bay and through the passage between Louth and Rabbit Islands and the coastline. These processes will act to impede the accumulation of fine organic and carbonate-rich fractions. In contrast, the decrease in mean grainsize and increase in sorting values at sites north of Cape Donington suggest that this area is a depocentre for these fractions (Figure 3.10). These fine sediments have large mineral surface areas that help protect and stabilise organic matter (Keil et al., 1994; Mayer, 1994), explaining the correlation between OC, TN and mineral grain fractions <125 μm, clays in particular. Bathymetrically controlled grainsize sorting, in which fines preferentially accumulate in deeper, less energetic water, as well as tidal flows, could help redistribute fine-grained sediments across the farming zone. Although we lack high-resolution bathymetry, the low-resolution bathymetry suggests that this area overlies a relatively gently sloping seafloor in comparison with sites south of Rabbit Island where the change in topography is more abrupt (Figure 3.1). Grzechnick (2000) simulated the dispersion of particles around Boston Island, and the results of the model corroborate the idea that fine particles are likely to converge and settle in the region north of Cape Donington.

The most likely sources of organic matter to these coastal sediments are plankton and seagrasses. The system is oligotrophic, with low chlorophyll-*a* levels (<1.5 μg L^{-1}) and plankton counts (<500 cells mL^{-1}) (Clarke et al., 1999; Clarke et al., 2000) (M. Fernandes, P. Lauer and A. Cheshire, unpublished data), and seagrass meadows cover the coastal protected embayments in the vicinity of the farming zone. The carbon isotopic signature of seagrasses is much heavier than that of other marine primary producers because seagrasses assimilate bicarbonate, an isotopically heavier source of inorganic carbon compared with CO_2 (Lepoint et al., 2004). The $\delta^{13}C$ values of seagrasses from the Port Lincoln area are typically around –8.3‰ (authors' unpublished data), while values for plankton in the gulf are generally less than –20.5‰ (Svane & Hall, 2000). Nitrogen isotopic signatures are also distinct between those two primary producers, with $\delta^{15}N$ values close to –0.1 for seagrasses in meadows close to the southern bluefin tuna farming zone (authors' unpublished data) and 4.1 for plankton in the gulf (Svane & Hall, 2000). Considering the higher C:N ratio of seagrasses (20-35) (Duarte, 1990) in comparison with plankton (6-7), any contribution of seagrass detritus to sediments will impact on $\delta^{13}C$ values but less so on $\delta^{15}N$ values.

We used a stable isotope mixing model corrected for different carbon and nitrogen contents of the sources adapted from Faure (1986) and Philips & Koch (2002) to calculate the contribution of plankton and seagrasses to sediments. The model calculates the contribution of each source to carbon and nitrogen in the sediments according to the mass balance equations (Phillips & Koch, 2002):

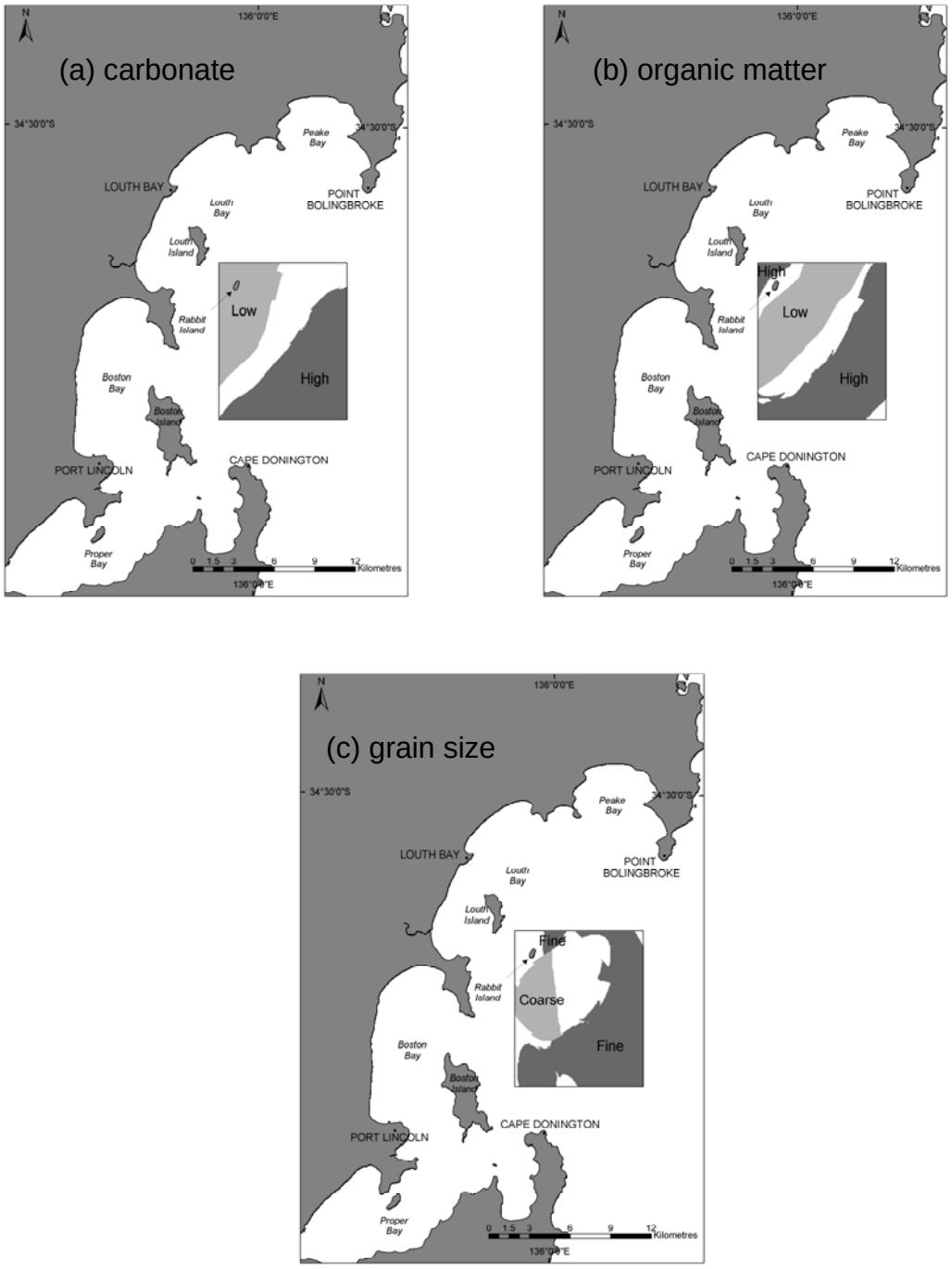


Figure 3.10. Prediction maps showing the distribution of (a) carbonate, (b) organic matter and (c) mineral grainsize in sediments offshore of Boston Island obtained by applying the kriging interpolation method. High carbonate contents are >74% and low carbonate contents <62%; high organic matter contents correspond to OC values >0.52% and TN values >0.06%; low organic matter contents to OC values <0.52% and TN values <0.06%; coarse sediments have mean grainsize >160 μm and sorting values <5.8, while fine sediments have mean grainsize <160 μm and sorting values >5.3. Unlabelled white zones in the study area correspond to intermediate values.

$$\delta^{13}C_{OM} = f_{P,C}\,\delta^{13}C_P + f_{S,C}\,\delta^{13}C_S \quad [3]$$

$$f_{P,C} + f_{S,C} = 1 \quad [4]$$

$$\delta^{15}N_{OM} = f_{P,N}\,\delta^{15}N_P + f_{S,N}\,\delta^{15}N_S \quad [5]$$

$$f_{P,N} + f_{S,N} = 1 \quad [6]$$

where $\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$ represent the carbon and nitrogen isotopic signatures for sedimentary organic matter (OM subscript), and similarly for plankton (P subscript) and seagrasses (S subscript), and $f_{P,C}$, $f_{S,C}$, $f_{P,N}$ and $f_{S,N}$ represent the fractions of carbon (C subscript) or nitrogen (N subscript) from each of these sources in the sediments. The model also calculates the contribution of each source to total organic matter in the sediments taking into consideration that the proportion of carbon derived from a carbon-rich source will be higher than the proportion of nitrogen derived from that source (Faure, 1986):

$$f_{P,OM} = \frac{[C]_S\,(\delta^{13}C_S - \delta^{13}C_{OM})}{[C]_P\,(\delta^{13}C_{OM} - \delta^{13}C_P) + [C]_S\,(\delta^{13}C_S - \delta^{13}C_{OM})} \quad [7]$$

$$f_{P,OM} + f_{S,OM} = 1 \quad [8]$$

where $[C]_S$ and $[C]_P$ are the carbon concentrations in seagrasses (32.6%, authors' unpublished data) and plankton (45.6% on an ash free basis, average from Parsons et al. (1961) and Hedges et al. (2002)), respectively. $f_{P,OM}$ and $f_{S,OM}$ can be calculated just as easily using data for nitrogen. However, the isotopic values for nitrogen in this system appeared to deviate from the binary mixing curve (Figure 3.9) leading to unrealistically high contributions of seagrasses when nitrogen is used to calculate $f_{S,OM}$. This deviation could result from several factors but most likely reflects the contribution of a third source with distinct $\delta^{15}N$ values and N content, and/or post-depositional alteration of the signature of sources as a result of organic matter decomposition. We used data for carbon to calculate $f_{P,OM}$ and $f_{S,OM}$ as this is the element with the most distinct signatures between sources and a much higher content in the sediments.

The use of the model described above indicates that the organic matter accumulating in these sediments is mostly derived from plankton (84-97%), with more than 88% of carbon and 76% of nitrogen coming from this source. This is consistent with C:N ratios averaging 7.5. The small increase in $\delta^{13}C$ values found at sites closer to shore (Figure 3.11a), particularly to

the north, suggests a slightly higher contribution of seagrass-derived organic matter to these sediments, calculated to vary between 10 and 16%, as opposed to 3-8% in the other areas.

The concomitant increase in OC and TN contents (Figure 3.10) and $\delta^{15}N$ values (Figure 3.11b) north of Cape Donington suggests a greater contribution of higher trophic levels (i.e. fauna *vs* plankton) and/or that organic matter in these sediments has been reworked and is likely to include a larger fraction of refractory compounds (Freudenthal et al., 2001). ^{14}N is preferentially utilised during metabolic processes leading to an accumulation of ^{15}N with increasing trophic position (Davenport & Bax, 2002). Similarly, the light isotope is preferentially remineralised during microbial degradation leading to the release of ^{15}N-depleted inorganic nitrogen and build-up of ^{15}N in the remanent organic matter. The overall increase in $\delta^{15}N$ values for these sediments suggests that the amount of remineralised inorganic nitrogen trapped in clay lattices is a minor fraction of TN. The distinct spatial patterns for nitrogen in comparison with carbon isotopes suggest that the distribution of these elements is decoupled and most likely determined by different geochemical and ecological processes.

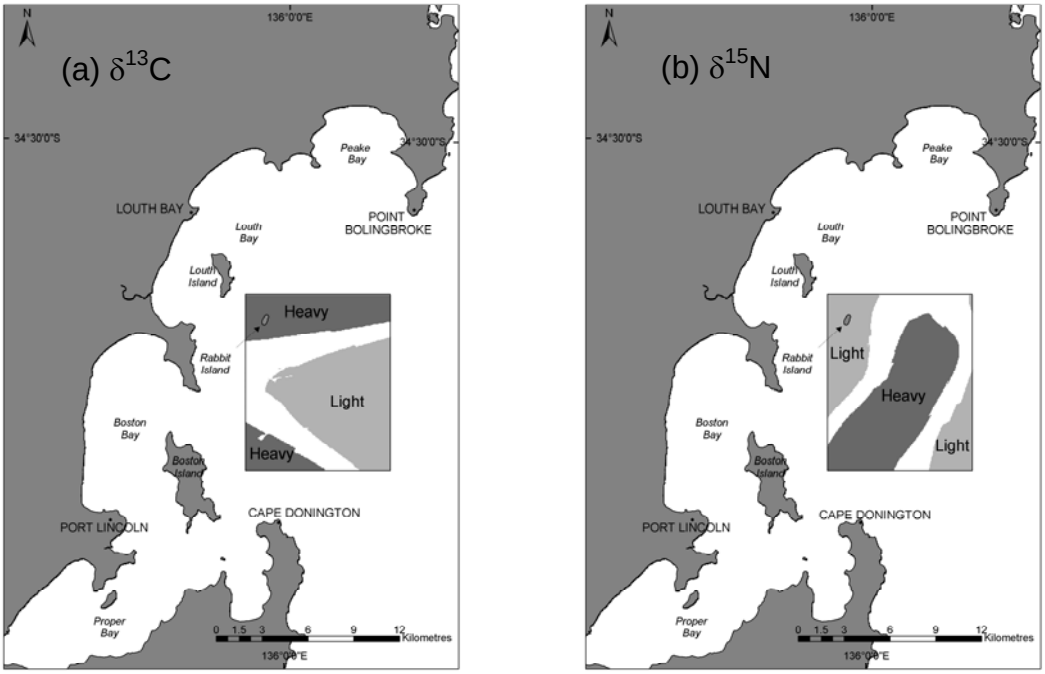


Figure 3.11. Prediction maps showing the distribution of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values of organic matter in sediments offshore of Boston Island obtained by applying the kriging interpolation method. Sediments with a light signature have $\delta^{15}N < 3.5$ and $\delta^{13}C < -20.0$, while sediments with a heavy signature have $\delta^{15}N > 3.9$ and $\delta^{13}C > -19.7$. Unlabelled white zones in the study area correspond to intermediate values.

3.4.2. Implications for southern bluefin tuna aquaculture

The varying sediment types described suggest that the benthic assimilative capacity of farmed sites will depend on their location in the farming zone. The area north of Cape Donington appears to be a natural depocentre for fine sediments, and higher carbonate and organic matter background levels will mask accumulation of farm-derived wastes. Phosphorus is the only element likely to show a clear pattern of enhanced accumulation as a result of farming because it is sequestered by carbonate (De Kanel & Morse, 1978; Koch et al., 2001), and we found significantly higher carbonate levels in this area.

Sites south of Rabbit Island, in contrast, would not be as "pre-conditioned" to organic matter accumulation, perhaps with less adapted microbial and infaunal assemblages leading to a slower response in remineralising farm-derived wastes. However, this area appears subject to a strong flushing regime that would have an opposite effect, winnowing out fine wastes and contributing to recovery of sediments underneath the pens through dispersal. Coarser sediments will also mean greater aeration of porewaters and reduced surface area for organic matter stabilisation, probably acting to promote remineralisation and diffusion of nutrients from sediments back into the water column.

Distinct sedimentary environments will not only affect the benthic assimilative capacity of the sites but may also influence the health of the stock. Sediment resuspension might increase the risk of irritation to southern bluefin tuna gills associated with respiratory difficulties, and increase infestation from sediment-dwelling parasites. Abnormal southern bluefin tuna mortalities in 1996 were accompanied by excessive mucus production in the gills, with the mucus containing heavy loads of fine silts (Clarke, 1996).

Silts and fine sands make up more than half of the sediments close to Cape Donington. Sediments in this area are not only finer but also lighter due to a small siliciclastic component (generally <20%). These sediments can be mobilised off the seafloor and into the water column given winds and swell of sufficient energy, implying that farmed sites here may be affected by sediment resuspension during major storm events. Current velocities necessary to mobilise fine sediment off the seabed are far greater than those to maintain these sediments in suspension. Silts and clays in particular form a compact sediment layer when deposited on the seafloor, and current speeds in excess of 20 cm s^{-1} are necessary for resuspension according to the simplified Hjulström diagram, which shows the relationship between current velocity and sediment size of particles picked up, transported and deposited (Rowell & Ryan, 1996b; Rowell & Ryan, 1996a). Current speeds of this magnitude are not uncommon offshore of Boston Island. Once disturbed, these fine particles will remain in suspension for long periods of time with settling rates of less than 0.01 cm s^{-1} for quartz according to Stokes Law. Given an average water column depth of 20 m in the offshore southern bluefin tuna farming zone, fine sediments would remain in suspension for periods of at least 2 days, probably much longer given swell and tidal energy in the area. Longer residence times in the water column will also increase the likelihood of these fine sediments spreading over a larger area than where resuspension initially occurs.

Acknowledgements

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Chapter 4: Dissolved nutrient release from solid wastes of southern bluefin tuna (*Thunnus maccoyii*, Castelnau) aquaculture

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Abstract

Finfish pens are point sources of dissolved nutrients released from fish metabolism or degradation of solid wastes. Nutrients leaching from uneaten feed and faeces are not usually quantified in mass budgets for these systems, leading to an overestimation of fish retention or deposition to the seabed. In this study we investigated nutrient leaching from pellets and baitfish feed as well as faeces of southern bluefin tuna (*Thunnus maccoyii*) into seawater. Faeces were nitrogen-depleted (51-54 mg N g^{-1}dw) and phosphorus-enriched (62-72 mg P g^{-1}dw) compared to feeds (83-111 mg N g^{-1}dw and 17-21 mg P g^{-1}dw). Less phosphorus was available for leaching from pellets and faeces of pellet-fed tuna (5-6%) than from baitfish and faeces of baitfish-fed tuna (17-21%). The proportion of soluble nitrogen in pellets (15%) was also lower than in baitfish and faeces (35-43%). Leaching loads for a feed conversion ratio of 5 were estimated as 22 and 26 kg N $tonne^{-1}$ growth when baitfish or pellets are used as feed, respectively. Phosphorus loads were estimated as 15 and 4 kg P $tonne^{-1}$ growth, respectively. More than 90% of nitrogen loads, and approximately 50% of phosphorus, are likely to be released into seawater before solid wastes reach the seafloor.

4.1. Introduction

Aquaculture production accounted for 7% of the world's food fish supply in 1970, whereas it represents over 36% nowadays (FAO, 2003). This expansion of aquaculture activities in general, and finfish farming in particular, has raised questions regarding the environmental effect of farming operations (Wu, 1995; Crawford, 2003). A major concern is the increased loads of dissolved nutrients, which are released into water by direct fish metabolism or from degradation of solid wastes. Water nutrient enrichment can accelerate algal growth, which not only produces an undesirable disturbance in the biological balance of aquatic ecosystems but can also affect the health of farmed fish (Wu, 1995; Brooks et al., 2002).

Research suggests that more than half of the total nitrogen and phosphorus fed to fish in commercial farms is released into the surrounding environment (Gillibrand et al., 2002; Green et al., 2002; Hardy & Gatlin III, 2002; Satoh et al., 2003; Krogdahl et al., 2004; Strain & Hargrave, 2005; Mente et al., 2006; Roque D'Orbcastel & Blancheton, 2006). For nitrogen, between 70 and 90% of this environmental loss occurs in dissolved form, mostly directly as urinary excreta but also from remineralization of organic matter accumulating in sediments (Foy & Rosell, 1991a; Hall et al., 1992; Enell, 1995; Gillibrand et al., 2002; Strain & Hargrave, 2005). Comparatively less phosphorus is lost in dissolved form, with 30 to 80% of environmental losses believed to occur in particulate form and accumulate in the sediments (Foy & Rosell, 1991a; Holby & Hall, 1991; Enell, 1995). However, nutrient mass budgets developed for finfish aquaculture do not usually quantify the fraction of nutrients that is lost to the water column as a consequence of leaching from solid wastes, leading to an overestimation of the amount retained in fish tissues or deposited on the seabed.

One of the aquaculture sectors of highest commercial value in Australia is southern bluefin tuna (SBT), contributing to more than 20% of the gross value of production in 2004-2005 (EconSearch Pty Ltd, 2002; Newton et al., 2006). Most of its value comes from the sea-ranching of approximately 5,000 tonnes of wild SBT caught each year off the coast of western Eyre Peninsula, South Australia, and farmed in coastal waters off lower Spencer Gulf (van Barneveld et al., 1997; Glencross et al., 2002b; Jeffriess, 2004). Despite its importance, limited quantitative information is available on the loss of nutrients from SBT aquaculture to the surrounding environment. Although values from salmonid research are informative and can be used as an indication, major differences between species, feed type and environment preclude its use for tuna industry management and risk assessment. In this study we performed laboratory-based simulations to estimate the rate and amount of nitrogen and phosphorus in solid wastes from SBT aquaculture that is lost as dissolved products when immersed in seawater. Pellets and baitfish feed, as well as faeces were investigated. The significance of the results is discussed in terms of settling rates of the wastes, nitrogen and phosphorus leaching loads per tonne of growth and contribution of leaching to total nutrient loads in modelling exercises.

4.2. Materials and Methods

4.2.1. Samples

Samples consisted of pellets and baitfish feed, as well as faeces from SBT fed on baitfish (baitfish-faeces) and on pellets (pellet-faeces). Feed samples were obtained from commercial operators and stored frozen at -30 °C in zip lock bags. The pellets were manufactured by Skretting (Hobart, Australia) from fish meal, fish oil and plant protein meal, with added vitamins, minerals and antioxidants. These pellets were approximately 30 mm long and 25 mm in diameter suitable for feeding to 15 to 40 kg SBT. Baitfish consisted mainly of 10 to 20 cm long sardines (mostly locally caught Australian sardines, *Sardinops neopilchardus*). SBT faeces were collected following harvest by gentle stripping of the distal section of the intestine of the fish. Each sample was a composite of faeces from two fish. Stripping motion stopped 3 to 5 cm before the end of the intestine to minimize contamination from epithelial cells. Samples showed no visible contamination from blood or mucous and were stored frozen at -30 °C in glass jars.

4.2.2. Total phosphorus, nitrogen and water contents

Samples for determination of nutrient contents were freeze-dried and homogenised. Aliquots for the determination of total phosphorus contents in baitfish (n=9), pellets (n=5), baitfish-faeces (n=2) and pellet-faeces (n=6) were digested with nitric acid, hydrogen peroxide and hydrochloric acid following USEPA Method 200.3 and analysed in a Varian Vista Axial ICP-AES. Aliquots for the determination of total nitrogen contents in baitfish (n=12), pellets (n=8), baitfish-faeces (n=2) and pellet-faeces (n=6) were analysed by Continuous-Flow stable Isotope Ratio Mass Spectrometry (CF IRMS) using a Europa Scientific ANCA-SL elemental analyser coupled to a Geo 20-20 Mass Spectrometer. All phosphorus and nitrogen concentrations are reported in mg g^{-1} dry weight (dw). Baitfish (n=5), pellets and faeces (n=2) used for the determination of water content were oven dried at 105 ^{o}C for at least 16 h.

4.2.3. Leaching simulations

SBT are messy eaters and smaller scavengers also consume uneaten feed in the water column and on the seafloor (Cheshire et al., 1996b; Svane & Barnett, 2006). Underwater video observations and sediment collection in the vicinity of stocked commercial pens indicated that feed debris on the seafloor are generally <20 mm long. To best simulate these conditions, frozen baitfish and pellets were thawed at room temperature and cut into 10 to 20 mm pieces using a scalpel. Faeces samples were also thawed and gently homogenised in their glass storage jars. Approximately 1 g of pellets, 1-4 g of baitfish, or 450 mg of faeces were weighed and placed into a jacketed reaction vessel containing 300 mL of artificial seawater. Sample sizes were defined based on phosphorus contents initially but the same amounts proved adequate for experiments investigating leaching of nitrogen. Artificial seawater was prepared by dissolving the following salts in Milli-Q water (Grguric, 2000): KCl (0.78 g L^{-1}), $CaCl_2.2H_2O$ (1.54 g L^{-1}), Na_2SO_4 (4.09 g L^{-1}), $MgCl_2.6H_2O$ (11.01 g L^{-1}) and NaCl (24.38 g L^{-1}). The temperature of the water in the reaction vessel was maintained at 18.0 ± 0.5 ^{o}C by passing water from a thermostatted water bath (20 L min^{-1}) through an external jacket. Water temperatures recorded in the coastal area used for SBT farming in South Australia vary between 13 and 22 ^{o}C, with an average during the farming season of approximately 17-18 ^{o}C

(Fernandes et al., 2004). Each leaching experiment was conducted in duplicate over a period of 24 h to minimize microbiological growth. The suspensions were stirred continuously (75 rpm) using a Teflon coated stir bar. Ten mL samples were taken at time intervals of 0, 1, 2, 5, 10, 30, 60, 120, 240, 360 and 1440 min (24 h) for the determination of phosphorus. Separate experiments were run for nitrogen and 20 mL samples were taken at time intervals of 0, 5, 30, 60, 240 and 1440 min. All samples were taken using Terumo single use eccentric syringes and filtered into a glass vial using 30 mm Bonnet syringe filters (DIA non-sterile, 1.0 μm prefilter, 0.45 μm acetate filter). Orthophosphate was determined using the molybdate blue colorimetric method (Vogel, 1987). Total nitrogen, ammonia and nitrates/nitrites were analysed by flow injection analysis in a QuickChem 8000 Automated Ion Analyser (APHA-AWWA-WPCF, 1998c; APHA-AWWA-WPCF, 1998b; APHA-AWWA-WPCF, 2001). Nutrient concentrations were corrected for background concentrations in the artificial seawater, measured in the same way as the samples.

4.2.4. Settling rates

Settling rates were determined using a clear acrylic tube (diameter 0.20 m, length 1.83 m) fitted into an aquaculture fish tank filled with seawater. The acrylic tube was open at the top and bottom, and the bottom fitted with an exchangeable plastic collector. The water column in the tube varied between 1.46 and 1.64 m. Samples were defrosted and pre-weighed. Pellets and sardines (whole or cut into 10-20 mm pieces) were placed on the water at the top of the acrylic tube. Pellet-faeces aliquots were transferred into a beaker before being gently poured at the water surface. There was insufficient baitfish-faeces to run in the settling experiments.
The settling rates of fast, average and slow sinking material were recorded in three different runs. In the first run a diver visually established the time necessary for the bulk of the sample to reach the bottom. This was considered as the average sinking time and the material reaching the collector kept for gravimetric analyses. The fast settling fraction was then measured by weighing the material reaching the collector in approximately ¼ (faeces) or ½ (feeds) of the average sinking time. The slow settling material was measured by recovering the material reaching the collector in 3 min. Each run was repeated three times. All experiments were run at an ambient water temperature of 17 ^{o}C.

Baitfish and pellets reaching the collector were separated into three size fractions. Large pieces were removed using tweezers, transferred to a pre-weighed aluminium tray and oven dried at 50 ^{o}C. The water in the collector was then sieved into a 63 μm sieve, the material retained by the sieve washed onto a pre-weighed glass fibre filter (MFS GF-75, 0.7 μm, 47 mm diameter) and filtered under vacuum to determine the coarse fraction (>63 μm). The filtrate was also drawn through glass fibre filters to determine the fine fraction (<63 μm). Faeces samples were filtered directly onto pre-weighed glass fibre filters. Filters were placed in separate glass petri dishes, covered with a glass lid and oven dried at 50ºC. Before gravimetric analyses, samples were placed in an oven at 50 ^{o}C for at least 3 h and placed in a desiccator with silica gel for 1 h to cool. Results were corrected for salt that impregnates the filters. The weight of material recovered in each of the size fractions was combined and results reported as the percentage of each sample falling through the tube as a function of settling rates.

4.3. Results

4.3.1. Phosphorus

The phosphorus content of baitfish was higher than the phosphorus content of pellets (Table 4.1). Significantly more phosphorus leached from baitfish than pellets (Figure 4.1), the maximum amount corresponding to 21% of the total in baitfish, but only 5% in pellets (Table 4.2). Leaching from feeds was relatively fast and experiments reached equilibrium within the first hour.

The content of phosphorus in faeces was 3-4 times higher than in feeds (Table 4.1). The amount of phosphorus available for leaching was also 3 times higher than in feeds (Figure 4.1). The maximum amount that leached from pellet-faeces was similar to pellets, or 6% of the total (Table 4.2). The maximum from baitfish-faeces was slightly lower than from baitfish, or 17% of the total. Phosphorus leached faster from pellet-faeces (mostly 1-2 h) than baitfish-faeces. Figure 4.1 indicates that phosphorus concentrations may not have reached equilibrium and residual leaching from faeces may continue beyond 24 h, particularly for baitfish-faeces.

Table 4.1. Water, nitrogen and phosphorus contents of baitfish, pellets and faeces from SBT aquaculture. Values are reported as the mean (SD).

Sample	Water (%)	Nitrogen (mg N g^{-1} dw)	Phosphorus (mg P g^{-1} dw)
Baitfish	71 (6)	111 (12)	21 (2)
Pellets	23 (1)	83 (10)	17 (1)
Baitfish-faeces	84 (4)	54 (15)	72 (0)
Pellet-faeces	86 (2)	51 (11)	62 (12)

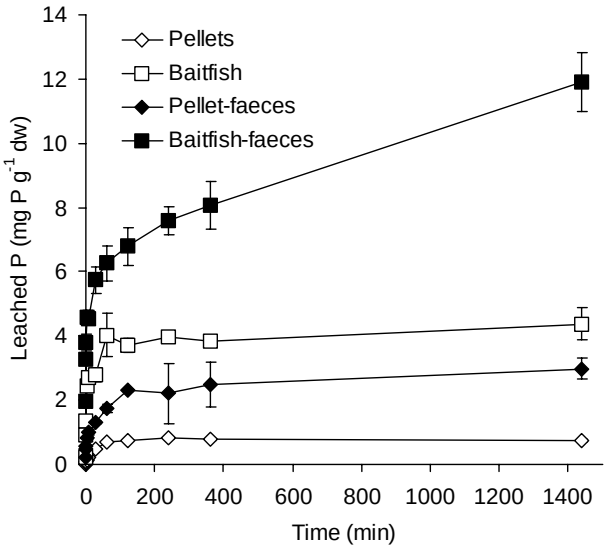

Figure 4.1: Amount of phosphorus that leached into seawater from baitfish, pellets, baitfish-faeces and pellet-faeces as a function of time. Values are reported as the mean, bars indicate standard deviation.

4.3.2. Nitrogen

The nitrogen content of baitfish was highest, followed by pellets (Table 4.1). More nitrogen leached from baitfish (Figure 4.2), with up to 41% of total nitrogen leaching into seawater, against a maximum of 15% from pellets (Table 4.2). Organic nitrogen accounted for almost all nitrogen leaching from feeds (92-100%), ammonia generally for less than 4% and nitrates/nitrites for less than 1%. Most soluble nitrogen in feeds leached between 1 and 4 h. Baitfish had an unusual pattern in that the amount leached after 24 h was lower than the amount leached after 4 h.

Faeces had significantly lower nitrogen contents than feeds (Table 4.1). The nitrogen in faeces was highly soluble (Table 4.2) and leached primarily in organic forms (94-96%), with ammonia accounting for less than 5%, and virtually no nitrates/nitrites. Leaching of nitrogen from faeces occurred at a faster rate in comparison to feeds, with equilibrium attained in the first 5 min.

Table 4.2. Maximum fraction (%) of total phosphorus and total nitrogen contents available for leaching from baitfish, pellets and faeces from SBT aquaculture. Values are reported as the mean (SD).

Sample	P (%)	N (%)
Baitfish	21 (1)	41 (2)
Pellets	5 (0.2)	15 (2)
Baitfish-faeces	17 (1)	35 (1)
Pellet-faeces	6 (1)	43 (3)

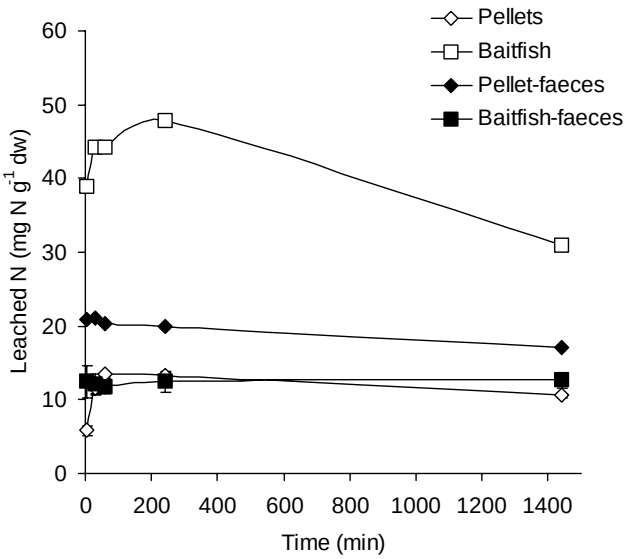

Figure 4.2: Amount of nitrogen that leached into seawater from baitfish, pellets, baitfish-faeces and pellet-faeces as a function of time. Values are reported as the mean, bars indicate standard deviation.

4.3.3. Settling rates

Pellets had the fastest settling rates (Table 4.3). Whole pellets sank at 15 cm s^{-1}. When cut into 10-20 mm pieces, most pellets reached the bottom at 9 cm s^{-1}. Large pellet pieces accounted for the majority of the settling material, coarse particles (>63 μm) to 2% and fine particles (<63 μm) to 1% or less. Baitfish settling rates were lower than for pellets, with whole fish sinking at 7 cm s^{-1}. The bulk of 10-20 mm baitfish pieces (73%) sank at a rate of 8 cm s^{-1}, and the remainder at 5 cm s^{-1}. The average settling rate calculated from these values was 7.2 cm s^{-1}. Some pieces of baitfish (heads and tails in particular) floated. Fine particles comprised between 4 and 6% of baitfish reaching the bottom, and coarse particles approximately 0.5%. Pellet-faeces formed a cloud when entering the water column and sank at comparatively slower rates. The majority (approximately 62%) sank at rates of less than 0.9 cm s^{-1}. Considering that 14% of faeces sank at 5 cm s^{-1}, 16% at 1.3 cm s^{-1} and the remainder had maximum rates of 0.9 cm s^{-1}, the maximum values for settling rates of faeces were calculated to fall in the range 1-2 cm s^{-1}.

Table 4.3. Fraction of total weight of baitfish, pellets and faeces sinking through the water column as a function of settling rates.

	Settling rate (cm s^{-1})	Fraction (%)	Cumulative (%)[1]	
Baitfish	7	100		
Baitfish pieces[2]	8	73	73	(27)
	5	27	100	(26)
Pellets	15	100		
Pellet pieces[2]	9	100	100	(12)
Faeces[3]	5	14	14	(25)
	1.3	16	30	(20)
	0.9	8	38	(21)
	<0.9	62	100	

[1]mean (SD).
[2]baitfish or pellets cut into pieces < 20 mm.
[3]pellet-faeces.

4.4. Discussion

4.4.1. Total nutrient contents

Nutrient contents of pellets (Table 4.1) were in the higher range of values for other finfish feeds (Pettersson, 1988; Phillips et al., 1993; Talbot & Hole, 1994; Enell, 1995; Hillestad et al., 1999; Ruohonen et al., 1999). Nutrient contents of baitfish were higher still, similar to other baitfish and fish in general (Holby & Hall, 1991; Ackefors & Enell, 1994; Enell, 1995; Ruohonen et al., 1999; Aguado et al., 2004; Ellis & Rough, 2005). These values might vary slightly with baitfish species, location sourced and season, but are in agreement with averages reported by Ellis & Rough (2005) for the three main species fed to SBT: Australian sardines (*Sardinops neopilchardus*), American sardines (*Sardinops sagax*) and Californian sardines (*Sardinops caeruleus*).

SBT faeces were mostly composed of water (Table 4.1), similarly to Atlantic bluefin tuna faeces (84-89%) (Aguado et al., 2004). On a dry weight basis, faeces were nitrogen-depleted

and phosphorus-enriched in comparison to feeds. This is likely a consequence of the higher digestibility of protein by SBT (65-90%) (Carter et al., 1999; Buchanan & van Barneveld, 2004) in comparison to phosphorus (25%) (Jeff Buchanan, personal communication). SBT faeces had slightly higher nutrient content (Table 4.1) than faeces of other aquaculture species (25-40 mg N g^{-1} dw, 10-36 mg P g^{-1} dw) (Penczak et al., 1982; Pettersson, 1988; Kibria et al., 1997; Chen et al., 2003; Coloso et al., 2003; Aguado et al., 2004). To increase feed efficiency and reduce environmental losses, the trend in fish aquaculture is to produce diets with high fractions of digestible nutrients while reducing overall concentrations (Talbot & Hole, 1994; Enell, 1995). Our results suggest that the indigestible protein and phosphorus content is potentially high in nutrient-rich SBT diets.

4.4.2. Leached nutrients

Nutrients in baitfish were highly soluble, with comparatively more soluble nitrogen than phosphorus (Table 4.2). Nitrogen is organic-bound to water-soluble proteins in flesh tissues (Cowey, 1995), explaining its high leachability. Phosphorus is primarily found in the skeleton where it is locked into calcium phosphate crystals making up the bones (Ketola & Richmond, 1994; Schenau & De Lange, 2000; Yamada et al., 2002).

Pellets are exposed to varying degrees of heat, moisture and pressure to produce a water-stable feed with lower leachability of nutrients (Hilton et al., 1981; Talbot & Hole, 1994). The fraction of soluble nutrients in SBT pellets (Table 4.2) is similar to values reported for Atlantic salmon diets (9-16% for nitrogen and 3-8% for phosphorus) (Phillips et al., 1993; Chen et al., 2003). It is possible that once feed debris reach the seafloor, leaching of nutrients from uneaten feeds increases further as wastes are broken down in size by physical, chemical and biological processes.

Baitfish had high fractions of soluble nutrients that decreased in the faeces (Table 4.2) as a result of the concentration of more complex, indigestible forms, such as calcium phosphate in bones. Comparatively lower amounts of indigestible phosphorus in pellets result in similar proportions of soluble phosphorus in pellets and pellet-faeces. The increase in soluble nitrogen from 15% in pellets to 43% in pellet-faeces, and its fast leaching rates, might be explained by hydrolysis of feed protein into their amino acid building blocks during passage through the gut (Fernandez et al., 1998). The fraction of nitrogen available for leaching in faeces of Atlantic salmon is lower than 26% based on an immersion period of 10 min (Chen et al., 2003). The small particle size and paste-like consistency of SBT faeces is likely to facilitate leaching from slow settling SBT faeces (maximum 1-2 cm s^{-1}) compared to salmon (4-6 cm s^{-1}) (Phillips et al., 1993; Chen et al., 2003).

Leaching of inorganic phosphorus was higher than inorganic nitrogen (Table 4.4). However, inorganic nitrogen corresponded to only a small proportion (< 5%) of nitrogen leached from feeds and faeces. These results are supported by work of Kibria *et al*. (1997), who reported that 85-87% of faecal nitrogen from silver perch is organic-bound. Although inorganic nitrogen accounted for only a small fraction of the total nitrogen leaching from baitfish, the decrease of concentrations in the leachate after 24 h suggests that the organic nitrogen released into water is readily available to microbial uptake (Figure 4.2).

The molar N:P ratios of marine plankton (16) (Redfield et al., 1963) are typically higher than those found here for baitfish (12), pellets (11) or faeces (2). Since more nitrogen leaches out

of feeds and faeces than phosphorus (Table 4.2), N:P ratios of leached wastes will decrease to values of 4 in feeds, and less than 1 in faeces. These results suggest that the N:P ratios of sediments could be used as a qualitative tool for assessing fresh waste accumulation.

Table 4.4. Maximum leaching rates of inorganic phosphorus, inorganic and total nitrogen from baitfish, pellets and faeces from SBT aquaculture. Values are reported as the mean (SD).

Sample	Inorganic P (mg P g^{-1} dw)	Inorganic N (mg N g^{-1} dw)	Total N (mg N g^{-1} dw)
Baitfish	4 (0.3)	0.8 (0.1)	45 (2)
Pellets	0.8 (0.04)	0.5 (0.1)	12 (2)
Baitfish-faeces	12 (0.9)	0.4 (0.04)	12 (0.3)
Pellet-faeces	3 (0.3)	0.7 (0.1)	20 (2)

4.4.3. Significance of leaching

The SBT production cycle involves the capture of approximately 5,000 tonnes of wild SBT between December and March, followed by fattening over summer and autumn to obtain approximately 4,300 tonnes of weight gain (Jeffriess, 2004). Although pellets are now available to the industry, commercial farms currently use baitfish as feed because of its ready availability and price. Feed conversion ratios (FCRs) for baitfish typically vary between 3 and 5 on a dry weight basis (Gunn et al., 2003; Fernandes et al., submitted-b). FCRs for pellets are comparable, between 4 and 6 (Glencross et al., 2002a; van Barneveld et al., 2003). These high FCRs reflect the distinctly higher metabolic rates of tuna when compared to other aquaculture species (Korsmeyer & Dewar, 2001; Musgrove & Fitzgibbon, 2006; Fernandes et al., submitted-b). Feed wastage varied between 4 and 23% in the first years of the industry (Bruce, 1997) but is much lower nowadays (Jeff Buchanan, personal communication). For modelling of leaching loads, we assumed FCRs of 5, 3% uneaten feed and 25% of ingested dry feed excreted as faeces. Dry matter digestibility of baitfish by SBT has not been measured, but values for pellets are 75% (Buchanan & van Barneveld, 2004).

Based on the values above, faeces comprise 89% of solid dry wastes and uneaten feed 11%. Using maximum nitrogen leaching rates for baitfish and baitfish-faeces (Table 4.4), the nitrogen load from leaching was calculated as 22 kg N $tonne^{-1}$ growth, 69% of which from leaching of faeces. Similarly, using maximum phosphorus leaching rates for baitfish and baitfish-faeces (Table 4.4), the phosphorus load from leaching was calculated as 15 kg P $tonne^{-1}$ growth, 96% from faeces.

These nutrient loads represent maximum leaching from solid wastes occurring during transport through the water column but also after deposition. For a 20 m water column, typical of SBT farm sites, it would take 5 min for uneaten baitfish to reach the seafloor, and more than 15 min for faeces. The bulk of the soluble nitrogen in feeds and faeces would enter the water column in these time intervals, with the leaching load reaching 21 kg N $tonne^{-1}$ growth. For phosphorus, only about 50% would enter the water column before deposition, or 7 kg P $tonne^{-1}$ growth.

Leaching loads for pellet-feeding would be slightly higher for nitrogen, or 26 kg N $tonne^{-1}$ growth, but significantly lower for phosphorus, or 4 kg P $tonne^{-1}$ growth. The loads for

leaching through the water column were calculated as 25 kg N tonne^{-1} growth and 1.6 kg P tonne^{-1} growth. Values for other finfish fed manufactured diets are generally lower, e.g. 0.4-0.9 kg P tonne^{-1} growth for Atlantic salmon (Phillips et al., 1993).

Total nitrogen losses to the environment modelled for salmonids with a hypothetical FCR of 5 vary between 252 and 350 kg N tonne^{-1} growth (Talbot & Hole, 1994; Islam, 2005). These modelled loads are in the same range as measured for other carnivorous species of high FCR, e.g. 321 kg N tonne^{-1} growth for areolated grouper (Leung et al., 1999) and 158-274 kg N tonne^{-1} growth for yellowtail (Watanabe et al., 1993). The nitrogen leaching load calculated here for SBT wastes represent only 6-10% of modelled total loads.

Total phosphorus loads for fish with FCRs > 1.5 typically vary between 16 and 69 kg P tonne^{-1} growth (Foy & Rosell, 1991b; Hall et al., 1992; Watanabe et al., 1993; Satoh et al., 2004). Using the model in Islam (2005), the load for an FCR of 5 would be 58 kg P tonne^{-1} growth. One can assume that the total load for SBT with the same FCR will potentially be higher than 58-69 kg P tonne^{-1} growth. The phosphorus leaching load for SBT wastes would thus account for less than 6-26% of total loads. The range reported for other finfish fed commercial diets is similar, between 4 and 10% (Phillips et al., 1993).

The leaching loads modelled here were based on a static system and therefore represent a minimum value for open systems as water currents and resuspension could promote further leaching. Although leaching will release significant quantities of nitrogen and phosphorus directly into the water column, long term monitoring of the tuna farming zone in South Australia indicates that the loads under current stocking regimes are not sufficient to affect water column nutrient and phytoplankton levels (Clarke et al., 1999; Clarke et al., 2000; Bierman et al., 2005). Potential regional effects are now being considered through a follow up research project of the Aquafin Cooperative Research Centre. The quantification of the pathways of nutrient release to the water column is essential to model loads under different management strategies and to predict the ecological consequences (and through this the risks to stock) of the application of new technologies or mitigation solutions.

4.5. Conclusions

Nutrients in baitfish feed and faeces were highly soluble, whereas nutrients in pellets were sparingly soluble. Soluble nitrogen was mostly in organic forms, with less than 5% released as ammonia or nitrates. Significantly more inorganic phosphorus than inorganic nitrogen leached from farm wastes. The N:P molar ratios of these wastes are lower than the natural background and could be useful as indicators of waste accumulation on the seafloor.

Leaching loads were modelled as 22 kg N tonne^{-1} growth and 15 kg P tonne^{-1} growth when SBT are fed baitfish. These loads might vary if baitfish composition changes with species, location sourced and season, but represent a first estimate of environmental losses from leaching and give us an indication of the magnitude of inputs. More than 90% of leached nitrogen, and approximately half of phosphorus, would enter the water column during transit of wastes to the seafloor. The leaching loads for SBT fed on pellets were slightly higher for nitrogen, 26 kg N tonne^{-1} growth, but significantly lower for phosphorus, 4 kg P tonne^{-1} growth. Leaching loads are expected to comprise less than 10-26% of total environmental losses of nitrogen and phosphorus. These loads are large enough to warrant inclusion in

nutrient models to avoid overestimation of the amount of nutrients retained in fish tissues or deposited on the sediments, and underestimation of the amount released directly into the water column.

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Chapter 5: Effects of SBT farming on benthic metabolism

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Abstract

In order to determine whether SBT farming had significant effects on benthic metabolism, we measured sedimentation rates in the water column, oxygen uptake rates (OUR) and nutrient fluxes at the sediment-water interface, as well as nutrient concentrations in porewaters and standing stocks of organic carbon, total nitrogen and chlorophyll *a* in the sediments. Other benthic variables measured included macroinfauna abundance and biomass, redox potential and mineral grain size. Sedimentation rates measured near the seafloor at farmed sites (9-85 g m^{-2} d^{-1}) reached values up to 10 times those recorded at control sites (< 25 g m^{-2} d^{-1}). Despite this high deposition of wastes, the redox potential of sediments was always positive. Mean OUR measured at the farm sites (1,249-4,329 µmol m^{-2} h^{-1}) significantly exceeded natural variability by up to 7 times. This was particularly evident when feed inputs exceeded 0.5 kg m^{-2} d^{-1}. Less than 12% of the variance in OUR values was explained by macroinfauna data. Mean ammonia (194-9,963 µmol m^{-2} h^{-1}) and phosphate (227-2,177 µmol m^{-2} h^{-1}) fluxes adjacent to commercially stocked pens were 1 to 2 orders of magnitude the natural background. Although not as marked, the effect on mean nutrient porewater concentrations (1-11 mg N/L and 1-8 mg P/L) was also significant, with much lower values found in control sites (0.4-4 mg N/L and 0.05-0.4 mg P/L). However, other nutrient standing stocks in the sediments showed only marginal increases in the footprint of the pens. These results suggest that although inputs are significant, these are quickly metabolised by the system, reflecting in high flux rates but low accumulation. Effects of SBT farms on benthic metabolism were transitory rather than chronic and changes were reversible.

5.1. Introduction

The nature and fate of waste material generated by the holding and fattening of southern bluefin tuna (SBT) within pontoons provides the focus for the research within this Chapter. The data for this chapter were gathered over the 2003/2004 SBT farming season from within commercially operated SBT farms and control sites in the vicinity of the SBT farming zone off the coast of Port Lincoln. The types of interaction that the SBT farms have with aquatic environmental processes arise from the nutrient inputs that are part of the farming process. The aim of this chapter was to determine whether SBT farming had significant effects on benthic metabolism and, if so, what factors were connected to the changes observed.

From monitoring done as part of the Tuna Environmental Monitoring Program (TEMP) since 1997, little evidence of impacts from SBT farms have been found on either the benthic or pelagic environments around the lease sites (Clarke et al., 2000). The TEMP monitors the environment surrounding each company's lease sites, based upon compliance points, set-up 150 m from the edge of the lease sites' boundaries. The monitoring program uses video transects and macroinfauna analyses to monitor the level of impact of each company farming SBT, and from this, no company has exceeded the legislated limits since 1997. However, no data exist on the fate, magnitude and composition of the waste generated by individual SBT pontoons, let alone for the entire industry. Brown et al. (1987) found four zones of impact extending from the edge of the pontoons at salmon farms on the west coast of Scotland: beginning with an azoic zone directly beneath the pontoon; to a highly impacted zone within 3 m of the edge of the pontoon; to an intermediate zone comprising characteristics of both impacted and undisturbed sites (less than or equal to 25 m away); and finally to a low or non-detectable impact zone with characteristics of the background benthos (more than 25 m away). This pattern of impact has been noted elsewhere and reaffirmed since the study by Brown et al. (1987) was carried out. Holmer & Kristensen (1992) and Hansen et al. (2001) also found that azoic areas or white mats of sulfur-reducing bacteria (*Beggiatoa* sp.) may develop under marine finfish pontoons. However, Findlay & Watling (1997) and Karakassis et al. (2000) suggested that significant effects from farm derived particulate waste were generally confined to within 30 m of the pontoons and that, overall, impacts were characterised from no gross impact to highly eutrophicated. Thus, although there seems to be a tendency for a gradient of impacts, conditions specific to each aquaculture industry, including the environment and the marine fish species cultured, must be considered independently before making generalisations from other situations.

Previous research has shown that the sedimentation rate of carbon was correlated with the rate of benthic metabolism (Findlay & Watling, 1997). The severity of impacts of fish farming depends directly on the amount of waste material produced by a farm and the degree of dispersal (MacDougall & Black, 1999). Given previously documented effects of farming fish at sea in pontoons (Folke & Kautsky, 1992; Johnsen et al., 1993; Beveridge et al., 1994; Ervik et al., 1997; Hargrave et al., 1997; Elberizon & Kelly, 1998; Chen et al., 1999b; Dudley et al., 2000; Hargrave & Phillips, 2001), it was anticipated that the farming of SBT may contribute to enhanced levels of benthic organic enrichment and consequently, alter the rate of benthic metabolism. Oxygen uptake rates (OUR) are considered an integrative measure of aerobic metabolism by the benthos (Valdovinos & Figueroa, 2000) and as such they are likely to be a good indicator of altered metabolic processes associated with increased organic loading. OUR variability has been attributed to several physical, chemical and biological factors measured in the pelagic and benthic environments. Among other factors, these include seasonal changes in water temperature (Mazouni et al., 1996), sedimentation rate and

hence organic load (Holby & Hall, 1991; Kelly, 1995; Wu, 1995; Panchang & Newell, 1997; Hansen et al., 2001), redox state of the sediment (Jorgensen, 1977), benthic microalgae (Wenzhofer & Glud, 2004) and macrofaunal abundance (Rosenberg et al., 2001; Wenzhofer & Glud, 2004). Therefore measurement of other pelagic and benthic variables known to affect OUR was done concurrently, to help explain any differences observed in OUR among sites. Thus the benthic response to SBT farming activities was investigated in terms of sedimentation rates and sedimentary OUR, dissolved nutrient fluxes, porewater chemistry, macroinfaunal abundance, organic carbon (OC) and total nitrogen (TN) content, grain size, chlorophyll *a* and redox profiles. As the majority (i.e. about 60 to 70 %) of fish farm derived wastes have been shown to be in the dissolved fraction (Holby & Hall, 1991; Karakassis et al., 2000), we also measured water column nutrient concentrations (dissolved organic carbon, total nitrogen, ammonium, nitrate plus nitrite and phosphate).

The aim of this Chapter was to assess if the commercial activities at individual SBT pontoons had a measurable effect on benthic metabolism above natural variability. Initially, variation in OUR among sites and months was investigated through shipboard incubation of intact sediment cores. To explain some of the variation observed in OUR at the core level, analysis of sedimentary nutrient fluxes, chlorophyll *a* and redox potential was carried out. Due to incomplete sampling of macroinfauna, the relationship between macroinfauna and OUR was assessed independently from the other variables taken at the core level. Patterns of variation in OUR were also related to sedimentary (grain size, OC and TN) and pelagic variables (dissolved nutrients, sedimentation rates) at the site level.

5.2. Methods

5.2.1. Site descriptions

To determine the effect of SBT farming on benthic metabolism, two commercially operated pontoons, one research pontoon and two control sites were studied. For logistical and funding reasons, one of the commercial pontoons and the research pontoon shared a common control site. For commercial reasons, the names of the two SBT farming companies that took part in this study are withheld and sites are from hereon referred to by their site identification code (Table 5.1). The sites P04 and RC1 were in the northern part of the farming region, whereas P05, TRF and BC5 were in the southern area (Figure 5.1).

Both P04 and P05 were commercially-stocked SBT pontoons. TRF was a research pontoon stocked with one tenth the number of SBT as in P04 and P05. SBT in P04 and P05 were fed baitfish, whereas SBT in TRF were fed pellets. RC1 and BC5 were control sites that were at least 1 km from the edge of any SBT stocked pontoons, including pontoons that were not the focus of this research. The SBT farming companies supplied farm management information that included pontoon dimensions, stocking and harvesting dates, as well as average fish weights (Table 5.2). At the research pontoon TRF, 5 harvests occurred throughout the season (largest was 113 SBT on 19-8-04) before the final harvest, which accounts for the difference in stocking and harvest SBT numbers. The SBT farm management information was used to more accurately interpret the data gathered.

In March, P04 was the only pontoon site where SBT had been stocked, with this occurring 2 weeks prior to the first sampling (Figure 5.2). Feeding did not take place every day during the sampling periods and the feeding rates presented were averaged over the sampling period,

for feeding days only. Generally, SBT are fed 6 days a week and thus about 4 days every month SBT are not fed. During May and July, all pontoon sites were stocked with SBT and feeding rates were at their maximum. By November, the harvest was completed and no fish remained at any of the sites (Figure 5.2). In fact, P04 was harvested in July, P05 in August and TRF in September (Table 5.2). The ambient seawater temperature was highest in March (22 ºC) and lowest in July (14.5 ºC) (Figure 5.2). The predominant feed type throughout the season at the commercial pontoons was baitfish and sardines (mostly *Sardinops neopilchardus*) made up the majority of the daily quota. The average nutrient composition of sardines was 45% carbon, 11% nitrogen and 1.7% phosphorus. The pellets used at TRF consisted of 44% carbon, 8% nitrogen and 1.7% phosphorus.

Table 5.1. Site identification codes and general characteristics of site and pontoons.

Site code	Site	Type of pontoon	Water depth (m)	Pontoon diameter (m)	Pontoon net depth (m)
P04	Pontoon	Commercial	22	38	10
P05	Pontoon	Commercial	20	40	10
TRF	Pontoon	Research	19	40	10
RC1	Control	n/d	18	n/d	n/d
BC5	Control	n/d	18	n/d	n/d

n/d = no data.

5.2.2. Sampling

Sampling was conducted throughout 2004, in March, May, July and November. Unfortunately, due to unforeseeable logistical constraints, only 3 sites were sampled in November (BC5 and TRF were not sampled). The consequences on the completeness of the dataset should be noted. The general approach to this study was to measure a range of pelagic and benthic variables before, during and after the 2004 SBT farming season. The overall sampling design is described in Table 5.3. For each month sampled a series of 7 variables were measured and, unfortunately, due to logistical constraints (e.g. research vessel time, weather and safe access), uneven numbers of replicates may have been taken.

Ambient seawater was sampled from 3 and 10 m water depths using a modified messenger bottle. Samples were filtered using a pre-rinsed sterile syringe onto a 0.45 µm filter and stored shipboard (-20 ºC) before being transferred to laboratory storage (-30 ºC).

Sediments were collected with 67 mm (i.d.) stainless steel tubes using a HAPS Corer (KC Denmark). Upon retrieval, the overlying water in the tube was carefully discarded to minimise surface disturbance and the sediment extruded onto a clean stainless steel table. Four cores were collected for the analyses of OC and TN. The top layer (0-1 cm) of each core was sliced, transferred into a pre-combusted glass jar and stored frozen (-30 ^{o}C). Two cores were collected for the analyses of ammonium and phosphate in porewaters. The top layer (0-2 cm) of each core was sliced, transferred into a pre-weighed centrifuge tube and stored refrigerated (4 ^{o}C) for up to three hours before transfer to the laboratory. Two cores were collected for the determination of mineral grain size distributions. The top layer (0-4 cm) was sliced, transferred into a pre-combusted aluminium tray and stored frozen (-30 ^{o}C).

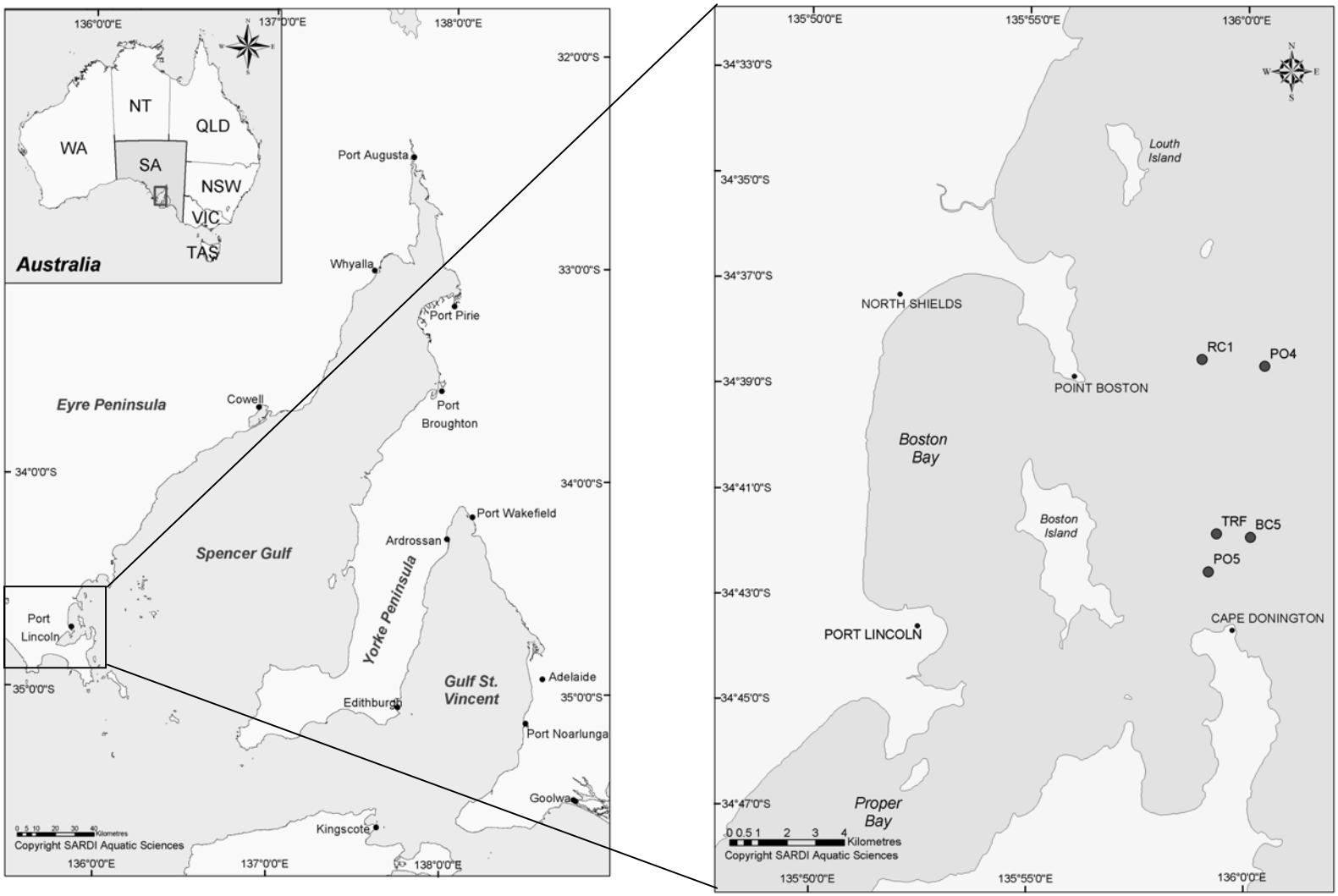


Figure 5.1. Map of the sampling sites within the southern bluefin tuna farming area off the coast Port Lincoln, South Australia.

Table 5.2. Summary of pontoon information from SBT companies for the 2003/4 season.

Site	Date stocked	Date harvested	No. of days stocked	No. fish when stocked	No. fish at harvest	Ave SBT weight when stocked (kg)	Ave SBT weight at harvest (kg)	Ave feed per pontoon per day (kg)
P04	2-2-04	20-7-04	170	1,794	1,742	16.61	27.05	3,630
P05	18-4-04	30-8-04	136	2,325	2,225	17	30.92	3,280
TRF	26-3-04	14-9-04	172	189	18	17	30.97	119

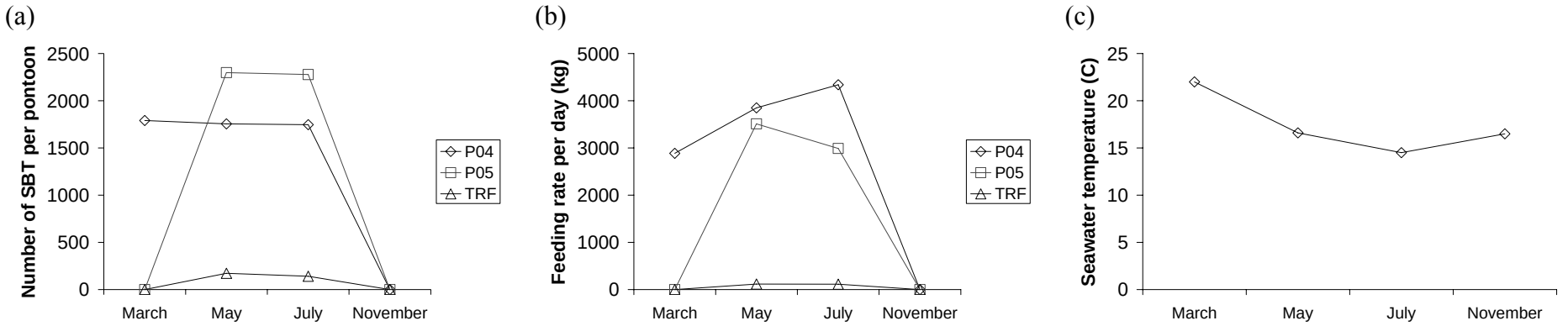


Figure 5.2. The (a) number of SBT per pontoon, (b) feeding rate per pontoon per day and (c) the ambient seawater temperature during the months samples were taken.

Table 5.3. Summary of the samples taken at each site throughout the 2003/4 season. For each variable measured the number of replicate cores/water samples and levels investigated are given (* denotes that TRF and BC5 were not sampled during November).

Variables	Replicates per site	Site	Month	Depth
Oxygen uptake rate	3 to 6	5	4*	n/d
Ammonium, phosphate and nitrate plus nitrite fluxes				
Porewater	3	5	4	n/d
Redox potential	3 to 6	5	4*	5
Chlorophyll a	3 to 6	5	4*	n/d
Macroinfauna	3 to 6	5	4*	n/d
Grain size	2	5	4	n/d
Organic carbon and total nitrogen	4	5	4	n/d
Sedimentation rate (3 distances)	2	5	4	2
Dissolved organic carbon, ammonium, nitrate plus nitrite, total nitrogen, phosphate	2	5	4	2

For determination of OUR and nutrient fluxes at the sediment-water interface, we collected sediments in 105 mm (i.d.) opaque PVC core tubes impervious to light. These cores were fitted with a bottom seal with double o-rings and a top seal with single o-ring. Three to six replicates were transferred into an incubation system for determination of nutrient fluxes. These cores had a visibly undisturbed sediment surface, a minimum of 800 mL of clear overlying *in situ* bottom seawater and at least 10 cm depth of sediment. At the conclusion of the incubation period, each sediment core had sediment redox potentials measured, sedimentary chlorophyll *a* samples taken and the remaining sediment was preserved for benthic macroinfaunal analysis.

Profiles of redox potential were measured by inserting a platinum electrode with a calomel reference (ORP, Phoenix) through holes pre-drilled into the PVC barrels at different sediment depths (2, 4, 6 and 8 cm). These holes were 12 mm in diameter, 20 mm apart (centre to centre), and sealed with rubber grommets. Surface redox measurements were made by vertically inserting the 10 mm platinum tip into the top of the sediment core. The redox probe tip was rinsed clean in seawater before each insertion and readings were allowed to stabilize for 20 seconds before measurements were recorded. Voltage output (mV) measurements were adjusted relative to the normal hydrogen electrode (Eaton et al., 1995).

For chlorophyll-*a*, the top 1.5 cm of the sediment was sampled using a modified 50 mL syringe (29 mm i.d.) with the sample being placed in a sterile, preweighed 50 mL centrifuge tube. Samples were immediately frozen shipboard at -20 ºC, prior to storage in the laboratory at -80 ºC. During storage and processing, samples were kept from exposure to sunlight by wrapping the tubes in aluminium foil.

For collection of samples for infauna analyses, the top 10 cm of the sediment core was extruded from the PVC barrel into a 2 L polypropylene jar. Previous macroinfauna sampling has shown that around 90 % of the individuals are found in the 0 to 5 cm sediment depth (Weston, 1990); this was similar for the Port Lincoln farming area as shown in preliminary trials that analysed the 0 to 10 cm depth. Formalin was then added to a final concentration of 10 % via dilution with seawater and the samples stored at room temperature. Macroinfaunal samples were collected for only the first 3 months, due to time restrictions associated with sorting and identification of samples.

Sediment traps consisted of two PVC tubes with a height to width ratio of 4.7 (height 400 mm, diameter 85 mm), lead weighted on the bottom (40 g) to ensure correct vertical orientation (Figure 5.3). These were placed 1 m (bottom) and 10 m (mid-water) above the seafloor (Figure 5.4). Three sets of sediment traps separated by 30 m were deployed at each site (Figure 5.4). This design gave us sedimentation rates at 0, 30 and 60 m from the edge of pontoons with two replicates per distance and depth. In order to estimate the area affected by sedimentation around the pens, we deployed a second line of sediment traps in one of the pens in May. This additional line gave us sedimentation rates at 100, 130 and 160 m from the edge of the selected pontoon. Sediment traps were deployed for at least 48 h but no more than 96 h. Upon retrieval, traps were spiked with $HgCl_2$ to a final concentration of 10 mg L^{-1} to prevent microbial degradation.

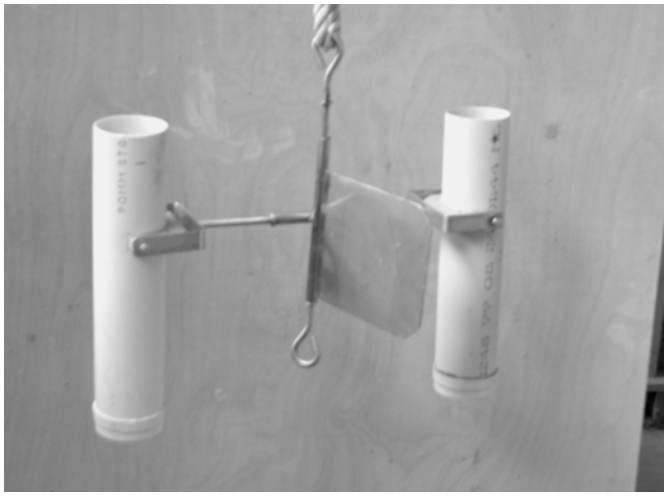


Figure 5.3. A pair of sediment traps (400 mm long) attached to a stainless steel frame.

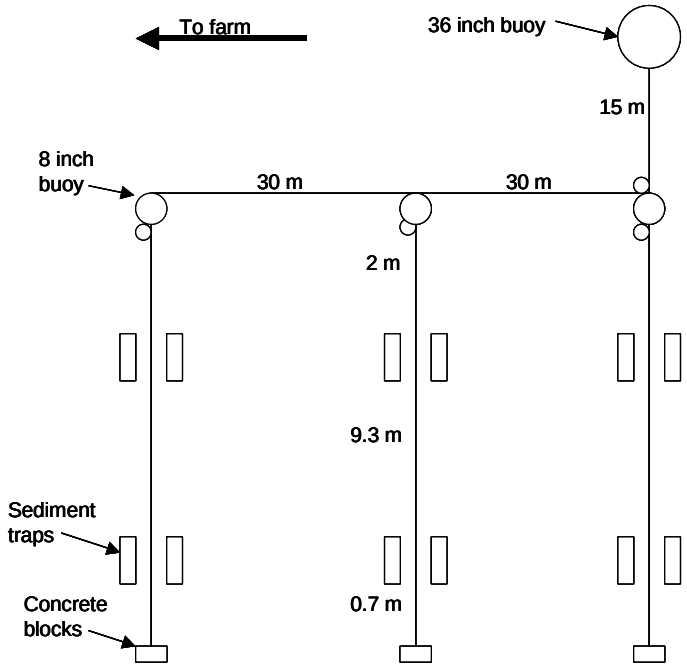


Figure 5.4. Schematic diagram representing a series of sediment traps deployed at each of the 5 sites.

5.2.3. Water column nutrients

Samples for the analysis of dissolved organic carbon (DOC) were acidified with HCl and purged before being analysed in a Shimadzu TOC-5000 elemental analyzer. Determination of nitrates/nitrites involved reducing nitrates to nitrites in a column packed with copper coated cadmium granules and measuring total nitrites colourimetrically by flow injection analysis (FIA) in a QuickChem 8000 Automated Ion Analyser (APHA-AWWA-WPCF, 1998b). Briefly, the sample is mixed with acidic sulphanilamide to form a diazo compound, which is then mixed with N-(1-Naphthyl)-ethylene diamine dihydrochloride to form a purple azo dye, the intensity of which is proportional to the sum of the nitrate and nitrite concentration measured spectrophotometrically at 520 nm. The automated phenate method was used for the determination of ammonia by FIA (APHA-AWWA-WPCF, 1998c). Ammonia reacts with hypochlorite to form monochloramine, which in the presence of phenol, nitropusside and excess hypochlorite, gives indophenol blue. The intensity of indophenol blue is proportional to the ammonia concentration in the sample and is measured spectrophotometrically at 630 nm. For determination of total nitrogen (APHA-AWWA-WPCF, 2001), the sample is digested for 45 min in an autoclave with an alkaline persulphate solution to convert N-containing compounds to nitrates which are determined as described above. The digestion process is repeated twice. The persulphate solution is prepared by dissolving 4.5 g of NaOH in 200 mL of MilliQ-water, this solution is cooled to room temperature, 20 g of $K_2S_2O_8$ added and the volume adjusted to 500 mL. FIA was also used to determine phosphate (APHA-AWWA-WPCF, 1998a). The sample is mixed with ammonium molybdate and antimony potassium tartrate to form phosphomolybdic acid, which is reduced by the addition of ascorbic acid to form a blue complex, the intensity of the colour being proportional to the concentration of phosphate. The absorbance is measured at 880nm.

5.2.4. Sedimentation fluxes

The contents of sediment traps were sieved onto a 1 mm mesh sieve to remove non-sedimentary matter (e.g. live fish) that were not part of the passive flux. Sieved samples were vacuum filtered through pre-combusted (450 ºC overnight) and pre-weighed glass-fibre filters (MFS GF-75, 0.7 µm, 47 mm diameter). Filters were placed in separate pre-combusted glass petri dishes, covered with a glass lid and oven dried at 50ºC. Before gravimetric analyses, the petri dishes containing the dried filters were placed in an oven at 50 ^{o}C for at least 3 h and placed in a desiccator with silica gel for 1 h to cool. The filters were then weighed using an electrobalance to five decimal places. Results were corrected for salt that impregnates the filters and sedimentation rates expressed in units of g (dw) m^{-2} d^{-1}.

5.2.5. Sediments

Sediment samples were freeze-dried, sieved to 500 µm to remove large shell fragments, and homogenized with a mortar and pestle. Samples were weighed into foil capsules and analysed for TN by Continuous-Flow stable Isotope Ratio Mass Spectrometry (CF IRMS) using a Europa Scientific ANCA-SL elemental analyser coupled to a Geo 20-20 Mass Spectrometer. TN concentrations are reported as a percentage of total dry sediment. Aliquots for determination of OC content were decarbonatated with 1N HCl and analysed by CF IRMS as described for TN. Weight percentages of OC were corrected for carbonate content and are reported as a percentage of total sediment. Carbonate contents were determined with a pressure transducer (Pressure tranducer from RS Components, part 348-8065, Iso-Tech

voltameter IDM 91) by measuring the increase in pressure generated by the CO_2 released after acidification of core aliquots with 5.5N HCl at room temperature.

Sediments collected for porewater analyses were centrifuged at 3,000 rpm for 10 minutes, the supernatant filtered (0.45 μm) and stored frozen (-30 ^{o}C). Ammonium and phosphate were determined spectrophotometrically as described above for water column nutrients.

Samples collected for infaunal analyses were decanted and sieved (1 mm mesh). The material retained was sorted for macroinfauna under a dissecting microscope (10x magnification, Leica MZ95). Results are expressed per number of individuals (no. m^{-2}) or total wet biomass (g m^{-2}).

Cores for the determination of mineral grain size distribution were thawed, oven-dried, homogenized and muffled at 350°C to remove organic matter. Muffled samples were dispersed with a solution of sodium hexametaphosphate, transferred and homogenized into a measuring cylinder, and the clay content (<4 μm) determined with a hydrometer after 2 hours of settling. The content of the cylinder was then wet sieved (63 μm) and dried at 100°C. An automatic sieve shaker (Endecotts EFL2000) with graded sieves of 2000, 1000, 500, 250, 125 and 63 μm mesh size was used to obtain sand fractions. The silt content (4-63 μm) was calculated as the difference between the muffled weight of the sample and the sand and clay fractions. Mean grain size was calculated with the software package GRADISTAT using the Folk and Ward graphical method (Blott and Pye, 2001).

5.2.6. OUR and benthic nutrient fluxes

OUR and nutrient fluxes were measured on board with a manually operated incubation system immediately after sampling**.** Incubations were designed to last for 2 to 4 hours to limit the likelihood of non-linear nutrient changes. The incubation system consisted of two temperature sensors connected to a data logger (DT 50, Datataker), six seawater stirrers to prevent stratification (single blade, 7 mm wide, 4 mm long) and a temperature-controlled water bath, thus allowing up to six sediment cores to be incubated at once. The magnetic stirrers were fitted with an o-ring and penetrated through the top seal of the cores. The water bath consisted of a 200 L PVC outer container filled with ice and a 60 L polystyrene inner container filled with freshwater. The PVC barrels sealed at both ends were placed in the inner container, which had a pump to circulate the water and an aquarium heater to maintain water temperature. The temperature was set at ambient bottom seawater temperature measured at the time of collection. The system maintained temperatures to ± 0.8 ºC of the set value.

Samples of the overlying seawater were taken in duplicate from each core at the start and end of the incubation, filtered (0.45 μm) and stored frozen (-30 ºC). Nutrient fluxes were determined from the change between initial and final concentrations in the overlying seawater. Samples were analysed for nitrates/nitrites (NO_x), ammonium and phosphate according to the methods described above for water column nutrients (APHA-AWWA-WPCF, 1998c; APHA-AWWA-WPCF, 1998b; APHA-AWWA-WPCF, 1998a). The duplicate samples were averaged and the change in nutrient concentration was then adjusted to account for the sediment surface area, duration of incubation and volume of overlying seawater to determine the rate of nutrient release or uptake. The resultant rate of change was expressed in units of μmol m^{-2} h^{-1}. For nutrient fluxes, positive measurements represented a

flux out of the sediments into the overlying water and negative measurements represented a flux into the sediments from the overlying water.

5.2.7. Statistical analyses

SPSS (SPSS Inc., Chicago) was used for univariate data analyses and PRIMER (PRIMER-E Ltd, Plymouth) for multivariate analyses. To assess the null hypothesis that there was no significant difference in OUR between sites or months, or from the interaction of site and month, a two-way ANOVA was carried out. Site and month were considered as fixed factors and Type IV sums of squares was used to account for the missing cells of TRF and BC5 in November. To determine which sites or months contributed to any significant main effects, *post-hoc* analysis (via Tukey's HSD) was conducted subsequently if required. To meet the assumptions of normality and correct the positively skewed distributions, the data for OUR were $\log_{10}$ transformed prior to analysis. To determine the potential for the ammonium, phosphate and nitrate plus nitrite fluxes, as well as sedimentary chlorophyll *a* concentrations and redox potential (at 4 cm), to predict OUR, a multiple regression was performed.

To explicitly describe linear relationships among ammonium, phosphate and nitrate plus nitrite fluxes, as well as the sedimentary chlorophyll *a* concentrations and the redox potential, principal component analysis (PCA) was used. PCA scores for principal components with eigenvalues greater than 1 were used to assess the null hypotheses that nutrient flux data and the standing stock measurements did not vary significantly with site or month or as a result of the interaction of site and month. The PCA scores, which are independent by definition, were separately analysed (two-way ANOVA). To determine which sites or months contributed to any significant main effects, *post-hoc* analysis was conducted subsequently if required (via Tukey's HSD). If required, the minimum whole integer required to make all PC scores positive was added, prior to $\log_{10}$ transformation.

To assess the null hypotheses that the number of infauna and total wet biomass did not significantly differ among sites or months, or from the interaction of site and month, two-way ANOVAs were carried out. *Post-hoc* analysis (via Tukey's HSD) was conducted subsequently if required. To assess the relationship between OUR and macroinfaunal abundance or biomass, linear regressions were carried out.

To test the null hypotheses that concentrations of ammonium and phosphate in the porewater did not significantly differ among sites or months, or as a result of the interaction of site and month, two-way ANOVAs were used. *Post-hoc* analysis (Tukey's HSD) was conducted subsequently if required. To meet the assumptions of normality, the data were $\log_{10}$ transformed prior to analysis.

To assess the null hypotheses that mean grain size and the percentage of silt and clay and OC and TN did not vary significantly with site or month or as a result of the interaction of site and month, two-way ANOVAs were used. *Post-hoc* analysis (via Tukey's HSD) was conducted subsequently if required. To meet the assumptions of normality, the data on mean grain size, OC and TN were $\log_{10}$ transformed prior to analysis.

Unfortunately, as some individual sediment traps were lost during the deployment period, several missing values were accumulated (16 missing out of 240 individual sediment traps, or

12 pairs with no value or single unreplicated values out of 120 pairs) and statistical analyses were not performed.

To explicitly describe the linear relationships between dissolved nutrient concentrations (organic carbon, ammonium, nitrate plus nitrite, total nitrogen, phosphate), PCA was used. PCA scores for principal components with eigenvalues greater than 1 were used to assess the null hypotheses, via three-way ANOVA, that dissolved nutrient concentrations did not vary significantly with site, month or depth, or as a result of their interactions. *Post-hoc* analysis (via Tukey's HSD) was conducted subsequently if required. If required, the minimum whole integer required to make all PC scores positive was added, prior to $\log_{10}$ transformation.

5.3. Results

5.3.1. OUR

The maximum mean OUR was recorded in July for the commercially stocked SBT sites of P04 and P05 (Figure 5.5). These measurements (4,329 and 3,453 µmol m^{-2} h^{-1}, respectively) were 3 to 7 times those recorded at the control sites at the same time. OUR at P04 and P05 increased by 2 to 3 times from March to July, before dropping again by November. The change in OUR throughout the year at TRF, RC1 and BC5 was less consistent. For example, BC5 and RC1 decreased from March to July by 2 to 3 fold, which corresponded with the onset of winter, with RC1 then increasing in November with warmer seawater temperatures. TRF showed an initial decrease in OUR from March to May before increasing in July. The differences between the commercial pontoons and the other sites in how OUR varied over time are reflected in a significant interaction of site and month.

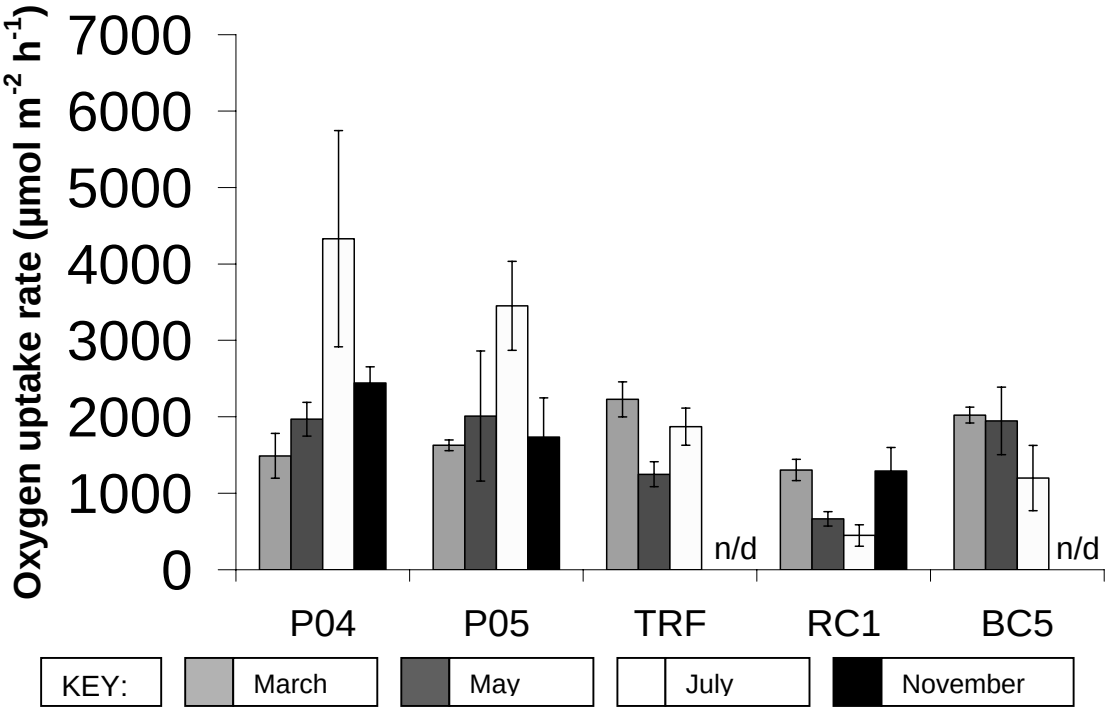


Figure 5.5. Oxygen uptake rates for the 2004 sampling year. Bars indicate means (n = 3 to 6 cores, ± SE) (n/d = no data).

5.3.2. Nutrient fluxes, redox potential and sedimentary chlorophyll *a*

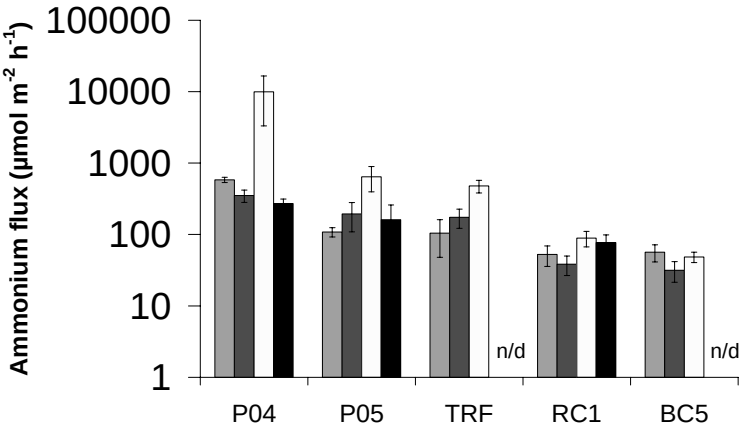
The mean ammonium and phosphate fluxes increased from March to July at both the commercially stocked pontoons (Figures 5.6 a, b). For TRF, only the ammonium flux followed this pattern. Ammonium and phosphate fluxes at RC1 and BC5 fluctuated among months. The highest mean ammonium and phosphate fluxes were recorded at P04 (9,962 and 2,177 μmol m^{-2} h^{-1}, respectively) and P05 (644 and 1,476 μmol m^{-2} h^{-1}, respectively) during July, and these were in excess of 10 times those recorded at either RC1 or BC5 (i.e. control sites) in any month. It is noteworthy that the high rates recorded at P04 and P05 during July were not observed in November. The nitrate plus nitrite fluxes exhibited different patterns to the ammonium and phosphate fluxes (Figure 5.6 c). The nitrate plus nitrite fluxes at RC1 and BC5 increased from March to July, whilst at the pontoon sites, there were more variable results. The highest mean nitrate plus nitrite flux was observed at P04 during November (122 μmol m^{-2} h^{-1}), and was approximately double the maximal rate observed at any other site for any month.

Generally, redox potentials remained positive, except for the November readings made at the commercial pontoon sites (i.e. P04 and P05), where below 2 cm depth, negative redox potentials were recorded (Figures 5.7 a, b). At the control sites (RC1 and BC5), the surface redox potentials remained above 200 mV for the 4 months (Figure 5.7 d, e). TRF showed a decrease in surface redox potentials below about 200 mV only for July (Figure 5.7 c).

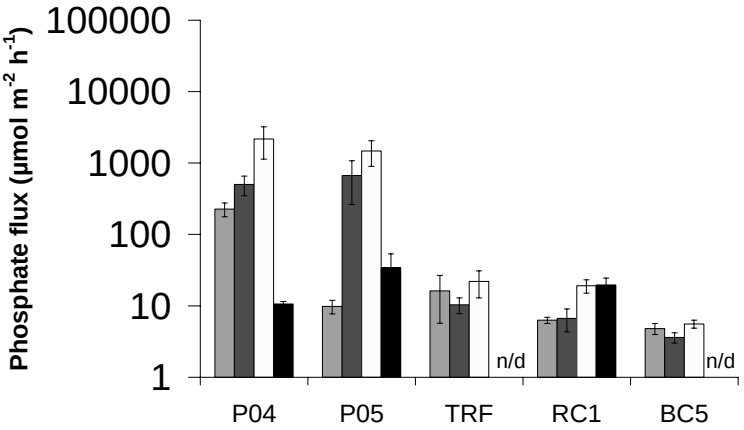
Table 5.4. Effect of spatial and temporal variability on OUR measured throughout 2004.

Source	df	Mean Square	F	Sig.
SITE	4	.575	12.827	.000
MONTH	3	.060	1.331	.273
SITE * MONTH	10	.179	3.988	.000
Error	57	.045		

(a)



(b)



(c)

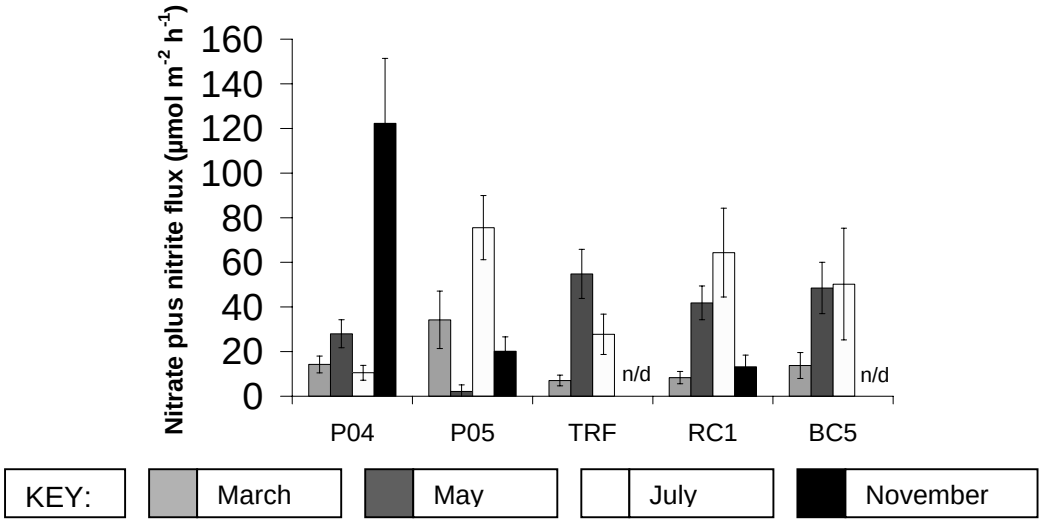


Figure 5.6. Nutrient fluxes of (a) ammonium, (b) phosphate and (c) nitrate plus nitrite recorded during 2004. Bars indicate means (n = 4 to 6 cores, ± SE) (n/d = no data).

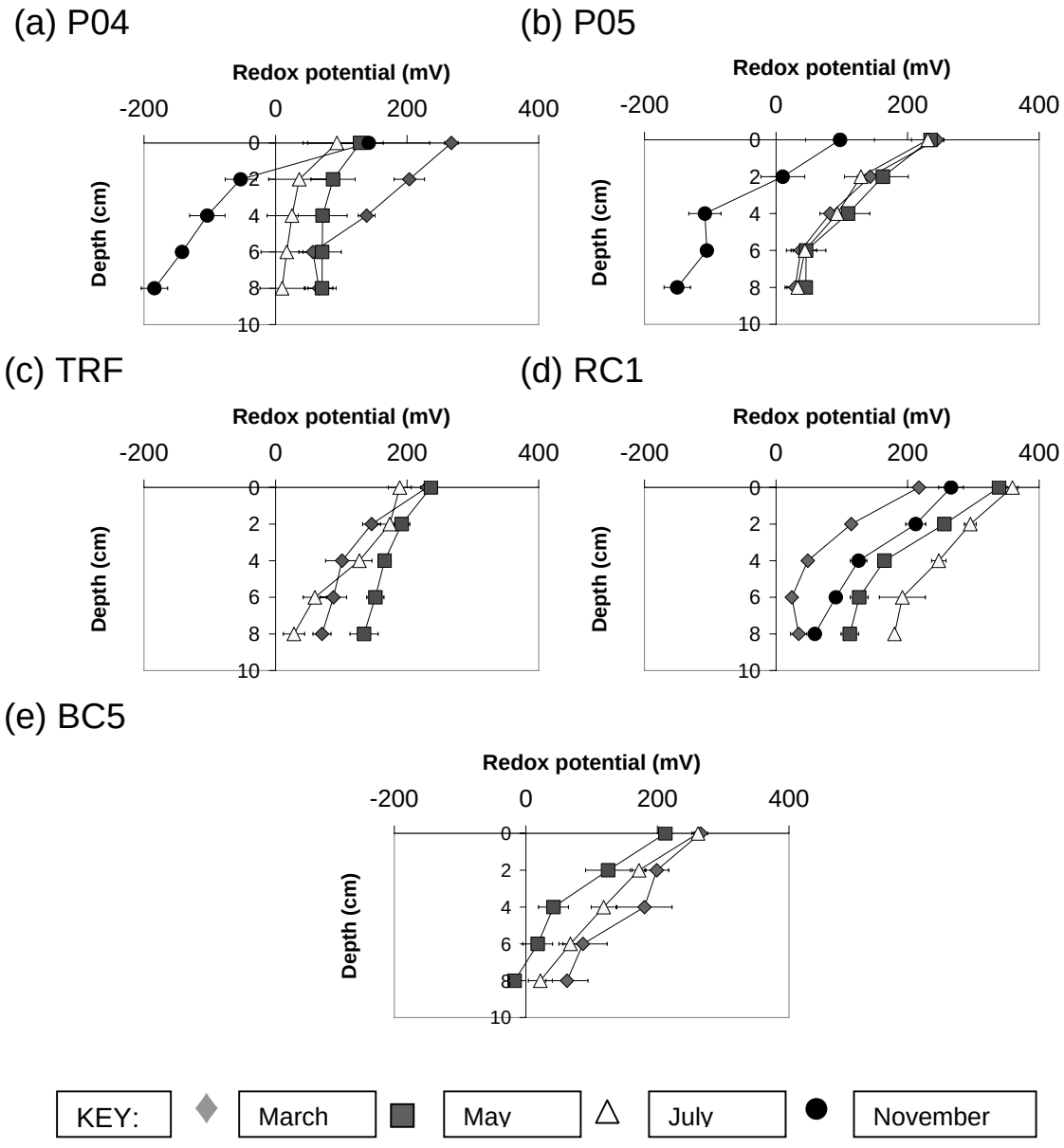


Figure 5.7. Redox potential profiles at (a) P04, (b) P05, (c) TRF, (d) RC1 and (e) BC5 sampled throughout 2004. Points indicate mean values (n = 3 to 6 cores, ± SE).

Chlorophyll *a* concentrations were the highest in March for P05, TRF and BC5 (101, 108 and 110 mg m^{-2}, respectively); and approximately double those at P04 and RC1 for the same month (Figure 5.8). P04 exhibited the largest fluctuations with the March values at P04 being similar to May (43 mg m^{-2}), before more than doubling to July (107 mg m^{-2}) and finally decreasing to November (79 mg m^{-2}). RC1 consistently recorded the lowest chlorophyll *a* concentrations (41 to 76 mg m^{-2}).

OUR was significantly related to the nutrient fluxes and the standing stock measurements (Multiple Regression, $r^2 = 0.757$, $p < 0.001$, $n = 61$). The equation generated was OUR (µmol m^{-2} h^{-1}) = 702.211 + 0.011 (ammonium flux µmol m^{-2} h^{-1}) -0.189 (nitrate plus nitrite flux µmol m^{-2} h^{-1}) + 1.049 (phosphate flux µmol m^{-2} h^{-1}) + 11.971 (chlorophyll *a* mg m^{-2}) – 1.881 (redox potential mV).

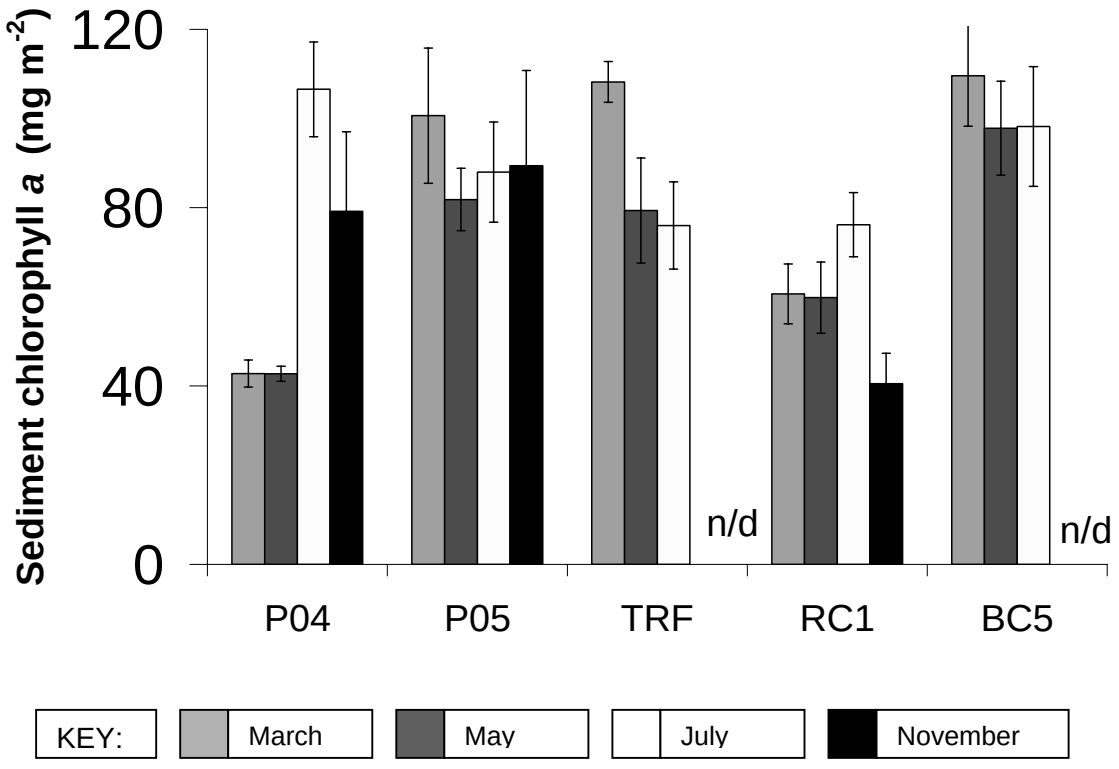


Figure 5.8. Sedimentary chlorophyll *a* concentrations at sites sampled throughout 2004. Bars indicate means (n = 3 to 6 cores, ± SE) (n/d = no data).

Despite some overlap, nutrient fluxes and standing stock measurements at the pontoon sites were generally separated from the control sites in the PCA ordination space (Figure 5.9). PC1 explained 38 % of the variation and PC2 explained 24 % (Table 5.5 a). From the loadings, negative scores on PC1 were generated by increasing phosphate and ammonium fluxes, while positive scores on PC2 were generated by increasing chlorophyll *a* and nitrate plus nitrite fluxes (Table 5.5 b). Significant differences among sites were found upon analysis of PC1 scores (Table 5.6), where P04 was significantly different from TRF, RC1 and BC5 ($p = 0.018$) and P05 was significantly different from RC1 and BC5 ($p = 0.004$). From the PC1 loadings (Table 5.5 b) and inspection of the raw data (Figure 5.6 a, b), the significant difference among sites was driven by higher phosphate and ammonium fluxes at the pontoon sites. A significant interaction between site and month was found on analysis of PC2 scores ($\log_{10}$ transformed) (Table 5.6). From the PC2 loadings (Table 5.5 b) the significant interaction of site and month on PC2 scores was driven by variability in chlorophyll *a* and nitrate plus nitrite fluxes.

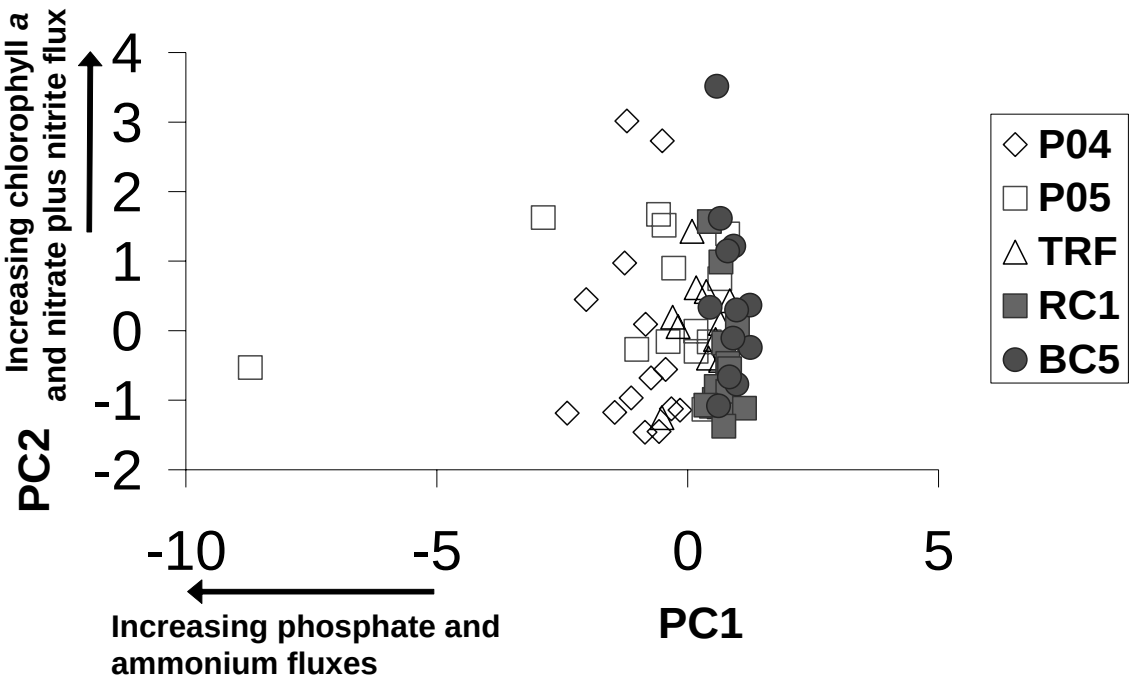


Figure 5.9. PCA plot of the nutrient fluxes and standing stock measurements for sites sampled in 2004 (pontoons sites are open symbols and closed symbols are control sites).

Table 5.5. (a) Eigenvlues and (b) loadings from the PCA of the nutrient fluxes and standing stock measurements from sites sampled in 2004. Bold type indicates variables with greatest loadings contributing the most amount of information to the ordination of points on the PCA plot.

(a)

PC	Eigenvalue	Variation (%)
1	1.9	38
2	1.21	24.1
3	0.94	18.8
4	0.75	15.1
5	0.2	4

(b)

Variable	PC1	PC2
Ammonium flux	-0.677	-0.027
Nitrate plus nitrite flux	-0.124	0.654
Phosphate flux	-0.670	-0.043
Chlorophyll *a*	0.143	0.703
Redox potential	0.241	-0.276

Table 5.6. Effect of spatial and temporal variability on PC1 and PC2 scores for the nutrient and standing stock measurements made in 2004.

Variable	Source	df	Mean Square	F	Sig.
PC1 scores	SITE	4	7.856	6.914	.000
	MONTH	3	3.081	2.712	.055
	SITE * MONTH	10	2.011	1.770	.091
	Error	51	1.136		
PC2 scores	SITE	4	.167	4.717	.003
	MONTH	3	.150	4.236	.009
	SITE * MONTH	10	.118	3.325	.002
	Error	51	.035		

5.3.3. Macroinfauna

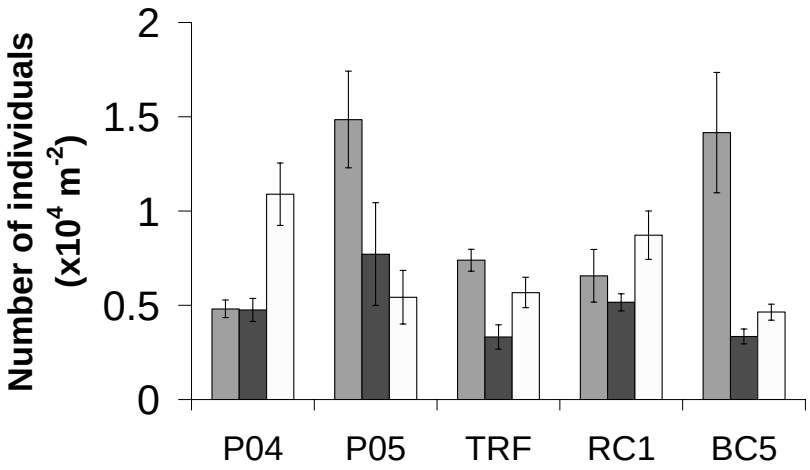
The number of individual macroinfauna at P04, TRF, RC1 and BC5 decreased from March to May, before increasing in July (Figure 5.10 a). The greatest number of individual macroinfauna was sampled at P05 and BC5 during March (1.49 x 10^4 m^{-2} and 1.42 x 10^4 m^{-2}, respectively). P04 and RC1 recorded their highest number of macroinfauna during July (1.09 and 0.87 x 10^4 m^{-2}, respectively). The lowest number of individuals was recorded in May for P04, TRF, RC1 and BC5 (range from 0.33 at TRF and BC5 to 0.52 x 10^4 m^{-2} at RC1). A significant interaction effect of site and month on the number of individuals was found (Table 5.7).

The biomass at P04, P05 and BC5 was the greatest during May (192, 338 and 237 g WW m^{-2}, respectively), which coincided with generally lower abundances of macroinfauna at each of these sites (Figures 5.10 a, b). The lowest biomass occurred in July for BC5 and RC1 (43 and 52 g WW m^{-2}, respectively). There was a significant main effect of site (Table 5.7) on the biomass, where RC1 was significantly lower than the other sites ($p < 0.02$). The biomass was also significantly different among months (Table 5.7), where May was significantly different to March ($p = 0.032$) and July ($p = 0.003$). Significant ($p < 0.01$) positive linear relationships were found between OUR and abundance ($r^2 = 0.11$, y = 0.0001x + 0.5125, n = 65) as well as biomass ($r^2 = 0.12$, y = 0.037x + 68.34, n = 65) (Figure 5.11 a, b).

Table 5.7. Effect of spatial and temporal variability on macroinfauna throughout 2004.

Variable	Source	df	Mean Square	F	Sig.
Number	SITE	4	.059	1.726	.155
	MONTH	2	.420	12.223	.000
	SITE * MONTH	8	.212	6.161	.000
	Error	66	.034		
Biomass	SITE	4	.655	8.002	.000
	MONTH	2	.519	6.337	.003
	SITE * MONTH	8	.152	1.853	.083
	Error	66	.082		

(a)



(b)

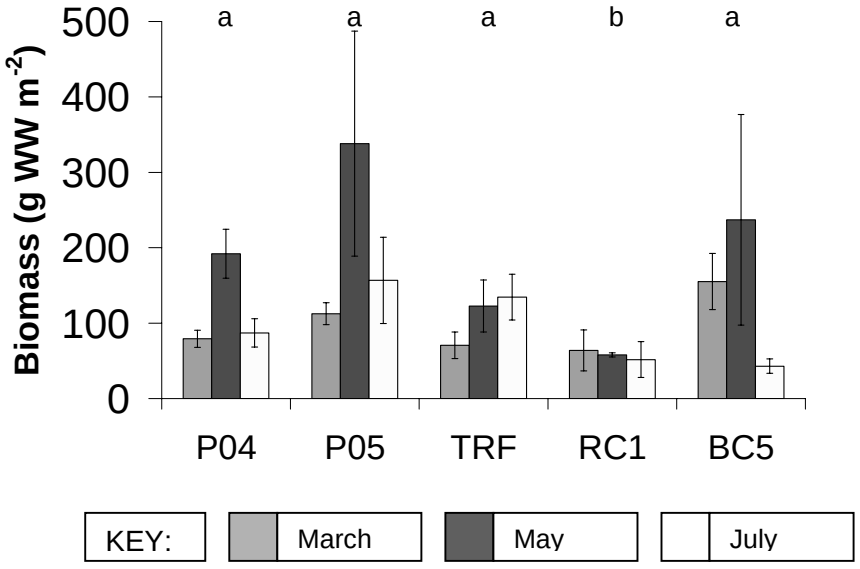
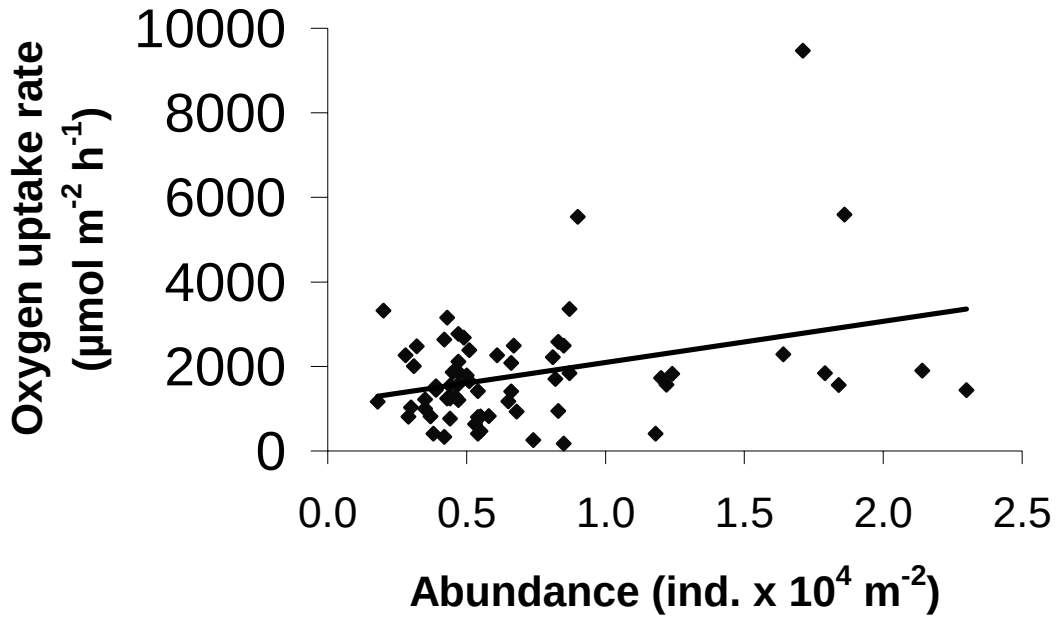


Figure 5.10. The number of (a) individuals and (b) wet biomass of the macroinfauna identified from the 2004 sampling season. Bars indicate means (n = 4 to 6 cores, ± SE). Letters denote significantly ($p < 0.05$) different sites.

(a)



(b)

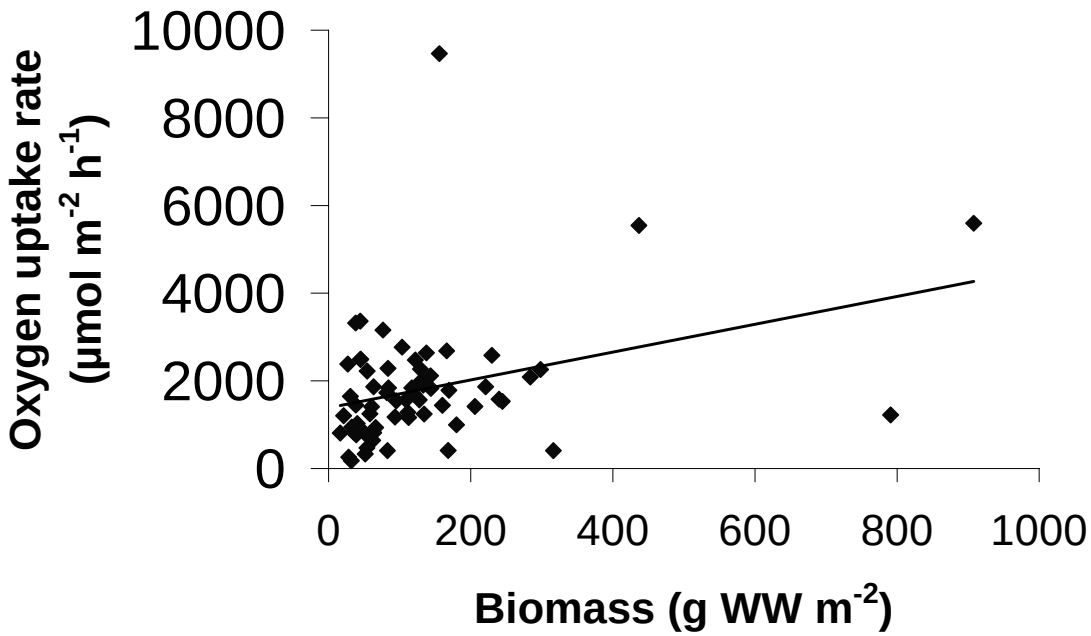


Figure 5.11. Regression of OUR on (a) individuals and (b) wet biomass of the macroinfauna identified from all months for the 2004 sampling season (*n* = 65 cores).

5.3.4. Porewater concentrations

P04 had the greatest increase in the concentration of ammonium in the porewater between March and July (156 to 617 μM NH_4^+- N) and these concentrations exceeded the range measured at RC1 and BC5 by a factor of 10 (Figure 5.12 a). It is noteworthy that the concentration of ammonium in the porewater at P04 halved (from 617 to 306 μM NH_4^+- N) between July and November. The concentration of ammonium in the porewater at P05 and TRF showed a general increase of about 30 to 40 % between March and November, whereas RC1 and BC5 were more variable between months. Porewater concentrations of ammonium varied significantly as a result of the interaction of site and month (Table 5.8).

The patterns in the concentrations of phosphate in the porewater were similar to those for ammonium. Again, the concentration of phosphate in the porewater at P04 (27 to 195 μM PO_4^{3-} - P) exceeded the control sites RC1 and BC5 (Figure 5.12 b), this time by a factor of 10 to 100. Both P04 and P05 experienced maximum concentrations of phosphate in the porewater during July, with a decrease in concentration of 4 to 6 times from July to November. The increase in the concentration of phosphate in the porewater between March and July was pronounced, with increases in concentrations at P04 of 3 times and 12 times increases at P05. Porewater concentrations of phosphate varied significantly as a result of the interaction of site and month (Table 5.8).

Table 5.8. Effect of spatial and temporal variability on the concentration of ammonium and phosphate in porewaters throughout 2004.

Variable	Source	df	Mean Square	F	Sig.
Ammonium	SITE	4	1.223	80.971	.000
	MONTH	3	.126	8.348	.001
	SITE * MONTH	12	.075	4.938	.001
	Error	20	.015		
Phosphate	SITE	4	3.714	96.403	.000
	MONTH	3	.212	5.493	.006
	SITE * MONTH	12	.160	4.161	.002
	Error	20	.039		

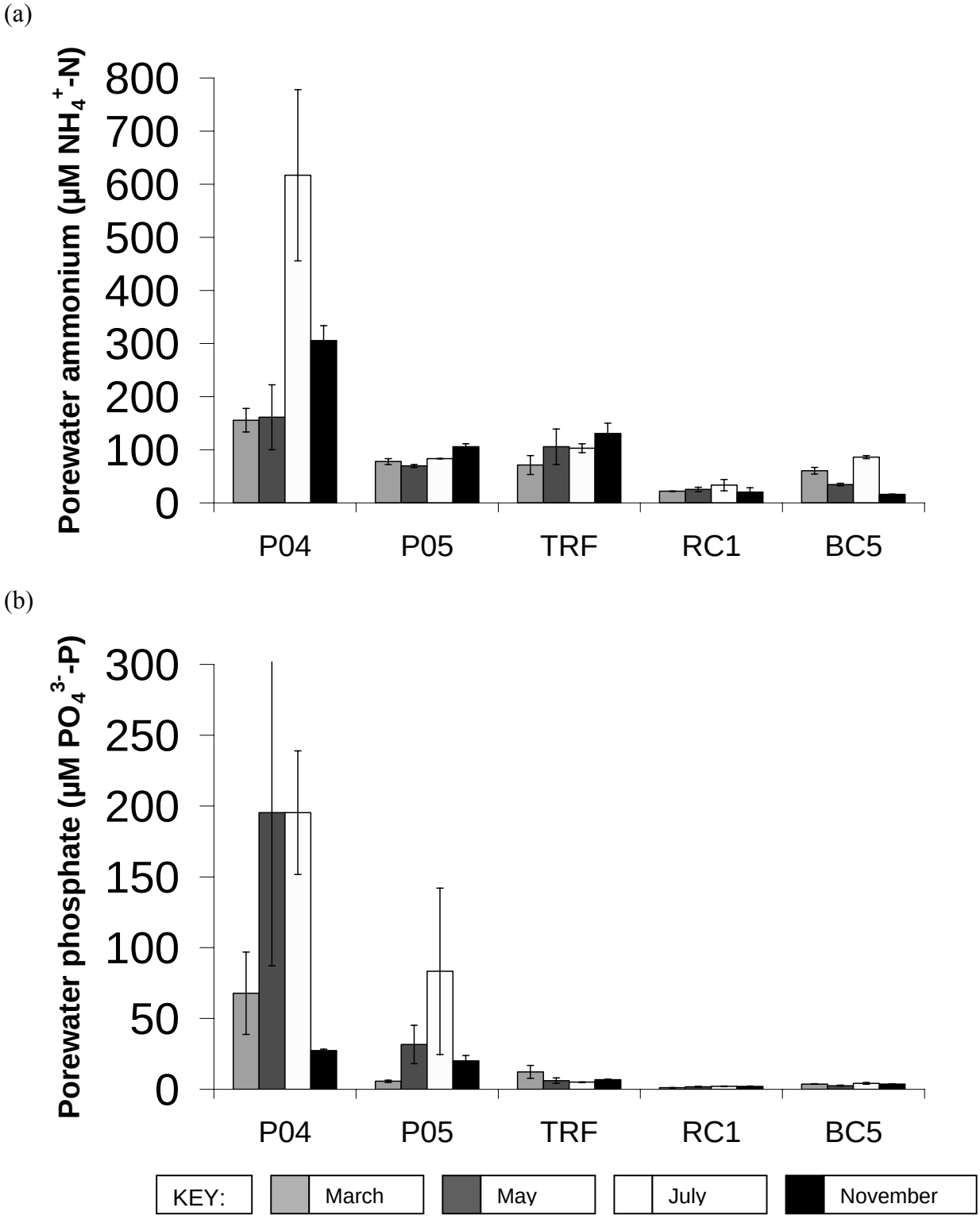


Figure 5.12. Concentrations of (a) ammonium and (b) phosphate in the porewater of the sediments. Bars indicate means (n = 2 cores, ± SE).

5.3.5. Grain size

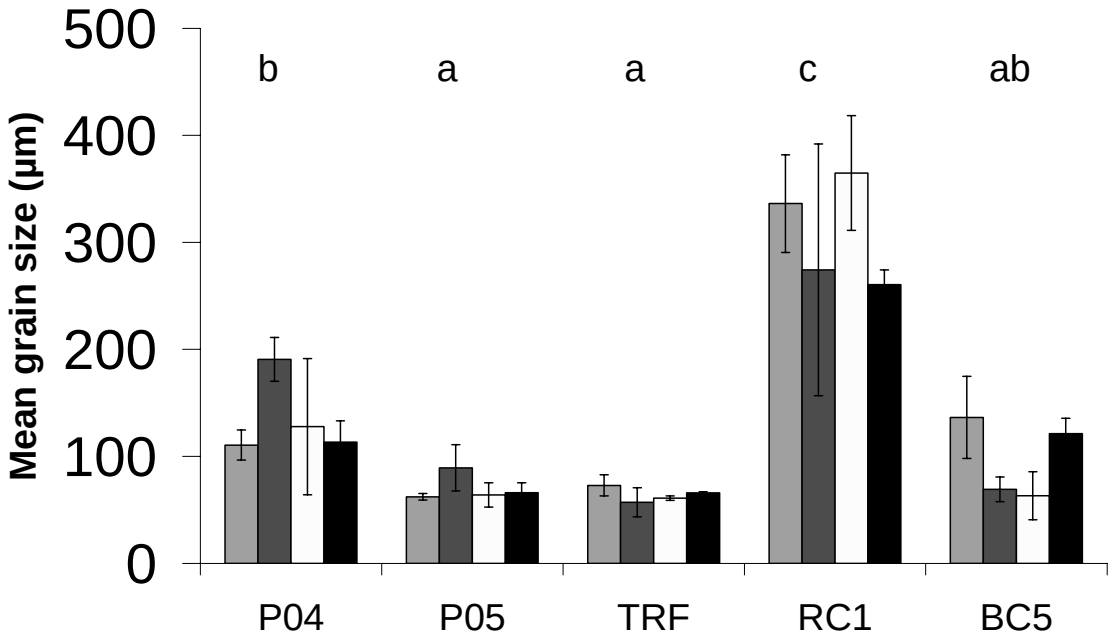
The mean grain size at RC1 was the largest (260 to 364 μm) (fine sand to medium sand), with P05 and TRF having the smallest mean grain size (57 to 89 μm) (very coarse silt to fine sand) (Figure 5.13 a). The mean grain size was significantly different between sites (Table 5.9). RC1 averaged 3 to 5 times the mean grain size found at the other 4 sites ($p < 0.001$). The mean particle size at P04 was less than double P05 ($p = 0.01$) and TRF ($p = 0.003$), whereas BC5 (63 to 136 μm) (very coarse silt to fine sand) was in between these two groups.

The largest change in the silt and clay fraction was found at P04 between the months of May and July, where an increase from 16 to 31 % was recorded; although measurements in March and November were intermediate (Figure 5.13 b). However, a similarly large change was observed at BC5, where an increase from 21 to 35 % was found between March and May. RC1 had 2 to 3 times less silt and clay (9 to 13 %) than the other sites and TRF had the highest percentage of silt and clay (32 to 40 %). The silt and clay content of the sediments varied throughout the year as an interaction of sites and month (Table 5.9).

Table 5.9. Effect of spatial and temporal variability on mean particle size and percentage of silt and clay in sediments throughout 2004.

Variable	Source	df	Mean Square	F	Sig.
Log_{10} mean particle size	SITE	4	.594	29.410	.000
	MONTH	3	.010	.476	.703
	SITE * MONTH	12	.026	1.297	.293
	Error	20	.020		
Percentage of silt and clay	SITE	4	759.141	50.191	.000
	MONTH	3	26.808	1.772	.185
	SITE * MONTH	12	45.363	2.999	.015
	Error	20	15.125		

(a)



(b)

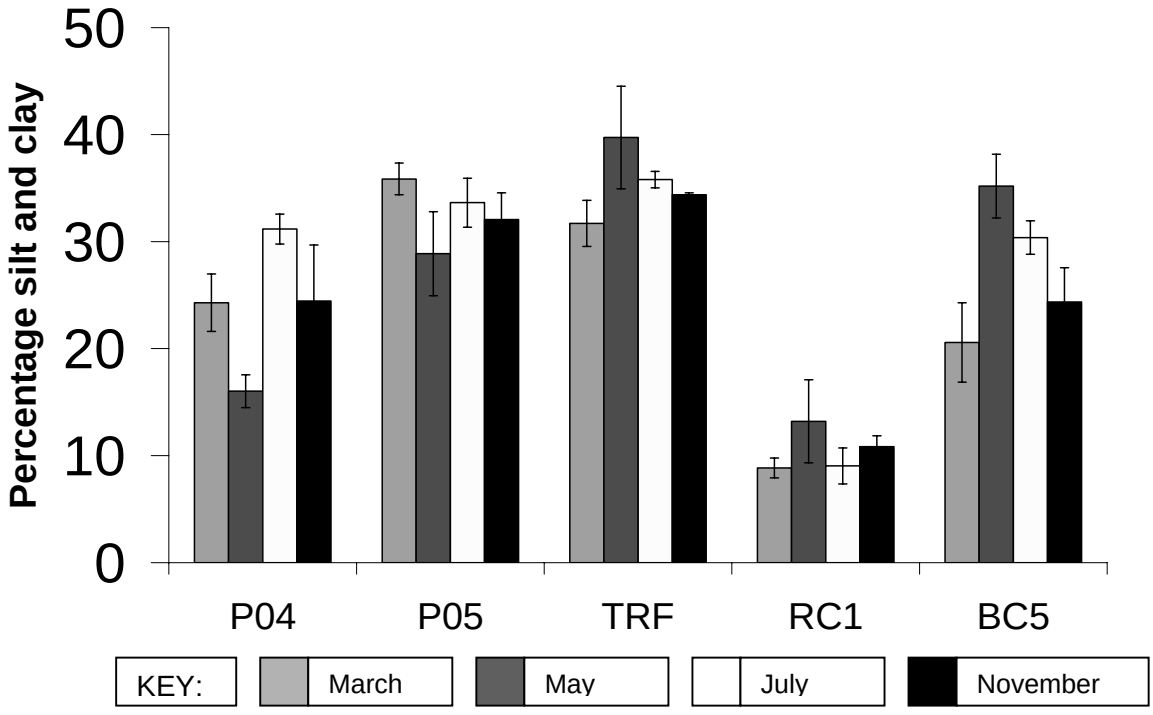


Figure 5.13. The (a) mean grain size and (b) percentage of silt and clay in sediments at the sites sampled throughout 2004. Bars indicate means (n = 2 cores, ± SE). Letters denote significantly ($p < 0.05$) different sites.

5.3.6. Organic carbon and total nitrogen

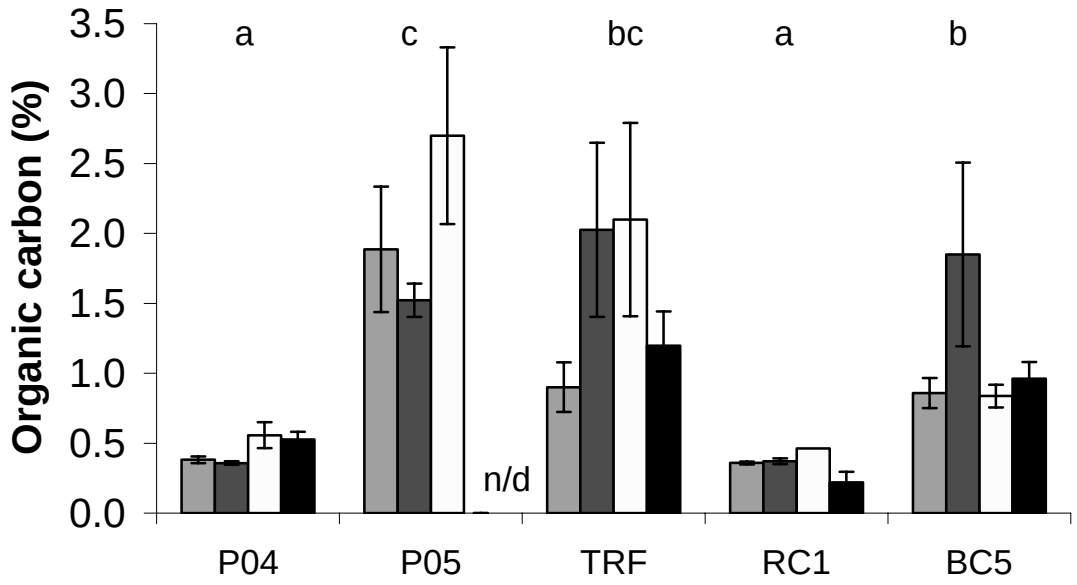
Organic carbon (OC) was lower at P04 and RC1 (range of 0.22 to 0.56 %) than at the other sites (0.84 to 2.7 %) (Figure 5.14 a). P05 showed the largest variation among months, from 1.52 % in May to 2.7 % in July. There was a significant effect of site and month on the OC content (Table 5.10). P05, TRF and BC5 had significantly higher ($p < 0.001$) OC contents than P04 and RC1, and P05 also had significantly higher OC contents than BC5 ($p = 0.002$). July was significantly different to March ($p = 0.027$) and November ($p = 0.038$).

Total nitrogen (TN) content was lowest at RC1 (0.03 to 0.08 %) and highest at P05 (0.1 to 0.17 %) (Figure 5.14 b). The TN content for sediments at P04, TRF and BC5 (0.06 to 0.13 %) were in between the values for RC1 and P05. For all sites, the pattern of change among months was the same, where TN content increased from March to July, before decreasing in November. There was a significant interaction of site and month for TN content (Table 5.10).

Table 5.10. Effect of spatial and temporal variability on sedimentary organic carbon and nitrogen content throughout 2004.

Variable	Source	df	Mean Square	F	Sig.
Log_{10} organic carbon	SITE	4	1.247	53.458	.000
	MONTH	3	.070	2.983	.040
	SITE * MONTH	11	.043	1.862	.067
	Error	52	.023		
Log_{10} nitrogen	SITE	4	.098	16.242	.000
	MONTH	3	.218	36.154	.000
	SITE * MONTH	12	.012	2.049	.036
	Error	56	.006		

(a)



(b)

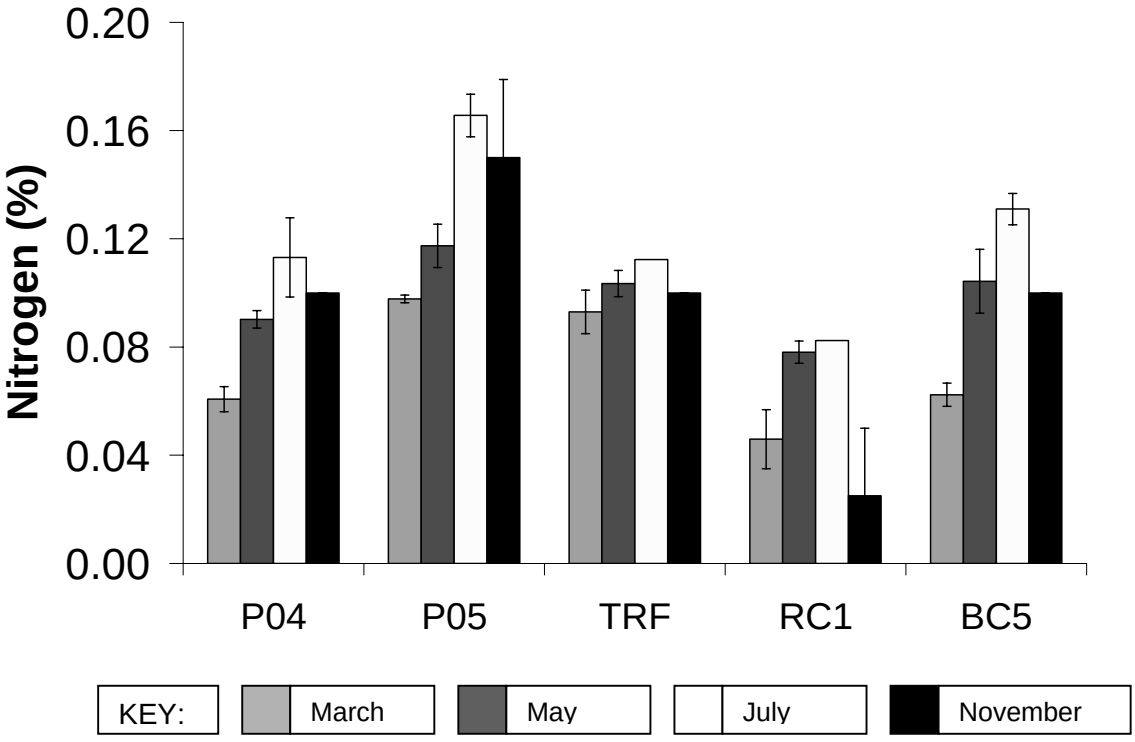


Figure 5.14. The (a) organic carbon and (b) nitrogen contents of the sediments throughout 2004. Bars indicate means (n = 2 cores, ± SE). Letters denote significantly ($p < 0.05$) different sites.

5.3.7. Sedimentation

The patterns in the sedimentation rates observed during the 4 months at the 5 sites in the bottom traps were virtually mirrored in the mid-water traps, although the sedimentation rates were approximately one third to a half as much in the latter (Figure 5.15). The sedimentation rates only exceeded 40 g DW m^{-2} d^{-1} for the bottom traps and 20 g DW m^{-2} d^{-1} for the mid-water traps at the pontoon sites; moreover, measurements of this magnitude were attained only while pontoons were stocked (Figure 5.15 a to f). P04 had the highest sedimentation rates in the bottom traps during March and July (above 79 and 50 g DW m^{-2} d^{-1}, respectively), while P05 recorded the greatest during May (above 31 g DW m^{-2} d^{-1}) (Figure 5.15 a, c and e). The sedimentation rates in the bottom traps at the commercial pontoon sites exceeded those at TRF, RC1 and BC5 by up to 10 fold for the same time periods (Figure 5.15 a, c and e). Generally, there was little difference among the sedimentation rates as a result of increasing distance from the edge of the pontoons.

5.3.8. Dissolved nutrients

For DOC, concentrations remained around 1 mg L^{-1} for all months, sites and depths except for July, when concentrations approached 1.5 mg L^{-1} (Figure 5.16 a, b). Total nitrogen concentrations remained around 0.1 mg N L^{-1} for the 5 sites during the 4 months with a slight decrease towards November (Figure 5.16 c, d), when a minimum concentration of 0.004 mg N L^{-1} was recorded for TRF at 3 m. A maximum total nitrogen concentration of 0.195 mg N L^{-1} was recorded at P05 at 10 m depth during May (Figure 5.16 d). Ammonium concentrations were mostly less than 0.01 mg N L^{-1} at all sites and depths, except at P04 where more than 10 times the amount of ammonium measured at 3 m (0.004 mg N L^{-1}) was found at 10 m (0.052 mg N L^{-1}) during May (Figure 5.16 e, f). Generally, the highest concentrations of ammonium were recorded during May. For nitrate plus nitrite, November had higher concentrations than any other month, ranging between 0.005 to 0.02 mg N L^{-1} (Figure 5.16 g, h). The control sites (RC1 and BC5) had the maximum concentration of nitrate plus nitrite (both 0.021 mg N L^{-1}) during November at 3 m. P04 only had measurable concentrations of nitrate plus nitrite during November. Again for P04, similarly to ammonium, phosphate at 3 m (0.003 mg P L^{-1}) was double that at 10 m (0.007 mg P L^{-1}) during May (Figure 5.16 i, j). P05 and RC1 generally had lower concentrations of phosphate than the other sites for any given month. March and July generally had lower concentrations of phosphate than May and November.

There was strong seasonal variation in overall dissolved nutrient concentrations indicated by clustering of months in the PCA (Figure 5.17). PC1 explained 34.5 % of the variation and PC2 explained 30.4 % (Table 5.11 a). From the loadings (Table 5.11 b), positive scores on PC1 were generated by increasing ammonium concentrations which were characteristic of May, while positive scores on PC2 were generated by increasing phosphate and decreasing DOC concentrations, associated with November. There was a significant interaction between site, month and depth from the PC1 scores (log_{10} transformed) (Table 5.12). From the PC1 loadings (Table 5.11 b) and inspection of the raw data (Figure 5.16), the significant difference in PC1 scores was driven by higher ammonium concentrations at P04, TRF and BC5 during May. Furthermore, the higher ammonium concentrations at 10 versus 3 m at P04 in May, and the lower concentrations in 10 versus 3 m at P04 in March contributed to variation at these depths among months. A significant interaction of site and month was found on analysis of PC2 scores (Table 5.12). From the PC2 loadings (Table 5.11 b) and inspection of the raw data (Figure 5.16), November was characterised by higher phosphate

concentrations at all sites compared with March, May and July, whereas March had lower phosphate concentrations than the other months. For DOC, higher concentrations were measured during July than the other months.

Table 5.11. (a) Eigenvalues and (b) loadings from the PCA of the dissolved nutrient concentrations for sites sampled in 2004. Bold type indicates variables with greatest loadings contributing the most amount of information to the ordination of points on the PCA plot.

(a)

PC	Eigenvalue	Percent variation
1	1.72	34.5
2	1.52	30.4
3	0.85	17
4	0.6	12
5	0.31	6.1

(b)

Variable	PC1	PC2
Dissolved organic carbon	-0.252	-0.531
Total nitrogen	0.478	-0.401
Ammonium	0.668	-0.072
Phosphate	0.426	0.568
Nitrate plus nitrite	-0.283	0.479

Table 5.12. Effects of spatial and temporal variability on dissolved nutrient concentrations.

Variable	Source	df	Mean Square	F	Sig.
PC1 scores	MONTH	3	.477	34.117	.000
	DEPTH	1	.031	2.185	.153
	SITE	4	.066	4.724	.006
	MONTH * DEPTH	3	.026	1.840	.168
	MONTH * SITE	12	.058	4.164	.002
	DEPTH * SITE	4	.008	.599	.667
	MONTH * DEPTH * SITE	11	.032	2.291	.045
	Error	23	.014		
PC2 scores	MONTH	3	19.643	56.450	.000
	DEPTH	1	.467	1.342	.258
	SITE	4	.492	1.413	.261
	MONTH * DEPTH	3	.355	1.021	.402
	MONTH * SITE	12	.903	2.595	.024
	DEPTH * SITE	4	.351	1.008	.424
	MONTH * DEPTH * SITE	11	.339	.975	.495
	Error	23	.348		

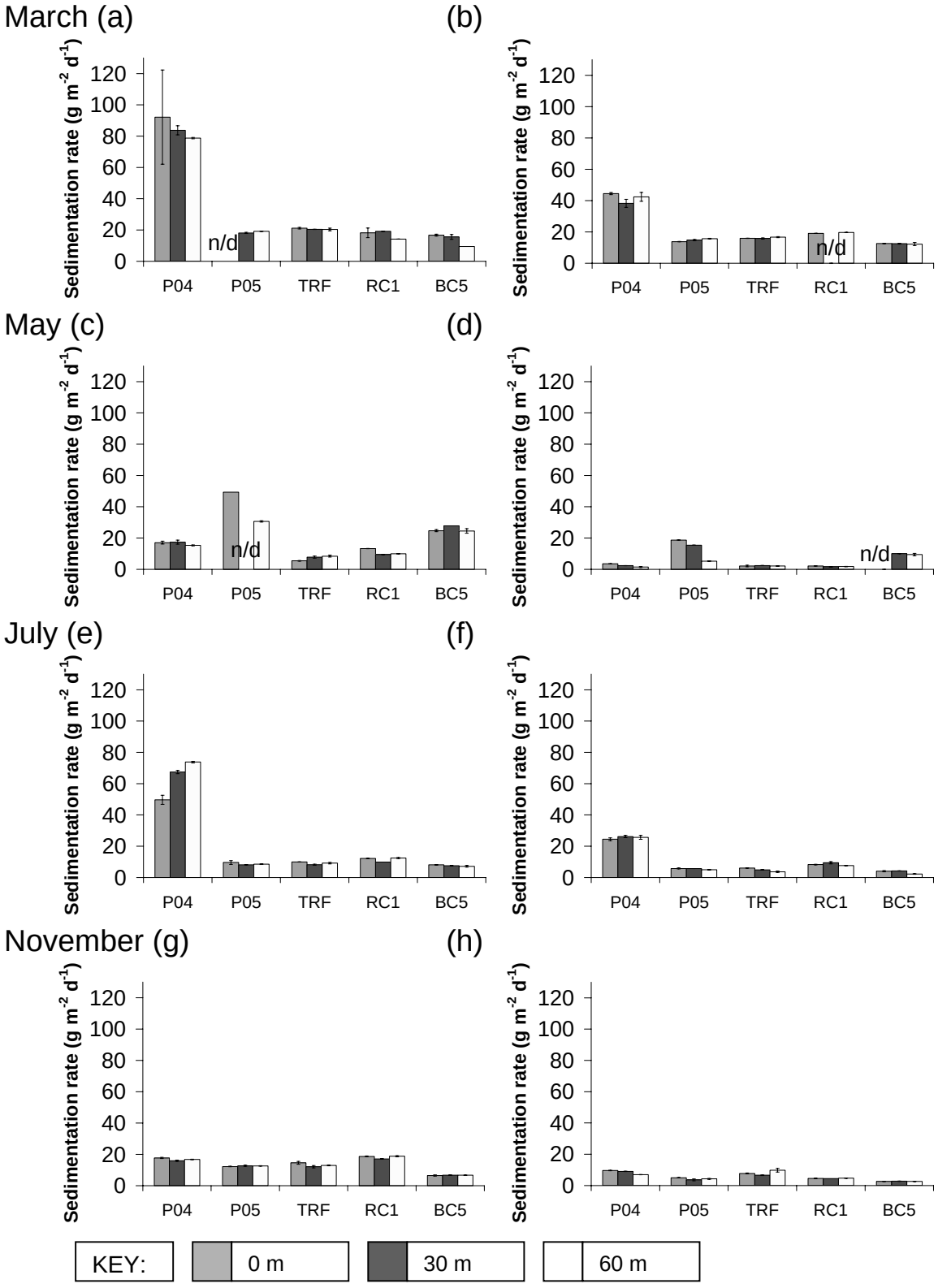


Figure 5.15. Sedimentation rates 1 m (bottom, left column) and 10 m (mid-water, right column) above the seafloor. Bars indicate means (n = 1 or 2 traps, ± SE) (n/d = no data).

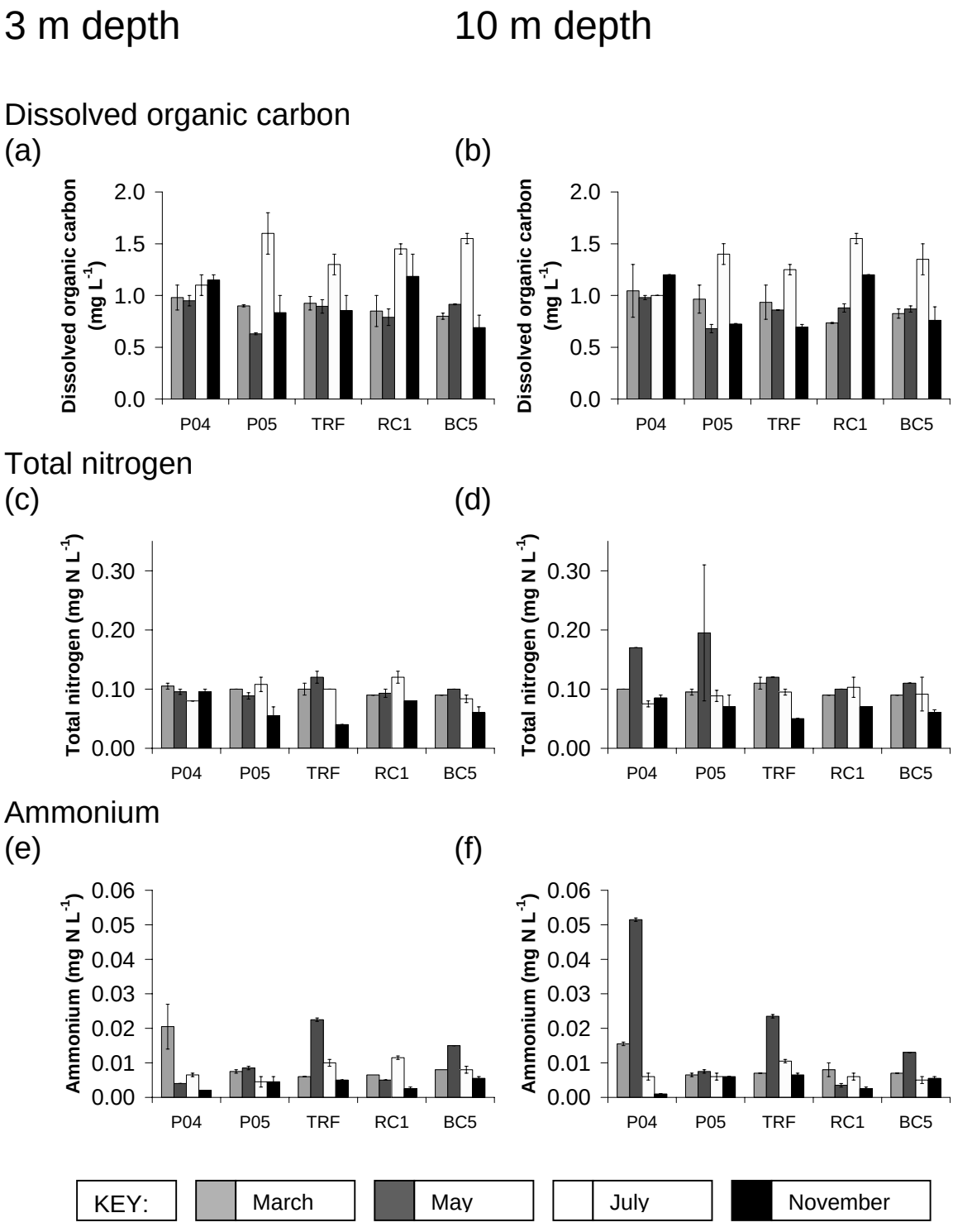


Figure 5.16. Dissolved nutrient samples taken at 3 and 10 m water depth. Bars indicate means ($n = 2$, ± SE).

Figure 5.16 continued

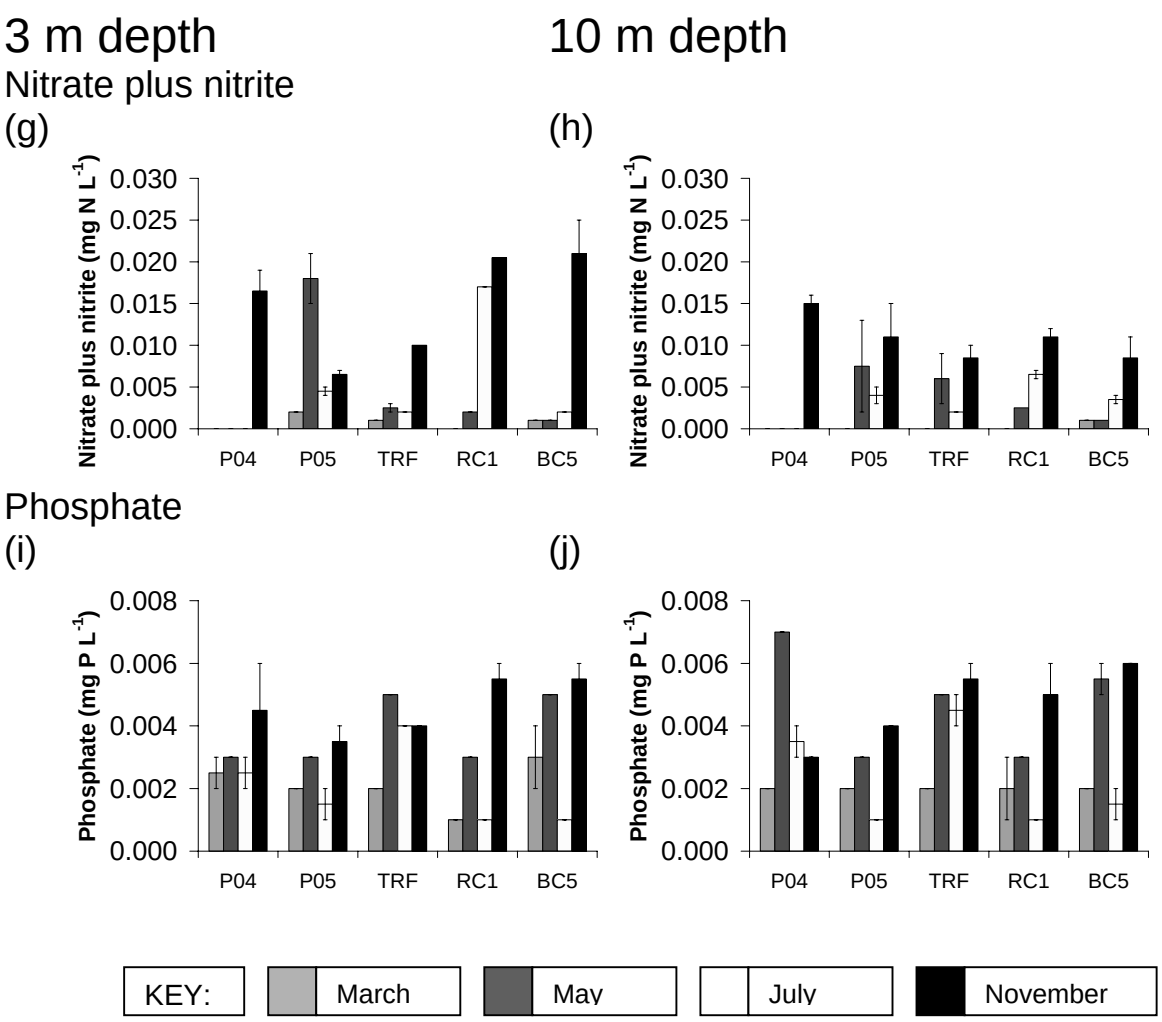


Figure 5.16 (continued). Dissolved nutrient samples taken at 3 and 10 m water depth. Bars indicate means (n = 2, ± SE).

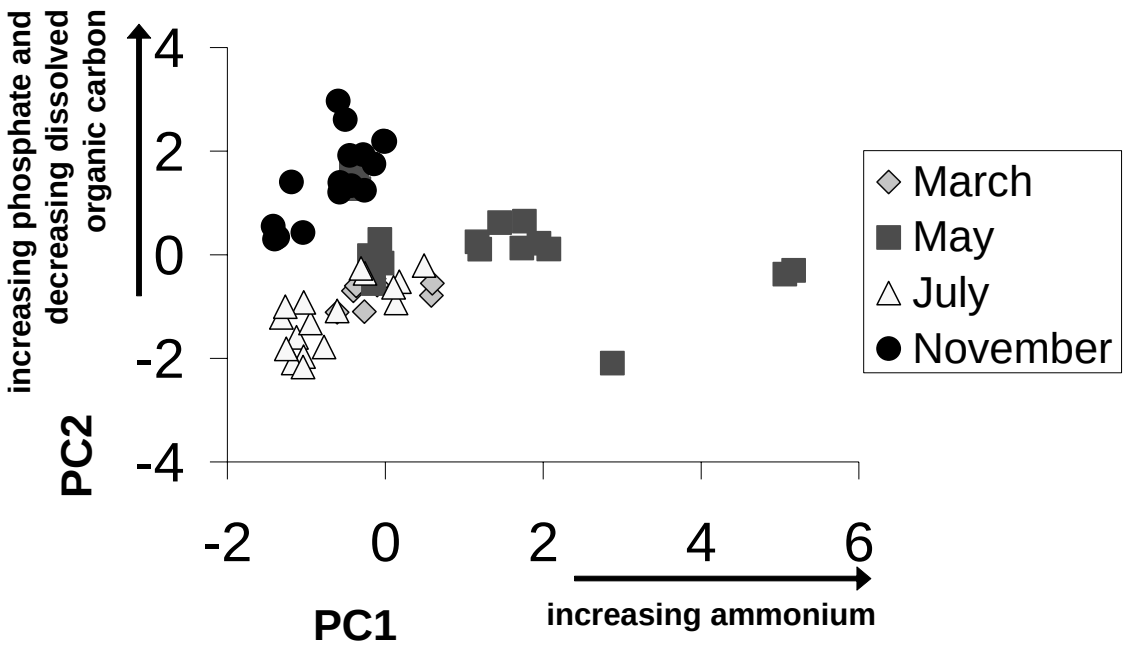


Figure 5.17. PCA plot of dissolved nutrient concentrations for the months sampled in 2004.

5.4. Discussion

5.4.1. OUR

OUR increased at the commercial pontoon sites throughout the farming season, before decreasing after harvest. In contrast the control sites and the research pontoon site showed only minor variations in OUR throughout the farming season. This pattern suggests that the benthic community responded to inputs from the commercial pontoons, and that there was some form of recovery after the pontoon sites were left vacant. Overall, maximal mean OUR at SBT pontoon sites during July (3,453 to 4,329 $\mu mol\ m^{-2}\ h^{-1}$) are comparable to measurements made by Hargrave et al. (1993) at active salmon pontoon sites in the Bay of Fundy (Canada) (maximal mean rate in September of 4,167 $\mu mol\ m^{-2}\ h^{-1}$). However, measurements by Findlay et al. (1995) off the coast of Maine (U.S.A.) and by Nickell et al. (2003) in Loch Creran (Scotland), both at pontoons of salmon farms, exceed OUR measurements made at SBT pontoon sites by 4 to 6 times (24,242 and 18,083 $\mu mol\ m^{-2}\ h^{-1}$, respectively). OUR measured at control sites for these aforementioned studies were comparable to that measured at the control sites within the SBT farming area. The maximal factor by which OUR at pontoon sites exceeded control sites for SBT was 3 to 7 times, during July; elsewhere this factor ranged from about 5 times (Hargrave et al., 1993) to about 40 times (Nickell et al., 2003).

Differences in stocking density of the fish, farming practices, current flows and other site specific differences such as water temperature must be considered when making comparisons of OUR among studies. For example, the environments in which SBT pontoons were situated and those described by Hargrave et al. (1993), Findlay et al (1995) and Nickell et al.

(2003) were dissimilar, with water depths ranging from 12 m at the sites studied by Hargrave et al. (1993) to 22 m for the SBT pontoons and salmon pontoons studied by Nickell et al. (2003). The sediment types ranged from silt-clay in the Bay of Fundy (Hargrave et al. 1993) to fine sands for the SBT pontoon sites. Also, ambient seawater temperatures varied from below 4 ºC in the Bay of Fundy (Hargrave et al. 1993) to 22 ºC at SBT pontoons off the coast of Port Lincoln. Feeding rates also varied, with 96 kg pontoon $^{-1}$ d^{-1} of semi-moist pellets (assuming fish weighed 1 kg each) reported by Findlay et al. (1995) and 80 kg pontoon $^{-1}$ d^{-1} of dry pellets reported by Hargrave et al. (1993), compared with 3 000+ kg pontoon $^{-1}$ d^{-1} of baitfish (75 % water content of 3 000 kg WW baitfish yields 750 kg DW) fed to SBT in pontoons off the coast of Port Lincoln. From the available information, there is no discernable trend in any one factor that may be highlighted from these studies as the main cause for stronger or weaker effects on OUR. Other information that may be useful to interpret differences in OUR associated with marine fish farming include: the frequency of sediment resuspension events, and current flows that may contribute to the export of accumulated wastes away from the vicinity of the pontoon sites (Findlay et al. 1995); whether accumulation of wastes occurs (Hall et al. 1990); the frequency of pontoon rotations or fallowing periods (Nickell et al. 2003); and the duration of site occupation. Some of this farm management information is supplied in the literature but generally not all the information is supplied for every study. The differences among farming situations contribute to the site specific variation of OUR observed between these aforementioned studies on salmon farms and SBT pontoons.

The range in mean OUR observed at the commercial pontoon sites throughout the farming season was greater than the natural range in mean OUR observed at the control sites within the farming area. In fact, OUR at the control sites decreased during the period March to July – consistent with a drop in water temperatures, whereas at commercial pontoon sites increases in OUR were observed during this period, consistent with stocking of the SBT pontoons (Table 5.2, Figure 5.2). Temperate studies have shown decreased OUR during winter associated with lower seawater temperatures when compared with summer periods (Mazouni et al., 1996; Hopkinson et al., 1999), which may explain the pattern of OUR observed at the control sites. However, increased OUR values at the commercial pontoon sites during the farming season were concurrent with farming activities.

Variation in mean OUR was observed among pontoon sites as a likely result of stocking density of SBT in pontoons. TRF was not a commercially stocked pontoon (Table 5.2) and the elevated rates of mean OUR observed at commercially stocked pontoons were not found at TRF. This observation suggests that the number of SBT farmed at this site, as well as the feeding regime, were not of sufficient quantity or duration to generate significant changes in mean OUR. Generally the 189 SBT at TRF received only 120 kg pontoon $^{-1}$ d^{-1} pellets (DW), whereas the 2,000 SBT in commercially stocked pontoons were fed 3,300 kg pontoon $^{-1}$ d^{-1} of baitfish (750 kg DW). Both of these amounts of feed were delivered over similar areas of the seafloor (40 m diameter pontoons = 1,257 m^2 seafloor area covered), meaning that more input of baitfish per square metre of seafloor was delivered to the SBT within the commercially stocked pontoon sites (not to mention faecal waste material generated by 10 times more SBT). Consequently, the stocking density of TRF and the associated farming activities were sufficiently small as to not produce a significant alteration in mean OUR compared with natural background variation in OUR. Thus, on a feed input basis only, the 0.1 kg m^{-2} of dried pellets delivered over the area of seafloor covered by the TRF pontoon did not significantly affect OUR but the 2.4 kg m^{-2} of baitfish (0.6 kg m^{-2} DW) delivered to the SBT in commercially stocked pontoons did. Furthermore, based on this interpretation of feed

input per area of seafloor covered by the pontoon holding the fish stock, comparable amounts of feed input reported by Findlay et al. (1995) (0.5 kg m^{-2} d^{-1} for 177 m^2 pontoons assuming 1 kg fish were stocked) and Hargrave et al. (1993) (0.8 kg m^{-2} d^{-1} for 100 m^2 pontoons) were associated with significantly greater OUR at pontoon sites versus control sites as well. Although site specific variation should also be considered to contribute to among site variation in OUR, this observation of significantly higher mean OUR associated with feed inputs exceeding 0.5 kg m^{-2} d^{-1} may be considered a common feature across these studies. Therefore, considering the amount of feed input per area of seafloor covered by the pontoon may be of practical use to management or regulatory bodies when evaluating the potential for a fish farm site to significantly alter mean OUR.

5.4.2. Variables measured with OUR at the sediment core level

OUR was significantly related to the nutrient fluxes and the standing stock measurements made at the core level of investigation, verifying previous conclusions that OUR represents the integrated measure of benthic community metabolism (Valdovinos & Figueroa, 2000). Ammonium fluxes from the sediments were the dominant form of dissolved nitrogen flux within the SBT farming area, particularly adjacent to active commercially stocked pontoons. Increased organic loading has been shown to increase the proportion of ammonium flux relative to the proportion of nitrification and denitrification (Blackburn & Blackburn, 1992a; Heggie et al., 1999). Grant et al. (1995) noted that the dominance of ammonium fluxes and a shift away from macroinfauna as an ammonium source was an important aspect of impacts from mussel aquaculture activities upon the local benthos. The maximal mean ammonium flux found adjacent to an active commercial SBT pontoon, P04 (mean of 9,962 μmol m^{-2} h^{-1}) exceeded, by a factor of 6, those reported elsewhere for finfish farming (Hall et al., 1992; Hargrave et al., 1993). However, the maximum mean ammonium flux found adjacent to the other active commercial SBT pontoon site was less than half of those values reported elsewhere. The variability in ammonium fluxes between sites adjacent to active commercial SBT pontoon sites within the SBT farming region is thus demonstrated, as well as the potential for activities at commercially stocked SBT pontoons to elevate ammonium fluxes. Moreover, ammonium fluxes adjacent to TRF were also elevated by the middle of the season in July, relative to the control sites, suggesting that ammonium flux was a more sensitive benthic indicator than OUR to detect the presence of active SBT pontoons, even at low stocking and feeding levels. Similarly, Stenton-Dozey et al. (2001) found that, although no conclusive inference could be made about impacts of a mussel aquaculture farm using OUR, ammonium fluxes were more sensitive.

Phosphate fluxes also showed a similar pattern to ammonium fluxes and OUR, where significant increases in phosphate fluxes were associated with stocking and feeding of SBT at active commercial pontoon sites and not at control sites. Phosphate fluxes measured adjacent to active commercial SBT pontoons were nearly double those recorded by Holby and Hall (1991) at a trout farm site in Gullmar Fjord on the Swedish coast. Unlike ammonium fluxes and comparable to OUR, phosphate fluxes at TRF were not similar to the other pontoon sites, but rather reflected the control sites in magnitude. The close association of phosphate fluxes and OUR has been reported previously; for example, Sundback et al. (1991) showed a significant correlation between phosphate fluxes and OUR (r^2 = 0.91). Mazouni et al. (1996) also observed maximal mean phosphate fluxes when OUR was highest in summer at an oyster farm in Thau Lagoon (France), related to reductive dissolution of ferric oxides and hydroxides.

The positive relationship between OUR and macroinfaunal abundance and biomass showed that increases in macroinfaunal abundance and biomass were associated with increased OUR. Stenton-Dozey et al. (2001) also reported a weak positive relationship (r^2 = 0.23) between OUR and macroinfauna biomass for subtidal marine sediments under active mussel aquaculture sites in South Africa. Linear relationships between OUR and macroinfaunal biomass have been used to estimate the contribution made by this part of the benthic community to total OUR measurements (Banse, 1982; Loo, 2001). Estimates of the contribution of macroinfauna to OUR range between 5 and 35 % (Smith et al., 1972; Smith et al., 1973; Loo, 2001) and these agree with the low correlation values for sediment within the SBT farming area (i.e. 11 to 12 % of variation in OUR explained by variation in abundance or biomass). The remainder of the OUR is a result of aerobic oxygen consumption by other organisms (i.e. meiofauna and microfauna) and oxidation of reduced compounds (Jorgensen, 1977; Sorensen et al., 1979; Sampou & Oviatt, 1991; Loo, 2001). Thus, the importance of these other oxygen consumers within the sediment matrix off the coast of Port Lincoln is demonstrated by a low contribution by macrofauna biomass and the relative contributions of these other factors to OUR may offer direction for further investigations. The macroinfaunal analysis showed that there was no evidence of azoic sediments occurring adjacent to active SBT pontoons as observed at some fish farming pontoon sites in the northern hemisphere (Lumb, 1989; Holmer & Kristensen, 1992; Yokoyama et al., 1997).

Positive redox potentials at the sediment surface measured throughout the farming season at all sites suggest that the very coarse silt to medium sand sediments within the farming area off the coast of Port Lincoln are well oxygenated. Interestingly, at P04 and P05, the surface redox potentials recorded in November after the farming season were not negative, despite readings of -100 to -200 below 2 cm depth. The negative redox potentials below 2 cm in November and not during the farming season (Table 5.2) may have been due to the large amount of shell debris that was found on the surface of the sediment in November. This 2 to 3 cm thick layer of debris from the cleaning of the pontoon's nets at the end of the season may have smothered the sediment surface, decreasing the availability of oxygen to the sediments below. However, because the surface layer was not compacted, some oxygen may have been available in the upper 2 cm, as evidenced by the positive surface redox potentials. Reports on redox potentials associated with finfish aquaculture concentrate on readings made at 4 to 7 cm depths (Brown et al., 1987; Weston, 1990; Hargrave et al., 1993). The upper 3 cm of the sediment may be more variable due to changes associated with the chemical and physical boundaries of the sediment –water interface (Pearson & Stanley, 1979). Therefore, if monitoring of redox potentials was carried out as a standard measurement of sediment condition within the SBT farming area, the results from this study suggest that sediment redox potential should be measured at the 4 cm depth interval within the profile, as measurements made only at the surface may misrepresent the redox potential at 4 cm.

The highest concentrations of chlorophyll *a* were consistently observed at sites with sediments that contained highest percentages of silt and clay (i.e. P05, TRF and BC5). In general, the biomass of benthic microalgae is greater in sheltered, muddy habitats than in exposed, sandy habitats (Mac Intyre et al., 1996). The lowest chlorophyll *a* concentrations were observed at RC1. The significantly larger mean grain size at RC1 compared to all other sites may reflect the current regime at this site, being of increased flow and consequently placing increased selective pressures on the benthic microalgal assemblage. Indeed sediment abrasion of benthic diatoms in high energy environments has been reported to decrease abundances (Miller et al., 1996). Site P04 showed a dramatic increase in sedimentary chlorophyll *a* between May and July. As vertical variability of nutrients including nitrate and

ammonium affect production of benthic microalgae (Mac Intyre et al., 1996) the observations at P04 may have been related to changes other than grain size within the sedimentary matrix – such as the increased nutrient fluxes, porewater concentrations and hence the availability of nutrients for benthic algae.

5.4.3. Sedimentary variation among sites

Significantly higher concentrations of phosphate and ammonium in the porewater at the commercial pontoon site P04 than the control sites, together with significantly higher phosphate concentrations at P05 during July, coincident with maximal mean rates of phosphate and ammonium fluxes, highlights the interchange across the sediment-water interface and the fact that the sediments are a source of nutrients into the pelagic system within the SBT farming area. The range in concentrations of ammonium within the porewater of the sediments within the SBT farming area (controls = 20 to 100 μM versus pontoons = 90 to 617 μM) are comparable to previous investigations of fish aquaculture sites. For example, Hargrave et al. (1997) used an ammonium electrode at salmon pontoon sites within the Bay of Fundy and found that ammonium concentrations in the porewater were greater than 200 μM at pontoon sites and less than 200 μM at control sites. Holmer and Kristensen (1992) reported relatively higher concentrations for rainbow trout farms in Kolding Fjord (Denmark), with porewater concentrations of ammonium attaining 1,200 μM at the end of the season under the pontoons *versus* 75 μM only 30 m away from pontoons. Given the high concentrations of ammonium in the porewater adjacent to active SBT pontoons, it would be of concern to the SBT farmers and managers if the porewaters were resuspended into the water column, potentially increasing the ambient concentrations of ammonium within the water column to toxic concentrations.

Patterns in sedimentary physical characteristics (i.e. grain size, silt and clay content) were not related to OUR. Similarly, Pamatmat and Banse (1969) found no relation of mean grain size or silt and clay fraction with OUR at various water depths (15 to 180 m) in Puget Sound Washington (U.S.A.) from *in situ* measurements. The data gathered here showed that these sediment characteristics were not subject to change as a consequence of SBT farming activities within a single farming season. It is not known if mean grain size or the percentage of silt and clay change significantly over longer time scales as a result of SBT farming activities. For example, Weston (1990) investigated salmon farming in Puget Sound, and found that the production of waste feed and faeces increased the percentage of silt and clay in the sediments adjacent to the salmon pontoons from 3 % at control sites to 8 % at pontoon sites. Given that Weston (1990) studied sediments with an average 97 % content of sand, a 3 to 8 % increase in the percentage of silt and clay in sediments may be noteworthy, although off the coast of Port Lincoln a 3 to 8 % percent change in silt and clay content for sediments with a 65 to 90 % sand content may be less significant. However, the links between grain size distributions and sediment characteristics have been shown to both affect and reflect other aspects of the environment from which they are sampled. For example, Argese et al. (1992) showed that on a mudflat in Venice Lagoon (Italy), slackness of water movement was exemplified by preferential deposition of finer particles and anoxic conditions of the sediment. Consequently, the higher percentages of silt and clay at P05, TRF and BC5, may reflect a depositional zone in the southern area and the significantly larger mean grain size at RC1 may reflect a well established erosional zone in the northern area (i.e. RC1 in north) (Figure 5.1).

Despite previous findings that marine finfish farming alters the OC and TN content of sediments adjacent to stocked pontoons (Hall et al., 1990; Weston, 1990), no such result was found at SBT pontoon sites. For example, Weston (1990) found that OC adjacent to stocked salmon pontoons in Puget Sound was 3 times that measured 45 m from the edge of the pontoon (1.26 versus 0.41 %, respectively) and TN contents were double (0.16 adjacent versus 0.07 % 45 m away). Since the OC accumulated in the sediment reflects the dynamic balance between supply and consumption rates, there need not be any correlation between the organic pool and actual metabolic rates (Jorgensen, 1977; Hargrave & Phillips, 1981). Furthermore, Jorgensen (1977) pointed out that, although fresh detritus may be continually supplied to the benthos, only the most resistant structures may persist for long periods. Therefore, given elevated OUR and nutrient fluxes associated with the sediments adjacent to commercial SBT pontoon sites but no significant accumulation of OC or TN within those sediments, I conclude that the sediments adjacent to SBT pontoons are able to turn over the material that arrives in the benthos.

5.4.4. Pelagic consequences of SBT farming

The highest sedimentation rates (above 40 g DW m^{-2} d^{-1}) were found only during the farming season and were only observed at active commercial SBT pontoon sites, thus demonstrating the increased input of particulate material associated with SBT farming. Increased rates of carbon sedimentation associated with finfish farming of other species have been previously reported in several studies (Brown et al., 1987; Hall et al., 1990; Holmer & Kristensen, 1992; Hargrave et al., 1993; Johnsen et al., 1993; Findlay et al., 1995; Tsutsumi, 1995; Findlay & Watling, 1997; Hargrave et al., 1997; Karakassis et al., 2000; Mazzola et al., 2000). Furthermore, the main process driving the various benthic impacts of finfish farming is the rate of sedimentation of particulate waste matter (Silvert & Sowles, 1996; Hansen et al., 2001). Therefore, increased sedimentation derived from commercial-scale SBT-farming activities is likely to be the cause of significantly higher OUR, nutrient fluxes and porewater concentrations at commercial pontoon sites relative to control sites. It is likely that the greatest contribution to increased sedimentation rates observed at commercial pontoon sites was derived from the feeding events and faecal material produced. It was noteworthy that only one commercial pontoon per month that was sampled showed sedimentation rates in excess of that measured at control sites. Sediment trap deployments were made over 3 days – placed on day one, left for a full second day and retrieved on the third day. Thus, given the variable nature of feeding events at SBT pontoon sites (e.g. either 0, 1 or 2 events per day, morning or afternoon feeding), it was quite possible that the traps were on site when no feeding event was taking place. Generally there was little difference between sedimentation rates measured at 0, 30 or 60 m from the edge of the active SBT pontoons. Video survey data has previously found waste food within 50 m of commercial SBT pontoons (Clarke et al., 2000). Therefore, evidence suggests that sedimentary material falls in an area around the SBT pontoons extending at least 60 m from the edge of the pontoon. This result has implications for the potential spatial distribution of measured impacts on OUR, nutrient fluxes and porewater concentrations associated with the commercial SBT pontoons. For example, future sampling designs that measure OUR, nutrient fluxes or porewater concentrations in sediments adjacent to SBT pontoons may investigate whether there is a gradient of change in any one of the variables with increasing distance from the edge of the pontoon, concurrent with patterns in sedimentation rates.

The sedimentation rates at control sites ranged from 7 to 28 g DW m^{-2} d^{-1}, whereas at

commercial pontoon sites the range was 8 to 92 g DW m^{-2} d^{-1}. Findlay et al. (1995) found significant differences between sedimentation rates measured at 1 m (68.4 g DW m^{-2} d^{-1}) from the edge of commercial salmon pontoons off the coast of Maine from those measured at 20 m (57.7 g DW m^{-2} d^{-1}). Findlay et al. (1995) measured sedimentation rates averaged over approximately monthly intervals, which may have led to more consistent daily averages than were found within the SBT farming area over several days. MacDougall & Black (1999) reported higher sedimentation rates than either of the previous studies, at a bream and sea bass farm facility in the eastern Mediterranean (Selonda Bay, Saronik Gulf) (100 to 200 tonnes capacity, fed 23 to 32 kg pontoon d^{-1}, 5 x 5 x 6 m pontoons of unstated fish density, over 20 to 40 m of water depth). Sedimentation rates measured at the pontoon sites ranged from 25 to 1,870 g DW m^{-2} d^{-1}, with the very high rates mainly caused by waste feed pellets (MacDougall & Black, 1999).

Sedimentation rates measured within the SBT farming area by the bottom traps were regularly double those measured by the mid-water traps. The observation of increasing sedimentation rates with depth has been made previously over a depth range of 20 to 110 m (Nickell et al., 2003). Conversely, Miquel et al. (1994) reported decreased sedimentation rates with depth, but this was observed over a depth range of 80 to 1,000 m. The increased transit time associated with greater depths of the water column permits more time for bacterial breakdown of the sedimentary material. Given this rationale, little degradation of the sedimentary material within the 20 m water column occurred within the SBT farming area. Other explanations for the observed doubling of the sedimentation rate at 1 m above the seafloor versus 10 m above the seafloor may include that resuspended sediments were deposited into the sediment traps, or that planktonic production within the lower 10 m of the water column was greater than the top 10 m. Future investigations may analyse the organic structure of the sedimentary material to better understand the relative contribution of the sources to the sedimentary flux within the SBT farming area.

Tracing nutrient dispersion from specific sources such as salmon or SBT farms may be difficult due to dilution within the receiving water body or rapid assimilation by microalgae (Lupatsch & Kissil, 1998), but nutrient measurements over time are useful and necessary to follow broad-scale changes in nutrient concentrations (Butler et al., 2001). Indeed the month that ambient seawater samples were taken within the SBT farming area was reflected in the PCA analysis of dissolved nutrient concentrations. This result highlighted the importance of seasonal changes in dissolved nutrient concentrations within the SBT farming area. A study by Kelly et al. (1996) found that the water chemistry (e.g. suspended solids, total phosphorus, biological oxygen demand) surrounding a freshwater Atlantic salmon farm in Scotland varied cyclically within a single day in response to daily fish feeding and activity periods. The finest temporal resolution investigated by Kelly et al. (1996) was hourly over a day. Temporal resolution of hours throughout the day may be worth addressing in future investigations to gain a better insight into the timing and magnitude of daily inputs of dissolved nutrients from commercial SBT pontoons.

5.5. Conclusions

Measurements of OUR, nutrient fluxes and porewater concentrations offered direct evidence for significant changes in the benthic environment associated with proximity to active SBT pontoons throughout a farming season. Sedimentation rates were up to 10 times higher at commercial SBT pontoon sites compared with control sites, indicating that pontoons were a

source of sedimentary material. Measurements of OUR and nutrient fluxes at the active commercial SBT pontoon sites showed changes that were significantly greater than those observed at the control sites, and hence we may conclude that the presence of the stocked pontoons increased OUR and nutrient fluxes. Interestingly, the increases observed as the farming season progressed were matched by decreases after the season had ended, suggesting that the effects of SBT farms on benthic metabolism were transitory rather than chronic and that the changes observed were reversible. Furthermore, sedimentary OC or TN contents of the sediments adjacent to stocked SBT pontoons were not significantly different from control sites, thus suggesting that material derived from SBT pontoons was not accumulating in the sediments. Therefore, the current management practices of the SBT pontoons investigated does not result in irreversible changes in benthic metabolism or sedimentary conditions.

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Chapter 6: Fish farms *versus* other anthropogenic activities: comparison of impacts on benthic metabolism

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Abstract

This chapter uses meta-analysis to quantitatively assess the impacts of different anthropogenic activities upon benthic metabolism, measured as oxygen uptake rates (OUR), in subtidal marine environments from temperate latitudes. The available data permit comparison of OUR measurements made at fish farms, mussel farms and sewage outfall sites. From comparison of OUR measured within the SBT farming area with other studies on finfish aquaculture, SBT farming has a relatively small effect. The evidence for this statement was drawn from comparison of difference in OUR between farming and control sites. Quantitative comparison of OUR measured at finfish and shellfish aquaculture and outfall stations showed that shellfish aquaculture had the least impact on OUR measurements. The larger effect on OUR produced by finfish aquaculture and outfall stations than shellfish aquaculture was linked to the level of organic input.

6.1. Introduction

This chapter uses a formal meta-analysis, supplementing data from this project with literature values, to quantitatively assess the impacts of different anthropogenic activities upon benthic metabolism, measured as oxygen uptake rates (OUR). Meta-analysis is a method to statistically combine the results from independent experiments to reach general conclusions (Gurevitch & Hedges, 2001). The available data permit comparison of OUR measurements made at fish farms, mussel farms and sewage outfall sites. The meta-analysis of these anthropogenic activities will place fish farming, and particularly SBT farming, within a wider context of OUR measurements based upon the level of impact observed. The spatial scale of Chapter 5 was the level of the farming region off the coast of Pt Lincoln, whereas Chapter 2 related to the larger Spencer Gulf region. The spatial domain of this chapter is global, but comparisons are limited to subtidal marine environments from temperate latitudes.

Sedimentation of organic material derived from aquaculture activities may artificially increase the sedimentation rate when compared with natural rates (as found in Chapter 5). Because OUR is a measure of benthic metabolism (Berg et al., 2003) and OUR may be affected by organic load (Hargrave, 1973; Kelly & Nixon, 1984; Sampou & Oviatt, 1991), the effects of fish farming, shellfish farming and sewage outfalls have often been investigated through measurement of OUR (Hatcher et al., 1994; Findlay et al., 1995; Mazouni et al., 1996; Findlay & Watling, 1997; Loo, 2001; Stenton-Dozey et al., 2001). Folke and Kautsky (1992) reported that intensive aquaculture systems generate, and are characterised by, the same attributes of a stressed ecosystem as caused by pollution from other anthropogenic activities.

General comparisons of OUR between studies are difficult and must be subject to cautious interpretation due to the differences in water depth (e.g. intertidal versus subtidal), salinity (e.g. freshwater, estuarine, marine), latitude (and hence seasonal temperature variation), sediment type (e.g. silt to sand), the method of measurement (*in situ*, lab or shipboard incubations; light or dark; oxygen profiles or core incubations, e.g. Aller et al., 1996), relative proximity to the source of pollution (either aquaculture farm or sewage outfall) and temperature-related effects (Hargrave, 1969; Andersen & Hargrave, 1984; Kemp et al., 1992; Mazouni et al., 1996). Furthermore, the extent to which an area is subject to an impact may range from a small localised area (e.g. under a finfish or shellfish farm) to a large regional area (e.g. an embayment surrounded by a city, industry or agriculture). Therefore, the objectives of any analysis comparing OUR from various independent studies must be qualified before any quantification of the treatment effects.

When comparisons of SBT farms are made with salmonid farms, there are several notable differences that should be considered. The farming of SBT in South Australia occurs in an open coastal environment, rather than the enclosed and generally more sheltered environments of salmonid farms (e.g. Hall et al., 1990, studied trout in Gullmar Fjord western Sweden and Nickell et al., 2003, studied salmon in Loch Creran, Scotland). The exposure of the site to wind, wave and tidal action determines current flow. Findlay & Watling (1997) suggest that a non-linear relationship exists between flow rate and diffusive oxygen supply to the benthos, where increases in flow produce an asymptote in diffusive oxygen supply. Therefore, given that exposure increases current flow, it would be expected that increased oxygen availability exists for SBT sites compared with sites within a less exposed fjord. Unfortunately though, quantification of current flows at a given site is not common in the literature.

In some cases, farm management data may be supplied in the literature and thus more meaningful comparisons may be made. For one of the 16 SBT farming companies operating in 2004 (site P04 from Chapter 5), the farm size was 0.63 km^2 with 10 stocked pontoons of 40 to 50 m diameter within that area; yielding about a 2 % coverage of pontoon area relative to the farm area. On average, the number of SBT within a 40 to 50 m diameter pontoon was 2,000 fish. Similarly, Hargrave et al. (1993) reported around 3,300 Atlantic salmon in each of the 36 pontoons of 11 m diameter within an area of 0.5 km^2; yielding about a 1 % coverage of pontoon area relative to farm area. In contrast, Hall et al. (1990) reported that for a trout farm, 15 square pontoons of 7 by 7 m (the areal equivalent of 8 m diameter) were contained within an area of 0.001 km^2 (25 x 40 m); yielding a 75 % coverage of pontoon area to farm area. The farming practice described by Hall et al. (1990) implies a much greater density of pontoons per unit area and consequently, more fish, feed and waste produced per unit area than for SBT studied in Chapter 5 or for Hargrave et al. (1993). However, differences between farms also exist in the total number of fish, the weight of individual fish, feeding practices and feed type.

Maximum feed delivery of 80 kg $pontoon^{-1}$ d^{-1} (i.e. 0.8 kg m^{-2} pontoon d^{-1} or 2,880 kg $farm^{-1}$ day^{-1}) as reported by Hargrave et al. (1993) was less than a tenth of the feed delivered to a commercial SBT pontoon (maximum 3,000 kg $pontoon^{-1}$ d^{-1} which equals 2.38 kg m^{-2} pontoon d^{-1} or 30,000 kg $farm^{-1}$ day^{-1}). It must be noted though, that in the former case dry pellets (water content not given) were fed to the salmon and in the latter baitfish were fed to SBT. This fact alters the comparison of the total amount of a given nutrient delivered to the fish in the pontoons. For baitfish based on a water content of 75 %, the figures stated for the SBT pontoon above may be reduced by three quarters to generate a per dry weight basis. With correction for the water content of baitfish, the comparison between SBT (750 kg $pontoon^{-1}$ d^{-1} which equals 0.6 kg m^{-2} pontoon d^{-1} or 7,500 kg $farm^{-1}$ day^{-1}) and Hargrave et al. (1993) becomes more equitable. Consequently, care must be taken to correctly interpret farm management information supplied for a given species, so that sensible and realistic comparisons are made.

Finally, consideration must be given to differences in the stocking density of the fish. Hall et al. (1990) reported that an average pontoon would hold 450 to 500 kg of trout (density of 3.4 to 3.7 kg m^{-3} pontoon) at the start of the season in April and by the end of season in December hold 1,700 to 2,300 kg (12.7 to 17.2 kg m^{-3} pontoon). In contrast, an average SBT pontoon would hold 30,000 to 40,000 kg (1.8 to 2.4 kg m^{-3} pontoon) at the start of the season in January and by the end of the season in August the mass of fish approaches 60,000 to 80,000 kg (3.6 to 4.8 kg m^{-3} pontoon). Consequently, the density (fish body weight per cubic metre of the pontoon volume) was much less for SBT by the end of the season when compared with the trout farm reported by Hall et al. (1990). Overall, there are a number of similarities and differences between the farming practices of various species of fish that must be considered in detail to understand observations made on OUR. Unfortunately, not all studies that investigate fish farming or other aquaculture activities and OUR report complete inventories of farm management data. To some extent, the lack of specificity of farm management information precludes accurate comparisons of the farming situations. General characteristics of the environment are more widely stated.

One of the most important decisions for this meta-analysis was the selection of data to compare. Englund et al. (1999) pointed out that, of the many decisions which may lead to conflicting conclusions among contemporaneous meta-analyses, the nature of the literature search and so the set of studies included in the meta-analysis is one obvious factor. To gain

insights into the general applicability of an impact or ecological process, some of the detail from individual studies may be overlooked in order to generalise across studies (Gurevitch & Hedges, 2001). That is, not all the data from all studies within an area of research may be suitably included in their entirety to make a general conclusion about the main hypotheses of the meta-analysis. When all the data from the available literature cannot be included in a meta-analysis, the criteria for inclusion should be clearly identified and be scientifically defensible (Gurevitch & Hedges, 2001).

The results from independent experiments may be used to place the present study into the wider context of the available literature. A narrative method of comparison was used in the discussions of the preceding chapters, whereas a quantitative method is adopted here. Meta-analysis has been used to investigate a variety of ecological questions, including the interactions between competition and predation (Gurevitch et al., 2000) and the effects of nutrient enrichment upon marine phytoplankton (Downing et al., 1999). The specific aim of this chapter was to investigate the impact of anthropogenic activities (fish farming, shellfish farming and sewage outfall) upon benthic metabolism, measured as OUR. The impact on OUR of these activities was compared both within similar categories of studies (fish farming or shellfish farming or sewage outfall) and among categories to determine the nature and magnitude of the variation.

6.2. Methods

OUR data from 13 studies that investigated potential anthropogenic impacts were used for the meta-analysis (Tables 6.1 and 6.2). Selection of the studies included for the meta-analysis was based on a number of criteria. Studies that focussed upon the impact on OUR from anthropogenic activities were sought. The studies had to assess an impact and control site (at least one of each) in the same experiment. This requirement was crucial to calculate the effect size, which is the difference between the means of the experimental and control group divided by their pooled standard deviation (Gurevitch & Hedges, 2001). Consequently, studies were required to have published data on the means of the experimental and control groups, the standard deviations about these means and the number of individual measurements (replicates) in each group (Gurevitch & Hedges, 2001). Measurement of OUR adjacent to the potential source of the impact was required, because distance from an impacting anthropogenic activity has been shown to affect OUR measurements (Loo, 2001). It was also required that the situation investigated was subtidal, marine and in a temperate latitude. Given these criteria, 13 studies from three categories of anthropogenic activity were selected. The 13 studies included seven on fish farming, three on shellfish farming and three on sewage outfalls. From Chapter 5, two pontoon sites at two different farm sites were studied and thus each pontoon was included as a separate study on SBT farming for the purpose of the meta-analysis. Other studies on fish farms that replicated pontoons within the study (Hargrave et al., 1993; Findlay & Watling, 1997; Hargrave et al., 1997) and did not differentiate between OUR measurements made at each pontoon site were not separated on a per pontoon basis. After the studies were selected, the data from within each study was chosen.

The selection of data from each study was done to minimise the number of methodological differences between the experimental approaches. Generally, the experimental designs of the

Table 6.1. Sources of data for the meta-analysis and environmental characteristics of the studies discussed in the text.

Category and study code	Reference	Location	Latitude	Depth (m)	Water temp. (ºC)	Sediment type	Site description
Fish farming (F1)	Hargrave et al., 1993	Bliss Harbour, Canada	N 45º 2.25'	12-14	13	Silt-clay	Embayment
(F2)	Hargrave et al., 1997	Bliss Harbour, Canada	N 45º 2'	7-20	13*	Silty mud	Embayment
(F3)	Findlay et al., 1995	Maine coast, U.S.A.	N 44º 22.8'	16	< 15	Muddy sand	Embayment
(F4)	Findlay & Watling, 1997	Maine coast, U.S.A	N 44º 53.88' and N 44 º 22.8'	11-14	15	Muddy sand to poorly sorted gravel	Open coast and embayment
(F5)	Nickell et al., 2003	Loch Creran, Scotland	N 56º 31.45'	15-22	n/d	Soft and muddy	Embayment
(F6)	This study (May)	P04 versus RC1, Spencer Gulf, S.A.	S 34º 38.637'	22	17	Very fine to fine sand	Open coast
(F7)	This study (May)	P05 and BC5, Spencer Gulf, S.A.	S 34º 41.812'	20	17	Very fine to fine sand	Open coast
Shellfish farming (S8)	Hatcher et al., 1994	Upper South Cove, Canada	N 44º 21'	7	20	Mud	Embayment
(S9)	Stenton-Dozey et al., 2001	Saldanha Bay, South Africa	S 33º 0.14'	12-15	10-18	Fine to medium sand	Embayment
(S10)	Mazouni et al., 1996	Thau, France	N 43º 24.6'	5	25	n/d	Embayment
Sewage outfall (O11)	Giblin et al., 1997	Boston harbour, U.S.A.	N 44º 22	7-12	18	50-55% silt-clay	Embayment
(O12)	Nicholson & Longmore, 1999	Port Phillip Bay, Victoria, Australia	S 38º 7.04'	9-24	n/d	soft	Embayment
(O13)	Loo, 2001	Gulf St Vincent, S.A., Australia	S 35º 11.94'	7	20	Fine sand	Open coast

*temperature from Hargrave et al. (1993) at same farming area and for same months sampled in Hargrave et al. (1997).
n/d = no data.

Table 6.2. Experimental characteristics of the studies included for the meta-analysis.

Category and study code	Reference	Species	Incubation method	Distance of control site to impact site (m)	Sample size for impact (I) or control (C)	Activity
Fish farming (F1)	Hargrave et al., 1993	Salmon	lab	50	I = 3; C = 2	-120,000 fish in 36 pontoons; 11 m diameter; fed up to 80 kg pontoon^{-1} d^{-1} dry pellets
(F2)	Hargrave et al., 1997	Salmon	lab	>50	I = 23; C = 23	-Farm sites occupied for at least 3 years
(F3)	Findlay et al. 1995	Salmon	lab	100	I = 10; C = 10	-8,000 fish pontoon^{-1}; 15 m diameter, 7 m depth; 0.012 kg d^{-1} semi moist pellets (96 kg pontoon $^{-1}$ d^{-1} at 1 kg fish^{-1}); 3 years production
(F4)	Findlay & Watling, 1997	Salmon	lab	100	I = 6; C = 5	-7,200 to 15,000 kg pontoon^{-1} to 250,000 kg fish biomass site^{-1}; production from less than 3 to 10 years
(F5)	Nickell et al., 2003	Salmon	*in situ*	1,000	I = 2; C = 2	-Consented to farm 1,500 tonnes; 16 x 22 m diameter pontoons, 15 m depth; on site over a decade with 2 year rotation of occupied to fallow
(F6)	This study (May)	SBT	shipboard	1,000	I = 4 ;C = 6	-34,000 kg pontoon^{-1}; 19 m diameter, 10 m depth; 4 tonnes baitfish per day; 1 year in production
(F7)	This study (May)	SBT	shipboard	1,000	I = 5; C = 4	-46,000 kg pontoon^{-1}; 20 m diameter, 10 m depth; 4 tonnes baitfish per day; 1 year in production
Shellfish farming (S8)	Hatcher et al., 1994	Mussels	lab	30	I = 4; C = 4	-400 mussels m^{-2}; 3 m depth of long lines; since 1970's
(S9)	Stenton-Dozey et al., 2001	Mussels	*in situ*	1,000	I = 4; C = 4	-6 m depth of long lines; 320 ropes per raft, 27 tonnes per raft, 74 rafts; 10 years of production
(S10)	Mazouni et al., 1996	Oysters	*in situ*	< 500 **	I = 5; C = 5	-n/d
Sewage outfall (O11)	Giblin et al., 1997	n/a	lab	3,500 **	I = 3; C = 3	-n/d
(O12)	Nicholson & Longmore, 1999	n/a	*in situ*	24,500 **	I = 3; C = 3	-520 ML d^{-1}; annual load of 2,600 t dissolved inorganic N, 1,000 t organic N, 750 t dissolved inorganic P, 180 t dissolved organic P, 6000 t C
(O13)	Loo, 2001	n/a	*in situ*	7,000 **	I = 6; C = 4	-27 ML d^{-1}; secondary treatment

** estimated from map in study or from Mapsource software.
n/d = no data.

13 studies included temporal measurements of OUR over various seasons (Hargrave et al., 1993; Hatcher et al., 1994; Mazouni et al., 1996; Giblin et al., 1997; Nicholson & Longmore, 1999; Stenton-Dozey et al., 2001). This was not surprising given the number of studies at temperate latitudes that have found considerable seasonal variation in OUR and sedimentary nutrient profiles (Gilbert, 1982; Jorgensen & Sorensen, 1985; Laima, 1992; Hargrave et al., 1993). Therefore, the data were chosen to focus upon the effect of the anthropogenic activity on OUR, rather than reporting on environmental consequences on OUR such as seasonal changes (e.g. Mazouni et al., 1996). To establish whether there was a significant relationship between either temperature or depth and OUR, Pearson's correlation analyses were conducted on the data selected.

OUR data from temperate latitudes of the northern and southern hemispheres were chosen from the summer/autumn period. The choice of this season relates to the absolute water temperature at the time OUR measurements were made. Given that some of the northern hemisphere sites experienced ice and water temperatures below 5 ºC during their winter (Hatcher et al., 1994) but southern hemisphere sites did not, a compromise had to be made. The rationale was that using OUR data across an entire year may introduce seasonality into the variation among sites. Moreover, OUR data was not specifically chosen to reflect the maximum effect of an anthropogenic activity observed but rather temporal selection of data was chosen to minimise environmental differences among categories of anthropogenic activities and within studies for each category. It should be noted here, again, that the main focus of this meta-analysis on OUR was the impact of 3 different anthropogenic activities, rather than natural seasonality. Thus, the range of ambient temperatures was small for the selected datasets (Table 6.1).

Generally, farm management data was not supplied in a consistent format across studies and categories (Table 6.2). For example, Hargrave et al. (1993) reported the number of fish, the number of pontoons, pontoon dimensions and the area occupied by the farm, but not fish weight. Much less information was provided by Nickell et al. (2003), with only the pontoon dimensions and the maximum allowed biomass of fish stated. Similarly, Findlay & Watling (1997) supplied estimates of fish biomass data but no measurements of pontoons or the number of pontoons within a given area. The shellfish farm management data were also incomplete or non existent (e.g. Mazouni et al., 1996). Consequently, the amount of data that may be used to describe the farming situations of the studies was limited.

Some of the specific choices made are addressed in the following. It was common that more than one control site was studied with a single impact site and generally control sites were closer for fish farm studies than for outfall investigations. From Nickell et al. (2003) the control site at station 3 was a similar distance from the farm site as that used for our SBT investigations (i.e. 1,000 m). From Findlay et al. (1995) there was no supplied information on the water temperatures at the times of sampling of the single pontoon and it was assumed there was not much variation within the 3 summer months sampled, so all months were included. Similarly, for Hargrave et al. (1997) OUR data from all 3 summer months were used. Hargrave et al. (1997) sampled the most number of pontoons (11), but OUR measurements were not separated on a pontoon basis on the scatter plot, so all pontoons were included as one sample from an impacted site. For Nicholson & Longmore (1999), sites 6 (at the outfall site of the Western Treatment Plant, Port Phillip Bay, Australia) and 37 (the control site near the centre of Port Phillip Bay) were used, despite different water depths. The other site studied by Nicholson & Longmore (1999) was at the mouth of the Yarra River. The Yarra River site was excluded because it neither constituted an aquaculture site nor an outfall

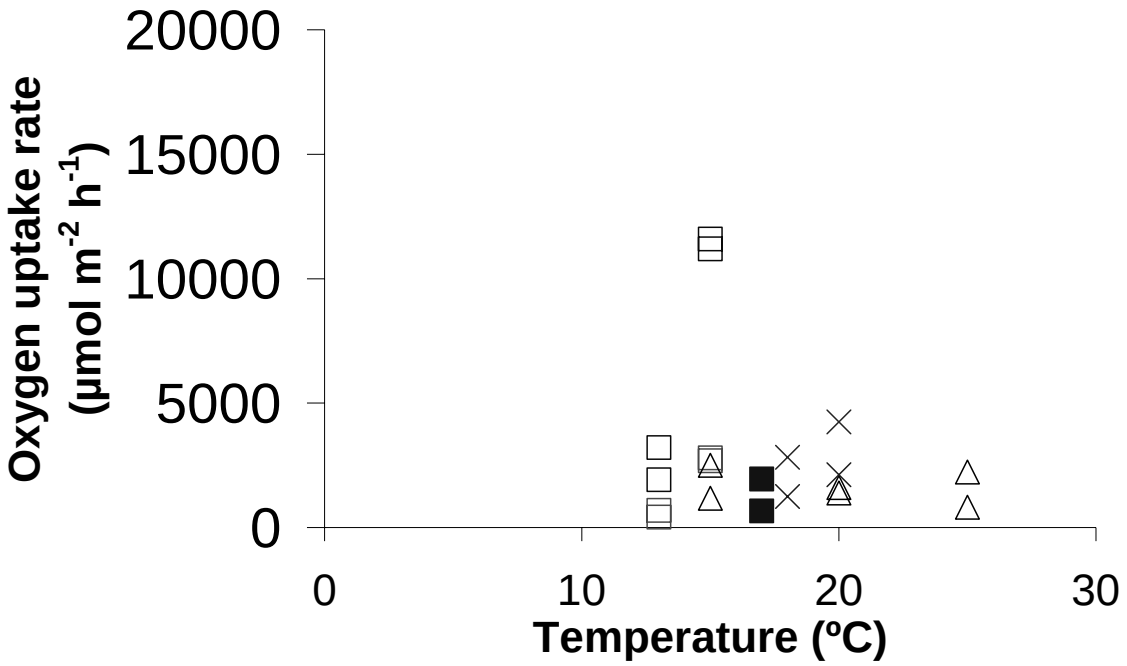
station. The samples chosen were from the dark incubations measured on February 1995. From Giblin et al. (1997), sites BH02 (at a previous outfall site stopped 1 year before sampling off Long Island, Massachusetts, U.S.A.) and BH03 (the control site nearest to the outfall site) were used. The other 4 control sites were dissimilar in terms of sediment type, water depth and organic content of the sediment (Giblin et al., 1997). The samples chosen were from August 1992, which corresponded to the 1st sampling event at which both sites were sampled after a hurricane event and nearest to the closing of the outfall station. For Stenton-Dozey et al. (2001), the culture site and B10 (a monitoring point of the industry for the preceding 10 years) were used and the samples from summer were chosen to align with the other studies included.

The meta-analysis followed the methods of Gurevitch & Hedges (2001). The type of meta-analysis used in the present study was a mixed model as opposed to a fixed model. The mixed model does not assume that all studies within a category share a single true effect size as for the fixed model, but rather that there is also some random variation among studies within a category in addition to sampling error (Gurevitch & Hedges, 2001). This approach was used in acknowledgement of potential differences within the environments which were studied (e.g. sheltered *versus* exposed waters) and differences in the measurement of OUR (e.g. *in situ* chambers *versus* shipboard incubations). Meta-analyses quantitatively synthesise a collection of studies (Gurevitch & Hedges, 1999), based upon the outcome of each experiment expressed as an index of the effect size (Gurevitch & Hedges, 2001). That is, the effect size quantitatively expresses the magnitude of the response to an experimental manipulation compared with a control group (Osenberg et al., 1999). For each of the 13 studies used in the present meta-analysis, replicate measurements of OUR from the impacted site and the control site were used to calculate the effect size. That is, the means of the OUR measurements, number of replicate measurements and standard deviations from each study were used to calculate the effect size for each category. The outcomes of meta-analyses address how large an effect is across studies, whether the results are consistent among studies and, if the effect is not equal, whether there are differences in the magnitude of the effect among categories of studies (Gurevitch & Hedges, 1999). Cohen (1969) provided a conventional interpretation of the magnitude of effect sizes (i.e. a dimensionless ratio), where 0.2 is a small effect, 0.5 is medium, 0.8 large and, from Gurevitch & Hedges (2001), greater than 1 is very large.

6.3. Results

Fish farms were generally situated in colder waters and greater depths than shellfish farms or outfall stations (Figure 6.1 a, b). The pontoons from the SBT farming area were in the middle of the temperature range and towards the deeper end of the water depths for the sites investigated (Figure 6.1 a, b). Shellfish farms were situated in more shallow and warmer waters than the other activities (Figure 6.1 a, b). Temperature and depth were not significantly correlated (Pearson's, $p > 0.05$, $r = -0.115$ and -0.036, respectively) with OUR when control sites were considered from studies across the three categories. There were two fewer sites for the scatter plot of OUR and temperature than for OUR and depth (Figure 6.1 a, b), because Nickell et al. (2003) and Nicholson & Longmore (1999) reported the depth of their sites but not ambient temperatures (presumably because they used *in situ* incubations).

(a)



(b)

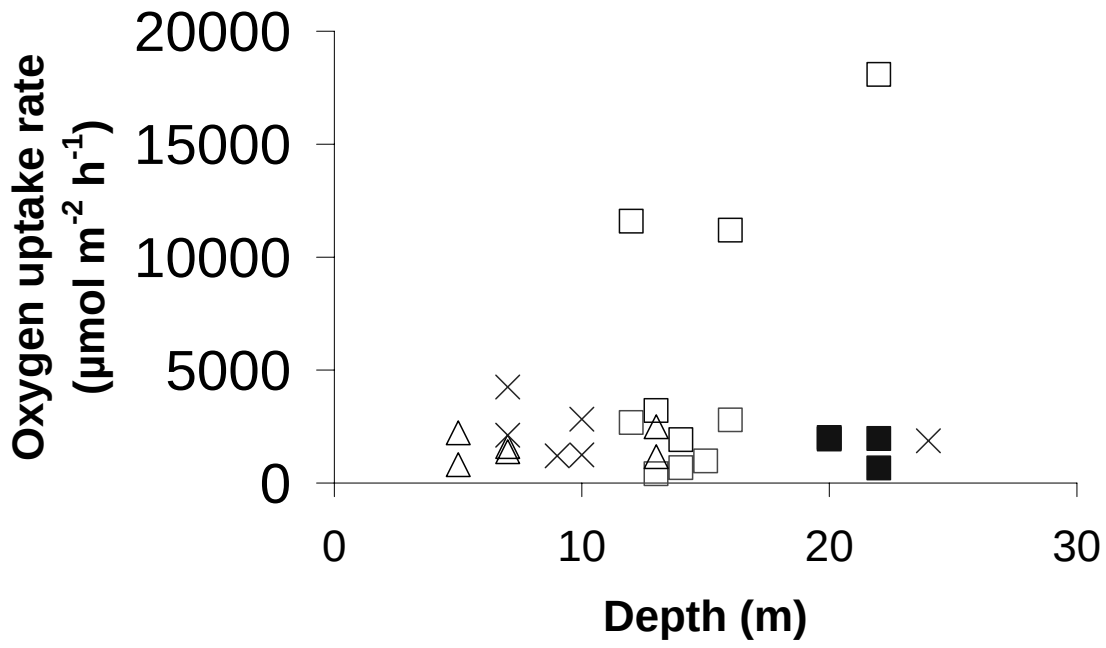


Figure 6.1. Mean OUR from impact and control sites against (a) temperature and (b) depth for the three anthropogenic activities (□= finfish farm, ■ = SBT farm, Δ = shellfish, x = outfall).

OUR from the various impacted sites were generally greater than the control sites, and the difference was most pronounced for fish farms (Figure 6.2). The fish farms studied by Findlay et al. (1995), Findlay & Watling (1997) and Nickell et al. (2003) generated the highest mean OUR at the impacted sites. For these studies mean OUR was above 10,000 $\mu mol\ m^{-2}\ h^{-1}$, although the standard deviations were about half the mean. Within the fish farming category, the smallest difference between impacted and control sites was recorded for one of the SBT farming sites (Figure 6.2). OUR measured at the outfall impacted sites was consistently greater than at the control sites. The smallest difference between the impacted and control sites was shown by the shellfish farms.

The effect of fish farms and outfall stations on OUR was significant across studies within each category, with mean effect sizes being greater than 1 and confidence intervals not overlapping 0 (Table 6.3). Shellfish farming produced a small mean effect size on OUR and the effect of shellfish farms was not significant across studies because confidence intervals overlapped 0 (Table 6.3). The total effect of the 3 anthropogenic activities on the measured OUR was very large (i.e. effect size > 1) (Table 6.3). The effect of the fish farms, shellfish farms and outfalls on OUR was significantly different among categories (Mixed model meta-analysis, Homogeneity statistic (Q_b) = 8.34, 2 df, $p < 0.005$).

6.4. Discussion

The impact on OUR from the three categories of anthropogenic activities studied in the present meta-analysis was conclusively demonstrated. Studies of environmental impacts may be considered to test the null hypothesis that a particular anthropogenic activity has no impact upon the environment (Fairweather, 1991). Indeed, the effect of the fish farms, shellfish farms and outfall on OUR was significantly different between categories, with shellfish farms generating the lowest mean effect size which was not significantly different to 0. Studies of aquaculture activities are sometimes put through qualitative interpretations into the larger environmental context of nutrient inputs that may lead to eutrophication. From this type of investigation it has been concluded that in some areas the addition of nutrients from aquaculture is negligible in comparison with other sources of eutrophication, like land run-off and atmospheric downfall (Ackefors & Enell, 1990). The result of the meta-analysis presented here, quantitatively demonstrates that these three anthropogenic activities have different effects on OUR.

The effect of finfish farms and outfall stations on OUR was significant across studies within each of these categories but this was not the case across studies of shellfish farming. The nature of these activities must be considered when interpreting these results. Previous studies have shown that increased organic loading to the sediments can stimulate benthic metabolism (Graf et al., 1982; Kelly & Nixon, 1984) and the actions of fish farming and outfalls are direct additions of particulate and dissolved organic and inorganic material into the environment (Pearson & Rosenberg, 1978; Grant et al., 1995). Shellfish culturing is different as there are no additional organic inputs, although there may be localized organic enrichment through deposits of faeces and pseudofaeces, as well as dislodged cultured shellfish and fouling organisms (Dahlback & Gunnarsson, 1981; Grant et al., 1995; Stenton-Dozey et al., 2001). Therefore, assuming that the material from these three sources was at least partly labile and the sediments were capable of aerobic decomposition processes, fish farming and sewage outfalls appear to present the greater risk to natural benthic metabolism.

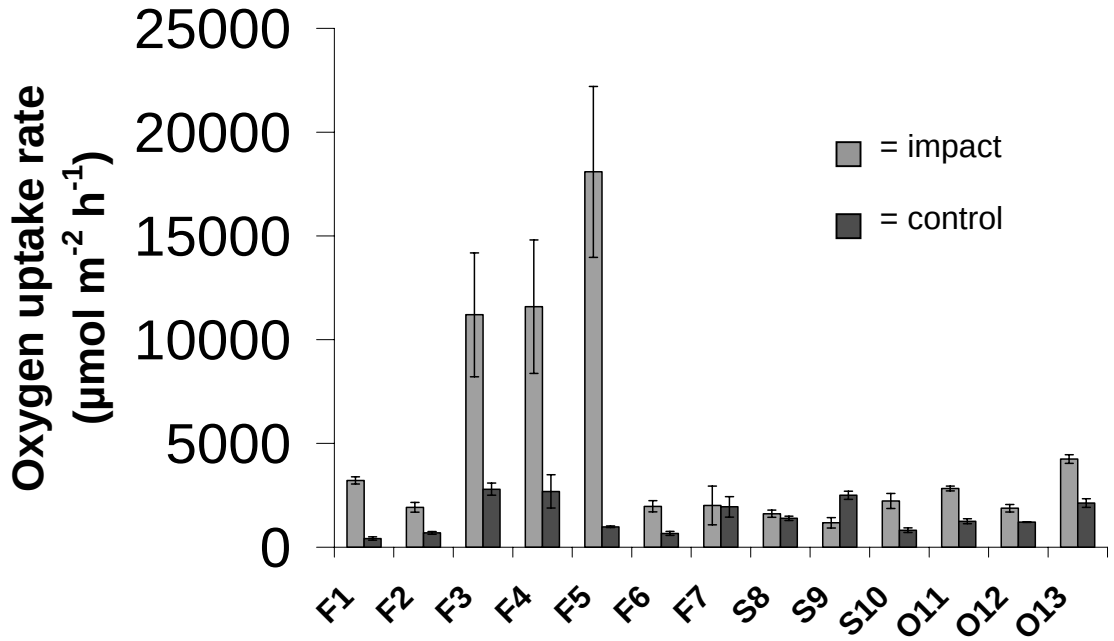


Figure 6.2. Mean OUR from impact and control sites for the three anthropogenic activities (study code as per Table 6.1). Bars indicate means (± SE).

Table 6.3. The effect sizes of the categories analysed from the meta-analysis.

Category	Mean effect size	SD	95% confidence interval
Fish	1.66	0.51	0.66 to 2.65
Shellfish	0.22	0.77	-1.29 to 1.72
Outfall	3.77	0.96	1.88 to 5.66
Total	1.63	0.39	0.87 to 2.39

Temperature and depth were not significantly correlated with OUR when control sites were considered from studies across the three categories. Both temperature and depth have been shown previously to correlate with OUR both within and among studies but the temperature range studied by Hatcher et al. (1994) was from 0 to 21 ºC and the depths studied by Kemp et al. (1992) were from 1 to 60 m. Hatcher et al. (1994) found a positive linear relationship between OUR and temperature and Kemp et al. (1992) reported that OUR was inversely proportional to depth. Despite these patterns in natural environments, such significant relationships with OUR were not observed among the multiple studies of the present meta-analysis, because there was a smaller (although still considerable) range of temperatures and depths across studies. Consequently, the method to select data from these studies with minimal environmental variation was successful. Thus, the magnitude of difference in OUR between impact and control sites, among studies, may be considered to be that derived from the anthropogenic activity, rather than merely a reflection of environmental variation between sites in which the anthropogenic activities were conducted.

Within the fish farming category, the lowest difference between impacted and control sites was recorded for one of the SBT farming sites. Fallowing of fish farming sites to allow recovery is a current method used for site rehabilitation after seasonal SBT farming activity. Fallowing has also been used elsewhere as a site rehabilitation process after the farming of marine finfish (Nickell et al. 2003). For some studies (Findlay et al., 1995; Findlay & Watling, 1997; Nickell et al., 2003), it was stated how long a fish farming area, or pontoon site was occupied prior to measurement (range of 3 to 10+ years) but only Nickell et al. (2003) reported a fallowing regime. The ongoing use of a site to receive anthropogenically derived organic inputs may have an effect on the sediment ecology, including OUR, as shown by the large effect on OUR by the outfall. For example, the outfall station at Christies Beach was first commissioned in 1971, approximately 30 years prior to measurement of OUR (Loo, 2001). The evidence from the literature suggests that fallowing regimes at the sites investigated by Nickell et al. (2003) did not prevent elevated OUR measurements at the impact site relative to the control site. In contrast fallowing regimes at SBT farms may have contributed to the lower range in OUR measurements between impacted and control sites. Findlay & Watling (1997) suggested that the non-linear relationship between increases in current flow and increases in diffusive oxygen supply was a major cause in an inability to predict impacts across a variety of sites. Other site specific differences than current flow, such as visible waste accumulation were noted as considerations for prediction of OUR at a given fish farm site (Findlay & Watling 1997).

To aid interpretation of the differences among studies and categories, detailed descriptions of the anthropogenic activity were required. The lack of specific information regarding the amount and types of input to the receiving water body precluded conclusions from being drawn about the intensity of some of the activities. For example, it may be assumed that sewage outfall stations are permanent structures and as such any organic enrichment would be more spatially and temporally consistent. This may be compared to finfish farms that undergo movement between growing seasons as a result of fallowing rotations (e.g. Nickell et al., 2003). Such a difference may have contributed to the larger, or more consistent, effect of outfall than finfish farming on OUR. Conclusive evidence of the duration of site occupation among studies from all categories was not supplied by the cited literature. Across studies, the amount of time a site was subjected to anthropogenic activities was not always stated (Hatcher et al., 1994; Mazouni et al., 1996; Nicholson & Longmore, 1999; Stenton-Dozey et al., 2001). Other environmental details may also have aided interpretation of the results. For example, generally no reference was made to the type of classification system used for

sediment type descriptions (e.g. Folk & Ward, 1957, used in Chapter 5). Thus, no quantitative measure of the mean grain size or the difference in sediment type among studies or categories was possible. For example, Hargrave et al. (1997) reported that the grain size distribution indicated that organic matter accumulated with the fine sediments at depositional sites defined by the hydrodynamic conditions. Therefore, grain size or sediment type information may have been used to evaluate the relative hydrodynamic conditions among the sites studied, but descriptions of sediment type such as soft (Nicholson & Longmore, 1999) offer little scope for meaningful qualitative comparisons.

6.5. Conclusions

The meta-analysis demonstrated that quantifiable differences exist in the effects of fish farming, shellfish farming and sewage outfalls on OUR measurements. Within studies, the largest mean effect size between OUR measured at impact and control sites was generated by outfall stations. The mean effect size of fish farming, including SBT farming, was also significant, but for shellfish farming the mean effect size was small. Consequently, the mean effect size of the fish farms, shellfish farms and outfall on OUR was significantly different among categories. The results of the meta-analysis highlighted the impact on OUR of anthropogenic activities that involve inputs of organic material into the marine environment.

6.6. References

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Chapter 7: Rates of N mineralization at the sediment-water interface adjacent to SBT pens

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Abstract

In this work we investigated perturbations to natural processes determining benthic nitrogen cycling as a result of finfish farming. Isotope dilution techniques were used to characterise the gross and net ammonium mineralisation rates over a 24-hour period for sediments taken from southern bluefin tuna farms and at control sites during spring. The isotope dilution technique permitted partitioning of the total amount of dissolved ammonium available to be released back into the pelagic environment from that retained in the sediments by the microbenthos or in standing stocks. Measurements of the extractable ammonium in the top 2 cm of sediments showed a significant build up as the season progressed of up to 5 μmol N cm^{-3}, suggesting that organic wastes were mostly remineralized in this upper sediment layer. As a result, ammonium mineralisation and incorporation rates at farm sites attained values (20 μg N g^{-1} d^{-1} and 13 μg N g^{-1} d^{-1}, respectively) that were more than double those at control sites. These results suggest fast ammonium turnover times within the sediments, between 1 and 4 days.

7.1. Introduction

This chapter further expands upon the results from Chapter 5, where rates of nitrogen mineralisation and nitrification by the benthos off the coast of Port Lincoln were found to respond to organic inputs from SBT farming activities. More specifically, in Chapter 5 differences in net ammonium mineralisation and nitrification were established between pontoon and control sites during a single SBT farming season. It was also found that ammonium mineralisation was the dominant flux of dissolved inorganic nitrogen from the sediments. In this chapter, isotope dilution techniques are used to characterise the gross and net ammonium mineralisation rates over a 24-hour period for sediments taken from pontoon and control sites during November, 2004. The isotope dilution technique permitted partitioning of the total amount of dissolved ammonium available to be released back into the pelagic environment from that retained in the sediments by the microbenthos or in standing stocks. Together with this, depth profiles of extractable ammonium from the sediments from pontoon and control sites are reported for the latter three sampling episodes of Chapter 5. Extractable ammonium includes ammonium reversibly sorbed onto sediment particles (i.e. it is exchangeable) as well as porewater ammonium dissolved in the interstitial spaces of the sediment, thus further building on the porewater analysis of Chapter 5.

Within the sediment matrix there are three main components that make up total nitrogen (Figure 7.1). Total nitrogen includes exchangeable ammonium bound to the sediment matrix, inorganic nitrogen in the porewater and organic nitrogen contained within the sedimentary matrix. Inorganic ammonium within the sediments exists in three phases, namely as porewater ammonium, exchangeable ammonium and fixed ammonium (Laima, 1992a). Although porewater may be separated from particulate sediments through centrifugation (Garber, 1984; Jensen et al., 1990), porewater and exchangeable ammonium are commonly extracted together using KCl (Blackburn & Henriksen, 1983; Garber, 1984; Mayer & Rice, 1992; Laima, 1992b; Vouve et al., 2000). This extractable pool of ammonium (i.e. porewater and exchangeable ammonium) is thought to be available to microbenthos (Laima, 1993).

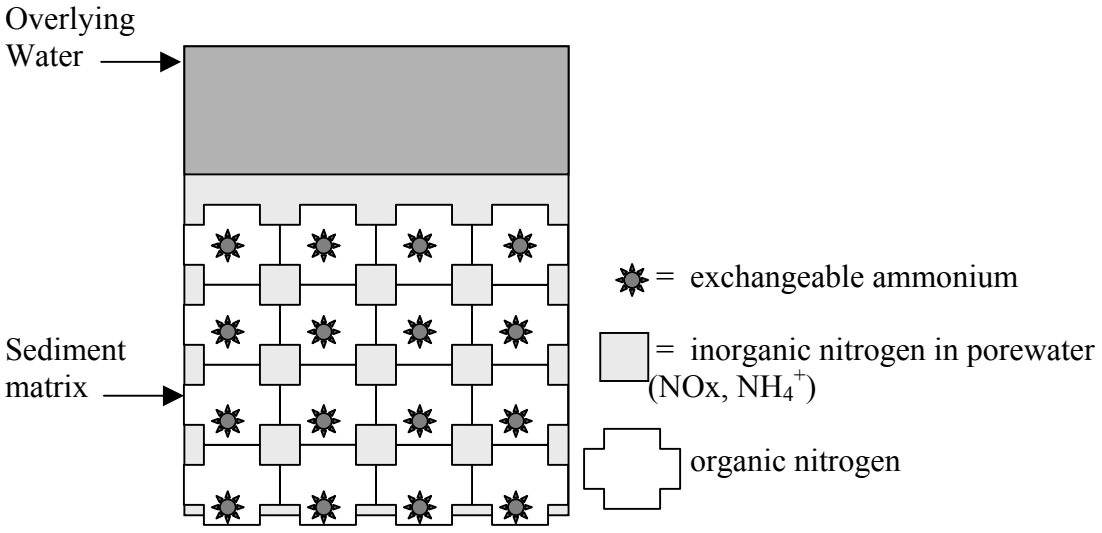


Figure 7.1. Schematic diagram of the nitrogen pools that make up total nitrogen within the sediment matrix.

Ammonium mineralisation may be either a simple or complex series of metabolic reactions depending on the structure of the organic matter (Herbert, 1999). Depending upon the pelagic and benthic environments, exchange of nitrogen between the overlying water and the sediment matrix can occur. Evidence of the benthic response to organic enrichment is indicated by sediment nitrogen fluxes (Grant et al., 1995). Furthermore, ammonium effluxes are characteristic of enriched sediments because ammonium is an end product of both aerobic and anaerobic mineralisation of organic matter (Hansen & Blackburn, 1991). Therefore a better understanding of the nitrogen cycle within the sedimentary environment off the coast of Port Lincoln may be gained through analysis of ammonium mineralisation.

Methods used to extract ammonium vary in the time allowed for extraction to occur, the temperature during the extraction, the number of sequential extractions, the concentration and type of extractant (e.g. 1 to 2 M KCl or NaCl) and the degree of agitation of the samples (Garber, 1984; Laima, 1994; Vouve et al., 2000). The extraction process aims to extract exchangeable and porewater ammonium from the sediment matrix, leaving the organic nitrogen component. The principle of the extraction is to mix the extractant with a sediment sample, the extractant displaces the extractable ammonium from the sediment matrix and the extract is then separated from the sediment matrix and analysed for the concentration of ammonium. Variations of the extraction process were explicitly tested by Laima (1994), where temperature, the type of extractant, the duration of extraction, and the sediment type were varied. The efficiency of this extraction method was examined by Laima (1994), where a labelled ammonium source was introduced into a sediment system and then attempts were made to recover the added ammonium. After addition of the labelled ammonium, the ammonium is partitioned into the various ammonium pools within the sediment matrix to certain degrees. Laima (1994) found that it took 2 hours for the added ammonium to gain equilibrium with porewater (29 to 49 %), exchangeable (9 to 30 %) and non-extractable organic bound (24 to 42 %) pools. Further, the ability to recover the labelled ammonium varied with the sediment type (Laima 1994). More specifically, over 97 % of added labelled ammonium was recovered with a 40 minute extraction at 0 ºC using a 2M KCl extractant at a ratio of 1:1 (extractant to sediment ratio w/w) for sand and sandy silt sediments, but this decreased to 20 % for fine silt sediments (Laima, 1994). Therefore, the method used to extract ammonium from the sediment matrix, and the applicability of experimental data from previous studies, should be based on a consideration of the sediment type under investigation. The sediment type found in the SBT farming region ranged from very coarse silt to medium sand (Chapter 5).

Stable isotopes are now frequently used in ecological research (Knowles & Blackburn, 1993; Waser et al., 1998; Kang et al., 1999; Riera et al., 1999; Davenport & Bax, 2002; Jennings et al., 2002). Many researchers have specifically utilised the stable isotope of nitrogen, ^{15}N (Holmes et al., 1998; Jennings et al., 2002) to advance knowledge of the nitrogen cycle in marine and fresh water ecosystems (Gilbert, 1982; Gilbert, 1992; Knowles & Blackburn, 1993), including subject areas such as trophic structure (Riera et al., 1999; Davenport & Bax, 2002; Jennings et al., 2002) and feeding preference (Waser et al., 1998; Kang et al., 1999; Riera et al., 1999). Blackburn (1979) was the first to use ^{15}N in ammonium mineralisation experiments with marine sediments and the technique described therein has been commonly used since the early 1980's (Blackburn & Henriksen, 1983; Laima, 1994; Tsutsumi, 1995; Vouve et al., 2000). The effect of temperature and sediment type on ammonium mineralisation within marine sediments was a focus for previous ^{15}N-based studies of ammonium mineralisation (Bowden, 1984; Vouve et al., 2000). There are several differences between isotope labelling experiments used to quantify ammonium mineralisation and whole

intact sediment core incubations (as done in Chapter 5). Differences may include the inhibition of nitrification (Laima 1994) that is a sink for mineralised ammonium under oxic conditions and the exclusion of macroinfauna species (Laima, 1993; Tsutsumi, 1995) that may be a source of ammonium through excretory products (Blackburn & Henriksen, 1983).

The principle of the isotope dilution technique is to add a known quantity of ^{15}N to a particular system and measure its dilution or incorporation into dissolved or particulate pools over time (Knowles & Blackburn, 1993) (Figure 7.2). That is, the movement of the labelled compound may be monitored through measurement of the change in the isotopic enrichment ratio (^{15}N: ^{14}N) in the dissolved and particulate fractions of interest (Knowles & Blackburn, 1993). If natural processes act to increase the concentration of natural unlabelled nitrogen (with natural ratio of ^{15}N: ^{14}N) in one compartment of the sedimentary matrix, the added ^{15}N will be diluted by the natural unlabelled ammonium, and the ratio of ^{15}N: ^{14}N will decrease. Also, if the added ^{15}N is assimilated into a specific compartment of the sedimentary matrix, ^{15}N will be incorporated and the ratio of ^{15}N: ^{14}N will increase.

An example of an experimental system constructed within a test tube and used to measure ammonium mineralisation is illustrated in Figure 7.2. The start of an ammonium mineralisation experiment corresponds to the direct addition of labelled ammonium into the overlying water and extractable ammonium pools. Dicyandiamide solution may be used as a nitrification inhibitor to ensure that ammonium is not lost from the system via nitrification (Laima, 1994). As nitrification may be blocked, the flow of the labelled ammonium that was added may be followed through the remaining processes (Figure 7.2). Net ammonium mineralisation rates can be assessed through measurement of the change in the extractable ammonium pool. Incorporation of the labelled ammonium into the organic nitrogen pool can be measured through mass spectrophotometric analysis of the nitrogen content of dried sediment samples. Diffusion from the sediment into the overlying water may be estimated through the combined measurement of the exchangeable ammonium pool and its isotopic dilution over time. Generally, the diffusion process is not assessed independently from measurement of the ammonium mineralisation rate (Blackburn, 1979; Blackburn & Henriksen, 1983; Vouve et al., 2000), because test tube systems are made with slurries and without excess overlying water components.

The same techniques used for extractable ammonium measurements are also used to remove the ammonium from the sediments for mass spectrophotometer analysis of the ^{15}N fraction. Dissolved ammonium in the extract is transferred into a solid substrate for accurate measurement (Holmes et al., 1998). Holmes et al. (1998) tabled a useful summary of the methods used for removal of labelled ammonium from solutions for mass spectrophotometer measurements, which include distillation and diffusion techniques. Briefly, the diffusion technique consists of placing the extract into an airtight bottle and adding magnesium oxide to raise the pH (Figure 7.3). Within the bottle, a filter pack is added directly after the addition of magnesium oxide powder. The filter pack consists of a glass-fibre filter impregnated with sulfuric acid that is sandwiched between two Teflon membranes and floats on the surface of the extract. The Teflon membranes provide a permeable seal between the glass-fibre filter and the extract. The addition of the magnesium oxide powder raises the pH, converting the ammonium into ammonia and the ammonia diffuses through the Teflon membrane and concentrates upon the acidified glass-fibre filter (Holmes et al., 1998). The glass-fibre filter serves to collect the dissolved ammonium into a particulate form that is amenable for quantification through mass spectrophotometric analysis.

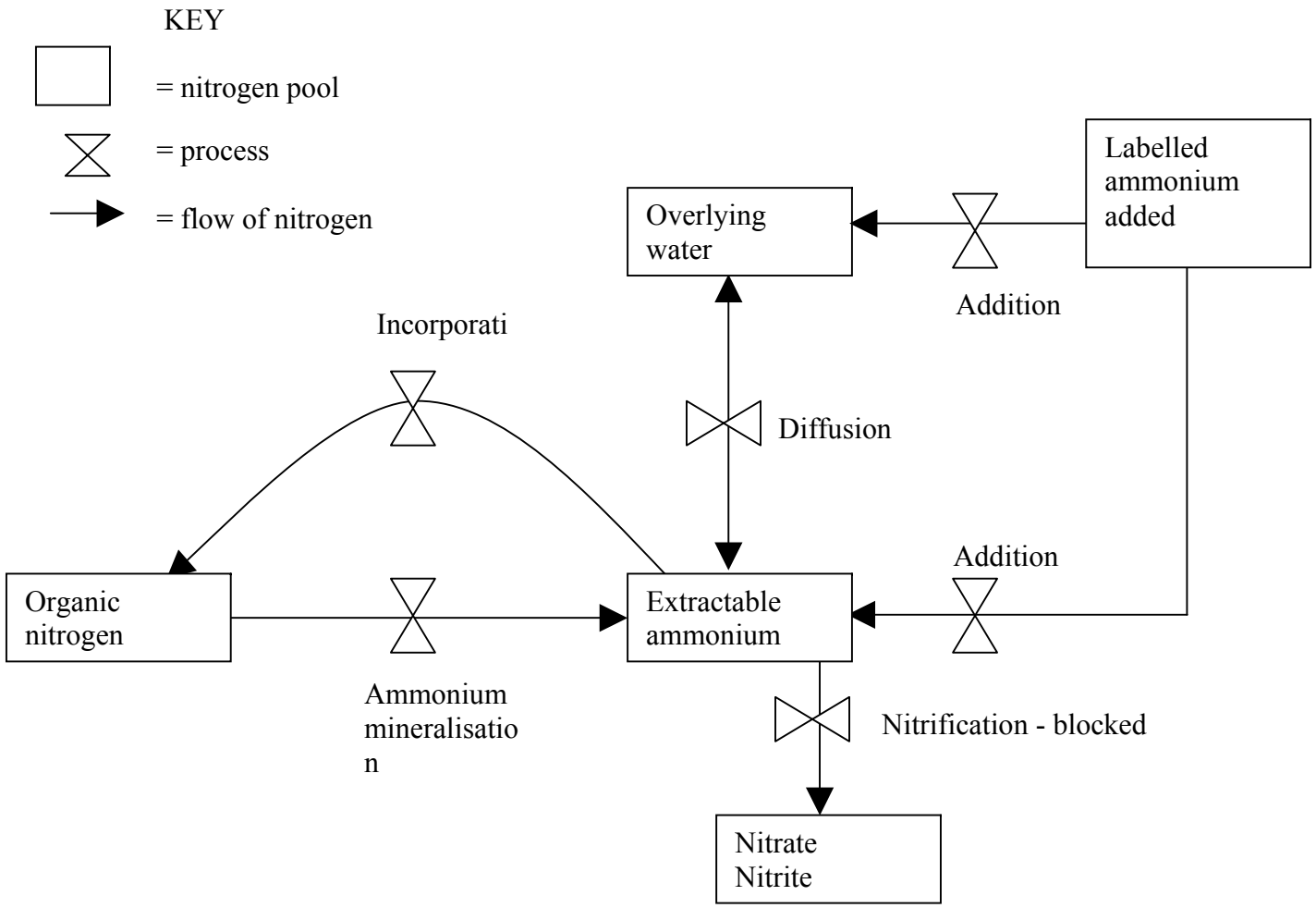


Figure 7.2. Schematic diagram of isotope dilution technique to experimentally assess ammonium mineralisation within the sediment matrix and the potential fate of the added labelled ammonium (bold type indicates measurement made on this pool).

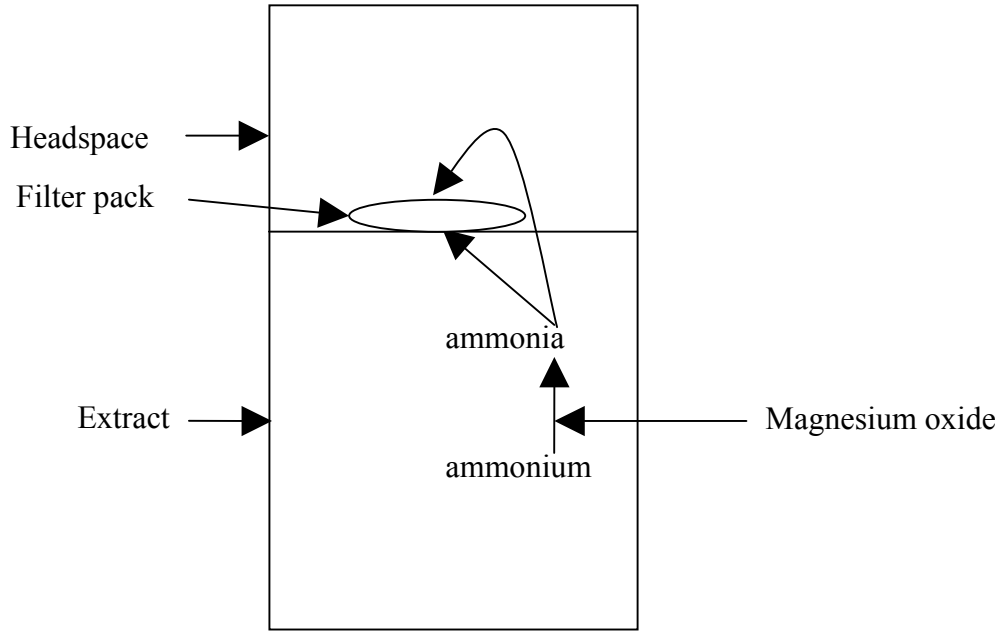


Figure 7.3. Diagram of the ammonium diffusion technique modified after Holmes et al. (1998).

Significantly higher rates of ammonium mineralisation were found adjacent to stocked pontoon sites compared with control sites in Chapter 5. For the purpose of the present chapter, it was necessary to understand the difference between the net and gross ammonium mineralisation rates to more accurately design models of the cycling of nitrogen in the SBT farming region. As farmers predominantly feed baitfish to their SBT stock, the high protein inputs constitute an influx of nitrogen into the system. For every kilogram of baitfish (wet weight, WW) fed to the SBT, an equivalent of 33 g of nitrogen is delivered into the environment, some of which is incorporated into the sediments. With data from this chapter, it may be possible to better refine model predictions about the expected fate of nitrogenous compounds in the environment around the SBT farms.

This chapter also investigates ammonium mineralisation and incorporation rates into sediments at relatively shorter time scales (i.e. hours) of ^{15}N labelled incubations than have been used in the literature (i.e. days) (Blackburn, 1979; Blackburn & Henriksen, 1983; Vouve et al., 2000). The rationale was to test how quickly microbial processes turn over ammonium in the sediments, as previous field experiments have shown that numbers of ammonifying bacteria increased rapidly in response to the collapse of spring phytoplankton blooms (Jensen et al., 1990; Herbert, 1999). For example, Middelburg (1989) (as cited by Kerner & Gramm, 1995) found that after a sedimentation event an adaptation period between 2 and 100 hours was required by the sediments before degradation gradually decreased. Time scales of investigation for ^{15}N labelled experiments may be in the order of hours (Blackburn et al., 1994; Tsutsumi, 1995) to days (Blackburn, 1979; Blackburn & Henriksen, 1983; Vouve et al., 2000). Generally, these studies calculate ammonium mineralisation rates from linear changes of labelled and unlabelled ammonium concentrations during the incubation period. Interestingly, Sumi & Koike (1990) selected the first sampling interval from among four or five sampling intervals to derive the rate of change, as the latter sampling intervals showed little change with time. None of these studies directly investigated changes in ammonium mineralisation and incorporation measured over short time-scale incubations (hours) and compared the conclusions with longer-term incubations (days).

The present chapter investigates ammonium dynamics within sediments at two commercial pontoon sites (P04 and P05), a research pontoon site (TRF) and two control sites (RC1 and BC5) (previously described in Chapter 5). The aims of this chapter were to determine if there was a difference in the ammonium mineralisation or incorporation rates between the pontoon and control sites and, furthermore, whether the conclusions from short-term incubations were consistent with long-term incubations. Together with this, extractable ammonium for the latter three sampling episodes of Chapter 5 is presented with the aim of evaluating among-site differences.

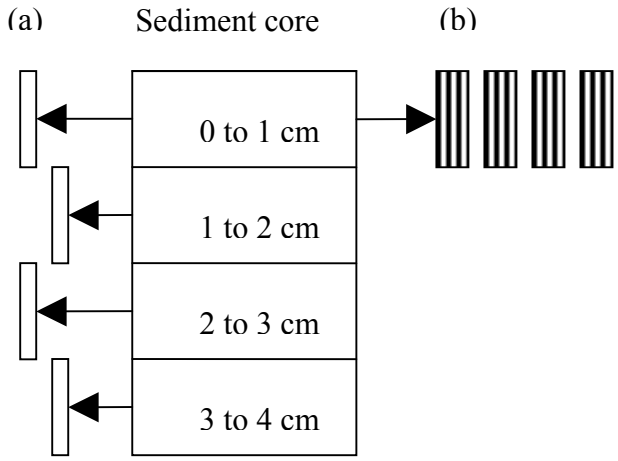
7.2. Methods

7.2.1. Extractable ammonium

To extract ammonium from sediment samples, the methods of Laima (1992a, b, 1994) were adapted. Quantification of extractable ammonium was carried out for three sampling events during the 2004 SBT farming season. Sediment cores used to measure the extractable ammonium were collected during the latter three sampling episodes described in Chapter 5 (i.e. May, July and November). PVC barrels (105 mm i.d.) were used to collect two sediment cores from the five sites studied in Chapter 5. The cores were refrigerated (4 °C) onboard the R.V. Breakwater Bay for up to three hours before transfer to the laboratory. The laboratory procedures for ammonium extraction are summarised in Figure 7.4 a. Once in the laboratory, each core was separately extruded onto aluminium foil and was sectioned (0 to 1, 1 to 2, 2 to 3 and 3 to 4 cm depths). The sediment from each section was then homogenised and any visible macroinfauna were removed (Figure 7.5). From each homogenised section, 6 g of the sediment was added to a 10 mL centrifuge tube; 6 mL of 2 M KCl was then added and the tubes were agitated at room temperature on an end-to-end rotary shaker for 40 minutes. The tubes were then centrifuged for 10 minutes (3,000 rpm), the supernatant was filtered (0.45 μm) and 5 mL was collected into a nutrient tube for analysis of the dissolved ammonium content (see Chapter 5 for method). For all depth sections, the bulk density and water content was determined (see Section 7.2.3). The concentration of extractable ammonium per volume of sediment was reported in the units of μmol NH_4^+-N cm^{-3}.

7.2.2. Isotope dilution technique

PVC barrels were used during November of 2004 to collect two sediment cores from each of the five sites studied in Chapter 5. These cores were the same cores used for the determination of extractable ammonium described in the previous section, for the November sampling only. An additional core was collected from a control site (BC5) to investigate whether ammonium adsorbs to the fine mineral fractions within the sediment matrix. Ammonium in sediments may be irreversibly bound to clay particles (Garber, 1984) and clay was known to be a constituent of the sediment matrix under investigation (Chapter 5). The top 1 cm of this additional sample was dried (16 hours at 60 ºC) and ashed (16 h at 450 ºC) to eliminate any organic material. Milli-Q water was added to the muffled sediment (designated sample CI) to restore its wet weight (36.5 % water content). The CI sample was then analysed along with the other 5 sites. Inclusion of this reconstituted sample permitted more stringent evaluation of ammonium mineralisation, through quantitative analysis of the loss of any added ammonium from non-biological processes.



(a)

(i) ▯ = 6 g of wet sediment into 10 mL centrifuge tube from each layer
(ii) KCl added, shake for 40 minutes, centrifuge for 10 minutes
Supernatant collected and extractable ammonium measured

(b)

(i) ▮ = 10 g of wet sediment into 50 mL centrifuge tube from top layer
(ii) Add salt solution, dicyandiamide and labelled ammonium
Incubate for 0, 6, 12 or 24 hours
KCl added, shake for 40 minutes, centrifuge for 10 minutes
5 mL of supernatant analysed for extractable ammonium
25 mL of supernatant collected for diffusion of ammonium from supernatant onto glass-fibre filter
Glass-fibre filter analysed via spectrophotometer for labeled extractable ammonium
Sediments dried and analysed via spectrophotometer for organic nitrogen and labeled fraction

Figure 7.4. Diagram of the experimental procedure used to measure (a) extractable ammonium and (b) ammonium mineralisation and incorporation from a sediment core.

Figure 7.5. Extrusion of the sediment core and transfer of homogenised sediment to centrifuge tubes.

Once in the laboratory, each core was separately extruded onto aluminium foil and the top 1 cm was sectioned, homogenised and any visible macroinfauna removed. The method used for the isotope dilution experiment is summarised in Figure 7.4 b. From the homogenate, 10 g aliquots were transferred into 4 separate 50 mL centrifuge tubes (i.e. 4 samples from each of the two cores per site); 40 mL of a salt solution (50 g NaCl L^{-1}), 300 µL of dicyandiamide (450 mg L^{-1}) and 1 mL of 0.026 mM $(^{15}NH_4^+)_2\ SO_4$ were then added to each tube. Each tube was shaken by hand to ensure complete mixing of the contents, then wrapped in aluminium foil and placed in a cupboard at room temperature for the duration of the incubation. The contents of the tubes were incubated for either 0, 6, 12 or 24 hours and analysed for the total content and labelled fractions of extractable ammonium, as well as the total content and labelled fractions of organic nitrogen in the sediment.

After the incubation period had elapsed, replicate 50 mL centrifuge tubes (one from each of the two cores) were centrifuged for 10 minutes (3,000 rpm) and the supernatant discarded. 30 mL of 2 M KCl was then added to each tube and they were shaken at room temperature on an end-to-end rotary shaker for 40 minutes. After this period of time, the tubes were centrifuged for 10 minutes (3,000 rpm), the supernatant was filtered (0.45 µm) and 5 mL was collected into a nutrient tube for analysis of the unlabelled extractable ammonium concentration. The total content of extractable ammonium per weight of sediment was recorded in the units of µg NH_4^+-N g^{-1} DW. To measure the labelled component of the extractable ammonium pool within the sediment sample, the diffusion method of Holmes et al. (1998) was adapted. The remaining 25 mL of the filtered supernatant was pipetted into a 50 mL leak-proof HDPE (high density polypropylene) bottle, 80 mg of MgO was added and the bottle swirled to mix the MgO with the supernatant. A filter pack was immediately added to each HDPE bottle and the bottle placed on a table shaker for 96 h. The filter pack consisted of two Teflon membranes (25 mm diameter) that sandwiched a glass-fibre filter (10 mm diameter) impregnated with 50 µL of undiluted, HPLC grade H_2SO_4. After 96 h, the filter pack was removed, placed in an ashed (16 h at 450 ºC) scintillation vial and allowed to

dry for 24 h within a desiccator (also containing an open 75 mL jar of H_2SO_4). The scintillation vial was sealed and stored at room temperature prior to analysis with the mass spectrophotometer. For analysis, the filter packs were removed from the vial, the Teflon membranes opened and the glass-fibre filter disc was removed and analysed. The content of ^{15}N as a percentage of total nitrogen on the filter paper was recorded. The total content and labelled fractions of the extractable ammonium pool were used to calculate the ammonium mineralisation rate as per Vouve et al. (2000); although here the calculations (Equations 1 and 2 below) represent net ammonium mineralisation and not gross mineralisation as suggested by Vouve et al. (2000).

The measurement of ammonium mineralisation from incubations of intact sediment cores (as per Chapter 5), constitute net ammonium mineralisation rates. Specifically, the measurement of the ammonium in the overlying water that emanates from the sediment is the amount of ammonium from mineralisation minus the incorporation rate (also note that nitrification and denitrification must be considered). Therefore, the gross ammonium mineralisation rate measured in a test tube incubation should be the sum of ammonium mineralisation (i.e. change in extractable ammonium pool over time) plus incorporation (Figure 7.2). In a test tube system ammonium mineralisation and incorporation are measured simultaneously. Thus, the change in the extractable ammonium pool may be considered as the net change in ammonium mineralisation, as incorporation has occurred. Calculations presented by Vouve et al. (2000) used to calculate the gross ammonium mineralisation rate do not include addition of the ammonium incorporation rate to the mineralisation rate. Furthermore, these calculations inappropriately suggest that the net ammonium mineralisation rate may be found through subtraction of the incorporation rate from the extractable ammonium pool that is measured (Figure 7.2). Presumably Vouve et al. (2000) assumed that the measurement of the extractable ammonium pool constituted measurement of the gross ammonium mineralisation rate, despite an obvious sink from incorporation. Therefore, the net ammonium mineralisation rate (referred to as ammonium mineralisation from here) was calculated as per Equations 1 and 2, for the 0 to 6, 6 to 12, 12 to 24 and 0 to 24 h periods. Firstly, Equation 1 was used to calculate the diffusion (Figure 7.2) from the system (d = μg NH_4^+-N g^{-1} DW d^{-1}):

$$d = \frac{P_1E_1 - P_2E_2}{t((E_1 + E_2)/2)} \qquad [1]$$

where, P_1 and P_2 were the concentrations of extractable ammonium per weight of dried sediment at the start and end of the incubation period (t = days). E_1 and E_2 were the corresponding % ^{15}N excesses of the extractable ammonium concentrations. Equation 2 was then used to calculate the net rate of ammonium mineralisation (a = μg NH_4^+-N g^{-1} DW d^{-1}):

$$a = d + \frac{P_2 - P_1}{t} \qquad [2]$$

The sediment remaining in the 50 mL centrifuge tube after the extraction of ammonium was dried (24 hours at 60 ºC) and stored at room temperature until mass spectrophotometric analysis of the organic nitrogen content. For analysis, the sediments were ground to a fine powder using a mill (Rocklabs, New Zealand) then 50 mg of the sediment was weighed and

analysed for the percentage content of ^{15}N in the sediment and the organic nitrogen content of the sediment. The total contents and labelled fractions of the organic nitrogen were used to calculate the ammonium incorporation rate (i = µg-N g^{-1} DW d^{-1}) (Equation 3) as per Vouve et al. (2000):

$$i = \frac{P_{org2}E_{org2} - P_{org1}E_{org1}}{t((E_1 + E_2)/2)} \quad [3]$$

where, P_{org1} and P_{org2} were the organic nitrogen content of the sediment at the start and the end of the incubation period (µg N g^{-1} DW). E_{org1} and E_{org2} were the corresponding % ^{15}N excesses of the sediment (where % ^{15}N excess = sample % ^{15}N – natural % ^{15}N). The incorporation rates were calculated for the 0 to 6, 6 to 12, 12 to 24 and 0 to 24 h periods. The same samples analysed at 0 h and 24 h for the short-term incubations were used to calculate the rate for the entire 24 h period (i.e. long-term). This was done for comparative purposes and, as these samples do not constitute true replicates, the difference was not statistically tested.

7.2.3. Dry bulk density and water content

The water content (%) of the sediment was determined from the weight loss after drying 30 g of sediment for 24 h at 60 ºC. The dry bulk density (g cm^{-3}) was assessed by filling a volumetric flask (10 cm^3) with dried sediment and then weighing it. The water content and dry bulk density were used to calculate extractable ammonium per gram dry weight of sediment in Sections 7.2.1 and 7.2.2.

7.2.4. Statistical analyses

To test the null hypothesis that there was no significant difference in extractable ammonium among sites or months or an interaction of these factors two factor ANOVA was used (SPSS Inc., Chicago). Tukey's HSD *post-hoc* tests were conducted subsequently if required. The two-way ANOVAs were conducted upon the 1 and 4 cm sediment layer of the sediment separately, as each depth sampled was from the same core and therefore each depth was not independent. To meet assumptions of normality and correct the positively skewed distribution, data on extractable ammonium were $\log_{10}$ transformed prior to analysis.

To test the null hypothesis that there was no significant difference in the ammonium mineralisation, incorporation rates or organic nitrogen among sites, incubation periods or from the interaction of these factors, two-way ANOVAs were used. *Post-hoc* analyses (Tukey's HSD) were conducted subsequently if required. To meet the assumptions of normality and correct the positively skewed distribution, ammonium mineralisation, incorporation rates and organic nitrogen were $\log_{10}$ transformed prior to analysis. Linear regression analysis was used to assess if there was a linear relationship between ammonium mineralisation and incorporation.

To test the null hypothesis that there were no significant differences among sites in ammonium mineralisation or incorporation calculated over the entire 24 h incubation period, one-way ANOVAs were used. Tukey's (HSD) *pos- hoc* analyses were conducted

subsequently if required. Linear regression analysis was used to assess if there was a linear relationship between ammonium mineralisation and incorporation.

7.3. Results

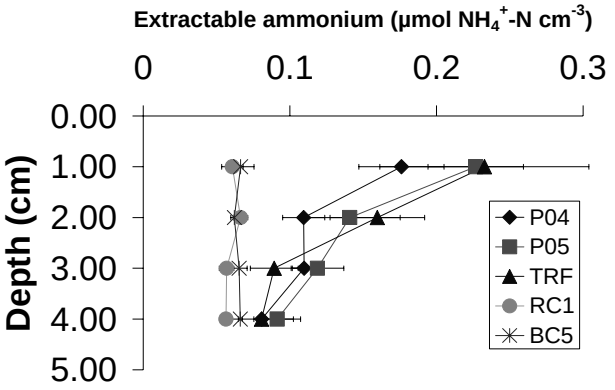
7.3.1. Extractable ammonium

The concentration of extractable ammonium generally decreased with increasing depth for P04, P05 and TRF (i.e. pontoon sites); whilst for RC1 and BC5 (i.e. control sites) extractable ammonium concentrations were less variable with depth (Figure 7.6). For P04, P05 and TRF the concentrations of extractable ammonium at 1 and 2 cm depths were generally greater than at 4 cm. Extractable ammonium concentrations at P04, P05 and TRF (i.e. pontoon sites) were generally in the upper range (> 0.1 μmol NH_4^+-N cm^3) compared with the lower concentrations at RC1 and BC5 (i.e. control sites) (< 0.1 μmol NH_4^+-N cm^{-3}). Most notably, high concentrations of extractable ammonium (2 to 5 μmol NH_4^+-N cm^{-3}) were measured at P04 during July (Figure 7.6 b), when compared with the other 4 sites (< 0.2 μmol NH_4^+-N cm^{-3}). The significant interaction of site and month found for both the 1 and 4 cm depths showed that the extractable ammonium measured at a given site was subject to change significantly with the month sampled (Table 7.1).

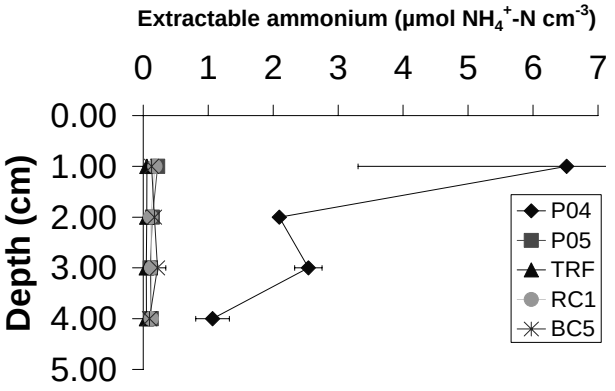
Table 7.1. Effect of spatial and temporal variability on extractable ammonium at both the 1 and 4 cm depth layers of the sediment.

Variable	Source	df	Mean Square	F	Sig.
Log_{10} 1 cm	SITE	4	.645	33.949	.000
	MONTH	2	.243	12.799	.001
	SITE * MONTH	8	.296	15.570	.000
Log_{10} 4 cm	SITE	4	.157	14.983	.000
	MONTH	2	.244	23.243	.000
	SITE * MONTH	8	.180	17.184	.000

(a)



(b)



(c)

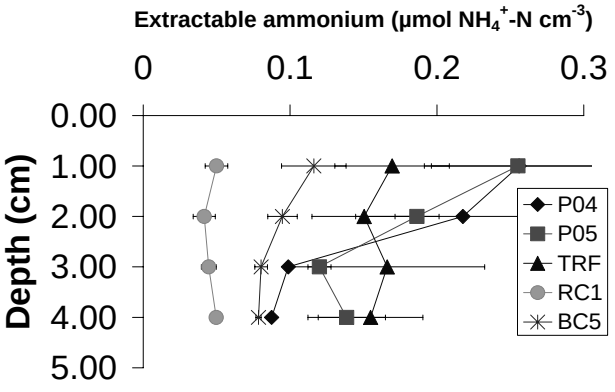


Figure 7.6. Extractable ammonium with depth at the 5 sites sampled in (a) May, (b) July and (c) November of 2004. Points indicate means (n = 2) (± SE).

7.3.2. Isotope dilution

Ammonium mineralisation varied between sites, with the highest rates measured at P04 and P05 approximating 19 μg NH_4^+-N g^{-1} DW d^{-1}, during the 6 to 12 hour period (Figure 7.7 a). The lowest rate was measured for the CI control, where an uptake of ammonium was found at a rate of -1.2 μg NH_4^+-N g^{-1} DW d^{-1} during the 6 to 12 hour period. The remaining sites had ammonium mineralisation rates in the range of 0.3 to 4.3 μg NH_4^+-N g^{-1} DW d^{-1} (Figure 7.7 a). Ammonium mineralisation changed at each site during the three incubation periods. P04 and P05 had the greatest ammonium mineralisation rate during the 6 to 12 h period. P04 and P05 showed the largest decreases from the 6 to 12 h period compared with the 12 to 24 h period, dropping from above 15 μg NH_4^+-N g^{-1} DW d^{-1} to below 5 μg NH_4^+-N g^{-1} DW d^{-1}. There were no significant differences in ammonium mineralisation among sites, incubation periods or as a result of the interaction of site and incubation period (Table 7.2).

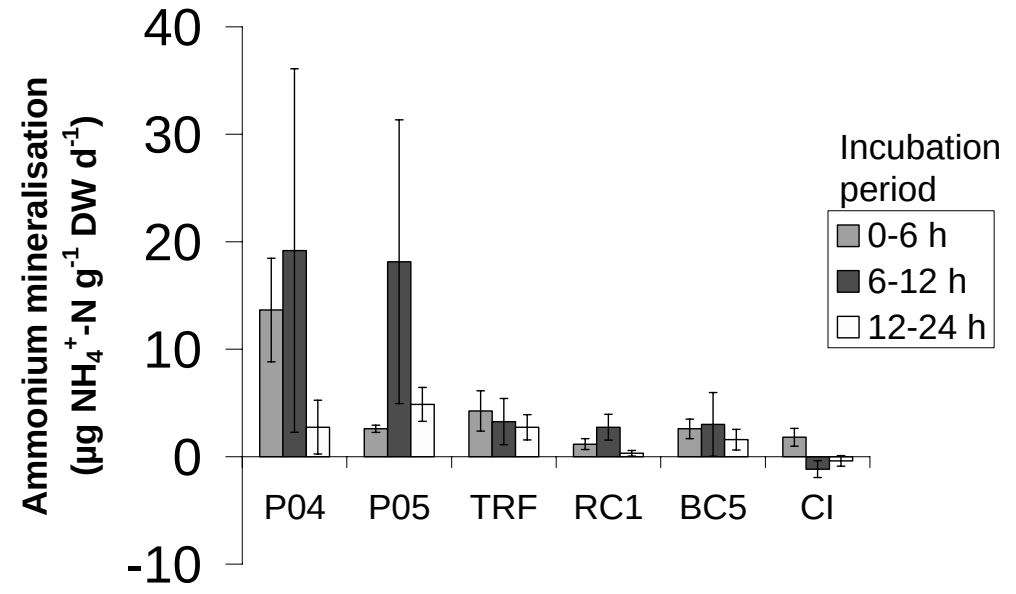
Table 7.2. Effect of spatial and temporal variability on ammonium mineralisation, incorporation and gross mineralisation rates.

Variable	Source	df	Mean Square	F	Sig.
Log_{10}	SITE	5	.905	2.533	.072
ammonium	PERIOD	2	.948	2.653	.101
mineralisation	SITE * PERIOD	9	.319	.893	.552
Log_{10}	SITE	5	1.589	15.228	.000
incorporation	PERIOD	2	.220	2.107	.164
	SITE * PERIOD	9	.074	.708	.693

Incorporation showed variation within sites over time and among sites (Figure 7.7 b). P04 and P05 recorded the highest incorporation rates averaging 8.5 and 12.6 μg N g^{-1} DW d^{-1} for the 6 to 12 h period, respectively. The lowest incorporation rate was from P05 during the 12 to 24 h period where -4.4 μg N g^{-1} DW d^{-1} was lost from the organic fraction. Interestingly, the highest incorporation rates during the 0 to 6 h period were recorded in the range of 2.5 to 4.5 μg N g^{-1} DW d^{-1} by P05, TRF and BC5. CI recorded the least variable incorporation rates and these values were consistently low, ranging from 0.07 to 0.37 μg N g^{-1} DW d^{-1}. There were significant differences in incorporation among sites (Table 7.2), where CI was significantly lower than all other sites ($p < 0.009$) and RC1 was significantly lower than P04 ($p = 0.048$) and P05 ($p = 0.009$). A significant positive linear relationship was found between ammonium mineralisation and incorporation ($r^2 = 0.22$, $p < 0.01$, $y = 0.2511x + 1.0337$, $n = 36$) (Figure 7.8). The linear relationship was driven by the high values measured at P04 and P05 during the 6 to 12 h period of the incubation.

The organic nitrogen content was highest in sediments from P05, averaging above 2,000 μg N g^{-1} DW (or 0.2 %) for the duration of the incubation (Figure 7.9). Similarly, the organic nitrogen content of sediments from P04 was also relatively high, ranging from 1,430 to 1,930 μg N g^{-1} DW throughout the incubation period. The content of organic nitrogen in the sediments from CI was the lowest, ranging from 11 to 106 μg N g^{-1} DW. The difference in organic nitrogen content among the sites was significant (Table 7.3). The organic nitrogen

(a)



(b)

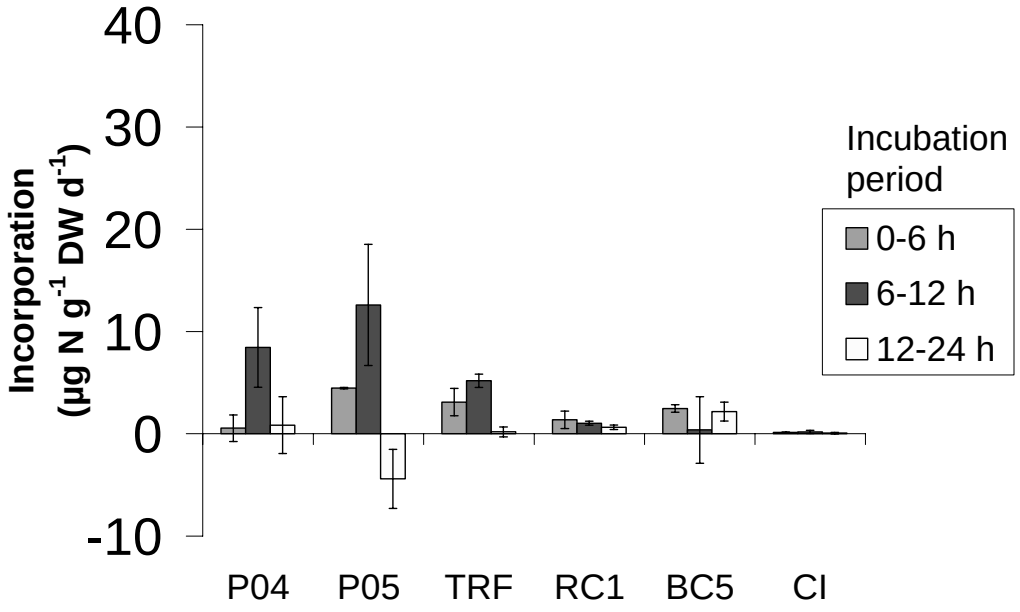


Figure 7.7. The (a) ammonium mineralisation and (b) incorporation rates measured during the incubation periods. Bars indicate means (n = 2) (±SE).

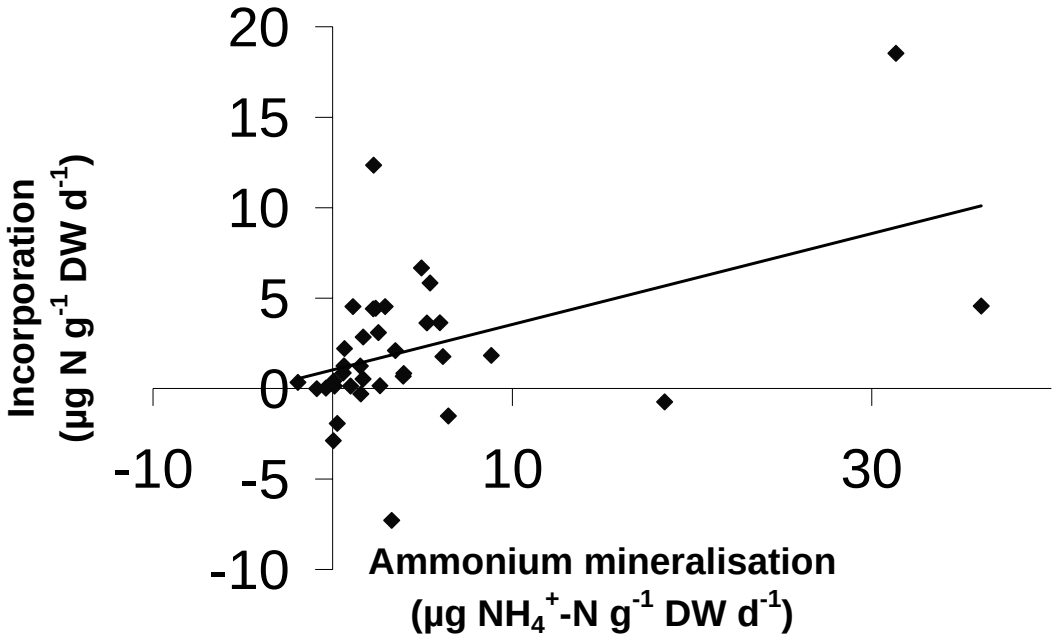


Figure 7.8. Correlation between ammonium mineralisation and incorporation rate for each sediment sample measured throughout the three incubation periods (n = 36).

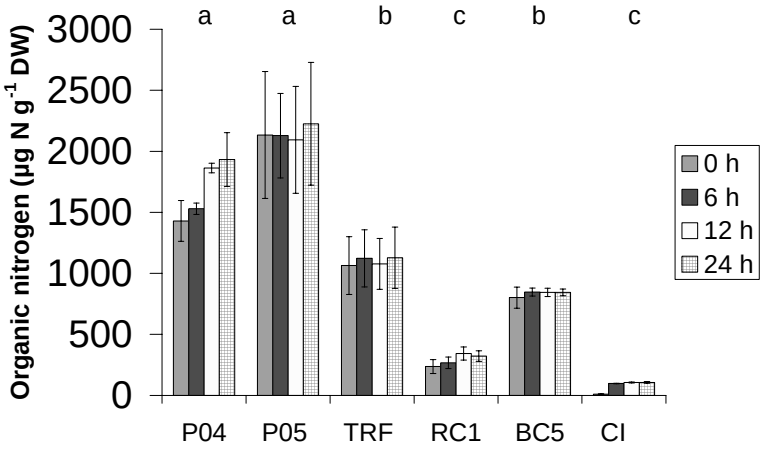


Figure 7.9. The change in the organic nitrogen content of the six sediment samples during the incubation period. Letters denote significantly different (p < 0.05) sites. Bars indicate means (n = 2) (±SE).

content at P04 and P05 was significantly ($p < 0.01$) higher than the other samples. The organic nitrogen content at TRF and BC5 was significantly ($p < 0.02$) higher than RC1 and CI. There were no significant differences among the incubation periods (Table 7.3).

When the ammonium mineralisation and incorporation were calculated over the entire incubation period (i.e. 0 to 24 h), except for P04, variation between replicate samples within sites decreased (Figure 7.10 a). The highest ammonium mineralisation rates were recorded at P04 (10.1 µg NH_4^+-N g^{-1} DW d^{-1}) and P05 (7.2 µg NH_4^+-N g^{-1} DW d^{-1}). The remaining 4 sites ranged from -0.03 to 3 µg NH_4^+-N g^{-1} DW d^{-1} at CI and TRF, respectively. There was no significant difference among sites in the ammonium mineralisation rate measured over the entire incubation period (Table 7.4). Incorporation was higher at P05 (3.3 µg N g^{-1} DW d^{-1}) than at the other sites (Figure 7.10 b). P04, TRF and BC5 had similar values (1.5 to 2.1 µg N g^{-1} DW d^{-1}), whereas RC1 (0.8 µg N g^{-1} DW d^{-1}) and CI (0.09 µg N g^{-1} DW d^{-1}) were relatively low (Figure 7.10 b). The difference in incorporation rates among sites was significant (Table 7.4), but means formed overlapping Tukey groupings (Figure 7.10 b). The relationship between ammonium mineralisation and incorporation was low as a result of one outlier ($r^2 = 0.16$, $p > 0.05$) (Figure 7.11).

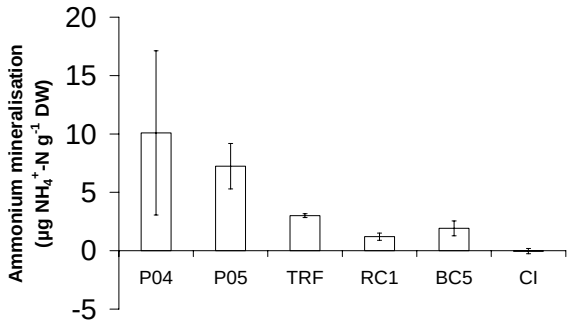
Table 7.3. Effect of spatial and temporal variability on organic nitrogen throughout the incubation period.

Source	df	Mean Square	F	Sig.
SITE	5	5067733	52.723	.000
PERIOD	3	49689	.517	.675
SITE * PERIOD	15	18067	.188	.999
Error	24	96120		

Table 7.4. Analysis of the incorporation rates calculated over the entire 24 h period of the incubation.

Variable		df	Mean Square	F	Sig.
Ammonium mineralisation	Between Groups	5	30.742	1.714	.265
	Within Groups	6	17.940		
	Total	11			
Incorporation	Between Groups	5	2.474	7.515	.015
	Within Groups	6	.329		
	Total	11			

(a)



(b)

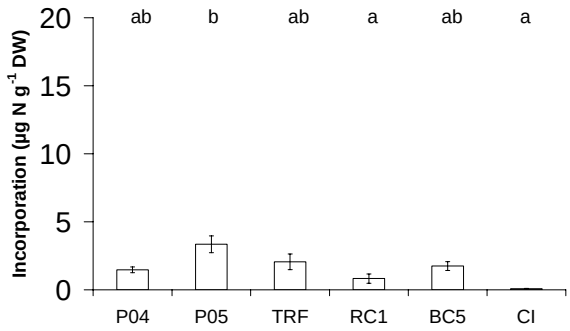


Figure 7.10. The (a) ammonium mineralisation and (b) incorporation rates calculated over the entire 24 h of the incubation. Letters denote significantly different ($p < 0.05$) sites. Bars indicate means ($n = 2$) (±SE).

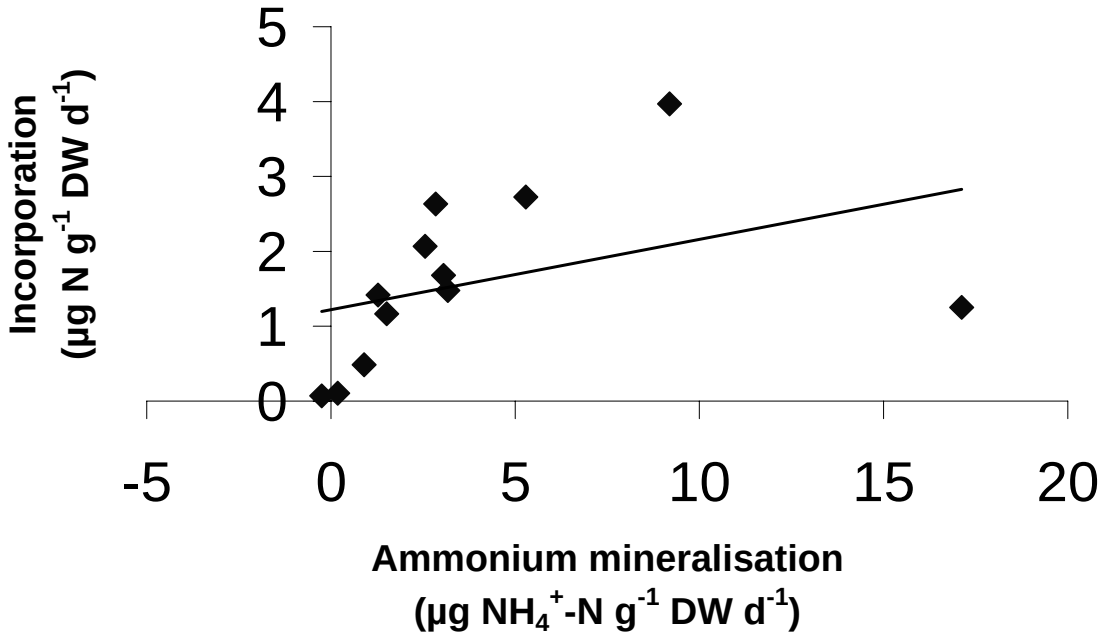


Figure 7.11. Correlation between ammonium mineralisation and incorporation rate for each sediment sample measured throughout the whole incubation period ($n = 12$).

7.4. Discussion

7.4.1. Extractable ammonium

Excluding the high extractable ammonium concentrations from P04 during July, the range of extractable ammonium found here (0.04 to 0.26 μmol NH_4^+-N cm^{-3}) was at the lower end of the range found by Laima (1994) for sandy to fine silt sediments (0.07 to 0.7 μmol NH_4^+-N cm^{-3}, respectively) from the Norsminde Fjord in Denmark. The higher concentrations of extractable ammonium were found in the more silty sediments by Laima (1994). Lomstein et al. (1990) found that extractable ammonium concentrations ranged from 0.03 to 0.2 μmol NH_4^+-N cm^{-3} in sediments of Aarhus Bight, Denmark, with the higher values associated with the settling of a phytoplankton bloom. Garber (1984) measured extractable ammonium in silty (60 to 70 %) sediments of Narragansett Bay, U.S.A. and found concentrations of 0.03 ±0.23 μmol NH_4^+-N cm^{-3}. The extractable ammonium concentrations at P04 during July (1 to 6 μmol NH_4^+-N cm^{-3}) are up to ten times values reported in the literature, even when potential measurement and technique differences are considered, the difference is large. Given the high concentrations of extractable ammonium within the sediments at P04 during July, a considerable build-up of ammonium was evident. The high concentrations of extractable ammonium at P04 during July were not observed at the other commercial pontoon site P05. The porewater concentrations measured at P04 and P05 in Chapter 5 agreed with the discrepancy in extractable ammonium concentrations between the commercial pontoon sites, as extractable ammonium includes both porewater and exchangeable ammonium (Laima, 1992a).

Extractable ammonium was found to vary significantly as a result of the interaction of site and month, with greater concentrations of extractable ammonium found at pontoon sites than control sites. The maximum concentrations of extractable ammonium coincided with maximum porewater concentrations of ammonium and ammonium fluxes from the sediment to the overlying water measured at P04 during July (Chapter 5). This observation highlights the link between these pools of ammonium within the sediment matrix. Jensen et al. (1990) has previously shown links between sediment porewater concentrations of ammonium and ammonium fluxes after a spring phytoplankton bloom in silty-mud sediments at Aarhus Bight, Denmark. Jensen et al. (1990) concluded that the increases in both the porewater ammonium concentrations and the ammonium fluxes were due to a sudden increase in the labile organic nitrogen pool and the consequent increase in nitrogen mineralisation. Given the farming cycle of SBT held within the pontoons, the resultant inputs of waste derived material to the sediments contributed to the observed increases in extractable ammonium within the sediment measured during July (i.e. near the end of the farming season). Interestingly, the maximum concentrations of extractable ammonium measured during July at P04 were not found in November, which was three months after the SBT were harvested. This observation further suggests that the concentration of extractable ammonium within the sediments adjacent to P04 was responding to the farming activities. The observations of Jensen et al. (1990), and those made here, highlight the link between the sediment history and the resultant changes in extractable ammonium within the sediment matrix.

Higher concentrations of extractable ammonium were generally recorded in the upper 2 cm of the sediment profile for pontoon sites. P04, P05 and TRF all showed decreases in extractable ammonium with increasing depth for the 3 months sampled; except for TRF where the gradient in November (after a period of fallowing) was less evident. The result demonstrated that within this upper 2 cm layer of the sediment either more ammonium

mineralisation was taking place or less mineralised ammonium was turned over at pontoons sites. Indeed, ammonium mineralisation rates at the pontoon sites showed a progressive increase during the farming season, compared with the control sites where comparatively less variation was observed (Chapter 5). These observations are in agreement with previous work. Laima (1992b) found seasonal gradients in extractable ammonium within a 9 cm profile of silty to sandy sediments from Danish fjords. Laima (1992b) found that the steepest gradient (0.8 to 0.4 μmol NH_4^+-N cm^{-3}) in extractable ammonium with depth occurred during January (i.e. winter), resulting from the build-up of ammonium pools during the warmer months. For example, the site with the steepest gradient was affected by drainage water from agricultural waste where summer growth of *Ulva* sp. was observed (Laima, 1992b).

7.4.2. Isotope dilution

Ammonium mineralisation rates at P04 and P05 were highest during the 6 to 12 hour period of incubation and both sites exhibited sharp decreases from the 6 to 12 to the 12 to 24 hour period. This showed that ammonium mineralisation was highest during the former period resulting from the maximal rates of microbial activity and slowing down during the latter period, perhaps from limitations imposed on microbial processes by the closed artificial system. A general decrease in ammonium mineralisation after 24 hours of incubation was shown by Vouve et al. (2000). Vouve et al. (2000) concluded that decreased ammonium mineralisation implied decreased substrate availability with time because of microbial utilisation and that the rates measured after this period no longer reflected *in situ* dynamics. Indeed, the negative incorporation rate measured at P05 during the 12 to 24 h period, together with positive ammonium mineralisation, showed that ammonium was remineralised during this period. Therefore, the short-term incubation periods used allowed us to identify the period of the incubation when ammonium remineralisation was maximal for this particular sediment sample and when substrate availability was limited.

The time dependent changes of ammonium mineralisation and incorporation were different for all sites, although generally maximal rates were recorded during the 6 to 12 h period of incubation. When the periods of the isotope dilution incubations are broken into shorter time intervals (e.g. 6 hours), the interpretation of the dynamics in the systems becomes more complex. The evidence here would suggest that it may not necessarily be assumed (as by, e.g., Vouve et al., 2000) that the variation in the isotopic excess of the inorganic nitrogen fraction during the incubation is linear for short time periods like a few days.

Overall, the greatest ammonium mineralisation and incorporation rates were recorded at the pontoon sites, with maximal rates reaching more than double those recorded at the control sites. The ammonium mineralisation rates calculated over the 24 h period showed the same feature, with higher rates at the pontoon sites. The incorporation rates calculated over the 24 h period did not show a clear difference between pontoon and control sites. The maximal rates measured during each incubation period were different. From the shorter periods of incubation the maximum rate approached 20 μg NH_4^+-N g^{-1} DW d^{-1}, whereas over the entire 24 hour period the maximum was 10 μg NH_4^+-N g^{-1} DW d^{-1}. Similarly, incorporation measured over the shorter time intervals reached a maximum of 13 μg N g^{-1} DW d^{-1} at P05, whereas the maximum obtained over the 24 h incubation period was 3 μg N g^{-1} DW d^{-1}. These results show rapid shifts in ammonium mineralisation and incorporation that have been overlooked by previous investigations using longer term incubations (Blackburn, 1979;

Blackburn & Henriksen, 1983; Vouve et al., 2000) possibly leading to underestimates of maximal system activity in previous studies.

The range in 24 h ammonium mineralisation and incorporation rates found in the present study (1 to 10 µg NH_4^+-N g^{-1} DW d^{-1} and 1 to 3 µg N g^{-1} DW d^{-1}, respectively) is similar to that found by Vouve et al. (2000). Vouve et al. (2000) studied silt-clay intertidal mudflats (0.86 to 1 % organic carbon) on the French Atlantic coast and recorded ammonium mineralisation and incorporation rates for the 0 to 2 cm layer over a 24 h incubation of 2 to 10 µg NH_4^+-N g^{-1} DW d^{-1} and 1 to 5 µg N g^{-1} DW d^{-1}, respectively. To compare more widely with the literature, the units of measurement were converted. Upon comparison, ammonium mineralisation measured in the present study falls at the lower end, and incorporation rates fall within the range of measurements reported in the literature for marine sediments (Table 7.5). Differences among these sites include organic matter and water content of the sediments, and differences among methods include temperatures at which incubations were conducted (Iizumi et al., 1982; Tsutsumi, 1995), both of which affect ammonium mineralisation rates (Grant et al., 1995; Vouve et al., 2000). For example, Iizumi et al. (1982) investigated eelgrass beds and as rooted macrophytes deposit plant material year round (Herbert, 1999), the higher rates observed at these sites may have resulted from the constant organic inputs of plant material into the sediments, as opposed to seasonal inputs from SBT farms. The turnover time of ammonium within the sediment (defined as the ratio of average extractable ammonium to ammonium mineralisation (Vouve et al., 2000) was 1 to 4 days. The turnover time for ammonium within the sediments off the coast of Port Lincoln is faster when compared to previous reports by Vouve et al. (2000) for an intertidal mudflat (3 to 15 days) and by Bowden (1984) for marsh sediments (7 to 14 days). Despite the lower ammonium mineralisation rates at Port Lincoln, relative to other subtidal coastal marine sediments (Table 7.5), the turnover time of ammonium was faster than the intertidal and marsh sediments studied. Ammonium mineralisation rates measured via the isotope labelling protocol (i.e. sediment slurries) and those from the incubation of intact sediment cores from Chapter 5 measured in November are similar (Table 7.6). These similar ammonium mineralisation rates show that the sediment slurries approximated the estimates made from the incubation of intact sediment cores.

Table 7.5. Comparison of ammonium mineralisation and incorporation rates using the isotope labeling method, measured in the present study (excluding CI) with the literature.

Reference	Location	Temperature (^oC)	Ammonium mineralisation (nmol N g^{-1} WW h^{-1})	Incorporation (nmol N g^{-1} WW h^{-1})
Iizumi et al., 1982	Alaska, U.S.A.	11 to 17	46 to 150	38 to 77
Iizumi et al., 1982	Japan	20 to 22	24 to 35	11 to 21
Sumi & Koike, 1990	Japan	6 to 24	6 to 220	3 to 110
Vouve et al., 2000	France	22	3 to 14	2 to 8
This chapter, control sites	Pt Lincoln, Australia	19 to 21	2 to 6	26 to 56
This Chapter, SBT pontoons	Pt Lincoln, Australia	19 to 21	5 to 29	47 to 107

Table 7.6. Comparison of mean ammonium mineralisation rates (μmol N m^{-2} h^{-1}) measured *via* test tube slurries (this chapter) and intact sediment cores (chapter 5).

Site	Slurry incubations	Intact core incubations
P04	319	271
P05	205	161
TRF	62	n/d[1]
RC1	44	77
BC5	38	n/d[1]

[1]no data.

There was a significant positive linear relationship between ammonium mineralisation and incorporation for the shorter-term incubation periods. This result identified a relationship between these processes, where increased ammonium mineralisation was associated with more nitrogen being incorporated within the sediment matrix. This relationship may provide useful information for modelling purposes. However, the positive linear relationship was driven by a few extreme values, corresponding to the high ammonium mineralisation and incorporation rates measured from separate replicates of P04 and P05 during the 6 to 12 h period of the incubation. Similarly, the non-significant linear relationship between ammonium mineralisation and incorporation calculated over the entire 24 h period was again driven by an extreme value at P04, despite an evident linear relationship among the remaining samples (i.e. r^2 = 0.83 without P04 sample). To clarify the linear relationship between ammonium mineralisation and incorporation rates more replication of measurements at this higher end of the scale are required.

Large variation between replicate samples from the same site was observed. Future experiments would benefit from increased replication to better understand the variation between samples from a given site. The time of the season when these samples were taken coincided with the end of the farming season and the start of cleaning of the SBT pontoons' nets. As a consequence of net cleaning, fouling organisms which include paper oysters smother part of the surface layer of the sediment within the sampling area at the previously active pontoon sites. Thus it was not possible to collect sediment samples at previously active pontoon sites without collecting some fouling organisms within the core barrel. The patchy distribution of the deposited fouling organisms that were included in the sediment samples at previously active pontoon sites probably contributed to the variation between replicate samples.

The highest incorporation rates during the 0 to 6 h period were recorded at P05, TRF and BC5. This may have been related to the sediment type. Higher percentages of silt and clay (10 to 25 % more than P04 and RC1) were observed at these sites (Chapter 5) and the extraction process used in the present study has been quantified as being less efficient at extraction of ammonium from silty sediments (Laima 1994). Because there was not a similar change in the incorporation rate for CI samples, which were defaunated sediment from BC5, it cannot be concluded the relatively greater rates were a result of sediment type alone. That is, a biological contribution to the incorporation rate must have taken place. It was noteworthy that the relatively high ammonium mineralisation rates and incorporation rates at P04 during the 6 to 12 h period resulted in increased organic nitrogen content measured after 12 h. Although the organic nitrogen content of the sediments was significantly different among sites, there were non-significant changes in the organic nitrogen content of the sediment during the incubation.

7.5. Conclusions

Estimates of extractable ammonium from the sediments reinforced earlier observations made on ammonium porewater and ammonium fluxes within the SBT farming region. The concentrations of extractable ammonium were higher at pontoon sites than control sites. Significant differences in extractable ammonium were recorded as a result of the interaction of site and month. Together these results showed that SBT farming produced significant changes in the concentration and distribution of extractable ammonium within the sediments.

The use of shorter incubation intervals for calculations of ammonium mineralisation and incorporation using isotope labelling allowed a more stringent appraisal of the time-dependent nature of these processes within the artificial sediment system. Moreover, the maximum rates of ammonium mineralisation and incorporation obtained exceeded those recorded for the entire 24 h incubation period. However, relative differences among different sites remained consistent, with higher rates at the pontoon sites compared to control sites observed using both methods of calculation.

Acknowledgements

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7.6. References

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Chapter 8: The occurrence of benthic scavengers and their consumption at SBT farms off Boston and Rabbit Islands, Port Lincoln, South Australia: a preliminary study

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Abstract

Farming of southern bluefin tuna (*Thunnus maccoyii*) at Port Lincoln, seaward of Boston and Rabbit Islands, involves feeding of fish in 130-150 cages, using primarily frozen Australian sardines (*Sardinops neopilchardus*) amounting to more than 40,000 t per year. A largely unknown amount of sardines is lost from the fish cages and available to scavengers. Cafeteria experiments were conducted during October 2002 and January 2003 at the tuna farms off Boston and Rabbit Islands, respectively. The experiments were carried out during day and night using underwater video, with sardines as bait. The most common scavengers were leatherjackets during the day, notably the Degens leatherjacket (*Thamnaconus degeni*) and sea lice (a group of voracious scavengers on carrion composed of several species of isopods, notably the isopod *Natotalana woodjonesi*, and amphipods) during the night. Scavengers arrived at the bait within minutes, but this was highly variable irrespective of site and day/night. Consumption was found to be inconsistent between periods. During October 2002, consumption was independent of site and day/night. During January 2003, a significant effect of site and time was found, showing that consumption was higher at Boston Island than at Rabbit Island and higher during the day than at night. In all trials, all bait was consumed within 25 minutes of deployment. The average consumption rate was estimated to be about 12 g per minute. The effects of excess feed available to scavengers and the ecological effects are discussed.

8.1. Introduction

The environmental impact of excess feed and faeces (solids) from marine aquaculture deposited on the seabed can be significant and constitutes wastage for the industry (Lopez, 1997; Ang & Petrell, 1997). However, the amount of excess feed available for consumption by scavengers and benthic consumers, and the extent to which it affects ecosystems, are not known. The likely ecological effect is on trophic linkages and food web dynamics. Excess feed provides increased feeding opportunities for scavengers feeding opportunistically on carrion. Carrion, however, is spatially and temporally an infrequent food resource, and a single meal may sustain individuals for long periods (Britton & Morton, 1994). In fisheries, discarded by-catch that settles on the bottom attracts a variety of scavenging species depending on the type of environment, and may constitute as much as 6-13% of the annual secondary production (Groenewold & Fonds, 2000). Densities of scavenger species up to 200 times that of the background populations have been reported around discarded by-catch, and aggregation of some species may persist for up to three days (Veale et al., 2000). While these studies are on discarded by-catch from fisheries, excess feed from aquaculture will have similar effects, depending on amount. Many animals inhabiting the deep sea floor are specialised as scavengers, such as the isopod *Natatolana borealis*, and rely on fall-out from the surface because of a lack of primary production (Castro et al., 2005; Collins & Bagley, 1999; Johansen, 2000).

Farming of southern bluefin tuna (*Thunnus maccoyii*) at Port Lincoln involves growing approximately 5,000 t of fish in 130-150 cages, situated seaward of Boston and Rabbit Islands. During the 3 to 6 months grow-out period, the tuna are primarily fed frozen Australian sardines (*Sardinops neopilchardus*) amounting to more than 40,000 t per year. Although the feeding of tuna is a controlled process without known significant environmental effects (Clarke et al., 1999; Clarke et al., 2000; Loo et al., 2006), a largely unknown amount of sardines is lost from the fish cages and available to scavengers at the surface, midwater or on the bottom. The purpose of this study was to conduct a series of so-called “cafeteria experiments” at tuna farms, using sardines as bait, and underwater video, to identify the most common benthic scavengers, and estimate their occurrence and feeding rates.

8.2. Material and methods

8.2.1. Study sites

The two selected experimental sites were located in the tuna farming area (aquaculture zones) off Boston and Rabbit Islands, respectively (see Chapter 11).

8.2.2. Cafeteria experiments

A digital video camera (Canon MV1) in an underwater housing was mounted vertically on a galvanised rig above a steel mesh grid measuring 1 x 1 meter (Figure 8.1). The distance from the focal plane to the grid base was 120 cm allowing a full photographic view using a wide-angle lens. During the night, light was supplied by a 50-watt underwater daylight photographic lamp controlled by an adjustable timer set to 5 minute intervals of light and darkness.

Field experiments on scavenger occurrence and consumption were conducted during day and night at each of the two sites. At each site and time, replicated (N=3) so-called "cafeteria experiments" were conducted with the rig deployed on the bottom. Australian sardines were used as bait and attached to the grid base using 1 mm steel wire. The camera was activated and the rig lowered over the side of the research vessel and placed on the bottom for 30 minutes before being retrieved.

Figure 8.1. Video-camera rig used for cafeteria experiments.

8.2.3. Consumption

Batches of four Australian sardines were pre-weighed in the laboratory, numbered and frozen. On board, the bait was thawed before use in the "cafeteria experiments". After 30 minutes exposure to scavengers, the rig was retrieved and the remaining bait packed and frozen in order to be re-weighed in the laboratory and the consumed weight subsequently calculated. This procedure was carried out because of the difficulties of weighing with any accuracy at sea.

8.2.4. Analyses

The 30 minute video recordings of the grid were analysed by recording the abundance (or percent cover of the bait in the case of sea lice, which could not be resolved to individuals) of each species observed in 2-minute intervals (12 observations). Night recordings were analysed by recording the number of each species present at two times during each 5 minutes of light with 2-minute intervals (6 observations). Time 0 was the moment when the cafeteria stand first contacted the sea floor. However, for the night trials, because the lamp was not always on when contacting the sea floor, time 0 was the moment the cafeteria stand first entered the water.

Consumption was analysed using three and two-way ANOVA's with period, site and time as fixed effects. All data sets were tested for normality and homogeneity of variances using the Levene's test as provided in the statistical package SPSS 14.0 (SPSS Inc., Chicago).

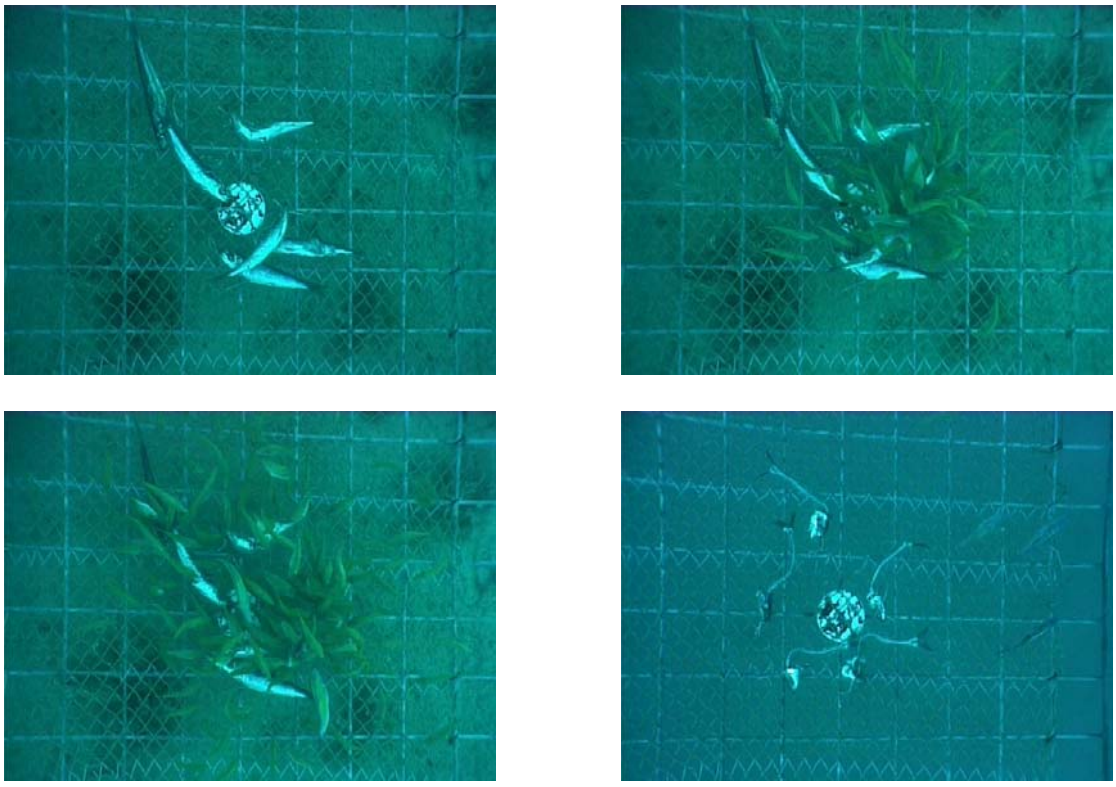

Figure 8.2. Video photos showing leatherjackets feeding at cafeteria experiments at Boston Island in October 2002. Start of experiment: above, left; after 10 minutes: above, right; after 15 minutes: below, left; retrieval after 25 minutes: below right.

8.3. Results

The most commonly occurring scavengers, irrespective of site, were leatherjackets, primarily the Degens leatherjacket (*Thamnaconus degeni*) during the day (Figure 8.2). Leatherjackets of the same species may differ significantly in appearance due to factors such as age and sex. However, almost all leatherjackets observed appeared to be the Degens leatherjacket. The only other species that was clearly different was the Chinamen leatherjacket (*Nelusetta ayraudi*), but this species occurred in small numbers only.

The most commonly occurring scavengers during the night were sea lice, a group of voracious and highly mobile crustaceans of the genus Natatolana (Isopoda) and the order Amphipoda (Figure 8.3). The dominating sea lice species was *Natatolana woodjonesi*. Other species were observed, such as Port Jackson shark (*Heterodontus portusjacksoni*), sand crab (*Ovalipes australiensis*) and spider crab (*Naxia aurita*), but these species were few in numbers. Occasionally, large numbers of Jack mackerel, or chows (*Trachurus declivis*), appeared over the cafeteria tray but did not feed (Figure 8.3). This species was probably attracted by the light, but generally feeds on smaller food items in the water column and can be observed in large numbers both inside and outside the cages when the tuna are fed. Leatherjackets were not observed to feed during the night.

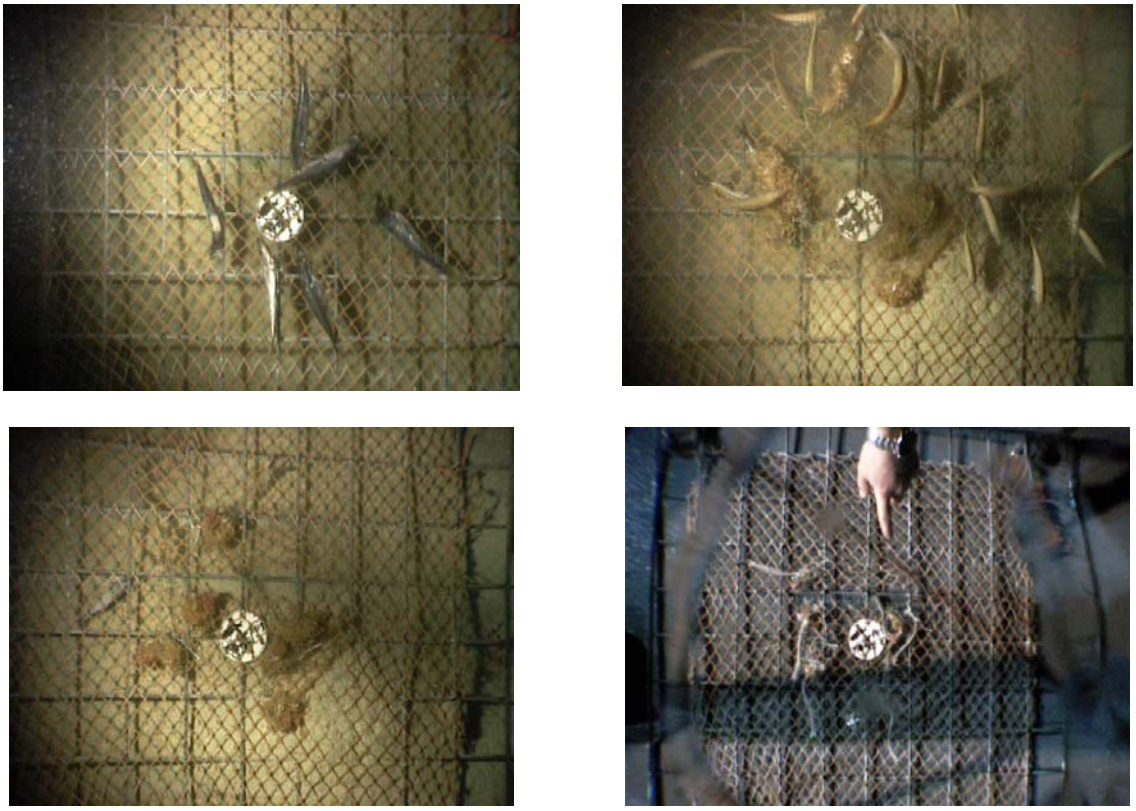

Figure 8.3. Video photos showing sea lice feeding (brown cover of Australian sardines) and the occurrence of Jack mackerels at cafeteria experiments at Boston Island during the night in October 2002. Start of experiment: above, left; after 10 minutes: above, right; after 15 minutes: below, left; retrieval after 25 minutes: below right.

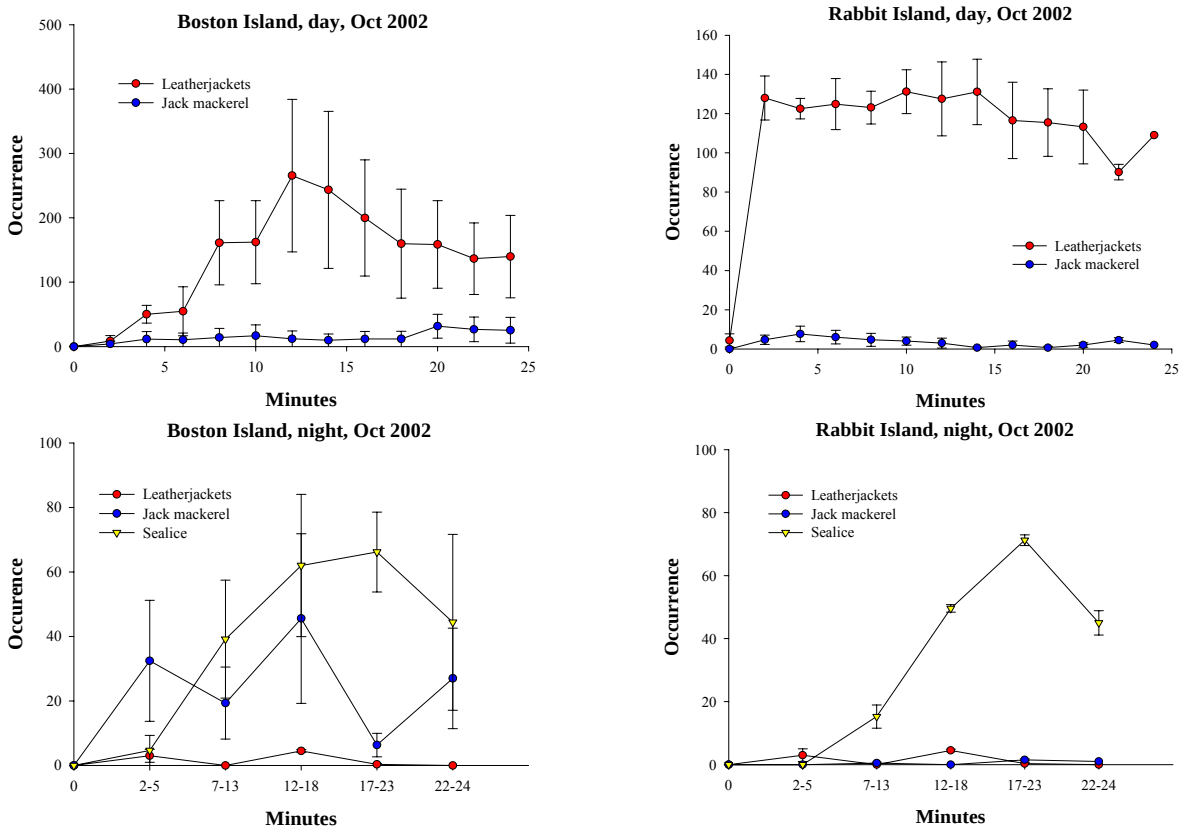

Figure 8.4. Occurrence of dominating scavengers at Boston and Rabbit Island during day and night in October 2002. Error bars are ± SE.

Figure 8.4 shows the occurrence of the dominating scavengers at Boston and Rabbit Islands during the day and night in October 2002. The analyses showed that during the day leatherjackets occurred at the bait within 5 minutes of it reaching the bottom. High variability between replicates was evident, particularly at Boston Island (Figure 8.4; above, left). At Rabbit Island, occurrence of leatherjackets was more rapid and relatively more consistent than at Boston Island (Figure 8.4; above, right). Jack mackerel were observed in low numbers but this species was not observed to actively feed on the bait. At Boston Island, leatherjacket numbers reached more than 300 while at Rabbit Island the maximal numbers observed were more than 140 per observation (Figure 8.4). During the night, the quantitatively most important scavengers were sea lice. These scavengers were observed to be highly active and occurred at the bait within minutes irrespective of site (Figure 8.4). The maximal cover of sea lice occurred after 17-23 minutes irrespective of site. Variability between replicates was high, particularly at Boston Island (Figure 8.4).

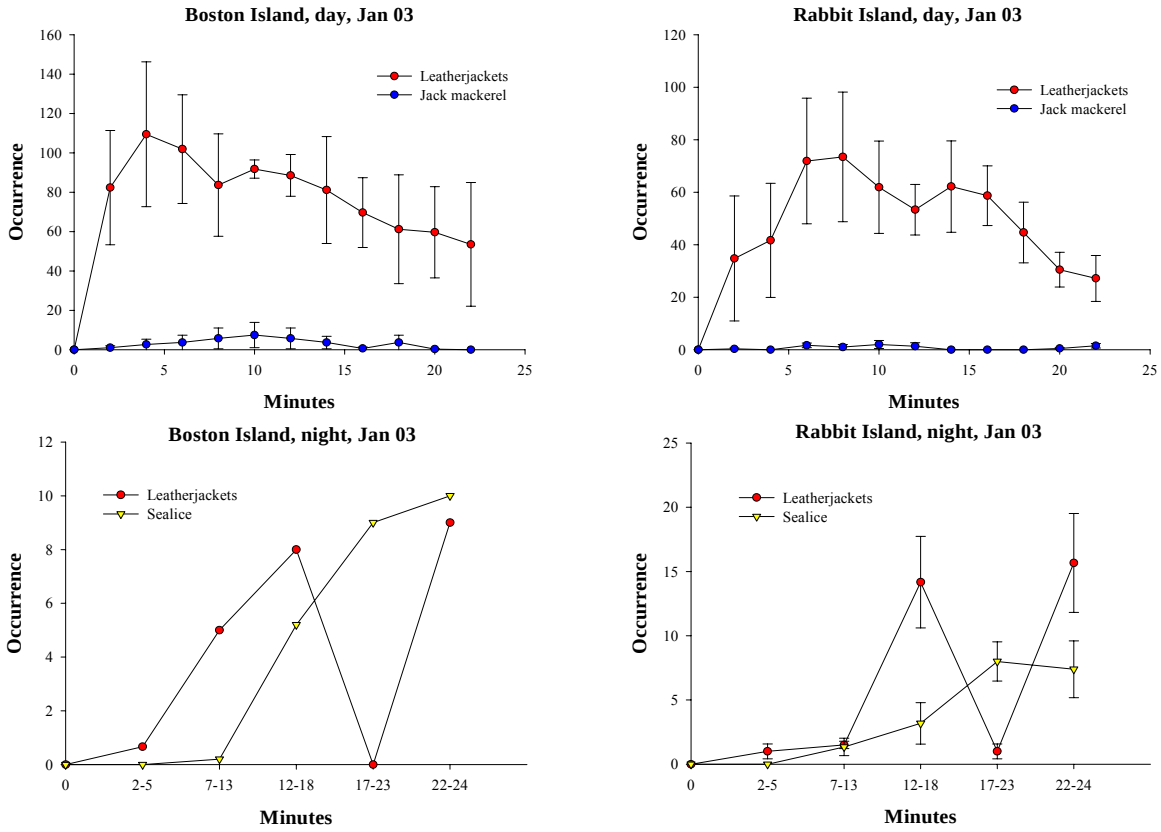

Figure 8.5. Occurrence of dominating scavengers at Boston and Rabbit Island during the day and night in January 2003. Error bars are ± SE.

Figure 8.5 shows the occurrence of the dominating scavengers at Boston and Rabbit Islands in January 2003. The analyses showed a similar pattern during the day with leatherjackets occurring within five minutes, but with lower maximum numbers (Figure 8.5). During the night, a similar pattern as during October was also evident, with a rapid occurrence of sea lice (Figure 8.5). However, the numbers, and cover of the bait, were much lower. During the night, more leatherjackets were observed, but they did not feed. At Boston Island, only one night observation was made because of malfunctioning equipment, and therefore no error bars are shown (Figure 8.5; above, left).

Consumption rates of Australian sardines during cafeteria experiments during the day and night at Boston and Rabbit Island during the two periods of October 2002 and January 2003, are shown in Figure 8.6. A three-way ANOVA with site, time and season as fixed effects showed significant interactions, demonstrating spatial and temporal inconsistency in scavenger consumption (Table 8.1). Variances were found to be homogeneous by Levene's test (P= 0.104). A two-way ANOVA with site and time as fixed effects was therefore carried out for each of the two periods. The data for the October 2002 experiments showed no significant effects of site ($F=0.065_{[1, 12]}$, P=0.804) and time ($F=2.839_{[1, 12]}$, P=0.126), but with a significant interaction between site and time ($F=9.390_{[1, 12]}$, P=0.013). Variances were found

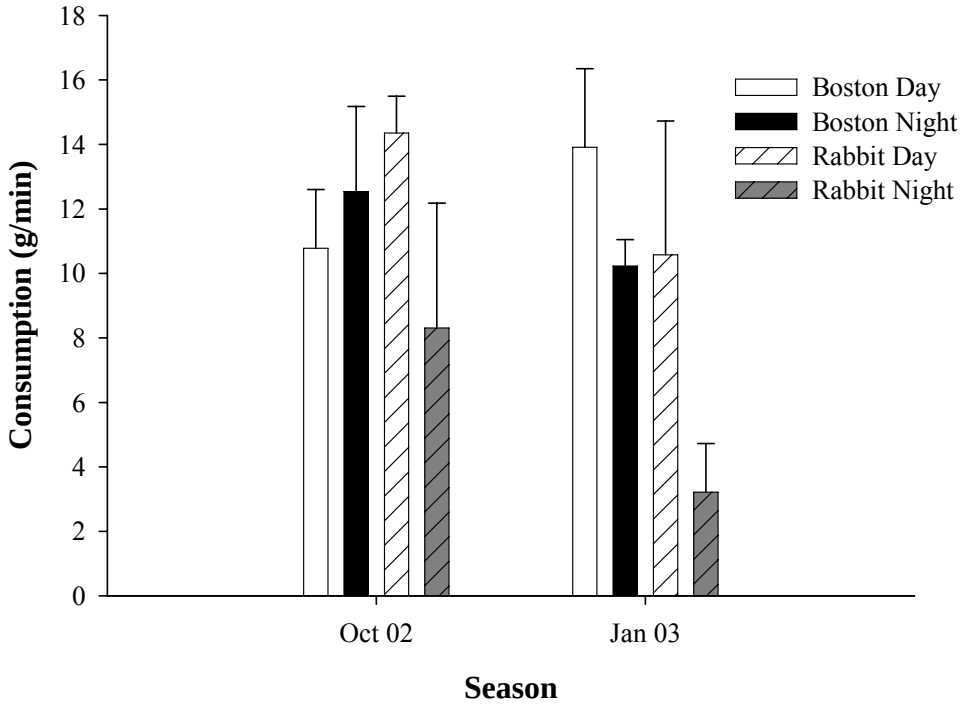

Figure 8.6. Cafeteria consumption rates of Australian sardines at Boston and Rabbit Islands during the day and night at two periods. Error bars are ± 95% CI.

Table 8.1. Three-way ANOVA with site, time and season as fixed effects on consumption rates of Australian sardines at cafeteria experiments.

Source	SS	df	MS	F	P
Site	46.828	1	46.828	9.104	0.008
Time	91.039	1	91.039	17.698	0.001
Season	25.099	1	25.099	4.879	0.041
Site * Time	51.049	1	51.049	9.924	0.006
Site * Season	36.420	1	36.420	7.080	0.016
Time * Season	17.658	1	17.658	3.433	0.081
Site * Time * Season	6.583	1	6.583	1.280	0.274
Error	87.448	17	5.144		
Total	3110.005	25			
Corrected Total	353.620	24			

to be homogeneous by Levene's test (P=0.153). The interaction is caused by inconsistency between the two sites in day and night consumption rates with higher consumption at Boston Island during the night, and higher at Rabbit Island during the day (Figure 8.6). The data for the January 2003 experiments showed a significant effect of site ($F=13.484_{[1,\ 12]}$, P=0.04, partial eta squared = 0.663), and time ($F=13.484_{[1,\ 12]}$, P=0.03, partial eta squared = 0.692) with no interactions ($F=13.484_{[1,\ 12]}$, P=0.196, partial eta squared = 0.200). Variances were found to be homogeneous by Levene's test (P=0.135). The results showed that consumption rates were significantly higher at Boston Island than at Rabbit Island and higher during the day than at night. The partial eta squared values show that the probability of type I error (rejecting the null hypothesis when it is true) is low because effect size is large giving the test

a power of 0.934 and 0.958, respectively, for the two factors. The capacity of the two dominating scavengers, leatherjackets and sea lice, to consume bait on the bottom was impressively fast and efficient. All bait was consumed during the 25 minutes of observation irrespective of time, leaving only sardine bones, heads and tails, which constituted 10-20% of the total weight. Under the experimental conditions, the average consumption rate was estimated to be about 12 g per minute.

8.4. Discussion

The introduction of carrion from fish farms through excess feed is likely to affect the energy flow and the ecosystem food webs by subsidising consumer populations (Ramsay et al., 1997). Carrion is a spatially and temporally infrequent food resource in the sea. In the absence of human interference, few marine animals die as a consequence of natural senescence, thereby becoming available as carrion for scavengers (Britton & Morton, 1994). In southern Spencer Gulf, the most common scavengers observed were leatherjackets, notably the Degens leatherjacket, and sea lice, a group of voracious scavengers on carrion composed of several species of isopods and amphipods (Britton & Morton, 1994; Moore & Wong, 1995; Ramsay et al., 1997).

Juvenile Degens leatherjackets are inhabitants of shallow seagrass beds of Spencer Gulf, while the adults school deeper and relatively close to the bottom. The literature provides little information regarding the feeding ecology of leatherjackets in general and no studies seem to have been carried out. Leatherjackets are important consumers in seagrass assemblages elsewhere and have been found to be generalists feeding on a variety of food items (Bell et al., 1978; Buchmore et al., 1984; Edgar & Shaw, 1995; Last, 1983).

It would be expected that there would be a considerable underestimation of leatherjackets in the larger counts, due to fish obscuring each other when large numbers were present. Counting was further confounded during the larger counts because when many fish were present, the bottom was stirred, resulting in a slightly cloudy image.

Sea lice constitute a group of marine scavengers composed of several isopod and amphipod crustaceans. Bird (1981) described the group as being eminently carnivorous, active swimmers and voracious scavengers active at night when they aggregate at carrion in swarms (Hale, 1976; Stepien & Brusca, 1985). In Spencer Gulf, the dominating species are isopods of the genus Natatolona (Cirolanidae), notably *Natatolana woodjonesi* and *N. viridis*. Bruce (1986) lists 31 species of Natatolona that are known to inhabit Australian waters. However, there is little information on the ecology of the Australian *Natatolana* species and most published work is on *Natatolana borealis*, a deep sea species occurring in the northern hemisphere (Bird, 1981; Johansen, 1996; Moore & Wong, 1995; Taylor & Moore, 1995; Wong & Moore, 1995). It has been reported that these isopods burrow in the sediment during the day, emerging primarily at dusk to feed and return before dawn (Stepien & Brusca, 1985). Sea lice may attack living animals by entering the body cavity through the gills or anus, consuming their prey from the inside out (Hammer & Zimmerman, 1979; Hammer, 1981). Sea lice are considered a pest in many fisheries, including the Spencer Gulf blue crab fishery, because they rapidly consume bait and may attack animals that are caught, whether in nets, pots or hooked.

In the video recordings it was difficult to count individual sea lice, due to poor visibility and insufficient resolution of the camera. Accordingly a value of cover of the bait was used, which probably underestimates abundance because many enter the carrion and are otherwise difficult to observe in video recordings, owing to their high swimming speed. Considerable variability in sea lice cover between individual Australian sardines was observed. This variability seemed to be due to damaged sardines attracting more sea lice than undamaged ones, probably because of the increased scent released of damaged sardines.

Johansen (2000) estimated that *Natatolana borealis* was attracted to bait from a maximum distance of 190 m and swam at a speed of 4.5 to 18.7 cm s^{-1}. The cafeteria experiments showed that the accumulation rate of *N. woodjonesi* was constant during a 30 minutes period. Because sea lice responded to bait within 2 minutes the maximum distance travelled to bait, using the swimming speed reported for *N. borealis*, could be about 75-300 meters within the 30 minutes period of bait deployment.

This study shows that benthic scavengers play an important role in the southern Spencer Gulf ecosystem. Consumption at cafeteria experiments largely reflects their abundance. By far the most important scavengers were the Degens leatherjacket during the day, and sea lice during the night. One Port Jackson shark was observed during the night. Fish farms are known to attract scavengers feeding on excess feed falling to the bottom (Lopez, 1997; Ang & Petrell, 1997). In addition, fish farms develop fouling assemblages (artificial reefs), which provide refuge for many algae, invertebrates and fish exploited by grazers and predators. Excess feed, which is not consumed by scavengers and subsequently transported away, remains on the bottom to be decomposed by smaller benthic infauna and bacteria. This process consumes oxygen and anoxia may develop with the risk of the release of hydrogen sulphide, which can be detrimental to fish farms. However, a build up of organic matter in the sediments at the tuna farms has not been observed in the benthic samples obtained for the Aquafin CRC environment programs, which can be attributed to the relatively strong currents at the farms, rational feeding practices, and, as has been demonstrated here, by the presence of voracious scavengers. It is self evident that rational and effective feeding at the farms constitutes good environmental practise, firstly to avoid build-up of organic matter on the bottom, and, secondly, to avoid contributing to the nourishment and establishment of large scavenger populations, such as seabirds, leatherjackets and sea lice.

The observations presented are likely to have wider implications for environmental management of fish farms because they point to the fact that the carbon contributions from fish farms are not necessary retained locally, but may be rapidly transported away and assimilated elsewhere in the ecosystem with possible regional effects.

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Chapter 9: Modelling of nitrogen environmental loads from southern bluefin tuna aquaculture

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Abstract

Farming of wild tuna in coastal areas is a relatively new aquaculture industry and little is known about the magnitude of nutrient discharges to the environment. In this work we modelled nitrogen loads for southern bluefin tuna (*Thunnus maccoyii*) pens in lower Spencer Gulf, South Australia. The small retention of nitrogen in fish tissues (7-12 % of feed inputs) was associated with high environmental losses (260-502 kg N tonne^{-1} growth). Considering Australian annual production of 4,380 tonnes over initial stocked biomass, total loads can reach 1,137 tonnes N per year, 86 to 92 % lost as dissolved wastes. These results reflect the distinctly higher metabolic rates of tuna when compared to other aquaculture species. The nature of wastes suggests low localized impacts at current stocking densities and holding periods.

9.1. Introduction

Coastal aquaculture has expanded considerably over the last three decades as the demand for seafood increases worldwide. Foremost amongst the threats to the development and sustainability of the industry in new areas of production is the discharge of wastes to the water column and sediments, and the subsequent potential for impact on the surrounding marine ecosystem. In order to establish sustainable production systems, it is necessary to quantify the amount of nutrients released as a result of farming and estimate the assimilative capacity of the surrounding environment. Delivery of nutrients above pre-determined trigger levels should be avoided to reduce adverse impacts on both the environment and farming operations, such as algal blooms, spread of diseases, and changes in biological assemblages (e.g. scavenger and opportunistic species) (Wu, 1990; Bron et al., 1993; Brooks et al., 2002; Nash, 2003; Edgar et al., 2005). Nutrient trigger levels need to be defined for each aquaculture industry taking into account not only the amount of waste generated by each farm, but also the local conditions responsible for the dispersion and assimilation of wastes at both local and regional scales.

Nitrogen, in particular, is usually considered the limiting nutrient for primary productivity in coastal waters (Hecky & Kilham, 1988; Carpenter et al., 1998; Herbert, 1999). Primary producers adapted to live in nitrogen-depleted conditions are likely to have a fast growth response to additional nutrients, and the ecological balance in such systems might be easily disturbed as a consequence. Russell et al. (2005) have suggested that an increase in nitrogen availability in the oligotrophic coastal waters of South Australia is likely to have a disproportionately large impact on local ecosystems when compared to areas chronically affected by higher nutrient levels along the more populated southeast Australian coast. Southern bluefin tuna (SBT) (*Thunnus maccoyii*) are farmed in South Australia in the lower southwestern corner of Spencer Gulf. This industry is one of the most valuable aquaculture sectors in the country, contributing to more than 20 % of the gross value of production in 2004/2005 (Love & Langenkamp, 2003; FRDC, 2004; Newton et al., 2006). Almost the totality of the Australian SBT quota of 5,265 tonnes is caught between December and March along the southern continental margin of Australia and transported to coastal waters off Port Lincoln for fattening over autumn and winter. Approximately 280,000 SBT are fed 50-60,000 tonnes of baitfish during the season to produce an additional 4,380 tonnes per year (average between 2000 and 2003, from Jeffriess, 2004). This baitfish is mostly sourced locally, with Australian sardine (*Sardinops neopilchardus*) catches in South Australia in the order of 40,000 tonnes per year (Rogers & Ward, 2005). Baitfish is usually delivered as frozen blocks suspended underwater in the centre of the pens in separate feeder cages. These blocks continually release feed as they defrost. A smaller fraction is delivered as fresh baitfish shovelled by hand into the pens or supplied by automatic feeders on feeding boats.

The regional effects of the nutrients released from this seasonal activity over the oligotrophic waters of Spencer Gulf are largely unknown. The first step to evaluate the influence of these inputs over ambient conditions is to estimate the magnitude of nutrient loads associated with SBT farming. This initial quantification of inputs should be followed by the analyses of loads in the context of local conditions and other natural and anthropogenic inputs to the system. Of particular interest is the fact that these farms differ from the majority of other coastal finfish operations, as they are located in a very well flushed, high-energy, open-water system where current speeds in excess of 10 cm s^{-1} are not uncommon, peaking at values above 25 cm s^{-1}

(Bierman et al., 2005). In contrast, salmonid operations are usually undertaken in sheltered systems where current speeds can be as low as 3-5 cm s^{-1} (Gowen & Bradbury, 1987).

In this study we were interested in defining the nitrogen loads derived from feeding over 50,000 tonnes of baitfish per year to SBT farmed in pens spread over a farming zone of 172 km^2. For this purpose, we determined the amount of nitrogen that is lost directly to the water column as soluble wastes, the rate of accumulation of solid wastes in the sediments, and the regeneration of nutrients at the sediment-water interface. These results were combined with estimates of fish metabolism to define nitrogen pathways, which were ultimately built into a model for nitrogen flow from individual pens. The amount of nitrogen released per tonne of production was compared to the loads reported for other finfish aquaculture industries and discussed in terms of the magnitude of discharges considering current production and other anthropogenic inputs. Although previous modelling exercises have attempted to quantify the impacts of SBT farming on the environment (Petrusevics, 1993; Sinclair Knight Merz Pty Limited, 2001; Collings et al., 2006), these lacked essential environmental and farming information valid in a local context. The loads calculated here thus constitute a first estimate of nitrogen losses from SBT farming in South Australia using actual data collected for the region.

9.2. Methods

9.2.1. Study area

The SBT farming zone is located in the lower Spencer Gulf in coastal waters off Port Lincoln, South Australia. The system is microtidal (< 2 m), marine (salinity 35-37) and well mixed as strong currents flow into the western entrance of Spencer Gulf from the Southern Ocean (Fuller et al., 1994). A summary of the natural background characteristics of the study area has been previously presented in Fernandes et al. (2006a).

Eleven operators have farms located in an area seaward of Boston Island where water depths vary between 18 and 25 m. Combined, these farmers have 130 to150 pens in the farming zone during the season with typical stocking densities of 2-3 kg m^{-3}. SBT are fed mostly baitfish and feeding rates are highest in summer after stocking but decline later in the year as SBT reach condition and water temperatures drop from approximately 22 to 14^{o}C (van Barneveld & Ellis, 2003).

Study sites included two commercial pens in farms of different companies and consequently subject to different management regimes (Table 9.1). These pens were 38-40 m in diameter with a net wall depth of 10 m, and were located in water depths between 20 and 22 m. Pen P1 was stocked with 1,794 fish in February and fattening lasted for 160 days with feeding rates averaging 2,890 kg d^{-1} until harvest in July. Pen P2 was stocked unusually late in April with 2,306 fish. These were fed an average of 2,514 kg d^{-1} during 135 days until August. The estimated initial weight of fish in both pens was 17 kg, determined from a sample of 40 fish collected from each tow pen used to transport fish to the farming area. Harvested whole fish weighed on average 32 kg (P1) and 33 kg (P2).

Table 9.1. Feed input, growth and feed conversion performance of SBT in commercial pens P1 and P2.

	P1	P2
Mean initial weight (kg)	17	17
Mean final weight (kg)	32	33
Number of fish stocked	1,794	2,306
Number of mortalities	54	75
Stocking (days)	160	135
Feeding (days)	151	115
Mean feed input (kg d^{-1})	2,890	2,514
SGR (%)[a]	0.41	0.48
FCR[b] (wet weight)	17	10
FCR[c] (dry weight)	4.9	2.7

[a]Specific growth rate, or weight gain per day.
[b]Feed conversion rate (wet weight feed/wet weight gain).
[c]Feed conversion rate (dry weight feed/wet weight gain).

9.2.2. Sampling

Baitfish feeds, consisting mostly of 10-20 cm long Australian sardines (*Sardinops neopilchardus*), were obtained from commercial operators and stored frozen at -30 °C in zip lock bags.

Environmental samples were collected in March, May and July 2004 at the edge of the commercial pens. Sediments were collected with 67 mm (i.d.) stainless steel tubes using a HAPS Corer (KC Denmark). Upon retrieval, the overlying water in the tube was carefully discarded to minimise surface disturbance and the sediment extruded onto a clean stainless steel table. Four cores were collected for the analyses of total nitrogen (TN). The top layer (0-1 cm) of each core was sliced, transferred into a pre-combusted glass jar and stored frozen (-30 ^{o}C). Two cores were collected for the determination of wet density. The top layer (0-2 cm) of each core was sliced, transferred into a pre-weighed centrifuge tube of known volume and stored refrigerated (4 ^{o}C) for up to 3 h before transfer to the laboratory.

For determination of nutrient fluxes at the sediment-water interface and sediment water content, we collected sediments in 105 mm (i.d.) opaque PVC tubes impervious to light (Lauer, 2005). The cores used for determination of nutrient fluxes were fitted with a bottom seal with double O-rings and a top seal with a single O-ring. Three to six replicates were transferred into an incubation system for determination of nutrient fluxes. These cores had a visibly undisturbed sediment surface, a minimum of 800 mL of clear overlying *in situ* bottom seawater and at least 10 cm depth of sediment. Two additional cores used for the determination of water content were sealed with rubber bungs and refrigerated (4 °C) for up to 3 h before transfer to the laboratory.

Sediment traps were placed 1 m above the seafloor. Each trap consisted of two PVC tubes with a height to width ratio of 4.7 (height 400 mm, diameter 85 mm), lead weighted on the bottom (40 g) to ensure correct vertical orientation. Three lots of sediment traps separated by 30 m were deployed at each site. This design gave us sedimentation rates at 0, 30 and 60 m from the edge of the pens with two replicates per distance. In order to estimate the area affected by sedimentation around the pens, we deployed a second line of sediment traps from one of the pens in May. This additional line gave us sedimentation rates at 100, 130 and 160

m from the edge of the selected pen. Sediment traps were moored for at least 48 h but no more than 96 h. Upon retrieval, traps were spiked with $HgCl_2$ to a final concentration of 10 mg L^{-1} to prevent microbial degradation.

9.2.3. Analytical procedures

Feed wastes

Samples (n = 6) used for the determination of water content were oven dried at 105 ^{o}C for at least 16 h and weighed using an electrobalance to five decimal places. Whole baitfish were minced in a glass blender, freeze dried and homogenised in a coffee grinder. Aliquots (n = 9) were weighed into foil capsules and analysed by Continuous-Flow stable Isotope Ratio Mass Spectrometry (CF IRMS) using a Europa Scientific ANCA-SL elemental analyser coupled to a Geo 20-20 Mass Spectrometer. TN concentrations are reported in mg g^{-1} dry weight (dw).

Sediments

Sediment samples were freeze-dried, sieved to 500 μm to remove large shell fragments, and homogenized with a mortar and pestle. TN was determined as described above for feed wastes and reported as a percentage of total dry sediment. The samples collected for determination of wet density were weighed and results are reported as g (wet weight) cm^{-3}. The samples collected for determination of water content were extruded onto aluminium foil, the top 0-1 cm sectioned, homogenised and any visible macroinfauna removed. 30 g of each homogenised section was transferred to an aluminium tray and oven-dried at 60 ^{o}C until constant weight. Results are expressed as the percentage of water in the wet sediment.

Sedimentation fluxes

The contents of sediment traps were sieved (1 mm mesh size) to remove material not part of the passive flux (e.g. mobile organisms such as zooplankton). Sieved samples were vacuum filtered through pre-combusted (450 ºC overnight) and pre-weighed glass-fibre filters (MFS GF-75, 0.7 μm, 47 mm diameter). Filters were placed in separate pre-combusted glass petri dishes, covered with a glass lid and oven dried at 50 ºC. Before gravimetric analyses, the petri dishes containing the dried filters were placed in an oven at 50 ^{o}C for at least 3 h and placed in a desiccator with silica gel for 1 h to cool. The filters were then weighed using an electrobalance to five decimal places. Results were corrected for salt that impregnates the filters and sedimentation rates expressed in units of g (dw) m^{-2} d^{-1}. The material collected in the traps deployed at the edge of the pens was gently scraped off the filters with a spatula, homogenized in a mortar and pestle, and analysed for TN according to the method described above for feed wastes. Nitrogen sedimentation rates were calculated using average sedimentation rates and nitrogen contents for each site and sampling time, and are expressed in units of mg N m^{-2} d^{-1}.

Benthic fluxes

Nutrient fluxes were measured on board with a manually operated incubation system immediately after sampling (Lauer, 2005). Incubations were designed to last for 2 to 4 hours to limit the likelihood of non-linear nutrient changes. The incubation system consisted of two

temperature sensors connected to a data logger (DT 50, Datataker), six seawater stirrers to prevent stratification (single blade, 7 mm wide, 4 mm long) and a temperature-controlled water bath, thus allowing up to six sediment cores to be incubated at once. The magnetic stirrers were fitted with an O-ring and penetrated through the top seal of the cores. The water bath consisted of a 200 L PVC outer container filled with ice and a 60 L polystyrene inner container filled with freshwater. The PVC barrels sealed at both ends were placed in the inner container, which had a pump to circulate the water and an aquarium heater to maintain water temperature. The temperature was set at ambient bottom seawater temperature measured at the time of collection. The system maintained temperatures to ± 0.8 ºC of the set value. Samples of the overlying seawater were taken in duplicate from each core at the start and end of the incubation, filtered (0.45 µm) and stored frozen (-30 ºC). Nutrient fluxes were determined from the change between initial and final concentrations in the overlying seawater. Nitrates/nitrites (NO_x) were determined spectrophotometrically at 520 nm by flow injection analysis with a QuickChem 8000 Automated Ion Analyser (APHA-AWWA-WPCF, 1998b). Ammonium was also determined spectrophotometrically by flow injection analysis using the automated phenate method with detection at 630 nm (APHA-AWWA-WPCF, 1998c). The duplicate samples were averaged and the change in nutrient concentration was then adjusted to account for the sediment surface area, the duration of incubation, and the volume of overlying seawater to determine the rate of nutrient release or uptake. The resultant rate of change is expressed in units of mg N m^{-2} d^{-1}.

9.3. Model development

9.3.1. Feed input

Total and daily feed inputs were calculated using the amount of baitfish fed to each pen per day (data supplied by individual farmers) and the average water and nitrogen contents of feed. Feeding occurred on most days (Table 9.1). The feeding rates employed by the farmers were as low as 1 % of body weight in winter and as high as 15 % in the first month of stocking. Mean baitfish TN contents were 111 ± 12 mg N g^{-1} dw whereas mean water contents were 72 ± 7 %. The average nitrogen input from fish feed varied between 78 and 91 kg N d^{-1} depending on the operator, the cumulative value over the season reaching 10-14 tonnes N. These modelled ranges of nitrogen feed input as well as SBT retention, excretion and environmental flows are depicted in Figure 9.1 as the minimum and maximum values observed for both pens.

Although the fraction of uneaten feed varied between 4 and 23 % in the first six years of the industry (Bruce, 1997), current video footage under the pens suggests much lower values. We used an estimate of 3 % of the total feed input for this component of the budget (Jeff Buchanan, personal communication). This corresponds to an environmental loss of 2-3 kg N d^{-1}, or 0.3-0.4 tonnes N over the season.

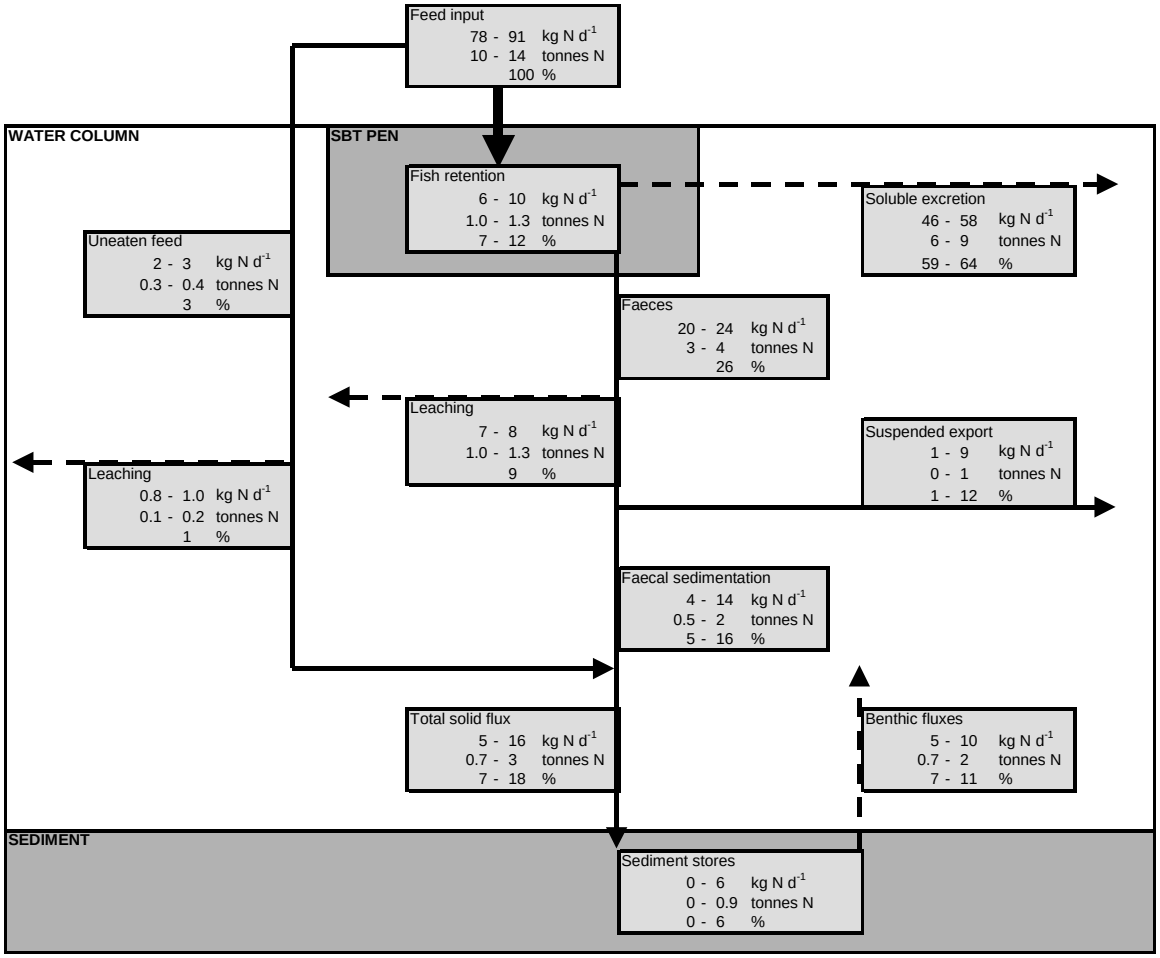

Figure 9.1. Model of environmental flows for nitrogen supplied with feed to a SBT pen in coastal waters off Port Lincoln. Values correspond to the range calculated for two commercial pens and are reported as daily (kg N d-1) and season (tonnes N) totals, as well as a fraction of total feed inputs (%). Particulate flows are depicted as solid arrows and dissolved flows as dashed arrows.

9.3.2. Fish retention and excretion

In order to calculate daily retention of nitrogen by SBT, we had to estimate the amount of nitrogen in fish biomass for each day of the season based on fish weights, water and nitrogen contents. Although there is some evidence in the literature suggesting that weight gain occurs primarily in the early stages of the SBT season (Smart, 1996; Glencross et al., 2002b), no growth curves are available for this species reared in commercial pens. As a consequence, SBT weight was estimated on a daily basis according to the specific growth rate (*SGR*) calculated from the mean initial (W_i) and final weights (W_f) for the season:

$$SGR = \frac{\ln W_f - \ln W_i}{t} \qquad [1]$$

where *t* corresponds to time in days. *SGR* for the whole season was 0.41-0.48 % weight gain per day (Table 9.1). We assumed that the protein content of SBT remains constant during the season at 23%, while water content follows a linear decrease from 66 to 57% between stocking and harvest to compensate for fat deposition (David Ellis and Robert van Barneveld, personnal communication). These results lead to a decline in nitrogen contents from 106 to 84 mg N g^{-1} dw over the farming season. Using these values, nitrogen retained daily for growth was calculated as the change in the total amount of nitrogen in each pen for any specific day in comparison to the previous day. Retained nitrogen averaged 6-10 kg N d^{-1} over the season. The cumulative value was estimated as the difference between the nitrogen recovered at harvest and with recorded mortalities, and the nitrogen in the initial stock. The cumulative value retained for growth over the season accounted for 1.0-1.3 tonnes N.

To calculate faecal nitrogen excretion, we used protein digestibility values. Preliminary results from current *in vivo* trials indicate that SBT digestibility of protein in pellet feeds might be as high as 90 % (Buchanan & van Barneveld, 2004). Previous studies have reported values as low as 38 % for protein in experimental fish meal feed (van Barneveld et al., 1997), while *in vitro* trials using SBT crude enzymes suggest that digestibility of baitfish protein fed to SBT varies between 73 and 83 % (Carter et al., 1999). We used the lowest value found in the literature for baitfish feed (73 %) to calculate maximum rates of faecal excretion. Therefore, nitrogen released with faeces was estimated as 27 % of nitrogen in ingested feed, with an average of 20-24 kg N d^{-1} leading to a total of 3-4 tonnes of faecal nitrogen over the season. We assumed that the amount of ingested nitrogen that is not retained for growth, or excreted with faeces, was metabolised and excreted as soluble excretion products. This corresponded to 46-58 kg N d^{-1}, with a season total of 6-9 tonnes N.

9.3.3. Leaching, dispersion and settling of wastes in the water column

Fernandes et al. (submitted-a) showed that approximately 41 % of nitrogen in baitfish and 35 % in faeces is soluble and available to leach into seawater. These authors also reported that 86 % of the soluble nitrogen in baitfish, and all of the soluble nitrogen in faeces, would enter the water column during the time these wastes take to settle to the seafloor in a 20 m water column typical of SBT farms. We used these values to estimate the amount of nitrogen that would leach from solid wastes into the water column before deposition. The combined daily average for leaching from both uneaten feed and faeces was 8-9 kg N d^{-1} and the season total 1.1-1.5 tonnes N.

The first step in determining the flux of nitrogen settling through the water column and reaching the sediments is to estimate the area of impact of sedimentation fluxes from the pens. Gowen & Bradbury (1987) suggested that the radius of impact around fish pens (*D*) can be determined from the following equation:

$$D = \frac{d \times V}{v} \qquad [2]$$

where *d* is water depth, *V* is current speed and *v* is settling velocity of the waste. The radius of impact calculated using this equation is 100 m assuming an average water column depth of 20 m, current speeds in the order of 10 cm s^{-1} (Bierman et al., 2005), and maximum faecal

settling velocity of 2 cm s^{-1} (Fernandes et al., submitted-a). The settling velocity of faeces was used as a proxy for the settling velocity of wastes because it is much lower than the settling velocity of baitfish and therefore will give the maximum area of impact. While we observed no significant decrease in sedimentation rates up to 60 m from the pens, data from an extra line of sediment traps deployed in May indicated that sedimentation rates dropped to background values between 60 and 100 m from the pens. For modelling purposes, we thus considered the area affected by sedimentation of farm wastes extending up to 100 m from the edge of the pens. We then calculated the average daily input of nitrogen with feed from the day prior to deployment until the day of retrieval of sediment traps using daily values of feed input. This average was used to estimate the fraction of the total input accounted for by sedimentation fluxes in the area of impact. Average sedimentation rates varied from 58 to 215 mg N m^{-2} d^{-1} in the footprint of stocked pens (Table 9.2). Considering the radius of impact up to 100 m from the pens, these sedimentation rates accounted for a maximum of 5 to 16 % of feed inputs depending on pen location. As large pieces of uneaten feed were never recorded in the material intercepted by sediment traps, we assumed that the geometry of the traps precluded the collection of the majority of uneaten feed debris so that sedimentation rates accounted primarily for faecal sedimentation. The maximum amount of nitrogen sedimenting with faecal matter was then estimated considering that 5 or 16 % of nitrogen in daily feed inputs will reach the seafloor depending on the pen under consideration. These faecal sedimentation fluxes delivered 4-14 kg N d^{-1} to the sediments up to 100 m from the pens, or 0.5-2 tonnes N over the season.

Table 9.2. Mean sedimentation and benthic fluxes (± SD) measured at pens P1 and P2.

	Sedimentation fluxes (mg N m^{-2} d^{-1})				**Benthic fluxes (mg N m^{-2} d^{-1})**			
	P1		**P2**		**P1**		**P2**	
February[a]	190	(111)	82[b]		201	(42)	48	(18)
May	58	(4)	141	(58)	127	(58)	66	(69)
July	215	(14)	62	(11)	3,351	(5,476)	242	(203)

[a]P2 was not stocked in February.
[b]Only one sample retrieved in February.

To calculate the amount of faecal inputs dispersed away from the area of impact by currents, we assumed that the difference between the total faecal input and the amount leached into the water column, and settling to the seafloor, was exported out of the system as fine suspended matter. Suspended matter exports out of the system varied between 1 and 9 kg N d^{-1} depending on farm location, with the seasonal total reaching a maximum value of 1 tonne N.

Although large pieces of uneaten feed were never recorded in the particles intercepted by sediment traps, these were sporadically observed in underwater footage of the seafloor in the vicinity of the pens. These observations lead us to assume that the total flux of solid wastes reaching the seafloor can be calculated as the sum of uneaten feed (after leaching) and faecal sedimentation if we ignore the consumption of uneaten feed by wild scavenger populations. As a consequence, the maximum amount of nitrogen delivered to the sediments with solid wastes amounted to 0.7-3 tonnes N over the season, with 5-16 kg N delivered daily.

9.3.4. Remineralization and accumulation of wastes in the sediments

The sum of ammonia and NO_x benthic fluxes was used to estimate total inorganic nitrogen fluxes from the sediments, with average values varying between 66 and 3,351 mg N m^{-2} d^{-1} in the footprint of stocked pens (Table 9.2). Benthic incubations were always aerobic, and only on a few occasions did fluxes reach values above 270 mg N m^{-2} d^{-1}. These unusually high values were invariably accompanied by uneaten baitfish pieces at the surface of the collected sediment core. The settling rate of uneaten baitfish (8 cm s^{-1}) is significantly higher compared to faeces (2 cm s^{-1}) (Fernandes et al., submitted-a), indicating that uneaten feed will disperse over a smaller area around the pens. Using Equation 2 above, uneaten baitfish will disperse up to a maximum of 25 m from the edge of the pens as opposed to the 100 m estimated for faecal matter. Therefore, sediments in the inner zone under and in the immediate vicinity of the pens will receive uneaten feed and faeces wastes, while the outer zone from 25 to 100 m from the pens will receive only faeces. We averaged all values of benthic fluxes measured at each site and sampling period and assumed for modelling purposes that these rates remained constant up to 25 m from the edge of the pens. We then assumed that sediments in the area between 25 and 100 m from the pens released lower amounts of nitrogen into the water column, or the average of sediment fluxes below 270 mg N m^{-2} d^{-1}. The daily release of nitrogen from sediments to the water column calculated in this way was relatively constant between February and May but peaked in July towards the end of the season (Figure 9.2). Lipid signatures in the sediments corroborate the idea that microbial biomass builds up over the season to attain maximum levels in July (John Volkman and Milena Fernandes, unpublished results). With the lack of additional data points, we assumed that values increased exponentially between May and July and remained constant up to harvest. Based on these assumptions, the daily amount of nitrogen remineralised in the sediments and released with benthic fluxes averaged 5-10 kg N d^{-1} over the season, with a cumulative total of 0.7-2 tonnes N.

The amount of nitrogen that actually accumulated in the sediments at the end of the season was estimated from the difference between the total reaching the seafloor with solid fluxes and the amount released from the sediments with benthic fluxes. Using this approach, less than 1 tonne N accumulated in sediments up to 100 m from the pens during the season, with a daily average of less than 6 kg N d^{-1}.

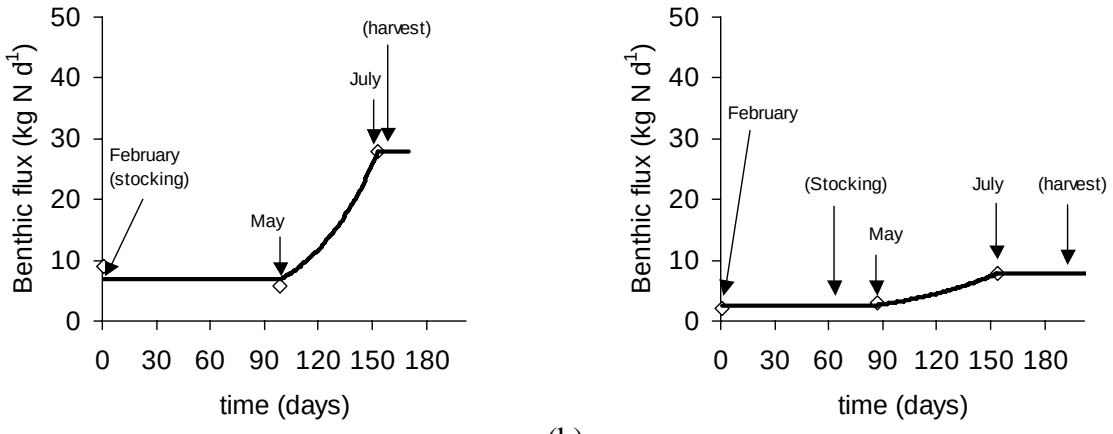

Figure 9.2. Predictive evolution of benthic fluxes of total inorganic nitrogen over the SBT stocking season (bold line) against values measured in the field in February, May and July (diamonds) for pens P1 (a) and P2 (b).

9.4. Discussion

The fraction of nitrogen retained for growth was overall small, varying between 7 and 12 % of feed inputs depending on the operator. Values for other well-studied aquaculture species are usually much higher (e.g. 20-40 % for salmonids) (Table 9.3). These low values of nitrogen retention for SBT are not surprising considering that the metabolic rates of tuna are at least three fold higher than in other species (Korsmeyer & Dewar, 2001). The high standard metabolic rates of tuna support an increased aerobic capacity and allow these fish to have faster metabolic functions than other species. The heat produced by metabolic functions is used to maintain comparatively high body temperatures necessary to enhance metabolic processes and power continuous and occasionally intense bursts of swimming activity (Graham & Dickson, 2001). It is often mentioned that tuna are "energy speculators" in that they maximize energy intake (rather than minimize expenditure) to increase the ratio between energy input and output (Stevens & Neill, 1978). The high metabolic rates in tuna lead to their high cost of locomotion (through higher swimming speeds), feed digestion and growth. Farmed SBT have the advantage over wild fish of spending no (or less) energy on recovery from short periods of high intensity swimming, and reproduction. For wild populations, it is estimated that less than 5 % of aerobic energetic costs are actually spent on growth (Korsmeyer & Dewar, 2001). As tuna mostly rely on protein for energy production (Aguado et al., 2004; Aguado-Giménez & García-García, 2005), these physiological adaptations would help explain the low values of nitrogen retained for growth modelled in this work. The fact that farmed SBT are generally much larger fish than other aquaculture species (2 to 4 years old juveniles weighing 16-20 kg at capture) (Jeffriess, 2004; Farley & Basson, 2005), would also act to reduce protein retention as nutrient utilization is known to be less efficient with older and/or larger fish (Lupatsch & Kissil, 1998; Strain & Hargrave, 2005).

The high enzymatic activity and turnover associated with the high metabolic rates of tuna would also explain why soluble excretion products accounted for the largest environmental loss, releasing 59 to 64 % of nitrogeneous feed inputs directly into the water as soluble excretion products. Similar values have been reported for other carnivorous fish fed baitfish, such as areolated grouper (*epinephelus areolatus*) (46-64 %) (Leung et al., 1999). In comparison to direct metabolism, leaching from solid wastes during settling to the seafloor and remineralization at the sediment-water interface delivered similarly small amounts of nitrogen to the water column, or a total of 17-22 % of feed inputs. When combined with branchial and urinary excretion losses, these flows indicate that most (76-86 %) of the nitrogen in feed inputs is released directly into the water column in dissolved form (Table 9.3). Although dissolved wastes greatly outweigh particulate wastes, the former are likely to represent a smaller fraction of total loads to the environment at the beginning of the season when retention in fish tissues is highest and benthic fluxes have not yet peaked.

Particulate wastes accounted for only a small fraction of environmental losses, between 8 and 12 % of feed inputs (Table 9.3). These losses occurred mostly through sediment accumulation (maximum 6 %) or export out of the system with current flows (maximum 12 %). The balance between sediment accumulation and particulate export was site specific, e.g. pen P2, characterized by comparatively lower sedimentation rates, had high rates of export of particulate matter but hardly any accumulation on the seafloor. Environmental conditions that promote particulate export might also play a part in the lower FCR achieved at this site, as higher flushing rates might be beneficial to the health and performance of stocks.

Table 9.3. Partition of nitrogen feed input into retention, soluble and particulate waste streams, and nitrogen loads per tonne of SBT production, as compared with data for some other species.

Species	Fate of nitrogen in feed inputs (%)			Nitrogen loads (kg N tonne^{-1} growth)			Diet	FCR	Source
	Fish retention	Solid wastes	Soluble wastes	Solid	Soluble	Total			
SBT	7	8	86	40	462	502	Baitfish	4.9[a]	This work
SBT	12	12	76	35	224	260	Baitfish	2.7[a]	This work
Areolated grouper	12	42	46	153	168	321	Baitfish	---	(Leung et al., 1999)
Yellowtail	---	---	---	---	---	177	Baitfish	2.3[a]	(Watanabe et al., 1993)
Yellowtail	---	---	---	---	---	265	Pellets	3.1[a]	(Watanabe et al., 1993)
Atlantic halibut	30	19[b]	50[b]	18	48	66	Pellets	1.3	(Davies & Slaski, 2003)
Atlantic salmon	38	15[b]	47[b]	14	43	57	Pellets	1.2	(Gillibrand et al., 2002)
Atlantic salmon	40	26[b]	34[b]	9	33	42	Pellets	1.1	(Strain & Hargrave, 2005)
Salmonids	20	28	52	43	80	123	Pellets	2.0	(Gowen & Bradbury, 1987)
Salmonids	28	16[b]	56[b]	17	61	78	Pellets	1.5	(Ackefors & Enell, 1990)
Rainbow trout	25	21	54	28	73	102	Pellets	2.1	(Hall et al., 1992)
Rainbow trout	19	7	74	11	114	125	Pellets	1.8	(Foy & Rosell, 1991b; Foy & Rosell, 1991a)
Seabass	---	---	---	---	---	65[c]	Pellets	1.2	(Kaushik, 1998)
Seabream	---	---	---	---	---	52[c]	Pellets	1.1	(Kaushik, 1998)
Gilthhead seabream	22	10[b]	68[b]	13	90	103	Pellets	1.8	(Lupatsch & Kissil, 1998)

[a]dry weight feed/wet weight gain.
[b]these values do not account for remineralization of solid wastes at the sediment-water interface.
[c]maximum estimates.

For both pens, between 40 and 80 % of nitrogen faecal inputs did not reach the seafloor and were lost through leaching into the water (35 %) and export of suspended matter out of the system (5 or 45 % depending on pen location). It is likely that the proportion of faeces exported out of the system with suspended matter is even larger than the values reported here. Fernandes et al. (submitted-a) observed that SBT faeces are fibrous and have a low density. These authors estimated that 62 % of faecal matter would remain in suspension for more than 35 min in 20 m of still seawater. Similarly, Vita et al. (2004) observed that the density of farmed Atlantic bluefin tuna (*Thunnus thynnus*) faeces is comparable to the density of seawater, making tuna faeces more "soluble" than faeces of other fish species. Adding to these observations, it has been suggested that fish faeces disintegrate and absorb water as

they settle so that settling velocities continually decrease during transport (Chen et al., 1999a). If the amount of nitrogen exported out of the system with suspended faecal matter is significantly higher than the value modelled here, faecal production would need to be adjusted to a higher value to balance the sedimentation rates measured, suggesting that the digestibility of protein in baitfish might be less than the value of 73 % used here. This would also mean lower soluble losses, as a higher faecal production would reduce the excretion of soluble metabolic products.

Despite significant losses to the water column, faeces account for the majority (73-89 %) of nitrogen reaching the seafloor. The amount of nitrogen transported to the benthos with uneaten feed is comparatively much smaller. The value of 3 % used for feed wastage is low in comparison to some reports of other aquaculture industries using baitfish as feed (e.g. areolated grouper, up to 46 %) (Leung et al., 1999), but similar to more recent values for marine fish fed manufactured diets (5 %) (Gillibrand et al., 2002; Davies & Slaski, 2003; Doglioli et al., 2004). Underwater trials using a video platform with baitfish suggest that scavengers (mostly fish) in lower Spencer Gulf quickly consume uneaten feed on the seafloor (Chapter 8 of this report). The use of underwater cameras in our study made it clear that uneaten baitfish is not a prominent feature under the pens, except towards the end of the season when sparse accumulation was evident, concurrent with an increase in benthic nutrient fluxes. Whether these results are related to slower SBT metabolism during winter leading to lower consumption rates and an increase in the flux of uneaten feed, or as a result of seasonal changes in scavenging populations, it is not clear. In any case, the amount of uneaten feed calculated here corresponds to the maximum reaching the seafloor and should be taken as the upper value for this component of the model.

The sediment accumulation rates within a 100 m radius from the pens calculated in the model are small, less than 0.9 tonnes N (or 6 % of feed inputs), suggesting low localized impacts as a result of a significant export of particulates by currents and a fast turnover of organic matter in the sediments. Although small, these sediment accumulation rates were slightly higher than calculated using nitrogen contents measured in the sediments over the course of the stocking period. The latter marginally increased in the footprint of the pens towards the end of the farming season (Table 9.4). Using the values of TN, water content and wet density measured in July, we calculated the amount of nitrogen in sediments of the impacted area up to a 100 m from the pens assuming that the depth of impact is restricted to the first cm of the sediment profile. Subtracting from this value the average amount of nitrogen in sediments during February and May, we find that the accumulation of TN varied between 0.04 and 0.1 tonnes N. If we increase the depth of impact to 5 cm, maximum values are still below 0.5 tonnes N. Sediment resuspension and consumption of uneaten feed by scavengers might explain why accumulation measured *in situ* was lower than that predicted by the model. If scavengers consumed all of the uneaten feed and we excluded this component from the solid flow reaching the sediments, maximum rates of sediment accumulation in the model would be reduced to 0.6 tonnes N (less than 4 % of feed inputs). The difference between this value and maximum *in situ* accumulation might thus be related to resuspension, which would affect at least 10 % of nitrogeneous wastes reaching the seafloor, possibly much more.

According to the flows modelled above, more than 85 % of nitrogen in baitfish fed to SBT is expected to be lost to the environment, with total environmental loads varying between 260 and 502 kg N tonne^{-1} growth depending on the operator (Table 9.3). The high FCR values measured in this work (2.7 and 4.9 on a dry matter basis) would partially explain these high loads modelled for SBT. Typical FCR values across the SBT industry in South Australia are

reported to vary in a narrower range of 3-4 (David Ellis, personal communication) and therefore average nitrogen loads are expected to be skewed towards the minimum values reported here. Although high, these FCR values for SBT are lower than reported for other tuna species such as Atlantic bluefin tuna, where values are generally between 6 and 8 (Aguado et al., 2004; Aguado-Giménez & García-García, 2005).

Table 9.4. Mean (± SD) total nitrogen, wet density and water content of sediments collected under pens P1 and P2.

	Total nitrogen (%)		Wet density (g cm^{-3})		Water content (%)	
	P1	P2	P1	P2	P1	P2
February[a]	0.08	0.13	1.65	1.66	31	41
	(0.01)	(0.01)	(0.02)	(0.01)	(1)	(0.0)
May	0.08	0.12	1.72	1.63[b]	32	37
	(0.01)	(0.01)	(0.01)	(---)	(1)	(0.2)
July	0.11	0.18	1.65	1.59[b]	44	36
	(0.03)	(0.03)	(0.08)	(---)	(13)	(2)

[a]P2 was not stocked in February.
[b]Only one sample was available for measurement of wet density.

High FCR values are generally associated with the use of baitfish as feed (Gowen & Bradbury, 1987; Wu, 1995). As a consequence, carnivorous species fed baitfish have higher nutrient loads to the environment (> 170 kg N $tonne^{-1}$ growth) compared to other species fed manufactured diets (40-125 kg N $tonne^{-1}$ growth) (Table 9.3). However, FCR alone cannot account for these differences in nutrient loads, as it does not reflect assimilation of individual nutrients. In order to establish the effect of FCR alone, we calculated the hypothetical loads for salmonids using the same FCRs found for SBT in this work, based on models proposed by Ackefors & Enell (1990), Talbot & Hole (1994) and Islam (2005). Based on these models, maximum nitrogen loads for salmonids would be 198 and 387 kg N $tonne^{-1}$ growth for an FCR of 2.7 and 4.9, respectively. These loads are 23-24 % lower than the loads for SBT at equivalent FCR values, corroborating the idea that factors other than FCR will affect nitrogen losses to the environment. Among these, the high metabolic rates of tuna and the lower ratios of digestible protein to energy in baitfish compared with manufactured pellets might play a significant role.

Using the loads calculated above, and considering a production of 4,380 tonnes of SBT over initial stocked biomass, the total environmental release of nitrogen in the farming zone during the SBT stocking season would vary between 1,137 and 2,200 tonnes N depending on FCR. For comparison, the maximum annual discharge of nitrogen from salmon farms in the Bay of Fundy, Canada, occupying a much larger area compared to SBT farming in South Australia, varies between 1,816 and 1,870 tonnes (Strain & Hargrave, 2005). Considering an average FCR across the SBT industry of 2.7, and that between 86 to 92 % of these inputs would be delivered in dissolved form, a minimum of 983 tonnes of dissolved N and 154 tonnes of particulate N will be lost to the environment as a result of SBT farming. To put these data in perspective, the nitrogen standing stock in the water column of the SBT farming zone is only 310 tonnes N taking into account the 172 km^2 allocated for farming, an average water depth of 20 m and background total nitrogen concentrations of 0.09 mg L^{-1} (Lauer, 2005). However, the magnitude of flux rates between natural nitrogen pools in the system is not known. In terms of other anthropogenic inputs to the region, the local Port Lincoln wastewater treatment plant, which serves a population of 11,500, discharges 9.2 tonnes N per

year into the marine environment (SA Water, 2003), while the numbers associated with fish processors discharging into Proper Bay and stormwater from Port Lincoln are 23.8 and 3.6 tonnes N per year (as organic nitrogen and ammonia), respectively (Sinclair Knight Merz Pty Limited, 2001). The impact of these loads from aquaculture in a regional context is subject of current research by the Aquafin CRC. Earlier monitoring studies suggest low or undetectable impacts in the farming area (for a review see Fernandes et al., 2006b).

9.5. Conclusions

The loads calculated here constitute a first estimate of nitrogen losses from SBT farming in South Australia using actual management and environmental data. Although the model relies on many simplifications, the level of uncertainty of total loads is small as these were based on inputs (stock and feed) and outputs (mortalities and harvested fish) supplied by the industry. The calculation of daily loads during the course of the season would greatly benefit from more information detailing how feed intake, growth and body composition vary with size of fish and water temperature. This level of detail would allow calculation of loads on more suitable time scales (e.g. months) to pinpoint periods of increased susceptibility of disturbance to natural processes.

Nitrogen losses per tonne of SBT growth using a diet of baitfish were found to be more than double values for other aquaculture species fed manufactured pellets. Some reduction of nitrogen loads could be achieved by improving feeding strategies and associated technologies, and producing diets with an optimal protein/energy ratio so that a significant proportion of energy requirements are met by non-protein sources. However, these strategies are unlikely to achieve drastic reductions in nitrogen loads comparatively to other aquaculture species given the unique metabolic characteristics of tuna.

The extremely high metabolic rates of these endothermic fish not only account for high nutrient loads to the environment, but also to a different partition between solid and dissolved wastes. High rates of nitrogen excretion in urine and through the gills explain the low nitrogen retention in fish tissues, high losses of dissolved wastes to the water column and proportionally lower losses as faecal matter. These processes, combined with the low settling velocity of tuna faeces, and the effects of scavenger feeding, lead to minimal impacts to the benthos. The nature of the wastes (dissolved or faecal with low settling velocity) however, suggests that these will not be confined to the footprint of the pens and might spread over a large area. Potential regional effects were considered insignificant in earlier government regulatory environmental monitoring studies. These are now being considered through a follow up research project of the Aquafin CRC.

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Chapter 10: Effects of fallowing on macrobenthic assemblages in sediments adjacent to southern bluefin tuna cages

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Abstract

This study focuses on the effect of fallowing of southern bluefin tuna farms in southern Spencer Gulf, South Australia, on macrobenthic assemblages, comparing spatial and temporal patterns of distribution and abundance at eight control sites and eight fallowed pontoon sites, during the period October 2002 to October 2003. Two stations at each site were sampled five times throughout the year with four replicates. Polychaetes were the most abundant organisms, both at control sites (76.4%), and fallowed pontoon sites (80.5%). Surface deposit feeders dominated both control and fallowed sites, followed by carnivores. The relative abundance of sub-surface deposit feeders (mostly capitellid polychaetes) increased gradually as a function of percent organic carbon, but corresponding biomass decreased markedly owing to the dominance of small-body size opportunistic taxa. However, there was no reduction in trophic diversity as organic carbon increased. Lumbrinerids and spionids are suggested to be the best taxa for assessing the level of disturbance at fallowed sites. Univariate and multivariate analyses were used to assess patterns and rates and degree of recovery. Most of the variation in both the infaunal and sediment parameters analysed occurred at the site level (hundreds of square meters), and thus few differences could be detected between zones (Boston Island *versus* Rabbit Island), or between fallowed and control sites. However, more detailed examination of patterns of variation over time suggests that there are differences between fallowed and control sites, with most fallowed sites not showing the consistent temporal patterns seen at the control sites. Multiple *k*-dominance curves and Abundance Biomass Curve (ABC) plots were employed to assess the level of disturbance between sites over time. A general trend of separation between control and fallowed pontoon sites was more apparent using second-stage than first-stage of the dendrogram and MDS plots. The rates and degree of recovery of the assemblages are discussed. Seasonal fluctuations caused by natural variability, hydrodynamic conditions, sediment characteristics, and organic carbon are likely to be responsible for the observed changes of the assemblages over the study period.

10.1. Introduction

Despite its benefits, fish farming characteristically involves enclosing fish in high densities, which results in discharge of waste products (Naylor et al., 2000) and tends to cause organic enrichment (Cheshire, 1996; Emerson, 1999; Lorenzen et al., 1997; Merceron et al., 2002; Pawar et al., 2001; Yokoyama, 2002). Fish farms generate large amounts of solid wastes in the form of faeces and uneaten feed. These materials are generally deposited in the area surrounding the culture structure (Cho & Bureau, 2001; Pawar et al., 2001). On average, it is estimated that 80% of the feed is deposited, constituting 20% excess feed, 10% fish excretions as faeces and 50% as urine (ammonia) (Cheshire, 1996; Yokoyama, 2002). As a result, for every 1 kg production of salmon in a coastal area, 0.5 – 0.7 kg of particulate organic waste is generated. The deposition of organic matter eventually can result in physical and chemical changes in the natural sediments including decreased redox potential, increased sediment oxygen consumption, increased concentrations of total volatile solids, total organic carbon, sulphides, nitrogenous compounds and phosphates (Weston & Gowen, 1988; Cheshire, 1996). The extent of nutrient waste from aquaculture is influenced by feed ingredients and uptake efficiency, fish density, and location and design of cage facilities (Naylor & Burke, 2005).

Accumulation of aquaculture waste can result in qualitative and quantitative changes in benthic environments. The accumulation of organic-rich sediments under culture facilities and the consequent depletion of oxygen in the sediment porewater may result in changes in the infaunal assemblages. The depletion of oxygen is mainly caused by increased consumption by bacteria and other organisms for organic waste degradation. Opportunistic species that are resistant to low oxygen may attain numerical dominance (Cheshire, 1996; Cho & Bureau, 2001). For example, Weston & Gowen (1988) reported dramatic changes in the macrofaunal assemblages in the Calm Bay salmon farm, Washington, which became dominated by opportunistic species.

Southern bluefin tuna sea-ranching in southern Spencer Gulf occurs in oligotrophic waters where the bottom substrate generally consists of sandy sediments exposed to relatively strong tidal currents. In growing 5,265 t of tuna to about 9,000 t in cage farms, at least 45,000 t of feed in the form of Australian sardines and other baitfish is added. This implies that about 3,600 t of carbon is released to the environment (0.2 conversion of wet weight/dry weight, 40% carbon). This carbon results in an environmental load and impacts on sedimentary habitat, the composition of which is one of the most important factors in structuring macrobenthic infauna assemblages (Dernie et al., 2003).

In Port Lincoln, tuna farmers have developed a system of growing out tuna since 1992. Fish are purse-seined and towed to Port Lincoln where they are transferred to floating cages (pontoons). During the growing period, a diet consisting mostly of sardines is used for three to six months before harvest. Tuna farms operate from December to August and are fallowed during the remaining period. Rotation of cage positions allows for an approximately 2 year fallowing period.

The study presented here is a comparison of structural variables of the benthic assemblages at fallowed pontoon sites and control sites at two localities off Port Lincoln, east of Boston Island and Rabbit Island, respectively. The study focuses on the effect of fallowing southern bluefin tuna farms on the macrobenthic assemblage, comparing spatial and temporal patterns,

and allows conclusions to be made about the effectiveness of fallowing as a waste mitigation strategy.

10.2. Materials and methods

10.2.1. The study sites

The sampling sites were located between 135° 58.25' to 135° 59.82' E and 34° 35.41' to 34° 42.43' S, in southern Spencer Gulf, South Australia, where farming of southern bluefin tuna (*Thunnus maccoyii*) takes place (Figure 10.1). The farms consist of a series of pontoons 40-50 m in diameter, with a 15 m deep net. Pontoons are stocked at rates of 1.5 – 2.5 kg m^{-3} (Cheshire et al., 2005), and are situated in areas with relatively strong microtidal (<2 m) currents with an average current velocity of 5-10 $cm.s^{-1}$ (Petrusevics, 1993; Bierman, 2005). The seawater temperature fluctuates from 14°C in winter to 25°C in summer (Edyvane, 1997). Fallowed sites were sampled through the 2002 farming season after all fish and pontoons had been removed. Control sites were at least 1 km from any lease site.

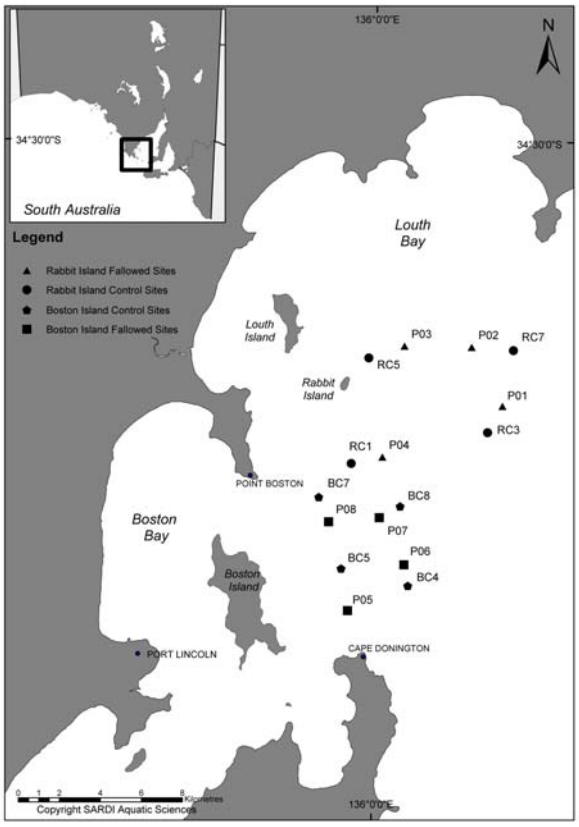


Figure 10.1. Map of sampling sites representing eight control sites and eight fallowed pontoon sites adjacent to Rabbit Island and Boston Island. P= pontoon site; C= control site; B=Boston Island; R=Rabbit Island.

10.2.2. Sampling procedures

Samples were collected five times during the period from October 2002 to October 2003. Sediment samples were taken using a HAPS bottom corer equipped with a corer of 67 mm in diameter and 315 mm in length, operated from the research vessel RV Ngerin (Figure 10.2). The depth of sediment collected varied between 25 – 75 mm (mean = 40.9 mm) at control sites and between 22 – 85 mm (mean = 44.9 mm) at fallowed sites. Cores were collected at eight fallowed pontoon and control sites: four each within the Rabbit Island and Boston Island aquaculture zones (Figure 10.1).

Figure 10.2. Sediment samples collected by using a HAPS corer operated on the research vessel Ngerin. Inset: the coarse sediment collected by the corer at a control site.

From the two zones of sampling located adjacent to Boston Island and Rabbit Island, 160 cores were collected for each sampling time. At each fallowed pontoon site, the first sampling station was located 10 m towards the "centre" from the edge of where the pontoon was located, and the second was a further 25 m (i.e. 5-15 m from the opposite edge). At each of these two sampling stations, 4 replicate cores were collected (64 cores in total). The same sampling strategy was used for each control site (64 cores in total). An additional sediment core was taken at each station for sediment grain size analysis (32 cores). During October 2002, an additional four samples were collected at each station to determine sediment chemical characteristics.

Water quality measurements were carried out using a Horiba multi-probe and a Seabird Conductivity-Temperature-Depth (CTD) profiler. The parameters recorded were pH, salinity, and temperature measured just above the sea bottom and at the surface (~ 5 m depth) at each sampling site.

10.2.3. Laboratory procedures

Sorting, fixing, and preserving benthic animals

Sediment samples taken from the HAPS corer were fixed in Bennett's solution and stored in 2 L plastic jars. The samples were then sieved through a 1.0 mm mesh. Macrobenthic animals were sorted from the sediment retained by the sieve under a binocular microscope. The fauna were preserved in 70% ethanol for further analyses. Enumeration and identification of benthic animals was carried out at family level for polychaetes and bivalves. Other animals were identified to higher taxa.

Ash free dry weight (AFDW) analysis

Biomass (AFDW) was measured for each family or taxonomic group of macrobenthic animals. Polychaetes were removed from their tubes and rinsed in water before weighing to remove as much preservative as possible. Bivalves were crushed to open the shells allowing the flesh to be exposed and separated from the shells as biomass. The organisms were placed in preheated and pre-weighed aluminum cups and then oven-dried at 60^{o}C for 24 hours to constant weight. Samples were cooled at room temperature and then weighed on a microbalance (Mettler UMT2; level of accuracy: 0.001mg) for dry weight. There were no corrections for weight loss as a result of fixation in Bennett's solution and subsequent preservation in 70% ethanol. Samples were then incinerated at 500-520^{o}C in an oven for one hour until weight constancy was reached. Samples were kept in a desiccator for about 1 hour before reweighing, while cooling down to room temperature after oven drying and removal from the muffle furnace.

Sediment grain size analysis

Individual cores were thawed, oven-dried overnight at 105 ^{o}C and homogenized. A 50 g aliquot of each core was muffled for 12 h at 350 °C to remove organic matter and allowed to cool. This sample was stirred with a dispersing agent (40 g L^{-1} sodium hexametaphosphate in MilliQ water) for 15 minutes and left to soak overnight. Blank hydrometer (Calton Glass Marketing) readings were noted for the dispersing solution. The sample was stirred for 10 minutes, transferred into a 1L-measuring cylinder and the volume made up to 1L using MilliQ water. The cylinder was then inverted until the sediment was evenly suspended throughout the water column, and placed on a level surface. A hydrometer and temperature reading were taken exactly 2 hours after this placement to determine clay content (<4 μm). The contents of the cylinder was then wet sieved through a 63 μm sieve and the retained fraction was dried at 100°C. A stacked series of graded sieves comprising 2000, 1000, 500, 250, 125 and 63 μm mesh size were used to obtain sand fractions. The sample was dry sieved using an automatic sieve (Endecotts EFL2000) set at 5 min. The silt content (4-63 μm) was calculated as the difference between the muffled weight of the sample and the sand and clay fractions.

Other sediment analyses, which are organic carbon, total nitrogen and carbonate contents, were provided by Fernandes et al. (2006) from another related project.

10.2.4. Data analyses

Number of taxa (S) and Margalef's index were used to compare taxon richness between sites and times. The diversity of the macrobenthic assemblages was analysed using the Shanon-Wiener index (H') after log (x+1) transformation. Pielou's evenness index was used to express equitability. All indices were presented graphically as plots of means and 95% confidence intervals within sampling times (Clarke & Warwick, 2001).

Because of the complexity in functionally grouping the benthic animals (Fauchald & Jumars, 1979), infauna were categorised based on six major trophic groups: carnivores, herbivores, omnivores, suspension feeders, surface deposit feeders, and subsurface deposit feeders. The proportion of each trophic group was then calculated for each sampling site and sampling time.

Permutational Multivariate Analysis of Variance (PERMANOVA – Anderson 2001) was used to compare the differences in sediment grain size characteristics between sampling sites. Euclidean distances were used on untransformed data, with 4,999 permutations under a reduced model to obtain probability values. To display differences between sites visually, a principal components analysis (PCA) was then undertaken using Primer (Primer-E Ltd, Plymouth, UK). Sediment chemical characteristics (percent organic carbon, total nitrogen and carbonate) were each analysed by univariate ANOVA in SPSS (ver 14.0, SPSS Inc, Chicago). Due to missing values for each variable, it was decided not to do a MANOVA beforehand, as too many samples had missing values. Zone (Boston vs Rabbit Island) and distance from lease (control vs fallowed) were treated as fixed factors, while site (nested in zone x distance) and station (nested in site) were random. Arcsine transformed data met the assumptions of normality (QQ plots) for all variables, but Levene's test could not be satisfied, thus results should be interpreted cautiously. Total macrofaunal abundance (ln transformed), diversity (species richness, Margalef's Index, Pielou's Index and Shanon-Wiener Index), as well as the abundance of the five dominant taxa (Capitellidae, Cirratulidae, Lumbrineridae, Nephtyidae and Spionidae, all ln transformed) were all analysed similarly. All variables satisfied the assumption of normality (QQ plots), but Levene's test was only satisfied for Total Abundance, Species Richness, and Nephtyidae, so results for all other variables should be treated cautiously, although balanced ANOVA is known to be robust to deviations from homogeneity of variances (Underwood, 1997).

Changes in the dominance pattern of macrobenthic assemblages based on both abundance and biomass were assessed using the Abundance/Biomass Comparison (ABC) method (Clarke & Warwick, 1994) with *k*-dominance curves for both abundance and biomass plotted on the same graph (Warwick, 1986). Depending on the level of disturbance, the biomass curve may lie above the abundance curve (for undisturbed areas) or under the abundance curve (for heavily/grossly disturbed areas) or they may be closely coincident for their entire length or may cross each other one or more times (for moderately disturbed areas) (Clarke & Warwick, 2001). The ABC method is used to determine a shift in the proportions of different phyla and in relative distributions of abundance and biomass among taxa between control sites and fallowed pontoons sites and over time. '*W*' shown in the graphs is Clarke's W statistic describing the degree and direction of separation of the curves (Warwick & Clarke, 1994) and is calculated as:

$$W = \sum_{i=1}^{S} \frac{(B_i - A_i)}{[50(S-1)]} \qquad [1]$$

where S is number of species, A_i is abundance of species i, and B_i is biomass of species i. The value of W is in the range between –1 and +1, with W → +1 for even abundance across species but biomass dominated by a single species (undisturbed), and W → -1 in the converse case (severely disturbed) (Clarke & Warwick, 2001).

The extent and rate of recovery were then evaluated by a comparison of the samples between sites and times. A variety of different multivariate analyses were used to assess the patterns using PRIMER 6.1.5. The dominance (and intrinsically diversity) of the assemblages was graphically illustrated by cumulative *k*-dominance curves for each sampling time. This graphical method is generated by plotting cumulative ranked abundances against log species/taxa rank (Lambshead et al., 1983). Analysis of similarities (ANOSIM) performed on the resemblance matrix of dominance dissimilarities (DOMDIS) was employed to test the differences in slope of the dominance plots among sites. Non-metric Multi Dimensional Scaling (MDS) of Bray-Curtis similarities on untransformed data was used to provide a visual representation of differences between sites over time. To reduce noise associated with intra-site variability, samples from each site at each survey were pooled. The initial analysis incorporates all sites at all times to determine if there was any tendency for separation between control and fallowed sites, and between the Boston Island and Rabbit Island zones. The dominant taxa were then superimposed on the MDS plots to assess their roles in configuring the ordinations. Based on this ordination, some of the stations that had high variability in their configuration over the study time were selected. A trajectory line was used to link between stations at different times. PERMANOVA was used to assess the differences of the assemblages between sites and times, following the procedure described for sediment grain size, with the exception that Bray-Curtis distances were used.

Second-stage hierarchical cluster analyses and MDS plots were utilized to assess the consistency in spatial patterns of the assemblages over times and contrast them with the first stage cluster analysis and MDS plots. First, a first-stage resemblance matrix of Bray-Curtis similarities was created by averaging the log(X+1) transformed abundance data for the control and fallowed sites over times. The matrix was analysed using cluster analysis and MDS to create first-stage dendrogram and MDS plots. A single Bray-Curtis similarities matrix of log (X+1) transformed data with factors in a two-way crossed layout was then used to perform a second-stage analysis. A sub-matrix of five sampling times for control sites and five sampling times for fallowed sites was defined as an outer factor, whereas a sub-matrix of eight sites was defined as an inner factor to produce a second-stage resemblance matrix of Spearman (ρ) correlations (Clarke and Gorley, 2006; Somerfield & Clarke, 1995; Clarke & Warwick, 2001; Clarke et al., 2006a; Clarke et al., 2006b).

10.3. Results

10.3.1. Hydrography, water chemistry and sediment structure

The hydrographical conditions and water chemistry of southern Spencer Gulf varied little with the location of the stations sampled. Water temperatures at the surface and bottom

varied between 13.9 – 21.2 ^{o}C and 13.8 – 21.2 ^{o}C, respectively. Salinity was 36.08 – 36.11 psu in winter, and 36.73 – 37.13 psu in summer. The depth of sampling sites varied between 18 and 23 m, and pH between 7.88 and 8.35.

The sediments at control sites adjacent to Rabbit Island were dominated by fine, medium, and coarse sands while sediment grain size composition at the control sites adjacent to Boston Island appeared somewhat finer. The dominant fractions at Boston Island were silt and very fine sands. Compared to control sites, sediments at fallowed pontoon sites had less coarse sand, but more silts and clays. Figure 10.3 shows the average proportion of each grain size class at control and fallowed sites in each zone at each sampling time. PERMANOVA revealed that the variation in sediment grain size was primarily at the site level, as only the Site and Site x Time terms were significant (Table 10.1). This variation is clearly shown in the PCA (Figure 10.4), which indicates that the two most disparate sites are both from the Rabbit Island control group. Nearly all of the variation (69.8%) is on the first axis of the PCA, with sites lying on the right of this axis having fine sediments as opposed to coarse sediments on the left. High values on axis 2 indicate sediments with an intermediate size, while low values correspond to coarse sediments.

Similarly to grain size, organic carbon, total nitrogen, and carbonate content of the sediments all varied primarily as a function of site (ANOVA – Carbon: $F_{12,16}$=6.4, p<0.001; Nitrogen: $F_{12,16}$=6.9, p<0.001; Carbonate: $F_{12,16}$=77, p<0.001), with neither zone (Carbon: $F_{1,12}$=0.17, p=0.69; Nitrogen: $F_{1,12}$=0.11, p=0.74; Carbonate: $F_{1,12}$=2.6, p=0.13) nor distance (Carbon: $F_{1,12}$=0.39, p=0.54; Nitrogen: $F_{1,12}$=0.51, p=0.49; Carbonate: $F_{1,12}$=0.38, p=0.55), or their interaction (Carbon: $F_{1,12}$=0.07, p=0.80; Nitrogen: $F_{1,12}$=0.15, p=0.71; Carbonate: $F_{1,12}$=0.08, p=0.79) having significant effects. The lack of zone and distance effects is shown in Figure 10.5.

Table 10.1. PERMANOVA results for sediment grain size.

Source	df	MS	F	P
Zone	1	455	1.45	0.24
Distance	1	299	0.95	0.38
Site (ZxD)	12	314	7.71	<0.0002
Time	4	120	0.40	0.93
ZxD	1	21	0.07	0.99
ZxT	4	163	0.55	0.81
DxT	4	597	2.02	0.057
S(ZxD)xT	48	295	7.27	<0.0002
ZxDxT	4	55	0.19	0.99
Residual	80	41		

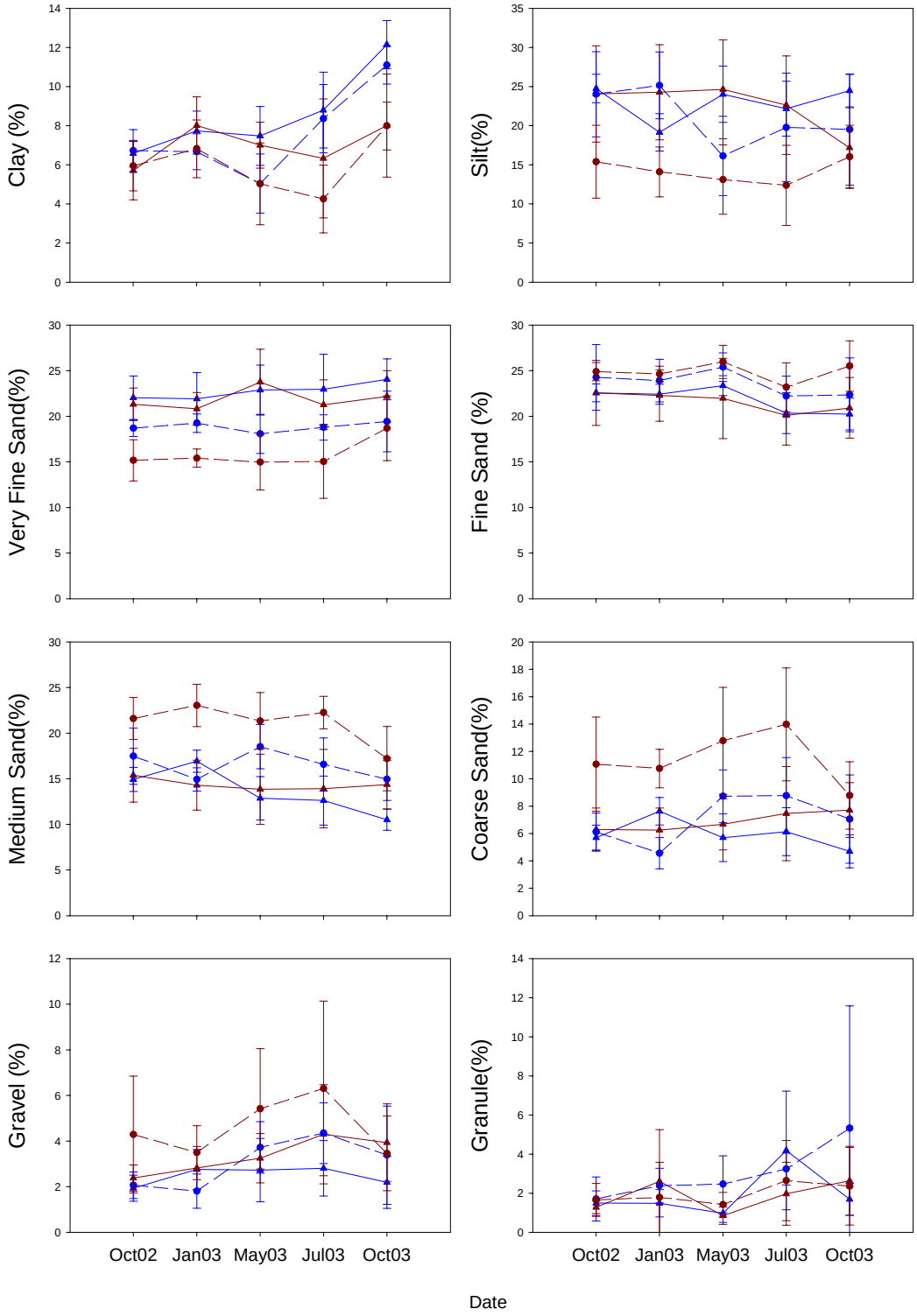


Figure 10.3. Sediment grain size at control and fallowed pontoon sites over the study period (error bars are 95% CI). Continuous lines represent Boston Island sites, dashed Rabbit Island. Red represents control sites and blue represents fallowed sites (data from Fernandes et al., 2004).

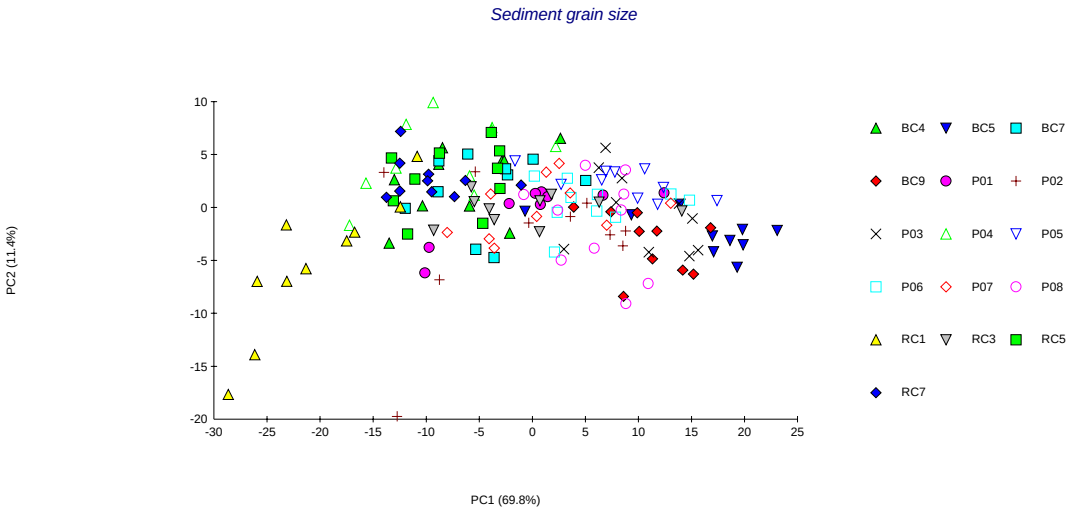


Figure 10.4. Principal components analysis showing separation of sites according to grain size characteristics.

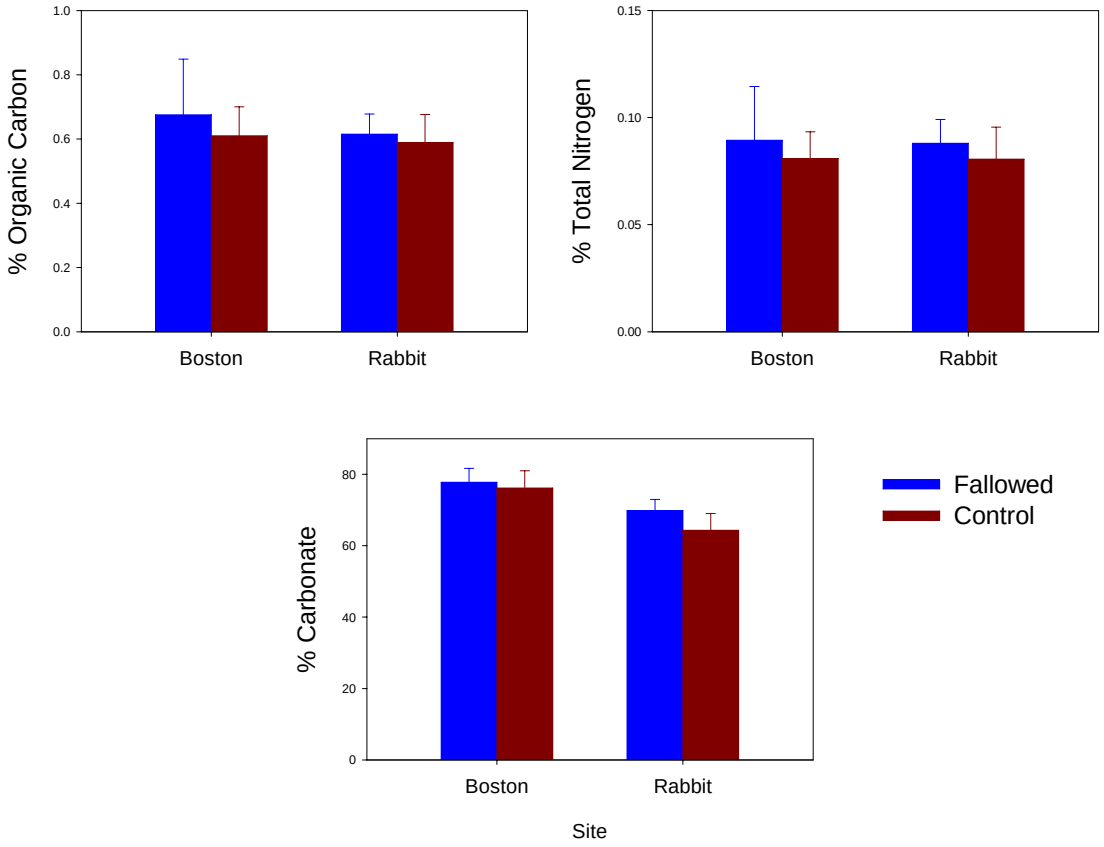


Figure 10.5. Sediment chemical characteristics in October 2002. Error bars are 95% CI.

10.3.2. General trends of macrobenthic structure

Most of the variability in total infaunal abundance was associated with Site and Time, and interaction effects involving these terms (Table 10.2). Rabbit Island control sites showed a fairly consistent increase in abundance over the study period, whereas only one fallowed site showed a similar trend, with the other three maintaining constant abundance (Figure 10.6). At Boston Island, only a single control site showed an increase in infaunal abundance, with the other three sites showing little change. There was much more variability within and among the Boston Island fallowed sites, with one increasing, one decreasing, and two showing no net change.

Table 10.2. ANOVA results for total infaunal abundance (ln transformed).

Source	df	MS	F	P
Zone	1	3.09	0.98	0.34
Dist	1	4.52	1.43	0.26
Time	4	3.50	7.58	<0.001
ZxD	1	0.01	0.004	0.95
ZxT	4	1.74	3.76	0.01
DxT	4	1.87	4.05	0.007
ZxDxT	4	0.25	0.53	0.71
Site(ZxD)	12	3.17	8.16	<0.001
TxS(ZxD)	48	0.46	1.96	0.006
Station(S(ZxD))	16	0.16	0.69	0.80
TxStation(S(ZxD))	64	0.24	1.13	0.24
Residual	480	0.21		

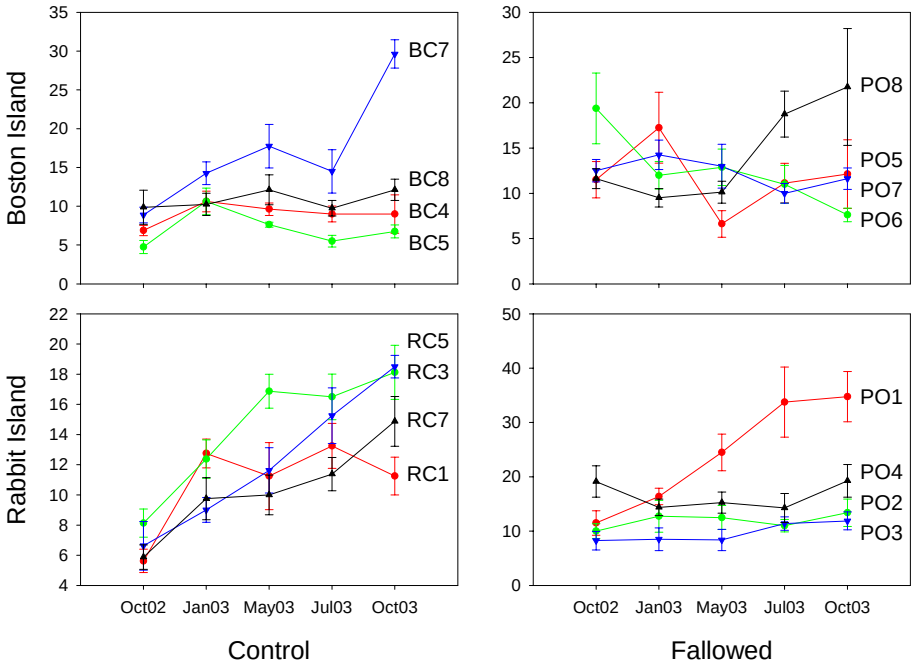
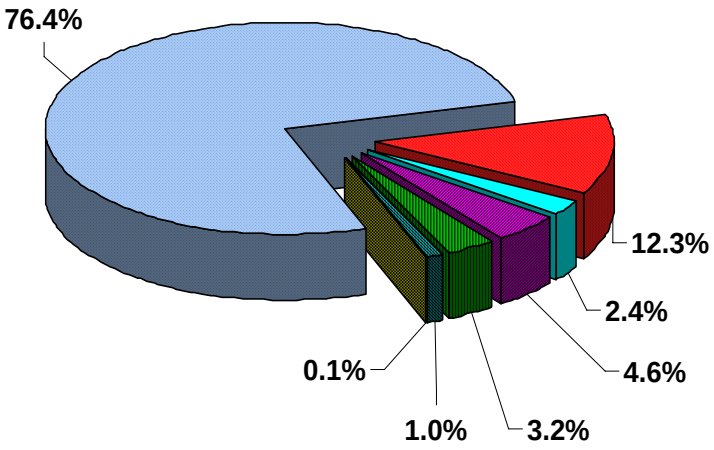
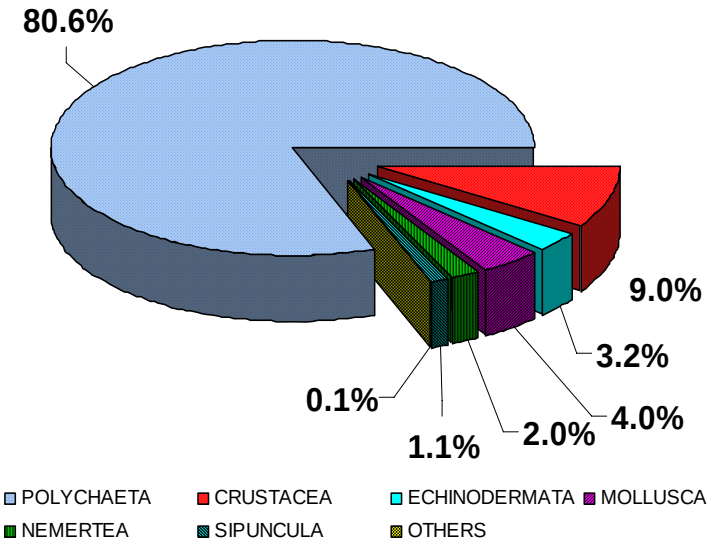


Figure 10.6. Total infaunal abundance at control and fallowed pontoon sites over the study period (error bars are SE).

The macrobenthic assemblages were dominated by 28 families of polychaetes at both control and fallowed sites (Figure 10.7). By numbers, the proportion of Polychaeta at control and fallowed pontoon sites was 76.4% and 80.5%, respectively. Other major taxa in the assemblages were Crustacea, Echinodermata, Mollusca, and Sipuncula. The second most abundant group of animals was the Crustacea, which was relatively more abundant at control sites by 3.3% compared to the fallowed sites. Seven families of bivalve molluscs were recorded during the sampling period. Other phyla were relatively rare and varied little between fallowed and control sites.



(a)



(b)

Figure 10.7. The total proportion of major macrobenthic taxa at control sites (a) and fallowed pontoon sites (b) during the sampling period.

10.3.3. Taxonomic richness, evenness and diversity

The diversity, evenness, and taxonomic richness across both sites and sampling times were comparatively different but with high variability. At the last sampling time, higher values were observed for all indices at the control sites compared to the fallowed sites (Figure 10.8). The greatest changes were seen in taxonomic richness, as opposed to the diversity indices. Again, ANOVA showed that the majority of the variability was related to site and time, and that little, if any, variability was associated with either zone or distance from leases (Table 10.3). The patterns in taxonomic richness (Figure 10.9) were broadly similar to those in total abundance (Figure 10.6). Rabbit Island control sites showed an increase in richness over time, with three of the fallowed sites showing smaller increases and the other remaining constant. At Boston Island, three of the control sites showed some increase, while all four fallowed sites were highly variable over time, showing no consistent trends.

Table 10.3. ANOVA results for infaunal taxonomic richness and diversity.

Source	df	MS	F	P	MS	F	P
		Taxonomic Richness			**Margalef's Index**		
Zone	1	38.0	0.60	0.45	0.44	0.14	0.71
Dist	1	25.6	0.41	0.54	0.057	0.018	0.90
Time	4	75.8	8.37	<0.001	3.64	5.11	0.002
ZxD	1	28.9	0.46	0.51	6.06	1.93	0.19
ZxT	4	15.6	1.72	0.16	0.54	0.76	0.56
DxT	4	33.0	3.65	0.01	1.33	1.87	0.13
ZxDxT	4	1.3	0.14	0.97	0.016	0.022	0.99
Site(ZxD)	12	63.1	10.91	0.001	3.14	5.78	0.006
TxS(ZxD)	48	9.1	1.10	0.35	0.71	1.09	0.37
Station(S(ZxD))	16	4.9	0.60	0.87	0.48	0.74	0.74
TxStation(S(ZxD))	64	8.2	1.41	0.03	0.65	1.42	0.023
Residual	480	5.8			0.46		
		Pielou's Index			**Shannon's Index**		
Zone	1	0.018	0.46	0.51	0.20	0.19	0.67
Dist	1	0.090	2.27	0.16	0.001	0.001	0.98
Time	4	0.015	0.80	0.53	1.51	7.15	<0.001
ZxD	1	0.087	2.21	0.16	2.14	2.01	0.18
ZxT	4	0.022	1.19	0.33	0.21	0.98	0.43
DxT	4	0.012	0.63	0.65	0.50	2.35	0.068
ZxDxT	4	0.005	0.24	0.91	0.014	0.07	0.99
Site(ZxD)	12	0.039	1.86	0.068	1.07	7.00	0.006
TxS(ZxD)	48	0.019	4.37	<0.001	0.21	1.01	0.49
Station(S(ZxD))	16	0.007	1.56	0.108	0.15	0.72	0.76
TxStation(S(ZxD))	64	0.004	0.70	0.96	0.21	1.67	0.002
Residual	480	0.006			0.13		

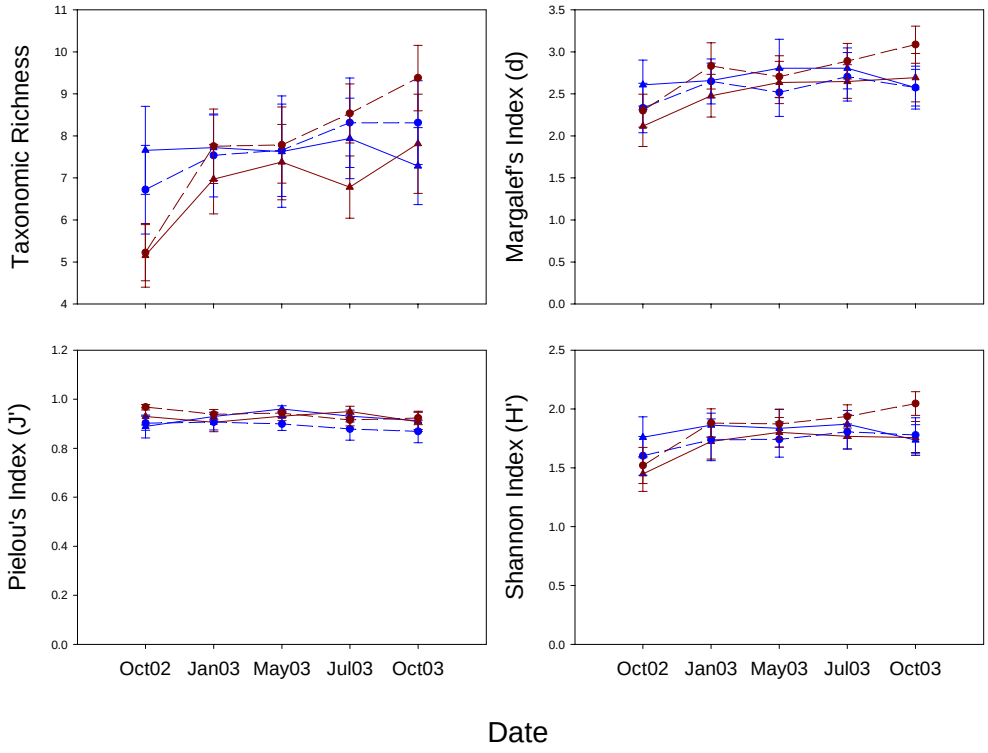


Figure 10.8. Infaunal taxonomic richness and diversity at control and fallowed pontoon sites over the study period (error bars are 95% CI). Continuous lines represent Boston Island sites, dashed Rabbit Island. Red represents control sites and blue represents fallowed sites.

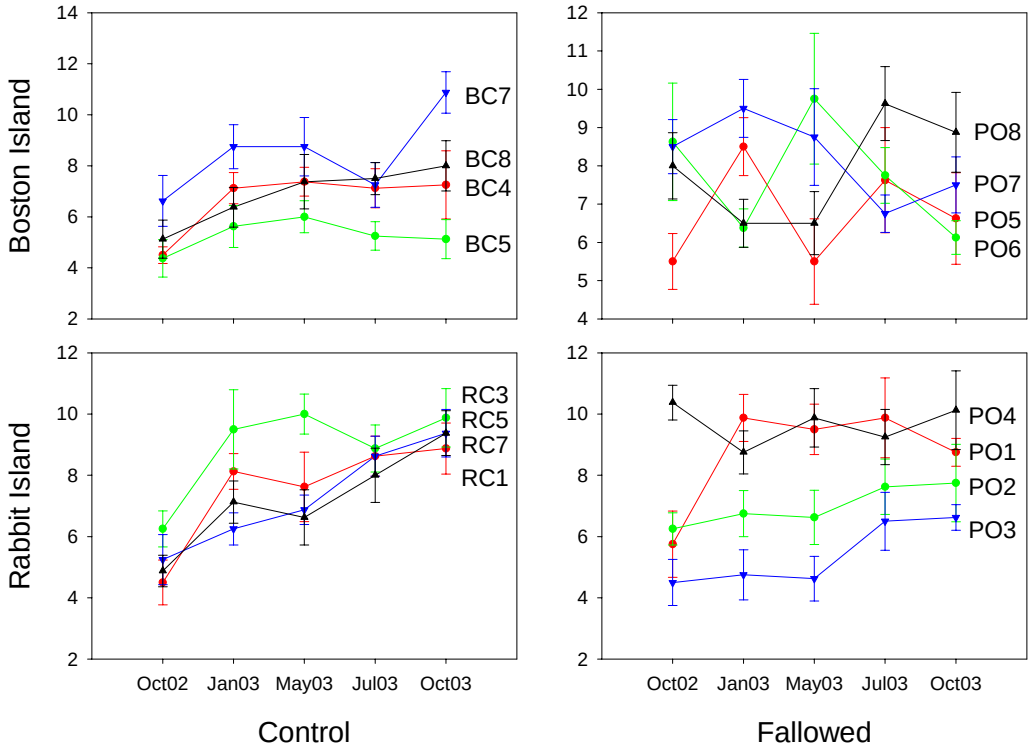


Figure 10.9. Infaunal taxonomic richness by site over the study period (error bars are SE).

10.3.4. Abundance-Biomass Comparison (ABC) curves

The results of the ABC analyses from control sites and fallowed sites for all sampling times are shown in Figure 10.10. At the beginning of the study, the biomass curve lies above the abundance curve at control sites for its entire length, indicating an undisturbed area. Conversely, the abundance curve at fallowed pontoon sites lies above the biomass curve from the starting point to the middle of the curves where they cross, indicating a moderately disturbed area (Warwick, 1986). From January to May 2003, the curves at both control and fallowed sites are fairly similar. In July 2003, the abundance curve at the control and fallowed pontoon sites again lies over the biomass curve from the starting point to the middle where they cross, indicating a moderately disturbed situation. By the end of the study, the abundance and biomass curves at both sites are fairly similar and are indicative of moderately disturbed areas by Warwick's criterion. The results also show seasonal variability in levels of disturbance with little consistency between sites.

10.3.5. Response of trophic groups

Surface deposit feeders (SDF) dominated both control and fallowed sites over the study period, followed by carnivores (Figure 10.11). The proportion of SDF increased from 42% in October 2002 to 59% in October 2003 at control sites, and at fallowed pontoon sites from 37% to 64%. However, the proportion of sub-surface deposit feeders (SSDF) was relatively low at both control and fallowed sites ranging between 5 and 6% at control sites and 5 and 12% at fallowed sites. The proportion of carnivores at control sites fluctuated between 18 and 25%, while at fallowed pontoon sites it decreased from 27% in October 2002 to 19% in October 2003. The highest percentage of omnivores occurred in January 2003 at both sites. Suspension feeders (SF) decreased at both control and fallowed sites by 8% and 6%, respectively, throughout the sampling period. The smallest trophic group was herbivores with 0.1% represented by *Asselota* (Isopoda).

The distribution of abundance and biomass of the trophic groups as a function of sediment organic content is shown in Figure 10.12 (October 2002 samples only). At low levels of organic carbon, SDF (mostly sipunculans, terebellids, and sabellids) dominated macrobenthic abundance (52.4%), and SSDF had the lowest proportion (4.8%) while carnivores (mostly eunicids and lumbrinerids) and SF (mostly bivalve molluscs) were recorded as having the highest (47.0%) and the lowest (14.3%) biomass respectively. At high levels of organic carbon, however, three detritivore feeding groups (SF, SDF, and SSDF) dominated numerically, contributing about 67% of total abundance. At the same level of organic carbon, the biomass was dominated by nearly equal proportions of SF (31.8%) and omnivores (32%), while SSDF (mostly capitellid polychaetes) showed the lowest proportion of the total biomass. The abundance of SSDF increased gradually as a function of percent organic carbon, but decreased markedly in biomass proportion owing to the dominance of small-body size opportunistic taxa. Echinoids were the largest omnivores. Other large animals recorded over the sampling period were suspension feeding mytilids and surface deposit feeding holothuroids.

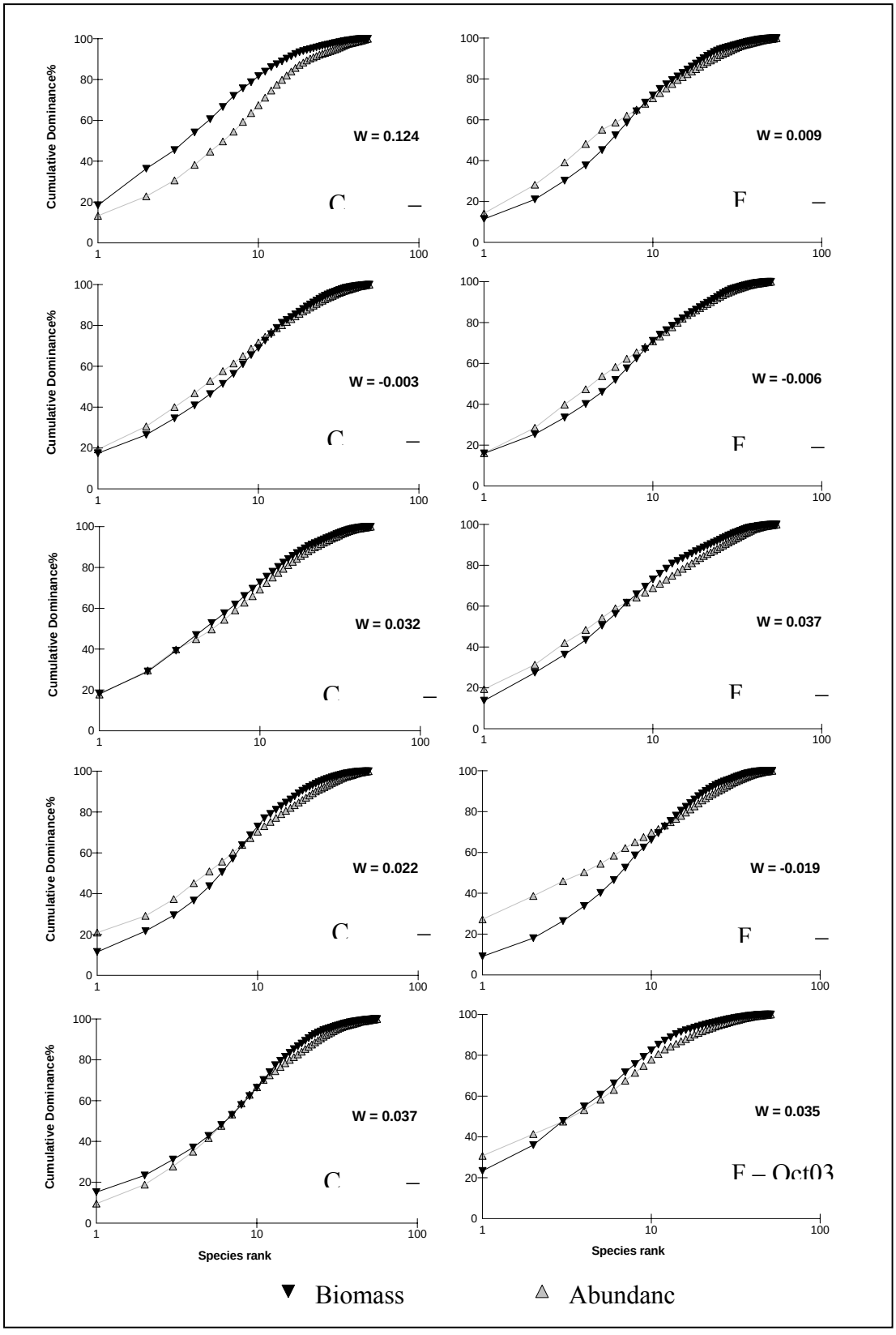


Figure 10.10. The ABC curves at control and fallowed pontoon sites plotted for each sampling time (**'**C' = Control sites; 'F' = Fallowed pontoon sites).

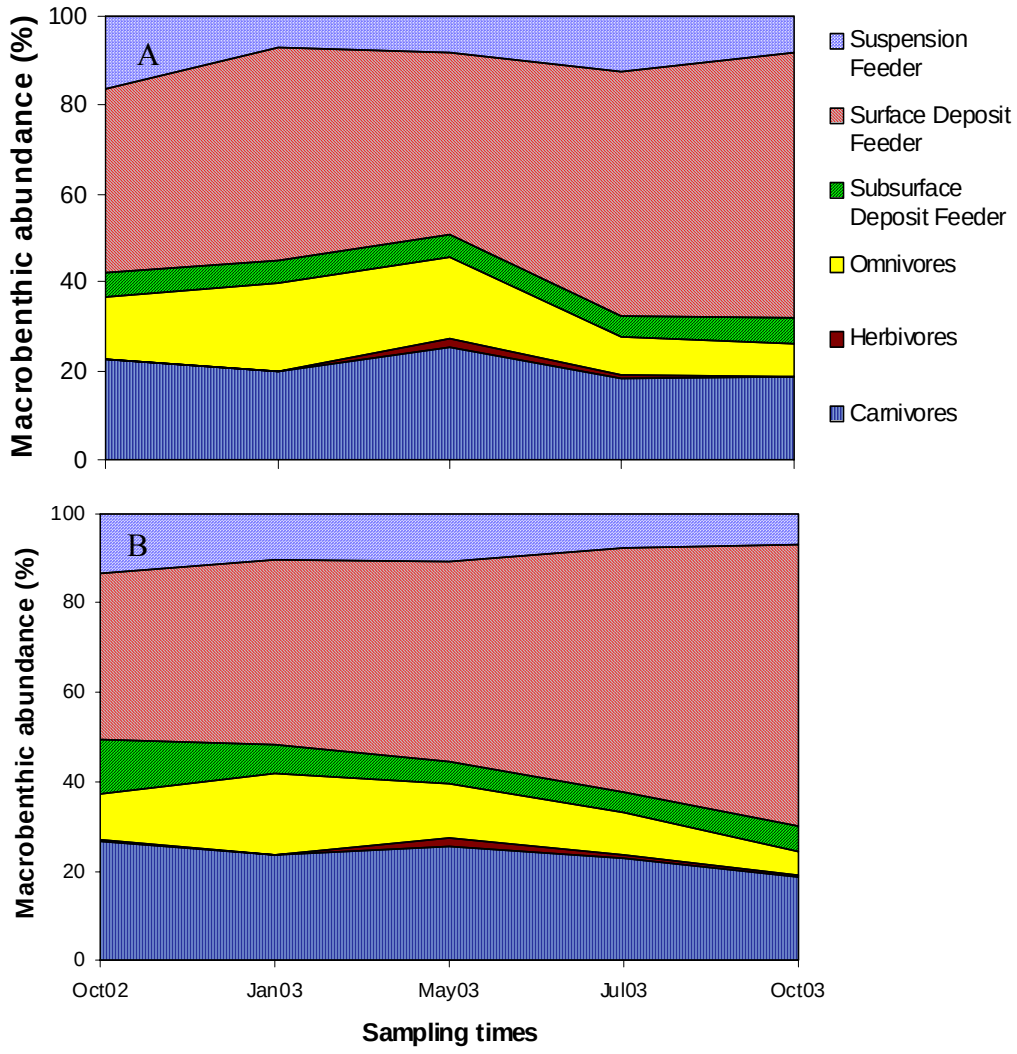


Figure 10.11. The proportion of trophic groups of the fauna at control (A) and fallowed (B) sites over the sampling period.

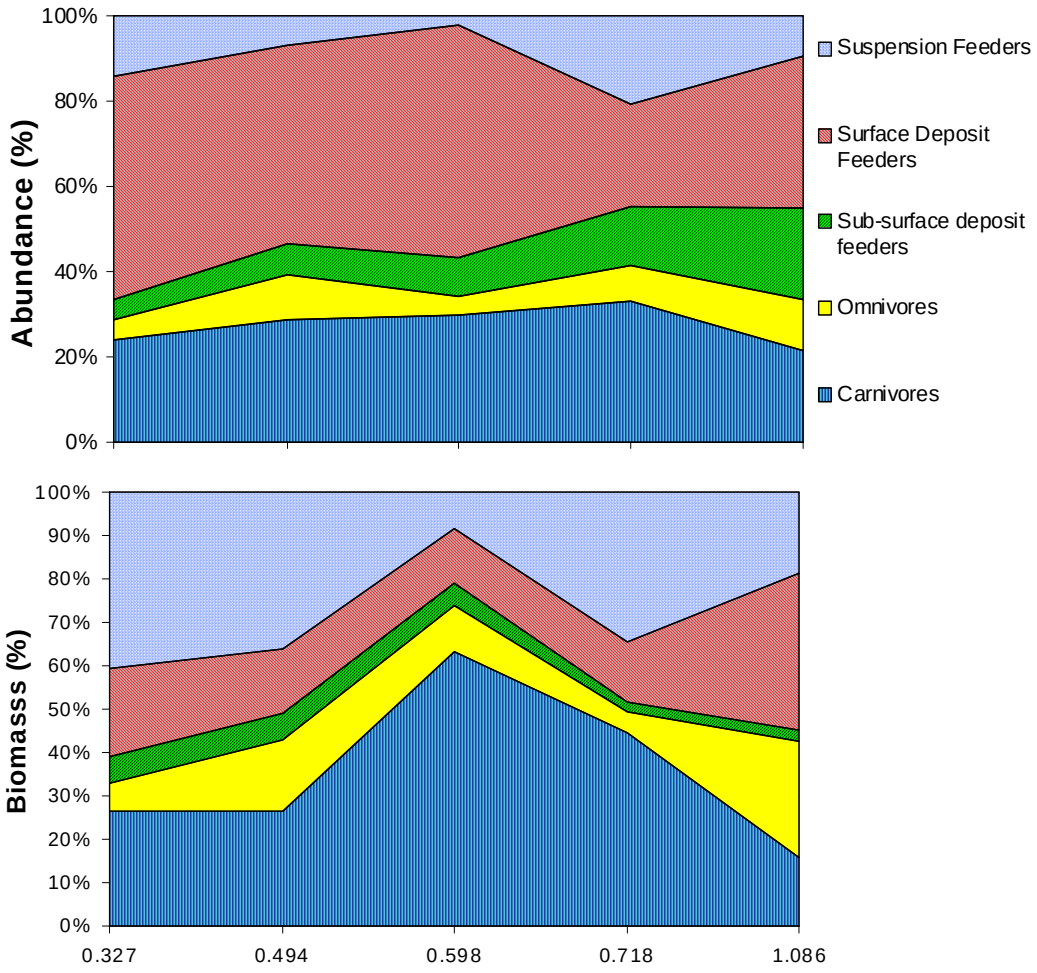


Figure 10.12. Abundance and biomass of macrobenthic fauna as a function of increasing level of organic carbon (organic carbon data from Fernandes et al., 2004).

10.3.6. The dominant animals of the assemblages

Five taxa, all polychaete families, dominated macrobenthic abundance during the study. These were Capitellidae, Cirratulidae, Lumbrineridae, Nepthyidae, and Spionidae. The total number of individuals in each taxon varied with time and site (Figure 10.13). The main obvious trends are seasonal changes in abundance for Nephtyidae, with abundance peaking in January 2003, and annual changes for Spionidae, which increased from October 2002 to October 2003. For both taxa, these patterns were obvious in both zones, and at both control and fallowed sites. Again, the ANOVAs showed that most of the variability in each taxon was associated with site and time, or their interactions, with the exception of the Lumbrineridae, which only varied with site (Table 10.4).

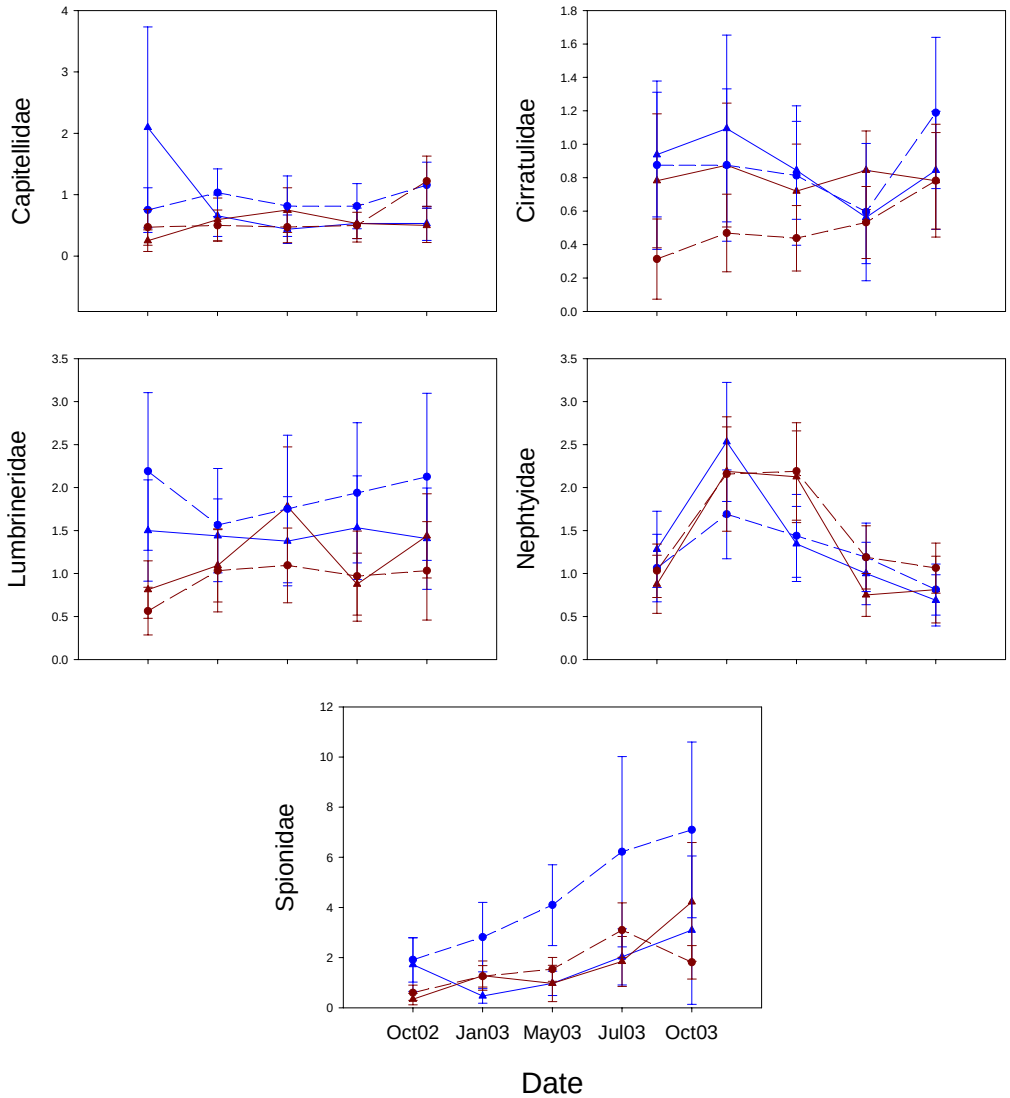


Figure 10.13. Total number of individuals m^{-2} $site^{-1}$ with 95% CI of the dominant taxa for each sampling time, sampled during October 2002 to October 2003 at control and fallowed sites. Continuous lines represent Boston Island sites, dashed Rabbit Island. Red represents control sites and blue represents fallowed sites.

Table 10.4. ANOVA results for abundance of dominant infauna taxa (all ln transformed).

Source	df	MS	F	P	MS	F	P	MS	F	P
		Capitellidae			Cirratulidae			Lumbrineridae		
Zone	1	1.29	4.03	0.068	1.43	0.79	0.39	0.14	0.02	0.89
Dist	1	1.57	4.88	0.047	0.66	0.37	0.56	5.99	0.85	0.37
Time	4	0.40	0.88	0.49	0.29	0.71	0.59	0.21	0.68	0.61
ZxD	1	0.039	0.12	0.74	0.64	0.36	0.56	1.59	0.23	0.64
ZxT	4	0.79	1.75	0.15	0.25	0.62	0.65	0.079	0.26	0.90
DxT	4	0.57	1.26	0.30	0.36	0.89	0.48	0.54	1.77	0.15
ZxDxT	4	0.46	1.02	0.41	0.015	0.038	0.99	0.20	0.64	0.63
Site(ZxD)	12	0.32	0.79	0.66	1.80	4.59	0.001	7.02	39.1	0.001
TxS(ZxD)	48	0.45	1.81	0.013	0.41	1.87	0.01	0.31	0.98	0.52
Station(S(ZxD))	16	0.20	0.81	0.67	0.21	0.95	0.53	0.19	0.59	0.88
TxStation(S(ZxD))	64	0.25	1.12	0.25	.022	1.12	0.26	0.31	1.19	0.16
Residual	480	0.22			0.19			0.26		
		Nephtyidae			Spionidae					
Zone	1	0.097	0.17	0.68	25.45	4.80	0.049			
Dist	1	0.58	1.03	0.33	9.22	1.74	0.212			
Time	4	5.62	13.71	<0.001	6.59	4.33	0.005			
ZxD	1	0.60	1.07	0.32	7.32	1.38	0.26			
ZxT	4	0.47	1.14	0.35	1.18	0.78	0.55			
DxT	4	0.72	1.75	0.16	1.18	0.78	0.55			
ZxDxT	4	0.069	0.17	0.95	1.19	0.78	0.54			
Site(ZxD)	12	0.56	2.18	0.13	5.30	3.91	0.001			
TxS(ZxD)	48	0.41	1.22	0.23	1.52	4.93	<0.001			
Station(S(ZxD))	16	0.18	0.54	0.91	0.15	0.47	0.95			
TxStation(S(ZxD))	64	0.34	1.38	0.033	0.31	0.86	0.76			
Residual	480	0.24			0.36					

10.3.7. Multiple *k*-dominance curves

The multiple *k*-dominance curves of macrobenthic abundance for each sampling time at control and fallowed sites are shown on Figure 10.14. For the control sites, the differences in dominance or diversity of the assemblages are not apparent and the shapes of the curves for these sites are not different. The only curve that was initially lower is October 2002 (C1), indicating higher diversity than others, as also shown on the ABC plots (Figure 10.10). The pattern at fallowed pontoon sites was more variable. It is apparent from the graph that the assemblages from the last two sampling times at the fallowed sites (Figure 10.14) differed from the other curves. The change in the shape of P4 and P5, indicating an increase in dominance (and consequently lower diversity), is clear, especially compared to the assemblages from the previous sampling times at the same site (P3). To assess the level of differences between the two assemblages, the dominance curves from each of the 8 fallowed pontoon sites are shown in Figure 10.14 B1 and B2. However, analysis of similarities (ANOSIM) performed on the resemblance matrix of dominance dissimilarities (DOMDIS) between P3 and P4 showed no significant difference (global R: -0.097; $p>0.05$). A similar result was observed between P3 and P5 (global R: -0.004; $p>0.05$). This implies that the difference between the two sampling periods is entirely due to a reduction in taxon diversity at a single site, P07, in July 2003 compared to the other sites, and the dominance of certain taxa at this site, especially Spionidae and Lumbrineridae.

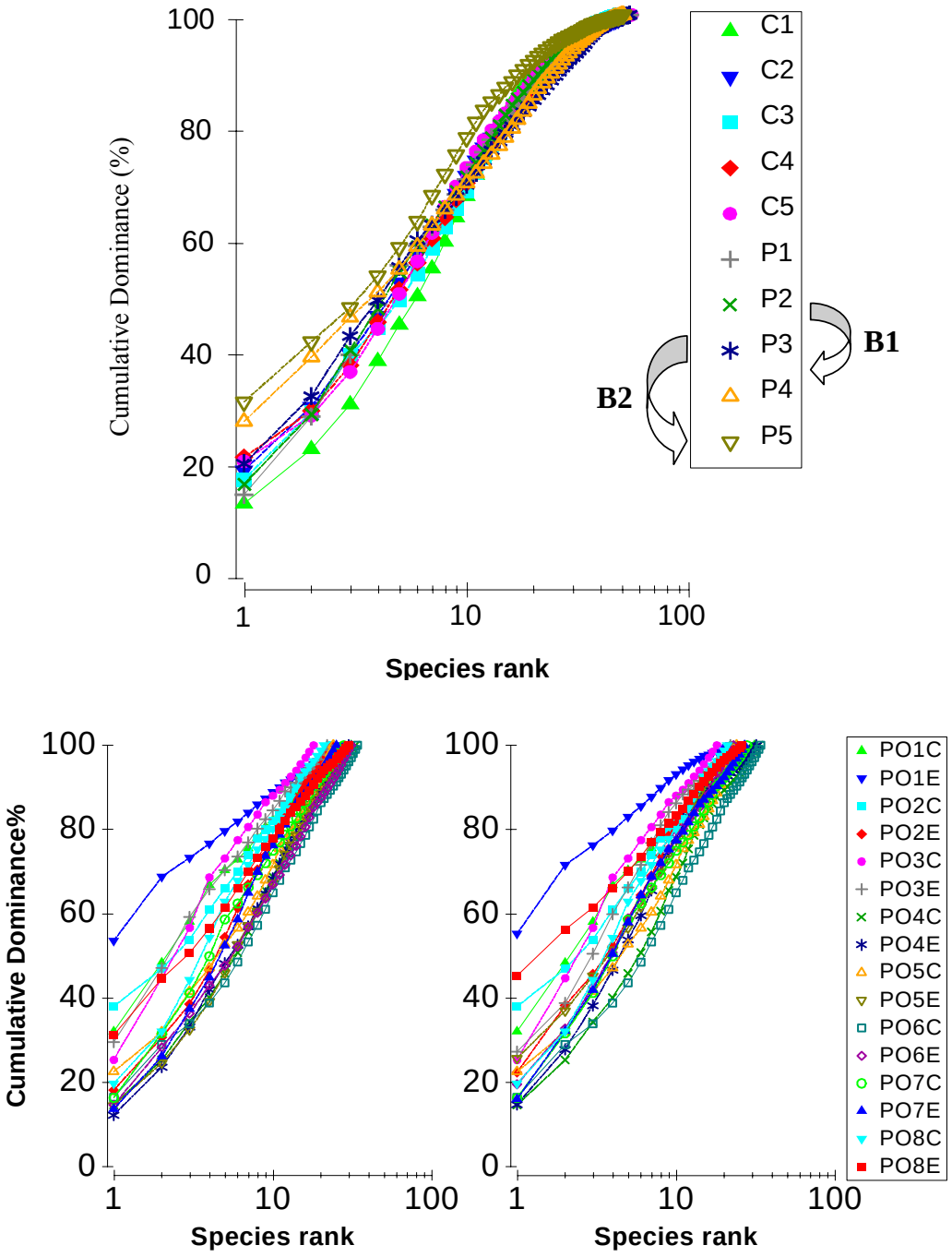


Figure 10.14. (A) Multiple *k*-dominance curves of macrobenthic abundance for each sampling time averaged over all control (C) and fallowed (P) sites. (B) The subsequent changes in slope of the dominance plot compared between P3 and P4 (B1) and between P3 and P5 (B2) of the two different sampling times. Terminal codes for graph A: 1 = Oct02, 2 = Jan03, 3 = May03; 4 = Jul03, 5 = Oct03; for graph B1&B2: 01-08 = sampling sites; C = May03, E = Oct03.

10.3.8. Multi-Dimensional Scaling (MDS) plots

Ordination (Figure 10.15) shows a separation between control and fallowed pontoon sites, suggesting a difference in assemblage composition between the two sites over the sampling period but with overlap. There is also a separation between sites located at Boston Island and Rabbit Island indicating that the Boston Island zone caused most of the overlap between control and fallowed sites. This pattern shows that control sites and fallowed sites are more similar at Boston Island than at Rabbit Island indicating that both treatments at Boston Island may be influenced by sediment structure and / or organic enrichment.

Figure 10.16 shows "bubble plots" displaying how the abundances of the five dominant taxa relate to overall assemblage differences between sites as shown by the MDS. The highest abundance for all dominant taxa occurred at fallowed pontoon sites, suggesting that opportunistic taxa dominated the assemblages at these sites. Capitellid worms were the most abundant at fallowed station P06 in October 2002 and responsible for the separation of P06 from the other stations. All other taxa were more evenly distributed between samples.

The differences in relative proportions of lumbrinerids and spionids are responsible for the horizontal spread of stations in that the higher proportions of these taxa were plotted to the top right and the bottom right of the configurations, respectively. No clear trends, however, were found for nephtyids or cirratulids, with high abundance tending to occur in the middle of the configuration.

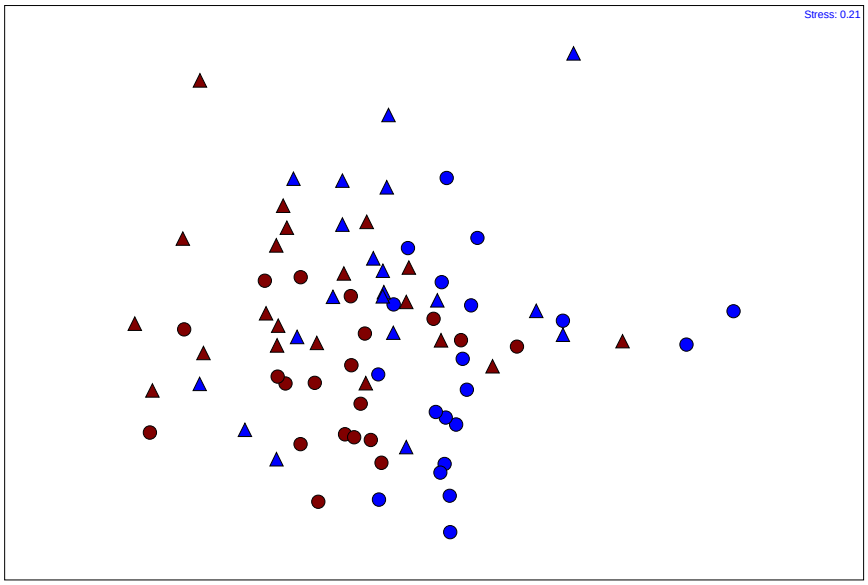


Figure 10.15. MDS plots of macrobenthic abundance showing separation between control (brown) and fallowed (blue) sites and between zones located at Rabbit Island (circles) and Boston Island (triangles). (Resemblance matrices: Bray Curtis similarity; untransformed data; 2D MDS).

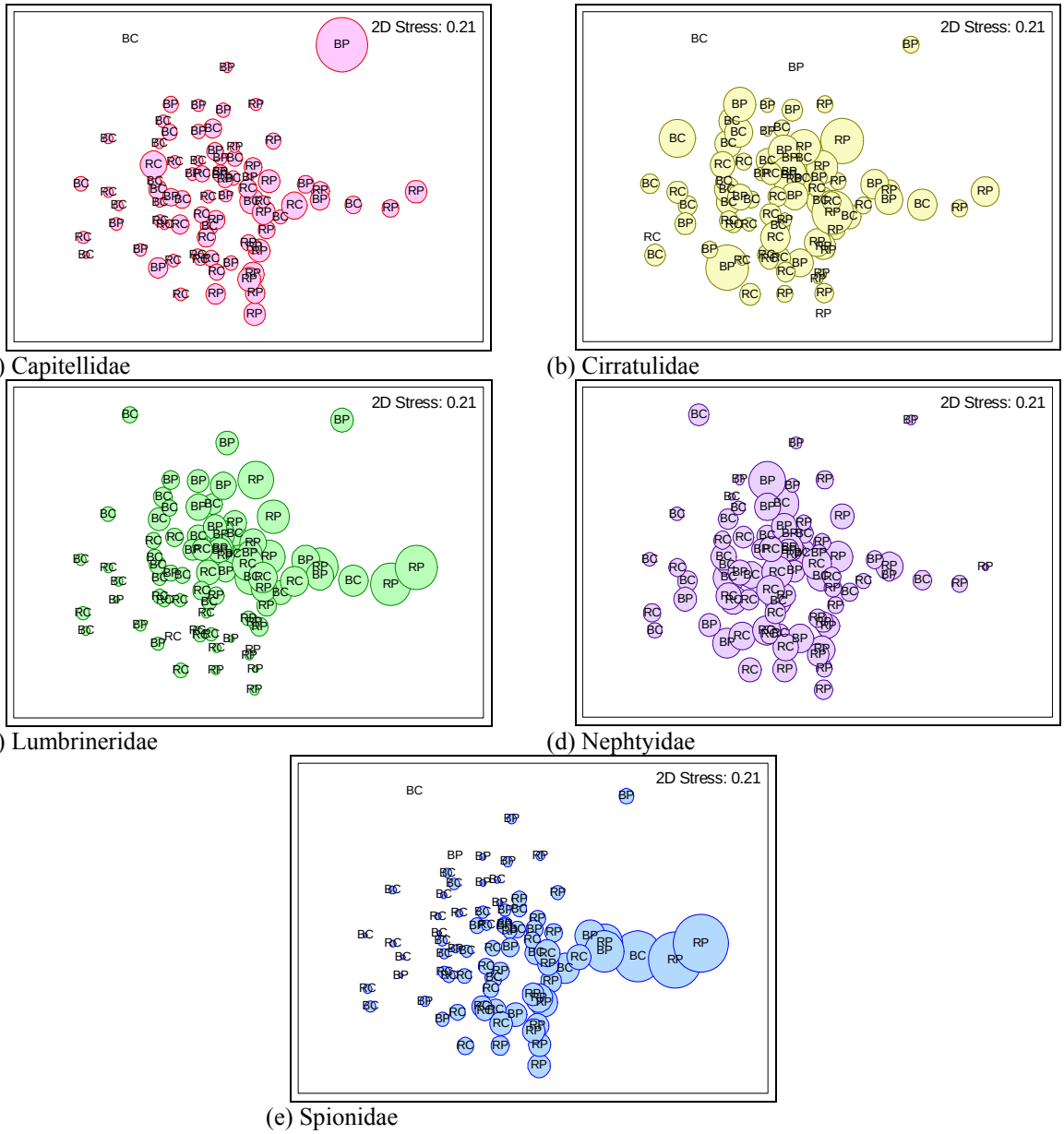


Figure 10.16. Bubble plots of the MDS displaying the relative abundance of five dominant taxa superimposed on the overall macrobenthic composition. Codes: BC = Boston-control sites; RC = Rabbit-control sites; BF = Boston-fallowed sites; RF = Rabbit-fallowed sites.

Four sites (2 control – BC7 & RC3, and 2 fallowed – P01 & P06) showed major changes in their location on the MDS plot over time (Figure 10.17). The direction of movement of each site over time was highlighted using a trajectory line. Sites that were not selected (shown as dots on the graphs) did not show any major changes in their structure over the sampling period.

Sites BC7 and RC3 (control sites) move gradually from the middle-left to the right of the configuration over time, indicating a further disturbance upon macrobenthic structure over the sampling period. Station P06 (fallowed) moves towards the left where control stations are mostly located, indicating that its taxonomic composition became more similar to those at

control stations by the end of the study. This suggests a major recovery of P06 from disturbance. However, site P01 moved to the right towards the end of the study indicating further disturbance.

The consistency in spatial patterns of the assemblages over time was assessed using second-stage hierarchical cluster analyses and MDS plots (Clarke and Gorley, 2006). These were then compared to the first stage cluster analysis and MDS plots. Figure 10.18 shows first-stage and second-stage dendrograms and MDS plots in 2 dimensions of macrobenthic abundances for control and fallowed sites over the studied period. As shown from the graphs (Figure 10.18 a&c), the control sites at all sampling times are not well separated from the fallowed sites using first-stage cluster analysis and MDS, indicating that the first stage matrix (generated from averages of abundance data) is less sensitive in detecting the potential impact of the disturbance. In contrast, fallowed sites are well separated from the control sites (stress level of 0.04) over time using second-stage cluster analysis and MDS (Figure 10.18 b&d), indicating more similar spatial patterns of benthic assemblages across the sampling times at fallowed sites than at control sites. This also implies that the second-stage matrix is more sensitive in detecting the potential impacts, focusing only on the consistency in spatial pattern over time.

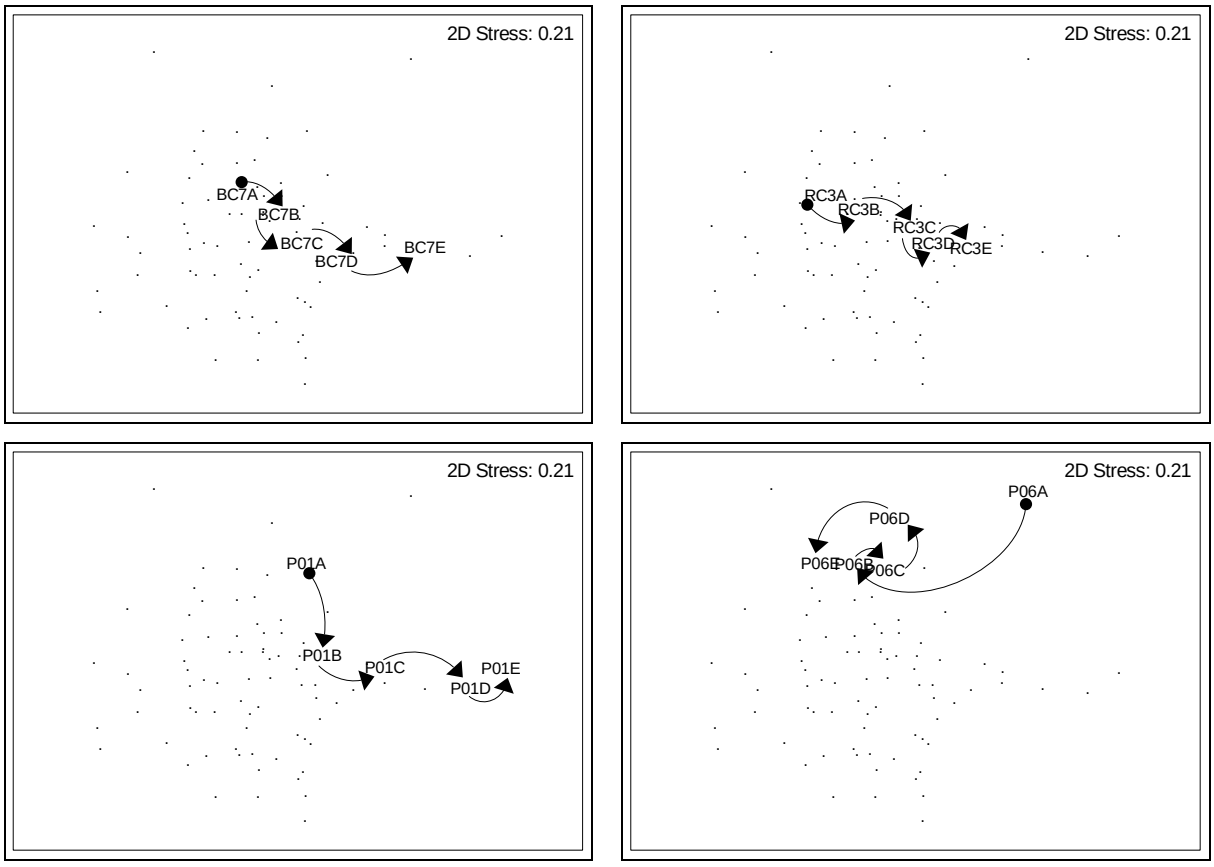


Figure 10.17. The MDS by Bray-Curtis similarities for untransformed macrobenthic abundance showing the shifts in direction of two selected control and two fallowed stations over the sampling period (BC7 and RC5= control stations; P01 and P06= fallowed stations; terminal codes: A= Oct02, B= Jan03, C= May03; D= Jul03, E= Oct03). Highlighted stations in time series are linked by trajectory lines. Positions of other stations are indicated by dots.

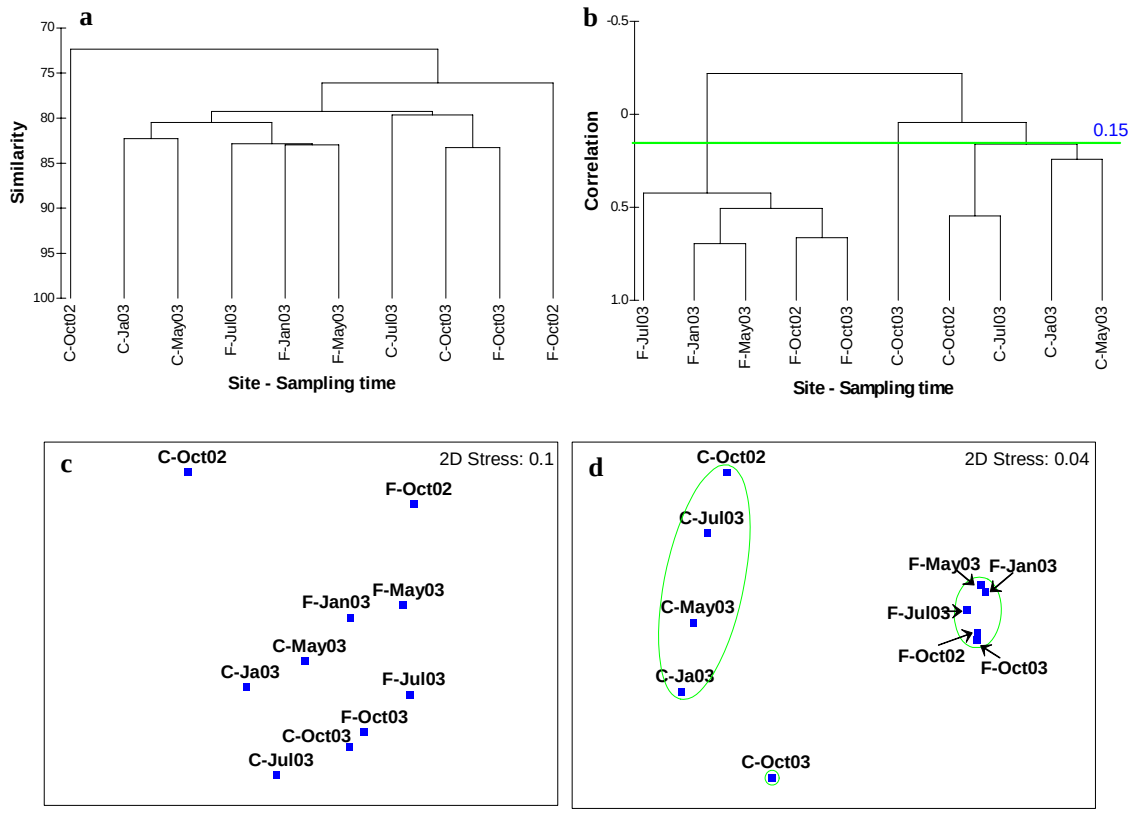


Figure 10.18. First-stage and second-stage dendrograms and MDS plots in 2 dimensions of macrobenthic abundances for control and fallowed sites over the studied period: a) first-stage cluster analysis dendrogram and c) first-stage MDS from averaging the macrobenthic abundances at both control and fallowed sites for each sampling time; b) second-stage cluster analysis dendrogram and d) second-stage MDS derived from a single Bray-Curtis similarities matrix of log (X+1) transformed data with factors classifyied in a two-way crossed layout (outer factor: sampling time; inner factor: site). The first capital letters indicate control (C) and fallowed (F) sites followed by sampling times. Green circular lines derived from cluster analysis indicate an arbitrary correlation level of 0.15, distinguishing control and fallowed sites.

10.4. Discussion

10.4.1. Water quality, sediment structure and the benthic assemblages

During the sampling period, the hydrographical conditions of southern Spencer Gulf (see Nunes, 1986) can be considered typical for this period at both control and farm sites. The values of water temperature are relatively close to a representative mean temperature for this region, which is ~22^{o}C (Nunes & Lennon, 1986), and are still in the range of the fluctuation between winter and summer observed by Edyvane (1997).

Sediment grain size structure, and chemical composition, both varied primarily as a function of site, as well as the interaction between site and time for the former. There was no large-

scale variation detected related to either farming zone (Boston vs Rabbit Island) or between fallowed versus control sites. These results suggest that tuna farming, as it is currently practiced in Spencer Gulf, doesn't have a detectable impact on the sediments, although they could also mean that sediment structure and composition recovers within a matter of weeks after the cessation of farming. There are other large-scale patterns in sediment structure that were not obvious in the data analysed here, however. Fernandes et al. (2006) observed that sediments at Cape Donington close to Boston Island were finer and lighter due to a small siliciclastic component (<20%). Furthermore, the dominance of silts and very fine sands in this region may be caused by the presence of highly weathered biogenic fragments covered with a coating of fine particles (Fernandes et al., 2006). In general, sampling sites off Rabbit Island appear to be in a higher energy environment than those off Boston Island. High-energy environments are typically characterised by strong bottom flows, coarse sandy sediments, and low organic and microbial content. In contrast, low-energy environments are characterised by weak currents, greater vertical flux of organic matter, and fine muddy sediments (Snelgrove, 1999).

10.4.2. Structural pattern of the macrobenthic assemblages

The patterns of variation in total infaunal abundance were complex, with much of the variability attributed to site level effects. However, there was also a significant distance by time effect, indicating that fallowed and control sites exhibited different overall patterns with time. While five of the control sites showed substantial increases in abundance over the course of the year, only one fallowed site showed a similar pattern. In addition, the only decreases in abundance occurred at fallowed sites. The increase at control sites over the year could indicate two things. Firstly, control sites were actually impacted, and recovered over the year. This can be discounted on the basis that while farming does cease over summer, it recommences again early in the year, and the impact on control sites should be the same in October of 2002 and 2003. Thus, it appears that there has been an overall system-wide increase in infaunal abundance that is unrelated to aquaculture, but is probably caused by some natural factor (e.g. annual variation in climate). The lack of a corresponding change at fallowed sites suggests that these sites are impacted by aquaculture, and that even one year later they have not recovered sufficiently to show natural patterns of variation in abundance.

The changes in the dominance pattern of abundance and biomass may be used as an indicator of community disturbance. Shifts in the proportions of different phyla and in relative distributions of abundance and biomass among species occur with increasing levels of disturbance. In theory, macrobenthic assemblages under stable conditions or low levels of disturbance are competitively dominated by conservative species, which have a "K-selected" life history. These species are characterised by large-body size and long life span and dominate in biomass but not in numbers. When pollution disturbs the assemblage, "r-selected" organisms or opportunistic species dominate the assemblage, while conservative species are less favoured so that the opposite situation may occur. Depending on the level of disturbance, the biomass curve may lie above or under the abundance curve, or they may be closely coincident along their entire length or may cross each other one or more times (Clarke & Warwick, 2001). Warwick (1986) proposed three different types of *k*-dominance curves (Figure 10.19). In undisturbed areas, the biomass is dominated by few large species elevating the biomass curve but, because each species is represented by few individuals, the abundance curve is low. Based on the hypothetical *k*-dominance curves proposed by Warwick (1986), the fallowed sites can be categorized as moderately disturbed, due to the elimination of large

competitive dominants and a decrease of inequality between abundance and biomass. At the end of the study, a larger number of individuals were recorded at the fallowed sites compared to control sites. However, the biomass was lower, indicating a dominance of small-body sized taxa.

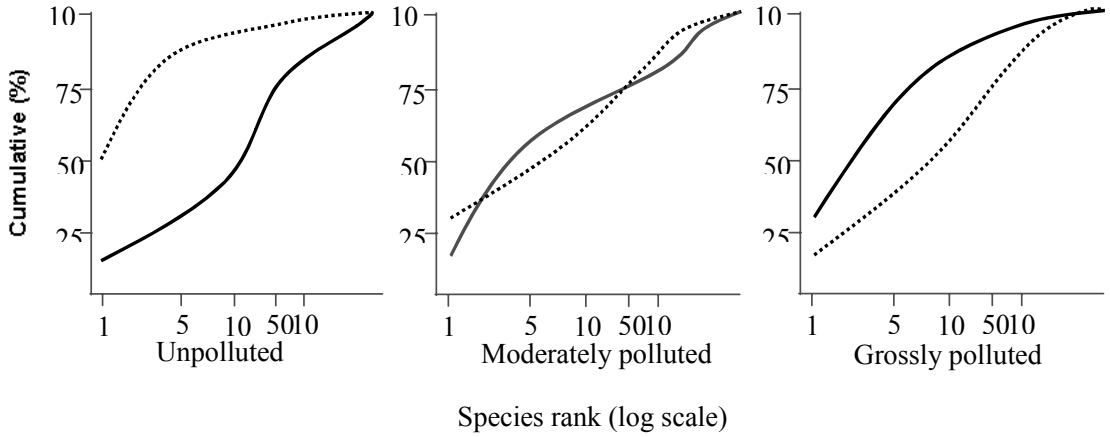


Figure 10.19. Hypothetical cumulative k-dominance curves for species biomass (dashed lines) and abundance (continuous lines) (modified from Warwick, 1986; Rosenthal, 2002).

The curves at control sites, however, do not represent undisturbed areas but rather moderately disturbed areas using Warwick's *k*-dominance criteria, particularly the January to October 2003 curves (Figure 10.10). This result suggests that the entire area is subject to a moderate level of disturbance, except for control sites in October 2002. This is either because of the extension of impacts of farming to the control sites, given that the current velocity in this region is relatively strong, or because of other factors. Most of the dominant taxa are considered as opportunistic species, which have a small-body size, resulting in the slope of the biomass curves, and grow rapidly in response to a disturbance, especially organic enrichment (Pearson & Rosenberg, 1978; Diaz & Rosenberg, 1995; Warwick, 1996). In addition, statistical analyses showed that there were no significant differences in the abundance of these taxa between control and fallowed sites, except for Capitellidae. These results lead to the similar patterns of the ABC curves between control and fallowed sites, especially in biomass curves, owing to the relatively similar proportion of the dominant taxa.

Despite previous successful use of the ABC method (Kaiser et al., 2000; Smith & Simpson, 1998), inconsistency in results has also been reported (Weston, 1990; Warwick et al., 1990; Dauer et al., 1993; Clarke & Warwick, 2001). The ABC method is strongly influenced by the presence of a small number of large-size species (Dauer et al., 1993) and is over-dependent on the single most dominant species (Clarke & Warwick, 2001). Weston (1990) suggested that some conceptual revisions are needed.

10.4.3. Response of the benthic fauna to organic enrichment: trophic groups approach

Species such as *Capitella capitata* (Capitellidae), *Polydora ciliata* (Spionidae) and *Hydrobia ulvae* (Bivalvia) have previously been used as indicators of organically enriched sediments (Pearson & Rosenberg. 1978). However, these species can be found in areas with low organic content. The relative spatial and temporal abundance of groups of species is considered to be more useful to assess the level of organic enrichment than individual species.

Although most macrobenthic species are relatively unselective in their food requirements and rely on spatial partitioning of the habitat (Dernie et al., 2003), species may still be functionally grouped based on their feeding habits. Fauchald & Jumars (1979) categorized polychaete families into several major feeding guilds and grouped polychaete families as filter feeders (8 families), surface deposit feeders (19 families), subsurface deposit feeders (13 families), herbivores (10 families), carnivores (19 families), and a few families as omnivores. However, assessing trophic groups of macrobenthic assemblages can be complicated. Overlapping food selection can occur in macrobenthic fauna, especially suspension feeders (Roth & Wilson, 1998), as they may switch feeding strategies depending on environmental factors (Snelgrove and Butman, 1994). It has been suggested that stability, water and organic content, oxygen content, particle size and microbiomass of sediment are all factors associated with trophic composition (Gaston, 1997).

Each trophic group may respond differently in response to an environmental disturbance (organic enrichment, hypoxia or anoxia etc). Diaz (1995) suggested that sediment contamination leads to greatly reduced trophic complexity in which benthic assemblages are dominated by opportunistic species such as Capitellidae and Spionidae. These groups are adapted to rapidly re-colonize disturbed areas and establish a large population within a relatively short time (Boesch & Rosenberg, 1981; Pearson & Rosenberg, 1978; Lu & Wu, 1998). Based on laboratory experiments, the depletion of nitrogen-containing food can affect the population dynamics of several deposit-feeders, decreasing individual growth, abundance and/or reproductive output (Rossi, 2003). Weston (1990) observed that suspension feeders disappeared and surface deposit feeders markedly declined at 45-90 m from a salmon farm.

Attempts have been made to relate organic matter content and the abundance of different trophic groups of macrobenthos. Rakoncinski *et al*. (2000) showed that the proportion of subsurface deposit feeders increased along organic-chemical contamination gradients, whereas carnivores, filter feeders, and surface deposit feeders decreased. Surficial sedimentary organic carbon correlated with deposit feeding species richness, whereas total particulate and food particulate variety correlated with their species diversity (Whitlatch, 1981). Weston (1990) observed that trophic diversity was reduced with proximity to a salmon farm as a result of increasing organic matter. Suspension feeders constituted 10% of the assemblages at 450 m from the farms, but disappeared at 45-90 m from the farm. Surface deposit feeders markedly declined, whereas sub-surface deposit feeders increased directly under the farm. Pearson & Rosenberg (1978) emphasized that a simple trophic system composed of only non-selective deposit feeders and carnivores is established in sediments where input levels of organic matter are noticeably high.

In this study, the responses of benthic fauna to organic matter (assessed using % organic carbon) are in accordance with the theory of macrobenthic successional stages proposed by

Pearson & Rosenberg (1978). The main trophic groups seem to respond in the classical way to organic enrichment of sediments at the pontoon sites. As the amount of organic material on the sediment surface increases, the larger and deeper burrowing species are gradually eliminated and replaced by greater numbers of small surface deposit feeders.

Nonetheless, the composition of opportunistic taxa in the sampled assemblage was spatially and temporally variable over the study period. For instance, Capitellidae were most abundant only at station P06 in October 2002 and decreased in numbers at the end of the study. Despite hydrographical condition (relatively strong in this region) that may influence the availability of organic matter over time, it is likely that this deposit-feeding organism was efficient in decomposing organic matter generated from fish farms, as has been observed by Chareonpanich et al. (1994) using *Capitella sp. I*. As there is no clear trend found for nephtyids, it is suggested that they are an unresponsive organism to organic enrichment (Grall & Glemarec, 1997). This group is always present in relatively low densities with low variability over time. Therefore, it is reasonable that nepthyids were present at both control and fallowed stations as shown in Figure 10.16 D. However, the ordinations failed to show that cirratulids are accountable for horizontal spread of the configurations, considering they are suggested to be second-order opportunistic taxa in disturbed areas (Grall & Glemarec, 1997; Borja, 2000). The low abundance of cirratulids recorded may be due to competition among surface deposit feeders for food resources, especially with spionids.

In this study, a reduction of trophic diversity did not occur as organic carbon increased. Beside high variability during the sampling time, the low levels of organic carbon recorded may be the main reason for this. Therefore, it is likely that organic loading from southern bluefin tuna farms in this region is relatively low in comparison to fish farming situations that have been studied elsewhere (see Chapters 5 and 9 of this report). However, given the high spatial and temporal variability of the accumulation of organic matter beneath fish cages (Porello et al., 2005), further analysis using data over a full year may be useful to assess fauna-organic matter relationship, thus provide a better understanding surrounding the issue.

10.4.4. Rates and degree of recovery

Fallowing, or periodic abandonment, has been suggested to be one of the best management tools for the sustainability of salmon farming in cold-water environments (Carroll et al., 2003). This involves rotation of the fish pontoons around a lease farm area for a certain period of time in order to allow the sediments underneath to recover from farm waste deposits. The recovery rates of macrobenthos after a fallowing period have been discussed by McGhie et al. (2000), Pereira et al. (2004), Crawford (2001), Lu & Wu (1998), Morrisey et al. (2000), and used as environmental quality criteria for managing marine aquaculture in several countries, i.e. Japan (Yokoyama et al., 2002), Tasmania-Australia (Crawford, 2003), Norway (Carroll et al., 2003), and Hong Kong (Gao et al., 2005). Dernie et al. (2003) suggested that less stable habitats (coarse, clean sands) had the most rapid recovery rate following environmental disturbance, whereas stable habitats (muddy sands and mud) had the slowest. Reductions in species richness, biomass, and abundance with increasing proximity to salmon farms have been reported by Weston (1990). At a semi-enclosed marine fish culture zone with 4.5 $kg.m^{-3}$ of stock density of groupers, snappers and sea breams, Gao et al. (2005) observed a considerable reduction in species diversity under fish cages compared to reference stations, which were 600 m away from the farms. A reduction of environmental stresses with increasing distance from the cage farms was also reported. The occurrence of small bivalves

Tellinides sp. and brittle star *Aphioplus sp.* in larger numbers at intermediary and reference stations than at farm stations was suggested as an indication of reduction in farming impacts. Aure & Stigebrandt (1990) showed that only about 10% of the organic matter deposited from fish farms on the sediment was broken down annually, but recovery was variable and depended upon environmental variables and farming practices.

The multiple *k*-dominance curves of macrobenthic abundance for each sampling time at control sites show that the differences in dominance or diversity of the assemblages are not apparent. However, variability was higher at the fallowed sites, especially for the last two sampling times compared to the other sampling times. The curve for site P01 (for July and October 2003) is separated from the other curves and located on the top of the graph, compared to the other sites, indicating a reduction in taxonomic diversity and the dominance of certain taxa at this site, especially Spionidae and Lumbrineridae. Results from biotic indices showed that Shannon-Wiener diversity index (H') and Pielou's evenness index (J') for site P01 were the lowest compared to the other sites and sampling times (in July 2003: H' = 1.9; J'= 1.58; in October 2003: H'= 1.7; J'= 0.56).

The shifts in direction through time shown in the MDS plots (Figure 10.16) for some stations were due to the changes of abundance and number of taxa throughout a year (Table 10.5), with opportunistic taxa (mostly surface and sub-surface deposit feeders) dominating the assemblages. These surface-dwelling organisms have an r-selected life history characterized by a small-body and a rapid life cycle, high reproductive potential and early maturation, and are tolerant of a wide range of environmental stressors, including hypoxia (Diaz & Rosenberg, 1995). The five dominant taxa recorded at both control and fallowed sites are all polychaetes, which have been shown to be the marine organisms most tolerant to stress associated with organic enrichment and low oxygen levels (Pearson & Rosenberg, 1978; Yokoyama, 2002; Levin, 1998). The dominance of deposit feeders, such as capitellids, spionids, and cirratulids, at moderate levels of organic enrichment has been reported elsewhere (Gao et al., 2005; Chareonpanich et al., 1993; Chareonpanich et al., 1994).

By superimposing the dominant taxa in Figure 10.16, it is apparent that Spionidae and Lumbrineridae are responsible for the horizontal spread of the MDS configuration, with samples having high abundances of these taxa being plotted to the right side. Lumbrinerids are generally thought to be unresponsive to organic enrichment and always present in low densities with non-significant variations with time (Grall and Glemarec, 1997; Borja et al., 2000). In this study, however, lumbrinerids responded positively to a moderate level of the disturbance. Lumbrineridae were well connected with position on the MDS plot. Samples with higher abundance were consistently plotted in the upper-right of the ordinations, while the lower values plotted to the bottom-left. Because a higher proportion of silt and clay at fallowed pontoon sites than at control sites was observed in this study, it is likely that finer sediments are favorable for this taxon. Furthermore, it is likely that they are co-dominant with opportunistic species, as reported by Pearson and Rosenberg (1978). They found that *Lumbrinereis latreilli* was among eight species on the edge of an anoxic area, and that *L. minima* was abundant at stations where *Capitella* was dominant. As these taxa are characteristic of moderately to grossly disturbed areas, the shifts in plotted positions of the stations (to the left or to the right) over the study period along the horizontal axis of the MDS plots can thus be justified to assess the status of the disturbance and degree of recovery.

Table 10.5. Control and fallowed sites that have shifted over the sampling period caused by the changes of abundance, number of taxa and dominant taxa. B=Boston Island; R= Rabbit Island; C=control; P0=pontoon=fallowed sites.

Stations	Abundance (ind. / m^2)*		Number of taxa		Dominant taxa	
	Oct 02	Oct 03	Oct 02	Oct 03	Oct 02	Oct 03
BC7	2,517	8,403	22	29	Lumbrineridae Nepthyidae	Spionidae Lumbrineridae
RC3	2,305	5,141	24	25	Nepthyidae Lumbrineridae	Spionidae Lumbrineridae
P01	3,261	9,856	26	22	Lumbrineridae Eunicidae	Spionidae Lumbrineridae
P06	5,495	2,162	22	23	Capitellidae Dorvilleidae	Cirratulidae Lumbrineridae

* Diameter of the corer = 67 mm; replication: 8 cores.

The use of multivariate analyses and distributional/graphical techniques to assess the response of macrobenthic assemblages to environmental disturbance made it possible to detect effects at fallowed farms based on the structure of the benthic assemblages. The second-stage hierarchical clustering and MDS, in particular, separated fallowed sites from the control sites. This also means that the similarity of the patterns of macrobenthic assemblages over sampling times is well detected. The sensitivity of the second-stage MDS in detecting similarities between samples within each sampling time has been reported by Clarke *et al.* (2006). They observed that the second-stage ordination removes the main-effect differences, which are main inter-time (time-to-time) differences. These effects are usually displayed by a conventional/first-stage plot. The analyses of the second-stage plot thus concentrates only on the relationships among samples within each sampling time, by excluding all the similarities between samples at different sampling times. As shown in Figure 10.18, a general trend of separation between control and fallowed pontoon sites was clearly more apparent using the second-stage dendrogram and MDS than first-stage anaylses, indicating more similar spatial patterns of benthic assemblages across the sampling times at fallowed sites than at control sites.

The great variability between stations within each sampling time has resulted in a high complexity in rates and degree of recovery. Recovery from disturbance is likely dependent on the location where the samples were collected. Depending on sites, a major recovery or further disturbance upon macrobenthic structure over the sampling period was observed. In particular, a major recovery may have occured at site P06 as the site shifted from the upper-right to the middle of the MDS configuration, owing to similarity of taxa composition to those at control stations by the end of the study. Result from 'the bubble plots' (Figure 10.15) suggest that capitellid worms were the most abundant at this site in October 2002. However, further disturbance may have occurred at site P01, BC7 and RC3 as they shifted to the right towards the end of the study. For instance, site P06 (fallowed site) recovered from disturbance, in which the benthic structure became relatively similar to the control sites over time as shown on the MDS plots, while others remained moderately disturbed at the end of the study. Results from the ABC plots indicated that stations at fallowed sites were categorized as moderately disturbed areas, suggesting that the current aquaculture practices in southern Spencer Gulf are unlikely to have a major impact on the sedimentary habitat. However, some of the control sites were also detected as moderately disturbed, suggesting that organic matter may be dispersed to areas at least 1 km away from the fish cages. This

result coincides with a finding by Modica et al. (2006) in the Gulf of Castellammare, Meditteranean, where fish farm facilities generated an organic enrichment of the water column up to at least 1,000 m downstream from the cages. This location had mean water current velocities throughout the year of about 10 - 12 cm s^{-1}, relatively similar to southern Spencer Gulf, at 8 – 10 cm s^{-1}. This implies that a constant downsteam flux of particulate organic matter may be generated in such conditions. Thus, hydrodynamic condition with relatively strong current velocity (8 – 10 cm s^{-1}), as in southern Spencer Gulf, may affect the dispersion of particulate matter and cause an extended zone of impact.

Based on the variability of macrobenthic abundance over time, which also responded differently in their patterns between the first six months and the last six months, the availability of organic matter may vary, whether because of variations and/or the difference in harvesting time of the farms, or organic dispersion by relatively strong water current velocity in this region. It is suggested that the tendency of decreasing abundance at fallowed sites over the first six months may indicate the direct impact of farming activities on the sediment structure during this period. The recovery process of the assemblages may be started six months after the fallowing period. By the end of the study, however, the recovery process is still underway, in which some of the areas are still in a moderately disturbed condition caused by the farming activities.

10.5. Conclusions

Changes in macrobenthic abundance were significantly different between the control and fallowed pontoon sites, suggesting that impacts from aquaculture prevented the fallowed sites from following natural trajectories of abundance. Abundances at at least some fallowed sites were considerably higher than at control sites at the start of the study, and while most fallowed sites remained high, abundances at some control sites increased to a similar level by the end of the study, especially in the Rabbit Island zone. However, most of the variation in taxonomic richness and diversity was at the site level, with no impacts of aquaculture detectable. Similar results were found for sediment composition. Five dominant taxa (Capitellidae, Cirratullidae, Lumbrineridae, Nephtyidae, and Spionidae), all relatively tolerant to organic enrichment, also did not differ greatly between control and fallowed sites. The responses of the main trophic groups to organic matter (assessed using % organic carbon) are in accordance with Pearson & Rosenberg (1978) in that larger and deeper burrowing species are gradually replaced by greater numbers of small suspension- and surface deposit feeders as organic matter increases. However, a reduction in the number of trophic groups did not occur, implying moderate levels of disturbance at the studied sites.

Multivariate analysis was more sensitive in detecting the response of the macrobenthic assemblages in relation to organic enrichment than univariate analysis. Second-stage hierarchical clustering and MDS analyses, in particular, separated fallowed sites from the control sites.

The great variability between stations within each sampling time in this study resulted in a high complexity in rates and degree of recovery as a result of the spatial and temporal changes in the infaunal structure. Thus, recovery from disturbance is likely to be dependent on the location where the samples were collected. In particular, site P06 shows recovery from disturbance from moderately disturbed to undisturbed, in which the benthic structure became relatively similar to the control sites. Conversely, a further disturbance is suggested for site

P01, BC7 and RC3. Seasonal fluctuations caused by natural variability, hydrodynamic conditions, sediment characteristics, and organic matter are likely to be responsible for the observed changes of the assemblages over the study period.

It appears that after 12 months of fallowing, most of the fallowed sites had returned to similar levels of infaunal abundance as the control sites (6 out of 8). However, one fallowed site in each zone continued to have elevated levels of abundance. It is difficult to determine if this was due to a lack of recovery, as one of the control sites also increased in abundance to similarly high levels (BC7). Overall, it seems that fallowing does work as a waste mitigation strategy, but that 12 months may not be sufficient time for all sites to return to normal.

The high site-to-site variability observed also has implications for future monitoring. If this variability is not considered when designing a monitoring program, then it becomes very difficult to detect even large changes, as they are hidden by the other sources of variability. However, to account for this in monitoring programs requires that the source of the variability is understood, which is not the case here. Further analyses might show that it is related to environmental characteristics such as grain size and chemical composition, however, a preliminary cluster analysis of sites based on grain size did not show any clear separation of sites. Instead, the two major groupings contained samples from most sites, with sites changing group depending on time of sampling (J. Tanner, unpublished analyses). Unfortunately, other sediment parameters (organic carbon, total nitrogen and carbonate) were only sampled once, and hence cannot be incorporated into this analysis. Thus, while it is suggested that the geographic distinction between Boston Island and Rabbit Island may not be the most useful way to determine zones, we do not currently know enough about what is driving infaunal composition to be able to provide a better classification.

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Chapter 11: Fouling assemblages on SBT nets and the efficacy of an antifouling treatment

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Abstract

A test of the antifouling properties of Wattyl NetClear® was conducted on fish farms containing southern bluefin tuna (*Thunnus maccoyii*) during a six-month period (February-July). Wattyl NetClear® is a water-based synthetic latex-based coating where the active antifouling agent is a mixture of two isothiazolinones likely to affect both biochemical conditioning and bacterial colonisation. The development of fouling was monitored using underwater stereo-photogrammetry on three treated and three untreated net cages with the main factors being treatment, depth and cage nested within treatment. A significant treatment and depth effect was found but variable through time and in some instances with significant cage effects. By the end of the study the difference in fouling load between treated and untreated nets was 14.7%. In total 72% of all free-space was on treated nets. The dominant fouling organisms were *Entermorpha sp*. and sponges with low settlement of blue mussel and paper oysters. Passive deposition of tuna faeces contributed significantly to the cover. The largest cover of fouling was observed in April-May with a dominance of sponges in June-July. *Enteromorpha sp.* dominated shallow depths while sponges dominated at deeper. Tuna faeces were distributed independent of depth but varied with time. The results showed that fouling of fish cages consists of both active settlement and passive deposition, the later independent of antifouling treatment.

11.1. Introduction

The farming of southern bluefin tuna (*Thunnus maccoyii*) in Port Lincoln, South Australia has occurred since 1989. About 130-150 sea-cages (from 30 - 50 m diameter) are situated seaward of Boston and Rabbit Islands (Figure 11.1). Licence holders are restricted to a maximum of 6 tonnes of tuna per ha and must not exceed 4 kg m^{-3} allocated according to the quota held (1 ha for every 3 tonnes introduced to the site) (see http://www.pir.sa.gov.au/byteserve/aquaculture/policy/lower_eyre.pdf).

A total quota of 5,265 tonnes southern bluefin tuna is caught in purse seines in the Great Australian Bight and subsequently towed to Port Lincoln to be transferred to the sea cages. Here they are kept from December-January to August-September and grown to about 9,000 tonnes. During this period, the tuna are fed at least 45,000 tonnes of sardines. This implies that about 3,600 tonnes of carbon are excreted to the environment (0.2 conversion of wet weight/dry weight, 40% carbon).

Oxygen supply is one of the key issues in intensive aquaculture systems such as sea cages, where a reduction in the dissolved oxygen content of the water is a common problem (Braithwaite & McEvoy 2005; Edwards & Edelston, 1976; Madenjian, 1990; Silvert, 1992). A number of factors including the oxygen demands of the fish, the plankton, the microfauna and the microbial flora of the sediments accumulating beneath the cage may interact to deplete oxygen concentrations to levels below that required to optimise fish growth. In addition, mass water flow provides for the exchange of oxygen-depleted water from inside the cage with oxygenated water from outside the cage. The effectiveness of this exchange is substantially reduced when net fouling impedes currents (Inoue, 1972; Lee et al., 1985; Yokoyama et al., 2004) and can lead to a reduction in oxygen concentrations to levels that negatively impact upon farmed fish. This depletion may in turn lead to increased stress and susceptibility to disease.

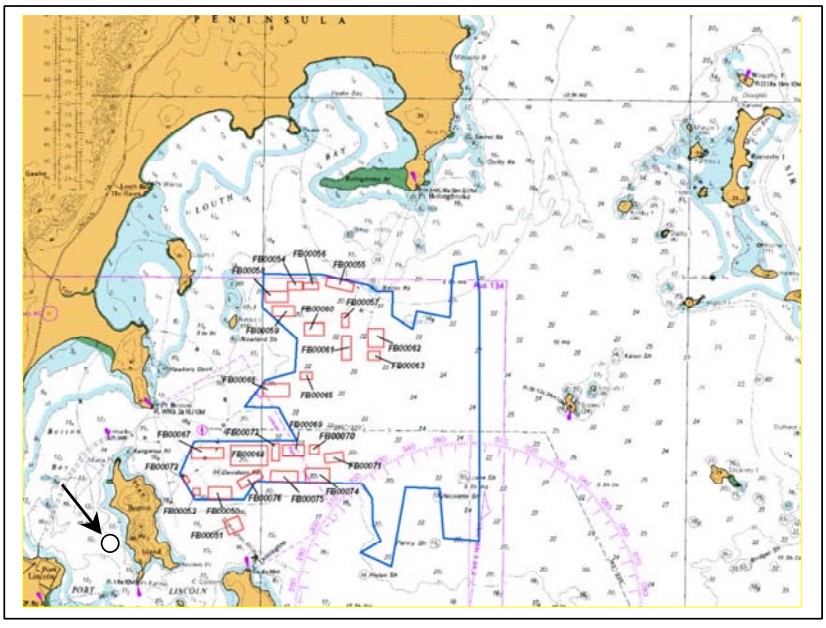

Figure 11.1. Map showing the tuna-farming area at Port Lincoln, South Australia, off Boston and Rabbit Islands in 2002. Arrow indicates position of experimental fish cages.

The nets used for the cultivation of finfish in marine cages introduce an artificial substratum, which together with the high level of input of waste products in the form of food and faeces, enables the proliferation of an abundant fouling assemblage. Historically, nets in Boston Bay have shown obvious fouling after only 4 weeks, quickly becoming heavily encrusted (Bond, 1992), and in a previous study (Cronin et al., 2000), typical fouling rates of between 2 and 4 kg wet weight (WW) m^{-2} were observed on cages. Such fouling can cause considerable drag on a net cage leading to deformation, stress on anchorage and low water exchange (Sliskovic & Jelic, 2002).

There is a need for effective antifouling agents to prevent settlement and growth of marine organisms, in particular for agents that are biodegradable and harmless to the environment (Boxal et al., 2000; Evans et al., 2000; Yebra et al. 2004). For many years tributyltin (TBT) has proven effective as the active agent in paint formulations but due to its toxicity it has been banned and replaced with copper boosted with biocides. To date the chronic effects of these are unknown and difficult to determine (see Konstantinou & Albanis, 2004).

In this study, six sea cages, which were elements of a SARDI research farm located in Boston Bay, west of Boston Island (Figure 11.1), were used to evaluate the extent to which net treatment with the anti-fouling agent, Wattyl NetClear, results in a reduction in the load of fouling biota on nets of tuna sea-pontoons, and provide an estimation of the likely impacts of net fouling on water exchange between the cage and the external environment.

11.2. Materials & Methods

11.2.1. Study site and experimental net cages

Net cages for tuna, measuring 12 m in diameter with a mesh size of 3-4 inches (7-10 cm, corner to corner stretched measurement), were situated on the South-West side of Boston Island, Port Lincoln, South Australia (Fig. 10.1). A total of 6 nets were selected for sampling and 3 of these treated with the antifouling agent and 3 left untreated as controls. Each cage contained between 5 and 20 fish throughout the trial period. The tuna were fed using a standard commercial pellet with variable nutrient content. Water flows at the study site are tidally driven in a north-south direction. The mean water temperatures at 10 m depth during the experimental period in 2002 were 20.1^{o}C (March), 19.0^{o}C (April), 17.5^{o}C (May), 14.7^{o}C (June) and 13.2^{o}C (July).

11.2.2. Anti-fouling treatment

Three net cages were treated with Wattyl NetClear on land. Wattyl NetClear is a water-based synthetic latex-based antifouling coating for aquaculture nets and ropes. The active agent in Wattyl NetClear (product code 107302) is a mixture of two so-called isothiazolinones. The most frequently used are 2-methyl-4-isothiazolin-3-one (MI) with CAS No. 2682-20-4 and 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) with CAS No. 26172-55-4. These two substances are used as a mixture in the preservative product with the commercial name Kathon 886 (CAS No. 55965-84-9), which contain 3.8% MI and 10.1% CMI = 13.9% active ingredients. Kathon 886 is generally used as a preservative, biocide and disinfectant. In addition to isothiazolinones, Wattyl NetClear also contains diethylene glycol monobutyl ether and ammonium hydroxide embedded in acrylic polymer latex (www.chemwatch.net).

11.2.3. Sampling procedure

Nets were deployed during the first week of February 2002. On each cage, 2 vertical transects were established by marking the depth levels 2, 4, 6, 8, 10 m with coloured cattle ear tags. UW stereo-photographs (Fuji-chrome Sensia 100) were taken of the netting by scuba divers at each depth on each of the 12 transects, ensuring that the same area was photographed each time by placing the cattle tag in the right upper corner of the photographic frame. The area photographed at each depth was 0.25 m^2. Photos were taken on March 12th, April 11th, May 2nd, June 5th and July 17th 2002. The underwater stereo-camera consisted of two Nikonos V's mounted with a stereo-base of 20 cm, manually synchronised shutters and 15 mm wide-angle lenses. The cameras and the electronic flash system were mounted on a specially designed rig attached to a 50 x 50 cm photographic frame with a 67 cm maximum distance from the focal plane. Lighting was controlled by four DYFO UW flash units. Stereo-photographs taken with this camera allow a three-dimensional view of the net with a resolution of up to 2 mm depending on water clarity.

11.2.4. Fouling analyses

The stereo-photographs were analysed using two aligned Nikon dissecting microscopes to obtain a three-dimensional view. The photographed netting was analysed for cover of fouling organisms and free space by applying 100 regularly spaced points within the photographic frame. Cover was identified as both primary and secondary substratum and was not separated. Species visible in the stereo-photographs were identified to the highest taxonomic level possible. No samples were taken for further identification.

11.2.5. Statistical design

The experimental design is a split-plot ANOVA with treatment, cage and depth (2, 4, 6, 8, 10 m) as fixed factors repeated over time (February-July). The model was designed with the between subject factors Treatment and Cage (Treatment), and within subjects factors Depth, Treatment * Depth and, Cage (Treatment)*Depth, analysed separately for each time (see Quinn and Keough 2002). The data (percentage cover) were arcsine transformed and the analyses were performed using the statistical package SPSS for Windows v. 13.0. Levene's test was used to test for homogeneity of variances. Additional analyses were done using the non-parametric Kruskal-Wallis Test and Mann-Whitney U-Test.

11.3. Results

11.3.1. Effects of treatment, depth and cage

The results of the ANOVA of the fixed factors treatment, depth and cage are shown in Table 11.1. In order to meet the assumption of an ANOVA, all variables were tested for normality and homogeneity of variances. In several cases these assumptions were not met and further transformations could not solve this problem. Because ANOVA is considered robust against non-homogeneous variances the analyses were carried out regardless (see Underwood, 1997). A significant treatment effect through time, with the exception of March, is evident with a greater cover of open space (water) for treated nets than untreated ones in April, June and July and reversed in May (Figure 11.2). A significant effect of depth is evident throughout the whole period with the exception of June (Figure 11.3). The effect of cage (Cage nested within Treatment) is an error term describing whether the treatment effect is consistent between cages of each treatment. The results showed no effect of cage in April, May and June but a significant effect in March and July indicating that the significant treatment and depth effect in July was not consistent among cages. In March, there was no significant effect of treatment, but there was of depth, although this effect was not consistent among cages. No significant interactions were evident with the exception of April indicating that the significant treatment effect was not consistent across depths for this month. The relationship between percentage cover of free space (water) of untreated and treated nets is shown in Figure 11.4. The data points are the means of 3 replicates. In total 72% of all free-space (all time x depth combinations) was on treated nets. The cover of net (net visible in the photographs) is shown in Figure 11.5. The graph shows that during March-April, more net was visible in treated than untreated cages. The cover of visible net declined rapidly during the first two months and remained low throughout the test period (Figure 11.6).

Table 11.1. Result of the test of fixed effects over time (ANOVA). Significant P-values are in bold.

	df	March	April	May	June	July
Treatment	1	0.627	**0.000**	**0.008**	**0.000**	**0.000**
Depth	4	**0.016**	**0.000**	**0.000**	0.448	**0.000**
Cage (Treatment)	4	**0.000**	0.055	0.056	0.060	**0.000**
Treatment * Depth	4	0.183	**0.001**	0.607	0.845	0.268
Depth * Cage (Treatment)	16	0.670	0.173	0.852	0.151	0.171

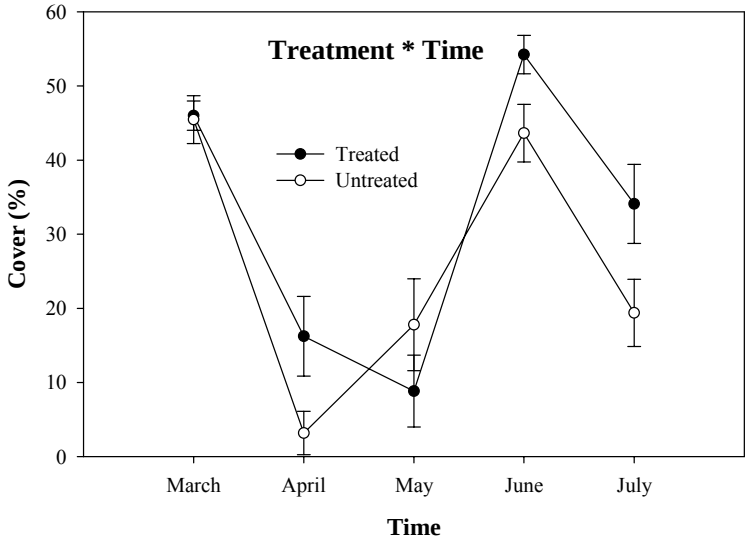

Figure 11.2. Percentage cover of free space (water) as a function of treatment and time. Error bars are 95% CI.

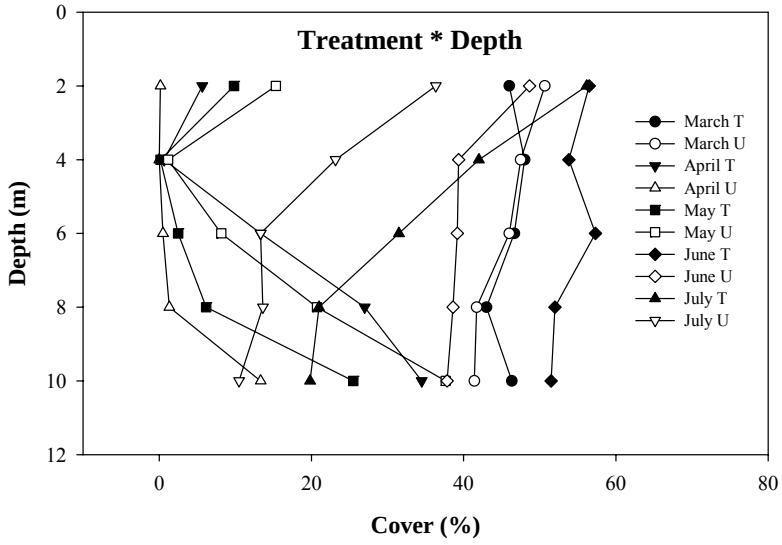

Figure 11.3. Percentage cover of free space (water) as a function of treatment and depth. Error bars are not included for clarity.

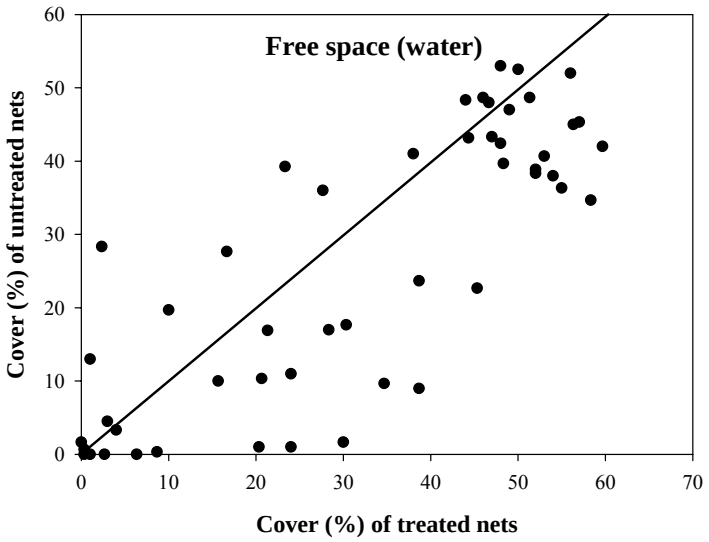

Figure 11.4. The relationship between percentage cover of free space (water) of untreated and treated net. Data points are means of 3 replicates. The line is the 1:1 ratio of cover of untreated and treated nets.

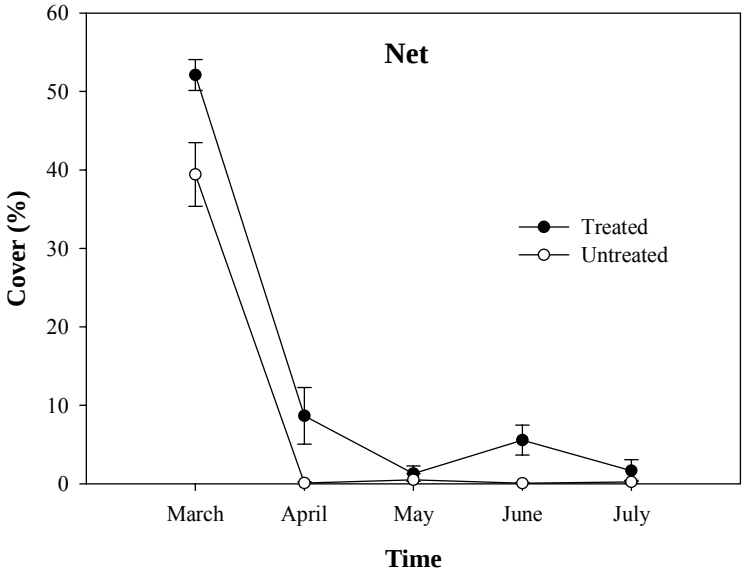

Figure 11.5. Percentage cover of net as a function of treatment and time. Error bars are 95% CI.

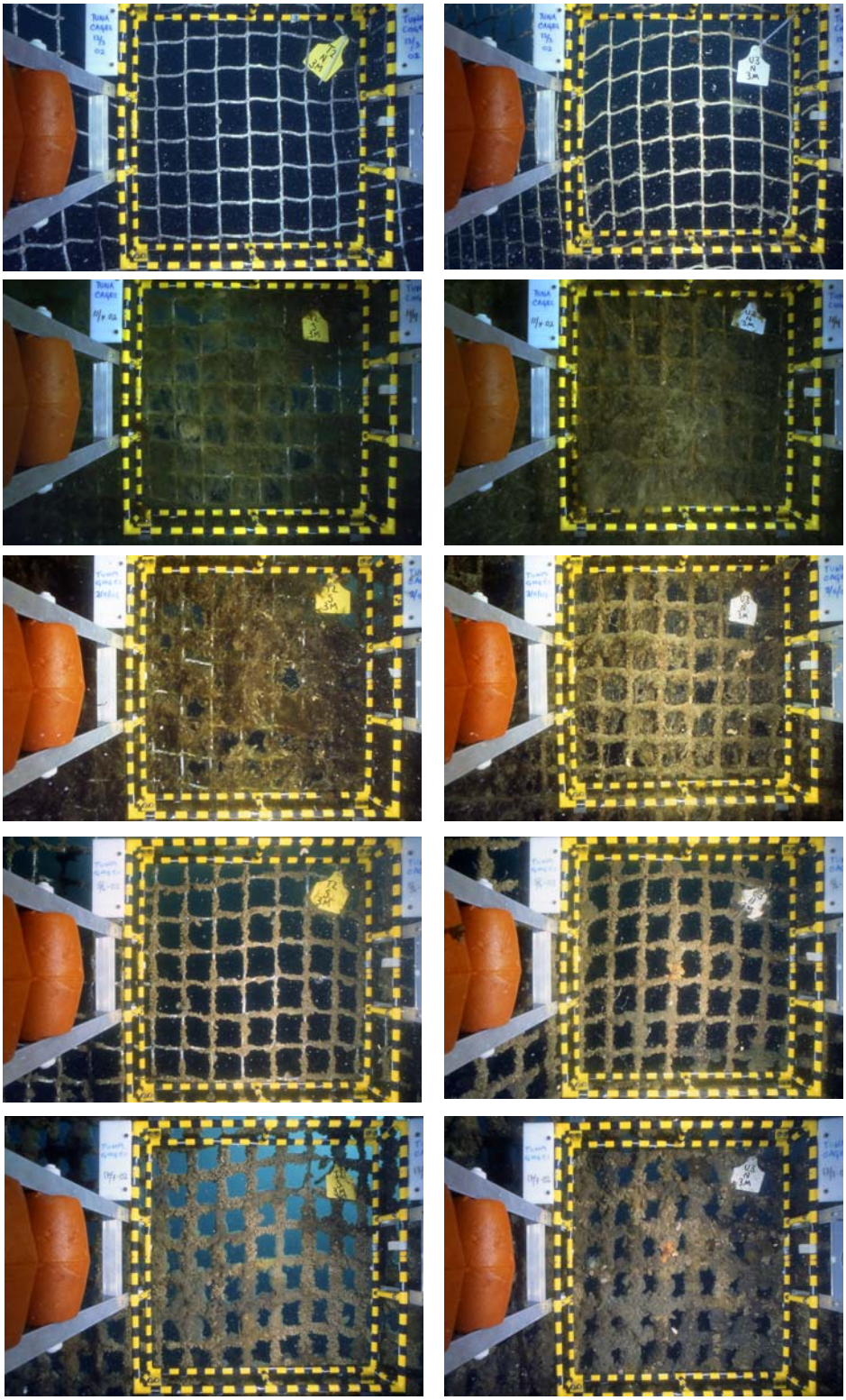

Figure 11.6. Development of fouling on plots at 4 m depth from March to July. Treated nets are to the left and untreated to the right. Frame size is 50 x 50 cm.

11.3.2. The fouling assemblage

The diversity of the fouling assemblage was generally low, particularly at depths greater than 2 m. The most abundant organisms during the trial were the green alga *Enteromorpha sp.* (*prolifera*) and sponges (possibly of the genus *Verongia*) primarily occurring on the deeper areas of the nets. Settlement of the paper oyster (*Electroma georgiana*), blue mussels (*Mytilus galloprovincialis*) and hydroids took place at the shallower depths, but only with a marginal cover and these species will therefore not be considered further.

Effects of time

The fouling assemblage on the nets varied significantly through time and constituted in March a low and patchy occurrence of small thread-like red algae (not identified), which subsequently disappeared. From March to June, the nets were colonised extensively by the green alga *Enteromorpha sp. (prolifera),* which dominated during April- May with up to 100% cover (Figure 11.7). In April, significantly more *Enteromorpha sp*. was observed on untreated compared to treated nets (Mann-Whitney Test, $Z=-4.443$, $p<0.00$). In May and June, however, this trend was reversed with significantly more *Enteromorpha sp.* on treated nets than untreated (Mann-Whitney Test, May: $Z=-2.522$, $p=0.012$; June: $Z=-2.090$, $p=0.037$). From May onwards, *Enteromorpha sp.* and nets were densely covered with particulate tuna faeces (Figure 11.8). No significant effect of treatment on percentage cover of tuna faeces was evident at any time. The cover of sponges developed from May onwards, with significantly greater cover on untreated than treated nets in the June (Mann-Whitney Test, $Z=-5.012$, $p=0.00$) and July (Mann-Whitney Test, $Z=-2.515$, $p=0.012$) samples (Figure 11.9).

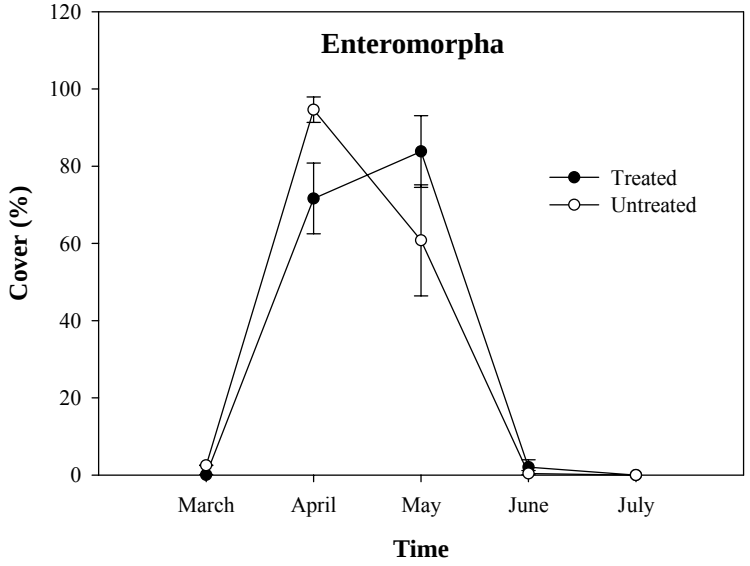

Figure 11.7. Percentage cover of *Enteromorpha sp*. as a function of time. Error bars are 95% CI.

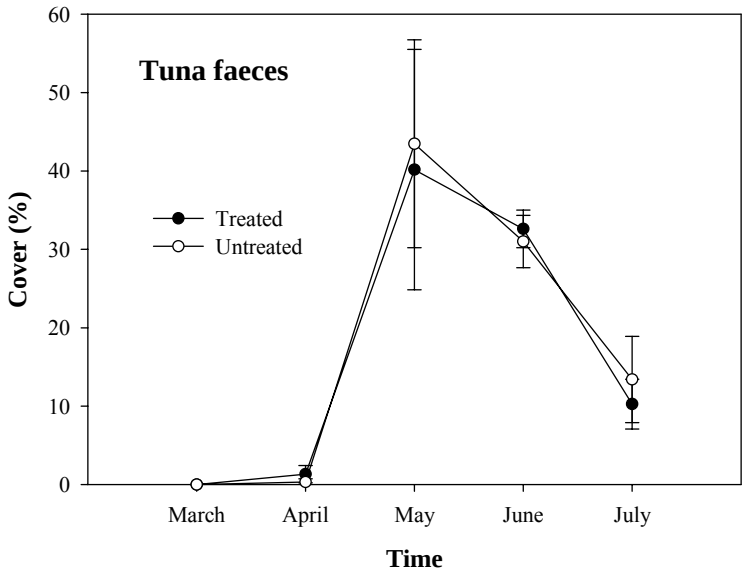

Figure 11.8. Percentage cover of tuna faeces as a function of time. Error bars are 95% CI.

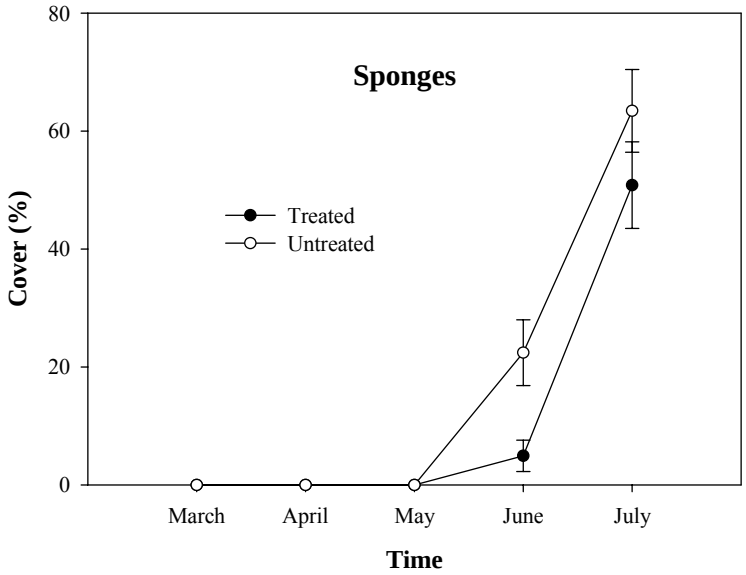

Figure 11.9. Percentage cover of sponges as a function of time. Error bars are 95% CI.

Effects of depth

The fouling assemblage on the nets varied significantly with depth. The mean cover of *Enteromorpha sp.* with depth and time is shown in Figure 11.10. The *Enteromorpha sp*. cover was largest at 4 m depth being almost 100% in the April-May samples. Untreated nets in April had a mean cover of nearly 100% from 2 to 8 m depth with less cover for treated nets with the exception of the 4 m level (Figure 11.10). During May and June, cover of *Enteromorpha sp.* was successively reduced but was consistently larger on treated than on untreated nets at all depths. For treated nets, significant effects of depth on the cover of *Enteromorpha sp.* were observed in April, May and June (Kruskal-Wallis Test, χ^2=22.329, 16.664, and 10.739; p=0.000, 0.002 and 0.03; df=4), while for untreated nets a significant effect of depth was observed in April and May only (Kruskal-Wallis Test, χ^2=16.774 and 14.448; p=0.002 and 0.006, df=4).The cover of tuna faeces was fairly similar at all depths during the June-July period with about 30-35% and 10-15% cover, respectively (Figure 11.11). In May, however, tuna faeces had a variable cover of 40-60% at all depths with the exception of 4 m where the cover was marginal. A significant effect of depth on cover of tuna faeces was observed in April for treated nets (Kruskal-Wallis Test, χ^2=13.358, p=0.010, df=4) and in April and May for untreated nets (Kruskal-Wallis Test, χ^2=112.341 and 13.777, p=0.015 and 0.008, df=4). Sponges appeared in the June samples and the cover developed further in the July samples reaching more than 50%, particularly at water depths greater than 4 m (Figure 11.12). Sponge growth constituted sheets of tissue covering the netting and in a few cases developed over extensive areas of more than 0.25 m^2 at the deeper sites. Tuna faeces periodically covered the sponges. A significant effect of depth was observed for treated nets in both the June and July samples (Kruskal-Wallis Test, χ^2=11.542 and 18.572, p=0.021 and 0.001, df=4) and for untreated nets in the July samples only (Kruskal-Wallis Test, χ^2=10.205, p=0.037, df=4). By the end of the test period, the difference in fouling load between treated and untreated nets was 14.7%, with about 65.9% cover of fouling (34.1% free space) in treated nets compared to untreated nets with about 80.6% cover (19.4% free space).

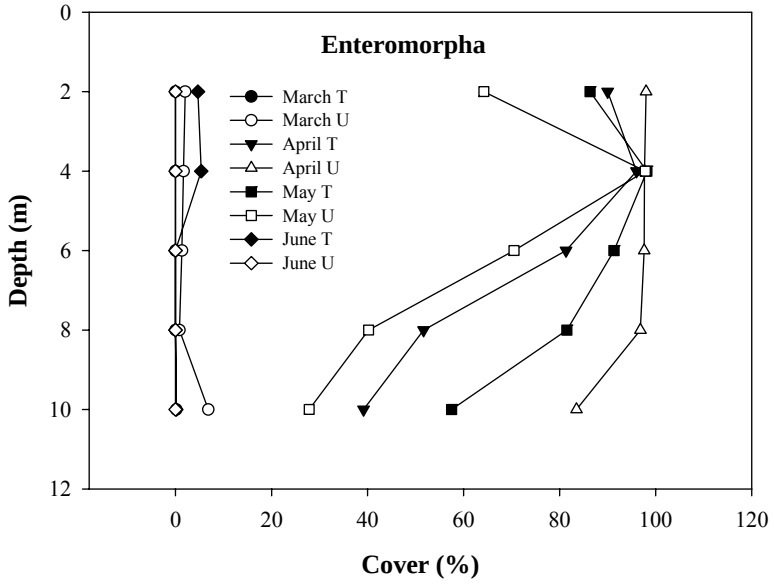

Figure 11.10. Percentage cover of *Enteromorpha sp*. as a function of depth. July data for both treated and untreated nets were zero and not shown for clarity. T = treated with NetClear; U = untreated. Error barsnot included for clarity.

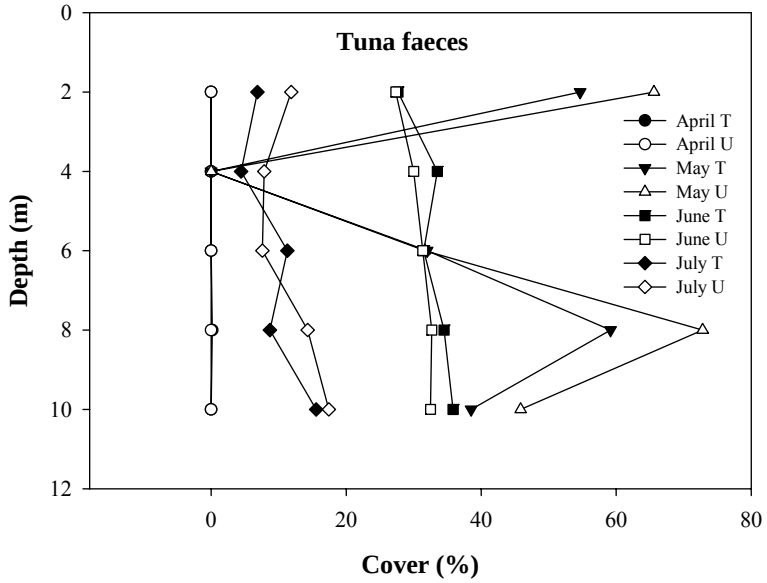

Figure 11.11. Percentage cover of tuna faeces as a function of depth. March data for both treated and untreated nets were zero and not shown for clarity.T = treated with NetClear; U = untreated. Error bars not included for clarity.

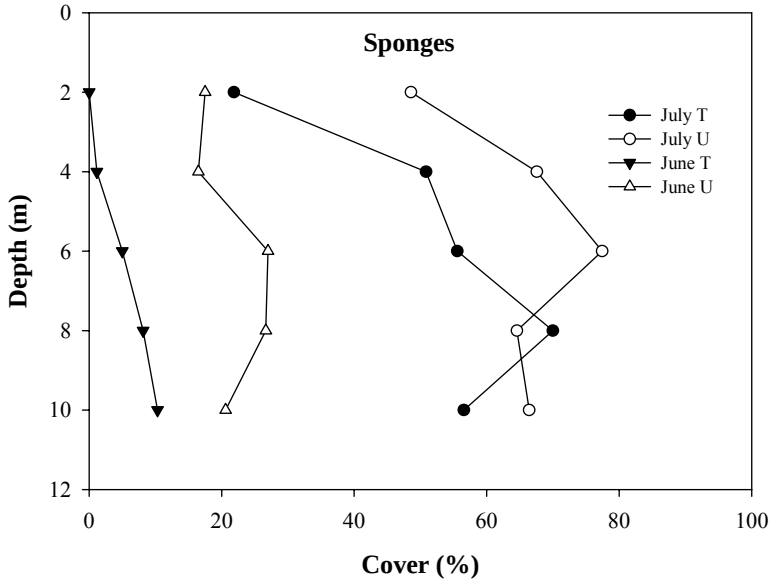

Figure 11.12. Percentage cover of sponges as a function of depth. March, April and May data for both treated and untreated nets were zero and not shown for clarity. T = treated with NetClear; U = untreated. Error bars not included for clarity.

11.4. Discussion

The establishment of fouling assemblages on marine artificial substrata such as fish cages is a complex process starting with an initial biochemical conditioning followed by bacterial colonisation. After bacterial priming, colonisation by unicellular eukaryotes forms the substratum necessary for successive colonisation of multicellular eukaryotes. This process has been described by Wahl (1989) as a "fouling sequence model". Surfaces of fish cage nets subjected to fouling seem to follow this model. Nets are firstly fouled by microbial organisms such as diatoms, hydroids, algal spores and marine bacteria (Chea & Chua, 1979; Hodson & Burke, 1994), followed by macro-organisms including algae, barnacles, bivalves, marine worms and ascidians (Milne, 1975). An effective anti-fouling treatment would accordingly be best directed towards prevention of biochemical conditioning and bacterial priming (development of microbial bio-films) (see Steinberg et al., 2002).

Since the ban on TBT (tributyltin) as an antifouling agent, the use of alternative coatings containing copper combined with organic booster biocides to prevent growth of bacteria and subsequent settlement of higher invertebrates has increased (Konstantinou & Albanis, 2004). The use of toxic antifouling treatments, such as metal-based agents on the netting of fish cages, has proven effective, but is less desirable because of their environmental effects and possible effects on the caged fish leading to lower market prices and an unfavourable image of the industry (Lewis, 1994). This has led to the development of silicone-based coatings, which act to prevent biochemical conditioning, provide poor adherence for settling organisms, and make nets easy to clean (Hodson et al., 2000, Yebra et al. 2003).

Wattyl NetClear is a water-based synthetic latex-based coating where the active antifouling agent is a mixture of two so-called isothiazolinones, which are a class of broad spectrum biocides used in low doses as preservatives in a range of shampoos, household and industrial products and are particularly effective against bacteria and fungi (www.sci-toys.com/ingredients/isothiazolinone.html). Wattyl NetClear does not contain metal-based antifouling agents and thus prevents biochemical conditioning in combination with biocide effects on unicellular eukaryotes.

To test antifouling impregnation of netting the common experimental design is to use panels constructed from netting stretched across frames (Hodson et al., 2000). However, such a design does not expose experimental nets to the full effects of fish kept in net cages (nutrients, particulate matter, faeces) and the hydrodynamic forces, which will act on a full-scale fish cage. In the experiment reported here, replicated treated and untreated fish-cages containing southern bluefin tuna were used to test the efficiency of the antifouling coating Wattyl NetClear in a real-farm situation. The results showed that the development of fouling on fish cage netting is complex and consists of two processes: 1) passive accumulation of particulate matter (tuna faeces) and algal spores, and 2) active settlement of fouling organisms.

Accumulation of tuna faeces was found to be independent of treatment with Wattyl NetClear (Figure 11.8) indicating passive deposition. Fouling by *Enteromorpha sp.* is likely to occur in two ways, either as settling spores or attachment of drifting threads. The results showed that "settlement" of *Enteromorpha sp.* was inconsistent with treatment, because in the April census the cover was observed to be significantly larger on untreated than on treated nets but with reversed effect in May and June (Figure 11.7). In either case, *Enteromorpha sp.* spores

show considerable adhesive and cohesive strength (Finlay et al., 2002) and the developed algae can cause considerable drag on the netting (Romano, 2003). Treatment by Wattyl NetClear may initially exercise some control over settlement of *Enteromorpha sp.* but other processes affecting growth and development take over. This includes the antifouling properties of the alga itself because extracts of *Enteromorpha sp*. have been shown to have antifouling properties (Young Cho et al., 2002). The development of the cover of *Enteromorpha sp.* increased rapidly with nearly 100% on untreated nets at all depths in April but with a reduction with depth in May (Figure 11.10) and subsequent removal in June (Figure 11.7). *Enteromorpha sp.* showed the most prolific development at 4 m depth, which can be explained by the effect of the tuna in the cages, which are generally swimming at that depth when not feeding. Currents and the operation of the feeding boat may also be a factor affecting *Enteromorpha* growth because propeller action removes tuna faeces allowing light to reach the alga (Figure 11.11, May samples).

The low cover of *Enteromorpha sp.* in June-July allowed settlement and subsequent development of sponges as the dominant fouling group (Figure 11.9). At this time there was a clear treatment effect of Wattyl NetClear with significantly greater cover on untreated nets. However, the difference in fouling between treated and untreated nets by the end of the trial period constituted only 15%, which is likely to be irrelevant in terms of cleaning cost.
The composition of the fouling assemblages found on the cages in this study was largely comparable to those found by Cronin et al. (2000) with the exception of the rapid proliferation of *Enteromorpha sp.* That study comprised an assessment of assemblages on cages from Rotten Bay, which is only a short distance from the site used for this study.

Fouling of netting of fish cages has two main effects that may affect the operation of the farm and the health of the fish. Firstly by reducing water flow with subsequent reduction in oxygen, and secondly by increasing drag. The use of non-toxic antifouling treatments of netting for fish cages, such as silicone products or low-level biocides in synthetic latex-based coatings, requires regular cleaning of the nets to avoid build-up of fouling organisms. If nets are cleaned annually, direct reduction in oxygen by fouling is likely to be minor. Cronin et al. (2000) found that in comparison to other oxygen sources and sinks in a fish cage system, the fouling assemblage consumes less than 3% of the available oxygen.

Drag on fish cages caused by tides, waves and current can be substantial and expressed as $D=½\ C_D\rho SU^2$, where D=drag, C_D=drag coefficient, ρ=viscosity (density of fluid), S=surface area over which the force is applied, U=current velocity (Vogel, 1981). C_D is a function of Reynolds number (see Zahn et al., 2002). Viscosity (ρ) of seawater is a function of temperature and salinity. At 35 psu and 10ºC ρ=1.39, 20ºC ρ=1.09, 30ºC ρ=0.87. According to this model there are only two variables to consider, namely C_D, which is a function of the shape of the cage and S, which is the surface area facing the incoming current and is proportional to the cover of free space (pore size) (Figure 11.2). C_D is likely to vary between 1.17 and 2.3 for a theoretical model. Only flume simulation experiments validated by *in situ* experiments can determine C_D accurately (see Lader et al., 2001; Frederikson et al., 2003.). In this study, drag was pronounced and influenced by the cover of *Enteromporpha sp*. during April-May and sponges in July (Figure 11.6, 10.7 and 10.9).

11.5. Conclusions

In conclusion, nets treated with Wattyl NetClear had a significant reduction in fouling relative to untreated nets. By the end of the study this represented a 14.7% (65.9% *vs* 80.6%) reduction in fouling load. The fouling load changed over the course of the study being highest at the second sample time (April-May) when levels peaked at 75-95%. Much of the fouling, particularly early in the study, related to seasonal blooms of macroalgae, which in many cases appeared to be from material caught on cages (rather than having recruited and grown onto cages). It is unlikely that any anti-foulant will be able to prevent this sort of material becoming entangled in lines and nets. Not withstanding this, the underlying level of recruitment appears to have been less on treated cages. To avoid fouling by seasonal blooms of macroalgae like *Enteromorpha sp.*, location of fish cages further offshore (as has happened at Port Lincoln) is likely to be beneficial (see Benetti et al., 2001). Results from this component of the project provided the basis for development of the project "Aquafin CRC - Southern Bluefin Tuna Aquaculture Subprogram: net fouling management to enhance water quality and Southern Bluefin Tuna (Thunnus maccoyii) performance" (FRDC 2003/226), currently underway.

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Chapter 12: Evaluation of waste management strategies for the Southern Bluefin Tuna industry

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Abstract

The southern bluefin tuna (SBT) industry operating off the coast of Port Lincoln is Australia's most valuable aquaculture sector (FRDC, 2004). The farming of SBT produces excess nutrients in the form of uneaten feed, faecal matter and other excretion wastes, which are released into the surrounding ocean environment. Polyculture has been proposed as a method to utilise the waste material produced by monocultures such as SBT. It involves integrating cultures of nutrient-extractive species that can be exploited commercially with a primary culture, in this case SBT, to reduce nutrient loading while providing the added benefits of increased profitability through crop increase and diversification. The aim of this review was primarily to assess the potential of using polyculture techniques in the culturing of SBT in the waters off Port Lincoln. We have also briefly addressed engineering solutions currently available for use in waste mitigation. This study involved conducting a literature survey on current open ocean polyculture studies, a literature investigation into the marine biodiversity of the Port Lincoln area, and a discussion on the major issues presented by polyculture. This information was then combined to suggest potential models that could be used by the SBT industry.

12.1. Introduction

Open ocean aquaculture of southern bluefin tuna (SBT, *Thunnus maccoyii*) forms a significant component of Australia's aquaculture industry and plays a major role in supporting the coastal community of Port Lincoln on South Australia's Eyre Peninsula. As with most intensive farming systems, the farming of SBT produces excess nutrients in the form of uneaten feed, faecal matter and other excretion wastes, which are released into the surrounding ocean environment in both dissolved and particulate form.

Currently, fallowing is used as the single waste management tool for SBT and involves relocating pens at the end of each season to prevent the accumulation of wastes within a particular area. While this technique has proven to be successful, with sediments showing little impact 12 months after fallowing (Fernandes et al., 2004; Putro & Svane, 2005), the impact of excess nutrients at a regional scale is harder to quantify and may become increasingly significant if stocking densities, holding period and the number of farms increase in the future.

On a worldwide scale aquaculture is expanding rapidly to fill the discrepancy between the increasing demand for seafood and the decreasing provisions afforded through wild fisheries. In order to accommodate this growth environmentally, there is a general consensus in the literature that aquaculture companies will need to become financially responsible for the waste produced by their ventures. This is particularly important to operations carried out in publicly owned environments, such as the ocean. Through this 'user pays principle' a strong emphasis will be placed on waste reduction.

Polyculture has been proposed as a method to utilise the waste material produced by monocultures and can provide the extra benefits of increasing profitability through producing a second product stream. It involves integrating cultures of nutrient extractive-species that can be exploited commercially with a primary culture, in this case SBT. Cultures of the extractive species utilise the particulate (e.g. mussels, oysters) or inorganic soluble wastes (e.g. seaweeds) produced by the fish farm, reducing nutrient loadings through conversion into valuable biomass. To date, the greatest success of polyculture can be seen in land-based systems, where the complexities of the natural environment can be largely controlled and manipulated to maximise the productivity of each unit. Ocean-based examples are less common, in part due to the uncertainties of working in the open sea. Successful examples do exist however, and research into commercial scale systems continues to grow, forming an innovative and timely area of new research.

The aim of this review was primarily to assess the potential of using polyculture techniques in the culturing of SBT in the waters off Port Lincoln. We have also briefly addressed engineering solutions currently available for use in waste mitigation. This study involved conducting a literature survey on current open ocean polyculture studies, a literature investigation into the marine biodiversity of the Port Lincoln area, and a discussion on the major issues presented by polyculture. This information was then combined to suggest potential models that could be used by the SBT industry.

12.2. The concept of polyculture

A recent review of polyculture conducted by Neori et al. (2004) highlights the shortage of published commercial scale open ocean polyculture studies. While economic models exist for land-based polyculture they are yet to be developed for ocean-based systems. A literature review has shown that the number of polyculture studies conducted within Australia is limited to three, with only one of these carried out in an open ocean environment. While ocean-based polyculture can be difficult due to the complexities of the open environment, it has been suggested that progress has been halted by political rather than technological shortcomings. The fact that aquaculture companies are not legally accountable for the wastes produced by their operations has also slowed research in the area (Neori et al., 2004). Nevertheless, there are a number of commercial scale ocean trials currently underway, designed to be applicable to a variety of different culturing species and environments.

12.2.1. Shellfish

Most open ocean polyculture studies involve growing mussels/oysters and/or seaweeds adjacent to ocean salmon farms (Figure 12.1). Jones & Iwama (1991) studied the growth of the Pacific oyster (*Crassostera gigas*) cultured within Chinook salmon (*Oncorhynchus tshawytscha*) farms in British Columbia, Canada. The shell height of oysters suspended at salmon farms was found to be 3 times greater than at control sites and growth rates declined with distance from farms. Both chlorophyll and particulate organic matter (POM) was found to be significantly higher within the pens. Correlation analysis demonstrated that shell growth was most dependent on chlorophyll levels, while growth rate was dependent on the concentration of POM. The Pacific Oyster was chosen due to its commercial importance, availability and hardiness. The temperature of the water in this study ranged from 14 to 21°C, which is similar to the range found within Port Lincoln waters. This study was unique in that oysters were suspended within the fish pens themselves, using a lantern net suspended by buoys 1 m from the surface.

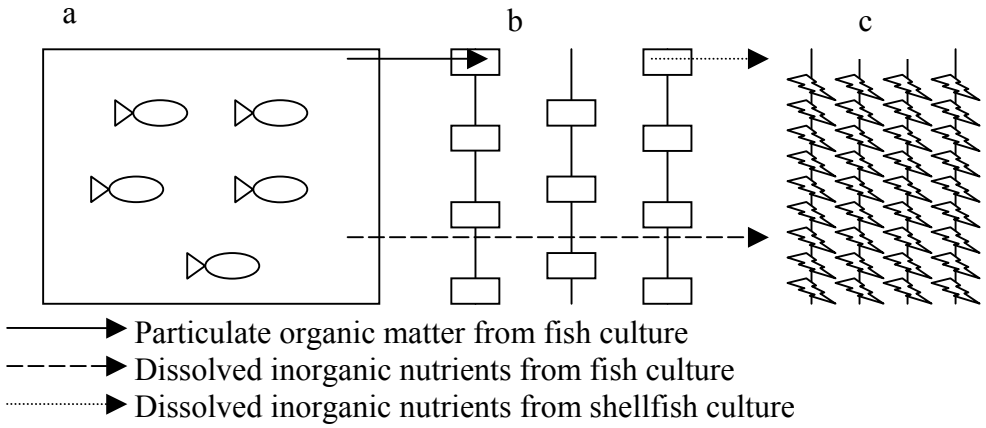

Figure 12.1. The classic open-ocean polyculture model. Particulate organic matter from fish pens (a), drives production in adjacent shellfish cultures (b), with dissolved inorganic nutrients from both (a) and (b) used in the photosynthetic growth of adjacent seaweed cultures (c).

An Australian study highlighted the potential of pearl oyster aquaculture in controlling waste nutrients (Gifford et al., 2004). In this model it was estimated that up to 19 kg of nitrogen could be removed from coastal waters per tonne of pearl oyster harvested per year. By exploiting the high volume of water pumped by the pearl oyster (the highest of any bivalve) and the subsequent bioaccumulative capacity, it was suggested that this species could be used as a bioremediator in impacted sites. Advantages of incorporating the pearl oyster into a polyculture system include its wide natural distribution and the fact that the value lies in the pearl and not the flesh, so food safety issues would not be of concern. One potential disadvantage discussed in that study, which is applicable to any commercial scale shellfish culture, is the adverse impact of biodeposition of faecal and pseudofaecal material from the shellfish on the surrounding environment.

Mazzola & Sarà (2001) used stable isotope analysis to determine the dominant carbon sources available to mussels (*Mytilus galloprovincialis*) and clams (*Tapes* sp.) cultivated around fish pens (*Dicentrarchus labrax* and *Sparus aurata*) in the Mediterranean. Results suggested that particulate organic carbon (POC) waste from fish feed provided 80% of the adult clam diet for clams cultivated in baskets adjacent to fish pens, at 9 m depth (1 m from the seafloor). POC waste from fish feed was also said to provide up to 50% of the dietary needs of mussels suspended on long lines approximately 3 m from the surface adjacent to farms. It was concluded that in the oligo-mesotrophic, calm (<10 cm s^{-1}) Mediterranean waters of the study site, mussel and clam cultivation around pens provided two economic benefits. They were shown to firstly reduce the environmental impact of pens and secondly, to increase profitability by extending the yield through incorporation of multiple species.

These results are supported by studies on mussel-salmon polyculture undertaken by Stirling & Okumus (1995) in Scotland. The growth of mussels (*Mytilus edulis*) grown adjacent to salmon farms was compared to growth at neighbouring mussel monoculture sites. In the first year of the study, 1 year old mussels from the monoculture site were stocked in 5 m long high tensile polypropylene and cotton mesh socks. Three socks at each site were suspended from a mussel raft and from walkways in between small wooden salmon pens (7 x 7 x 5 m). The following year scallop culture lantern nets were used to prevent uncontrolled losses from drop off and predation. Three tray lantern nets were used with approximately 426 mussels per lantern. All mussels were suspended from 2 m below the surface. POM and chlorophyll-*a* levels were higher at the salmon farms and this was said to support energy retention during winter for mussels at these sites. Shell length growth was found to be higher than at the mussel farm but was only significant for one of the two years of the study.

While the above studies found mussel co-cultivation to be successful, there are a number of studies in the literature in which mussel growth has shown no benefit from cultivation adjacent to fish farms. Taylor et al. (1992) found that mussels (*Mytilus edulis*) deployed on long lines at 3, 15 and 75 m from salmon farms did not show enhanced growth relative to controls. POM and chlorophyll-*a* levels were not found to be significantly higher within the farms and hence did not provide additional nutrients for growth. The authors concluded that fouling mussels on the nets (with an estimated 1.2 million mussels per salmon net) could possibly be utilising the additional POM before it has a chance to reach the adjacent mussel cultures.

The results above are in agreement with those conducted on the Tasmanian Blue Mussel (*Mytilus planulatus*), which was cultured near salmon farms (*Salmo salar*) in Northwest Bay, Tasmania, in the only published example of ocean polyculture in Australia (Cheshuk, 2001;

Cheshuk et al., 2003). Mussels were cultured for 14 months suspended from 4 longlines positioned at 70, 100, 500 and 1,200 m away from salmon pens. There were no observed differences in growth of mussels with distance from pen, or relative to controls. The similarities in environmental parameters amongst the long lines, including ambient phytoplankton and POM, was said to suggest that solid waste loadings (feed particles and faeces) from the farm were too diluted to significantly enhance the particulate food concentration above ambient. It was also suggested that mussels may have been cultured too far (closest 70 m) to intercept particulate wastes generated by the farm.

In a review of mussel culture in polyculture, Troell et al. (1999) highlighted that suspended solids from fish pens can be significantly diluted by the large volume of water passing through the pens. The authors also proposed that many studies may have failed to document enhanced phytoplankton production around pens due to the water exchange rate being much higher than the doubling time of phytoplankton in open waters. It was proposed that POM derived from fish aquaculture only contributed significantly to a given environment during periods of low background plankton production and/or POM concentrations, which tend to occur primarily during winter. At this time mussels positioned close to pens could benefit from the additional POM and overcome restrictions in growth associated with winter.

Abalone represents another possibility for polyculture in the open ocean. Land-based abalone culturing techniques dominate within Australia, however open ocean culture is commonly used throughout the world with barrels and cages placed in the sea and secured to the seafloor via longlines and anchors attached to rafts (PIRSA, 2000). The barrels and cages are covered by mesh to permit water circulation while keeping the abalone contained and protected. Seaweeds are placed within these structures for feeding. Studies conducted by Neori et al. (2000) recognise that abalone culture is limited worldwide by the availability of suitable macroalgae. Through a land-based integrated tank system they were able to demonstrate that when ammonia-rich waters from fish cultures flow into adjacent tanks of the macrophytes *Ulva lactuca* and *Gracilaria conferta*, the algae are able to utilise the high ammonia content resulting in high growth rates. The macroalgae were fed to adjacent abalone cultures and were found to be of sufficient quality to promote good abalone growth. Only 30% of fish food was released to the environment with wastes (compared to 70-80% in monocultures) and the production of 2-3 tonnes of abalone was potentially supported per tonne of fish. A daily input of 1 kg of nitrogen with 13 kg of fish feed would produce over 7 kg of fish valued at AUD $67 per kg and 5 kg abalone valued at AUD $310 per kg within this system.

12.2.2. Macroalgae

A huge market exists for macroalgal aquaculture globally, with seaweed aquaculture production exceeding that of its animal counterparts (Chopin et al., 2001). Macroalgae provide the benefits of high nutrient uptake to reduce wastes and are also commercially valuable as food products for both humans and other animals, including highly valuable aquaculture species such as abalone and sea urchins.

Seaweeds and shellfish have been grown together in ocean polyculture in China for centuries, where mussel culture ropes and scallop culture net pens are co-located with seaweed cultures of *Laminaria* and *Undaria* suspended on ropes from floating lines (Brzeski & Newkirk, 1997). The *Laminaria* is said to create a mini ecosystem, providing shelter, releasing oxygen

and improving water health. Despite initial investment costs, the polyculture brings in 58% more market return than monocultures of each component for this system.

Additional studies with *Laminaria* include a model developed by Petrell et al. (1993) using a hypothetical *Laminaria*- salmon polyculture based in British Columbia. *Laminaria*, known as kombu, is sold as food and as a source of iodine and alginate. In this model *Laminaria* was cultured on ten 60 m ropes on each end of a salmon sea pen. It was estimated that the payback period for the initial investment of AUD $68,000 is 6 years after which a net profit of AUD $23,000 per year could be achieved.

Seaweeds have been used in a number of field-based polyculture studies, with results indicating that they can successfully utilise inorganic wastes when grown adjacent to fish pen aquaculture. Flat sheet seaweeds such as *Ulva* are the most productive of the macroalgae with rapid uptake accommodated by their thin sheet morphology (Chopin et al., 1999). Whilst *Ulva* presently has limited value after harvest (Neori et al., 2004), *Porphyra*, otherwise known as nori, shares many of the same characteristics and is an important source of food for humans. The majority of *Porphyra* cultivation takes place in Asia and is valued at AUD $2.15 billion per year (Chopin et al., 1999).

In their studies, Chopin et al. (1999) investigated the growth patterns of *Porphyra* in the Gulf of Maine, USA, adjacent to salmon (*Salmo salar*) farms. Rapid growth was observed (<40 days from seedling to first harvest in net cultures) permitting repeated harvesting of net grown crop every 9-15 days. It was estimated that 27 nori nets would be required per tonne of fish per year for the complete assimilation of phosphorus from farm wastes. The amount required for the assimilation of nitrogen from farm wastes was slightly less, or 22 nori nets per tonne of fish per year. Previous attempts to grow *Porphyra* as a monoculture within the bay at locations away from pens were limited by a decrease in ambient nutrients during summer. However, when grown adjacent to farms this seasonal low was buffered by the farm.

Another commercially important seaweed is the red macroalga *Gracilaria*. Troell et al. (1997) cultivated *Gracilaria chilensis* on ropes 10 m, 150 m and 1 km from salmon pens in Southern Chile over two months in summer. This species was chosen for its economic value, natural occurrence and its previous success at nutrient uptake in laboratory-based fish tank cultivation. 10 bundles (each 25-30 g wet weight) of *Gracilaria chilensis* were attached to frames on a rope using rubber bands at 1, 3 and 5 m depth. Ropes 10 m from the pens had up to 40% higher growth rate than the 150 and 1 km counterparts. Additionally, algal nutrient content and agar production was highest at 10 m. Epiphytic cover was low at all distances. Extrapolating the data, 1 ha of *Gracilaria* cultivation could potentially remove at least 5% of dissolved inorganic nitrogen (DIN) and 27% of dissolved inorganic phosphorus (DIP) released by the fish farm. This would produce an annual harvest of 34 tonnes (dry weight) of *Gracilaria* worth AUD $46,000. The ability of this species to assimilate the additional nutrients released by the farm in pulse events was vital to the success of this system.

12.2.3. Sustainable coastal production systems (SCPS)

Most research to date has involved utilising filter-feeding shellfish and photosynthetic macroalgae to utilise wastes. One problem with using shellfish is the biodeposition of filtered material and pseudofaeces. In addition, questions about the amount of farm-derived POM

reaching adjacent shellfish cultures exist. It has been suggested in a number of studies that polyculture techniques could benefit through the inclusion of additional trophic levels including detrital feeders (Cheshuk, 2001; Neori et al., 2004). These species scavenge on waste material that accumulates on the sediment floor directly underneath or adjacent to fish pens and could be used as additional crops if the appropriate species were chosen.

Newkirk (1996) and Brzeski & Newkirk (1997) introduced the potential of a multiple species integrated system in their theoretical discussions on sustainable coastal production systems (SCPS). In SCPS the local ecology dictates the aquaculture practices of a region. The concept behind SCPS is an expansion on the traditional polyculture model, with importance placed on the selection of additional marketable benthic species that capitalise on the organic energy settling out of the fish pens and adjacent shellfish cultivations. Suggested species that could fill this niche include sea cucumbers, sea urchins, pearl oysters, flounder, bait worms and detritus-consuming bivalves such as clams. These species may be retained within the fish pen, could be independently caged or be free to roam such that they were able to position themselves in areas where waste tended to accumulate. It is apparent that the viability of this complex multi-species approach needs to be validated through experimentation in a diversity of settings with a variety of species. It could, however, present a significant innovative step forward for all forms of ocean aquaculture.

12.2.4. Current research initiatives

In summary, a number of successful open water polyculture studies exist but these vary in scope and location and mainly consist of the integration of only two species (fish and one extractive species). As reviewed in Neori et al. (2004) there are three major projects currently underway in Europe and North America to conduct polyculture research at a practical commercial scale and to produce successful models that can be applied universally. These projects aim to bridge the gap between theoretical and commercial integrated ocean aquaculture.

SEAPURA is a European initiative to develop and test the cultivation of high value seaweed species not yet used in polyculture systems and for which the food market, extraction of pharmaceuticals and use in fish feed are being investigated.

GENESIS is a collaborative project between Israeli, French and Scottish scientists who are transforming traditional European monoculture systems into innovative polyculture units using technologies and practices developed in Israel.

The final project, AquaNet, sees the integration of salmon (*Salmo salar*), mussel (*Mytilus edulis*) and kelp (*Laminaria saccharina*) at several pilot sites in the Bay of Fundy, Canada. It investigates the productivity of each culture to determine the appropriate portions needed to counterbalance the metabolic processes occurring in the system. Additionally, the sites are being used to introduce and train net pen farm staff in the techniques of polyculture. Food safety is an essential component of the study, with mussels and kelps being monitored for therapeutants and phycotoxins. In over two years of monitoring, the cultures have shown no traces of drug residues (AquaNet, 2005).

12.3. Issues impacting open ocean polyculture

There is not a clear cut recipe to follow for successful commercial scale open ocean polyculture but it is possible to recognise key factors to consider when designing such a system. These can be split into three interrelated groups, environmental, economic and social, as summarised in Figure 12.2.

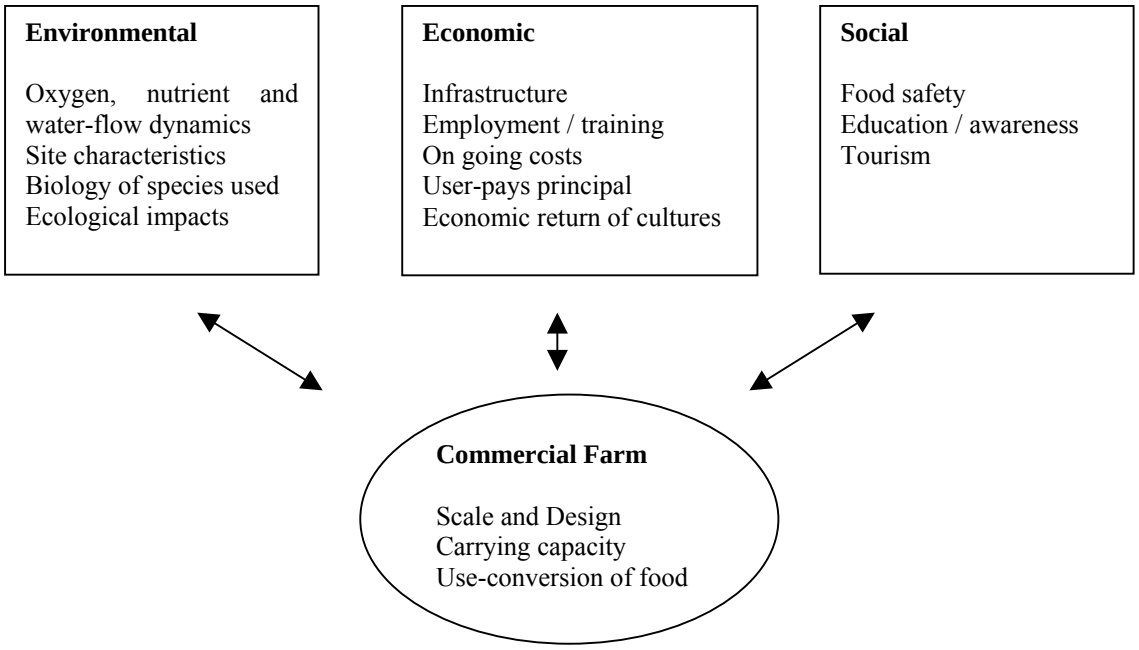

Figure 12.2. An overview of the environmental, economic and social issues impacting on the success of commercial scale polyculture.

12.3.1. Environmental factors

The oxygen budget of the primary fish culture may be affected by additional adjacent cultures. Heterotrophs such as shellfish may increase the oxygen demand in a system, while macroalgae consume oxygen at night during respiration. Neori et al. (2004) point out that daytime oxygen production is much higher than subsequent nighttime consumption by macroalgae. This is supported by land-based tank studies in which 1 kg of *Ulva* produced enough daily oxygen to support 2 kg of fish stock (Hirata et al., 1994). Modelling work conducted by Petrell et al. (1993) also suggests that oxygen availability is not greatly reduced in a *Laminaria*- salmon farm polyculture. It would therefore be necessary to incorporate an assessment on the oxygen dynamics of any polyculture model.

The dispersal patterns of both particulate and inorganic dissolved nutrients within the polyculture system would need to be well understood in order to maximise uptake efficiency and determine nutrient budgets (Troell & Norberg, 1998). The effects of each culture component on sedimentation and resuspension, nutrient uptake and production would need to

be quantified to determine the success of the system as a bioremediator. The impact of additional chemicals such as fertilizers and pesticides (if required) would also need to be accounted for.

The importance of maintaining the ecological integrity of a polyculture site requires an understanding of the biodiversity of the region and the interactions between the integrated cultures. The system could potentially impact the surrounding ecology in a variety of ways, resulting in changes to community structure and food chain patterns.

The importance of understanding the biology of the species used in polyculture is also evident from the literature. For example, macroalgae species should have high tissue nitrogen content and growth rates, be resistant to epiphytes and easy to cultivate (Neori et al., 2004). Preliminarily laboratory studies that quantify variability in nutrient uptake rate, yield and protein content for macroalgae species used in polyculture should be undertaken (Troell et al., 2003). Also important is understanding the influence of site-specific characteristics such as light, temperature, nutrient concentration and flux on each culture unit, and biological characteristics including life history stages and reproduction dynamics. Chopin et al. (2001) emphasised the benefits of cultivating marketable native species. Utilising endemic species makes use of the natural adaptation these organisms have made to the culturing site and overcomes the complexities of introducing foreign species to the environment.

12.3.2. Economic factors

Economic models of commercial scale polyculture (both land and open-ocean) are largely absent in the literature. In their review of polyculture, Troell et al. (2003) estimate that only 7% of studies include aspects of economics. The economic advantages offered by polyculture are two-fold. Firstly, through the utilisation of monoculture wastes, polyculture offers a means to increase profit per unit feed via the additional crops produced by multiple cultures. Secondly, there is a general consensus in the scientific literature (e.g. Troell et al., 2003) that a user-pays principle should be brought into legislation, requiring aquaculture companies to be financially accountable for the wastes emitted by their farms. This would involve determining the monetary cost of pollution and would promote strategies to reduce waste, one of the key outcomes of polyculture. Chopin et al. (2001) modeled the internal environmental costs of pollution to a land-based salmon-seaweed polyculture in Chile. It was found that the production of 250 tonnes of salmon had an environmental cost of AUD $269,512, where cost is quantified based on the amount of nitrogen and phosphorus produced in the form of solid and dissolved wastes. When the macroalgae *Gracilaria* was integrated into the operations this cost was reduced to AUD $85,639. This was due to reductions in the phosphorus and nitrogen loadings from the salmon culture via conversion into *Gracilaria* biomass.

Inherently, the costs of a particular polyculture system will vary depending on the design and scale but will include the costs of infrastructure, production, training, employment and processing (Neori et al., 2004). In regard to open-ocean polyculture, it is not possible to draw on economic examples from the literature, as none are available. Neori et al. (2004) discuss the economics of an operational land-based seabream-*Ulva*-abalone/sea urchin integrated farm, called SeaOr Marine Enterprises, operating in Israel. While this system differs substantially from an open ocean farm, it gives some indication of the annual costs involved based on a farm using 500 tonnes of feed per year. The model does not however, incorporate information on initial investment costs, or detail expenditures. Table 12.1 summarises the

budget of this integrated system and demonstrates a profit of AUD $2.8-4.4 Million yr^{-1}. Much of the income is generated through the sale of either abalone or sea urchin.

Table 12.1. Economic profile of a land-based polyculture system modelled off the SeaOr Marine Enterprises farm in Israel (Neori et al., 2004).

Species	Yield (tonne yr^{-1})	Revenue (AUD $)	Costs (AUD $)	Profit (AUD $)
Seabream	265	1.7M	N/A	
Ulva	2,215	0[1]	N/A	
Abalone	185	10.4 M	N/A	
Sea Urchin	275	8.8 M	N/A	
Totals	450-550	10.5-12.1 M	7.7 M	2.8-4.4 M

[1]used as feed for abalone/sea urchin cultures.

12.3.3. Food Safety and Social Opinion

Overcoming public perceptions of products generated by polyculture as being unsafe or substandard may also be an issue of significance. Since cultures are converting waste material into biomass it is essential that all products are monitored for food safety. Both shellfish and macroalgae grown adjacent to fish farms could potentially be contaminated with therapeutants given to the primary fish culture (Stirling & Okumuş, 1995). Shellfish have been shown to bioaccumulate heavy metals, phycotoxins and bacteria pathogenic to fish and humans (Carmody et al., 1996; Griscom & Fisher, 2004). The AquaNet polyculture project (Chopin et al. 2004) has incorporated a monitoring program of both the blue mussel (*Mytilus edulis*) and kelp (*Laminaria saccharina)* culture units and has found no traces of harmful substances.

The available research would suggest that with successful production and good monitoring protocols polyculture products have the potential to be as good as, if not better than standard equivalent monocultures. Raising public awareness of the environmental (waste reduction) and economic (employment, training, innovation) benefits of polyculture to the community as a whole is essential to the success of this approach.

12.4. Polyculture potential for the Southern Bluefin Tuna (SBT) industry

Occurring in the waters offshore from Port Lincoln, in South Australia's Spencer Gulf, the SBT industry is Australia's most valuable aquaculture sector (FRDC, 2004). Since its inception in the 1990s the industry has grown rapidly and currently sees 130-150 pens stocking each up to 2,200 individual SBT within a season (Steven Clarke, personal communication).

Tuna pens form a point source of nutrients to the surrounding environment in the form of uneaten feed, fish faeces and other soluble excretion products. The impact of fish farming on Port Lincoln waters was documented in the early days of the industry, when operations were carried out within the calmer waters of Boston Bay (Blackburn et al., 1992). Observed impacts included eutrophication of sediments immediately beneath pens and changes in

benthic species composition, with scavenger species, such as spider crabs, sea cucumbers and sea urchins replacing natural benthic assemblages.

In 1996, unusual storm activities promoted resuspension of sediments and led to mass SBT mortalities (Clarke, 1996; Grzechnik, 2000). Subsequently, all operations were moved outside of the bay and into Spencer Gulf where the environment is influenced by open water oceanic conditions. Within this environment fallowing has been considered successful as the single management tool used to control the environmental impact of SBT pens, which are moved at the end of each season to allow the underlying seabed to recover.

Currently, open ocean polyculture does not occur in South Australia. Regulation states that within the SBT lease zone, SBT and other finfish may not be farmed together under the same lease, unless for the purpose of approved research, with other forms of polyculture requiring assessment by PIRSA aquaculture on an individual basis (PIRSA Aquaculture, 2003). In reports conducted on the SBT industry from the 1990s there is considerable reference to the potential of combining other cultures with SBT (Blackburn et al., 1992; Hone, 1994). In the 1992 Port Lincoln Aquaculture Management Plan (Blackburn et al., 1992) it was suggested that deep-water mollusc and algae culture research should be encouraged within the area and reseeding with native oysters and abalone was proposed. Also highlighted was the potential of using mixed fish cultures within pens and the convenience of utilising the existing resources such as boats, farming operations, factory and processing facilities at Port Lincoln in this process. It was concluded that there were no technical reasons why mixed cultures should not be considered.

A review of South Australian aquaculture by Hone (1994) concurs with this discussion. The report highlighted the poor food conversion ratio of the SBT industry (17 tonnes of baitfish to produce 1 tonne of SBT) as a significant management issue. It was suggested that there is a need for innovative and adaptable management that takes advantage of waste foods and converts them into product. Polyculture was recommended as a successful solution by incorporating scallops and/or mussels or by utilising the incidental occurrence of other fish species (e.g. tommy ruff) associated with SBT culture.

12.4.1. Potential commercial species established in monocultures

The Port Lincoln area already supports aquaculture industries other than finfish (Figure 12.3), including ocean-based pacific oyster and blue mussel leases and two land-based abalone farms (Edyvane, 1999). The oyster industry is the second largest aquaculture sector in South Australia, valued at AUD $16.1 million in 2002/2003 (PIRSA, 2001a). Cultivation takes place in sheltered coastal waters between Ceduna and Coffin Bay and involves two species, the Pacific oyster (*Crassostrea gigas*) and the native flat oyster (*Ostrea angasi*) (Hone, 1994). The introduced Pacific oyster has since been found in the wild and soopen ocean aquaculture of the species may require a precautionary approach. The method of cultivation varies with location and includes growth on rack/rail systems, long-line systems or hybrid systems that suit particular growing areas, which are aimed at allowing oysters greatest access to food (PIRSA, 2001a).

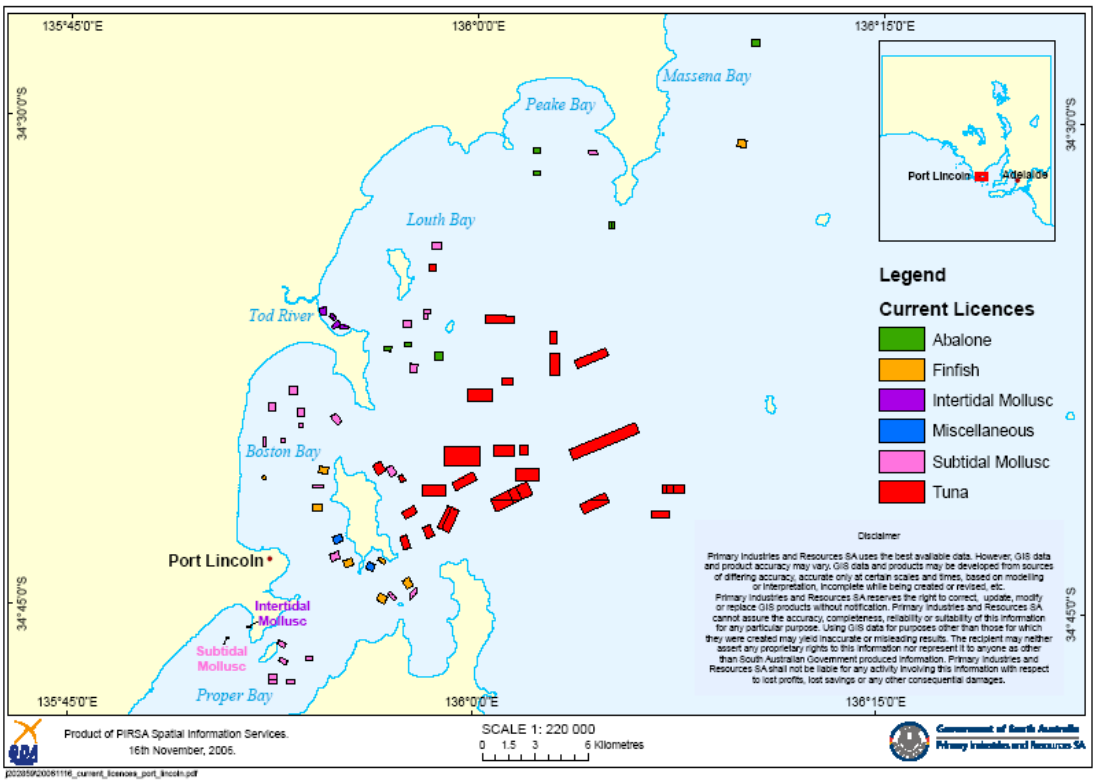

Figure 12.3. Aquaculture leases in the Port Lincoln area (PIRSA Aquaculture, 2005).

Cultivation of the blue mussel (*Mytilus edulis*) is a new aquaculture venture operating predominantly off the coast of Port Lincoln. Still a relatively new industry, but developing strongly, it is predicted that it will become a significant sector in the future (PIRSA, 2001b). The Port Lincoln-based South Australian Shellfish Quality Assurance Program (SASQAP) operates in conjunction with the shellfish industry, providing microbiological testing of shellfish in the interest of public health. The local infrastructure to monitor toxic substances in shellfish could be used to monitor other products produced by polyculture.

Abalone aquaculture occurs in land-based systems operating on the coasts of Port Lincoln, Streaky Bay and Kangaroo Island. Abalone is a highly sought after product and has a high market value. It involves culturing species of the genus *Haliotis*, predominantly greenlip (*Haliotis laevigata*) and blacklip (*Haliotis rubra*) abalone (PIRSA, 2000). Both species naturally inhabit rocky surfaces, crevices and caves around the coastline or on reefs in moderate to rough waters. Greenlip abalone is generally found in open environments including open waters on rocky patches 10-20 m deep and tends to range deeper than blacklip abalone (Shepherd, 1973). Both species are largely sedentary but will move locally in search of food. Water movement stimulates feeding, with red algae making up approximately 80% of the abalone diet (Shepherd, 1973). Studies on feeding by Shepherd (1973) showed that greenlip abalone was dependent on drift algae for feed, while blacklip abalone tended to graze more on epiphytic algae.

Hone (1994) identified that a major limitation to the abalone industry is the availability of cheap feed. Growth of these animals is highest when fed on red algae and related seaweeds

but the large volumes required to commercially produce abalone and associated ecological, harvesting and storage issues restricts utilising natural sources with most farms using artificial feed. Hone (1994) also referred to work on developing the red alga *Gracilaria confervoides* as a food source for abalone. This reference was brief but made mention of determining the economic feasibility and culturing conditions required to successfully produce *Gracilaria confervoides* for abalone feed. Hone (1994) also identified sea-based cage culturing of abalone as a low cost alternative technique to current land-based farming methods.

At this stage, sea cage abalone culture in South Australia is still largely in its infancy but the industry is developing with a farm operating off Waldegrave Island near Elliston and a second farm under development off Goat Island near Ceduna (Simon Bryars, personal communication). Not much information is available on the farming techniques used by these operators or additional licensees. The industry is currently very small and still predominately in the experimental stage, trialing a diverse array of technologies (Stephen Madigan, personal communication). Some farmers are modifying existing fish farm pontoons with 'hides' specifically developed to accommodate abalone, while others are using customized cages containing internal shelving units, suspended below surface buoys (Stephen Madigan, personal communication). Manufactured feeds developed for land-based abalone cultures are being used and attempts are also being made to grow algae naturally as feed. The success of each of these variables remains to be seen as the industry continues to develop, with predictions of 5 to 10 years before the industry fully reaches commercial scale (Stephen Madigan, personal communication).

12.4.2. Potential native species

A number of farm related benthic surveys were carried out during the 1990-1996 period when SBT pens were located in protected waters inside Boston Bay (Blackburn et al., 1992; Blackburn, 1995) and an investigative survey was carried out to test potential farm sites seaward of Boston Island in 1996 (Hone et al., 1997). Since this time there have been no published benthic biodiversity surveys specific to SBT operations other than infauna (> 1 mm) data collected annually as part of the Tuna Environmental Monitoring Program (TEMP). Tables 12.2, 12.3 and 12.4 summarize the major species of potential economic interest for polyculture found during these local studies, with additional regional scale information provided by Edyvane (1999) and Bryars (2003) .

Seagrass meadows and rocky reef macrophytic communities dominate the intertidal areas around Cape Donington to Port Boston and the intertidal zones of Boston and Taylor Islands. These environments support a diverse array of organisms significant to a number of South Australian commercial fisheries. Seagrass meadows are most dense in 1-3 m of water becoming sparse in depths up to 7 m. Rotten Bay and Spalding Cove are sites with some deep water seagrass communities (10-12 m) (Blackburn et al., 1992). Brown macrophytes such as *Ecklonia*, *Sargassum* and *Cystophora* sp. are said to dominate inter and subtidal reef areas (<3 m) with red (e.g. *Botryocladia obovate*) and green (e.g. *Caulerpa spp, Ulva*) macroalgae attached to broken rock and cobble substrates in deeper waters (Blackburn, 1995).

Table 12.2. Macroalgae diversity in lower southwestern Spencer Gulf.

Species	Algal Type	Habitat Type	Reference
Cystophora monoliformis	Brown	Rocky shore	Edyvane (1999)
Cystophora silquosa	Brown	Rocky shore	Edyvane (1999)
Cystophora monolifera	Brown	Rocky shore	Edyvane (1999)
Zonaria spp.	Brown	Sheltered coasts	Edyvane (1999)
Sargassum spp.	Brown	Sheltered coasts	Hone et al. (1997), Edyvane (1999)
Scaberia spp.	Brown	Sheltered coasts	Edyvane (1999)
Lobophora spp.	Brown	Sheltered coasts	Edyvane (1999)
Osmundaria spp.	Red	Shallow waters (<5 m)	Hone et al. (1997), Edyvane (1999)
Haliptilon spp.	Red	Shallow waters (<5 m)	Edyvane (1999)
Ecklonia radiata	Brown	Deeper rocky reef	Blackburn et al. (1992), Edyvane (1999)
Plocamium spp.	Red	Deeper rocky reef	Edyvane (1999)
Phacelocarpus spp.	Red	Deeper rocky reef	Edyvane (1999)
Carpophyllis spp.	Red	Deeper rocky reef	Edyvane (1999)
Carpopeltis spp.	Red	Deeper rocky reef	Edyvane (1999)
Peysonnelia spp.	Red	Deeper rocky reef	Edyvane (1999)
Sonderopelta spp.	Red	Deeper rocky reef	Edyvane (1999)
Caulerpa spp.	Green	Deeper rocky reef	Blackburn et al. (1992), Edyvane (1999)
Acrocarpia paniculate	Brown	Deeper rocky reef	Edyvane (1999)
Ulva spp.	Green	SBT farming zone	Blackburn et al. (1992), Blackburn (1995), Cronin (1995)
Botryocladia obovate	Red	SBT farming zone	Blackburn et al (1992), Hone et al (1997)
Gloiosaccion brownii	Red	SBT nets	Matt Hoare, personal communication

Table 12.3. Invertebrates of potential commercial value occurring naturally in lower southwestern Spencer Gulf.

Common name	Species	Habitat Type	Reference
Sand crab	*Ovalipes bipustulatus*	Unvegetated soft bottom, impacted areas	Blackburn et al. (1992), Blackburn (1995), Bryars (2003)
Queen scallop	*Equichlamys bifrons*	Unvegetated soft bottom, impacted areas	Blackburn et al. (1992), Blackburn (1995), Hone et al. (1997), Bryars (2003)
Razor fish	*Pinna bicolor*	Unvegetated soft bottom	Blackburn et al. (1992), Hone et al. (1997), Bryars (2003)
Southern keeled octopus	*Octopus berrima*	Unvegetated soft bottom	Bryars (2003)
King scallop	*Pecten meridionalis*	Unvegetated soft bottom	Blackburn et al. (1992), Bryars (2003), Hone et al. (1997)
Baitworm	Not specified	Sheltered beach	Bryars (2003)
Sea cucumber	*Lipotrapeza vestiens* *Holothuria hartmeyeri* *Paracaudina luticola* *Paracaudina australis* *Stichopus mollis* *Stichopus ludwigi*	Seagrass meadows, unvegetated soft bottom, tuna pen areas Boston Bay 7-15 m Impacted sediments	Shepherd (1975), Blackburn et al. (1992), Blackburn (1995), Hone et al. (1997), Keuskamp (2003)
Greenlip abalone	*Haliotis laevigata*	Onshore reef	Hone et al. (1997), Bryars (2003)
Blacklip abalone	*Haliotis rubra*	Onshore reef	Hone et al. (1997), Bryars (2003)
Sea urchin	Not specified	Onshore reef (Boston Bay 7-15 m)	Blackburn (1995), Bryars (2003)

Table 12.4. Fish species of potential commercial value for polyculture occurring naturally in lower southwestern Spencer Gulf (from Bryars, 2003; personal communication).

Common name	Species	Habitat Type
King George Whiting	*Sillaginodes punctata*	Onshore reef, seagrass meadow, unvegetated soft bottom
Snapper	*Chrysophrys auratus*	Onshore reef, seagrass meadow
Western Australian Salmon	*Arripis truttacea*	Onshore reef, seagrass meadow, unvegetated soft bottom
Tommy Ruff	*Arripis georgiana*	Onshore reef, seagrass meadow, unvegetated soft bottom
Southern sea garfish	*Hyporhampus melanochir*	Onshore reef, seagrass meadow, unvegetated soft bottom
Yelloweye mullet	*Aldrichetta forsteri*	Onshore reef, unvegetated soft bottom
Trevally	*Pseudocaranx spp*	Onshore reef, seagrass meadow, unvegetated soft bottom
Yellowtail kingfish	*Seriola lalandi*	Onshore reef, seagrass meadow, unvegetated soft bottom
Leatherjacket	*Acanthaluteres spp*	Onshore reef, seagrass meadow
Snook	*Sphyraena spp.*	Onshore reef, seagrass meadow, unvegetated soft bottom
Sea sweep	*Scorpis spp.*	Onshore reef
Gummy shark	*Mustelus antarcticus*	Onshore reef
Flathead	*Leviprora spp.*	seagrass meadow, unvegetated soft bottom
Red mullet	*Upeneichthys vlamingii*	Seagrass meadow, unvegetated soft bottom
Flounder	*Ammotretis rostratus, Rhombosea tapirina Pseudorhombus jenynsii*	Unvegetated soft bottom
School whiting	*Sillago flindersi*	Unvegetated soft bottom

Moving into areas deeper than 7 m is usually accompanied by a drop in benthic diversity with studies both inside the bay and the adjacent gulf indicating that the benthos is largely dominated by patches of the red alga, *Botryocladia obovate,* and low densities of sea cucumbers, scallops, sand and spider crabs (Blackburn et al., 1992; Hone, 1994). Exceptions occurred at sites offshore on the eastern sites of Boston and Taylor Islands, where the abundance and diversity of the benthic community was still significant at these depths, with the red alga *Osmundaria prolifera* found in abundance at Taylor Island.

Red macroalgae and *Ulva* were found to be the dominant macroalgal assemblages in investigations into the composition of the fouling community on a research SBT pen located in the area of the commercial sites in Spencer Gulf (Cronin, 1995). *Ulva* was dominant at 1 m depth during May, while the filamentous *Rhodophytes* were abundant at 2-3 m during August. The southern side of the nets received the most sunlight and supported the greatest photosynthetic biomass, which was prolific even at depths greater than 12 m. This study demonstrated the ability of these macroalgae to grow in abundance in the vicinity of SBT pens.

Observations would suggest that areas adjacent to fish pens are naturally defined by low macrobenthic diversity due largely to the depth of the sites and the fact that sediments are unvegetated. Studies by Blackburn et al. (1992) found an initial drop in the percentage of red algae found at the old farm sites within Boston Bay, with a concurrent increase in the abundance of opportunistic species including sea cucumbers and *Ulva*. Within 6-12 months of stocking, the only macrobiota present in sediment beneath the sea pen was spider crabs, which were found in abundance. Changes in benthic community structure were also seen in studies conducted by Shepherd (1975) within the nearby Proper Bay, where sea cucumbers, spider crabs, sand crabs and razor fish were found to be significantly more abundant in sites impacted by sewage and waste discharge sites. These invertebrates appear to flourish in impacted sites including SBT pen areas and show strong commercial potential if cultured in sufficient numbers. Additional commercial invertebrates that are found within the region that could be considered in polyculture trials include the Southern keeled octopus, the Western King Prawn, rock lobsters, baitworms, scallops, abalone and sea urchins (Table 12.3).

The response of native fish species to tuna pens is not well documented, although Blackburn et al. (1992) stated that the pens act as a 'fish aggregating device', with significant numbers of pelagic fish including pilchards, anchovies, salmon trout and yellow-eyed mullet commonly seen in the locality of farms. Table 12.4 outlines locally found commercial fish species of potential polyculture value. Of these, King George whiting, snapper, Western Australian salmon and the tommy ruff offer the most profitability in terms of product value (Simon Bryars, personal communication, see Table 12.4 for scientific names) but the logistics of culturing these species to capitalise on SBT waste would need to be further investigated. Incorporating commercial bottom-dwelling scavenger species such as flathead and flounder may be more effective since they could be cultured in separate pens immediately underneath SBT pens.

12.4.3. Potential polyculture systems

Tables 12.2, 12.3 and 12.4 provide a stock take of species of potential commercial value defining the natural ecology of the Port Lincoln region. At this stage additional research on the biology and ecology of any given species is necessary before a polyculture model for

SBT can be proposed, but the tables form a starting point for future research and polyculture model development. Having been vigorously tested in scientific research and given the success of current monoculture practices within the region, the traditional polyculture model of integration of mussel and/or oyster and SBT could be an option. The model could be modified slightly to include cultures of locally found razor fish and/or scallops. The impact and success of these additional cultures on the surrounding environment and the SBT would need to be well understood and some caution may be required if culturing introduced species such as the Pacific oyster.

The most successful and commonly used macroalgal families in polyculture models (*Laminaria*, *Porphyra* and *Gracilaria)* are largely lacking from these local surveys. *Laminaria* is a northern hemisphere genus but temperate Australian species from both *Porphyra* and *Gracilaria* do exist and their range does include this region. Whether the species found locally are commercially viable would need to be investigated or alternatively, the commercial counterparts of each genus used overseas could be trialed in the region.

What the surveys do show is the abundance of red macroalgae within the region. Hypothetically, an integrated system of SBT, red macroalgae and abalone/sea urchins seems promising and would appear to suit the area well with the additional cultures addressing a current deficiency (i.e. red macroalgae for abalone/sea urchin aquaculture). In feeding studies conducted by Shepherd (1973) a number of the red algal genera identified in the tables were the preferred food source of abalone. This model would utilise the high diversity and abundance of red macroalgae within the region and market it as a highly desirable food resource in the culturing of abalone and sea urchins. These herbivores could be farmed in land-based monocultures or could be integrated into the SBT-macroalgal system in the open ocean using models currently being tested by the sea cage abalone industry, as outlined previously.

The concept of sustainable coastal production systems (SCPS) as introduced by Newkirk (1996) could be applied to a polyculture model of SBT at Port Lincoln. The surveys show the abundance of scavenging species including spider crabs and sea cucumbers in the vicinity of the SBT pens, these species could be exploited commercially to remove depositing wastes from SBT and/or shellfish cultures and then be harvested. Additional bottom-dwelling fish species of commercial importance such as flathead and flounder and profitable invertebrates including the rock lobster could be placed in separate cultures beneath the pen, to take advantages of the detritus falling out underneath the pens.

In summary, research into the local ecology and aquaculture of Port Lincoln suggests that local species and already existing monocultures should be further investigated as potential cultures to integrate with pre-existing SBT monoculture in the region. The culture of red algae in the vicinity of SBT farms would intercept soluble wastes and produce an alternative feed source for local land-based abalone farms. The growth of locally farmed mussels could also be enhanced by correct positioning in relation to SBT farms to intercept solid wastes. This would add new dimensions to the industry that would need to be well researched and understood prior to application in the open environment.

12.5. Engineering solutions

Unlike polyculture, references to engineered waste treatment systems available to sea aquaculture are largely absent from the scientific literature. Information is predominantly only available from the companies producing these systems themselves. A small number of systems exist and these have primarily been tested in reasonably calm, freshwater environments. The most comprehensive summary of systems available to salmon farmers can be found in a report prepared by G3 Consulting Ltd. (2000).

Three systems are available to open net pen cultures. The first of these systems, the Akva Lift-UP is a feed and dead fish collector (Lift-UP-Akva Ltd., 2005). It consists of a plastic "china hat" that is lowered into the bottom of a pen and fitted with a compressor delivering compressed air, which sucks up feed and dead fish into a bucket on the surface. The second system, developed by NorAm Aquaculture, involves a plastic inverted cone-diaper, which attaches to the bottom of the pen and collects fish waste and uneaten feed in a septic-tank type container underneath the cone (G3 Consulting Ltd, 2000). The waste material collected in this way is subsequently pumped through plastic pipes into holding tanks on shore. This system is said to function efficiently in protected, non-tidal waters but is not thought to succeed in the open marine environment.

The third and most tested system, also potentially the most relevant to SBT culturing and waste management, is the enclosed bag SEA System^{TM} developed in Canada (Future SEA Systems Inc., 2005). The waterproof fabric bag system is isolated from the adjacent aquatic environment, with each bag containing a pump that draws in water from a chosen depth outside the pen. This allows control over water quality, current speed and water temperature. The newer Sea System II^{TM} is stand-alone and incorporates a flotation ring that can be expanded to fit bags from 8 to 19 m in diameter and a hydraulic or electric pumping station (Figure 12.4). One of the big advantages offered by the SEA Systems is the incorporation of a waste trap, which has been said to remove at least 80% of solid wastes produced by the fish culture (Future SEA Systems Inc., 2005), although this remains to be tested by an independent source (G3 Consulting Ltd, 2000). The bag uses a cone shaped bottom that creates a vortex directing wastes into it and removing faecal pellets before they decompose.

Over 50 Sea Systems operate globally, with the company stating that their technologies have proven successful in the culturing of Atlantic, Chinook and coho salmon, rainbow trout, arctic charr and steelhead. Of particular significance is the work they have conducted with Aquatas Pty. Ltd. in Tasmania, the second largest salmon farming operation in Australia and the only reference to a SEA System installed in seawater (G3 Consulting Ltd, 2000). The Aquatas site was in Northwest Bay, south of Hobart, and is the same farm discussed in the section on shellfish polyculture (Cheshuk, 2001). Although the location is not heavily affected by tides, it is subject to 1 m waves and occasional winds of up to 100 km h^{-1} (G3 Consulting Ltd., 2000). The site utilised 8 bags with waste traps and waste concentrators. It also used a converted US navy barge as a service platform to run hydraulics, generators, controls and monitoring gear and includes living quarters. The waste concentrator at the bottom of each bag was serviced by a boat which pumped out the waste and then discharged into a sewage pump truck on land. The collected sludge can then be treated or used in composting (Future SEA Systems Inc., 2005). The estimated cost for the SEA System was AUD $1.85 million with the additional cost of the barge (AUD $0.4 million), resulting in a

total cost (including shipping and duty taxes) of approximately AUD $2.25 million. Although operational costs are estimated to be 4 times those of traditional net pen counterparts, the system is thought to reduce mortalities, increase growth rates and allow higher stocking densities so that it could buffer this increased cost.

Based on his work on mussels, Cheshuk (2001) suggested that the enclosed bag culturing system offered by Future SEA Systems Inc. could potentially offer a better solution to waste mitigation than a polyculture system. It was suggested that the ability of the bags to concentrate fish wastes could offer more potential for integration of bivalves and seaweeds. Although the SEA system was incorporated into the culturing of salmon at the Aquatas farm, information on its success is sparse and it would appear that these systems generally require independent trialing and validation at large scale commercial sites. An additional limitation of these systems is their inability to remove dissolved nutrients and so a significant proportion of wastes (estimates of > 70% for nitrogen) is still released into the surrounding environment.

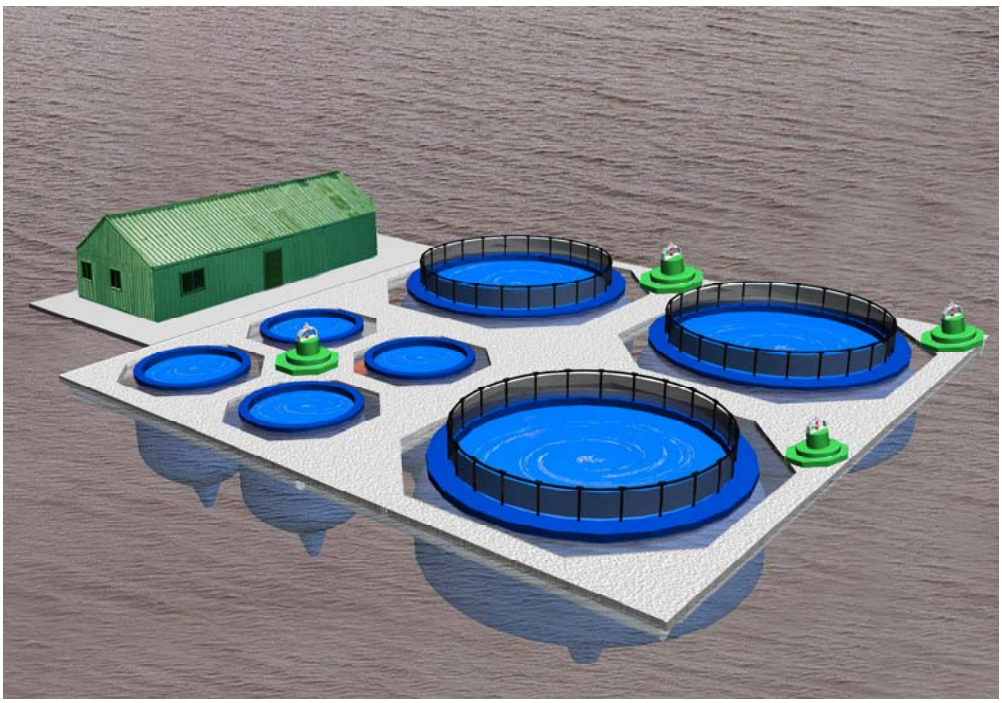

Figure 12.4. The SEA System IITM is an enclosed bag culturing system that incorporates a waste trap to collect finfish waste (Future SEA Systems Inc., 2005).

12.6. Conclusions

The development of open-ocean polyculture for commercial scale application is an innovative and exciting field of aquaculture. The success of integrated culturing techniques can be seen in models currently being tested overseas, in which environmental impacts are being reduced with a concurrent increase in profits through increased yield. The AquaNet project in Canada is one such model and its development will be one to watch.

Existing in a complex open water environment poses many challenges with successful models so far being built on detailed preliminary research carried out on the species being cultured and the culturing environment of these areas. To apply a polyculture model to the SBT industry at Port Lincoln would require a similar degree of understanding for this particular environment and species being cultured. Currently this information is largely lacking and detailed regional research and development into the environmental, economic and social issues, as outlined in this review, is critical to any future development.

The potential of the area to support polyculture is significant. Successful monocultures of two well tested polyculture species, the blue mussel and Pacific oyster, already exist in the region and the infrastructure to support the processing and marketing of each of these units is in place. By positioning these species appropriately in relation to SBT farms, growth could be enhanced. This would require investigations into the nutrient dynamics of the farms and the region generally which is being addressed by through the CRC project "Risk and Response - Understanding the Tuna Farming Environment" (FRDC 2005/059).

The abundance of native species of potential commercial value within the region is also promising for polyculture development. A variety of models could be tested on a smaller scale to determine which cultures work. They could also include delicacy species such as abalone, sea urchins and rock lobsters in SBT farm integrations to reduce wastes and increase profitability. The successful growth of red macroalgae within the region could be capitalised upon to reduce inorganic farm wastes and overcome feed limitations in abalone aquaculture.

The applicability of engineered systems for waste mitigation in SBT aquaculture is questionable at this stage since the technology still requires testing and validation in open ocean environments. In contrast, while much remains to be tested regarding the applicability of polyculture in the farming of SBT, the suitability of the area to polyculture and the advantages it offers in waste reduction, increased profitability and regional growth/development are significant and warrant future considerations. It would put the region on the map as being at the forefront in environmental management and aquaculture innovation.

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Chapter 13: Conclusions

13.1. Benefits and adoption

This project provided essential information for the development of initiatives to minimise the environmental impacts of SBT aquaculture and the implementation of comprehensive monitoring programs to ensure access to sites and security of tenure for finfish farmers, thereby benefiting both industry and regulators. The mapping of the distribution of sediment types in the offshore SBT farming zone highlighted how farm location might have important consequences for benthic recovery and the health of the stock. The comparison with other regions of Spencer Gulf pointed out the unique characteristics of the system. This study also provided a comprehensive understanding of the dynamics of waste production within SBT pens. The quantification of nitrogen flows associated with farming allowed the assessment of the loads released to the environment per tonne of production. It provided a model of the relationship between waste production and composition, stocking density, feed type and environmental parameters. These results will be useful for the new Aquafin CRC project investigating the regional effects of farming: "Risk and Response - Understanding the Tuna Farming Environment" (FRDC 2005/059). The model highlighted differences between SBT farming and other finfish industries and the need to adapt monitoring strategies to account for these differences. Among those, the low accumulation of particulate detritus on the seafloor is of note, with most nutrients being released directly into the water column. The effect of farms on the seafloor was considered transitory rather than chronic and changes were reversible. Although some level of disturbance was still noticeable within 12 months of fallowing, the low accumulation on the benthos suggests that the current fallowing regime over 24 months might be adequate if current stocking densities and holding periods are maintained. Considering the high release of nutrients to the water column, and the economic potential of many native species, polyculture was identified as a suitable strategy for the industry to improve management of waste, bringing benefits to both stock and the environment. Dissolved wastes and fine particulate wastes are likely to spread over a large area and monitoring programs should target regional effects.

The flow of benefits was partitioned between the commercial sector in South Australia (90%) and non-fisheries beneficiaries such as the ecotourism industry and public aesthetic enjoyment (10%). The primary beneficiary in the commercial sector is the tuna industry, with data generated in this project being instrumental for the identification of suitable mitigation strategies, facilitation of aquaculture development approvals, refining regulatory frameworks by government agencies responsible for licensing and managing the industry, and improving community perceptions of aquaculture developments. Results also have the potential to be useful for other marine finfish industries in South Australia, as well as Australia more broadly.

13.2. Further development

This research unravelled the localized effects of SBT farming to the benthic and pelagic environments, but little information is available on the spatial and temporal gradient of impacts. We currently have no clear idea of how organic matter accumulation and remineralization at the sediment-water interface varies with distance from stocked pens according to sediment types and water circulation. Data on infaunal communities is currently

the only monitoring technique used to detect environmental impacts of the industry, and yet we have little information on how these communities are affected as a result of sediment type distribution and changes in nutrient availability with distance from pens. Effects on a regional scale are also unclear, but are being investigated, together with the development of a biogeochemical model for the region, as part of the Aquafin CRC project "Risk and Response - Understanding the Tuna Farming Environment" (FRDC 2005/059). In order to refine nutrient budgets, we need more information on baitfish digestibility and the amount of uneaten feed. We also do not understand how waste inputs from SBT pens vary during the course of one day or with season. The calculation of daily loads during the course of the season would greatly benefit from more information detailing how feed intake, growth and body composition vary with size of fish and water temperature. This level of detail would allow calculation of loads on more suitable time scales (e.g. months) to pinpoint periods of increased susceptibility of disturbance to natural processes.

13.3. Planned outcomes

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

1. A system for quantitatively assessing the impact from alternative farming processes that will provide greater certainty in planning and thereby help to secure tenure and access to sites for aquaculture industries in marine environments.

The project highlighted how farming activities alter flux rates at the sediment-water interface but have a relatively minor impact on standing stocks in the water column or sediments. Nutrient porewater concentrations, nutrient fluxes from the sediments and to a lesser extent, benthic oxygen uptake rates, clearly showed the effects of farming over time. The analysis of abundance and diversity of benthic assemblages was also identified as a powerful mechanism to highlight disturbances to the natural background. These variables provided a system to quantitatively assess impacts and the efficacy of recovery strategies such as fallowing.

2. Reductions in environmental impacts that will lower mortality and improve feed conversions, growth rates and product quality.

While a reduction in environmental impacts has not been directly achieved in this project, the dataset obtained has important implications for management of the industry to achieve greater environmental sustainability and increased profitability. Despite minor localized impacts, results suggest that currents disperse wastes over a large area. Correct positioning of pens would act to minimize carry over between leases possibly benefiting the health and performance of stock. Changes in sediment characteristics within the farming zone also indicate that some sites are more likely to be affected if sediment resuspension occurs, a factor that can lead to irritation to gills and respiratory difficulties, and increase infestation from sediment-dwelling parasites.

3. An ability to model changes in waste under different feeding mechanisms and/or mitigation strategies that will allow predictions about the ecological consequences (and through this the risks to stock) of the application of new technologies or management regimes.

The project produced a model of nutrient loads to the environment according to feed conversion ratios and environmental conditions. The model allows the determination of direct inputs to the water column as dissolved nutrients, accumulation on the seafloor and export out of the system as particulate matter. These constitute a first estimate of the partition of loads to the environment and have important consequences to the implementation of mitigation strategies and the refinement of regulatory and licensing frameworks by government agencies.

4. Benefits to other users of coastal waters in the tuna aquaculture area that will accrue from a sustainable approach to tuna aquaculture.

A clear understanding of the environmental effects of SBT farming will help develop management alternatives to minimize impacts and improve community perceptions of SBT aquaculture developments.

The project has also contributed to the achievement of the following outcomes in the Aquafin CRC Commonwealth Agreement:

1. Improved monitoring of the environmental performance of cage aquaculture operations.

The current environmental monitoring program is well designed to monitor local impacts of leases. The nature of SBT wastes indicates that environmental monitoring could be improved by also targeting regional effects.

2. Widespread adoption of sustainable cage aquaculture practices.

Results suggest that the current management regime ensures that sustainable aquaculture practices are widespread within the SBT industry. However, a few growers still clean nets *in situ* at the end of the grow-out season and have difficulty in matching feeding rations with declining SBT intake when water temperature drops between May and July. These practices need to be improved to increase sustainability of operations.

13.4. Conclusions

A summary of the project's key findings according to the objectives in the original application is detailed below.

1. To determine the type and quantity of waste produced by sea-cage operations across a range of management and environmental regimes.

Wastes produced by SBT sea-cages are primarily in the form of dissolved nutrients. Most of these dissolved losses are associated with urine and gill excretory products as a consequence of the extremely high metabolic rates of SBT. Nutrients in uneaten feed and faeces are highly soluble and a significant fraction is also lost through leaching from solid wastes, particularly when SBT are fed baitfish. Leached nitrogen is mostly released in organic forms and therefore not readily available to primary producers.

Despite high sedimentation rates in the vicinity of sea-cages, up to 10 times higher than the natural background, particulate nutrients comprise only a small fraction of wastes. Uneaten baitfish are mostly consumed by scavengers. SBT faeces have slow settling velocities and are easily dispersed by currents. Faeces and uneaten feed that are not consumed by scavengers or exported out of the system with currents are quickly metabolized at the sediment-water interface and reintroduced back into the water column as dissolved inorganic nitrogen and phosphorus. The accumulation of nutrients from solid wastes in the sediments is minor. These wastes have significantly higher molar ratios of nitrogen to phosphorus in comparison to the natural sedimentary background, suggesting that molar ratios might be used to monitor the accumulation (if any) of uneaten feed and faeces on the seafloor.

We quantified the production of wastes from commercial pens in farms of different companies and consequently subject to different management regimes. Feed conversion ratios (FCRs) of 10 (wet weight feed/wet weight gain), which are considered representative of the industry as a whole, were associated with nitrogen losses of 260 kg N tonne^{-1} growth. These losses are more than double values for other aquaculture species fed manufactured pellets. One company with an unusually high FCR of 17 was associated with losses of 502 kg N tonne^{-1} growth. Considering Australian current production of 4,380 tonnes of SBT per year over initial stocked biomass, total annual loads to the environment can reach 1,137 tonnes N, including 983 tonnes N released as dissolved products.

The study covered a full grow-out cycle from February to July and assessed changes with environmental conditions. Although dissolved wastes greatly outweigh particulate wastes, the former are likely to represent a smaller fraction of total loads to the environment at the beginning of the grow-out season when retention of nutrients in fish tissues is highest and benthic fluxes have not yet peaked. Sparse accumulation of uneaten baitfish on the seafloor was only observed towards the end of the grow-out season, concurrent with an increase in benthic nutrient fluxes. Whether these results are related to slower SBT metabolism during winter leading to lower consumption rates and an increase in the flux of uneaten feed, or as a result of seasonal changes in scavenging populations, it is not clear.

The study also investigated the dispersion of wastes from sea-cages across a range of benthic environments. Wastes from sea-cages located in an erosional area south of Rabbit Island are likely to be quickly winnowed-out by wave and tidal action. The refractory matter not assimilated by the system is potentially transported and buried in a depositional area north of Cape Donington.

2. To develop and validate a model or modify an existing model (e.g. DEPOMOD) of the waste dynamics of sea-cage operations incorporating information on stocking density, feed type and a number of environmental and management parameters to quantify the extent and intensity of localised impacts.

Waste dynamics was investigated in a model of nitrogen flow in stocked sea-cages. The model was based on environmental and farm management data, and estimates of fish metabolism. It indicates that the extremely high metabolic rates of these endothermic fish not only account for high nutrient loads to the environment, but also to a different partition between solid and dissolved wastes. Most of the nitrogen in feed (76-86%) is lost to the water column as dissolved wastes and only a small fraction (7-12%) is retained for growth. These results are primarily a consequence of the high rates of nitrogen excretion in urine and through the gills (59-64% of nitrogen inputs with feed). Other dissolved losses include

leaching to the water column (10%) and remineralization in the sediments (7-11%). Particulate wastes account for a comparatively smaller fraction (8-12%) of environmental losses, occurring mostly through sediment accumulation (maximum 6 %) or export out of the system with current flows (maximum 12 %). The importance of dissolved wastes combined with the low settling velocity of SBT faeces and the high scavenging activity in the area lead to minimal impacts to the benthos at current stocking densities and holding periods. The effects on benthic metabolism are transitory rather than chronic and changes are reversible as a result of fast turnover periods. Meta-analysis corroborates the idea that benthic impacts under current stocking regimes are small compared with other finfish aquaculture.

The nature of the wastes however, suggests that these will not be confined to the footprint of the pens and might spread over a large area. The abundance and diversity of macrobenthic assemblages suggests that the entire area might be subject to some disturbance, particularly evident during the farming season. Sediments in the farming zone are finer, with localized higher levels of organic carbon and total nitrogen, as well as water column phosphate, compared to other locations in Spencer Gulf. Potential regional effects are now being considered through a follow up project of the Aquafin CRC ("Risk and Response - Understanding the Tuna Farming Environment", FRDC 2005/059).

3. To obtain information on the composition of the fouling community on and adjacent to tuna cages with reference to potential polyculture species with an assessment of their potential biological and economic viability.

The dominant fouling organisms on SBT sea-cages are green algae (*Entermorpha sp.*) at shallow depths and sponges (possibly of the genus *Verongia*) at deeper depths, with low settlement of blue mussels (*Mytilus galloprovincialis*) and paper oysters (*Electroma georgiana*). Green algae and paper oysters have no economic value, while sponges have poor wholesale market returns and low growth rates in temperate waters. These organisms have low potential for polyculture with a species of such high profitability as SBT. Other native species (e.g. abalone) not necessarily associated with fouling have much higher economic viability (see below).

Nutrients discharged by finfish sea-cages have the potential to promote the growth of harmful algae that would pose a risk to the health of shellfish consumers. As a consequence, the South Australian Shellfish Quality Assurance Program (SASQAP) and PIRSA Aquaculture do not recommend the culture of filter feeders such as blue mussels in association with finfish. Blue mussels were thus considered of low viability for polyculture with SBT. However, the aquaculture industry in Port Lincoln includes a number of mussel licences that would benefit from correct positioning in relation to the movement of particulate wastes from SBT farms to increase production.

4. To develop a sampling program for benthic assemblages exposed to waste from tuna farming operations with reference to the efficacy of fallowing as a waste remediation process. This will form the basis for consideration of alternative waste mitigation strategies and for recommending improvements to environmental monitoring regimes.

The examination of macrobenthic assemblages indicates that sediment recovery during fallowing is slow, with some fallowed sites remaining moderately disturbed after 12 months. These sites have finer sediments and higher infauna abundance, with assemblages characterized by low diversity, number of taxa and evenness. Sediment recovery was site-

dependent, indicating that benthic assimilative capacity will vary according to location and sediment type. Lumbrinerids and Spionids were identified as the best macrobenthic taxa for assessing the level of disturbance at fallowed sites.

The current environmental management regime requires operators to fallow farmed sites for two years. This study only evaluated the first year of fallowing and the actual period necessary for complete environmental recovery remains unknown. However, results suggest that albeit slow, fallowing does lead to recovery. Organic detritus are quickly assimilated in the first few months of fallowing and the only signs of disturbance after 12 months are a higher fraction of fine sediments and associated changes in infauna assemblages. Site selection could act to improve the time necessary for recovery by minimizing accumulation of fine sediments through improved dispersion.

Current stocking densities lead to minimal impacts to the benthos, and these impacts are localized and restricted to the lease boundaries. Feeding should be carefully monitored to match SBT intake, particularly at the end of the grow-out season when sparse accumulation of uneaten feed was observed. Solid wastes produced by SBT are mostly in the form of faeces. More effort should be placed in producing diets of high digestibility that will minimize the release of faeces. Since SBT faeces have very low settling velocities, they can spread over a large area. Increasing the number of filter feeding licences in the region could potentially act to minimize regional effects.

Other solid wastes released by SBT pens include biofouling dislodged from the nets. These can contribute to localized impacts to the seafloor, particularly when nets are cleaned *in situ* at the end of the grow-out season. This practice should be discouraged and nets brought to shore for cleaning. Effective and environmentally friendly anti-fouling alternatives are needed to minimize the problem.

The adoption of diets with lower phosphorus content and lower protein/energy ratio so that energy requirements are met by non-protein sources, could help reduce nutrient loads to the environment. These loads are mostly in the form of dissolved nutrients. Incorporating the culture of macroalgae would act to reduce dissolved loads to the water column (see below). The nature of wastes (dissolved or faeces with low settling velocity) suggests that monitoring programs should also target regional effects. The high site-to-site benthic variability should be considered when revising programs monitoring localized impacts.

5. To test the potential of integrated farming in mitigation studies. This involves the use of benthic filter feeders (e.g. blue mussels) benthic surface feeders (holothurids and crabs) and bioturbators (e.g. stingrays, fish or native oysters).

We were unable to find an industry partner to test the potential of integrated farming. Instead, we conducted a desktop study to evaluate the species of economic interest occurring naturally in and around commercial farms. Polyculture appears as a promising waste mitigation alternative with the added benefit of increasing profitability. An integrated system combining SBT, abalone/sea urchins and red macroalgae would be beneficial on both economical and environmental terms. Abalone/sea urchins intercept particulate wastes while macroalgae uptake dissolved wastes. Other native benthic scavengers such as spider crabs and sea cucumbers could also be used to reduce benthic impacts. On a regional level, production of mussels and/or oysters could be enhanced by correct positioning in relation to the movement of particulate wastes from SBT farms.

6. To evaluate potential applications of technological approaches (e.g. diaper systems) to waste mitigation.

At this stage, engineering solutions for waste mitigation in such open water systems would be prohibitive as a consequence of high cost and the need to test and validate available technologies in open ocean environments.

Appendix 1: Intellectual Property

This report will be made freely available to the public via the Aquafin CRC, FRDC and SARDI.

Components of the project were the subjects of two PhD theses:

Lauer, P. (2005). Benthic metabolism adjacent to Southern Bluefin Tuna (*Thunnus maccoyii*) pontoons in South Australia. PhD Thesis, Flinders University, Adelaide, 210 pp.

Putro, S. (submitted). Studies on spatial and temporal structures of macrobenthic assemblages inhabiting coarse sediments under southern bluefin tuna (*Thunnus maccoyii*) cages. PhD Thesis, Flinders University, Adelaide.

Appendix 2: Project Staff

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