Environmental assessment of zinc coated wire mesh sea cages in Botany Bay NSW

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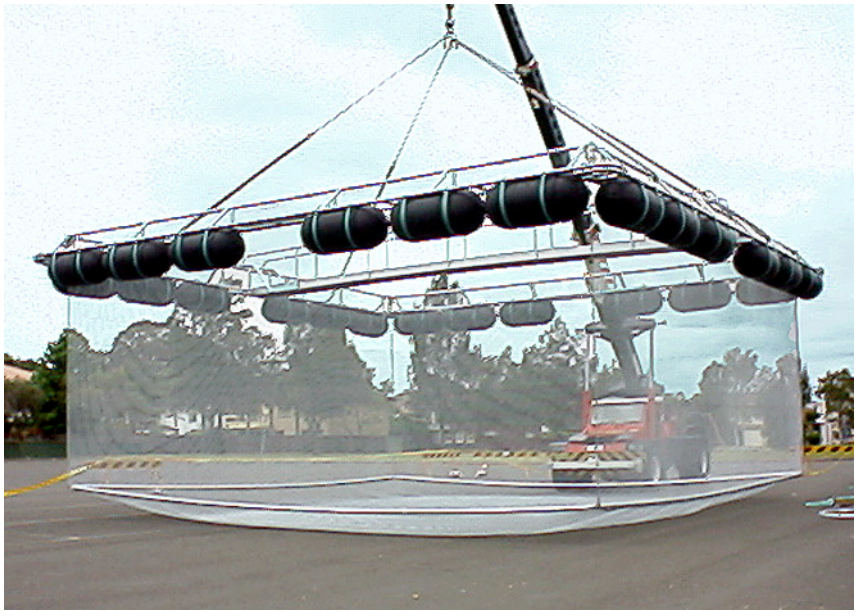

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This report is the product of a combined contribution and effort by OneSteel (formerly BHP, Australia), NSW Fisheries, Silver Beach Aquaculture (SBA) and the University of Canberra (UOC).

In particular we would like to thank Matt Condon, George Niccoli and the late Henry Zakrzewski for their input to the design, construction and placement of the MarineMesh™ seacages. John Hedison, proprietor of SBA, is thanked for his support and assistance with monitoring. Daniel Spooner UOC performed the analysis of sediments and oyster samples.

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Karen Astles assisted with statistical analyses and John Matthews produced the front cover and numerous other figures within the report.

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NON-TECHNICAL SUMMARY

Background

In the marine aquaculture industry the reduction of biofouling in sea cage aquaculture is important for economic and environmental reasons. Problems arising from biofouling result in great costs to farmers because the removal and constant cleaning of net pens are labour-intensive. The presence of large amounts of biofouling on seacage netting may also provide a habitat for pathogens to fish posing a potential threat to the fish occupying the cage. The removal of this waste material can also pose environmental concerns if disposed of incorrectly.

OneSteel Limited, formerly part of BHP (Broken Hill Proprietary Company Limited) is a steel products company that recognised the potential advantages of their wire-coated MarineMesh™ products to the surrounding environment and the aquaculture industry with regards to physical strength and the antifouling properties of zinc. OneSteel was also aware of the need for monitoring the possible environmental implications that might result from extensive use of its coated wire products. As a result OneSteel provided funding for NSW Fisheries to conduct independent research to determine whether there were any adverse impacts on the fish or surrounding environment resulting from the depletion of the zinc coating. NSW Fisheries carried out four related studies in this project that examined: (1) whether snapper *Pagrus auratus* farmed in the MarineMesh™ seacages and grown to marketable size, bioaccumulated zinc above levels considered safe for human consumption; (2) whether the depletion of zinc coating from the MarineMesh™ seacages caused detectable increases in the levels of zinc within the water column; (3) whether the depletion of zinc coating from "MarineMesh™" caused detectable increases in the concentration of zinc in sediments; and (4) the degree of fouling by algae and invertebrates of MarineMesh™

Effects on the concentration of zinc in the tissues of snapper

The concentrations of zinc in the muscle and liver tissues of snapper did not increase significantly from their placement in the MarineMesh™ seacages after a period of 22 months.

The mean concentrations of zinc in fish muscle and liver tissue did not exceed 0.0456 and 0.1619 mg/kg, respectively. The maximum concentrations of zinc in the fish muscle tissue was less than 1% of the safe concentration for human consumption of 150 mg/kg (Australian & New Zealand Food Authority standards code A12 - metals & contaminants in food).

Effects on the concentration of zinc in the sediments

The concentrations of zinc in the surficial sediments in Botany Bay ranged from 2.58 to 282.87 mg/kg and fluctuated in an inconsistent manner in space and through time. The observed concentrations of zinc were also well within the range of zinc concentrations known to occur in sediments in the Sydney region. The concentrations of zinc in the surficial sediments under the MarineMesh™ seacages did not increase significantly compared to control sites.

Effects on the concentration of zinc in the water column

Invertebrate molluscs, such as oysters, are filter feeders and are known to accumulate metals. They have been used as biological indicators in other studies to assess the accumulation of metals in marine waters. The Sydney rock oyster *Saccostrea glomerata* was used to examine the effects of the MarineMesh™ seacages on the concentration of zinc in the water column because it is endemic to this area and has been used successfully as a biological indicator of water quality in numerous other studies.

The mean concentration of zinc in oyster tissue did not differ significantly among sites. The concentration of zinc in the oysters attached to the MarineMesh™ seacages did not significantly increase through time compared to the oysters at the control sites after the 6 and 12 weeks of deployment. In fact, the oysters attached to the MarineMesh™ seacages lost zinc during the period of the study demonstrating that no detectable accumulation of zinc occurred as a result of the seacages.

Effects on the fouling by algae and invertebrates

The "soft" netting currently used by the aquaculture industry has to be changed at about 6-weekly intervals because of the fouling by algae and invertebrates. Prior to this study, the antifouling properties of the OneSteel MarineMesh™ had not been assessed. However, the antifouling properties were likely to be substantially greater because of the zinc-coated wire. To assess the effectiveness of the antifouling properties and to quantify the reduction in mesh-size due to biofouling, unused MarineMesh was compared to the mesh in the seacages that had been deployed for about 16 months.

The clean, unfouled MarineMesh™ had a mean mesh size of 36.5 mm. The 16 month deployment resulted in reductions of mesh-sizes to between 27.52 and 31.92 mm, representing reductions in the range of 25 – 13% of the original mesh-size. The reduction in mesh-size also varied between sides of the seacage and between the top and bottom areas of each side.

A comparison was also made of the degree of biofouling on samples of MarineMesh™ and Nylon mesh over a 16-week period of submersion. The mean reduction in mesh-size of nylon mesh was from 36.5 to 5.9 mm, representing a reduction of 81% of the clean mesh size. MarineMesh™ had a reduction in mesh size from 36.5 to 33.1mm, representing a reduction of only 9% of the clean mesh size. It is clear that from the images taken of the biofouling on each mesh type, and from statistical analyses of results, that the degree of fouling by algae and invertebrates on the MarineMesh™ was substantially less than that occurring on the "soft" nylon netting currently used by the aquaculture industry.

Overall conclusions

The results of the four studies show that the OneSteel MarineMesh™ seacages had no detectable adverse environmental effects arising from the loss of zinc and, that the degree of biofouling was substantially reduced in comparison to nylon netting over the period of this study.

1. INTRODUCTION

1.1. Background

In 1998 OneSteel identified a potential market for its uniquely galvanised wire products in the aquaculture industry. OneSteel provided funding for NSW Fisheries to conduct independent research involving environmental monitoring to determine if any effects or impacts could be detected as a result of the gradual depletion of the wire coating.

This study and OneSteel's interest in using zinc coated wire seacages was instigated by the discovery that wire cages, made from cyclone fencing wire, had been used successfully in offshore Japanese fish farms. Observations of these cages revealed that the cages were regarded highly by the farmer, as they are robust enclosures that offered protection from predators and maintain their shape, virtually unaffected by tidal and wave actions. Farmers described the wire cages as "fish friendly" as the fish grown within appeared less stressed by the constant shape provided by the wire cage. The cages also offered a degree of biofouling protection, with little fouling occurring after the twelve-month submersion period. Unfortunately the cages only had a life span of approximately twelve months before corrosion had damaged the cage, questioning the economic value of the product.

OneSteel have developed seacage netting known as MarineMesh™, which is woven from a high quality wire called MarineWire. This gives the mesh significantly longer life in a Marine environment than ordinary wires. MarineWire is a special wire that has been galvanised using OneSteel's patented Advanced Galvanising Process. This allows OneSteel to coat the wire very accurately and very uniformly with coating weights in excess of 610 g/m^2. Ordinary fence wires have coating weights around 50 g/m^2 for similar wire diameters and even normal heavy galvanised wires only have coating weights around 250 g/m^2. Another significant advantage of MarineMesh™ over ordinary fence meshes is the edge finish, known as Marine Knuckle. Tests show that this double knot provides more than 3 times the edge strength than ordinary chain mesh (OneSteel pers. Com.).

The use of metal-based coatings as antifouling agents is not a new practice for the preservation of structures in aquatic marine applications. For example metal-based paints are used extensively in the shipping industry to protect ship hulls and other marine structures from biofouling. The reduction of biofouling in the marine sea cage industry is important for both economic and environmental reasons. Problems arise from biofouling that can result in great costs to farmers because the removal and constant cleaning of the net pens is labour intensive. The presence of large amounts of biofouling on seacage netting may also provide a habitat for fish pathogens posing a potential biological threat to the fish occupying the cage. The removal of the waste material generated after cleaning is costly to farm operators and can pose environmental concerns if disposed of incorrectly. The NSW Environmental Protection Authority (EPA) prohibits the disposal of wastes, such as biofouling material, into waterways (EPA 1998).

The MarineMesh™ seacages may overcome this problem as they have the potential to greatly reduce the accumulation of biofouling organisms, reducing the need to de-foul nets, remove and dispose of biological waste material. In Japan, some fish farmers have already used low-grade zinc coated wire for sea cages in marine waters. The cages were found to be robust enclosures that were particularly good in rough seas as they held their shape, unlike conventional soft netting. After approximately one year's sink time (expected lifetime) the Japanese sea cages were removed and observations revealed that although the cages were corroded, very little biofouling material

had grown on the galvanised wire netting (BHP pers. Com.). MarineMesh™ is manufactured using the similar materials to conventional wire however it has a thicker and more evenly distributed coating of zinc that may possibly lead to increased life span and resistance to biofouling organisms.

Metal-based coatings have been developed to protect traditional nylon sea cage netting. However these products have not yet been tested and approved for use in Australia. As the metal-based coatings deplete they may contribute to an increase in the levels of metals in sediments beneath the fish cages, posing environmental concerns. In Scotland the use of metal-based coatings as antifouling agents on seacage nets used in salmon farms has been blamed by local media for catastrophic changes to benthic communities located within sediments and other biota in surrounding farm sites. However, a literature review of the subject revealed no published evidence to substantiate this claim. The SEPA (Scottish Environmental Protection Authority) Internet web-site provides information regarding fish farming practices, including monitoring of sediments. No reference concerning the monitoring of sediments for metals was found. The only data referenced concerned nutrients from biological matter produced from fish feeds and faeces.

Various metal coatings have been used as antifoulants in marine industries. Organotin antifoulants were first used by the Australian Navy in the 1970's, and are still used for antifouling protection of their underwater hulls (Lewis, T. 1994). As a result of worldwide concerns about the impact of the antifouling biocide tributyltin on non-target species, detailed studies have been done to evaluate alternative antifouling technologies (Lewis, J. 1994). The use of zinc coating as an antifoulant could be considered as a possible threat to non-target species such as local fish communities and the fish grown within the cages. However, fish grown are unlikely to be affected as toxicological information indicates that marine fish are relatively resistant to zinc at all stages of life, compared with larval invertebrates such as crustaceans, bivalves, molluscs and polychaete worms (Mance 1987).

OneSteel have recognised the potential advantages of their unique galvanised wire coated products to the aquaculture industry with regards to strength and antifouling, and the importance of testing and monitoring the possible environmental implications that may result from extensive use of its coated wire products. These implications include concerns that the fish being farmed may be affected by exposure to the zinc coating of their cage, or that the depletion of the zinc coating might cause problematic increases in the levels of zinc within the water column and sediments within close proximity to the seacage. An increase in zinc could adversely affect biota residing on or around the sea cage structure.

1.2. Objectives

This project had the following key objectives:

Objective 1. To identify and test environmental indicators to assess possible increases in zinc (Zn) in the water column and in sediments resulting from the use of MarineMesh™ seacages.

Objective 2. To monitor fish being grown within the cages to ensure they are unaffected and safe for human consumption.

Objective 3. To identify and test methods to assess the degree of biofouling accumulated on MarineMesh™ when used in a commercial application.

2. DEPLETION OF ZINC COATING

2.1. Introduction

OneSteel manufacture zinc coated wire (MarineMesh™) that has a far greater loading of zinc than traditional coated wires and the method of production ensures the coating is more efficient. The depletion of zinc will still occur and the life span of the product is still limited as with traditional coated wire, however if the life span of MarineMesh™ is greatly increased then too will be that of the cages, rapidly increasing the economic value to the farm operators.

The basis of this study was to examine key environmental issues to determine if any adverse environmental impacts had occurred as a result of the introduction and use of MarineMesh™ seacages on the fish farm. To establish and confirm the depletion and loss of zinc from the MarineMesh™ it was necessary to do a basic assessment of the zinc coating remaining on the MarineMesh™ after it had reached its expected life span.

In order to estimate the depletion of zinc coating lost, pieces of wire mesh were removed from MarineMesh™ cages after a period of 26 months and examined to determine the amount of zinc remaining on the wire itself. At this time it was considered that the MarineMesh™ cages had almost reached their full life span. The mass of zinc lost and the approximate rate of depletion can be easily estimated. The factors are important in the overall environmental assessment of the cages as the lower the release rate of zinc ions into the water the greater the flux of zinc from water currents.

2.2. Materials and Methods

The MarineMesh™ sea cages are comprised of five panels of wire mesh. Each of the four side walls was virtually submersed, approximately 1m of mesh was held above the waterline to stop fish from escaping. Samples were collected by divers who removed sections of wire from the north-facing panel of a cage. The sampling strategy was established by dividing the cage side into nine sections. Three equal vertical sections (A, B, C) from left to right and three zones horizontally being 1, above the water, 2, and 3. The upper and lower halves of the submersed section of the panel (Fig. 1).

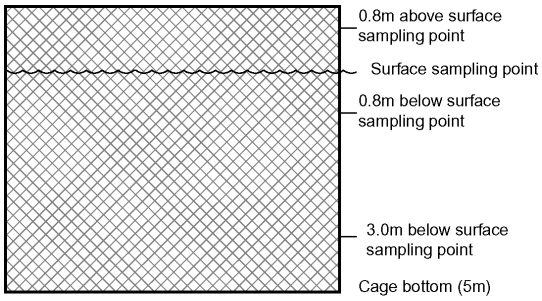

Figure 1. Side wall of MarineMesh™ seacage showing sampling locations.

2.3. Results

Results indicate that the zinc coating had depleted to a low level with some samples revealing virtually all the coating had depleted (Fig. 2). As expected, the sections of cage above the water line had also corroded but had substantial quantities of coating remaining. The fact that some coating is still present on the wire indicates that the cage is close to the end of its useable life span.

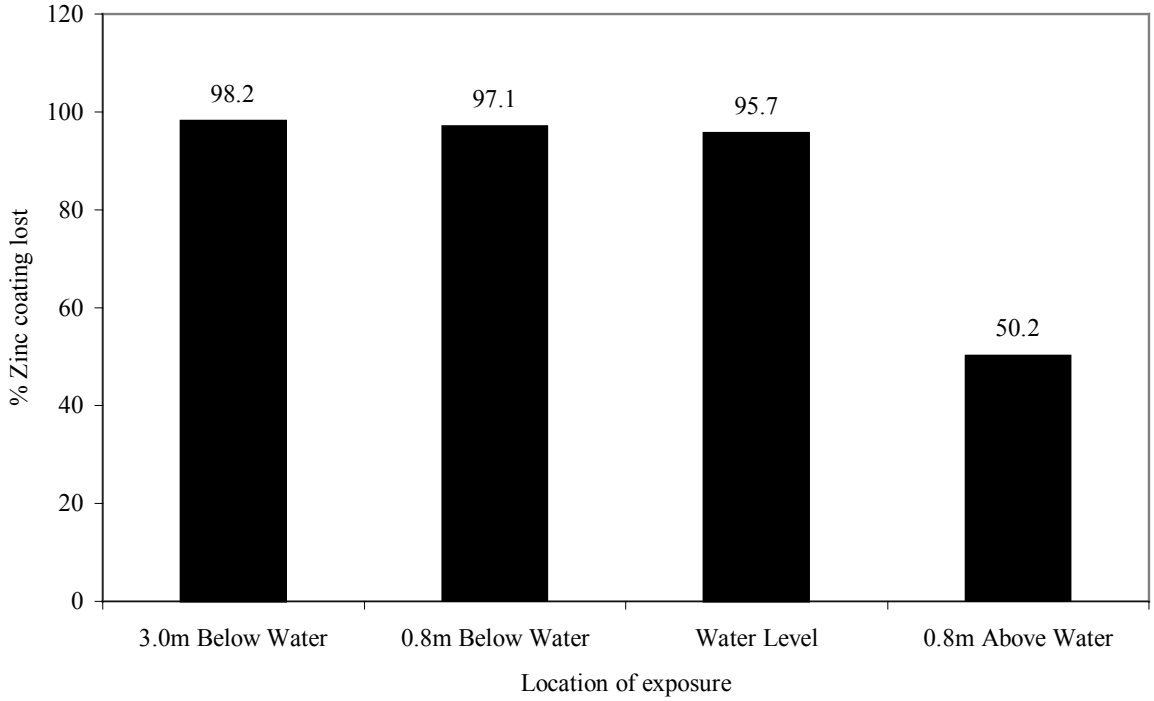

Figure 2. Plot of % zinc lost from each observed region of MarineMesh™ seacage.

2.4. Discussion

The life-span of standard zinc coated wire used by fish farmers in Japan was approximately 12 months. Although the conditions in where the sea cages are situated in Japan may be different to those where the MarineMesh™ cages were studied in Botany Bay, Australia, it would be prudent to say that the MarineMesh™ seacages would generally have a far greater life expectancy than standard zinc coated wire mesh. It is also possible that the addition and use of a cathodic protection system could further increase the life expectancy of the MarineMesh™ seacages.

3. SEDIMENT ANALYSIS

3.1. Introduction

The study of sediments and their associated soft-bottom, macro-invertebrate communities is a common practice in the assessment of operations such as fish farms which can increase the nutrient loading in the sediments (e.g. Wu *et al.,* 1994). The monitoring of levels of sediment nutrients such as phosphates, nitrites and organic carbon may indicate an accumulation of organic waste generated beneath the sea cages. Large and consistent amounts of waste deposition beneath sea cages can effect the sediment chemistry (Ye *et al.,* 1991). Elevated levels of sediment nutrients beneath fish farms can result in an azoic zone, which is devoid of macrobenthic organisms (Ye *et al.,* 1991). The reduction of these organisms underneath and in the areas surrounding the sea-cages can result in an anaerobic process occurring with the production of reduced compounds such as ammonia, hydrogen sulfide and methane. Increases in the levels of nutrients in the sediments may also affect the fish being grown in the seacages and the reduction of waste compounds can generate hydrogen sulfide gas which is toxic to fish (Wu *et al.* 1994). Monitoring environmental changes in sediments beneath a fish farm is regarded as a standard procedure in this industry. The commercial fish farm used for this study has an obligation to ensure that wastes generated from the farm do not impact on the surrounding environment. These wastes include solid and dissolved nutrients from fish faeces and uneaten fish food, biofouling material removed from seacage and fish net pens, and physical and chemical impacts from moorings and seacage structures.

In recent years, fish farmers have begun using wire-mesh sea-cages instead of the "more traditional" soft netting materials because of the strength of the wire mesh and the reduced levels of biofouling as a result of anti-fouling materials. Prior to the use of wire-seacages, operators had to change (and then clean) the nets regularly to maintain an adequate flow of water through the sea-cages. This task requires a substantial input of staff time and hence can increase running-costs greatly. The reduction in biofouling on wire-seacages, while eliminating the need to change nets, has posed its own potential problems. These are mainly related to the loss of the anti-fouling material, its accumulation in the sediments, and its bioaccumulation and/or toxic effects on surrounding biota. Consequently, it is important to monitor the changes in the concentration of the antifoulants in the sediments. Many metals in sediments occur naturally or accumulate as a result of industrial activities (e.g. Phillips 1977, Scanes 1996). Therefore, any monitoring of changes associated with wire-mesh sea-cages must also be done in reference to the existing conditions at other locations within the same general area.

This section of the study examined whether there had been a depletion of the zinc coating from the two zinc-coated wire sea-cages situated in Botany Bay NSW, and whether this has accumulated in the sediments surrounding the fish farm.

3.2. Materials and Methods

Botany Bay is an area with a long history of industrial disturbance and foreshore reclamation. Developments such as the Sydney international airport, Sydney container terminal and the Caltex Oil Refinery have been established on the foreshores of the Bay. Apart from the oil refinery wharf, which lies adjacent to the site of the fish farm, sampling sites for sediment studies were located in areas as far away from these industrial activities as possible.

To detect any possible change in the levels of zinc in sediments beneath the site of the wire cage, it was necessary to collect samples of the surface sediment at this and other reference sites, over a reasonable time, prior to and after the deployment of the wire cages. Fortunately, NSW Fisheries and Silver Beach Aquaculture P/L had been monitoring sediments at all sea-cage locations or sites within the commercial lease area and at seven control sites (see Fig. 1). A sampling strategy had been adopted where sediments were collected three times a year (or every four months) just prior to the movement of the sea cages among lease sites. The original sea cages were moved in a rotational pattern around the site to allow a period of vacancy for recovery for a used site. Monitoring at all locations commenced some eight months prior to the placement of the MarineMesh™ sea cages providing sediment data (collected on two occasions) from all study sites including sediments from beneath the MarineMesh™ sea-cage sites. The MarineMesh™ sea cages were placed in a fixed site for the duration of this study. Samples were also collected on two occasions after the placement of the cages in November 1998. The data collected documents the levels of zinc in the surficial sediments at the location of the cages, and at seven other control locations around Botany Bay at eight months and at four months before and at four and at eight months after the placement of the MarineMesh™ sea cages at Silver Beach Botany Bay.

The levels of zinc found within the sediments at the seven control sites provide an assessment of typical levels of zinc found within sediments in Botany Bay. A comparison of sediments levels at all control sites with those from beneath the MarineMesh™ seacages would indicate if any changes have occurred at these locations and secondly, if a change was detected whether similar changes have occurred in other locations within the Bay. If a change was detected in cage sites which did not occur at any of the control sites then it could be concluded that the introduction of the MarineMesh™ sea-cages had contributed to, or was the direct cause of, this change. Alternatively, if a change occurred at the seacage sites and one or more of the control sites, it could be concluded that this change could be the result of a factor, other than the coated wire cages. If no significant difference or change was detected at the cage sites or control sites, it could be concluded that no detectable changes had occurred to the levels of sedimentary zinc as a result of the introduction of the zinc coated wire seacages. Under this latter scenario it would seem unlikely that the depletion of zinc from the wire cages poses any environmental threat to the surrounding sedimentary habitat.

3.2.1. Positioning of Sites

The method used for position fixing was a differential global positioning system (DGPS). This method was used for the positioning of all sampling sites to ensure the exact locations of sites were recorded and relocated at each sampling time (Table 1). DGPS positions are accurate to within 5 meters. Distances from landmarks were also recorded and used to locate sampling positions at control sites. The mooring blocks for each cage site located the positions of sample sites beneath the seacages.

Table 1. Position data for all control sites.

Control Site	Location	Depth	Latitude	Longitude
1	Frenchman's Bay	6m	33^0 59' 17." 194207916	151^0 13' 43." 894326842
2	Silver beach	5m	34^0 00' 18." 880696575	151^0 11' 25." 655354695
3	Bonna Point	6m	34^0 00' 19." 944901524	151^0 11' 37." 116276487
4	Towra Point	4m	34^0 00' 07." 183064476	151^0 10' 39." 078427196
5	Ramsgate	7m	33^0 59' 08." 060648523	151^0 08' 52." 738693702
6	Brighton Le-Sands	6m	33^0 57' 46." 954659183	151^0 09' 23." 931017513
7	Kyeemagh	5m	33^0 57' 13." 184710809	151^0 09' 50."402614961

The two sea-cages were situated in fixed locations, on the fish farm adjacent to the refinery wharf, Silver Beach, Kurnell. Seven control sites were selected in various locations around Botany Bay (Fig. 1), with similar characteristics to the sea cage site, excluding the airport and container terminal areas, as these had recent sediment disturbances due to construction works. A more detailed description of control site location characteristics is provided in appendix 1. Sediment samples taken from these areas gave an indication of the spatial variation of sediment zinc levels within Botany Bay.

Time, depth, tide level and weather conditions at time of sampling were recorded at each location, at each time period. SCUBA divers collected three replicate core samples of surface sediment from each of the sea cage sites and all seven control sites for analysis. Sediment cores were collected using plastic specimen containers. Each container was cored to a depth of approximately 20-mm, collecting only surficial sediments. The sediment samples were frozen until sampling at all times was complete. All samples were then transferred to University of Canberra (UOC) for chemical analyses.

3.2.2. Chemical Analysis

The chemical analyses of sediment samples were done by the UOC using standard laboratory methods. Briefly, the sediments were acid digested following the methods of Baldwin *et al*. (1994) and Deaker *et al*. (1995). A Perkin-Elmer Elan 6000 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was then used to determine the concentration of zinc in the sediment digests. Standard Reference Material (SRM) was also routinely digested throughout the analytical phase to document the recovery rates and ensure quality control.

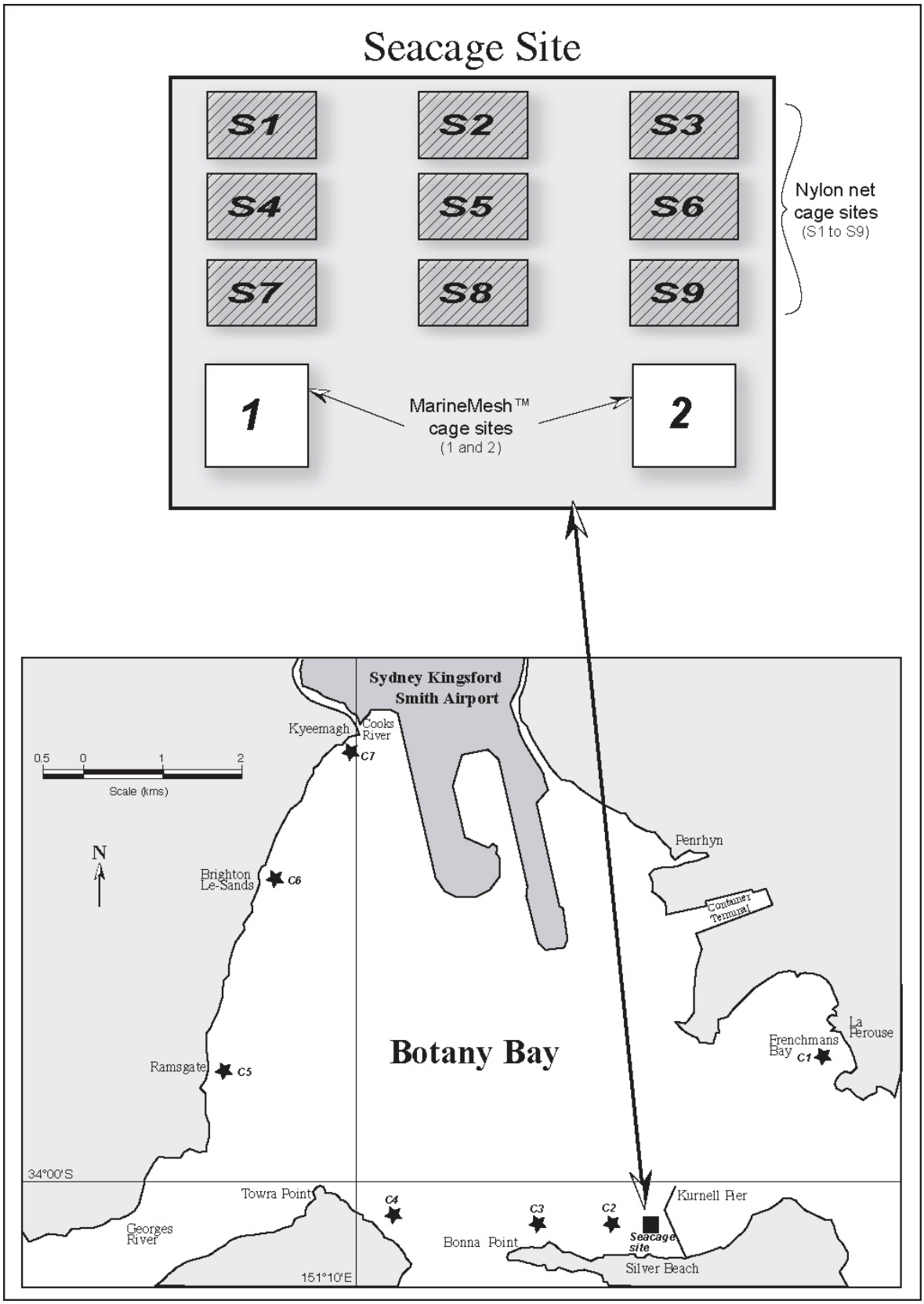

Figure 3. Map of Botany Bay showing control and sea-cage sites.

3.2.3. Statistical Analysis

Ideally, an assessment of any possible impacts of zinc from the wire sea-cages would be based on replicated control and putatively impacted sites. However, as there was only one putatively impacted site, the design became asymmetrical with respect to impact sites. Control sites should be, and are, replicated in this design and can have a great influence on the power of the tests for impact. Table 2 shows the design that was used to analyse the temporally-replicated data. Note that all terms denoted with an asterisk involve an asymmetry in the levels of the particular factor.

This design has several important features that overcome problems that have beset approaches to impact assessment in the past (Otway 1995, Otway *et al*. 1996a, b). First, the design incorporates spatial and temporal replication thus overcoming problems of pseudo-replication (Hurlbert 1984). Second, temporal replication (before and after the disturbance) is done at a time before and at several times after, to identify temporal trajectories (also see discussion in Stewart-Oaten *et al*. 1986). Third, the design detects whether disturbances, in this case the loss of zinc from the wire sea-cages, causes detectable changes in the variable of interest at the putatively impacted site (i.e. the fish farm site). Last, the design detects impacts that occur at different temporal scales i.e. as short-term 'pulse' or sustained (longer-term) 'press' changes (Bender *et al*. 1984).

The repartitioning of the asymmetrical analysis of variance in Table 2 provides for temporal interactions with an *a priori* orthogonal contrast between the single putatively-impacted site and the control sites before and after the disturbance begins. It is this feature that permits tests for impact. The detection of impact depends on the duration of the changes caused by the disturbance and the space-time interactions, which occur naturally, i.e. in the absence of an anthropogenic disturbance. The detection of impacts at different temporal scales requires several tests and these are described below. First, a persistent impact is detected using the *F*-ratio of MS B v A x SC v Controls / MS B v A x Among C giving a test with 1 and 6 degrees of freedom (i.e. with 7 control sites sampled). Second, if there is no significant variation from 'before' to 'after' among the control sites (there are no short-term temporal interactions among the control sites) i.e. the *F*-ratio of MS (B v A) x Among C/ MS Residual is not significant at $P = 0.25$, then the MS (B v A) x Among C term can be eliminated (pooled with the Residual) from the analysis. This then results in a test with substantially more power, as the MS Residual and its associated degrees of freedom (an order of magnitude greater) are then used in the *F*-ratio for assessing impact.

An intermittent impact can be detected in one of two ways. First, when there are no short-term temporal interactions among the control sites after the disturbance begins, i.e. the F-ratio MS Times (After) x Among Controls / MS residual is not significant in Table 2, a short-term impact is indicated when the *F*-ratio of MS Times (After) x SC v Controls / MS residual is significant and the two-tailed *F*-ratios of MS Times (After) x SC v C / MS Times (Before) x SC v C and MS Times (After) x Among C /MS Times (Before) x Among C are significant and not significant, respectively. Second, when there are significant short-term temporal interactions among the control sites after the disturbance begins, i.e. the *F*-ratio MS Times (After) x Among C / MS residual is significant in Table 2, a short-term impact is evident when the F-ratio of MS Times (After) x SC v C / MS Times (Before) x Among C is significant and the two-tailed *F*-ratios described above are significant and not significant, respectively.

While the design is a substantial advancement on the previous BACI (Before-After/Control-Impact) designs of Green (1979), Bernstein & Zalinski (1983) and Stewart-Oaten *et al*. (1986), there may still be some problems. Some of the tests may have low statistical power and thus, the probability of making a Type II error may be large. This could be overcome by relaxing the Type I error-rate of 0.05 to 0.10 as this will result in increased power and make the detection of impacts less conservative.

Table 2. Asymmetrical analysis of variance assessing the possible impacts of zinc from the wire sea-cages.

The design involves one putatively impacted site and multiple control sites contemporaneously sampled (with replication) through time before and after deployment of the zinc-coated sea-cages.

Source of variation	df	Denominator for *F*
Before vs After = B vs A	**1**	MS B vs A x Among C
Times (Before vs After) = Times (B vs A)	**2**	MS Times (B vs A) x Among C
Sites	**7**	
Sea-cage vs Controls = SC vs C	**1**	MS Among C
Among Controls = Among C	**6**	MS Residual
B vs A X Sites	**7**	
B vs A x SC vs C	**1**	MS B vs A x Among C [1]
B vs A x Among C	**6**	MS Residual
Times (B vs A) x Sites	**14**	
Times (B vs A) x SC vs C	**2**	
Times (B) x SC vs C	**1**	
Times (A) x SC vs C	**1**	Times (B) x SC vs C [2]
Times(B vs A) x Among C	**12**	
Times (B) x Among C	**6**	MS Residual
Times (A) x Among C	**6**	MS Residual
Residual	**28**	
Total	**55**	

Note:

1. If B vs A x among C is not significant and can be pooled, then the denominator for B vs A x SC vs C becomes the MS Residual.
2. If Times (A) x Among C is not significant and can be pooled, then the denominator for Times(A) x SC vs C becomes the MS Residual.

3.3. Results

The concentration of zinc in the sediments at the sea-cage site was significantly greater in January 1998 compared to May 1998 (Table 3). There was a significantly greater concentration of zinc in the sediments at the sea-cage site compared to the control sites in the "before" period (i.e. prior to the deployment of the sea-cage) as evidenced by the significant *F*-ratio for the Times (Before) x SC vs C term (Table 4a). Moreover, the greater overall mean concentration of 168.90 mg/kg at the sea-cage site in the "before" period was significantly greater than the mean of 71.59 mg/kg at the control sites and responsible for the significant B v A x SC v Controls *F*-ratio (Table 4b).

The concentrations of zinc in the sediments at the sea-cage site compared to the control sites after the deployment of the wire sea-cage did not differ significantly (Tables 3 & 4). There was also significant short-term temporal variation in the concentration of zinc in the sediments at the control

sites as indicated by the significant Times (After) x Among C *F*-ratio (Table 4a). The concentrations of zinc at the control sites varied between 10.84 mg/kg and 177.42 mg/kg in January 1999 and 2.58 mg/kg and 282.87 mg/kg in May 1999.

Table 3. Mean concentrations of zinc (mg/kg) in the sediments at the sea-cage site and control sites sampled twice before and twice after deployment of the MarineMesh™ sea-cage.

Time		All Control Sites	Sea-Cage Site
Before			
	1	60.83	280.23
	2	79.98	57.56
After			
	1	46.49	97.31
	2	95.92	74.20

Table 4a. Asymmetrical analysis of variance comparing the concentration of zinc in the sediments at the sea-cage site with all control sites sampled twice before (B) and twice after (A) deployment of the MarineMesh™ sea-cage.

Source of variation	df	MS	*F*	*P*
Before vs After = B vs A	**1**	2255		
Times(Before vs After) = Times(B vs A)	**2**	10508		
Sites	**7**			
Seacage vs Controls = SC vs C	**1**	33547	0.55	ns
Among Controls = Among C	**6**	61547	29.00	**
B vs A X Sites	**7**			
B vs A X SC vs C	**1**	18497	14.68	**
B vs A X Among C	**6**	1260	1.42	ns
Times(B vs A) X Sites	**14**			
Times(B vs A) X SC vs C	**2**			
Times(B) X SC vs C	**1**	76752	74.66	**
Times(A) X SC vs C	**1**	738	0.28	ns
Times(B vs A) X Among C	**12**			
Times(B) X Among C	**6**	1028	1.17	ns
Times(A) X Among C	**6**	2669	3.03	**
Residual	**64**	882		
Total	**95**			

Table 4b. Tests for Intermittent Impact

1-tailed test: MS Times(A) x SC vs C / MS Times(A) x Among C = 0.28 ns

2-tailed tests:
1. MS Times(A) x SC vs C / MS Times(B) x SC vs C = 0.01 ns
2. MS Times(A) x Among C / MS Times(B) x Among C = 2.60 ns

3.4. Discussion

Over the 24-month period of this study the MarineMesh™ seacages are known to have lost a considerable mass of zinc into the waterway (see Chapter 2). It is possible that some of this lost zinc may have accumulated within the sediments beneath the seacages.

The results showed a range of zinc concentrations in the sediments at the control sites and cage site through time. These levels were well within the range of zinc concentrations known to occur in the sediments in the Sydney region (Davies 1978, Gray 1995) and elsewhere (Chester & Stoner 1975, Everaarts 1989, Batley & Brockbank 1990). The levels of zinc in the surficial sediments beneath the sea-cage were consistent and showed no significant increase through time compared to the seven control sites and were within the range of zinc concentrations exhibited at these latter sites. This strongly suggests that within the proximity of the commercial fish farm the fluctuations in the concentrations of zinc in the sediments at the control sites are likely to be the result of natural spatial and temporal variation in the levels of zinc in the sediments in Botany Bay.

The pelletised feed used by the operator also has varying amounts of zinc present (see Chapter 5). Consequently, any pellets that are not consumed by the fish and sink to the seabed may also contribute to the overall concentration of zinc in the sediments. The degree to which this "waste" contributes to the concentration of zinc in the sediments will be directly related to feeding practices and sediment dynamics. Nevertheless, there were still no detectable increases in zinc in the sediments.

4. ZINC BIO-ACCUMULATION IN OYSTERS

4.1. Introduction

The corrosion of the zinc coating on the wire seacages occurs at the interface of the coating and the water. This corrosion of the coating is due to electrolysis slowly removing zinc ions from the surface of the coating. The detection of the depleted zinc and its possible effect on the levels of zinc found in the water column at the cage sites, at any given time, is difficult to assess and quantify.

Invertebrate molluscs, such as oysters, are filter feeders and are known to accumulate metals, including zinc and have been used as biological indicators in other studies assessing the accumulation of metals in marine waters. The Sydney rock oyster *Saccostrea glomerata* is endemic to this area and has previously been used successful as a biological indicator (see Scanes 1992, 1996) in the Sydney region and was chosen to see if bio-accumulation of zinc was greater in oysters grown near the MarineMesh™ cages.

Using a similar method of analyses to that used for sediments in chapter three, a comparison was made of the levels of zinc found in oysters grown at control sites with those grown at (and attached to) the MarineMesh™ seacage sites.

The laboratory-based analytical work and subsequent statistical analysis of the data for this study formed part of a Honours project by Mr. D. Spooner of the University of Canberra under the supervision of Associate Professor Dr. Bill Maher. The subsequent sections of this chapter are summaries of the results already presented in Spooner (1999).

4.2. Materials and Methods

4.2.1. Cage and Control Sites

The sea-cage sites are located within the fish farm lease area, adjacent to Kurnell oil refinery wharf, Silver Beach, Kurnell (see Fig. 3). The control sites were located around Botany Bay (see Fig. 3). At the sea cage sites the sampling units were attached directly to the MarineMesh™ seacage wire in a central position on the side walls of each sea cage. The control sites utilised stationary objects, such as a permanent navigational mark or jetties to permit the attachment of the oyster baskets.

4.2.2. Biological Indicator Sydney Rock Oyster, Saccostrea glomerata

Oysters were purchased from a commercial oyster farmer in Quibray Bay, adjacent to Botany Bay. The farmer provided history of the oysters, which showed that they were wild caught spat that had been initially grown in the Hawkesbury River, NSW. They were then transferred to Quibray Bay for final grow out to market size. The oysters were removed from the Bay and purged for 24 hours in re-circulating filtration tanks, a standard harvesting procedure.

Twenty-five oysters were placed in each of nine cylindrical plastic oyster baskets with an approximate volume of 5000 cm^3 and mesh size of 20 x 20cm, that were clip-locked to ensure that oysters were not lost. Divers attached baskets to physical structures (e.g. pylons) at each control

location and at the two zinc-coated MarineMesh™ sea-cages using plastic cable ties. Baskets were placed at a depth of 3.5 m below the mean tide level. The oysters were deployed at all the control and sea-cage locations on 15/5/99. Ten oysters were retrieved from each basket on each of two occasions (i.e. 30/6/99 and 15/8/99) separated by about six weeks. On each occasion, all oyster baskets were retrieved and the 10 randomly chosen oysters removed from each basket. Each basket was then returned to its original location. The oysters that were removed from each basket were placed into appropriately labelled plastic bags. Oysters from each location were then placed in separate 120-litre aquaria and purged in water filtered to approximately 100 microns for 24 hours. This process was established to remove excess sediments from within the oyster shells and improve analytical procedures.

4.2.3. Laboratory Analyses

Oysters were shucked, rinsed and weighed (wet weight). Each oyster was then freeze dried for 48 hours. The freeze-dried oysters were then digested with acid, following the methods of Baldwin et al. (1994) and Deaker et al. (1995). A Perkin-Elmer Elan 6000 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was then used to determine the concentration of zinc in the oyster tissue digests. Standard Reference Material (SRM) was also routinely digested throughout the analytical phase to document the recovery rates and ensure quality control.

4.2.4. Statistical Analyses

The resulting data were analysed using two 1-factor analyses of variance to examine the spatial variation in zinc concentrations after the 6 weeks and 12 week's deployments. Homogeneity of variance was examined prior to analysis of variance and where variances were heterogeneous the data were transformed following Underwood (1981). When the Sites term was significant in the analysis of variance, differences among means were identified using Dunnett's multiple comparison test with a Type I error-rate of 0.05.

4.3. Results

The levels of zinc within oyster tissues did not differ among sites (Spooner 1999, Tables 4.6 and 4.7, $P > 0.05$). However, the level of zinc within tissues of oysters tended to decrease over time at some locations (e.g. Control Site 1, see Fig. 3), whereas other sites remained virtually unchanged. There was no evidence of an increase in the concentration of zinc in the oysters at the MarineMesh™ sea-cage site compared to the oysters at the control sites after 6 or 12 weeks of deployment. It is important to note that the results of the analyses of the 10 randomly-chosen oysters sampled prior to the initial deployment in Botany Bay revealed high levels of zinc and other metals such as arsenic.

4.4. Discussion

The Sydney rock oyster has been successfully used as a biological indicator in relatively shallow open waters to observe accumulation and rate of effect over similar time period to those used in this study (see Scanes 1992, 1996). It was anticipated that the oysters purchased and selected for sampling would initially have had relatively low levels of zinc. However, the oysters were found to have fairly high initial levels of zinc. As the analyses of all samples was done after the sampling process was complete, this problem was not detected before rectifiable. Therefore results obtained from this component of the study may not be conclusive. The reduction in tissue metal levels indicates that the oysters were previously grown in a location exposed to greater metal ion concentrations compared to the locations chosen (for this study) within Botany Bay. However, the levels of zinc found in oysters attached to the MarineMesh™ cages did not significantly

increase through time in comparison with those of the oysters at the seven control locations. This suggests that the loss of the zinc from the cages occurred at a rate that did not cause increases in the concentration of zinc above those already present in the oyster tissues.

It is important to note that no detectable changes occurred in the levels of zinc in the superficial sediments analysed (see Chapter 3). Moreover, the depletion of the zinc coating of the MarineMesh™ resulted in a considerable mass of zinc being lost into the surrounding environment (see Chapter 2). It would be logical to conclude that the depleted zinc particles or ions may have been dispersed in the water column by the current flow (with a range of 8 – 12 cm/sec) (Quartararo 1996) occurring at the sea-cage site. Visual observations made by researchers collecting sediment samples revealed that obvious and sometimes dramatic movements of sediments had occurred, particularly after extreme weather conditions had been experienced resulting in heavy wave action within Botany Bay. It was estimated that the depth of sediments at a particular location could vary or alter up to 300 mm in depth between quarterly sampling periods. These movements were obviously occurring from strong currents throughout the water column, supporting the theory that it is most likely the zinc was dispersed in the water column by current flow.

5. MONITORING OF ZINC LEVELS WITHIN FARMED FISH

5.1. Introduction

Food surveillance authorities require strict quality control and assurance to help protect consumers from unsafe foods. The seafood industry is difficult to regulate in terms of quality, as most of its product is harvested from the wild or wild fish stocks.

The aquaculture industry has the ability to supply a product that has been cultured in known conditions including location, housing, water and feed quality. The product itself can be routinely monitored to ensure that it is within the Australian & New Zealand Food Authority (ANZFA) guidelines for safe consumption. For fish, the concentration of zinc in muscle tissue should not exceed 150 mg/kg (ANZFA food standards code A12 - metals and contaminants in food).

OneSteel has developed a zinc-coated wire product, MarineMesh™ that may have several advantages for the fish farmer. However, before this product is used by industry it is vital that monitoring is done to ensure that the fish grown within the zinc-coated MarineMesh™ cages are unaffected by possible loss of the zinc from the wire and are safe for human consumption. The objectives of this part of the study were: (1) to document the concentration of zinc in the muscle and liver of fish cultured in zinc-coated wire cages; and (2) to determine if these were within the regulations and regarded safe for consumption. The study also investigated the amount of zinc in the pelleted fish diets. This was done to determine whether the pelleted diets were a possible source of zinc to the tissues of the fish.

5.2. Materials and Methods

5.2.1. Field Processing

Fish (Snapper – *Pagrus auratus*) were grown on the farm site from an approximate mean weight of 5 g to a mean weight of 305.4 g (SD = 38.3) in soft, nylon-mesh sea-cages and then transferred into the MarineMesh™ sea-cages. Prior to their transfer, 10 fish were supplied by the operator in March 1999 and analysed for zinc in their muscle and liver tissues. A further 10 fish were supplied every 4 weeks over the period April – June, 1999. A final sample of 10 fish was collected in February 2001 after which the fish had successfully grown over a considerable period of time in the cages.

Two types of commercial fish pellets were used to feed the fish prior to and during this study. Randomly chosen samples of both feeds (hereafter referred to as Feed 1 and Feed 2) were collected by the operator and stored in labelled plastic bags for analyses.

5.2.2. Laboratory Processing

The fish were weighed and samples of muscle and liver removed. The tissue samples were then freeze dried and acid digested following the methods of Baldwin *et al*. (1994) and Deaker *et al*. (1995). A Perkin-Elmer Elan 6000 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was then used to determine the concentration of zinc in the fish muscle and liver samples. Standard Reference Material (SRM) was also routinely digested throughout the analytical phase to document the recovery rates and ensure quality control.

Commercial Feeds 1 and 2 (fish pellets) were processed in a similar manner to the fish tissues and then analysed for the concentration of zinc in an ICP-MS (for further details see Spooner 1999).

5.2.3. Statistical Analyses

The data were analysed using single factor analyses of variance. Prior to analysis of variance the data for the concentrations of zinc in the liver and muscle tissues were tested for homogeneity of variance using Cochran's test (Underwood 1981). The data for the concentrations of zinc in liver were heteroscedastic, but homogeneity of variances was achieved after log (x+1) transformation of the raw data. The data for the concentrations of zinc in muscle tissue also yielded heterogeneous variances which, unfortunately, could not be stabilised using a variety of transformations. However, as analysis of variance is robust to heterogeneous variances (Underwood 1998) and there were a large number of replicates, analyses were done using the raw data.

5.3. Results

5.3.1. Zinc in Fish Tissues

The concentrations of zinc in muscle and liver tissues of the fish differed significantly through time (Tables 5a & 6a, analyses of variance, $P < 0.05$). The concentration of zinc in muscle tissue initially decreased from before to after placement in the MarineMesh™ seacage, but then increased and fluctuated through time (Table 5, SNK, $P < 0.05$). In contrast, the mean concentration of zinc in the liver of the fish was significantly greater from before to after their placement in the MarineMesh™ seacage (Table 6, SNK test $P < 0.05$). Following placement in the marine mesh seacage, the mean Concentrations of zinc in liver tissue fluctuated significantly through time (Table 6, SNK, $P < 0.05$).

Analysis of Variance (muscle tissue)

Table 5. Analysis of variance of the concentrations of zinc in the muscle tissue of Snapper *Pagrus auratus* grown in zinc-coated MarineMesh™ sea-cage.

Note: data transformed to log (x+1).

Source of variation	df	MS	F	P
Times	4	1.220067	7.320644	2.13E-04
Residual	45	0.166611		
Total	49			

Results of SNK test (muscle tissue)

March 99 > April 99 < May 99 > June 99 > Feb 01

Analysis of Variance (liver tissue)

Table 6. Analysis of the concentrations of zinc in the liver tissue of Snapper *Pagrus auratus* grown in zinc-coated MarineMesh™ sea-cage.

Note: untransformed data used in analysis.

Source of variation	df	MS	F	P
Times	4	1.112818	7.322272	2.13E-04
Residual	45	0.151977		
Total	49			

Results of SNK test (liver tissue)

March99 > Feb01 > June99 > April99 > May99

Table 7. Mean (+ SD) concentrations of zinc in the muscle and liver tissues of Snapper (*Pagrus auratus*) grown in zinc-coated MarineMesh™ sea-cage; n = 10.

Note: the ANZFA food standards code A12 states that for safe human consumption of fish the concentration of zinc in muscle tissue should not exceed 150 mg/kg.

DATE	MUSCLE mg/kg		LIVER mg/kg	
	Mean	SD	Mean	SD
MARCH/99	0.0321	0.0134	0.1619	0.1178
APRIL/99	0.0163	0.0036	0.0743	0.0059
MAY/99	0.0240	0.0152	0.0691	0.0334
JUNE/99	0.0456	0.0391	0.0903	0.0251
FEBRUARY/01	0.0380	0.0090	0.1261	0.0161

Note: The initial sample, March/99 was taken prior to the placement of the fish in the MarineMesh™ sea-cage, the following samples were all taken after.

The mean (± SD) concentrations of zinc in fish muscle and liver (Table 7) did not exceed 0.0456 (± 0.0391) mg/kg and 0.1619 (± 0.1178) mg/kg, respectively. More importantly, the concentrations of zinc in the fish muscle tissue were 3 orders of magnitude below the levels of zinc recommended for safe consumption by the Australian and New Zealand Food Authority.

5.3.2. Zinc in Pelleted Feed

The mean (± SD) concentrations of zinc in the commercial fish feeds 1 and 2 had 181.5 (± 18.3) mg/kg and 54.5 (± 1.32) mg/kg of zinc, respectively.

5.4. Discussion

The significant decrease in the mean concentration of zinc in the liver tissue of the snapper *Pagrus auratus* is contrary to what would be predicted if the fish were accumulating zinc from the MarineMesh™ sea-cage or from the pelleted food. While the mean concentrations of zinc in the muscle tissue of Snapper *Pagrus auratus* significantly differed through time, the mean concentration never exceeded the ANZFA guideline. These results provide and demonstrate compelling evidence that the OneSteel MarineMesh™ sea-cage had no detectable effect on the concentration of zinc in the muscle and liver tissues of the Snapper *Pagrus auratus* after almost 2 years of growth in the MarineMesh™ sea-cage.

The analyses of fish feeds 1 and 2 indicated that both feeds contained reasonably high levels of zinc. As the zinc concentrations in fish tissues showed no significant increase, it is likely that any additional (unused) zinc in the pelleted feeds was passed through the gut of the fish without being accumulated.

It is also possible that the feeding behaviour of the snapper may have contributed to the very low levels of zinc found in the animal's tissues. Moreover, as zinc is mainly accumulated in body tissues by ingestion (Rainbow 1992) and snapper are opportunistic feeders (Henry 1988) and often graze on the invertebrates living on sea-cage netting, it is conceivable that the fish may have directly ingested some zinc from the corroding zinc-coated wire whilst feeding on these invertebrates. However few, if any, invertebrates were present on the wire sea-cages (see Chapter 6) and thus it is likely that little foraging occurred on the wire. Consequently, it is highly unlikely that the snapper would have ingested zinc directly from the wire over the duration of the study.

The other main objective of this part of the study was to examine whether fish grown in zinc-coated MarineMesh™ sea-cages were safe for human consumption. The levels of zinc found in the liver and muscle tissues were substantially less than that specified by the ANZFA standards and the snapper grown in the OneSteel MarineMesh™ sea-cages were clearly safe for human consumption with respect to concentrations of zinc.

6. QUANTIFYING BIOFOULING OF MARINEMESH™ SEACAGES

6.1. Introduction

The fouling of seacage nets by marine organisms poses substantial problems for fish farmers around the world. Traditional seacage netting used on aquaculture farms is woven from multi-filament nylon strands, with each strand consisting of hundreds of nylon fibres. Although this configuration provides the net with great strength, the increased surface area and roughness of these strands almost appear to be designed to facilitate colonisation of fouling organisms (Lewis, 1994). An excess of biofouling on a cage net can restrict the flow of clean water through the net, potentially reducing the dissolved oxygen levels within the cage. It can also provide an ideal habitat for parasites and other pathogenic organisms. For example, parasitic diseases pose problems in the culture of sparids, as well as other fish species (Roubal *et al*., 1992). Consequently, the settlement and subsequent rapid growth rates of microalgae, macroalgae and invertebrates on immersed surfaces such as nets is one of the major problems facing marine aquaculture ventures in Australia and throughout the world (Lewis, 1994).

Settlement of biofouling organisms such as algae, barnacles, oysters and other invertebrates is often inhibited on surfaces coated with active metals such as copper and zinc. Zinc products are commonly used in marine environments to protect structures from corrosion and biofouling. While many different agents have been developed to try to reduce the settlement and/or growth of biofouling on traditional seacage netting, the application of these agents has generally focussed on netting impregnated with, or painted with, anti-fouling compounds. Unfortunately, these approaches have had limited success and a majority of aquaculture operators have resorted to the changing and cleaning of the nets on a regular basis. The changing and cleaning of nets is a labour-intensive process and contributes greatly to the overall production costs in finfish aquaculture. Furthermore, the waste material generated (i.e. the fouling organisms) must be disposed of in an environmentally friendly manner resulting in further costs to the farmer. For example, the cost of controlling biofouling in the Australian aquaculture industry has been estimated to be approximately AUS$1,000,000 per annum (e.g. Lewis, 1994).

Biofouling organisms are classified as either micro-fouling or macro-fouling organisms. Micro-fouling organisms include bacteria, diatoms, protozoa, etc. and these form a thin layer or slime over submerged substrata. Macro-fouling organisms are further sub-divided into soft-fouling and hard-fouling organisms. Hard-fouling organisms are those that secrete calcium carbonate tubes, shells or skeletons. These include animals such as bivalves, barnacles, tubeworms, bryzoans and corals (Lewis, 1994). Soft-fouling organisms are those that lack such hard structural components. These organisms include most of the algae, and animals such as hydroids, sponges and ascidians (Lewis, 1994).

The commercial fish farm at the entrance to Botany Bay, was originally established by NSW Fisheries who used traditional, soft-mesh netting to hold finfish. The present proprietor of the farm uses the same nets and maintains them under the same maintenance regime as NSW Fisheries researchers. These soft-mesh nets are reported to require changing and cleaning every 10-28 days depending on the season and mesh-size (Quartararo, 1996). As described in previous chapters, the MarineMesh™ cages are located close to the traditional cages used on the fish farm and are, therefore, exposed to similar environmental conditions.

The objective of this part of the study was to quantify any reduction in the mesh-size (i.e. the size of the hole) of the MarineMesh™ resulting from biofouling. The mesh size of sea cage nets is gradually reduced as biofouling organisms such as algae, soft and hard invertebrates attach themselves and grow on the nets. The greater the amount of biofouling the greater the reduction in mesh size and consequently a reduction in water flow through the mesh. The reduction of water flow through the mesh can affect the available dissolved Oxygen of the water within the net. As it is desirable for farmers to densely stock sea cage nets, the availability of dissolved Oxygen to the fish being farmed is an important factor.

6.2. Materials and Methods

Two zinc coated wire seacages were placed in operation on the fish farm site and were immersed in salt water for approximately 16 months (30/10/98 to 25/2/00), respectively. The cage that had been stocked with fish for the longest period of time was chosen to carry out experiment 1 (see, Fig. 3). It had been subjected to nutrients from fish feed and faeces that may enhance algae growth and contribute to biofouling and corrosion of the mesh, as often occurs with seacages utilising soft netting (Lewis 1994, Quartararo 1996). Consequently, this seacage was sampled to assess the reduction in mesh size due to biofouling. This was done in two ways. Firstly, casual observations of the appearance of the seacage were made intermittently throughout its deployment whilst carrying out the other sampling detailed elsewhere in this report. Secondly, the seacage was sampled quantitatively after the 16 months of deployment. To this end, photographic images (slides) were taken of 0.1 m^2 quadrats of the biofouled mesh and clean mesh quadrats adjacent to one another. The clean mesh quadrat was made by placing a piece of new mesh on a quadrat with black shade cloth backing to assist with photographic resolution (Fig. 4). Each side of the cage, (i.e. N, S, E, and W) was stratified into top and bottom regions. Three replicate 0.1 m^2 quadrats of biofouled mesh were chosen at random in each of the top and bottom regions of each side and labelled for subsequent identification. These were then photographed together with a 0.1 m^2 quadrat of clean (unfouled) mesh.

In the laboratory, the developed images were illuminated through a standard slide projector ensuring that the projector was level and perpendicular to the screen to reduce any optical distortions and/or errors. On examination of the images, 42 holes or “squares of mesh” could be observed in each 0.1 m^2 quadrat. Ten mesh squares were then selected at random from each of the control (i.e. unfouled) and treatment (i.e. biofouled) meshes in each slide and the mesh-size of each “square of mesh” was measured. The actual measurement of each individual square mesh hole was made across the diamond, as the horizontal width, to the nearest mm. These measurements were then converted to actual size, using the measurement of mean actual mesh size. All the results are reported in mm.

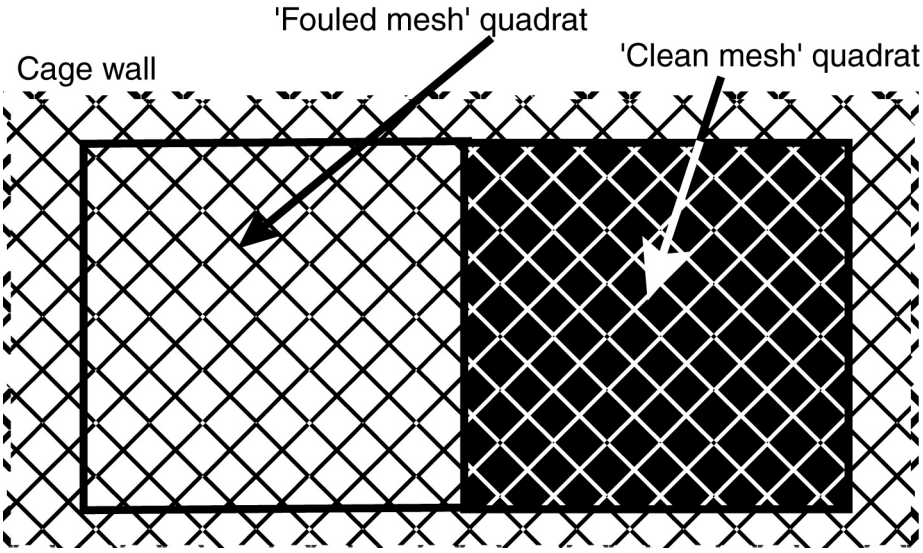

Figure 4. Profile of cage wall with "fouled mesh" and "clean mesh" quadrats.

Statistical analyses of the mesh-size were done using a partially-nested, partially-orthogonal 4-factor analysis of variance with the factors: Unfouled versus Biofouled (fixed), Side of cage (fixed), Top versus Bottom (fixed), and Quadrats nested within Side of cage, Top versus Bottom and Unfouled versus Biofouled (random). Preliminary tests for homogeneity of variances were done using Cochran's test (Underwood, 1981, 1997; Winer *et al*., 1991) and, when variances were heterogeneous, data were transformed using procedures outlined by Underwood (1997).

6.3. Results

The photographic images of quadrats of unfouled and biofouled mesh proved to be a robust and reliable means of documenting mesh-size. The unfouled wire mesh had a mean mesh-size of 36.5 mm (SD = 1.08 mm) and exhibited relatively little variation among replicate areas. After the 16 month deployment, the mesh-size of the biofouled mesh varied among the replicate quadrats and this resulted in heterogeneous variances (Cochran's test, $P < 0.05$). Various transformations of the data were examined, but were unable to stabilise the variances. However, given that there were numerous replicates and that analysis of variance is robust to heteroscedasticity and departures from normality (Eisenhart, 1947; Underwood, 1997), the analysis of variance was done using the untransformed data.

Following the 16 month deployment, the minimum reduction in mesh-size was 4.6 mm (i.e. from 36.5 to 31.9 mm, Table 8) representing a maximum reduction of 12.6 % of the clean mesh size. This reduction in mesh-size was evident in the top region of the eastern wall of the seacage. The maximum reduction in mesh size resulting from biofouling was 9 mm (i.e. from 36.5 to 27.5 mm, SD = 1.93 mm, Table 8). This result represented a reduction of 24.7 % of the original mesh-size and was evident in the top region of the northern wall of the seacage. In spite of the varied reduction in mesh-size, the analysis showed that there were no significant differences in the reduction of mesh-size among sides of the cage nor between the top and bottom regions (Table 9, $P > 0.05$).

Table 8. Mean (+ SD) mesh-sizes (mm) of clean and biofouled wire mesh at different positions on the seacage.

Note: clean (unfouled) mesh had a mean (+ SD) mesh-size of 36.5 (1.08) mm.

POSITION	CAGE WALL			
	North	East	South	West
Top	27.50 (1.93)	31.90 (1.99)	27.80 (1.77)	28.30 (1.84)
Bottom	29.70 (2.24)	30.10 (2.05)	28.80 (2.06)	29.90 (2.51)

Table 9. Analysis of variance comparing the mesh-sizes of the MarineMesh™ at various positions on the seacage after 16 months of deployment.

(see text for further details)

Source of variation	df	MS	F	*P*
Mesh Type = M	1	5.78E-03	3.20E-02	0.859245
Sides = S	3	1.61E-03	8.92E-03	0.998819
Position = P	1	0.554528	3.066306	8.95E-02
M x S	3	2.30E-02	0.12735	0.943193
M x P	1	4.33E-03	2.39E-02	0.878015
S x P	3	1.07E-02	5.90E-02	0.980873
M x S x P	3	4.09E-02	0.877639	8.77639
Quadrats(M x S x P)	32	0.180846	0	0
Residual	432	1.83E-03		
Total	479			

These observations of clean MarineMesh™ and the potentially fouled MarineMesh™ seacage wall have provided evidence regarding the accumulation of biota on the MarineMesh™ over the 16 month period, quantifying the effect of biofouling by observing the reduction in hole size of mesh after fouling.

In order to directly relate this apparent reduction in biofouling of MarineMesh™ to that of traditional nylon seacage netting it was necessary to make further observations and a direct comparison of each mesh type. Three panels of both MarineMesh™ and traditional nylon seacage netting (0.2 m^2 respectively) were tightly stretched and attached to a frame and suspended from the side of the seacage structure under the same conditions, at a depth of two metres, for a period of sixteen weeks. After this period photographs were taken of the panels to assess the degree of biofouling occurring on each mesh type, (see Fig. 5). Using similar methods to those described in

6.1 above, these images were then used to collect further data to examine statistically the difference in biofouling between MarineMesh™ and soft nylon over a sixteen week period of submersion. Statistical analyses of these results are presented in Table 10 and Figure 6.

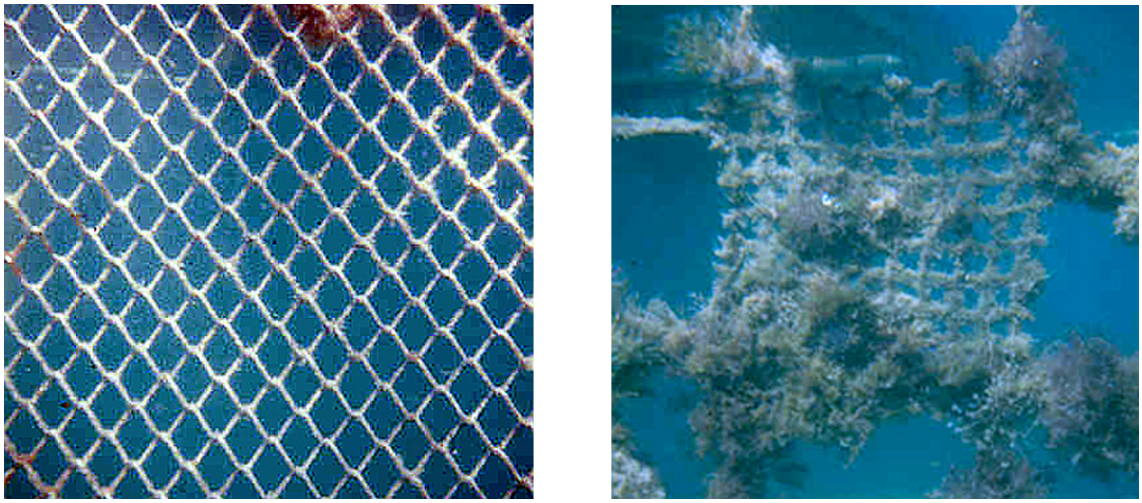

Figure 5. Image showing accumulation of biofouling on MarineMesh™ (left) and nylon mesh panels (right) after sixteen weeks of submersion.

The data obtained was analysed using two factor, nested analyses of variance, with factor 1 (net type, fixed) and factor 2 (frames, nested in net type). Prior to analysis of variance the data for each mesh type were tested for homogeneity of variance using Cochran's test (Underwood 1981). The data yielded heterogeneous variances which, unfortunately, could not be stabilised using a variety of transformations. However, as analysis of variance is robust to heterogeneous variances (Underwood 1998) and there were a large number of replicates, analyses were done using the raw data.

These heterogenous variances are most likely a result of the spatial variation of fouling organisms growing on the nylon net frames. The biofouling on the nylon mesh was comprised of large species of algae with leaves that completely blocked some mesh holes. In comparison the MarineMesh™ had a small amount of biofouling which more evenly covered the wire in a film of biota. Data from each frame of mesh was tested (see SNK test Table10) to establish if any differences could be found among replicate frames of each mesh type. Results indicate significant differences among nylon mesh frames and no significant differences amongst MarineMesh™ frames, suggesting there is far more variability amongst the fouling of nylon mesh. Analysis of variance results indicate a significant difference between Nylon and MarineMesh™ with respect to the reduction in mesh hole size resulting from biofouling.

Table 10. Analysis of variance comparing the mesh-sizes of the MarineMesh™ and soft nylon netting after submersion for16 week a period.

Source of variation	SS	df	MS	F	*p*	F vs
Mesh Type	10597.45	1	10597.45	110.1527	<0.001	Frames(mesh)
Frames (M)	384.8273	4	96.20683	4.599962	<0.001	RES
Residual	1129.394	54	20.9147			
Total	12111.67	59				

Results of SNK test (frames of mesh)

MarineMesh™	frame 1 =	frame 2 =	frame 3
Nylon mesh	frame 1 <	frame 2 =	frame 3

Following the 16 week submersion period of the mesh frames, the mean reduction in mesh-size of nylon mesh was from 36.5 to 5.9 mm, representing a reduction of 81% of the clean mesh size. MarineMesh™ had a reduction in mesh size from 36.5 to 33.1mm, representing a reduction of 9% of the clean mesh size. These results are presented as a % reduction in mesh size for each mesh type (see Fig. 6).

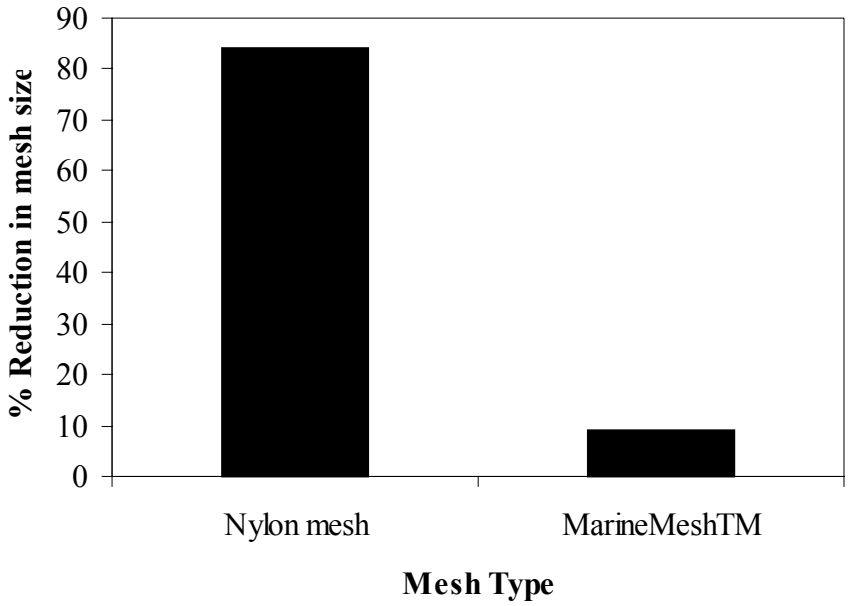

Figure 6. Plot of % reduction in mesh hole size of MarineMesh™ and nylon netting.

6.4. Discussion

This study used a visual, photographic method combined with statistical analyses to assess the reduction of biofouling occurring on the MarineMesh™. These observations provided information for each side wall of the MarineMesh™ seacage. Each side of the cage, (i.e. N, S, E, and W) were stratified into top and bottom regions to possibly detect any difference in the degree of biofouling occurring, possibly affected by factors such as depth or exposure to sunlight. Results clearly showed that statistically there was no significant difference between fouled and clean mesh after the 16 months of deployment. Nor were there any detectable differences between side walls at any depth. This indicates that there was no detectable difference in the degree of biofouling over the study period, using this method of assessment.

Results from a direct comparison of Nylon and MarineMesh™ made over a sixteen week period, clearly show that the MarineMesh™ was far less affected by biofouling organisms than traditional soft nylon netting. The reduction in mesh hole size of nylon netting was significantly greater than that of the MarineMesh™. Visual observations were also made by the author on S.C.U.B.A and from the surface. These casual observations made intermittently throughout the 16-month deployment of the seacage showed that there was little, if any, biofouling of the wire mesh during the first 9 months as evidenced by the still metallic shine of the wire. During the following 7 months, a very thin, algal film became evident in patches on all sides of the cage. While not quantified, the film appeared to reach maximal coverage during late spring, 1999. Soon after the algae appeared to simply fall off with current and wave action.

The fact that the MarineMesh™ seacage was still in operation after 16 months use is significant in determining the functional qualities of the cage. It was necessary for the operator of the fish farm to change and clean the traditional soft mesh net cages used on the farm every 10-28 days, as also experienced by NSW Fisheries (Quartararo, 1996). In terms of biofouling reduction the MarineMesh™ seacages required no maintenance or cleaning over their period of use and were in good condition at the end of this study.

The degree of biofouling a structure will obtain is relative to a number of factors including its exposure to settling biofouling organisms (Lewis 1994). Future observations or studies of MarineMesh™ may determine the biofouling reduction properties to be very different to what was found in this study. However the location of this study site was at the entrance to Botany Bay (see Fig. 3) which is a renowned catchment area for Sydney rock oyster and Mussel spat (Nell 2002) and other molluscs. In addition, the seacage floatation structures used on this farm experience a tremendous amount of biofouling comprising a diversity of organisms. Considering these factors the chosen study site and period of observation this study should provide a sound observation of the typical biofouling that could be expected to occur using MarineMesh™ in a similar temperate marine environment.

7. GENERAL DISCUSSION

7.1. Achievement of Objectives

Objective 1. To identify and test environmental indicators to assess possible increases in zinc (Zn) in the water column and in sediments resulting from the use of MarineMesh™ seacages.

The detection of metals in marine waters and habitats has been investigated in other studies in NSW. Methods currently available to identify environmental indicators were examined to decide the most cost effective and appropriate method to assess possible impacts resulting from the use of zinc in the coated wire mesh seacages. The depletion of zinc is an inevitable process. The rate of depletion of zinc from the wire cages was unknown, but it is likely that the depleted zinc would be dispersed into the water column and/or the sediments beneath the seacages. It is also possible that the fish being grown within the cages may absorb some of the zinc by means of ingestion, whilst grazing on biota growing or residing on the seacage.

Sydney Rock Oysters *Saccostrea glomerata* were chosen as biological indicators to detect and possibly assess the impact of zinc being dispersed into the water column. The possible detection of an increase in zinc in oyster tissues from samples taken beneath the wire cages could indicate two things: (1) that zinc levels within the water column were higher than those in other areas within Botany bay, suggesting that the wire cages or zinc contained within the fish feed had contributed to this increase; and (2) that biota, such as molluscs, residing within close proximity to the wire cages, are affected by this increase of zinc in the water column. This method has been used successfully to determine the levels of metals found in waters surrounding deep-water ocean sewage outlets of Sydney, NSW (Scanes 1996).

An unexpected situation occurred with this component of this study, in that the oysters obtained for the study were already laden with metals including zinc (which is not unusual for bivalves – see Rainbow 1992 for a review). This made the interpretation of results more difficult. However, statistical analysis of results indicated that there was no significant increase in zinc within oyster tissues at the seacage sites compared to the control sites. These results suggest that the amount of zinc depleted from the wire sea-cages was outside the detection limits of this study, and unlikely to cause any environmental concern.

The monitoring of sediments over time has traditionally been used to indicate possible increases in metals polluting aquatic habitats. Previous collections of sediments within the farm lease area and at other sites around Botany Bay had taken place as part of the farm's required environmental assessment program. This provided data for twelve months prior to the introduction of the MarineMesh™ cages. A sampling strategy was devised to collect sediments beneath the wire sea cages prior to their placement on the farm and from other control locations within Botany Bay. Sediments from all locations were analysed and statistical analyses of results revealed no detectable increases in the level of sedimentary zinc beneath the sea cages through time.

Objective 2. To monitor fish being grown within the cages to ensure they are unaffected and safe for human consumption.

To ensure fish grown within the wire sea-cages were unaffected by the depleting zinc and are safe for human consumption with respect to metal contamination, fish tissue samples were collected and analysed. Farmed snapper *Pagrus auratus* grown on the farm site were analysed prior to their placement in the wire sea-cages and periodically thereafter. Results indicated that there was no significant increase in the level of zinc within fish tissues observed in this study. The ANZFA (Australian and New Zealand Food Authority) provide standards regarding the level of zinc allowable in food for human consumption in Australia. The mean level of zinc found within fish grown in the wire sea-cages was less than 1% of the maximum legal limit for safe human consumption.

Objective 3. To identify and test methods to assess the degree of biofouling accumulated on MarineMesh™ when used in a commercial application.

To assess the degree of biofouling accumulated on the MarineMesh™, a method was devised using photographic images of the wire mesh sidewalls of the sea-cage. Biofouling reduces the mesh hole size of the seacage, resulting in a subsequent reduction in water flow through and a possible reduction in dissolved oxygen levels within the cage. Macroscopic observations indicated that the MarineMesh™ had accumulated very little biofouling during the first twelve months of use (see Fig. 5). The assessment of biofouling material and its impact upon mesh size was done after the sea-cages had spent approximately 16 months in the water. This assessment was made by measuring mesh hole size within quadrats (see methods 6.2). Results indicated that no significant differences in mesh size had occurred relative to the mesh size of non-immersed zinc-coated wire mesh after this period.

A direct comparison was also made between nylon and MarineMesh™, results clearly show that MarineMesh™ had a far greater resistance to biofouling than nylon mesh over the sixteen week submersion period. Statistical analysis of these results indicate a significant difference between the fouling of each mesh type (see Table10). MarineMesh™ and nylon mesh had a mean reduction in mesh hole size of 9% and 81% respectively (Fig. 6).

The absence of biofouling on the wire mesh is considered to be a function of the zinc coating. It is likely that the reduction in biofouling has occurred due to the gradual depletion of the zinc coating, which makes the surface of the wire undesirable or inadequate as a site for the attachment and establishment of biota.

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APPENDICES

Appendix 1. Control Site Characteristics

CONTROL SITE 1	
Location	La Perouse in Frenchman's Bay.
Depth	Approximately 6 meters deep.
Sampling location	A commercial mooring is the landmark used to identify the exact location for sampling, being 10meters due South of the mooring block.
Sediment characteristics	Mainly coarse grained sand, consistent for first 5-10 cm of sediment across sampling site.
Biological characteristics	
Flora	Mainly barren with very sparse patches of sea grass *Halophila* Sp. The abundance of which varies as it is affected by heavy wave action and consequent sediment movements.
Fauna	No fish or mammals were sighted during sampling.

CONTROL SITE 2	
Location	Silver beach Kurnell.
Depth	Approximately 5 meters.
Sampling location	The exact sampling location is found by using the alignment of two visual marks being the fourth rock groin off Silver Beach and the sea cage sites 1 to 3 on the most northern front of the fish farm.
Sediment characteristics	Mainly coarse grained sand, some fine sand and very little silt, consistent for first 5-10 cm of sediment across all of Control site.
Biological characteristics	
Flora	Sparse patches of sea grass *Halophila* Sp. The abundance of which varies as it is affected by heavy wave action and consequent sediment movements.
Fauna	Some fish were sighted at this location during sampling.

CONTROL SITE 3	
Location	Bonna point, Silver beach.
Depth	Approximately 6 meters deep.
Sampling location	The exact sampling location is identified by measuring a distance of 237 meters due North from a special navigation mark, parallel to the sea cage site from silver beach.
Sediment characteristics	Mainly coarse grained sand, some fine sand and very little silt, consistent for first 5-10 cm of sediment across all of Control site.
Biological characteristics	
Flora	Mainly barren with very sparse patches of sea grass *Halophila* Sp. The abundance of which varies as it is affected by heavy wave action and consequent sediment movements.
Fauna	No fish or mammals were sighted during sampling.

CONTROL SITE 4	
Location	Towra point at the Southern entrance to the George's river.
Depth	The depth is approximately 4 meters at mean low water.
Sampling location	The exact location is found by the alignment of a number of visual landmarks in three directions around the site.
Sediment characteristics	Mainly coarse grained sand, some fine sand and some silt, consistent for first 5-10 cm of sediment across all Control site.
Biological characteristics	
Flora	Patches of sea grass *Halophila* Sp. The abundance of which varies as it is affected by heavy wave action and consequent sediment movements.
Fauna	No fish were observed at this site during sampling.

CONTROL SITE 5	
Location	Ramsgate beach.
Depth	The depth across the farm site is 7 meters at mean low water.
Sampling location	The exact location for sampling is 10 meters due east of the most northern corner pylon of the baths.
Sediment characteristics	The sediments are mainly fine-grained sand with some silt present.
Biological characteristics	
Flora	Sparse patches of sea grass *Halophila* Sp. The abundance of which varies as it is affected by heavy wave action and consequent sediment movements.
Fauna	No fish were observed during sampling.

CONTROL SITE 6	
Location	Brighten Le-Sands beach.
Sampling location	A large State Emergency Service buoy is used to identify the location for sampling. The exact position is 10 metres due east of the mooring block for the buoy.
Depth	The depth is 6m at mean low water.
Sediment characteristics	The sediments are fine-grained sand and silt.
Biological characteristics	
Flora	The seafloor is baron.
Fauna	No fish were observed during sampling.

CONTROL SITE 7	
Location	Kyeemagh near the entrance to the Cooks River.
Sampling location	The most southern pylon of the swimming baths, the exact position being 10 meters due east of this pylon.
Depth	The depth across the farm site is 5 meters at mean low water.
Sediment characteristics	The seafloor at this location is fine silty sand.
Biological characteristics	
Flora	No sea grasses present.
Fauna	No fish were observed at this sight during sampling.

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