Final Report

Aquafin CRC – FRDC Southern Bluefin Tuna Aquaculture Subprogram:

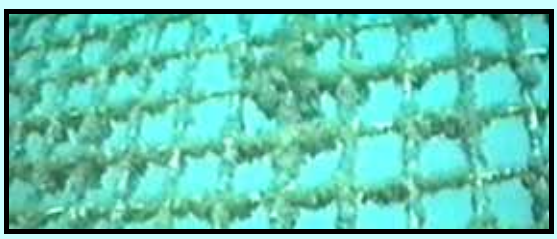
Net Fouling Management to Enhance Water Quality and Southern Bluefin Tuna (*Thunnus maccoyii*) Performance

Kirsten Rough, Rocky de Nys, Maylene Loo and David Ellis

May 2009

Aquafin CRC Project 4.5

FRDC Project No. 2003/226



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OBJECTIVES

1. Document current industry knowledge and methods used to control bio-fouling on nets and associated structures (both physical and chemical means) for various marine finfish species cultured in Australia and overseas.
2. Co-ordinate the tuna industry's approach on antifoul treatments.
3. Review currently available commercial antifoulant products, including the mechanisms by which they reduce fouling and the regulations involved in their use.
4. Determine efficacy (through reduction in fouling growth and impact on net integrity) of antifoulant products identified by objective 3 with net panels in the local environment where tuna are currently ranched.
5. Identify the development pattern of fouling communities on commercial tuna cages that are subject to the current standard industry practices, and relate this to oxygen levels monitored on the outside and inside of these nets.
6. Establish relationship between the percentage cover of fouling communities and water flow, net weight and net drag.
7. Enhance the dissolved oxygen diffusion model to provide predictive capacity for industry to evaluate fouling management systems.
8. Field test the most effective anti-foul treatment identified by objective 4 on a commercial tuna cage with the typical industry regime of tuna stocking density, feeding and net maintenance. Effectiveness of the antifoulant will be assessed utilising methods developed and used in objectives 4 and 5.
9. Test the chemical residue status of tuna and shellfish within the cage and the sediment beneath the net for the treated cage and compare these to tuna, shellfish and sediment of an untreated control.
10. Assess the health status of tuna in the treated cage by comparing it with that in two control/untreated cages (health status incorporates behaviour, mortality, parasite burdens and histopathology).
11. Disseminate results to industry on a regular basis through verbal, written and electronic communication.

NON TECHNICAL SUMMARY

Outcomes Achieved:

This project has greatly increased our knowledge of biofouling, the problems associated with its presence in the sea-cage culture of finfish and the various options utilised throughout the world to manage it. The development of biofouling on the nets of the southern bluefin tuna (SBT) aquaculture industry in Lower Spencer Gulf, South Australia was investigated using small experimental net panels and commercial sea-cages. The effects of net fouling on dissolved oxygen levels and current flow were also assessed.

By coating small experimental net panels with a variety of non-copper based antifouling treatments and sequentially retrieving them through the farming season, we were able to determine the impact that biofouling has on the weight of nets and which coatings were effective at reducing biofouling growth. These net segments were assessed to see if biofouling or antifouling coatings reduced the strength of the net. Also, by deploying small experimental net panels with various amounts and types of biofouling growth in a flume tank, we were able

to demonstrate how net fouling impacts on water flow. Oxygen consumption of various biofouling types and densities was determined in a closed tank. The results of these experiments enabled the development and refinement of a computer model that can be used by farm managers to investigate operational strategies to optimise the production of tuna by enhancing water exchange within sea-cages.

An entire commercial size SBT sea-cage net was coated with an antifouling treatment to appreciate the logistical, handling and regulatory issues associated with using antifouling products, as well as determine whether the treatment affected the tunas' health, accumulated in the tuna flesh or in the environment (bivalve net fouling organisms and seafloor sediments).

The main aim of the 'Aquafin CRC - FRDC Southern Bluefin Tuna Aquaculture Subprogram: Net Fouling Management to Enhance Water Quality and SBT Performance' project was to better understand the impact of net fouling in sea-cage culture, specifically within the South Australian southern bluefin tuna (SBT) farming industry and to investigate antifouling treatment as an option to mitigate these.

Reviews of the international scientific and technical literature on biofouling and sea-cage culture of fin-fish were undertaken. These suggested that biofouling is a significant problem in fin-fish aquaculture world wide. Biofouling adversely effects water quality, water flow, waste accumulation, fish productivity, fish health, and can also cause the deformation of cages and structural fatigue of infrastructure. Biofouling development and the types of fouling communities present can be influenced by the physio-chemical environment (eg. salinity, light, depth, water quality, nutrients), as well as farm practices including the characteristics of the netting (e.g. mesh size, mesh structure and mesh material). The range of currently available antifouling technologies were reviewed, including directions for future research.

The development pattern of the biofouling community in the local environment was determined on two commercial tuna sea-cage nets. The inshore site, with white sea-cage netting, had more diverse fouling assemblages with 14 taxonomic groups present across all depths. The fouling assemblages were dominated by hydroids in autumn, moving to mixed algae and encrusting organisms in winter and climaxing with colonial ascidians at the end of the farm season in spring. The offshore site, with black netting, had less diversity with 9 taxonomic groups; but followed the same developmental pattern through time. Depth differences were apparent, with algae dominating in the shallower depths, and encrusting organisms in the deeper depths of both sea-cages; bivalves were recorded from mid season but were not in high density.

A disruption to water exchange through the net as a result of increased biofouling was demonstrated using the water quality data collected. The dissolved oxygen concentration within the sea-cage decreased as net occlusion increased concurrently with fouling growth.

Small net panels were deployed at sea to grow biofouling for testing in a flume tank. This technique was very effective at establishing the relationship between percentage fouling cover and water flow, and determining oxygen consumption by biofouling communities. Biofouling assemblages and densities both influenced water flow and oxygen consumption.

Low fouling net cover (occlusion 40%) was capable of inducing turbulence if it contained hard shelled invertebrates. Dense fouling net cover (70-80% occlusion) entirely consisting of algae restricted water flow at low current speed. Dense fouling net cover (70-80% occlusion) of encrusting organisms restricted water flow at low and high current speeds. Encrusting organisms were primarily responsible for the dry weight gain of biofouling on nets. Oxygen consumption rates were influenced by the amount and type of biofouling.

A computer model "OxyTuna" was developed to assist farm managers in making better decisions about fouling management of fin-fish cage systems, particularly the relationship between net fouling and dissolved oxygen concentration in the sea-cage and its response to intervention (e.g. net cleaning). The model provides a quantitative prediction of changes in dissolved oxygen concentration through time for different sea-cage configurations (cage size, net type, stocking density and fish species) in response to changes in ambient conditions (temperature, salinity, ambient oxygen concentration and current speed). The dynamic nature of the model allows users to better understand the interplay of factors that control dissolved oxygen concentration in a sea-cage and is therefore useful as both a management and teaching tool.

The project tested three types of antifouling agents Lanolin (Lanotec™), latex with booster biocide Sea-nine 211 (Net Clear™), and a paint containing the heavy metal zinc oxide with booster biocide zinc pyrithione (Net Clear ZPT™) on panels of netting in the local environment. None of these totally prevented the development of fouling, but both the latex and paints, Net Clear and Net Clear ZPT, were significantly effective at delaying the onset of and reducing the overall amount of biofouling at depths of 2 and 9m, compared with untreated small experimental net panels. Also, coating net panels with Net Clear and Net Clear ZPT was found to maintain or improve the tensile strength of the netting irrespective of deployment time or depth. Lanotec significantly reduced the breaking strain of the net mesh compared with new and untreated net mesh.

Applying an antifouling treatment, Net Clear ZPT, to an entire commercial size sea-cage net, with the sea-cage stocked with commercial quantities of SBT, was logistically challenging. Constraints encountered in this project such as humidity delaying paint application, as well as the delays and costs associated with transporting nets to the dipping site in Tasmania would be largely resolved if a dipping site was established in South Australia. Once the coated net was in Port Lincoln it did not require any alteration to normal industry practice. The use of Net Clear ZPT, which is zinc based, on the commercially stocked sea-cage net did not result in elevated levels of zinc within SBT muscle or skin tissue, nor in shellfish on the net, or the sediment under and surrounding the sea-cage. All zinc residue testing results from SBT flesh were within the range of values found naturally in wild SBT and those farmed without the use of any antifouling treatment. Results from shellfish samples collected from the treated net were not significantly different from those of the untreated control net, and sediment values in the vicinity of both nets were comparable to those of a survey of the SBT farming zone in 2002 (prior to this antifoulant experiment).

SBT within the treated commercially stocked sea-cage net did not display any adverse behaviour, and 24% less mortality was recorded compared with the untreated control net sea-cage. Specific SBT health tests, including parasite checks, histopathology and haematology demonstrated no detrimental effect on SBT contained in a sea-cage with a Net Clear ZPT treated net.

There were indications that the SBT in the treated net sea-cage had better food conversion ratios, but these facts need to be validated through further replicated trials.

An economic analysis comparing the use of antifoulant treated nets as compared to untreated nets demonstrated that the use of antifouling compounds was beneficial to the SBT industry.

This research provides the foundation for further development and adoption of antifouling technologies.

Keywords: Tuna aquaculture, southern bluefin tuna, biofouling, antifouling, oxygen, water quality, water flow.

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* The tuna industry
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BACKGROUND

In the marine environment fouling organisms have the potential to significantly impact on farming operations, because:

1) fouling increases the weight that farming structures have to support when in the water. For example, a tuna industry farming net from a 40m diameter sea-cage with a depth of 10m will weigh approximately 1200 kilograms when placed in the water and after 6 months the fouling will have increased the weight of the net approximately 30 fold. This increase in weight places a significant stress on farming structures. Cleaning nets is a significant operating expense and OHS&W issue.

2) fouling affects the sea-cage integrity by causing the net to hang deeper in the water. The result of the net hitting the seafloor or stirring up sediments is deterioration of water quality, and high ammonia and low dissolved oxygen potentially negatively effect production.

3) fouling clogs nets and reduces water exchange from the surrounding waters into the sea-cage farming environment potentially leading to reduced dissolved oxygen in the vicinity of the tuna, which can again potentially negatively affect production. Suspended sediments trapped within net fouling can also affect water quality when disturbed by rougher weather conditions.

4) fouling clogs the nets and increases net drag, which potentially places increased stresses on moorings and negatively effects net shape. If this is addressed by adding more net weights then infrastructure costs are increased.

5) fouling on the net can harbour pathogens and enhance conditions for their survival and ability to infect fish. *Uronema*, one of the few diseases causing tuna mortalities on farms at present, is an example of such a pathogen.

6) as net changes are difficult in large tuna farm operations, the build-up of net fouling under current farming conditions makes longer-term holding of tuna a more problematic undertaking.

From these points it is clearly evident that marine fouling can significantly affect aquaculture production. As a result, marine finfish farmers attempt to minimise the impact of fouling by regular cleaning or complete changing of structures used for farming. This can have a stress effect on the farmed fish and therefore anti-fouling coatings have been developed to deter or eliminate fouling organisms. In the past, the coatings used in marine operations have raised concerns about residues in the fish and in the general environment. These coatings have been primarily copper based and there is now a global shift away from this type of anti-foul treatment in farming operations. Wattyl Pty Ltd has developed new anti-foul treatments which are not copper based and one of these recently (2004) became available for full commercial trials.

The tuna farming companies, M.G. Kailis Pty Ltd and Stolt Sea Farm Pty Ltd, performed small-scale trials in 2002 to evaluate a range of anti-foul treatments. The Kailis trial identified one treatment new to the market that appeared to perform well under experimental conditions and is not copper based. Similar treatments were used on small pontoons on the research farm in the year 2002 and the product showed promise (Svane et al, 2006). Since this trial, the product has been improved and is reported to be more suited to the local Port Lincoln environment (Hodson pers.comm.).

The trial performed on the SARDI Tuna Research Farm in 2002, reported by Svane et al (2006) demonstrated that the treatment reduced fouling by 14% on average for three old nets.

However, even a change as small as this can be significant if the output of a preliminary dissolved oxygen model developed by Prof. Anthony Cheshire, SARDI, proves correct.

It should be noted that there were significant differences between the trial performed on the SARDI Tuna Research Farm and the proposed trials performed in this project and are as follows:

1) The Research Farm Trial (RFT) was performed in the sheltered waters of Boston Bay where water temperature and fouling communities are quite different from those of the zone east of Boston Island where tuna farming now occurs.

2) The age of the nets was between 2-10 years.

3) Product development has progressed through 2002 and 2003 so that release rates and level of activity are more suited to the fouling communities of Spencer Gulf.

Whilst the research farm demonstrated that there was potential in the use of anti-foul coatings, the next step was to trial the best of all currently available products in a rigorous and scientific manner to better quantify and qualify the impact of fouling on a tuna farm.

NEED

For tuna ranching to continue to develop it must improve the farming environment by providing optimum water quality to the tuna. This will improve the performance of farming operations and deliver quality products to the market and ultimately maintain Australian farmed tuna's competitive edge.

Also the next major step in the industry's sustainable expansion strategy is longer term holding (eg. 15 months). The above planned outcomes of the anti-foul project are important prerequisites to successful long-term grow out.

The need for this project is quite obvious. If the culture environment is improved by the use of an antifoul coating, more than likely the following will occur:

- Increased water flow through the nets
- Reduction in weight on farming structures
- Reducing the re-suspension of sediments during rough weather
- Reducing surface area for potential pathogens
- Improving net handling techniques
- Potential to increase longevity of nets
- Reduce or eliminate the need for diving to clean equipment.
- Improve cage integrity.

This project aimed to integrate and coordinate the industry's approach on anti-foul treatments and ensure this meets with regulatory requirements. Furthermore, it was necessary to find out the efficacy of anti-foul treatments by monitoring key biological and farm husbandry parameters through trials on commercial farms. As mentioned previously, the research farm identified that the single product tested showed promise, but this trial only tested the product efficacy, and did not include fish health or measure environmental impacts (eg. residues). This product and others were further developed and the formulations were improved by the manufacturer, after field trials were undertaken through 2002 and 2003 by the M.G. Kailis Group in lower Spencer Gulf. These altered formulations to improve performance in Spencer Gulf need to be tested in the current tuna farm environment.

Economically it was important to find out how long a single treatment would provide a reduction of fouling organisms under the current operating procedures of the industry. For example, whether nets need to be treated every season, or whether one treatment would decrease fouling over two seasons. The cost to treat a net is significant but if the objectives are achieved then the benefits should outweigh the costs.

There was a need to provide confidence that the active constituent found in the anti-foul treatment is not absorbed by the farmed tuna, is not found in the sediments and is not taken up by other marine organisms that are located nearby.

OBJECTIVES

1. Document current industry knowledge and methods used to control bio-fouling on nets and associated structures (both physical and chemical means) for various marine finfish species cultured in Australia and overseas.
2. Co-ordinate the tuna industry's approach on antifoul treatments.
3. Review currently available commercial antifoulant products, including the mechanisms by which they reduce fouling and the regulations involved in their use.
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11. Disseminate results to industry on a regular basis through verbal, written and electronic communication.

Chapter 1 : SUB-PROJECT 1: IMPACTS OF BIOFOULING ON MARINE FINFISH AQUACULTURE

This chapter was authored by Bronwyn Houlden (James Cook University) and may be cited as:

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Note:

This report is based on an original review of the impact and control of biofouling by Dr. Stephen Hodson in 1998. The original document has been re-written and updated and has been submitted as a manuscript to the journal Aquaculture with the authorship de Nys R, and Guenther J.

This report is part of a review series of biofouling in aquaculture including reviews of 'Legislation and control of antifouling chemicals in aquaculture in Australia' and 'Methods and efficacy of biofouling control in sea-cage aquaculture'.

ABSTRACT

We review the impact of fouling of netting and cages in finfish aquaculture. The large surface area and structure of netting material, particularly multifilament mesh, is highly suitable for colonisation and growth of fouling. Furthermore, fouling growth is often rapid because the waters surrounding mariculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and excretory material) generated by high-density fish populations. Biofouling of fish-cage netting is a significant operational problem for mariculture. The occlusion of mesh and the resulting restriction in water exchange, can adversely affect fish health by the reduction in dissolved oxygen (DO) and potentially by accumulation of metabolic ammonia.

Fouling is of further concern because it significantly decreases cage flotation, increases structural fatigue and cage deformation, and may act as a reservoir for pathogens. The impacts of fouling vary dramatically depending on season and location, and are also influenced by farming methods and practices. The impacts of these factors are reviewed and highlighted. The overall outcome is that there are few published comprehensive quantitative studies of fouling or its impacts on sea-cage aquaculture, and this significantly impairs the ability to develop the most appropriate mitigation strategies to control fouling.

INTRODUCTION

The finfish aquaculture industry

Principal sectors

Over half of the total world aquaculture production in 2002, worth US$59.9 billion, was attributable to finfish (25.7 million tonnes, US$31.9 billion) (FAO, 2004). Of this the major

share of the total world finfish crop was freshwater carp (16.7 million tonnes), which were produced and consumed mostly in China and India (FAO, 2004).

Finfish dominated 2002 aquaculture production in freshwater (96.9 %), are a major sector in brackish water (43.2%), and constitute 9.4% of mariculture (FAO, 2004). In 2002, freshwater fish production (21.9 million tonnes) was valued at US$21.3 billion, diadromous fish production (2.6 million tonnes) was valued at US$6.5 billion, and marine fish production (1.2 million tonnes) was valued at US$4.1 billion (FAO, 2004).

In Australia, finfish aquaculture production is small on a global scale, but is a major component of the total aquaculture production. Finfish production was valued at A$437.8 million in 2002/03 (O'Sullivan & Savage, 2005). Finfish aquaculture is based principally on sea-cage culture of three species, Southern bluefin tuna (*Thunnus maccoyii*) and Yellowtail kingfish (*Seriola lalandi*) in South Australia, and Atlantic salmon (*Salmo salar*) in Tasmanian waters. These species (industry sectors) accounted for approximately A$377.2 million, or 86.2% of finfish aquaculture in 2002/03(O'Sullivan & Savage, 2005). Southern bluefin tuna are wild-caught under a quota system, and cage reared (ocean ranched) for 3-5 months off Port Lincoln, for the sashimi market in Japan. This is the valuable aquaculture sector in Australian, at A$255.6 million in 2002/03 (O'Sullivan & Savage, 2005). The second most valuable Australian industry is Atlantic salmon, worth A$117.5 million in 2002/2003 (O'Sullivan & Savage, 2005). During that year, over 14,000 tonnes of Atlantic salmon, produced mostly in Tasmania, were consumed primarily by the domestic Australian market, with a small export market to Japan, Indonesia, Hong Kong and Singapore (FAO, 2004; O'Sullivan & Savage, 2005). Aquaculture of yellowtail kingfish is in its infancy in South Australia, but the industry is expanding rapidly. In 2002/2003, production reached 225 tonnes, worth A$4.1 million (O'Sullivan & Savage, 2005).

Australian aquaculture is relatively small on a global scale. Production figures for 2002 demonstrate that the marine salmonoid aquaculture is dominated by Norway (548,992 metric tonnes, US$1,142 million), Chile (478,812 metric tonnes, US$1,450 million), United Kingdom (especially Scotland) (146,698 metric tonnes, US$442 million), and Canada (127,621 metric tonnes, US$321 million). The industry is also valuable in Ireland (24,119 metric tonnes, US$75 million), United States (12,734 metric tonnes, US$28 million), Japan (8,023 metric tonnes, US$36 million) and New Zealand (6,989 metric tonnes, US$17 million) (FAO, 2004). The principal salmonoid species reared in mariculture (sea-cages) are Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*).

Farm practices

Mariculture is undertaken in enclosed natural lochs, fjords or bays (enclosures), in pens (man-made structures enclosed on all sides with the bottom formed by the seabed pens) or cages (man-made structures enclosing all submerged surfaces) (Beveridge, 2004). The size of facilities ranges enormously, but enclosures and pens are larger (0.1 ha to > 1000 ha) compared to cages (1 m^2 to 1000 m^2), and come in four basic designs: fixed, floating, submersible and submerged (reviewed in Huguenin & Ansuini, 1978).

Cages for intensive commercial finfish culture are typically multifilament netting-bags suspended from a floating frame. Circular cages of 40 to 70m circumference are the most common design (Beveridge, 2004), but larger 80 to 120m cages are used for salmon culture in Australia (Isles, 1998; Douglas-Helders *et al.*, 2003), 90m to 120m cages for salmon culture in Norway (Guldberg *et al.*, 1993) and 125m to 160m cages for tuna culture in Australia (Cronin *et al.*, 1999). Square cages are also frequently used, and are produced commercially in a range

of sizes from 6m² to 25m². The depth of cages is limited by cage diameter, depth of the farm site, and ease of maintenance. The depth of fish cages ranges from just 2m (Lee *et al.*, 1985) to 20m (Hodson & Burke, 1994). Deeper cages (10-15m) are typical for large-scale finfish culture.

The stocking density of cages is dependent on the cultured species, cage size and environmental conditions. In Australia for example, Atlantic salmon are cultured at 10-15 kg/ m³ (eg. 12,000 x 2.5 kg salmon in a 65m cage) and southern bluefin tuna at 4 kg/ m³ (eg. 2000 x 23 kg tuna in a 125-160m cage[1]). Given the intensity of these aquaculture practices, it is evident that farms using a high number of cages are required to manage a significant volume of enclosed water and large populations of fish. Good husbandry techniques are required to maintain optimum culture conditions, and protect such a sizeable monetary investment. In particular, a high standard of water quality must be maintained by water exchange, which is dependent on water current, and which in turn is influenced by salinity, temperature and topography of the site.

Impact of fouling

Water quality and water exchange are strongly influenced by fouling on all submerged marine structures. Fouling of sea cages leads to the occlusion of netting mesh, and the resulting changes in water flow adversely affect water quality, limiting oxygen availability and waste metabolite removal (Hodson & Burke, 1994). Fouling also increases the risk of disease in the farmed stock. Fouling organisms can act as a reservoir of *Neoparamoeba pemaquidensis*, which causes amoebic gill disease in Atlantic salmon in Tasmania (Tan *et al.*, 2002; Douglas-Helders *et al.*, 2003), and harbour the toxin responsible for "netpen liver disease" (Andersen *et al.*, 1993; Kent *et al.*, 1996). The fouling biota can also harbour the intermediate stages of the metacestode *Gilquinia squali* which causes eye disease in farmed salmon (Kent *et al.*, 1991). Finally, fouling can cause deformation and structural fatigue of sea-cage nets, as a consequence of decreased mesh size and increased mesh surface area and weight (Milne 1975a). Current forces on fouled nets are over 12 times those of clean nets (Milne 1975a). The changes to water flow can distort the cage shape and decrease cage volume, and increase stress on the cage collar and moorings. The increased biomass can lead to net failure (Huguenin, 1975; Buchanan, 1977), and makes net changing cumbersome and onerous.

The control of fouling incurs a major cost to the aquaculture industry, since net cleaning is labour-intensive and capital-expensive, and disruptive to the fish. The frequency of net cleaning is site dependent. In the absence of chemical antifoulants, Tasmanian nets used in the Atlantic salmon industry must be removed and cleaned every 5-8 days in summer (Hodson *et al.*, 1997). In Japan, the average interval is 14 days (Milne 1976), whereas in Norway, net may be changed only a few times a year (Moller, 1979). The frequency of net cleaning is ultimately dependent on the rate at which the biofouling community develops, and this is influenced by local environmental factors.

ECOLOGY OF BIOFOULING

Community composition and temporal variation

The ecological progression of biofouling in marine environments is universally applicable to submerged surfaces, and is well understood (reviewed in Little, 1984; Wahl, 1989;

[1] Note this figure was calculated from one tuna cage in 1995; from the year 2000 to 2006 the average weight of sbt stocked into cages was 17.39 kg (Australian Fisheries Management Authority, from Trade Information Data supplied by the Conservation Commission for Southern Bluefin Tuna)

Maki, 1999; Holmström & Kjelleberg, 1999). An organic conditioning film composed of proteins, proteoglycans and polysaccharide compounds precedes bacterial adsorption (Loeb & Neihof, 1975; Lewin, 1984). Within hour's bacteria settle, and irreversible adhesion and growth occurs on the solid surface. This ultimately leads to formation of a macroscopic slime film (Wahl, 1989). Within days or weeks, diatoms and spores of macroalgae and protozoa colonise the surface. After a further 2-3 weeks, larvae of the macrofoulers including tunicates, coelenterates, bryzoans barnacles, mussels, and polychaetes settle and metamorphose (reviewed in Holmström & Kjelleberg, 1999). Thus, fouling involves organisms from nearly every invertebrate phylum.

The development and composition of fouling communities on fish cages have been described for many types of mariculture in a number of countries, including Scotland (Milne 1975a, b), Australia (Cronin, 1995; Hodson & Burke, 1994; Cronin *et al*., 1999), China (Chengxing, 1990), India (Santhaman *et al.*, 1983), Japan (Kuwa, 1984), Malaysia (Cheah & Chua, 1979; Lee *et al.*, 1985), Tanzania (Bwathondi & Ngoile, 1982) and the USA (Moring, 1973; Moring & Moring, 1975). There are also some studies of cage fouling in freshwater ponds and lakes (eg. Pantastico & Baldia, 1981; Greenland *et al.*, 1988; Dubost *et al.*, 1996).

Multi-filament netting material is an ideal surface for fouling, and the succession of organisms that colonise aquaculture netting has been evaluated specifically (Milne 1975a, b; Hodson & Burke, 1994). Generally, macroalgae are the most serious type of fouling on cages immersed for short periods (< 1 month) (Milne 1975a, b; Hodson & Burke, 1994), The dominant macroalgae reported on fish cages include *Gracilaria* sp. (Cheah & Chua, 1979), *Ulva* spp. (Moring & Moring, 1975; Cronin, 1995; Cronin *et al*., 1999), *Antithamnion* sp. (Hunter & Farr, 1970) *Enteromorpha* spp. and *Ectocarpus* spp. (Milne 1975a, b; Wee, 1979).

In general, bivalves and ascidians are predominant on cages immersed for longer periods (Milne 1975a, b), but can also cause significant fouling in short periods, particularly during times of high larval settlement (Sutterlin & Merrill, 1978). Bivalves reported as major net-cage foulers include the wing shell *Electroma georgiana* (Cronin *et al*., 1999), the mussels *Mytilus edulis (*Koops, 1971; Milne 1975a, b; Moring & Moring, 1975; Paclibare *et al.*, 1994), *Modiolus* sp. and *Perna viridis* (Cheah & Chua, 1979; Lee *et al.*, 1985), and the oysters *Crassostrea* spp. and *Pinctada* spp. (Cheah & Chua, 1979; Bwthondi & Ngoile, 1982).

The major fouling ascidians include solitary species such as *Styela picata* (Chengxing, 1990), *Ascidiella aspersa* and *Ciona intestinalis* (Milne, 1975b), and colonial genera including *Botryllus, Botrylloides, Symplegma* and *Tridemnum* (Cheah & Chua, 1979). Significant mesh occlusion by filamentous (tube-dwelling) diatoms has also been reported (Moring & Moring, 1975; Hodson & Burke, 1994).

Spatial variation

1. Between sites

Studies of fouling on mariculture netting revealed spatial variation over a wide geographical range, with test sites located in Scotland (Milne & Powell, 1967; Milne, 1969, 1970, 1975a, b), Hawaii (Rothwell & Nash, 1977), Hong Kong (Tseng & Yuen, 1979; Mak, 1982), Maine and Massachusetts (Huguenin & Ansuini, 1975, 1978). Spatial variation may represent differences in environmental conditions (Santhanam *et al.*, 1983) or abundance of larval stages (Bwathondi & Ngoile, 1982), as discussed below.

Salinity

Fouling communities on polyethylene netting differ between cages immersed in brackish and marine waters. Cages in brackish water (24.5 - 33.8 ‰) were colonised by the algal genera *Enteromorpha* and *Ectocarpus*. However, cages in marine conditions were colonised by bivalves (*Avicula vexillum*, *Dasychone*, *Crassostrea madrasensis*, and *Pinctada* sp.), sea anemone, solitary and colonial ascidians, algae (*Caulerpa* spp, *Codium* sp. and *Gracilaria* sp.), amphipods (*Corophium* spp.), barnacles (*Balanus amphitrite variegatus*), and polychaetes (*Serpula* sp.) (Santhanam *et al.,* 1983).

Abundance of larval stages

Bwathondi and Ngoile (1982) found different age classes of bivalves fouling fish cages, and identified the frequency and time of settlement of different species. They identified eight age groups of an *Ostrea* sp., four groups of a *Pinctada* sp. and three groups of *Pinctada vulgaris* on cages immersed for 103 days. The number of individual per age group was dependent on environmental conditions, and greater settlement of *Ostrea* sp. occurred during spring tides (the time of greatest plankton abundance), and greater settlement of *Pinctada* spp. occurred with high rainfall.

Haegele *et al.,* (1991) recorded the abundance of fouling invertebrates at numerous sites, and at various depths within sites, at salmon farms in British Columbia. Mussels, isopods and pycnogonids were frequently observed, but their abundance varied greatly between sites on different sampling dates. Further, species such as polychaetes that occurred in low abundance were only found at a few sites.

2. Within site variation

Light and depth

Fouling mass and species diversity have been found to vary between sides of cages at the same depth, and this microenvironment difference is directly related to light intensity. Cronin *et al*., (1999) found the southern side of a tuna cage (which received direct sunlight) had a greater photosynthetic biomass than other sides, and the highest total biomass over most depths. Variation between cage sides is only detectable near the surface where light intensity differences are most pronounced, to the extent that variation in fouling that was significant at 0.5 m was not significant at a depth of 2.0 m (Moring & Moring, 1975).

Significant differences between sides were also noted for specific organisms. Ascidians comprised a greater proportion of the community on the sunny southern side than any other side, and rhodophytes were most abundant on the southern side (Cronin,1995; Cronin *et al*., 1999). In contrast, bryozoans were least abundant on the southern and western sides (Cronin,1995; Cronin *et al*., 1999). Lee *et al.,* (1985) observed significant differences in the mass of algae and invertebrates between cage sides, and the two faces that had the greatest mass of bivalves *(Modiolus* spp and *Perna viridis)* had the lowest mass of marine worms and algae.

Reduction in light intensity also causes significant variation in species diversity and abundance between depths. Overall, fouling mass decreases significantly with increasing depth (Moring & Moring, 1975). The upper portion of fish cages are fouled with *Ectocarpus* spp., *Enteromorpha* spp. and other algae, whilst bivalves including *Mytilus edulis, Electroma georgiana*, oysters, hydroids and amphipods predominant at lower depths (Wee, 1979; Santhanam *et al.,* 1983; Cronin, 1995; Cronin *et al*., 1999).

Increased fouling growth around the top of cages, particularly of algae, is also shown by measurements of mesh occlusion. Fukuda *et al.,* (1965) reported fouling growth and mesh occlusion increased with distance above the base of a cage. Haegele *et al.,* (1991) reported a gradual decrease in mesh occlusion from 50% to 10%, over 0.3 to 9.1 m depth. Consequently, restriction in water exchange and the associated degradation in water quality are also likely to vary with depth, and could result in aggregation of the fish at specific depths to avoid unfavourable conditions (Gormican, 1989).

Orientation

The orientation of submerged surfaces affects fouling development, and significant differences occur between vertical and horizontal substrates (Harris & Irons, 1982). For example, Lee *et al.,* (1985) found a greater mass of bivalves on the bases, rather than the walls, of 2 m deep cages. To some extent these observations reflect a change in fouling with depth, but they also represent an orientation effect. This was demonstrated in a comparison of vertically and horizontally mounted net panels. The vertical panels were fouled more rapidly, developed a greater mass of fouling, and had increased abundance of compound ascidians and tubeworms (Cheah & Chua, 1983). However, barnacles and oysters were more abundant on the horizontal frame (Cheah & Chua, 1983).

The increased mass on the vertical panel was thought to reflect a greater interception of horizontally moving planktonic larvae and thus increased larval settlement. However, it is also likely that an increase in collisions with suspended material would increase nutrition of filter-feeding organisms. Communities on horizontal surfaces are subject to greater siltation and predation than vertical surfaces, and upright or mounding species are favoured. However, colonial growth is more effective on vertical surfaces where competition for space is critical and predation pressure is less (Harris & Iron, 1982).

Variation in fouling composition has been observed between cages and between the outer and inner surfaces of cages (Bwathondi & Ngoile, 1982). After 103 days immersion of two adjacent 0.5 m³ cages, 9 groups of bivalve species were identified, with 672 bivalves on one cage and only 315 on the other. In addition, the relative abundance in numbers of individual bivalve were recorded, and more individuals were found growing on the outer (503) than inner surfaces (169) of a cage. This effect was principally due to the preferential settlement of *Ostrea* spp. on the outside of the cage, but the significance of both observations is impossible to evaluate given the limited (n=1) sampling design (Bwathondi & Ngoile, 1982).

DYNAMICS OF BIOFOULING

Water quality and nutrients

Fouling growth is often rapid because the waters surrounding mariculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and excretory material) generated by the high-density fish populations (Gowen & Bradbury, 1987; GESAMP, 1991). The increased carbon, nitrogen and phosphorus levels in the waters immediately surrounding mariculture farms favour the growth of annual filamentous algae (Rothwell & Nash, 1977; Ruokolahti, 1988). The rapid fouling growth in the nutrient enriched waters of Pearl Harbour resulted in the complete blockage of netting mesh within 2 months, whereas the majority of panels immersed at 2 sites with minimal nutrient enrichment had only 0-10% blockage after 3 months immersion (Rothwell & Nash, 1977). In fact, the growth of algae around fish farms has spurred the commercial integration of seaweed culture in marine aquaculture systems (reviewed

in Chopin *et al.*, 2001), and this development has the potential to mitigate many of the environmental impacts caused by mariculture operations (Neori *et al.*, 2004).

Netting characteristics

Fouling of mariculture structures differs from that of many other marine industries in terms of surface characteristics, which are typically rough, non-toxic, and rarely coated with antifoulants. They are also not subject to the high water velocities associated with ship hulls or the internal surfaces of pipes. Early studies of fouling on mariculture netting showed netting material and mesh size to significantly affect fouling rate, mesh occlusion, and density and abundance of fouling species (Milne & Powell, 1967; Rothwell & Nash, 1977). From this data, and observations of mesh deterioration, materials were rated for their suitability in the construction and maintenance of fish cages. More recently, the effects of net angle (Cheah & Chua, 1983) and of microfouling development on multi-filament mesh (Hodson & Burke, 1994) have also been investigated.

Effect of mesh size

A variety of mesh sizes are employed for commercial finfish culture, ranging from 12-40 mm for salmon cages, through 60-90 mm for bluefin tuna cages to 100-150 mm for predator fences. The larger meshes are often of thicker gauge, but generally the smaller the mesh size the greater the surface area per m². Consequently, smaller meshes typically support a greater number of fouling organisms and total biomass (Milne, 1975a; Cheah & Chua, 1983). Cheah and Chua (1983) found the rate of fouling, mass of fouling, species diversity and species abundance to increase with a decrease in mesh size. For example, mesh sizes of 38 mm, 25 mm and 13 mm were fouled by 1, 3 and 5 species of colonial ascidian respectively. Small mesh sizes are also blocked by a relatively low mass of fouling, whereas larger mesh material (>50 mm) can support large fouling communities but maintain a significant open area (Milne & Powell, 1967). Consequently, to maintain acceptable water exchange small mesh nets must be cleaned far more frequently than larger meshes (Cheah & Chua, 1983).

Small mesh netting (15 mm) is particularly prone to accumulation of suspended sediment, and often has significantly less fouling for this reason alone (Mak, 1982; Lai *et al.*, 1993). In contrast, Cheah and Chua (1979) found high silt loadings on nets provided an excellent substrate for settlement and growth of fouling, particularly *Gracilaria* species. The accumulation of sediment due to the size of the netting is exacerbated by the rough surface of multifilament mesh.

Comparisons between different mesh sizes are affected by twine thickness because this changes the total surface area. Mak (1982) quantified fouling on mesh panels after 3, 6 and 9 months immersion, and found 25 mm and 50 mm multifilament meshes supported a greater biomass than 9 mm, 63 mm and 88 mm single-filament meshes. Tseng and Yuen (1979) found no significant difference in fouling mass on 50 mm, 38 mm, 20 mm and 19 mm mesh nets, which were woven from 36, 27, 9 and 4 filaments, respectively. Thus, mesh size and total surface area interact to influence biofouling development.

Whilst short-term fouling development (< 3 months) appears dependent on available surface area, long-term fouling mass (particularly of filter-feeding invertebrates) is dependent on the area in which the organisms can expand and feed. That is, smaller meshes supported the greatest fouling biomass after 3 months immersion, but larger meshes supported the greatest biomass after 9 months immersion (Mak, 1982). These communities are dominated by invertebrates and more than 75% of the 9-month community was composed of solitary ascidia.

Similarly, Milne (1975a) found large mesh sizes eventually developed mussels of a greater size than small mesh, and suggested that the water flow through larger mesh improved feeding. Thus, large mesh netting ultimately has a larger carrying capacity for biofouling communities.

Effect of mesh structure

The microtopography of multi-filament netting affects the distribution and type of initial fouling (Hodson & Burke, 1994). The cylindrical shape of mesh bars leads to differences in light intensity between the upper and lower surfaces of bars immersed horizontally. Consequently, horizontal bars develop a community dominated by phototrophs (eg. diatoms) on the upper surfaces, and heterotrophic protozoan communities on the lower surfaces (Hodson & Burke, 1994).

The large crevices and many filaments of the netting are likely to aid colonisation, either through entrapment of suspended material or because larvae of some fouling invertebrates, and spores of common fouling organisms such as *Ectocarpus* spp. and *Enteromorpha* spp., preferentially settle in small depressions (Crisp, 1984). The use of monofilament netting would obviously reduce problems associated with crevices, but it has significantly less strength than multifilament mesh. Furthermore, monofilament nets must be constructed with knots at the mesh intersections, which results in increased abrasion damage to nets during on-shore handling and increased abrasion of fish during culture.

Fouling development on netting is influenced by the 3-dimensional structure of mesh. Preferential colonisation at mesh intersections has been noted in many studies (eg. Milne, 1975a, b; Rothwell & Nash, 1977; Tseng & Yuen, 1978). Milne (1975a, b) observed that mussels developed large aggregations at intersections, and Tseng and Yuen (1978) reported bryozoans, barnacles, and green algae primarily occurred at knotted intersections. This preferential settlement presumably results from the greater surface area and changes in turbulence at these regions. Milne (1975b) also noted that the netting structure led to entanglement of drifting algae. This type of fouling can quickly block netting, because it is entangled rather than directly attached to the surface.

Effect of mesh material

A number of materials are suitable for the construction of fish cages, and these have varying degrees of fouling resistance. In this regard several studies have demonstrated the relative performance of many types of netting: multifilament-polymer mesh, extruded polymer mesh, metallic hardware cloth, and extruded metallic mesh (eg. Milne & Powell, 1967; Milne, 1969; Rothwell & Nash, 1977). Milne and Powell (1967, 1970) compared 10 mesh types at 4 sites in Scotland, and found polymer-fibre nets were the most susceptible to fouling and galvanised meshes the least. After 4 months immersion growth of mussels (*Mytilus edulis)* completely blocked polymer-fibre netting and the weight of test panels (0.4 m^2) had increased from 5.5 kg (clean) to more than 15.5 kg. In comparison, reasonable water flow still occurred through galvanised materials, and panel weight had increased from approximately 7 kg to 9 kg.

Nine types of netting were assessed in a 6-month trial at 3 locations in Hawaii (Rothwell & Nash, 1977). Netting panels were compared to determine time interval before cleaning and by the total fouling mass after 5 months. Nylon and polyethylene meshes were found to foul at a significantly greater rate than metal meshes, and after 5 months polyethylene mesh had the greatest fouling and galvanised mesh the least (Rothwell & Nash, 1977). The composition of the fouling community also differed between mesh types. Initially algae colonised the majority of net types, but became most abundant on nylon netting and netting with an ineffective

antifouling paint. After 5 months, serpulid tubeworms were abundant on all panels, but were least prevalent on extruded polymer mesh and PVC-coated chain-link, on which barnacles were abundant (Rothwell & Nash, 1977).

The colour of the mesh netting strongly impacts biofouling dynamics. White netting had significantly more fouling than black netting, as a consequence of preferential fouling by algae (Hodson *et al*., 2000).

Fouling composition and biomass

Fouling communities on cages are often characterised by a large biomass. For example, a 4-month old fouling community had an almost identical species composition to a 2-month old community, but had double its weight (Cheah & Chua, 1979). Wee (1979) quantified biomass change over time, and found an increase from 1.85 kg/ m² to 2.84 kg/m² and 4.98 kg/m² after 52, 77, and 106 days immersion respectively. Biomass in the range of 1-5 kg/m² (wet weight) is typically reported (Lee *et al.*, 1985; Chengxing, 1990; Cronin, 1995), although one study showed that 58% of the total fouling mass of 4.5 kg/m² was silt (Lee *et al.*, 1985). This degree of fouling constitutes a significant load since a mean biomass of 4-5 kg/m² on 90 m circumference net tuna cage would equate to a total mass of 6.5 tonnes (Cronin, 1995).

Atypical and very large values for biomass production have also been reported. Rothwell and Nash (1977) reported a total fouling mass of 13 kg/m² on nylon netting after 1 month in Pearl Harbour, but in excess of 80 kg/m² after 3 months. Similarly, Milne (1975a) found that 25 mm nylon mesh could support a mussel biomass of up to 140 kg/m².

THE EFFECTS OF FOULING ON FINFISH CULTURE

Restriction of water exchange

The predominant concern with fouling of fish cages is the occlusion of netting mesh and the changes in water quality resulting from restriction of water flow. A number of studies have demonstrated the extent of flow restriction through clean and fouled mesh (Hisaoka *et al.*, 1966 in Japanese; Wee, 1979). The flow of water through cages is generally measured as transmission: the current speed inside the cage expressed as a percentage of the current outside the cage. The transmission of clean nets is related to mesh size, but typically varies from 50% to 80%. Transmission is also affected by the external current velocity (Edwards & Edelsten, 1976) and the angle of the mesh to the current flow (Gularte & Huguenin, 1984). Differences in measurement of transmission may arise from the method used to quantify current, the stocking density of the cage and circulating currents created by the fish (Inoue, 1972; Wee, 1979).

Transmission has been shown to significantly reduce with fouling of mesh and grouping of cages. Transmission for clean 13 mm mesh (57.5%), was reduced to 23.4%, 18.7% and 13.1% after 52, 80 and 120 days in the sea, corresponding to fouling weights of 1.85 kg/m², 2.84 kg/m² and 4.98 kg/m² respectively (Wee, 1979). Similarly, Gormican (1989) measured current speed inside and outside a salmon cage and found a 65% transmission decrease at depths with significant fouling. The significant flow restriction through clean nets necessitates good fouling control in order to maintain adequate water exchange.

Flow decreases serially when cages are grouped in a row parallel to the current. Across three 9 mm mesh cages the transmission was found to drop from 70% in the first cage to 35% and 18% in the second and third cages respectively (Inoue, 1972). Across three 24 mm mesh cages the transmission was found to drop from 80% in the first cage to 50% and 35% in the second and third cages respectively (Inoue, 1972). When cages are aligned in a series, and when

netting becomes fouled, the effects combine to reduce water exchange (Aarsnes *et al.*, 1990). Beveridge (2004) thus recommended that although groups of 8-10 cages may be oriented perpendicular to the current, there should be no more than 2 or 3 cages in a series parallel to the current.

Water quality

Water exchange is critical for replenishment of dissolved oxygen and removal of excess feed and waste products. A reduction in oxygen concentration from the outside to the inside of cages, and a relationship between oxygen reduction and short-term water exchange, has been demonstrated in many studies (Hisaoka *et al.*, 1966; Inoue, 1972; Wee, 1979). In addition, increasing stocking density increases the rate of oxygen consumption in cages (Kadowaki *et al.*, 1978). Consequently, a combination of low current flow, significant mesh occlusion, and a high stocking density of fish, may reduce dissolved oxygen rapidly to critical levels (Edwards & Edelsten, 1976).

Kennedy *et al.*, (1977) reported fish mortality due to anoxia in a heavily fouled cage in which the dissolved oxygen (DO) concentration fell below 4.0 mg/1. This low DO concentration was directly attributed to poor water exchange, and was increased to 8.25 mg/1 after installation of a clean net. Oxygen concentrations of > 7 mg/1 are recommended for salmon farming, whilst concentrations < 5 mg/1 negatively impact on fish growth and respiration, and levels < 2 mg/1 can result in mortality (Boyd, 1982).

A number of factors contribute to the total supply and consumption of dissolved oxygen within sea cages, and the relative importance of these has been calculated through modelling (Edwards and Edelsten, 1976; Silvert, 1992; Løland, 1993; Silvert, 1994; Cronin, 1995). Oxygen supply is largely through water exchange, but also from photosynthetic fouling communities and atmospheric diffusion. Oxygen is primarily consumed by the fish, but to some extent also by the biochemical oxygen demand of the immediate environment and the fouling communities. The model identifies the most important factors as the respiratory demands of the fish and the mass of water exchanged.

The maximum stocking density of fish is almost completely dependent on water exchange and can be calculated based on the rates of oxygen consumption and supply. The model also allows calculation of tolerable mesh occlusion levels for existing stocking densities. For example, Cronin (1995) found that commercial tuna cages (30 m diameter, 15 m deep, 800 mm mesh, 840 x 25 kg tuna) require a transmission of at least 42% in spring (15°C water) and 80% in summer (22°C water) to maintain satisfactory oxygen levels. These latter figures also demonstrate the effect of decreased oxygen solubility with increased water temperature. However, these data are species-specific to some extent, and in Cronin's (1995) model respiration rates were based on salmonids, which are significantly lower than for tuna[2].

Whilst oxygen levels within cages are primarily controlled by water exchange, oxygen production or consumption by fouling communities can affect oxygen concentration (Wildish *et al.*, 1993; Cronin, 1995; Cronin *et al*., 1999). Diurnal changes in oxygen concentrations at salmon farms suggest that respiratory activity of phytoplankton and fouling macroalgae significantly affected cage oxygen concentration (Gormican, 1989; Wildish *et al.*,1993). Cronin (1995) found fouling communities on tuna cages to be net consumers of oxygen, because of a greater proportion of non-photosynthetic to photosynthetic biomass. However, Cronin *et al*.,

[2] This model was refined as part of this project (Cheshire and Loo, 2008; subproject 5, Chapter 7) to include new information from Aquafin CRC-FRDC project numbers 2003/228 and 2005/200 (Musgrove and Fitzgibbon, 2005; Fitzgibbon et al, 2008).

(1999) stated that the fouling community had minimal impact on the cage oxygen levels (less than 3% of the total oxygen exchange) relative to the processes of fish and sediment respiration and of mass water exchange.

A reduction in water exchange may also impact on fish health because increased levels of ammonia have been found within cages, compared to surrounding waters (Gormican, 1989; Wildish *et al.,* 1993). Detrimental levels of ammonia have not yet been reported in sea-cages because of sufficient water exchange (Gormican, 1989; Wildish *et al.*, 1993), but this is potentially a problem and acute ammonia toxicity has caused mortality in salmonids farmed in ponds (Lumsden *et al.,* 1993). Gowen and Bradbury (1987) estimated that 78% of nitrogen consumed by salmon is lost as faecal and excretory nitrogen, which equated to 32 kg of ammonium produced per tonne of fish food consumed. A 450 m^3 cage, holding 8 t of fish, would produce 1120 mg ammonia/m^3/h over an average 8 month growing season (Wildish *et al*., 1993).

Disease risk

Fouling communities may present a health risk to the cultured species because they could act as reservoirs for pathogenic microorganisms harboured by macrofouling species or existing in the extensive microbial communities on cage netting. Viral pathogens of finfish may accumulate and persist for long periods within shellfish. The viruses identified as finfish pathogens isolated from bivalves included 13p2 reovirus and the related chum salmon virus, JOV-1 Japanese oyster virus, infectious pancreatic necrosis strains, infectious hematopoietic necrosis virus (Leong & Turner, 1979; Meyers,1984). In addition, a number of bacterial agents that cause disease in finfish are also common to bivalve's tissues (eg. *Vibrio* sp).

Marine aquaculture may lead to infections with unusual parasites, either due to culture in new geographical areas, or in net pen environments (Kent, 2000). Amoebic gill disease, caused by the marine amoeba *Neoparamoeba pemaquidensis*[3], affects Atlantic salmon, *Salmo salar* and rainbow trout, *Oncorhynchus mykiss*, and causes significant mortality in Ireland (Rodger & McArdle, 1996), Chile, France, New Zealand, Tasmania aquaculture industries, particularly in summer (Clark & Nowak, 1999). Atlantic salmon and coho salmon, *O. kisutch* have also been affected in the USA (Kent *et al.,* 1988). Biofouling was reported to be a risk factor for amoebic gill disease outbreaks, since *N. pemaquidensis* was detected on macrofouling species (especially the bryzoan *Scupocellaria bertholetti* and the ascidian *Ciona intestinalis*), in the microbial biofilm layer and in the water column (Tan *et al.,* 2002). However, the level of the pathogen on fouled nets containing uninfected salmon was not investigated, so the pathogen may be ubiquitious in marine environments. Furthermore, exposure to lightly biofouled netting, or fouled netting washed in freshwater did not induce gill disease, indicating that the presence of the amoeba on netting may be necessary but not sufficient to cause disease in salmon.

The occurrence of disease in caged fish has also been linked to the consumption of fouling organisms by the cultured species (Kent, 1990; Andersen *et al.,* 1993). Netpen liver disease (NLD) was thought to be caused by a hepatotoxin that may be produced by algae, during summer (Kent, 1990). The toxin isolated from affected liver has been identified as microcystin-LR, a protein phosphatase inhibitor (Andersen *et al.,* 1993). In addition, injection of microcystin-LR is sufficient to re-create the pathologic changes of the disease in Atlantic salmon (Andersen *et al.,* 1993). Furthermore, the fouling biota of the salmon cage is a reservoir of the microcystin (Andersen *et al.,* 1993), and the disease is likely to be contracted by feeding on net biota. The organism responsible for producing microcystins has not been identified, but

[3] Note that recent research has shown *Neoparamoeba pemaquidensis* is not the cause of Amoebic Gill Disease (Professor B. Nowak, University of Tasmania, pers.comm. 2008)

the toxin is produced by freshwater cyanobacteria, and it has been detected in mussels collected near a NLD outbreak (Chen *et al.,* 1993).

Fish farms can also disrupt the parasite life cycle, by increasing the host density and promoting transmission from wild to cultured stocks and *vice versa*. Infection by *Gilquinia squali* metacestodes has been implicated in the deaths of Chinook salmon smolts of fish farms in British Columbia, where 10% mortality was associated with the eye disease at a particular site (Kent *et al.,* 1991). The definitive host for the parasite is the spiny dogfish *Squalus acanthias*, which were prevalent in and around the affected net pen sites (Kent *et al.,* 1991). It is likely that, during one of its lifestages, an unidentified crustacean acts as an intermediate host, and that transfer to the definitive host (or the farmed salmon) occurs directly through ingestion (Kent *et al.,* 1991). For salmon, therefore, the crustaceans within the fouling biota are a reservoir of the parasite. However, it is not known if the parasite is sufficient to cause the observed morbidity and mortality associated with the eye disease.

Fouling communities may directly impact on fish by causing physical damage to cultured species. Gill lesions and mortality caused by the spines of diatoms in dense mixed algal blooms have been recorded for pen-reared Atlantic salmon in British Columbia (Kent *et al.* 1995). Heavily fouled nets can also support the existence of free-swimming stages of sea lice (*Lepeophtheirus salmonis*).

However, biofouling may also have some positive effects on disease risk. The potential for mussels *Mytilus edulis* to harbour the bacterial kidney disease bacterium *Renibacterium salmoninarum* has been ruled unlikely (Paclibare *et al.,* 1994). *R. salmoninarum is* shed in the faeces of infected salmon, and it was considered possible that the pathogen may be concentrated in the filter-feeder, which fouls the net cages and act as a continuous source of re-infection for salmon. However, the mussels killed the majority of *R. salmoninarum* during digestion, and in fact are likely to reduce the levels of the pathogen in the cage environment (Paclibare *et al.,* 1994).

Cage deformation and structural fatigue

An increase in mesh occlusion will significantly increase drag forces on netting. Milne (1970) determined current forces on clean and fouled nets at various current velocities, and showed that forces on a fouled net may be 12.5 times that of clean net. Consequently, unless cages are heavily weighted the shape of the cage may be severely deformed by current flow (Osawa *et al.,* 1985). Aarsnes *et al.,* (1990) calculated deformation rates for a 12,000 m^3 cage (with 400 kg of bottom weight) and found that the cage volume was reduced by 45% (to 6,600 m^3) under a current velocity of 0.5 m/s (1 kn), and by 80% (to 2,300 m^3) under a velocity of 1 m/s (2 kn). Wee (1979) observed a 50% reduction in volume of a heavily fouled in use cage. Reduced cage volume is likely to impact on fish health because oxygen consumption and ammonia production will increase per unit volume, and crowding is likely to stress the cultured fish.

Highly deformed nets increase the structural stress of the cage and, although increasing cage weight will reduce deformation, this adds to the structural stress (Anon, 1993). Tomi *et al.* (1979) reported that weight added to cage corners resulted in a two to six-fold increase in horizontal forces on the cage. With heavy weighting, waves will cause the floating frame to move upward whilst the weights pull the netting downward. Structural loadings and fatigue are likely to increase further when predator netting is attached to cages.

The static load of the net is also directly impacted on by the biomass of the fouling community, which may increase the weight of the net up to 200-fold (Milne, 1972 in Beveridge, 2004). This increased load must be taken into account when designing the floatation

and mooring systems. Failure to do so can result in net failures, which have been devastating in commercial enterprises (Huguenin & Ansuini, 1978, Huguenin, 1997).

CONCLUSION

Biofouling on sea-cages causes mesh occlusion and a resultant decrease in productivity and fish health, as well as structural fatigue and cage deformation. Consequently, biofouling is a significant management issue resulting in significant operational expenses. What is surprising, for an issue with such high impact, is the sparse information about the effects of fouling, the lack of quantitative information on impacts, and more significantly the effects of antifouling methods on fouling. Given the limited choice of products available to control fouling in aquaculture, quantitative studies on the efficacy of antifouling technologies, at the level of species, will assist industries to choose the most cost effective method for fouling control taking into account regional and seasonal variation.

The focus on biofouling has been heavily skewed to more traditional aquaculture industries, such as the northern hemisphere salmon industry, with little quantitative information on fouling in new aquaculture regions and in the tropics where fouling is most rapid. However, many of the most targeted and quantitative studies are from emerging Australian finfish industries, and the capacity to quantify and mitigate the impacts of fouling is available, particularly in temperate Australian waters.

CHAPTER 2 : SUB-PROJECT 1: METHODS AND EFFICACY OF BIOFOULING CONTROL IN SEA CAGE AQUACULTURE

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Note:

This report is based on an original review of the impact and control of biofouling by Dr. Stephen Hodson in 1998. The original document has been re-written and updated and has been submitted as a manuscript to the journal Aquaculture with the authorship de Nys R, and Guenther J.

This report is part of a review series of biofouling in aquaculture including reviews of 'Legislation and control of antifouling chemicals in aquaculture in Australia' and 'Impacts of biofouling on marine finfish aquaculture'.

ABSTRACT

Biofouling of fish-cage netting is a significant operational problem to mariculture. We review the current literature on biofouling control of netting and cages in finfish aquaculture. The development of effective fouling control is particularly difficult, given the high species diversity and spatial variation typical of many fouling communities on cages. However, the continual expansion of finfish mariculture is increasing demand for fish-cage antifouling technologies, and has expanded research opportunities and created a viable market for specific products. At the same time, the control and regulation of products available for use in aquaculture and the phasing out of many products mean that there are fewer antifouling products available than there were a decade ago. We describe the range of antifouling technologies currently available, including mechanical and mechanized cleaning, coatings and extruded polymers incorporating naturally occurring compounds or commercial biocides, and their alternatives. We review the effects of antifouling metal-based paints, including environmental effects. Recommendations are made for effective biofouling control and directions for future research are identified.

INTRODUCTION

Biofouling is the accumulation of undesirable organisms on artificial surfaces submerged in seawater. Reviews of the literature on biofouling research have focused principally on fouling of ship hulls, oil platforms and other marine industries (Evans, 1981; Yebra *et al.,* 2004). Until now, biofouling in finfish mariculture has been largely neglected, particularly in Western countries. Some large studies specifically aimed at fouling in the aquaculture industry have been conducted by postgraduate students and are not widely available (Wee, 1979; Mak, 1982; Gormican, 1989; Cronin, 1995). Furthermore, commercial research and development aimed at increasing mariculture productivity is often conducted directly by

farms, or small research groups sponsored by farms, and is rarely published. Consequently, there is a need to review the current state of knowledge on the control of fouling in the finfish mariculture industry. This has been recognized in recent publications on this subject (Beveridge, 2004; Braithwaite & McEvoy 2005).

The impact of fouling on the finfish aquaculture industry

Fouling of fish cages causes the occlusion of netting mesh, resulting in changes in water quality from restriction of water flow. This impacts on the availability of dissolved oxygen and the removal of waste metabolites, and can increase disease risk. In addition, biofouling can cause deformation and structural fatigue of sea-cage nets. Failure to remove biofouling can lead to net failure and loss of the crop (Beveridge, 2004; Braithwaite & McEvoy, 2005; Houlden *et al.*, 2005).

Fouling develops very rapidly on cages in many regions of the world, and frequent changing and cleaning of nets is critical to maintain cage water exchange. For example, nets must be changed as frequently as every 5-8 days in summer in Australia (Hodson & Burke, 1994), every 8-14 days in Japan (Milne, 1979) every 14 days in Malaysia (Lee et. *al.*, 1985), and every 3-4 weeks in Canada (Menton & Allen, 1991). Large mesh cages are changed less frequently because of the considerable amount of fouling required to significantly occlude the mesh. In Australia predator fences (100-150 mm mesh) are changed every 3-6 months, and tuna cages (60-90 mm mesh) are cleaned every 6 months (Cronin *et al.*, 1999). Some delay in the frequency of cleaning may be achieved by raising the top few metres of the cage out of the water (Needham, 1988), but this is only applicable where the fouling is restricted to the upper area of the cage. Whilst frequent net changing is common in temperate and tropical regions, cages immersed at off-shore sites and in very cold water can remain immersed for long periods without cleaning. For example, cages in northern Norway are changed only once per year, usually in July after the period of maximum ascidian and mussel settlement (Sutterlin & Merrill, 1978).

Net changing incurs a major cost to the industry, necessitating purchase of a large number of nets and provision of dedicated net-changing and cleaning teams. Moreover, frequent net changing also risks damage or loss of stock, and disturbs feeding regimes and therefore lowers growth rates. However, the extent of the economic consequences of fouling and fouling control to the aquaculture sector is largely unquantified.

METHODS FOR THE CONTROL OF BIOFOULING

Commercial fish farms operations usually employ a multifaceted approach to controlling net fouling, which typically involves utilization of fouling resistant or rotating cage designs, frequent net changing and cleaning, and chemical control.

Shore-based net cleaning

The removal of fouling communities from cages is generally achieved by replacing the fouled net, and transporting it to shore for manual or semi-automated cleaning (Lewis, 1994a). However, the frequent changing of netting on a standard floating cage is labour and capital-intensive, and boat-mounted hydraulic cranes are needed for large cages. During changing, the fouled net is partially raised and a clean net is peeled underneath and attached to the collar. The fouled net is then untied and removed, with the fish released into the clean cage. Fouled netting is usually left to compost for 1-2 weeks on-shore, followed by cleaning with high-pressure

water hoses or automated washing machines (Sutterlin & Merrill, 1978; Lewis, 1994a; Cronin *et al.*, 1999).

Unfortunately, washing procedures and net handling frequently cause damage to netting and reduce its life-span. Consequently, after cleaning nets are laid out for mending and replacement of damaged sections. At some farms nets are dropped to the seabed after removal from the cage, and the fouling is degraded biologically over a period of weeks (Sutterlin & Merrill, 1978). However, this latter technique is unsuitable when clean nets must be available within short-time periods, and the practice is also likely to increase benthic pollution.

Underwater net cleaning

Given the large expense involved in frequent net changing, it is surprising that little information is available on underwater cleaning of cages. A cleaner designed by Japan's Bridgestone Corporation is perhaps the only significant development of automated underwater cleaning for fish cages (Anon, 1994). The machine consists of a cleaning head (with rotating brushes) supported beneath a floating platform which moves around the cage perimeter. Two cables are used for raising and lowering the cleaning head. The machine was reported to clean 4 m^2 - 6.3 m^2 of net per minute (3 hours for a 15 m deep hexagonal cage with 16 m long sides) depending on the level of fouling. However, the design does not allow for removal of debris created during cleaning. Doedens (1992) reported on an earlier version of the machine, and quoted a purchase price of Aus$166,000. At that time only 10 units had been sold in its 2 years of commercialisation.

The Tasmanian Atlantic salmon industry has trialled the efficacy of an underwater net cleaner, which prevented fouling over a 10-week period during summer (Hodson *et al.*, 1997). However, fouling was not removed from the netting bars or crevices due to physical constraints, and this led to rapid recolonisation and regrowth of fouling.

Simpler forms of underwater cleaning are practiced, but often require SCUBA diving and are therefore more expensive and dangerous than shore-based cleaning. High pressure water hoses have been used to clean tuna cages in South Australia (Cronin *et al.*, 1999), and vacuum cleaning equipment has been used for salmon cages in Tasmania (Doedens, 1992). However, the latter technique was only effective on painted nets (because the fouling attached poorly), and was eventually abandoned because of the considerable amount of time required to clean an entire cage. Handheld units combining a rotating brush and high-pressure water jets have been offered commercially, but are probably only cost-effective for small cages. In general, in *situ* cleaning is unlikely to be viable unless fully automated; any fouling remnants left after cleaning are likely to regrow quickly and underwater cleaning may therefore be required at a high frequency (Moss & Marsland, 1976). Geffen (1979) suggested that brushing increases fouling problems because it scratches the mesh and encourages rapid recolonisation.

Biological control

An increase in profitability and sustainability could be achieved by use of herbivorous fish or invertebrates to control fouling (Beveridge, 2004), and benthic/detritus feeders to remove uneaten food (Angel *et al.*, 2002). The biological control concept is constrained by the great variation in types of algal and invertebrate fouling, which suggests that only herbivores and omnivores with a broad dietary range will be successful control agents. Furthermore, it is likely that continuous grazing will provide an environment *selective* for inedible species, and thus polyculture may only reduce the frequency of net changing.

Nevertheless, biological control using sea urchins and hermit crabs has proved effective for controlling fouling of suspended shellfish systems (Hasse, 1974; Littlewood & Marsbe, 1990; Lodeiros & Garcia, 2004; Ross *et al.,* 2004). For finfish, biological control of fouling has been successful with co-culture of other finfish: Mullet (*Mugil cephalus* at 0.5-0.78 kg/m³) in small cages of pompano (Swingle, 1972); Rabbit fish (*Siganid* sp.) in cages of grouper and carangids (Chua & Teng, 1977; Chua & Teng, 1980); rohu *(Labeo rohita)* in cages of carp (Sharma, 1979); and knifejaws (*Oplegnathus* spp.) in cages of yellow tail (Kuwa, 1984). The stocking density of the added herbivorous fish varies greatly, from 3% - 9% of the total cage biomass (Kuwa, 1984; Li, 1994) to densities of only 1 fish/5m³ (Sharma, 1979). However, there are potential negative impacts: knifejaws preyed on the tail and fins of sick yellow tail (Kuwa, 1984) and there may be a number of risks to the primary culture species, such as greater disease potential and increased demands on dissolved oxygen.

Detritivores like the red sea cucumber *Parastichopus californicus* have proved effective in significantly reducing fouling in salmon mariculture. One hundred sea cucumbers placed inside a 18 m, 7.5 m deep 5 mm mesh pen containing one million salmon maintained 58% of the transect line completely clean, whereas control pens were uniformly fouled (Ahlgren, 1998). However, sea cucumbers were negatively affected by wave-generated undulation and were unable to maintain their positions on the sides of cages suspended with buoys, although they were able to maintain positions throughout in rigid frame pens (Ahlgren, 1998). The advantage of polyculture with sea cucumbers is that they are a commercially important aquaculture crop in their own right, with strong demand for the product in Asia (Conand & Sloan, 1989).

Alternative cage designs

An alternative to both frequent net changing and underwater cleaning is the use of fully-enclosed rotating cages (e.g. Caillouet, 1972; Anon, 1979; Blair & Burgess, 1979; Geffen, 1979; Blair *et al.,* 1982; Menton & Allen, 1991; Willinsky *et al.,* 1991). These have either been horizontally-mounted cylinders which rotate on a central axle (Caillouet, 1972; Menton & Allen, 1991), or rectangular boxes with inflatable buoyancy devices in each corner. The rectangular cages are gradually rotated by sequentially changing the buoyancy of the corners (either by inflation and deflation, or displacement and filling with water). With rotatable cages no area of netting needs to be left submerged for long periods, and netting can be brought to the surface to air dry and hence kill attached fouling. Furthermore, the cage is easily accessible for fouling removal and netting repair, and by keeping the net immersed for short periods significant fouling growth can be avoided. Blair *et al.* (1982) found that a cage rotation of 90 degrees per week was sufficient to keep cages essentially free of fouling, and Geffen (1979) reported that cage rotation at 3-day intervals kept cages completely clean.

Despite other benefits of completely enclosed cages, such as prevention of bird predation and avoidance of storms and ice through cage submergence, rotating cages are not widely used. It would be necessary to construct very large rotating cages if they were to hold volumes of fish comparable to conventional floating collars of > 90 m circumference. Moreover, commercially available rotating cages are more expensive than conventional designs and continued exposure to direct sunlight can increase netting degradation (Beveridge, 2004).

Chemical antifoulants and paints

Chemical antifoulants in paints prevent the establishment of a marine biofilm through leaching of a biocide that produces a thin layer of toxic solution around the net. During the past 50 years antifouling paints and coatings have been intensively studied. Some of the earliest

published attempts at antifouling of fish cages were conducted in the Western Baltic Sea by the Institute for Coastal and Freshwater Fisheries and showed that an antifoulant (Wiedox VF 65001/green) kept nets completely clean for 5 months, during which time untreated nets became totally occluded (Koops, 1971).

However, products designed specifically for fish cages are scarce, and the industry has historically borrowed antifouling technologies from other marine industries, particularly shipping. Consequently, chemicals and heavy metals that are now clearly recognized as dangerous in the environment have been used in the aquaculture industry.

Tin

The organotin antifoulant tributyltin (TBT) is a broad-spectrum algicide, fungicide, insecticide and miticide and was one of the most widespread antifoulants used on ship hulls from the 1960s (Yebra *et al*. 2004). Because of its antifouling efficacy TBT was also used extensively on the netting of sea cages in mariculture of salmon. For example, fouling on cages (2 x 2 x 2m; 13 mm, 9 ply mesh) coated with an organotin antifoulant was reduced to 1 kg/net after 2 months submersion, whereas 91 kg/net was present on untreated cages (Lee *et al.*, 1985).

However, the use of tributyltin (TBT) antifoulants has exemplified the hazards of toxic coatings in mariculture (Ellis, 1991; Alzieu, 1998; Terlizzi *et al.,* 2001). TBT leaches out of impregnated nets and has been recorded in the waters around treated fish cages (Balls, 1987). Balls (1987) measured TBT release in newly painted salmon cages, and recorded 1 mg/m³ (pg/1 as Sn), 0.1 mg/m³, and 0.005 mg/m³ after 1 day, 2 weeks and 5 months, respectively. Similarly, Short and Thrower (1986) reported TBT concentrations from 0.007 to 0.026 mg/m³ Sn in treated salmon pens in the USA.

The use of TBT-impregnated nets in salmonid aquaculture can induce histopathological effects (Bruno & Ellis, 1988) and mortality (Lee *et al.*, 1985). Short and Thrower (1986) reported acute intoxication with a 96-h LC50 of 1.5 pg/1 for Chinook salmon. Behavioural abnormalities and pathological changes occurred in farmed Atlantic salmon (*Salmo salar*) that were transferred to a newly antifouled cage and feeding responses were dramatically reduced after 4 days (Bruno & Ellis, 1988). Salmon showed lifting of the gill epithelium and an increase in number of leucocytes in the retina, and the lens was opaque, inferring blindness, and after 7 weeks exposure hyperplasia was observed in the dermal layers of the skin, resulting in protruding scales, especially along the lateral line (Bruno & Ellis, 1988). These observations were interpreted as TBT interfering directly with the normal growth of salmon.

Salmon raised in treated nets also rapidly bioaccumulate TBT. Short and Thrower (1986) reported bioaccumulation after 3-4 days exposure to 1.5 pg/1. They recorded levels of 6.4, 1.9 and 0.3 pg TBT/g wet weight of liver, brain and muscle respectively. Similarly, Atlantic salmon exposed to 0.1-1.0 mg/1 TBT for 26 days had bioaccumulation in tissues with the highest concentration found in the liver (Davies & McKie, 1987). Bruno and Ellis (1988) reported that after 7 weeks exposure, TBT had bioaccumulated in the flesh, liver, gills and caeca. TBT was therefore able to enter the human food chain where it is toxic to humans. The WHO has set a limit of 3.2 mg/kg body weight for tin in humans, which corresponds to a daily consumption of 150 g salmon for a 70 kg person (WHO, 1999).

TBT also affects non-target organisms, particularly bivalves (Paul & Davies, 1986). In the early 1970s the deleterious effects of TBT in the environment were observed through shell malformations and reduced growth in the Pacific oyster, *Crassostrea gigas* (Alzieu *et al.*,1981; Alzieu *et al.,* 1986). The serious problems encountered in commercial oyster cultures in France were soon followed by reports of similar problems in the UK (Waldock & Miller, 1983). TBT

also induces imposex[4] in gastropods (Gibbs *et al.,* 1991), and has since been found in fish, seabirds and marine mammals (reviewed in Terlizzi *et al.,* 2001). Clearly, TBT antifouling products pose an unacceptable risk to non-target species that was unidentified when introduced into the market.

The adverse effects' resulting from widespread use of TBT has led to a ban on its use (Alzieu, 1991; Evans 1999). In 1986 the National Farmers' Union in Scotland introduced a voluntary ban on its use in fish cages, and in1987 its retail sale was prohibited by the Scottish authorities (Balls, 1987). In Australia, TBT antifouling is presently restricted to vessels greater than 25 metres in length, and in New Zealand there has been a complete ban on all TBT sales and use since December, 1993 (ANZECC, 1995). The International Maritime Organisation banned the use of TBT in paints from 2003 (Julian, 1999) and concordantly many governments have prohibited organotins in antifouling paints (Bell & Chadwich, 1994; Costello *et al.,* 2001).

The challenge for the aquaculture industry is not to repeat the TBT scenario in the future (Ellis, 1991). In the wake of the TBT ban, Lewis (1994b) recommended six criteria for antifouling strategies in the aquaculture industry. They should: (1) be effective against a broad range of fouling taxa, (2) be environmentally benign, (3) have no negative effects on the cultured species, (4) leave no residues in the cultured species, (5) be able to withstand on-shore handling and cleaning, (6) be economically viable.

Copper

In the void left by the ban on TBT attention soon re-focused on copper and copper-containing coatings which have a long history of use in shipping and mariculture (Lewis & Metaxas, 1991; Lewis, 1994b). For example, in 1998 Norway used 180 ton of copper for antifouling in the aquaculture industry (Solberg *et al.*, 2002). Copper adds approximately 20-25% to the cost of a knotless nylon cage (Beveridge, 2004) but it is a very effective antifoulant. In temperate regions nets must be coated each year, but antifouling with copper gives good protection for 6 months and is effective during summer when fouling is worst (Beveridge, 2004).

Copper-based antifoulants are currently approved for use in aquaculture in Europe and North America, and have also been used in Australia (Hodson & Burke, 1994; Douglas-Helders *et al.,* 2003). In Canada, there are six antifouling products registered with the Canadian Pest Management Regulatory Agency for use in aquaculture (containing only cuprous oxide, for cages and ropes and netting) (reviewed in Houlden & de Nys, 2005, Chapter 3). In the UK, there are twelve antifouling products registered for use in aquaculture (all containing exclusively cuprous oxide) (reviewed in Houlden & de Nys, 2005). None of these products are approved for use in aquaculture in Australia, although treated nets are used under research permits issued by the APVMA (Houlden & de Nys, 2005).

Copper leaches out of impregnated nets into the water column. The leaching rate of copper in paints is increased by the presence of zinc, usually in the form of zinc oxide (French *et al.,* 1984). Leaching rates of 10 and 20 mg/cm²/day are required to prevent the settlement of barnacles and diatoms, respectively (Callow, 1999). In Jervis Inlet, British Columbia, the concentration of copper inside a treated salmon net pen was 0.54 mg/l 2 days after net installation, and this concentration was present one month later (Lewis & Metaxas, 1991). About 700 meters away from the nets, the copper concentration was 0.38 mg/l, but the difference was not statistically significant (Lewis & Metaxas, 1991). Other studies demonstrate that copper from nets treated with Flexgard XI® was released into the environment at an

[4] a pseudo-hermaphroditic condition in female gastropods, manifested by the development of a false penis

exponential rate of 155 mg Cu/cm² until reaching a long-term rate loss of 37.6 mg Cu/cm² (Brooks 2000; Brooks & Mahnken 2003). Industry best practice is to introduce fish into nets one month after newly coated nets are in position, to minimise the potential for bioaccumulation.

Copper is highly toxic to many marine organisms, but particularly to the larval stages of invertebrates (Mance, 1987). Relatively low concentrations of copper are known to be harmful to fish and diverse effects have been reported from toxicity studies (Chapman, 1978; Chapman & Stevens, 1978; reviewed in Peterson *et al.,* 1991; reviewed in Brooks *et al.,* 2003). Acute copper intoxication 96-h LC50 occurs in adult salmonid fish at 60-680 mg Cu/1 (Sorensen, 1991), and the USA EPA chronic marine standard of 3.1 mg Cu/1 is a 4-day average that must not be exceeded more than once every 3 years. The UK environmental quality standard for dissolved copper in seawater is 5 mg Cu/1 (Voulvoulis *et al.,* 1999a), but in practice this value is often exceeded and may be having a detrimental ecological effect (Matthiessen *et al.,* 1999).

However, whether copper bioaccumulates as a consequence of aquaculture activities is unresolved. Voulvoulis *et al.,* (1999a) regard copper as showing "only a slight tendency for bioaccumulation". There are reports that salmon raised in copper-treated nets do not bioaccumulate copper in muscle or liver tissue (Petersen *et al.,* 1991; Solberg *et al.,* 2002), and there was no detectable bioaccumulation of copper in the brown seaweed *Ascophyllum nodosum*, the blue mussel *Mytilus edulis*, or the saithe *Pollachius virens* from fish farms (Solberg *et al.,* 2002). In contrast, intestinal copper levels in the green sea urchin *Strongylocentrotus droebrachiensis* were elevated at salmon aquaculture sites (Chou *et al.,* 2003), and copper has been shown to bioaccumulate in the hepatopancreas of lobsters sampled near salmon farms (Chou *et al.,* 2000). Furthermore, oysters growing around marinas have elevated levels of copper, which may be due to antifouling paints (Claisse & Alzieu, 1993). In addition, there are environmental concerns from the elevated concentrations of copper found in sediments around salmon farms (Miller, 1998). Copper accumulation in sediments is highly dependent on physical characteristics and sediment chemistry. An increase in average copper concentrations in the sediment, from 21 mg/kg at a reference site, to 49-430 mg/kg was found for four out of five farms using coppertreated nets (Solberg *et al.,* 2002). However, due to high variance within sites, the differences were not statistically significant. In another study, an approximate twofold increase in copper in the sediments was found in 117 farms using copper-treated nets (48.24 ± 27.00 mg Cu/g) compared to 39 not using copper-treated nets (26.27 ± 2.77 mg Cu/g), but again the difference was not statistically significant (Brooks & Mahnken, 2003).

Antifoulants are biocides and as such are not directly used on fish, and therefore do not fall under the MRL system. However, fish may be exposed to antifoulants for long periods, up to months. The use of toxic metal-based antifouling is therefore an undesirable aspect in an industry selling a food product from a "clean and green" marketing perspective. Most countries are now working towards a reduction in the use of copper-based antifouling in the short-term. No restrictions are presently enforced on copper-based antifouling, but alternative strategies are being reviewed by the Australian and New Zealand Environment and Conservation Council as part of its Strategy on Maritime Pollution. The European Commission is proposing to give copper a R50/R53 classification, based on the 67/548/EEC directive on dangerous substances, which recognizes that copper is toxic to aquatic organisms and may cause long-term adverse effects in the environment. The Norwegian aquaculture industry is moving towards a reduction of copper based on public perception of copper treatment as a negative environmental impact.

Booster biocides

Worldwide, there are a number of other biocides currently being used as antifoulants, albeit not necessarily in mariculture (Callow, 1999; Konstantinou & Albanis 2004), and these are potential candidates to replace the use of TBT and copper as antifoulants. The most commonly used biocides include Irgarol 1051, Diuron, Sea-nine 211, Dichlofluanid, Chlorothalonil, Zinc pyrithione, TCMS (2,3,5,6-tetrachlora-4- methylsulfonyl) pyridine, TCMTB (2-thiocyanomethylthiobenzothiazole), and Zineb (Callow, 1999; Konstantinuo & Albanis, 2004, Yebra *et al*. 2004). Products based on cuprous oxide containing chlorothalonil (Flexgard VI; Flexbar Aquatech Corporation, USA), and dichlofluanid (Hempel's Antifouling Rennot 7150; Hempel Paints Limited, Denmark) have been used in aquaculture in the UK. However, both products are now being phased out due to the minimisation of biocide use for aquaculture.

There is a potential danger that the biocides listed, which are in some cases are largely untested, may be less efficient and/or more harmful to the environment than either TBT or copper (Evans 1999). The known chemical and physical properties of the common biocides vary widely, and their properties, toxicity, environmental fate and gaps in knowledge in the aquatic environment has been extensively reviewed (Callow, 1999; Thomas *et al.,* 1999; Voulvoulis *et al.,* 1999a, 1999b; Thomas *et al.,* 2000; Thomas, 2001; Thomas *et al.,* 2001; Okamura *et al.,* 2002; Thomas *et al.,* 2002; Voulvoulis *et al.,* 2002a; Voulvoulis *et al.,* 2002b; Thomas *et al.,* 2003; Konstantinuo & Albanis, 2004). It is also clear that biocides will persist in the environment when associated with paint particles, released particularly during cleaning procedures (Thomas *et al.,* 2003). With many gaps in our knowledge of the longer-term effects of biocides, it is difficult to evaluate impacts and risks for the aquatic environment, and hence good environmental policy must be formulated according to the precautionary principle. A summary of key data on each biocide follows, and the reader is directed to a comparative environmental assessment of relevant biocides for detailed information (Voulvoulis *et al.,* 2002a).

Irgarol 1051(Ciba-Geigy)

Evidence is accumulating that Irgarol 1051 residues may become a ubiquitous contaminant in the marine environment (Readman *et al.,* 1993; Gough *et al.,* 1994; Tolosa *et al.,* 1996, Liu 1999; reviewed in Konstantinou & Albanis, 2004). Irgarol 1051 has been detected in both the water column and the sediment (Tóth *et al.,* 1996; Voulvoulis *et al.,* 2000; Thomas *et al.,* 2001). Irgarol 1051 degrades in seawater with a half-life of about 100 days (Ciba-Geigy, 1995), however its major degradation product M1 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) is even more stable.

Ecotoxicity studies indicate that both Irgarol 1051 and M1 are toxic to non-target organisms and could damage aquatic ecosystems at environmentally relevant concentrations (Okamura *et al.,* 2000a, 2000b). Irgarol does not exhibit high toxicity to fish (Okamura *et al.,* 2002), but as an *s*-triazine herbicide it inhibits photosynthesis and is highly toxic to algae, corals, and sea grasses (Dahl & Blanck, 1996; Owen *et al.,* 2002). Irgarol has been found to bioaccumulate in sea grasses, algae and mussels (Scarlett *et al.,* 1999; Nyström *et al.,* 2002).

Diuron

Diuron is a substituted urea-based herbicide that also inhibits photosynthesis, and has been used widely in agriculture throughout the world. It is persistent in seawater (Callow & Willingham, 1996), and has a toxicity pattern similar to that of Irgarol 1051 (reviewed in

Konstantinou & Albanis, 2004; Giacomazzi & Cocket, 2004). Diuron is toxic to fish (fathead minnow *Pimephales promelas*) with a 24 h LC50 of 23.3 mg/l. Concentrations above 78 mg/l affected hatchability and increased deformities in minnow eggs and fry (Call *et al.,* 1987; Nebeker & Schuytema, 1998).

Diuron is reported not to bioaccumulate (Call *et al.,* 1987). It has been detected in the environment in the UK (Voulvoulis *et al.,* 1999a, Boxall *et al.* 2000), and is a contaminant in waters in Denmark, Sweden, and Spain (reviewed in Evans *et al.* 2000). Diuron is no longer approved for use in antifouling paints on any vessel in the UK, and is restricted to boats > 25 m in length in Denmark and Sweden (Konstantinou & Albanis, 2004).

Sea-Nine 211 (Rohm & Hass)

Sea-nine 211 is a broad-spectrum bactericide, fungicide and algicide. It was registered in the USA for use in antifouling paints in 1994 (Bingaman & Willingham, 1994). Its efficacy has been demonstrated over the last decade on shipping worldwide. It is rapidly degraded in natural seawater in under 24 h, is irreversibly bound to sediment, and does not bioaccumulate (Jacobson & Willingham, 2000). Sea-nine 211 is acutely toxic to a wide range of aquatic organisms, including sea urchin eggs and embryos (Kobayashi & Okamura, 2002) and may adversely affect phytoplankton communities (Larsen *et al.,* 2003). Significant concentrations of Sea-nine 211 are now being reported in the environment (reviewed in Yebra *et al.,* 2004).

Dichlofluanid

There are few data available for this biocide, but after the boating season dichlofluanid was detected in the sediment, but not the water column, of an estuary in the UK (Voulvoulis *et al.,* 2000).

Chlorothalonil

Chlorothalonil is a fungicide used widely in agriculture, and it has occasionally been detected in surface and groundwater. In freshwater, chlorothalonil residues can usually be found in biota. Its half-life in a water/sediment system was found to be <2 h; however, residues were still detected 30 d later (Caux *et al.,* 1996). In soil, it persists, with a half-life of 1 to 2 months (Caux *et al.,* 1996). Chlorothalonil is highly toxic to aquatic invertebrates (eg. water boatmen *Sigara alternata* and it can immobilize *Daphnia magna* at a concentration of 1.8 mg/l); and to some fish (caged three-spined stickleback *Gasterosteus aculeatus* and channel catfish *Ictalurus punctatus*), but is not toxic to algae (Ernst *et al.,* 1991; Caux *et al.,* 1996). It can affect the renal system of rats at a level of 1.5 mg/kg/d (Caux *et al.,* 1996).

Pyrithiones: zinc and copper

Zinc pyrithione (zinc omadine) is an effective bactericide, and fungicide widely used in anti-dandruff shampoos. It is also an algicide with an EC50 for algae in the range 3-6 mg/l (Karlsson & Eklund, 2004). The EC50 value of zinc pyrithione for the marine diatom *Amphora coffeaeformis* was 0.03 mg/l (Turley *et al.,* 2005). It is rapidly biodegraded in water and sediments (Turley *et al.,* 2000; Turley *et al.* 2005). Zinc pyrithione was registered for use in antifouling in the USA in 1997 where it is often formulated with cuprous oxide or cuprous isothiocyanate (Callow, 1999). Copper pyrithione has been recently developed and its environmental fate and toxicity assessed (Turley *et al.,* 2005), but is not registered in the USA.

TCMTB (2-thiocyanomethylthiobenzothiazole)

TCMTB is principally a fungicide used in seed coatings and timber treatments. Data is extremely limited on its use as an antifouling biocide, but it has not been detected in marinas in the UK (Thomas, 1998) or the Mediterranean (Ferrer & Barcelo, 1999).

Zineb

Zineb is a fungicide that is synergistic with copper, enabling a reduction of copper in antifouling paints without loss of efficacy (Hunter & Evans, 1990, 1991a, 1991b). It does not bioaccumulate and has a short half-life in sea water (Thomas, 2001)

In summary, the herbicides Irgarol 1051 and Diuron persist in the water column, whereas Sea-nine 211, Dichlofluanid, Zinc pyrithione and Chlorothalonil disappear quickly (Thomas, 2001; Thomas *et al.,* 2002; Thomas *et al.,* 2003; Konstantinuo & Albanis, 2004). Diuron, Sea-nine 211, Zineb and Thiram do not bioaccumulate appreciably, whereas Irgarol does (reviewed in Konstantinuo & Albanis, 2004). Diuron and Irgarol 1051 show the least toxicity to chinook salmon *Oncorhynchus tshawytscha*, while pyrithiones showed high levels of toxicity (Okamura *et al.,* 2002). Overall, Zinc pyrithione and Zineb perform the best for environmental parameters, then Irgarol, Chlorothalonil, Sea-nine 211 and Diuron. Dichlofluanid, TCMTB, TCMS pyridine and TBT perform poorly, with TCMS pyridine and TCMTB demonstrating environmental characteristics similar to TBT (Voulvoulis *et al.,* 2002a). Clearly, there are impacts on the aquatic environment with all booster biocides, and no ideal replacement for either TBT or copper has been developed.

In the current regulatory environment, development and registration of toxic biocides is extremely expensive. For example, Rohm & Hass spent 10 years and 10 million dollars registering Sea-nine 211 in the USA (Bingaman & Willingham, 1994; Rittschof, 2000). It is considered uneconomical to develop future toxic booster biocides for biofouling control (Bingaman & Willingham, 1994). The focus in research and development has shifted to antifouling agents that are both effective and environmentally benign as a consequence of their chemistry (non-toxic coatings) or their physical properties (foul-release coatings and non-leaching biocides) (Yebra *et al*. 2004).

Non-toxic coatings

Natural products

Natural products have a long history in aquaculture. Prior to use of modern polymer-based netting, farmers in Malaysia soaked cotton nets with tannins extracted from the bark of mangrove trees (*Rhizophora* sp.) (Lai *et al.*, 1993). Tannins are toxic and act as natural biocides, but whilst these absorb well to traditional fibre nets, the absorbancy to synthetic materials is poor and effectiveness is short-term (Lai *et al.,* 1993).

In contrast to heavy metals and organic biocides, many marine antifouling chemicals act as chemical deterrents and deter fouling settlement at concentrations that are not toxic (Clare, 1996). Research and development has focused on those isolated from plant and animals species not fouled in the marine environment that have potential application to commercial biofouling control (reviewed in Bhadury & Wright, 2004; Fusetani, 2004; Yebra *et al.,* 2004). For

example, sponge extracts incorporated into paints prevented fouling by barnacles (Willemsen & Ferrari, 1993). However the active ingredient in the extract was not identified in this study. Similarly, paints containing extracts of the supernatant of *Pseudomonas* sp. have also deterred the settlement of barnacles and algae (Burgess *et al.*, 2003). In addition, 5,6- dichlorogramine, an analogue of 2,5,6-tribromo-1-methylgramine, has strong antifouling activity against barnacles and mussels (Kon-ya *et al.*, 1994). Its evaluation in the field was undertaken in Japan but has not been reported. Other promising compounds identified recently include furanones (de Nys & Steinberg, 2002), pukalide and the renillafoulins (from the sea pansy *Renilla reniformis*) (Price *et al.*, 1992).

Nevertheless, the identification of non-toxic antifoulants derived from natural products is only the first stage in developing a commercial product. The compound must be synthesized in large quantities at reasonable cost, incorporated into the paint matrix, and undergo the same regulatory evaluation by environmental agencies that biocides go through. The lengthy timeframes and the cost incurred in this process may be prohibitive (Yebra *et al.*, 2004), and these products are unlikely to be viable commercial alternatives to currently registered biocides. This realization has led to recent investigations demonstrating the effects of well-known pharmaceuticals (Rittschof *et al.*, 2003) and commercially available enzymes (Pettitt *et al.*, 2004) as antifoulants, and this approach may prove productive in the future.

Foul-release coatings

Biocide-free low surface energy siloxane elastomers and fluoropolymers may provide a non-toxic alternative to control biofouling (reviewed in Callow & Fletcher 1994; Yebra *et al.*, 2004). These "foul-release" coatings aim at reducing or preventing the adhesion of fouling. Silicone-based paints are not toxic to any organisms tested (Karlsson & Eklund, 2004). They are presently seen as an alternative to toxic paints for ship hulls, where the speed of the vessel produces the hydrodynamic shear needed for the loosely attached fouling to fall off (reviewed in Yebra *et al.*, 2004).

The hydrodynamic forces and hence the efficacy of "foul-release" or self-polishing coatings should be much reduced in a "stationary" aquaculture net. Nevertheless, nets and panels coated with non-toxic silicone coatings effectively reduce the initial stages of fouling development and make it easier to clean the net of fouling that does accumulate (Rittschof *et al.*, 1992; Swain *et al.*, 1992; Edwards *et al.*, 1994; Hodson *et al.*, 2000; Terlizzi *et al.*, 2000).

A number of commercial products are available for aquaculture, including Hyperkote AQ (Hyperblast Limited, UK), Biosafe (Wattyl Australia) and Intersleek 425 (International Coatings, UK). Recently, a fluorinated elastomer (HFA-PDI) performed well in a field trial over an entire fouling season, it fouled slowly and the fouling was easily removed, and the coating was durable (Brady & Aronson, 2003). This area is likely to spur commercial outcomes of great interest to the aquaculture industry in the medium to short-term.

Non-leaching biocides

Biocides irreversibly bound to the antifouling coating surface or net are known as non-leaching biocides (Clarkson & Evans 1993, 1995). While this approach offers advantages in terms of limitation of environmental contamination, it has not been successfully pursued, presumably because of technical issues and the broad range of fouling organisms, many of which may not respond to bound biocides. However, this is an area of technical promise with the move towards legislation restricting antifouling technologies to non-release mechanisms.

Microtexturing of surfaces

Research identifying physical defences used to combat fouling in specific plants and animals may also have commercial application to antifouling technology. This approach aims to characterize topography and microtextured surfaces that prevent settlement of common fouling organisms (Bers & Wahl, 2004). For example, a natural regular rippled surface has been characterized on the blue mussel *Mytilus edulis* (Wahl *et al.,* 1998) and *Mytilus galloprovincialis* (Scardino *et al.,* 2003). This structure significantly inhibits the development of fouling (Wahl *et al.,* 1998; Scardino *et al.,* 2003). This field is in its infancy, although it has potential for combating fouling on ships, with possible direct application to polymer based nets.

CONCLUSION

The methods for controlling fouling on nets and other aquaculture structures are restricted to a limited range of products based on the release of copper and zinc with the addition of booster biocides. This limited range of products is also likely to be reduced as copper and possibly zinc are phased out through legislation, and booster biocides become restricted in their use. This will leave specific "environmentally friendly" biocides such as zinc pyrithione and zineb as the only effective broadspectrum fouling control. There will however be the development of new products with a focus on foul-release antifouling technologies based on low-surface energy (foul-release) coatings, texturing and surface-bound compounds. Foul-release technologies rely on hydrodynamic force to remove fouling organisms with poor adhesion on the foul-release surface, making them less suitable for aquaculture. However, as the technology for vessels improves the transfer (trickle-down) of technology to aquaculture industries will become more feasible with product development targeted at larger aquaculture industries. Another alternative to metal and biocide-based technologies, that has yet to be demonstrated as having broad-spectrum efficacy in controlling fouling, is biological control. Although this will be industry and site specific, and it is difficult to envisage its broad application, it may offer significant benefits to some industry sectors. Alternatively as metal and biocide based technologies are removed from the market the aquaculture industry may have to return to the traditional methods of net changes and shore-based cleaning.

CHAPTER 3 : SUB-PROJECT 1: LEGISLATION AND REGULATION OF ANTIFOULING CHEMICALS IN AQUACULTURE IN AUSTRALIA

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Note:

The information presented and interpreted in this report was accessed principally from the Australian Pesticides and Veterinary Medicines Authority (APVMA) website: http://www.apvma.gov.au/. The report is submitted in a format whereby web links embedded in the text (which are underlined) enable direct access to that information (Pdf file on appendix disc). This report is part of a review series of biofouling in aquaculture including reviews of 'Impacts of biofouling on marine finfish aquaculture' and 'Methods and efficacy of biofouling control in sea-cage aquaculture'.

EXECUTIVE SUMMARY

• **WORLDWIDE**, there are about 18 biocides currently being used as antifoulant (Konstantinou and Albanis 2004). **IN CANADA,** there are **SIX** antifouling products **for use in aquaculture** registered with the Canadian Pest Management Regulatory Agency (containing only copper/cuprous oxide, for cages and ropes and netting). **IN THE UK**, there are **TWELVE** antifouling products registered **for use in aquaculture** (all containing exclusively copper/cuprous oxide). Detailed information for other countries is difficult to obtain.

• **IN AUSTRALIA** Commonwealth legislation and regulations (Appendix 3.1 and 3.2 govern the sale, supply, distribution and use of chemicals. It is illegal to import an unregistered product.

• **IN AUSTRALIA** there are currently **NO** chemical products registered **for use in aquaculture.** There are nine registered active constituents with antifouling activity (including cuprous oxide) and **54** agricultural products registered for antifouling use (on boats etc but NOT on nets, NOR for aquaculture) (Appendix 3.3, Appendix 3.4).

• **IN AUSTRALIA**, **TWO** trial research permits for use in aquaculture have been issued, but no information about the products is currently available to the public. A permit is required to use a registered chemical in a way that is different to the use for which it was registered ie. antifouling on boats *vs* aquaculture.

• **The routine use of copper-based antifouling paint (Hempel) on nets in the salmon industry in Tasmania has been reported (Douglas-Helders *et al.*, 2003)**.

• The **Australian Pesticides & Veterinary Medicines Authority** (APVMA) is responsible for the assessment, registration and regulation of pesticides and veterinary medicines. **Both the product and the active constituent must be approved by the APVMA**.

• A comprehensive 'data package' must be submitted to the APVMA to register a new product, a new use, or a major change in formulation (see Appendices 3.7-3.9), or to obtain a permit.

• Permits are not intended to be used to circumvent the normal process of registering products and approving the uses on their labels. Permits address licensing at a different scale, or for trial research purposes, and may precede registration. Obtaining a permit requires the applicant to satisfy the same criteria as for registration.

• Technical information on the product's chemistry and manufacture, toxicology, metabolism and toxicokinetics, residues, efficacy, occupational health and safety and environmental effects must be provided. In addition, information on the potential implications for overseas trade of residues from the product or active constituent is required. Note that there are specific guidelines on describing antifouling efficacy. Less data is required to register a product that is similar to an already registered product.

• Registration and/or obtaining a permit is a complex, lengthy and expensive undertaking.

SCOPE OF THE REVIEW

Outline the process of how biocides and coatings are registered for use in the aquaculture industry within Australia, with special reference to nets and coatings.

Specifically address:

1. Current registered chemicals for aquaculture: What chemicals are currently registered or available for use in aquaculture in Australia, and how is this process regulated.

2. Permits for a new use in aquaculture: Describe the process of obtaining permits to use:

• a chemical for aquaculture if it is registered for a different use, or

• a new chemical developed specifically for aquaculture in a research trial.

3. Registering new chemicals: How to register a product available overseas for aquaculture, or a new coating/biocide developed specifically for aquaculture, or to register a major extension in use (to aquaculture) of a currently registered product.

BACKGROUND

International context

• *General antifoulants***:**

Worldwide, there are about 18 biocides currently being used as general antifoulants (Konstantinou & Albanis, 2004). In Europe, antifouling compounds are considered as biocides, and fall under the EU Biocidal Products Directive (BPD) Directive 98/8/EC which was agreed by the Member States in 1998 and implemented in the Member States in 2000. In Canada, the Pest Management Regulatory Agency regulates and lists approved antifouling products, which are restricted to copper derivatives (**antifouling.pdf** Last updated: 2004-11-01). In the USA, pesticides registration and control of use is regulated by the Environment Protection Authority (EPA).

• Legislation & regulation of aquaculture:

EU policy, regulation, control and monitoring of aquaculture are moving towards “harmonization” (EC, 1991; FAR, 1993). Subsequently, “The derivation of scientific guidelines

for best environmental practice for the monitoring and regulation of marine aquaculture in Euope" (Read *et al.,* 2001) was developed as part of the Monitoring and Regulation of Marine Aquaculture in Europe (MARAQUA) project 1999-2001. However, currently, most countries in Europe still have their own registration procedures and approvals (reviewed by Fernandes *et al.,* 2000). Information (reviewed in Fernandes *et al.,* 2000) on the legislation, regulation and control of aquaculture in Europe, including control of veterinary medicinal products and pesticides is available for:

- United Kingdom (Bell and Chadwick, 1994)
- Scotland (Henderson and Davies, 2000)
- Ireland (McMahon, 2000)
- Norway (Maroni, 2000)
- Sweden (Ackefors, 2000)
- Finland (Varjopuro *et al.,* 2000)
- Denmark (Pedersen, 2000)
- Iceland (Jonsson, 2000)
- Netherlands (Smaal and Lucas, 2000)
- Spain (Sanchez-Mata and Mora, 2000)
- Portugal (Bernardino, 2000)
- France (Dosdat and De la Pomelie, 2000)
- Germany (Rosenthal and Hilge, 2000)
- Italy (Saroglia *et al.,* 2000)
- Greece (Papoutsoglou, 2000)

There is also literature, which may now be outdated, on the regulation of aquaculture in Chile (Barton, 1997).

• Antifouling chemicals in aquaculture:

Information on what chemicals are approved for use or used in aquaculture is generally unavailable for most countries. Some information is available for most countries that are members of the International Council for the Exploration of the Sea (ICES) (Alderman *et al.,* 1994). At this time, it was reported that some banned substances were widely used, and in 1994, a Paris Commission (PARCOM) Recommendation detailed Best Environmental Practice for the reduction of "Inputs of toxic chemicals from aquaculture use" (OSPAR, 1994).

In Europe, the harmonization process means that an increasing number of actives either have been withdrawn as of 2005, or will be withdrawn from the market within the next few years. For example, in Denmark, it was reported that in the major aquaculture industry (rainbow trout *Oncorhynchus mykiss)* nets are impregnated with a copperbased antifoulant "the same as that used in ships" before the start of the season (Pedersen, 2000). It is not known if this is still current practice.

In the UK many products have been withdrawn in the past 5 years (Henderson and Davies, 2000; Costello *et al.,* 2001). Current status of antifouling products in the UK as of 2/2/2005 can be found at: http://www.hse.gov.uk/pesticides/bluebook/section03.pdf. Currently, there are **TWELVE** antifouling products registered **for use in aquaculture** with the UK Health and Safety Executive. All contain only copper/cuprous oxide:

• AQUA-GUARD, (STEEN-HANSEN MALING AS);

• AQUA NET NET-GUARD (STEEN-HANSEN MALING AS);

• COPPER NET, (STEEN-HANSEN MALING AS);

• AQUALINE, (GJOCO A/S);

• AQUALINE W, (GJOCO A/S);

• BOATGUARD, (INTERNATIONAL PAINT LTD);

• INTERCLENE PREMIUM BCA 300 SERIES (INT. PAINT LTD);

• CARMYPAINT SV-881, (CARMYCO S.A.);

• FLEXIGARD VI-II WATERBASE PRESERVATIVE, (AQUATESS LTD);

• HEMPEL'S NET ANTIFOULING 715GB (HEMPEL PAINTS LTD)

• NETREX AF (TULLOCH ENTERPRISES)

Three chemicals – metallic copper, cuprous oxide and copper thiocynate are approved for use in antifouling products by the Canadian Pest Management Regulatory Agency (PMRA) www.pmra-arla.gc.ca/english/pdf/intern/antifouling.pdf . At least **SIX** antifouling products are listed **for use in aquaculture** by the PMRA: at www.eddenet.ca/4.0/4.0.asp (containing only copper/cuprous oxide, for cages and ropes and netting):

• AQUA NET, (STEEN-HANSEN),

• NET GUARD MARINE ANTIFOULANT, (562752 BC LTD),

• SOLIGNUM EX-84 WATERBASE PRESERVATIVE NET COATING, (SOLIGNUM INC)

• FLEXGARD XI WATERBASE PRESERVATIVE, (FLEXBAR CORP)

• FLEXGARD VI WATERBASE PRESERVATIVE, (FLEXBAR CORP)

• NETREX AF MICROCRYSTALLINE WAX, (MOBIL OIL AS)

The situation in the USA is difficult to gain information on, despite a Federal Joint Subcommittee on Aquaculture publication Guide to Drug, Vaccine, and Pesticide Use in Aquaculture (2004), which lists chemicals in Appendix B. EPA-Registered Pesticides for Aquaculture/Aquatic Sites. Pesticides are listed in four tables (algicides, fish toxicants, aquatic herbicides and invertebrate toxicants), but antifouling paints are not included in these lists. In addition to regulation at the Federal level, registration also operates at the State level, and information is available on http://state.ceris.purdue.edu/ .A search of the US EPA/Office of Pesticide Program at www.cdpr.ca.gov/docs/epa/m2.htm and the Californian Department of Pesticide Regulation database available at www.cdpr.ca.gov/docs/label/labelque.htm, reveals 96 and 129 products with antifouling in the product name respectively, although it is not possible to determine whether these may be used in aquaculture. However, a search for products listed for use in aquaculture in Canada (above) in the USA revealed that FLEXGARD XI WATERBASE PRESERVATIVE, (FLEXBAR CORP) and FLEXGARD VI WATERBASE PRESERVATIVE, (FLEXBAR CORP) are registered for use on boats (but not in aquaculture). In addition, FLEXGARD II WATERBASE WIRE TRAP/CRAB POT ANTIFOULING PAINT is registered in the USA. Registration for the other products was not found.

Antifoulants are biocides and are not directly used on food-producing fish. Thus they do not fall under the maximum residue limits (MRL) system. However, where used, fish are exposed to antifoulants for months. There have been scientific studies on the impact of treating nets with copper-based antifoulants in salmon aquaculture for both Flexgard XI® (Brooks, 2000) and ®Americoat 675 (not currently registered by the EPA) (Peterson *et al.*, 1991) in North America, measuring insignificant heavy metal contamination of fish flesh or the environment with copper. Similar results were reported in farms using Aqua-net, Cu-net and Netrex in Norway (Solberg *et al.*, 2002). Since copper from Flexgard XI® treated nets was released into the environment at an exponential rate of 155 µg Cu/cm² until reaching a long-term rate loss of 37.6 µg Cu/cm² (Brooks, 2000; Brooks and Mahnken, 2003) current practice is to introduce fish in nets one month after newly coated nets are in position to minimise bioaccumulation.

Copper and zinc, the major active components of antifouling coatings, are broad spectrum metal-based toxins, and are listed under the EU Dangerous Substances legislation. As such their release to the environment requires control, and their use may be controlled under discharge permits. These give rise to new environmental concerns from the elevated concentrations of copper found in sediments around these farms (Miller, 1998), and the potential for both the copper and booster compounds to inhibit primary production in the surrounding waters. In addition, consumer concerns can jeopardize "clean and green" market image. Most countries are now working towards a reduction in the use of copper-based antifouling in the short-term.

REGULATION IN AUSTRALIA

Current registered chemicals for aquaculture:

What chemicals are currently registered or available for use in aquaculture in Australia, and how is this process regulated.

Current situation

There are currently no chemicals registered for use to prevent biofouling in aquaculture in Australia.

Two trial research permits have been issued, but no information about the products to the public at the current time (Colin Burns, APVMA, pers. comm.) (see section 2 for information about the process of obtaining permits). You can search for existing permits online at www.apvma.gov.au/permits/permits.shtml

Nevertheless, research over a decade ago has shown that copper-based and silicone-based antifoulants delay microfouling development on salmon-cage netting in Tasmania (Hodson and Burke, 1994). Furthermore,it has been reported that "to reduce biofouling on nets, antifouling paints are commonly used on Tasmanian salmon farms" and the effect of copper-based antifouling paint (Hempel paint, NSW, Australia) in Tasmanian salmon aquaculture has been investigated (Douglas-Helders *et al.*, 2003).

Process

Commonwealth legislation and regulations (Appendix 3.1 and 3.2) govern the sale, supply, distribution and use of chemicals. Please refer to Appendix 3.1 and 3.2 for a summary of the Acts and Statutory Rules, and see http://www.apvma.gov.au/about_us/legislat.shtml for a thorough discussion of the current legislation and regulations.

The Australian Pesticides & Veterinary Medicines Authority (APVMA) (formerly the National Registration Authority) is responsible for the assessment, registration and regulation of pesticides and veterinary medicines. This body evaluates the safety and performance of chemical products for intended sale/application, and monitors the market for compliance. ***In addition to the product, the active constituent must be approved by the APVMA.*** Variations to the formulation, new patterns of use (i.e. for aquaculture) and new labels must also be approved. It is illegal to import an unregistered product into Australia.

Products classified as antifoulants are agricultural chemical products, defined as "any substance or organism used to:

- destroy, stupefy, repel, inhibit the feeding of, or prevent pests on plants or other things;
- destroy a plant or to modify its physiology;
- modify the effect of another agricultural chemical product; or
- attract a pest for the purpose of destroying it."

For a legal definition of what does/does not constitute an agricultural chemical product refer to the Agricultural and Veterinary Chemicals Code (the Agvet Code), scheduled to Agricultural and Veterinary Chemicals Act 1994**,** and the Agricultural and Veterinary Chemicals Code Regulations (no. 27 of 1995) (Appendix 3.1 and 3.2).

The APVMA only registers agricultural and veterinary chemical products. Industrial chemicals are registered through the National Industrial Chemicals Notification & Assessment Scheme, whereas drugs and pharmaceuticals are registered through the Therapeutic Goods Administration. The regulating associated with the registration of chemical products is designed to ensure that they are safe, effective, and environmentally benign. In addition, the process ensures that chemical products do not appear at unacceptable residue levels in foodstuffs.

Requirements, guidelines and manuals are described in detail and are available on the AVPMA website www.apvma.gov.au. A synopsis of registration of agricultural chemical products for applications to the aquaculture industry, based on the information available on the APVMA website, is presented in section 3.

<u>Permits for a new use in aquaculture:</u>

Describe the process of obtaining permits to use:

- a chemical for aquaculture if it is registered for a different use, or
- a new chemical developed specifically for aquaculture in a research trial.

Current situation

Two research permits have been issued for use in aquaculture, but no information is available from the APVMA.

There are 54 agricultural chemicals registered for antifouling use (for boats etc but NOT on nets, NOR for aquaculture) in Australia (Appendix 3.3). A summary table of registered active constituents with biofouling activity, derived from this list, is given in Appendix 3.4.

These chemicals have potential for use in the prevention of biofouling on nets in the aquaculture industry. Only products based on copper/cuprous oxide are registered for use in aquaculture in the UK and Canada, although other antifouling chemicals may be registered in these countries (Appendix 3.4). A number of the products listed in Appendix 3.3 are based on cuprous oxide (eg. 42603, 46921, 48965, 49610, 52864, 52961, 54048). The use of copper-based antifouling paints on nets in the Tasmanian salmon industry has been reported (Hodson and Burke, 1994; Douglas-Helders *et al.*, 2003). While these products may be candidates for permit applications, there are concerns about the residue levels and toxicity of heavy metals such as copper, particularly in close proximity to foodstuffs for human consumption. However, the chemicals listed below are in the APVMA MRL standard Table 5 where maximum residue limits are **not** necessary":

- copper oxide (antifouling treatment of nets in aquaculture)
- sea-nine 211 (antifouling paint on nets in aquaculture)
- zinc oxide (antifouling treatment of nets in aquaculture)
- zinc pyrithione (antifouling treatment of nets in aquaculture)

Process

A permit is required to use a registered chemical in a way that is different to the use for which it was registered ie. antifouling on boats vs aquaculture. **Obtaining a permit requires addressing the same criteria as the registration process.** See the APVMA Factsheets on permits and related information.

The process is complex and contains a number of different types of permits. To assist the reader in determining which permit category is relevant to his or her particular needs, a decision tree flowchart is reproduced from the APVMA as a guide (Appendix 3.5).

"A comprehensive 'data package' must be submitted to the APVMA to register a new product, a new use or a major change in formulation. Less data is required to register a product that is similar to an already registered product. A data package must supply information which demonstrates that the product:

- will be effective for the all uses described on the proposed label;
- will be safe to humans, target and non-target species; and
- will not pose unacceptable risks to the environment or trade with other nations" (APVMA, 2005).

"Technical information on the product's chemistry and manufacture, toxicology, metabolism and toxicokinetics, residues, efficacy, occupational health and safety and environmental effects must be provided. In addition, information on the potential implications for overseas trade of residues from the product or active constituent is required." (APVMA, 2005). Documentation and links to specific data requirements are given

below and are presented in summary in Appendix 3.6. See also “Use of a registered product”, below.

'Control of use' legislation

Legislation in each State/Territory dictates how registered products can be used *other than the uses specified on the approved label* and these uses termed **off-label use**. Permits issued to allow the use of a registered product contrary to the approved label are termed **off-label permits**. Differences between State legislation mean that an off-label use may require a permit in one State, but not require a permit in another. State Coordinators or the APVMA can confirm whether a permit is required for a given off-label use.

Role and types of **off-label permits**, and application forms

Off-label permits are not granted to evade the normal registration process, but to provide a mechanism for approving uses in the following situations:

• a minor use - of a product in a situation on a small scale; A ‘minor use’ as defined in legislation is “*a use of the product or constituent that would not produce sufficient economic return to an applicant for registration of the product to meet the cost of registration of the product, or the cost of registration of the product for that use, as the case requires (including, in particular, the cost of providing the data required for that purpose)*”. See also Guidelines for Determining Minor Uses. Minor use permit approvals are generally restricted to currently registered products (since the relevant scientific data has been accumulated and assessed during the registration process).

• an emergency use - such as outbreaks of contagious diseases or exotic pests for which no registered product exists; (Application form for minor use and emergency use Download).

• research purposes – to screen and generate data that supports product registration. (Application form for trial use permits Download).

Permits issued to allow the use of a registered product contrary to the approved label are termed “Off-Label Permits”, those issued to allow the use of an unregistered product are called “Supply/Use Permits” and those issued to conduct a trial are termed ‘Research Permits’. The APVMA will not grant permits if there is a registered product currently available for that purpose.

Use of registered products

“When a permit covers the use of a registered product there is generally no requirement to submit toxicology, metabolism, and chemistry and manufacture data. A registered product has already been assessed to ensure that:

• it contains an active ingredient that has been assessed in regards to human toxicology and has received appropriate poison scheduling; and

• has had its full formulation assessed in regards to stability and toxicology; and

• has had its specific uses assessed in regards to OH&S and safety to the environment; and

• if it is used on food crops or animals, it has had the appropriate maximum residue limits (MRLs) established plus trade considerations assessed; and

• contains an approved label which includes a use pattern plus appropriate first aid and safety directions" (APVMA, 2005).

For a permit application using a registered antifouling product, if the pest was identical to the label, but the hosts were different (ie food *vs* boats), the data required to support an application as detailed in Appendix 3.6 (APVMA, 2005) would include:

• **Residues & Trade**

• **Environment**

• **Efficacy and host safety**

• **OH&S**

The application process

Any suitable person or organisation can apply for a permit, but in the case where an offlabel use of a registered chemical is required, the main grower organisation would be the most suitable permit holder.

The application comprises a completed application form and detailed information on the chemical product and its use including:

• what products are to be used;

• how the products are to be used;

• where they are to be used; and

• who will use the products

See the APVMA Factsheets on permits and related information. Permit assessment is done on a fee for service basis and vary according to the amount of technical assessment and consultation required, which reflects the complexity of the situation.

Registering new chemicals:

How to register a product available overseas for aquaculture, or a new coating/biocide developed specifically for aquaculture, or to register a major extension in use (to aquaculture) of a currently registered product.

Registration procedure

Registration is a complex undertaking. Industry consultants may provide detailed advice. A list of consultants is available from AVcare Australia (National Association for Crop Production and Animal Health) at http://www.avcare.org.au/default.asp?V_DOC_ID=951 and a current list is provided as Appendix 3.7.

An application to register an agricultural chemical product requires

• the application form (available on the forms page)

• assemblage of a complete data package. The data required to support an application for a product are explained in the Guidelines (available at guidelines and other publications), and include the Ag Manual, Ag Requirements Series, and the Ag Guidelines.

"A data package must supply information which demonstrates that the product:

• will be effective for the all uses described on the proposed label;

• will be safe to humans, target and non-target species; and

• will not pose unacceptable risks to the environment or trade with other nations"

(APVMA, 2005).

"Technical information on the product's chemistry and manufacture, toxicology, metabolism and toxicokinetics, residues, efficacy, occupational health and safety and environmental effects must be provided. In addition, information on the potential implications for overseas trade of residues from the product or active constituent is required" (APVMA, 2005). **Note that there are specific guidelines on describing antifouling efficacy.**

The registration process accommodates 40 categories of chemical product.

Documentation and links to specific data requirements are given for three categories of product which are likely to be applicable to registering a chemical product for aquaculture:

1. **New agricultural chemical product - primary application (cat 1)**

• for a new chemical product, containing one or more new active constituents, not previously approved or registered in Australia (see Appendix 3.8)

2. **New product, approved active constituent, new situation (cat 14)**

• for a new product, where there is already a registered product with the same active constituent, used in a new situation (see Appendix 3.9).

3. **Variation to registered product: Major extension of use (cat 32)**

• includes all extensions to a new host or situation (eg. boats to nets); (or those involving a higher rate or frequency of use) (see Appendix 3.10).

All applications should be accompanied by the relevant application fee. For example,

• the fee for a new active (cat 1) is $20,620 currently, with a 15 month assessment period.

• Registration of a new product, approved active constituent, new situation (cat 14) costs $12,370 with an 8 month assessment period.

• A variation to registered products involving a major extension of use (cat 32) costs $10,310 with an 8 month assessment period.

Submissions undergo assessment prior to approval (see assessment of applications), where the APVMA "takes full account of the nature of the product, the amount and completeness of the data for review, and the extent of consultation required between the

APVMA, manufacturers, advisory agencies, and State and Territory departments" (APVMA 2005).

In addition, a draft label conforming to the current code of practice for labelling (Ag Labelling Code) must also be approved before product registration.

Post-registration

The APVMA (NRA) Approval Number must be displayed on all labelling for the product.

The APVMA must be informed of:

• any adverse experiences, or any information that indicates that the product may have an unintended harmful effect.

• any significant change in the chemical characteristics or the performance of the product, and what action they propose to take to correct this.

Registrants must renew their registration annually by 30 June. Renewal fees as permitted under the Agricultural and Veterinary Chemicals Code and the Code's Regulations, are based on a product's disposals for the previous *calendar* year.

In addition, the APVMA imposes levies on disposals of registered Agvet chemical products. Levy rates are based on a product disposal for each calendar year and are payable on a product's gross sales, exclusive of sales tax.

Chapter 4 : SUB-PROJECT 2: FIELD EVALUATION OF VARIOUS ANTIFOULING TREATMENTS UTILISING PANELS IN THE LOCAL TUNA RANCHING ENVIRONMENT OF SOUTH AUSTRALIA

This chapter was authored by Kirsten Rough (Australian Southern Bluefin Tuna Industry Association) and may be cited as:

Rough KM, and Ellis DC, (2007). Evaluation of antifouling treatments utilising panels in the local tuna ranching environment of South Australia. In Rough KM, deNys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp.

ABSTRACT

This study used 0.5m² panels of white 150mm (stretch mesh) nylon tuna netting to investigate the efficacy of 3 types of antifouling treatments for reducing biofouling growth over a typical tuna farm season (6-7 months). Panels were deployed at 2 and 9m depth in the farming area of lower Spencer Gulf, South Australia. The products tested were non copper based (this was the market preference as antifoulants have not been used so far in this industry), and currently available (through research or trial use permits) within Australia. Treatments included sheep wool grease Lanolin (Lanotec™), latex with booster biocide Sea-nine 211 (Net Clear™), and a paint containing the heavy metal zinc oxide with booster biocide zinc pyrithione (Net Clear ZPT™).

The application of antifoul coatings significantly increased the pre-deployment dry weight of all groups of treated panels, as well as altering the pliability and handling properties of the net. The coated panel groups decreased in weight for up to 64 days post deployment; beyond this time biofouling growth was apparent and therefore ongoing weight loss could not be determined.

Both the latex and the paint, Net Clear and Net Clear ZPT were significantly effective at delaying the onset of and at reducing the overall amount of biofouling growth at depths of 2 and 9m compared with untreated net panels through all sample times ($P<0.05$). A maximum dry weight increase of 536%, 1.29±0.45kg (mean ± standard deviation) due to biofouling growth occurred 126 days post deployment in the untreated group at 2m depth. Maximum mean dry weight gains of antifoul treated groups at 2m depth were 1.55±0.81kg (449%) for Lanotech; 0.05±0.03kg (12%) for Net Clear; and 0.06±0.04kg (15%) for Net clear ZPT (mean ± standard deviation). Maximum weight gain at 9m depth occurred at 126 days with the untreated control group increasing by 185%, or 0.44±0.15kg (mean ± standard deviation).

Occlusion values were derived from photographs and image analysis of the net meshes. These had little relationship to the dry weight gain of panels ($R^2 = <0.6$). However these analyses did demonstrate that Net Clear and Net Clear ZPT effectively reduced the potential problem of fouling growth restricting water flow.

Coating nets with Net Clear and Net Clear ZPT was found to maintain or improve the tensile strength of the netting irrespective of deployment time or depth. Lanotec significantly reduced the breaking strain of meshes compared with new and untreated net.

INTRODUCTION

Biofouling of aquaculture nets is the settlement and establishment of various biological organisms (bacteria, hydroids, micro and macro alga, invertebrates, ascidians, molluscs etc). It is a sequential event in that once the primary film of microscopic organisms is established; macroscopic organisms can colonise and flourish (Cheah and Chua 1979; Hodson and Burke 1994; Wahl 1989). The growth of the macroscopic organisms can become so dense that the mesh size of the net is effectively reduced or totally occluded (Hodson et al 1995; Svane et al 2006).

Fouling is known to impact fish farming in the following ways: the significant impediment of water flow decreases the supply of dissolved oxygen to the caged fish; the increased weight of the net and resistance to clear water flow increase structural fatigue of cage and anchor infrastructure; and fouling assemblages may harbour disease-causing organisms (Aarsnes et al 1990; Kent 2000; Braithwaite and McEvoy 2005; de Nys et al 2005a). The significance of biofouling in the tuna aquaculture industry is similar to that in other mariculture industries as it potentially influences the tuna's productivity and management's operating strategies.

Within the tuna industry antifouling trials on panels began in late April 2000 with Stolt Sea Farm deploying 6 panels at 3m depth for a period of 4 months in the tuna farming zone of lower Spencer Gulf (David Ellis pers comm.). Old and new untreated white knotless nylon net with the same netting type were coated with one of the following: a silicone net stiffener (Wattyl Net Safe), single and double applications of latex with sea-nine 211 (Wattyl Net Clear) and lanolin (Lanotech). At the completion of panel deployment in August the controls and the various treatments were visually compared. From photographs, treatments can be ranked as Lanotech (least fouled), then Net Clear double coat, Net Clear single coat and new untreated (equal), then Net Safe, and the old untreated net was most fouled.

In 2002 an antifoul treatment (Wattyl Net Clear, latex with Sea-nine 211) was tested on entire tuna nets at the research farm site inside Boston Bay (Svane et al 2006). Results of this trial showed that the fouling load on treated nets was reduced by 14.7% after 6 months deployment (February to July).

Further panel trials, were undertaken by the M.G. Kailis Group through 2002 and 2003 in the tuna farming zone of lower Spencer Gulf. These were primarily to refine and improve the formulations of Wattyl Net Clear and another water soluble product Wattyl Net Clear ZPT; by altering inclusion ratios and release rates of the active ingredients isothiazolinones (Sea-nine 211), zinc oxide and zinc pyrithione. Lanotech and untreated new net were included for comparison with previous trials. At all sample intervals panels coated with Wattyl products performed better than lanolin or untreated panels; but there were obvious differences in biofouling growth among the panels provided by Wattyl (pers. obs. 2003).

The present subproject aimed to robustly test the efficacy of antifoul treatments in the local environment where tuna are currently ranched (lower Spencer Gulf) by using net panels to field test the best currently available products. Including the time for which the antifoul treatment prevents or reduces fouling on the net, and also its impact on net strength and weight. The treatments tested included both physical and chemical deterrents to biofouling settlement and establishment, and did not include products containing copper. This subproject addresses objectives 2 and 4 of the overall project 2003/226.

METHODS

Experiments

2004

A new, 120m² piece of white Badanotti 150mm (stretch mesh) knotless nylon netting was sectioned into 240 panels. Each panel measured 0.6 by 0.9m (8 by 12 meshes) with an approximate area of 0.54m². All panels were individually weighed prior to labelling and application of antifoul treatments. Panels were reweighed after identification tags were attached and treatments were applied. Groups containing 48 panels each were treated with Lanolin, Net Clear™ (a latex coating containing booster biocide Sea-nine 211), or left untreated as controls; and two groups were treated with Net Clear ZPT™ (a paint containing the heavy metal zinc in the form of zinc oxide and booster biocide zinc pyrithione). The panels were sent to Wattyl for treatment with Net Clear ZPT in two batches, one was dipped onsite at their laboratories in NSW and the other at the commercial dipping site of Nets Pty Ltd in Tasmania. The first set to arrive back at Port Lincoln was from NSW and was immediately deployed to start the aging process. In the meantime the second set had been forwarded to Tasmania for dipping alongside a commercial salmon net. This latter set was first deployed in 2005.

To determine whether a single application of the water soluble antifoulant, Net Clear ZPT, would be effective over two farming seasons, one group of labelled panels were deployed in 2004. These panels were suspended in the marine environment for 114 days from July to October; at a depth of 2.5m. Panels were retrieved, air-dried and stored with the other groups of panels until redeployment in 2005. This group were identified as second season or "Aged ZPT" in the 2005 trial.

Water temperature at each depth was recorded every hour increments with Vemco data loggers throughout the panels deployment.

2005

Net panels prepared in 2004 were randomly assigned a position along four 48m rope frames (appendix 4.1); so that 120 panels (24 of each treatment type) were suspended at 2m depth and 120 at 9m depth. The rope frames were of sufficient length so that a 20cm gap was present between net panels to allow unimpeded water flow (Plate 4.1 and Plate 4.2; and Appendix 4.1 has full specifications). Panel lines were deployed so that panels had a northerly aspect to maximise biofouling growth by being well illuminated (Cronin et al 1999).

Plate 4.1: Stitching panels onto the rope frames for subproject 2

Plate 4.2: Close-up of panel lines showing the gap between individual panels to allow free water flow.

Panel lines were deployed on the 9th March, within the tuna farming zone but not on a lease site that was stocked with tuna. Panels were retrieved progressively along the lines according to the schedule detailed in appendix 4.1. Retrievals occurred approximately monthly (weather dependent) on 13th April, 12th May, 15th June, 13th July, 11th August and 27th September. Four panels from each treatment and depth were retrieved at these sample intervals; the sequence of retrieval is shown in appendix 4.1. As the lease site was approximately 1.5 hours from shore, retrieved panels were cut from the rope frames and suspended in a bin with gently flowing fresh sea water on the vessel while being delivered to the Tuna Boat Owners Association research facility onshore.

Upon arrival at the laboratory, panels were individually placed in a water bath to allow fouling to sit naturally, and were then photographed. Panels were hung until dripping ceased, to obtain a wet weight, and then placed individually into a pre-weighed, labelled cotton bag for air- and later, oven drying to obtain a dry weight. To oven-dry, bags were suspended in a car

crash repairers' commercial baking oven at 60ºC for 24 hours, (as per Norberg 1999) (Plate 4.3).

Plate 4.3: Panels suspended individually in bags within a commercial baking oven to dry at 60ºC for 24 hours, (pictured is Danielle Foote)

Net panels were assessed for percentage of fouling occlusion of meshes, by photography and image analysis. The digital photographs were cropped so that the net area in the resultant image was a uniform 3 by 3 meshes using Sony "picture motion browser" software. Images were further modified to reduce water surface reflection and maximise the contrast between the background and the fouling on the netting. These modified images were subjected to count/measure analysis using Image-pro software to obtain the area of the image occupied by the background (hence area allowing 'free water flow' through the net). Several images required further filters to be applied from the image pro software, to reduce the distorting effect of shading within some photos. A general assessment of the relative proportions of hard and soft fouling was made visually from the cropped photos.

Net strength of dry panels deployed for 98 and 202 days was determined using a tensiometer at a commercial net making premise, Quinn Marine, Port Lincoln.

Water temperature at each depth was recorded every hour with Vemco data loggers throughout the panels' deployment.

Data analysis

Data sets were assessed for normality using the Shapiro-Wild W test and homogeneity of variance by Cochrans test. Differences between treatments, depth and time were tested by analysis of variance (ANOVA). If the results of ANOVA were statistically significant, Fischer LSD test was used to assess which means were different. Percentage data were arcsine transformed prior to analyses. The results were considered statistically significant if $P<0.05$. Statview software was used for statistical analysis.

RESULTS AND DISCUSSION

Panels treated with Net Clear ZPT and deployed in 2004 had no macroscopically visible fouling growth when removed from the water after being submerged for 114 days. Panels deployed for this period decreased in weight by 19.6 ± 1.5 grams for the pre-labelled group to be deployed at 2m depth in 2005 and 17.5 ± 5.1 for those to be deployed at 9m in 2005 (mean ± standard deviation; Figure 4.1). The weight loss was statistically significant at both depths. There was no change in the weight of panels that remained dry in storage for this time; indicating that leaching occurred from the coating once submerged in the marine environment. Water temperature ranged from 12.2 to 19.7ºC through this time (Figure 4.2).

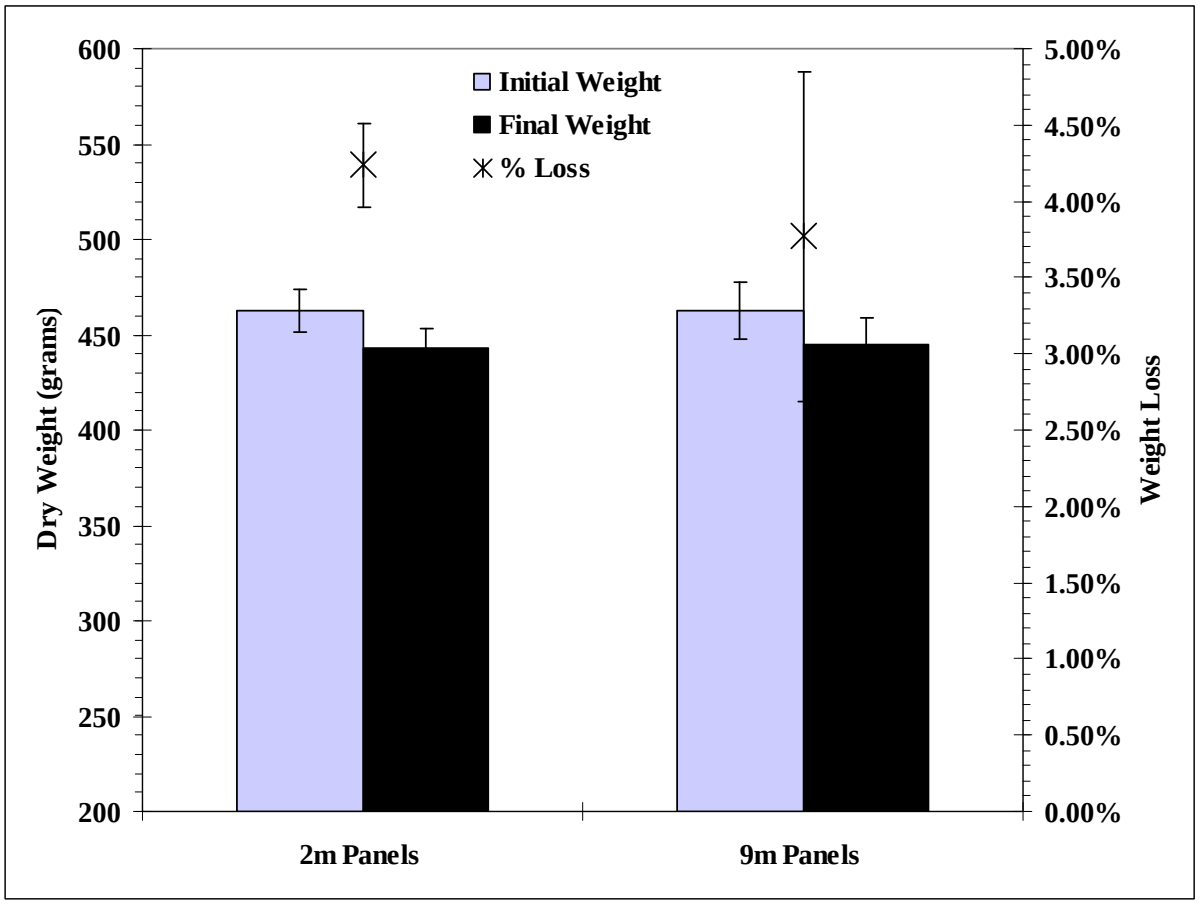


Figure 4.1: Dry weight and percentage weight loss of panels treated with Net Clear ZPT that were deployed at 2.5m depth for 114 days during 2004 (mean ± standard deviation)

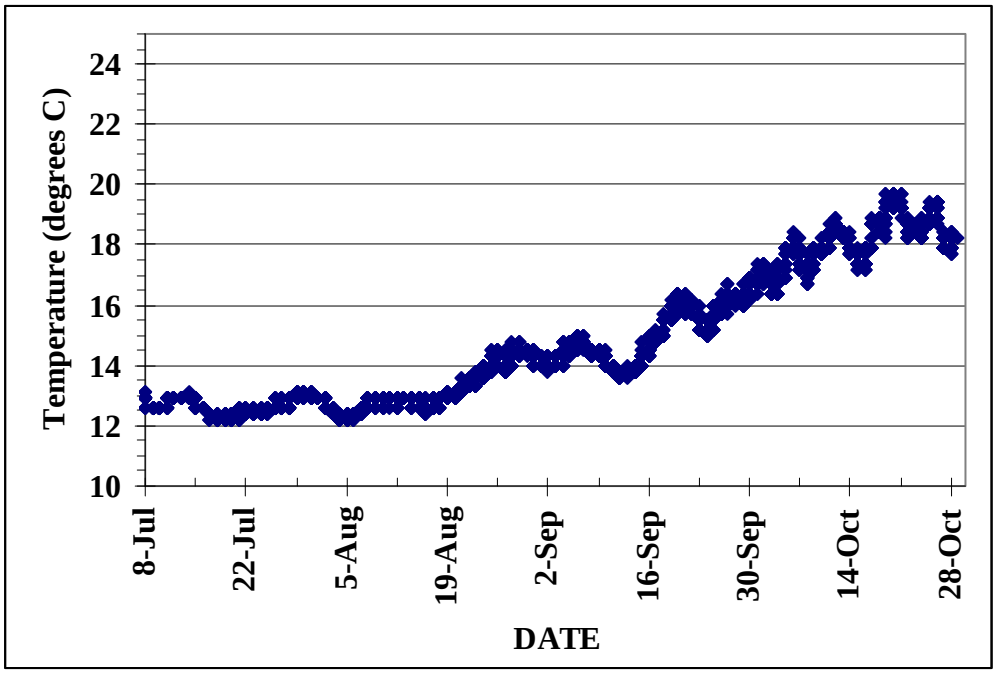


Figure 4.2: Daily water temperature recorded hourly during the panel deployment of 2004

All panels treated with an antifouling product increased in weight after the application of treatments (Figure 4.3). The antifoulant containing the heavy metal zinc had the highest weight gain after treatment: increasing by 182.87 ± 22.01 grams (mean ± standard deviation) for panels dipped commercially in Tasmania or 218.32 ± 13.53 grams for panels coated at the laboratory in NSW (gains of 75.8% and 89.5% respectively). The difference in panel weights between dipping locations post treatment was statistically significant ($P<0.05$) and could indicate that the laboratory treatment had either a higher inclusion of zinc oxide in the mix, a longer submergence time, or a second dip (details were not supplied). Despite the laboratory treated panels losing weight after the initial deployment, the start weights of the aged ZPT treated panels in the 2005 trial were still significantly higher ($P<0.05$) than those for the fresh treatment (commercially dipped in Tasmania). Panels treated with Net Clear latex coating increased in weight by 123.99 ± 9.2 grams, or 50.9% and those with lanolin by 100.27 ± 8.88 grams, or 41.3% (mean ± standard deviation).

In a commercial or farming situation the increase in weight due to coating a net with antifoulant could be beneficial in helping the net to 'settle and hang well' at an earlier time post deployment. In current farm practice once nets have a moderate amount of fouling growth together with suitably heavy lead line and counter weights, the walls hang vertical regardless of tidal conditions. An under-weighted and clean net can billow inwards with water movement from tidal flow and/or the swimming tuna. A net wall billowing inward decreases the internal volume of the net available to the fish and effectively increases the stocking density. When an antifouling treatment is used there may be a requirement to adjust the amount of counter weights applied to the net to ensure that the net shape retains its integrity. Clean nets allow the farm manager to have greater control over net shape and consequently the distance between the base of the net and the seafloor.

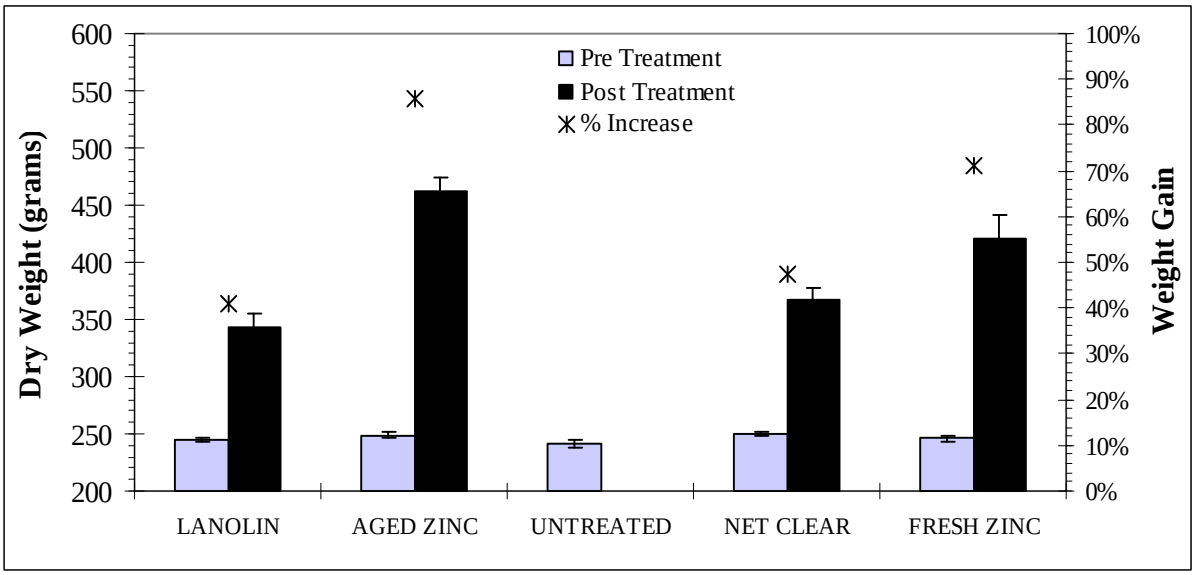


Figure 4.3: Weight of panels before and after treatment with antifoulant (mean ± standard deviation) and the average percentage increase in weight

Net characteristics following treatment

Lanolin gave the white net an oily appearance but it remained the most flexible of all types trialled; the handle-ability of these panels was comparable to untreated new netting. However this treatment does not dry and retained it's very greasy feel even after deployment. Dust and dirt readily adhered to the surface, changing the net colour during the period prior to deployment (Plate 4.4, Plate 4.5, Plate 4.6). This sticky grease easily rubbed onto any surface the net came in contact with, and required a detergent to remove it. An entire net treated with this product would require special handling at the factories and on the boats as concrete flooring or vessel decks would potentially become very slippery and an occupational hazard to the workers upon them.

The latex, Net Clear gave the panels a slight yellow hue and a pliable rubbery feel, the yellow colour became more apparent after panels were submerged, but unfortunately no photographs were taken at the time of deployment to demonstrate this in the report.

Net Clear ZPT paint turned panels a bright white and made the netting very stiff. This stiffness has proven advantageous to salmon growers as a deterrent to subsurface seal predation. But it does have the disadvantage that the netting requires nearly twice the space for storage (a point that needs to be considered particularly for transport to and from the dipping site in Tasmania). Furthermore the lack of pliability probably resulted in the cracking and dislodgment of paint flakes in transit, observed in panels with this treatment (Plate 4.7).

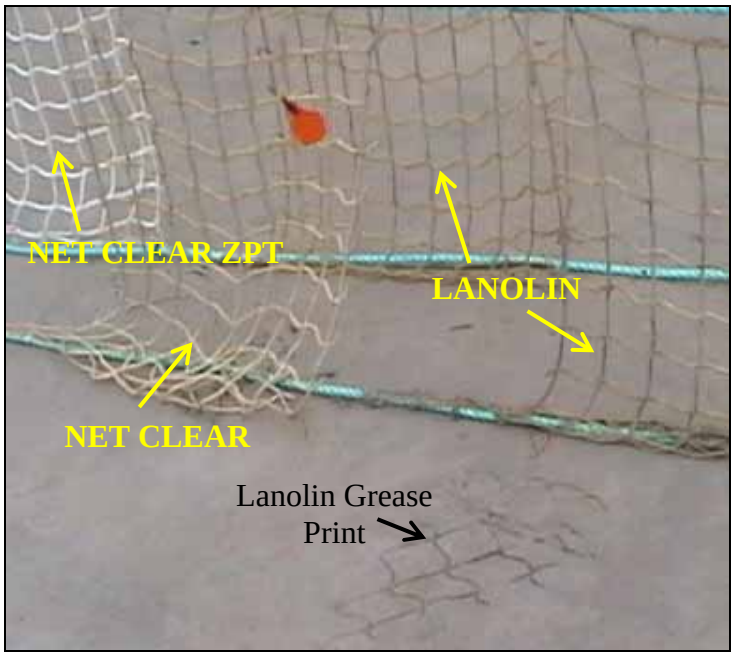


Plate 4.4: Close-up photograph of a section of two panel lines showing three treatment types and a contact grease print from the lanolin panel on the concrete in the foreground

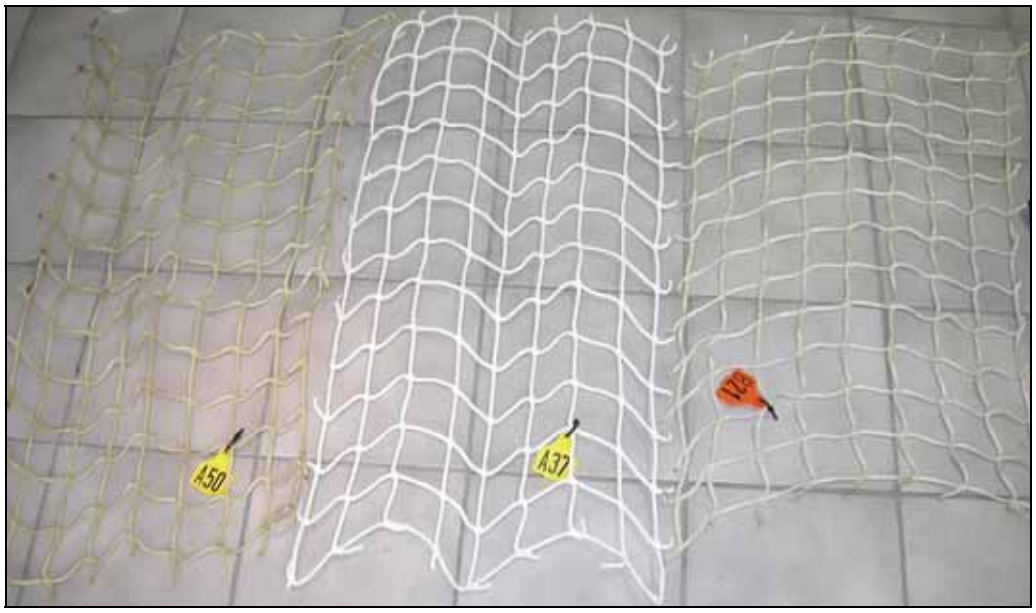


Plate 4.5: Comparison of treated panels, A50 is untreated; A37 is coated with Net Clear ZPT; and B21 is treated with lanolin (prior to any handling)

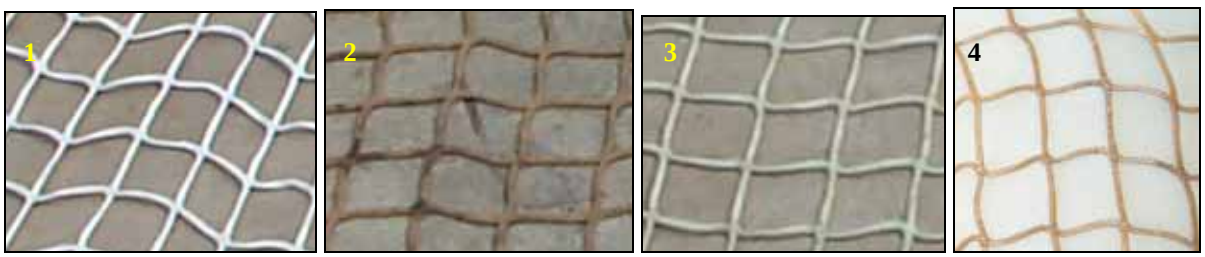


Plate 4.6: On the same background: 1. Net clear ZPT; 2. lanolin after handling; 3. and 4. Net clear on a grey and a white background to demonstrate the yellowish hue

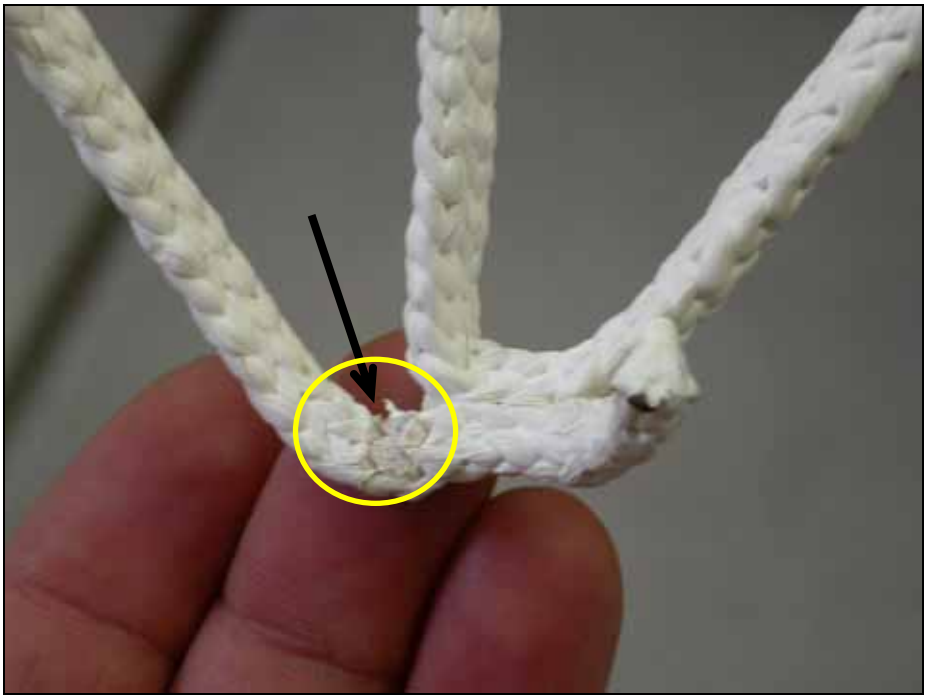


Plate 4.7: Cracking and flaking of paint on a panel coated with Net Clear ZPT prior to deployment

Biofouling weight

The efficacy of the various antifoulant types in inhibiting the colonisation and growth of biofouling varied with treatment type, depth of panels and their time submerged in seawater (comparative data in Figure 4.4 and Figure 4.5; actual data in Figure 4.6 to Figure 4.10 and Table 4.1 and Table 4.2). Wet weights of panels were difficult to obtain consistently, especially once biofouling was established; therefore only dry weight data are presented in this report. Generally panel weight increase due to biofouling growth was greater at the shallower of the two depths tested, and at both depths was greatest at 126 or 155 days post deployment.

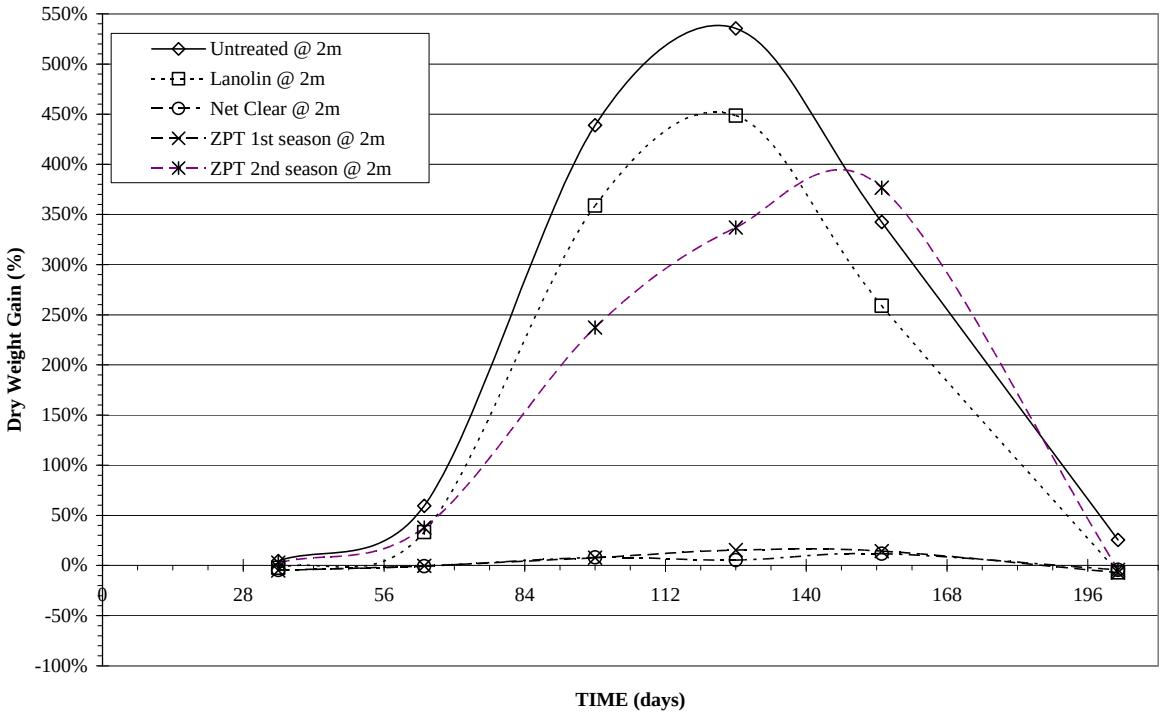


Figure 4.4: Comparative average percent weight increase of each treatment with time, for panels deployed at 2m depth

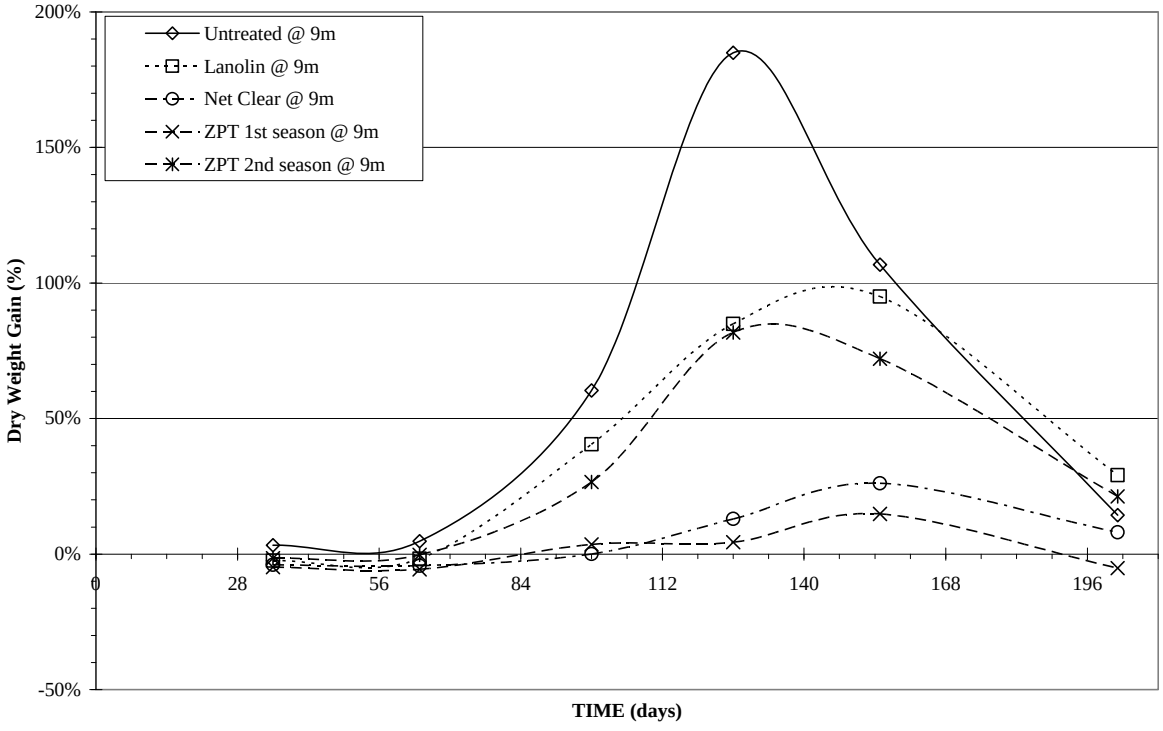


Figure 4.5: Comparative average percent weight increase of each treatment with time, for panels deployed at 9m depth

At two metres depth, differences in dry weight gain were statistically detectable between some treatments at 35 days post deployment (Table 4.1). This was principally due to the fact that the lanolin, Net Clear and Net Clear ZPT (1st season) treatments initially lost

weight. The latter two of these continued to lose weight at the second sampling, which was 64 days post deployment. The weight loss of treated panels was probably due to progressive weathering and leaching of constituents within the coating.

At 64 days, biofouling growth was evident on untreated panels as well as those dipped with lanolin, and with the second season Net Clear ZPT (Appendix 4.2). The weight gain of untreated control panels and of those treated with lanolin deployed at 2m depth was statistically different on only two occasions, the first and the final sample intervals (Table 4.1). The maximum weight gains for these two treatments occurred 126 days post deployment when the 0.5m² panels had increased in dry weight by 1.29 ± 0.45 and 1.55 ± 0.81 kg respectively (mean ± standard deviation). This weight of biofouling growth constituted a 536 ± 190 % weight increase for untreated panels and 449 ± 243 % for those treated with lanolin (mean ± standard deviation).

After the July sample interval (126 days post deployment), the weight of fouling on lanolin and untreated panels began to decline. The photographs show patchy removal of growth at this time (see Appendix 4.2). It is likely this occurred as a result of grazing by migrating fish (leatherjackets) and that the larger clumps of fouling became detached and fell off due to their weight. The latter scenario was probably compounded by the rope frame design as this would be more flexible and mobile than an entire net wall. After removal of mature fouling assemblages, further re-colonisation and growth would be slower due to the lowered water temperature and / or illumination at this time of year (Figure 4.13) (Bond 1992; Cronin et al 1999). At the final sample interval the panels were practically clean. This is most likely the result of fish grazing in combination with the dislodgement of heavy fouling clumps due to the unusually strong wind events experienced through the two weeks prior to panel collection; and the prevailing cool water temperatures, reduced illumination and shorter photoperiod through winter would have slowed re-colonisation and growth.

The second season Net Clear ZPT treated panels deployed at 2m depth demonstrated a similar pattern of weight gain to the untreated control panels and were statistically heavier at 155 days post deployment. At this time panels had gained 1.69 ± 0.3 kg dry weight of biofouling and had increased in weight by 377 ± 72 % (mean ± standard deviation). The maximum weight increase for this treatment was reached at this time. At the final sample collection panels in this treatment were lighter than at the start of the experiment this was most likely due to the combination of weathering and leaching of product, as well as fish grazing, fouling dislodgement, weather, water temperature and light as discussed previously.

Through all sample intervals the Net Clear and first season Net Clear ZPT panels, deployed at 2m depth, had weight gains that were statistically much less than those of the untreated panels (Table 4.1). Therefore these treatments will be compared to each other rather than to the control. At 2m depth these two treatments were statistically different from each other on only one occasion. At 126 days post deployment the Net Clear ZPT panels had significantly heavier biofouling growth. For the zinc based antifoulant Net Clear ZPT (first season use) the maximum weight gain due to biofouling growth occurred at 126 days post deployment, when panels had increased in dry weight by 0.06 ± 0.03 kg or 15 ± 9 % (mean ± standard deviation). The maximum weight gain due to biofouling growth on latex Net Clear treated panels was reached at 155 days post deployment when they had increased in dry weight by 0.04 ± 0.03 kg or 12 ± 7 % (mean ± standard deviation). Both of these groups of panels were lighter at the final sampling than at the start, due to the processes discussed previously.

Weight gain due to biofouling in all treatment groups (except Net Clear) was substantially lower on panels at 9m depth, compared to 2m (Table 4.1 and Table 4.2). The untreated panels had significantly less biofouling growth at 9m depth in all but the first and final sample intervals. Despite large variation between individual panels, the lanolin treated

group had significantly less growth at 9m depth 64, 98 and 126 days post deployment. With Net Clear there was generally less weight gain due to biofouling growth at 2m depth than at 9m depth. There was statistically less growth at 98 and 126 days post deployment; with the large variation between panels at 9m depth probably obscuring a statistical difference at 155 days (Figure 4.9). First season Net Clear ZPT showed little difference in fouling between 2 and 9m depth, however at the 64 and 126 day sample intervals the panels at 9m were significantly lighter than those at 2m depth. Second season Net Clear ZPT had significantly more growth on the panels at 2m depth at all sample intervals, except the final one where the variation between panels at 9m probably obscured any statistical difference.

At 9m depth, the untreated panels were the only group to increase in weight at the first and second sample intervals and were significantly heavier than all other treatment groups on both these occasions. From 98 days post deployment to the completion of the trial, there was no significant difference in weight gain due to biofouling growth between untreated panels and the groups treated with lanolin and second season Net Clear ZPT. After panels started to accumulate biofouling growth in June (98 days post deployment) Net Clear and first seaon Net Clear ZPT were statistically different from each other on only two occasions. At these times, 126 and 202 days post deployment, first season Net Clear ZPT had less weight gain due to biofouling. However the large variation between panels particularly within the Net Clear treatment at 155 days probably obscured statistical differences at this time (Figure 4.9).

Table 4.1: Actual dry weight gain (in grams) of panels deployed within the tuna farming zone in 2005 (mean ± standard deviation). The same superscripts within each row denotes no significant difference (P>0.05) between treatments

Time (days)	UNTREATED	LANOLIN	NET CLEAR	NET CLEAR ZPT 1st SEASON	NET CLEAR ZPT 2nd SEASON
Panels at 2m Depth					
35	10.9 ± 1.7 [a]	-7.8 ± 7.8 [b]	-16.7 ± 4.7 [bc]	-21. 1 ± 4.1 [c]	13.1 ± 10.3 [a]
64	143.1 ± 90.4 [ac]	114.8 ± 30.2 [a]	-2.2 ± 9.7 [b]	-1.3 ± 11.5 [b]	164.5 ± 23.6 [c]
98	1062.5 ± 234.5 [a]	1221.4 ± 638.0 [a]	29.9 ± 22.4 [b]	33.3 ± 18.0 [b]	1057.5 ± 154.8 [a]
126	1292.2 ± 454.3 [a]	1552.4 ± 808.3 [a]	19.5 ± 6.0 [b]	62.2 ± 32.5 [c]	1474.3 ± 162.7 [a]
155	832.0 ± 163.0 [a]	880.7 ± 446.7 [a]	43.8 ± 27.8 [b]	61.7 ± 16.3 [b]	1690.8 ± 300.2 [c]
202	61.7 ± 55.3 [a]	-23.7 ± 5.8 [b]	-15.6 ± 2.7 [c]	-30.9 ± 30.8 [bc]	-18.7 ± 4.5 [bc]
Panels at 9m Depth					
35	8.0 ± 1.3 [a]	-8.3 ± 3.6 [b]	-14.5 ± 1.3 [c]	-20.6 ± 1.7 [d]	-6.6 ± 6.6 [bc]
64	11.4 ± 4.1 [a]	-5.7 ± 6.9 [bc]	-15.9 ± 1.5 [b]	-23.8 ± 3.2 [d]	-1.1 ± 2.3 [c]
98	145.4 ± 36.7 [a]	137.8 ± 51.6 [a]	0.3 ± 3.8 [b]	15.1 ± 19.1 [b]	118.0 ± 25.2 [a]
126	443.4 ± 146.7 [a]	293.6 ± 212.9 [ab]	47.6 ± 19.3 [b]	18.5 ± 7.5 [c]	361.8 ± 181.1 [a]
155	254.9 ± 104.1 [a]	323.9 ± 299.3 [ab]	96.6 ± 57.4 [bc]	62.8 ± 32.6 [b]	324.4 ± 198.8 [ac]
202	34.4 ± 15.1 [a]	99.8 ± 117.7 [ab]	29.5 ± 33.9 [a]	-21.9 ± 16.1 [bc]	95.0 ± 102.1 [ac]

Table 4.2: Statistical comparison of weight gain within each treatment group due to biofouling growth on panels suspended at 2 versus 9m depth (NSD denotes P>0.05; * denotes P<0.05; ** denotes P<0.01; * denotes P<0.001)**

2m vs 9m depth	UNTREATED	LANOLIN	NET CLEAR	NET CLEAR ZPT 1st SEASON	NET CLEAR ZPT 2nd SEASON
35 days	NSD	NSD	NSD	NSD	*
64 days	*	***	NSD	**	***
98 days	***	**	*	NSD	***
126 days	**	*	*	*	***
155 days	***	NSD	NSD	NSD	***
202 days	NSD	NSD	*	NSD	NSD

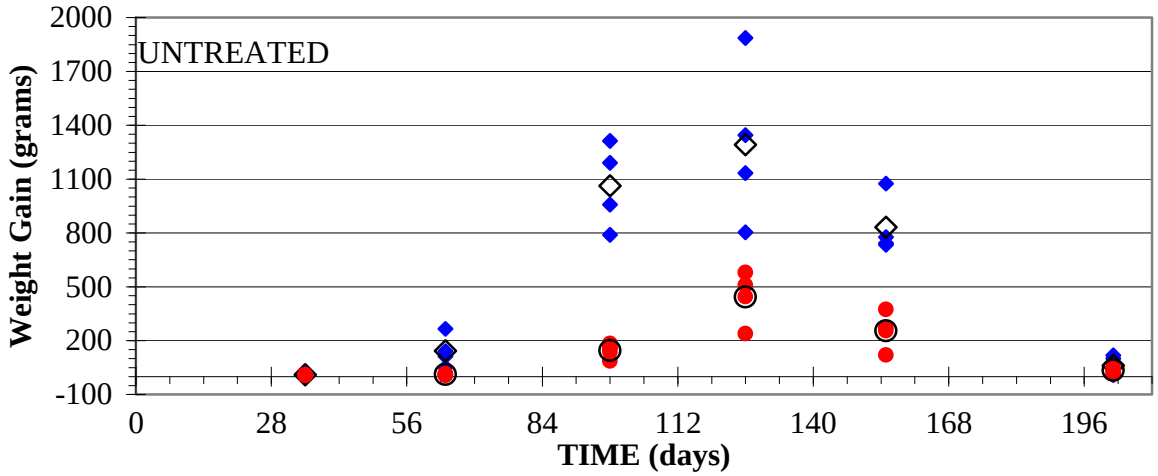


Figure 4.6: Dry weight gain of all untreated panels deployed at 2m ◆, and 9m ● depth (mean of panel subsets at 2m depth ◊; 9m depth ○)

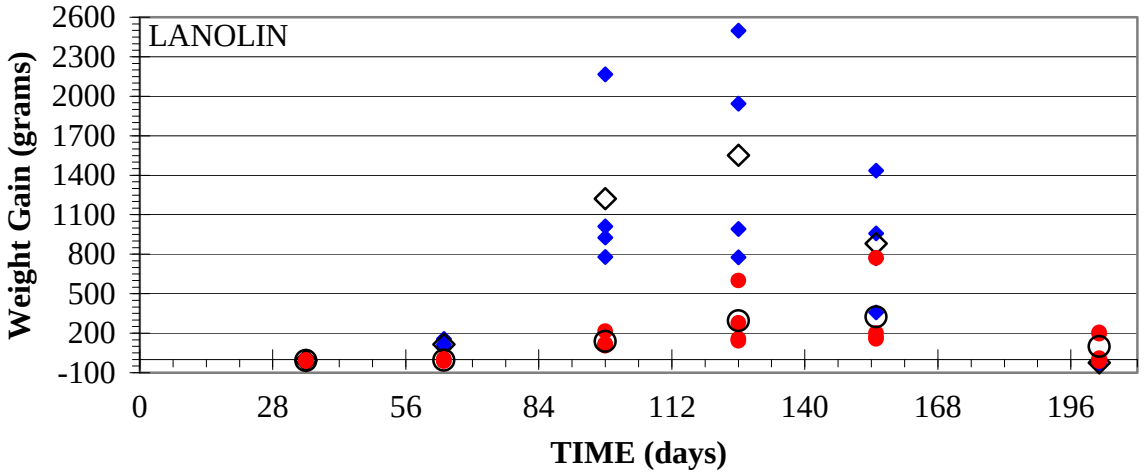


Figure 4.7: Dry weight gain of all panels treated with lanolin, deployed at 2m ◆, and 9m ● depth (mean of panel subsets at 2m depth ◊; 9m depth ○)

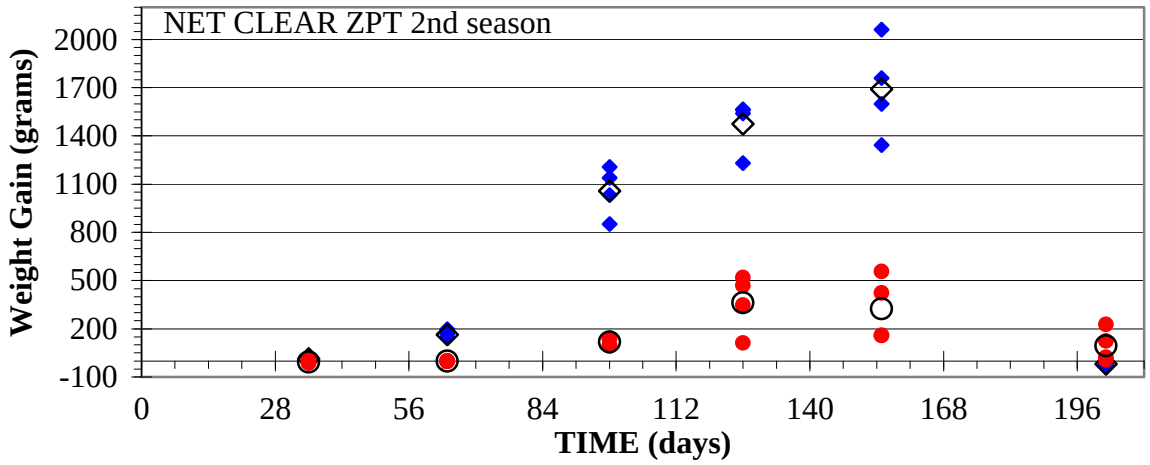


Figure 4.8: Dry weight gain of all panels treated with Net Clear ZPT and redeployed for their second season at 2m ◆, and 9m ● depth (mean of panel subsets at 2m depth ◊; 9m depth ○)

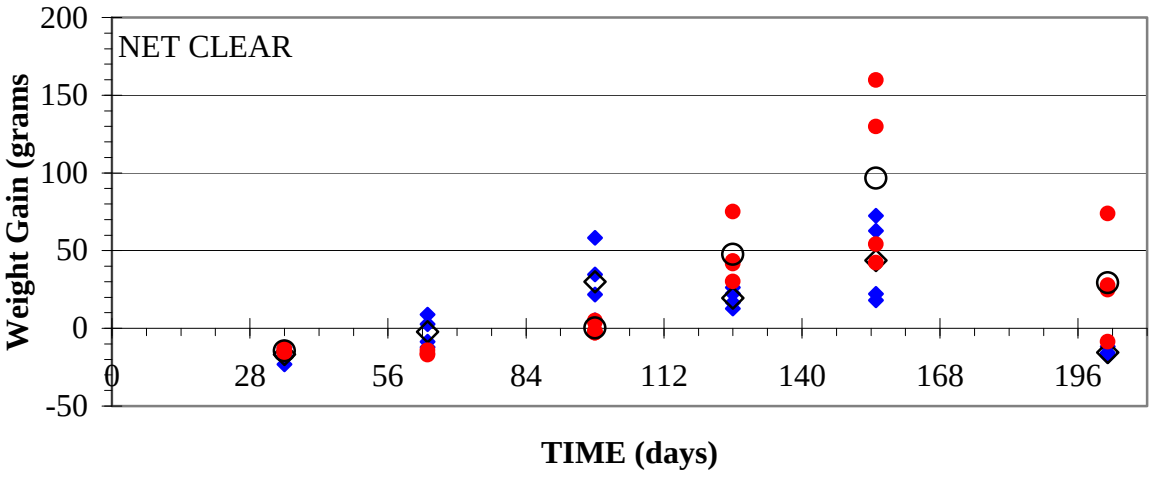


Figure 4.9: Dry weight gain of all panels treated with Net Clear deployed 2m ◆, and 9m ● depth (mean of panel subsets at 2m depth ◊; 9m depth ○)

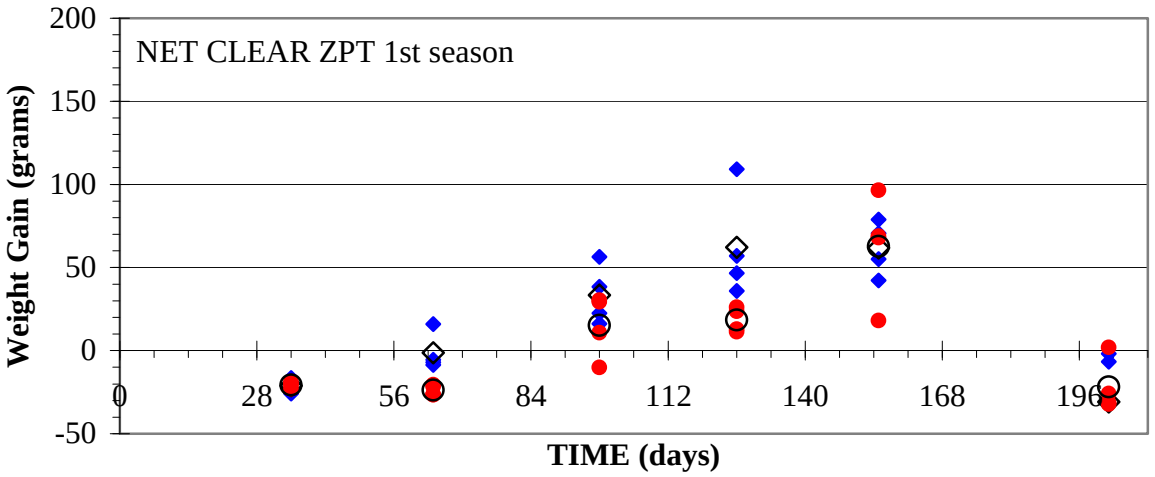


Figure 4.10: Dry weight gain of all panels treated with Net Clear ZPT, deployed at 2m ◆, and 9m ● depth (mean of panel subsets at 2m depth ◊; 9m depth ○)

Image analysis

The performance of the various antifoulants in their efficacy at reducing occlusion or mesh blockage of a 3 by 3 mesh subset on each panel varied with treatment type, depth, and time (comparative data in Figure 4.11 and Figure 4.12; actual data Table 4.3 and Table 4.4). As with weight gain, generally the percentage occlusion of meshes due to biofouling growth was greater at the shallower depth, 2m. The maximum value obtained from the entire experiment was from a panel at 2m depth in the second season Net Clear ZPT treatment group where 93.48% of the image was obscured by net and fouling; and this occurred at 64 days post deployment. The minimum value at 2m depth occurred 35 days post deployment in the first season, fresh Net Clear ZPT treatment group where the net obscured 20.16% of the image. When examining these data it must be noted that the occlusion value of an untreated new white Badinotti 150mm knotless nylon net ranged from 18 to 22%; and coated but never deployed netting ranged from 20 to 24%. A pictorial reference guide of occlusion values due to nets and biofouling was developed as part of these analyses (Appendix 4.3). Due to the removal of biofouling from net panels at the last sample interval, occlusion values were not determined for this photographic data set.

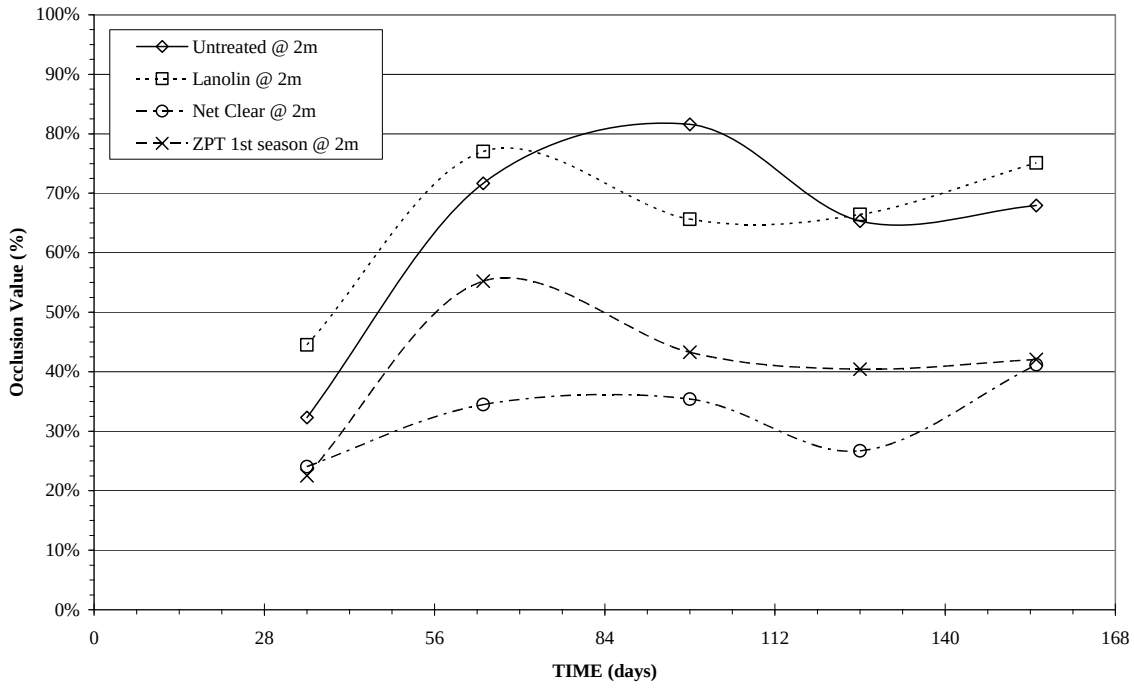


Figure 4.11: Comparative average percent occlusion of a 3x3 mesh subset from each panel of each treatment with time for panels deployed at 2m depth

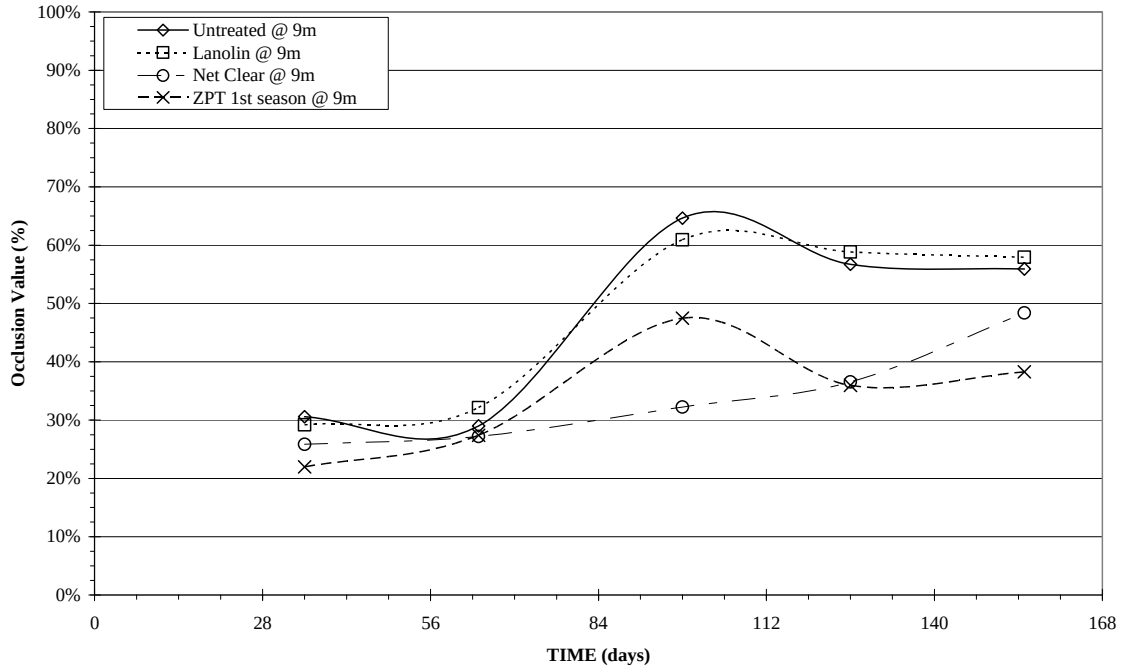


Figure 4.12: Comparative average percent occlusion of a 3x3 mesh subset from each panel of each treatment with time for panels deployed at 9m depth

Table 4.3: Percentage occlusion of a 3 x 3 mesh subset of panels deployed within the tuna farming zone in 2005, expressed as mean ± standard deviation; same superscripts within each row denotes no significant difference (P>0.05)

Time (days)	UNTREATED	LANOLIN	NET CLEAR	NET CLEAR ZPT 1st SEASON	NET CLEAR ZPT 2nd SEASON
Panels at 2m Depth					
35	32.3 ± 5.4^{a}	44.5 ± 5.8^{b}	24.1 ± 3.1^{c}	22.5 ± 2.0^{c}	38.0 ± 9.0^{ab}
64	71.7 ± 13.8^{ab}	77.0 ± 7.3^{a}	34.5 ± 10.6^{c}	55.2 ± 9.3^{b}	86.7 ± 7.5^{a}
98	81.6 ± 11.3^{a}	65.7 ± 9.6^{a}	35.4 ± 3.3^{b}	43.3 ± 6.0^{b}	83.5 ± 12.8^{a}
126	65.3 ± 5.8^{a}	66.4 ± 17.5^{ab}	26.7 ± 1.2^{c}	40.5 ± 4^{d}	80.5 ± 1.6^{b}
155	67.9 ± 8.0^{a}	75.1 ± 11.3^{ab}	41.2 ± 5.2^{c}	42.1 ± 8.3^{c}	86.5 ± 4.2^{b}
Panels at 9m Depth					
35	30.6 ± 3.1^{a}	29.2 ± 3.8^{ab}	25.9 ± 1.0^{b}	22.0 ± 1.5^{c}	33.5 ± 5.6^{a}
64	29.0 ± 2.7^{a}	32.1 ± 5.8^{a}	27.2 ± 3.1^{a}	27.4 ± 7.4^{a}	32.2 ± 5.3^{a}
98	64.7 ± 17.1^{a}	60.9 ± 9.9^{a}	32.3 ± 6.0^{b}	47.5 ± 15.6^{ab}	67.5 ± 6.8^{a}
126	56.7 ± 9.3^{a}	58.8 ± 11.5^{a}	36.5 ± 2.6^{b}	36.0 ± 5.1^{b}	58.0 ± 9.4^{a}
155	55.9 ± 10.4^{a}	58.0 ± 14.9^{ab}	48.4 ± 10.8^{ab}	38.3 ± 10.6^{b}	58.0 ± 23.0^{ab}

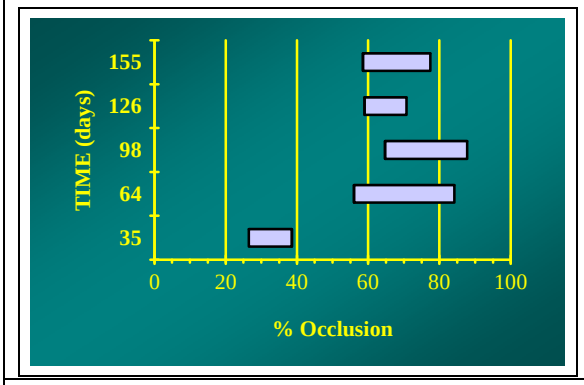
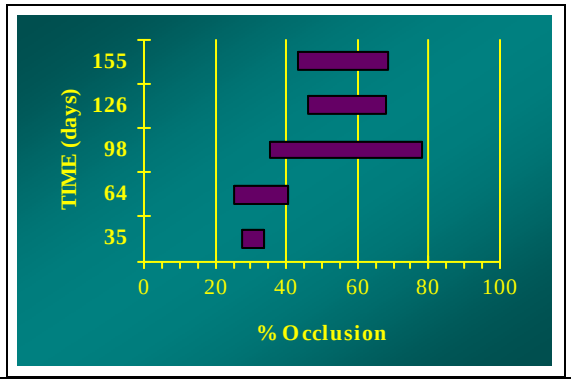
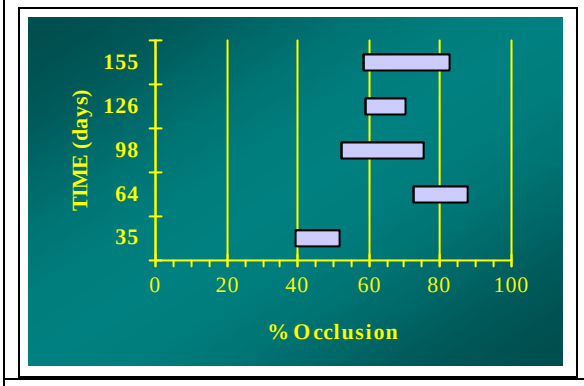
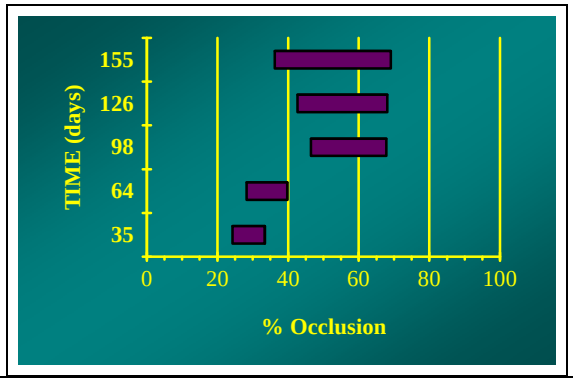
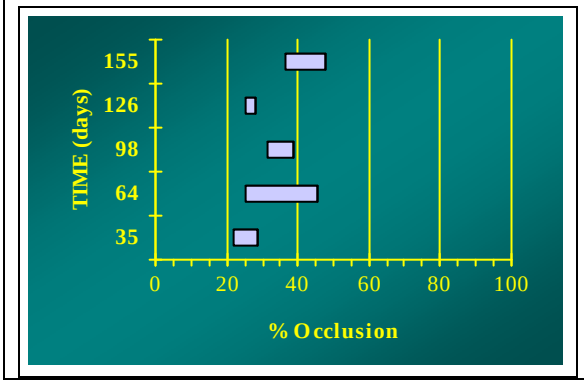
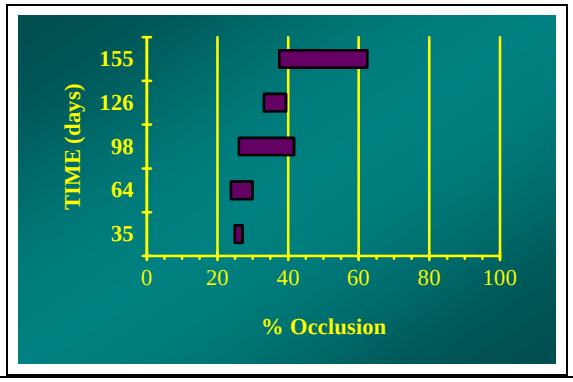
Statistically significant differences were evident between 2m deep treatment groups at 35 days post deployment (Table 4.3). At this time panels treated with lanolin had a higher occlusion value than untreated, Net Clear and first season Net Clear ZPT treatment groups. Through the remainder of the experiment panels treated with lanolin and deployed at 2m depth were not significantly different from those in the untreated or second season ZPT groups

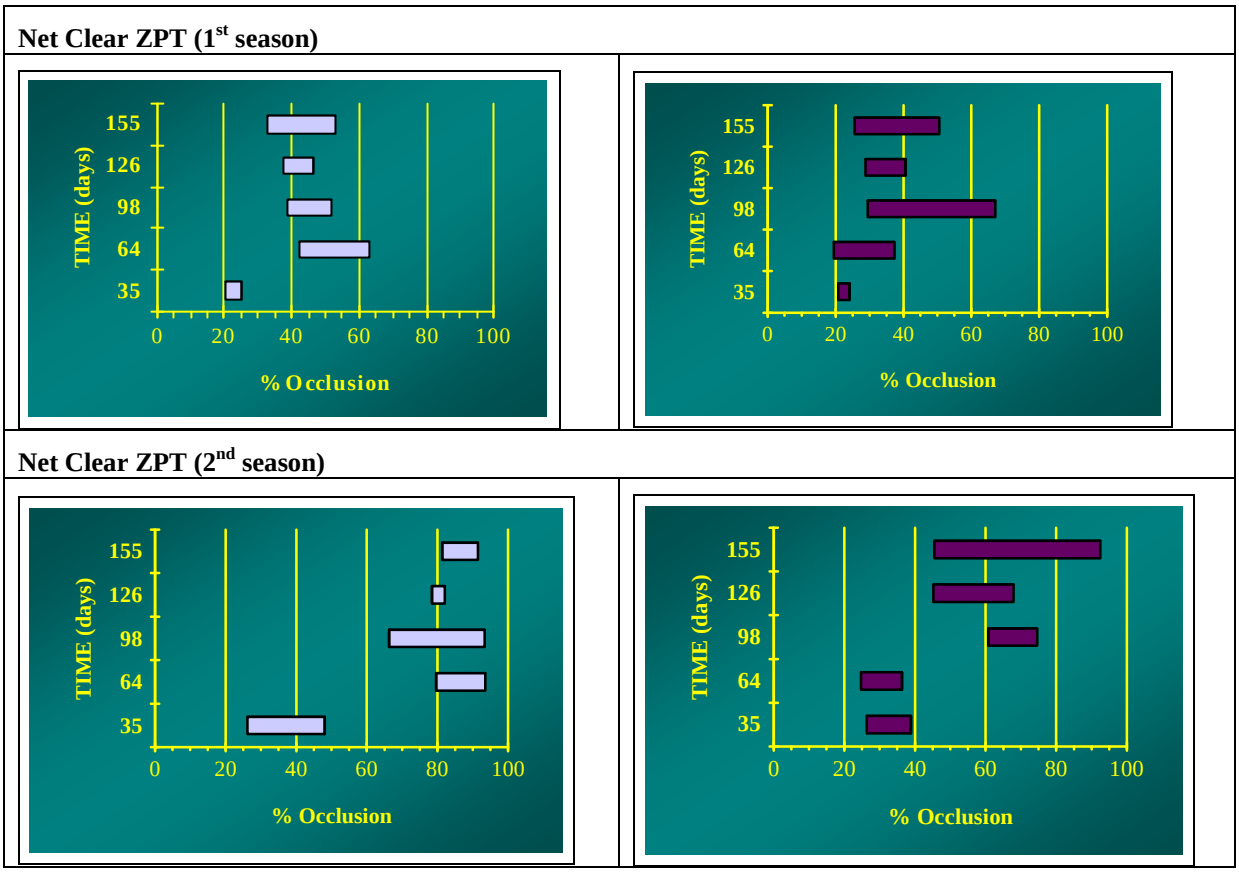
(P>0.05). There was no significant difference between these 3 treatment groups at any sample time for panels deployed at 9m depth.

Panels treated with Net Clear (sea-nine 211) were statistically comparable to those treated with first season Net Clear ZPT (zinc oxide and zinc pyrithione) throughout most sample intervals and at both depths. The exceptions were at 64 and 126 days post deployment at 2m depth when Net Clear had significantly lower occlusion values; and at 35 days at 9m when Net Clear values were significantly higher.

There was little or no relationship between occlusion values and dry weight gain for any treatment groups or depth (maximum R^2 value was <0.6 for Net Clear; all other treatments were <0.3).

Table 4.4: Percentage occlusion of a 3 x 3 mesh subset of panels deployed within the tuna farming zone in 2005, displayed as range of values

2m DEPTH	9m DEPTH
Untreated Control	
	
Lanolin	
	
Net Clear	
	



Anomalies were apparent in most data sets (eg lanolin 64 days at 2m, Net Clear 126 days at 2m; first season Net Clear ZPT 98 days at 9m) in that there are marked increases and decreases, especially in the maximum and range of values (Table 4.4). These were partly the result of changes in fouling types; but also were due to the way some fouling sat in the water bath when photographs were taken and the fact that the computer program distinguishes colour, and not consistency/density of the fouling. This highlights a limitation of the image analysis technique to assess biofouling growth on netting and especially to using these results to infer its influence on water exchange. Variations in the occlusion value occurred due to the shape of soft macrophytes under different prevailing water currents, a situation that is more pronounced if photographs are taken in-situ. This is particularly the case with the red and brown algae types that feature prominently in the fouling growth in this aquaculture area. These tend to align and compress with strong water currents but fluff out in zero and low water flow in real field conditions (pers. obs.). In an aligned and compressed state the occlusion value would be more similar to an un-fouled mesh (i.e. around 30%); but when fluffed out could register as more than 80% occlusion. However, it is likely that even when soft macrophytes are aligned with the current they may initiate turbulence, and hence decrease the velocity of water flow as it passes through the net mesh and effectively reduce the penetration of new water in to the net.

In this data set, similar occlusion values occur on several panels across treatment groups (Table 4.4), but this does not account for the actual type of fouling present which is more likely to influence on the behaviour of water passing through the net. For example the photographs in Plate 4.8 have an approximately equal occlusion value of around 80%. The solid, hard shell growth in the photograph on the left does not move with water flow and therefore effectively alters the mesh shape, reduces the size of the free water space effectively increasing the cord thickness of the netting. Increased cord thickness and decreased internal dimensions of the mesh are known to impede water flow (Aarsnes et al 1990; deNys et al 2005a (Chapter 1 this document)). The weed growth in the photograph on the right has an image analysis value of the

mesh being 80% blocked, as 80% of the image is a different colour to the background. However, this type of fouling growth is not rigid and aligns with water flow which effectively decreases the level of mesh occlusion.

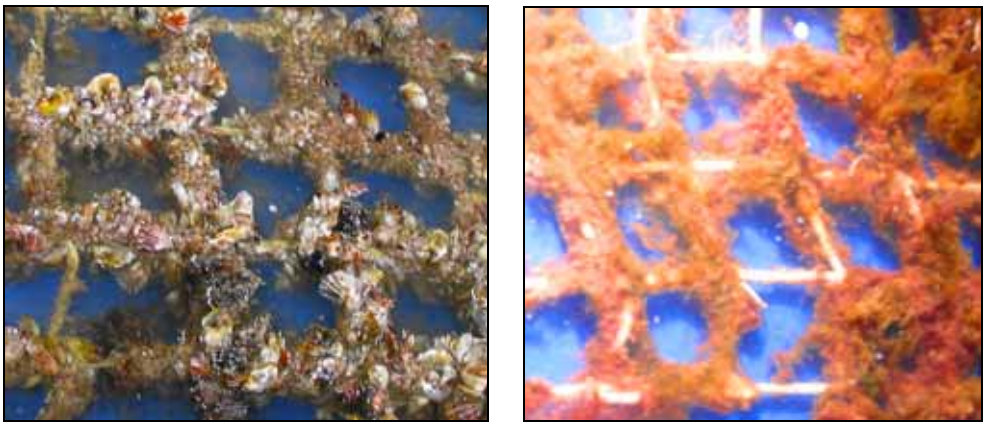


Plate 4.8: Net panels with similar occlusion values of 82% (left) and 80% (right), but due to the presence of different fouling assemblages

There were differences apparent in the general types of biofouling present as well as the density of growth with treatment types and depth (Table 4.5 and Table 4.6). For the purpose of these comparisons biofouling is only classified as: soft fouling (including micro and macro algae, small and flexible invertebrates such as worms, hydroids and amphipods etc); or sponge fouling; or hard fouling (including shell and "solid" invertebrates such as molluscs and ascidians). A detailed description of the development of biofouling communities on tuna netting with time, depth and orientation was undertaken by Loo (2008), Chapter 5 this report. This dealt with both the white 150mm Badinotti used in this trial, and the black 150mm braided type used for approximately 35% of industry nets in 2005).

The single image from each treatment depth and time included in Table 4.5 and Table 4.6 was generally of an area of maximum fouling on a panel from the clearest image in that subset; but photographs of all entire panels can be found in Appendix 4.2.

At the 98 day sample interval at 2m depth there was a change in biofouling type on the untreated control panels and on those treated with lanolin (Table 4.5). This was also seen on the panels in the second season Net Clear ZPT group; for simplicity images of this group were not included in this table but can be seen in Appendix 4.2. At this time shell (*Electroma sp.*) and sponge growth was very apparent, and the soft macrophyte fouling growth had declined or disappeared. This coincides with the first winter (June) sample interval when water temperature had undergone a rapid decline and was at or below 15ºC (Figure 4.13 and Figure 4.14) and probably also when illumination levels were decreasing (Cronin et al 1999). Generally this was when these treatments experienced rapid weight gains, up to 10 times that of the previous sample, and indicates that these advanced fouling assemblages are a substantial contribution to the increases in net weight seen throughout the industry.

A patchy film of fouling was apparent on the meshes of panels treated with Net Clear at 35 days post deployment. By 64 days soft macrophyte growth was apparent on panels treated with Net Clear and Net Clear ZPT at 2m depth. Growth on both Net Clear and Net Clear ZPT was generally confined to patches on the panels (Appendix 4.2). Macrophyte growth of red alga species was the main fouling type on each treatment through the experiment. At the July sample, 126 days post deployment, one panel of the latex Net Clear product contained a blue

mussel spat, and this was the only hard type of fouling on either of these treatments for the entire 202 days.

Panels at 9m depth contained surprisingly little hard shell biofouling, with sponge growth becoming the dominate type from July in the untreated and lanolin coated groups; and August for the Net Clear group (Table 4.6). Macrophytes and hydroids were the only types present on the Net Clear ZPT group. Growth on both Net Clear and Net Clear ZPT was generally confined to isolated patches on the panels (Appendix 4.2)

Table 4.5: Photographic display of biofouling growth and antifouling treatment efficacy with time on panels treated and deployed at 2m depth

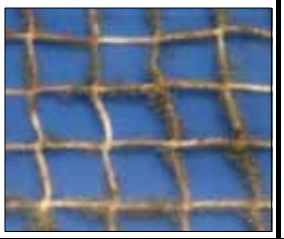
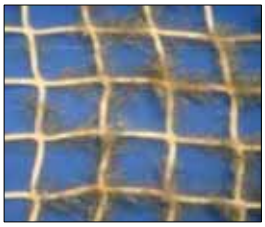
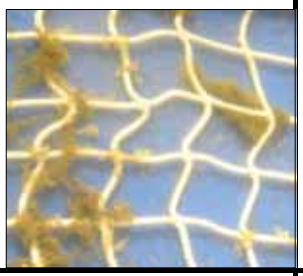
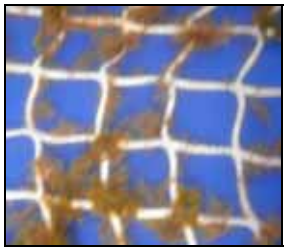
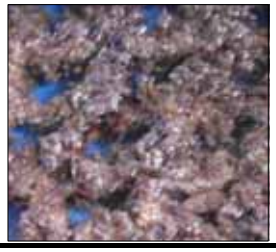
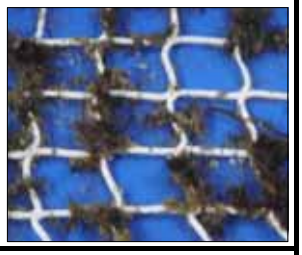
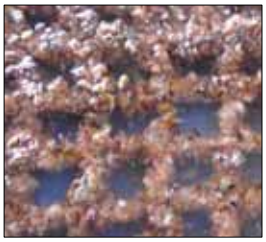
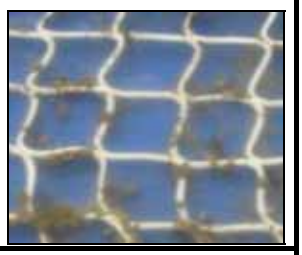
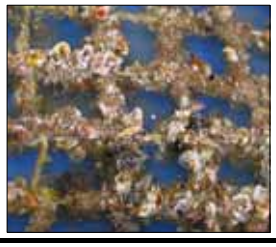
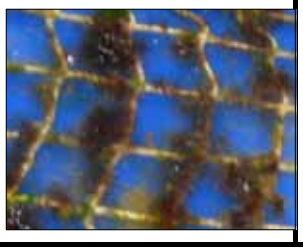
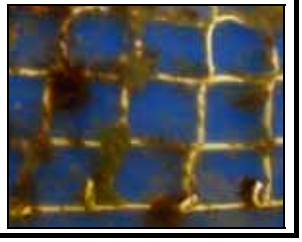
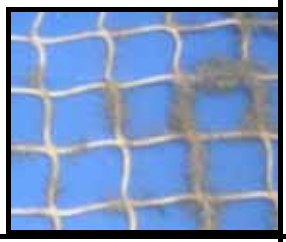
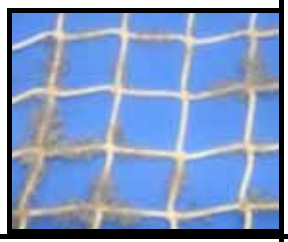
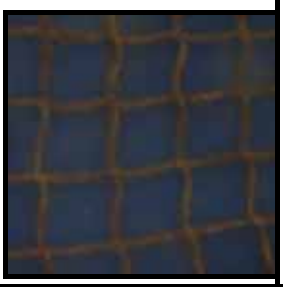
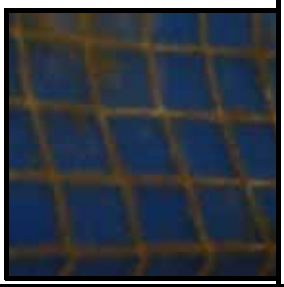
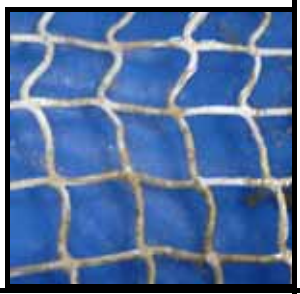
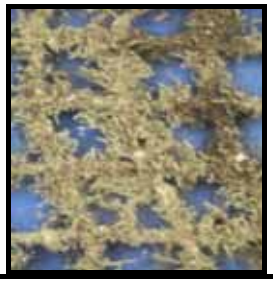
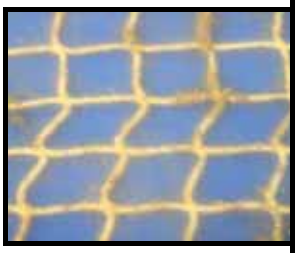
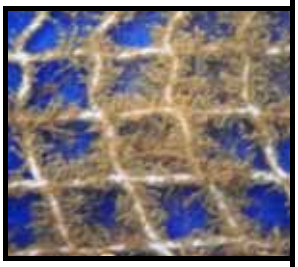
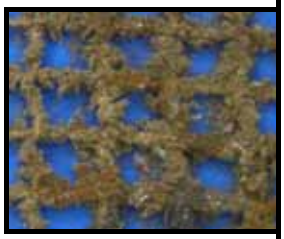
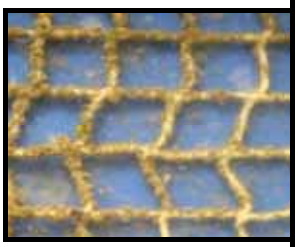
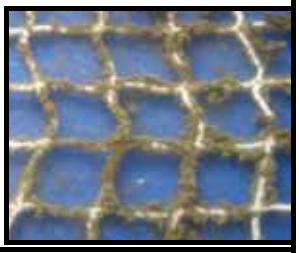
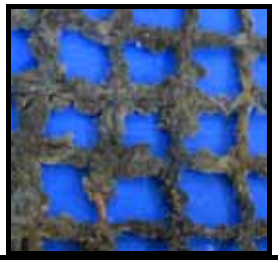
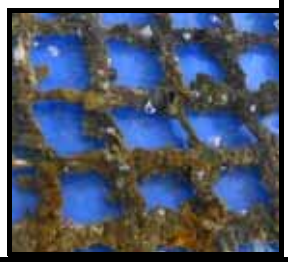
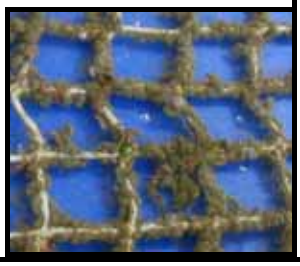
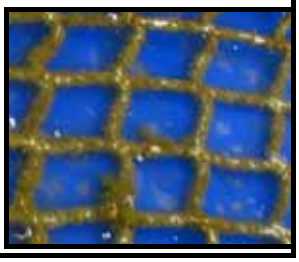
Days	UNTREATED	LANOLIN	NET CLEAR	NET CLEAR ZPT (1st)
35				
64				
98				
126				
155				

Table 4.6: Photographic display of biofouling growth and antifouling treatment efficacy with time on panels treated and deployed at 9m depth

Days	UNTREATED	LANOLIN	NET CLEAR	NET CLEAR ZPT (1st)
35				
64				
98				
126				
155				

Whilst the panels were deployed, the water temperature at 2m depth ranged from an autumn maximum of 21.0ºC (12th March) to a winter minimum of 13.0ºC (11th August) (Figure 4.13). At 9m depth the water temperature ranged from an autumn maximum of 20.6ºC (12th to 14th March) to a winter minimum of 13.0ºC (20th August) (Figure 4.14). The minimal differences in daily temperature between the two depths would suggest that differences in the degree of fouling growth are more likely to be related to other factors, such as light levels, and/or orientation as suggested by Cronin et al (1999).

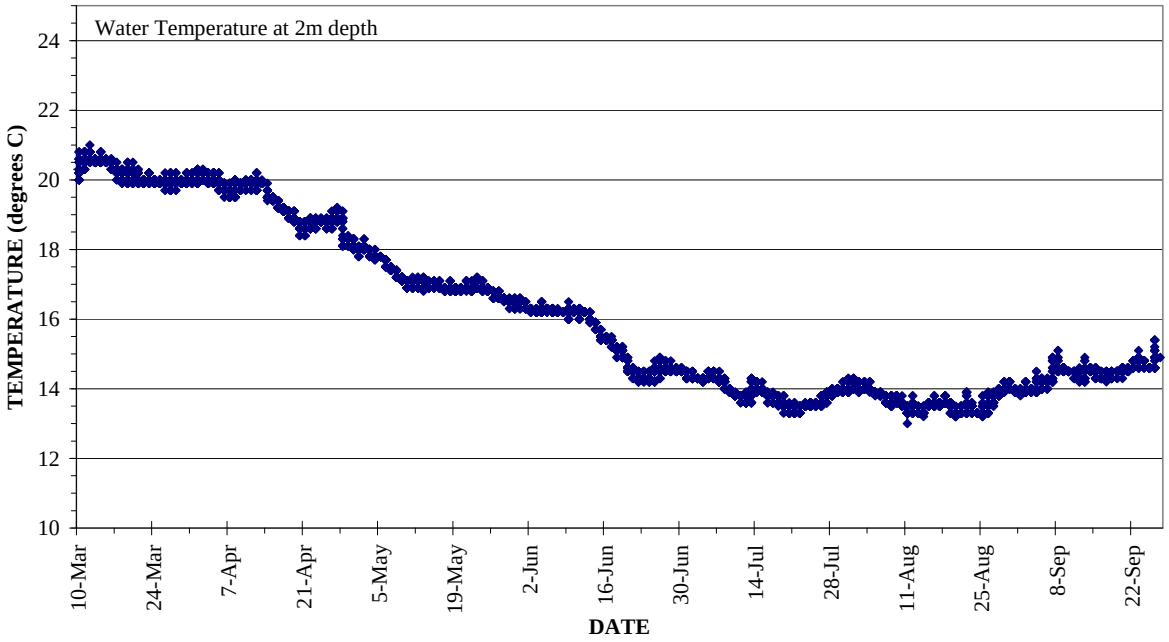


Figure 4.13: Daily water temperature recorded hourly with Vemco data loggers at 2m depth during the panel deployment of 2005

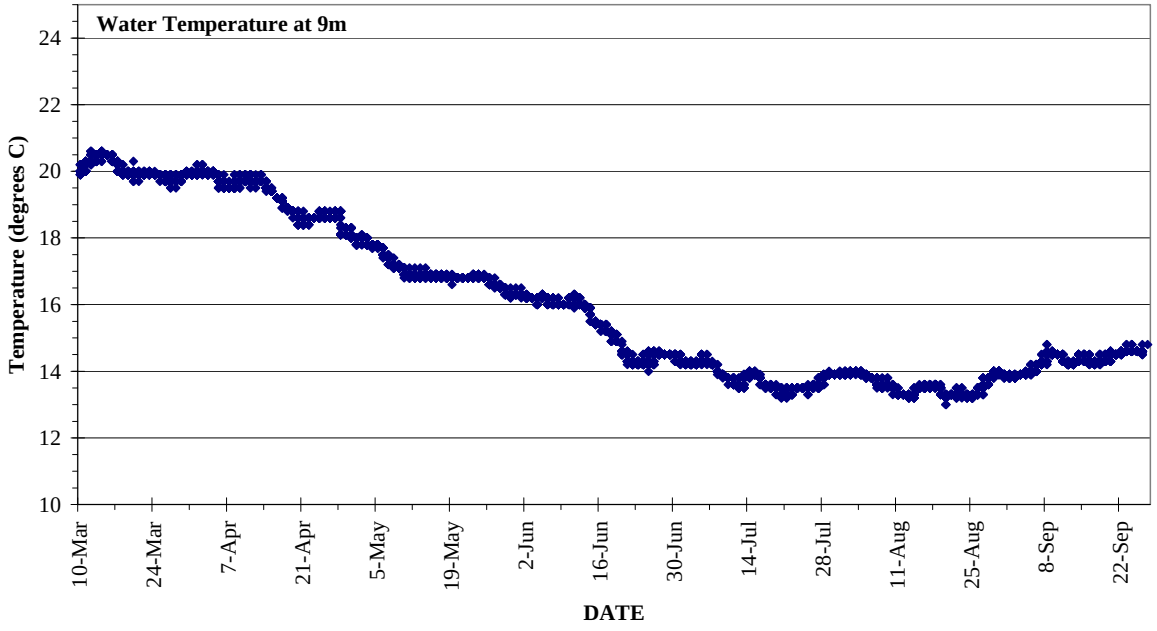


Figure 4.14: Daily water temperature recorded hourly with Vemco data loggers at 9m depth during the panel deployment of 2005

Strength tests

Another aspect to consider when contemplating the use of any coating on a fish farm net is whether the product will have any impact on the net's strength, and hence on its lifespan. Within the tuna industry, nets are decommissioned when any part of the netting at or below the water line has a breaking strain less than 200 to 250kg (this varies with type of net used and between operators). Under commercial conditions in the current farming zone,

decommissioning generally occurs when a normal untreated net is 4 to 5 years old (industry survey, pers. comm. 2004 – 2006). To assess net strength, panels retrieved on the 15th June and 27th September (ie 98 and 202 days post deployment) were tested for breaking strain on the tensiometer at a commercial net manufacturer's premises (Quinn Marine Port Lincoln). Results indicate that treatment, suspension depth and time in water all influenced the strength of the net panels (Table 4.7). Generally it appears that applying the coating forms of antifoulant to the netting (ie Net Clear and Net Clear ZPT in this trial) improves the nets strength and durability (Figure 4.17).

Untreated panels lost strength with increased time and increased depth, so that those deployed for 202 days at 9m depth had significantly lowered breaking strains compared to new netting. Within the group of untreated panels, those deployed at 9m depth for 202 days had significantly less strength than those suspended at 2m and 9m depth for 98 days ($P<0.05$). However it must be noted that these results are contrary to the observations of the tuna industry, where the weakest points of a net are at the waterline and surface sections of the net. It is likely that the untreated panels in this subproject reflect only part of the commercial situation; the upper sections of an entire net would have the added stress of biofouling weight, increased drag and the movement of the full wall and the base acting upon it. The breaking strain profile of a decommissioned 5 year old net, that was approximately 300kg when new; is 200kg at and above the water line, 220kg, 225kg, 247kg and 250kg at 1m, 2m, 5m and 10m respectively.

At 2m depth lanolin treated panels had significantly less strength than new netting and all other treatments at both 98 and 202 days post deployment. At 9m depth this treatment had significantly less strength than new netting at both sample times; but was not significantly different to panels in the untreated group or the Net Clear ZPT first season group at 98 days (Table 4.7). However, the high variability between panels of the latter group is most likely obscuring statistical differences with other treatments (Figure 4.15 and Figure 4.16). There was no statistical difference between groups of lanolin treated panels with depth or time.

Panels treated with Net Clear were significantly stronger than new netting, lanolin treated and the untreated panels at every sample depth and time interval. There were no significant differences between the latex Net Clear and the zinc paint based Net Clear ZPT panels (both first and second season) for all sample depths and intervals; except 202 days at 2m depth where the Net Clear treated panels were significantly stronger. There was no statistical difference between groups of Net Clear treated panels with depth or time.

Treating panels with Net Clear ZPT (both first and second season) made panels at least as strong as new netting for all sample times and depths. Panels treated and deployed for the first season were significantly stronger than new netting, lanolin treated and the untreated panels at only one sampling, 98 days at 2m depth. The high variability between panels of this group is most likely obscuring statistical differences with other treatments (Figure 4.15 and Figure 4.16). There was no statistical difference between groups of first season Net Clear ZPT treated panels with depth or time.

The group of second season panels treated with Net Clear ZPT were significantly stronger than new netting, lanolin treated and the untreated panels at every sample depth and time interval, except 202 days at 2m depth. There were no significant differences between the first and second season Net Clear ZPT panels for all sample depths and intervals. There was no statistical difference between groups of second season Net Clear ZPT panels with depth or time.

Table 4.7: Actual net strength (kilograms breaking strain) of panels deployed within the tuna farming zone in 2005 (mean ± standard deviation). The same superscript within each row denotes no significant difference (P>0.05) between treatments

New Net	Time	Untreated	Lanolin	Net Clear	Net Clear ZPT 1st season	Net Clear ZPT 2nd season
	Panels deployed at 2m depth					
336 ± 11 [a]	98 days	328 ± 23 [a]	244 ± 34 [b]	377 ± 15 [c]	372 ± 16 [c]	365 ± 21 [c]
336 ± 11 [a]	202 days	319 ± 27 [a]	258 ± 33 [b]	411 ± 24 [c]	333 ± 40 [a]	352 ± 23 [a]
	Panels deployed at 9m depth					
336 ± 11 [a]	98 days	309 ± 36 [ab]	285 ± 21 [b]	388 ± 9 [c]	313 ± 63 [abc]	386 ± 21 [c]
336 ± 11 [a]	202 days	276 ± 13 [b]	281 ± 17 [b]	384 ± 18 [c]	350 ± 41 [ac]	368 ± 25 [c]

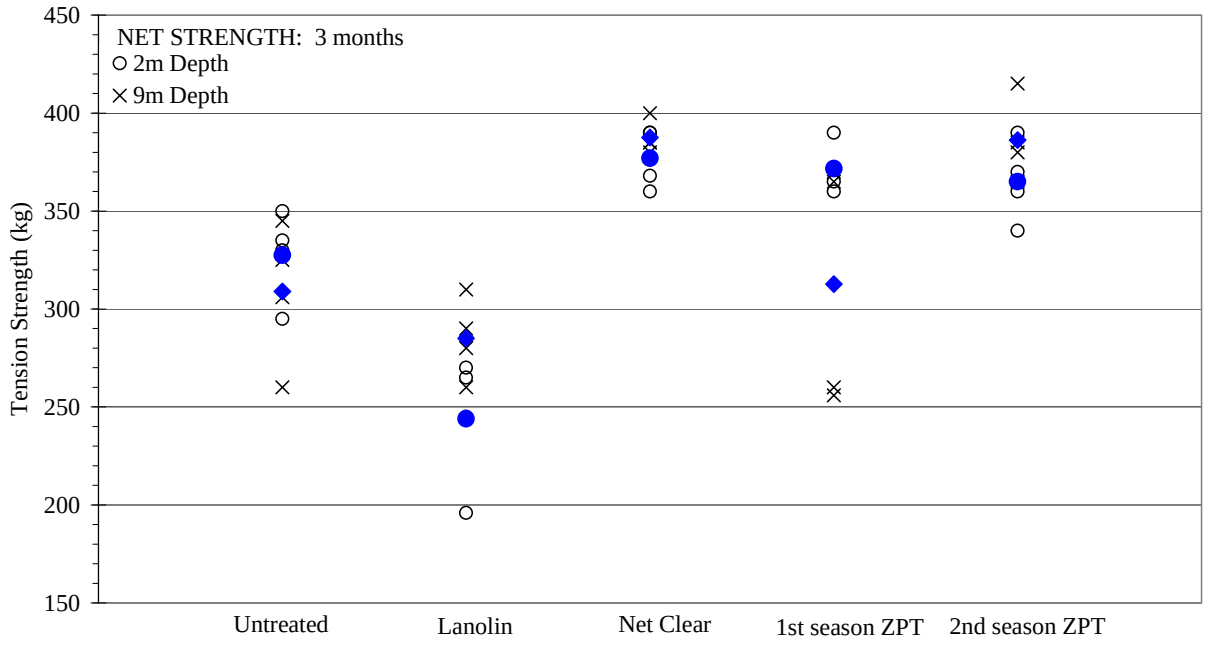


Figure 4.15: Breaking strain of netting for each treatment submerged for 3 months (individual panels at 2m ○ and 9m X; mean of panel subsets at 2m ●, and 9m ♦)

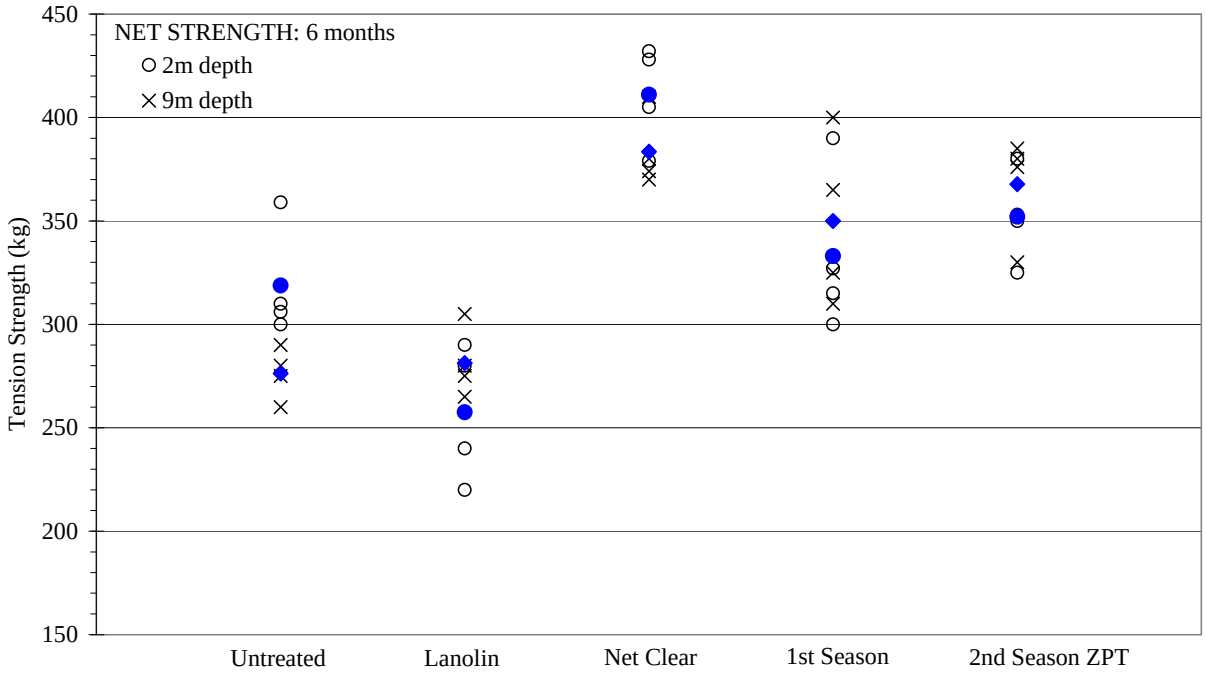


Figure 4.16: Breaking strain of netting for each treatment submerged for 6 months (individual panels at 2m ○ and 9m X; mean of panel subsets at 2m ●, and 9m ♦)

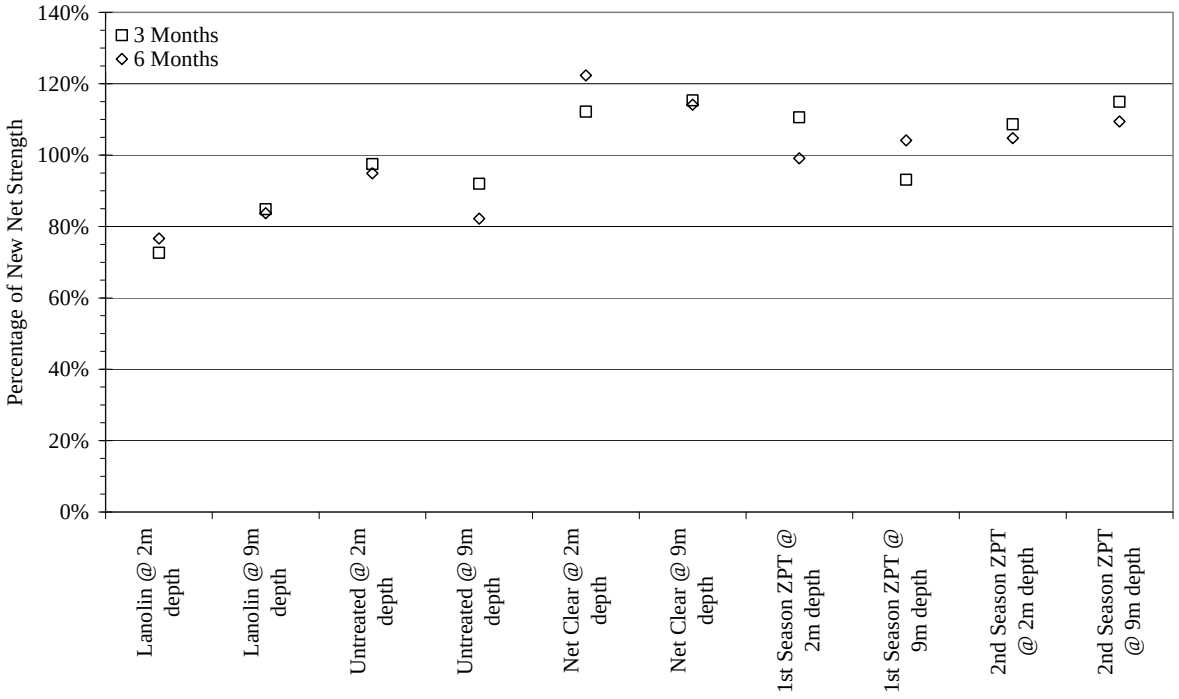


Figure 4.17: Comparative strength of net panels for all treatments after 3 and 6 months deployment; whereby a new untreated net is 100% (means only)

CONCLUSIONS

The antifouling products tested did influence the characteristics and handling qualities of the white, knotless nylon net type used in this trial. This net type was used on more than 50% of cages in the tuna industry in 2005. Lanolin increased the dry weight of the net by 41%;

gave it a greasy feel and was as pliable as untreated net; but decreased the breaking strain (strength) of meshes and hence potentially reduced the net's life. Net Clear increased the dry net weight by 51%; gave it a rubbery texture that had little influence on handling qualities and improved net strength. Net Clear ZPT increased dry net weight by at least 76%; turned the net bright white; made it stiff and inflexible and improved net strength.

None of the three antifouling treatments trialled totally prevented biofouling; however two, Net Clear (latex with Sea-nine 211) and Net Clear ZPT (zinc oxide and zinc pyrithione paint) were effective at delaying the start of and reducing the amount of biofouling growth on tuna netting. The types of organisms that made up the fouling assemblages varied with treatment and depth. Lanolin and untreated panels had heavy settlement of hard shelled organisms and much denser growth of soft algal and invertebrate communities. Fouling growth on the Net Clear and the Net Clear ZPT panels was confined to soft algal and invertebrate growth. This combined with the alteration to netting surface texture and topography provided by the application of the Net Clear and Net Clear ZPT coatings would most likely facilitate faster and more effective in situ net cleaning through the season if any cleaning was deemed necessary by management.

The inhibitory effect of the all the antifouling products tested was reduced during a single deployment and consequently would not be effective for subsequent farming seasons. This means that nets would need to be cleaned, coated and redeployed every year. However, if longer term holding of stock was implemented, in-situ clean(s) may be all that would be required to hold stock for the second season using Net Clear or Net Clear ZPT.

The formulation of the Net Clear product had been improved on the version used in the 2002 trial by Svane et al. (2006), as the difference in occlusion values between untreated and this product was greater than 15%. However this may also be due in part to the different species ratios and growth rates of fouling assemblages found in the sheltered, shallow environment within Boston Bay, the site of the 2002 trial.

At the completion of the panel trial there were two products that clearly showed potential for use in the pilot scale subproject, which was to apply an antifoul coating to an entire tuna net in the following season. Net Clear and Net Clear ZPT had comparable results for tests of biofouling weight gain, biofouling growth types, and mesh occlusion from biofouling growth and both at least maintained the strength of new netting; but Net Clear ZPT appeared to perform marginally better than Net Clear at depth. Either product would be suitable for the pilot scale subproject; however other factors in addition to the performance on panels need to be considered for the trial with an entire net. Product availability, in particular the 6000L stock solution that is required to ensure even coverage of the entire net, was the most important of these. At the completion of the panel trial 6000L of Net Clear ZPT was available at no cost to the project and the dipping facility was set up for this product. Moreover, this was the product that Wattyl wanted to explore further.

Chapter 5 : SUB-PROJECT 3: EVALUATION OF THE SEASONAL DEVELOPMENT PATTERN OF NET FOULING AND THE EFFECTS ON KEY WATER PARAMETERS IN THE LOCAL ENVIRONMENT OF SOUTH AUSTRALIA

This chapter was authored by Dr. Maylene GK Loo (South Australian Research and Development Institute, Aquatic Sciences, 2 Hamra Avenue, West Beach SA 5024 http://www.sardi.sa.gov.au) and may be cited as:

Loo, M. G. K (2008). Evaluation of the seasonal development pattern of net fouling and the effects on key water parameters in the local environment of South Australia. In Rough KM, deNys R, Loo, MGK, and Ellis DC, (Eds.) Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp.

SUMMARY

The main objective of this subproject was to identify the development pattern of the fouling community on commercial tuna sea-cages that are subject to the current standard industry practices, and relate this to oxygen levels monitored on the outside and inside of these cages.

The net fouling assemblages on the studied sea-cage located at DI Fishing Co. Pty Ltd (DI), comprised a range of 14 taxonomic groups (four animal phyla and three algal divisions) across all depths, being dominated by colonial ascidians mostly from the family Dideminidae and mixed algae from the divisions Rhodophyta, Chlorophyta and Phaeophyta. The net fouling assemblages on the sea-cage located at Australian Fishing Enterprises Pty Ltd (AF) were less diverse, with nine taxonomic groups (also from four animal phyla and three algal divisions) recorded. For the sea-cage at DI, the fouling assemblage was dominated by hydroids in March/April, moving to mixed algae and encrusting organisms in May/June and "climaxing" with colonial ascidians towards the end of the farming season in August/September. The seasonal development of fouling assemblages for the sea-cage at AF followed a similar trend. Depth differences were associated with dominance by algae in the shallower depths and encrusting organisms including bryozoans and ascidians in the deeper depths for both sea-cages. The bivalves *Electroma georgiana* and *Hiatella australis* were recorded from mid season (June) onwards, but not in high cover.

One of the main effects of net fouling on the management and operation of sea-cage systems is changing patterns of water flow through the nets, thereby affecting the supply of oxygen and removal of wastes from cages. This disruption of exchange through the net was demonstrated using the water quality data collected. The dissolved oxygen concentration within the sea-cage became lower as net occlusion increased, concurrently with increased fouling growth.

INTRODUCTION

The development of fouling communities on suspended-aquaculture fish sea-cages (biofouling) can result in added weight and drag to the sea cage, reduced water flow, and altered behaviour of the sea cage during rough seas and high currents (Swift *et al.* 2006). Reduced water flow is important as it can reduce oxygen concentrations to levels below those required for optimal fish growth (Edwards and Edelsten 1976, Madenjian 1990, Silvert 1992).

Various methods have been developed to control biofouling, but it remains a problem at aquaculture sites worldwide (Hodson *et al.* 2000 Braithwaite and McEvoy 2005, Braithwaite *et al.* 2007). The ecology and dynamics of biofouling, as well as its impacts on marine fish aquaculture, including effects such as restriction of water exchange, increasing disease risk and cage deformation and structural fatigue, have been reviewed as part of this project in Subproject 1 (de Nys *et al* 2005a, Chapter 1). de Nys *et al* (2005b) also reviewed the methods and efficacy of biofouling control in sea cage aquaculture (Chapter 2). An evaluation of a preferred antifouling treatment identified in Subproject 1 was carried out using panels, Subproject 2 (Rough and Ellis 2007, Chapter 4).

This study (Subproject 3) concentrated on the development of fouling communities. The main objective was to investigate the development pattern of the fouling community on commercial southern bluefin tuna (SBT) sea-cages within the tuna farming zone, and the influence of the fouling community on key water quality parameters, in particular, dissolved oxygen concentration between the inside and outside of sea-cages.

Most studies on biofouling involve deploying and photographing net panels over time, usually employing divers, to assess the development of fouling organisms (e.g. Hodson *et al.* 2000, Braithwaite *et al.* 2007, Greene and Grizzle 2007). Net panels are retrieved at the end of the study for removal and identification of fouling organisms. This method of collecting data can be time consuming, costly and potentially hazardous. In addition, the net panels may not be exposed to the effects of fish in commercial sea-cages, resulting in different patterns to what are obtained on commercial farms. To overcome these issues, remote video recording of fouling assemblages on active commercial sea-cages was employed in this subproject.

Video cameras have improved in quality and decreased in cost, making their use as an alternative method for collecting data in the marine environment more attractive. Due to decreased costs, time savings and the possibility for remote deployment, video recording has been extensively used in marine environmental assessment and monitoring (Charleton 1995 Berkelmans 1992 Leonard and Clarke 1993). In Australia, video has been investigated for assessing environmental impacts of fish farms (Cheshire *et al.* 1996, Crawford *et al.* 2001). They have also been used to investigate impacts of sand dredging on the seabed (Cheshire and Miller 1999, Fairhead *et al.* 2002). Previously, studies using video photography had been mostly qualitative, but with improvements in the quality of video cameras, footage can now be quantitatively analysed (Miller 1997, Crawford *et al.* 2001). In this subproject, images captured from video were analysed for percentage cover of fouling organisms and percentage net occlusion.

MATERIALS AND METHODS

Study site

The original project design was to use one stocked commercial SBT sea-cage in each of two farms, one within each of the two farming zones (Boston Island East and Rabbit Island Farming Zones), located off Port Lincoln, South Australia. However, due to changes in management, the two commercial farms that finally participated in the project were both located in the Boston Island East Farming Zone. However, one was located closer to Boston Island (DI Fishing Co. Pty Ltd) while the other (Australian Fishing Enterprises Pty Ltd) was located further offshore on the edge of the farming zone. Both commercial SBT sea-cages used measured 40 m in diameter and had nets with a mesh size of 75 mm (bar) hanging down to depths of 12 to 14 m.

Field sampling

A Canon MV200 digital video camcorder housed in an Amphibico "Dive Buddy" underwater video camera housing was used to record images. The Dive Buddy housing was attached to a sled with elliptical rails constructed of stainless steel and angled at 45 degrees. The sled with the camera was lowered via a rope system to the bottom of the side net walls of a sea-cage (approximately 12 m depth). The length of rope paid out was noted to determine the depth of the net. The sled and camera were then slowly pulled up the side net walls, with the rails of the sled keeping the camera at a fixed distance from the net. The sled was stopped every 2 m for one minute until it reached the surface. This process was repeated on the north, south, east and west sides of one sea-cage located within each farm. Weather permitting, the video transects were undertaken monthly from March 2005 until the end of the farming season in August/September 2005, using the same pontoon each month.

Laboratory analyses

In the laboratory, video recordings of each transect were analysed. At each of the 2 m stops, a video image was captured and a 100 x 100 mm grid divided into 20 x 20 mm squares was placed over the image. The image was then point scored for cover of net, bare space and sessile fouling taxa. For each 20 x 20 mm square, fouling organisms were scored if overall cover was greater than 50% otherwise it was scored as bare space. The fouling organisms were identified to genus or species for both flora and fauna, and to dominant growth forms when identification was not possible from the video images or when they were mixed assemblages. The growth forms and specific flora and fauna identified from the video images are given in Table 5.1. Point scores for the fouling assemblages were converted to percentage cover of each growth form or taxon identified.

Table 5.1: Taxonomic groups used in the analysis of video transects of fouling organisms on SBT sea-cage nets.

Flora	Fauna
Phaeophyta (brown foliaceous algae)	Bryozoan
Rhodophyta (red foliaceous algae)	Hydroid
Mixed algae	Colonial ascidian (mostly Dideminidae)
Gloiosaccion brownie (red alga)	*Herdmania momus* (solitary ascidian)
Giffordia species (brown alga)	*Electroma georgiana* (bivalve)
Ulva australis (green alga)	*Mytilus* species (bivalve)
Mixed encrusting	*Hiatella australis* (bivalve)

The captured video image for each stop was also analysed for net occlusion. The area of net aperture (i.e. area not covered by fouling organisms) was calculated using the software package ImageJ 1.39[5]. The percentage net occlusion was then calculated from the percentage net aperture of the netting before deployment and the percentage net aperture at each sampling time.

Collection of water quality data

[5] Source: http://rsb.info.nih.gov/ij/

The existing SBT telemetry-based environmental monitoring system used during the RESA[6] project, which records water quality (water temperature, dissolved oxygen and salinity) and weather patterns (wind speed and direction), was deployed on the DI pontoon. Another telemetry system, which also monitored water temperature, dissolved oxygen, and wind speed and direction, was purchased as part of this project and placed at the AF pontoon. Water quality measurements were taken inside and outside of each pontoon. However, the system deployed at AF encountered problems during the season and the data recorded were not reliable. Consequently, only data from the system deployed at DI were used.

Data analysis

The original experimental design involved sampling two sea-cages, four replicate transects on each cage, five/six depths for each transect, at monthly intervals. However, due to weather variability, there were no video recordings on certain dates. In addition, analyses of the fouling assemblages for some transects could not be carried out due to the low quality of the video recordings. Consequently, for the sea-cage at DI, two replicate transects were analysed for March, and four replicate transects were analysed for April, May, June, August and September. For the sea-cage at AF, two transects were analysed for May while four transects were analysed for April, June and August. No transects from either sea cage were analysed for July, and no video recording was undertaken at AF for September as all fish had been harvested and the net removed. Depth of nets recorded can vary, due to tidal currents and how securely the sea-cage is anchored, and transects may be as deep as 14 m. As the 12 m and 14 m stops occurred inconsistently, the maximum depth used was standardised to 10 m for all transects. As the experiment was dependent upon the participation of the SBT farming companies in the project, it was not possible to control the sea-cages used. The netting on each sea-cage was different in colour, with white netting on DI and black netting on AF, although the net apertures were the same (Figure 5.1). Surface colour has previously been found to affect the growth and composition of fouling communities, with larvae of many invertebrates demonstrating a phototactic response during settlement (e.g. Dahlem *et al.* 1984, Svane and Dolmer 1995, Swain *et al.* 2006). Due to the confounding of net type and location, data obtained from the two sea-cages were analysed separately.

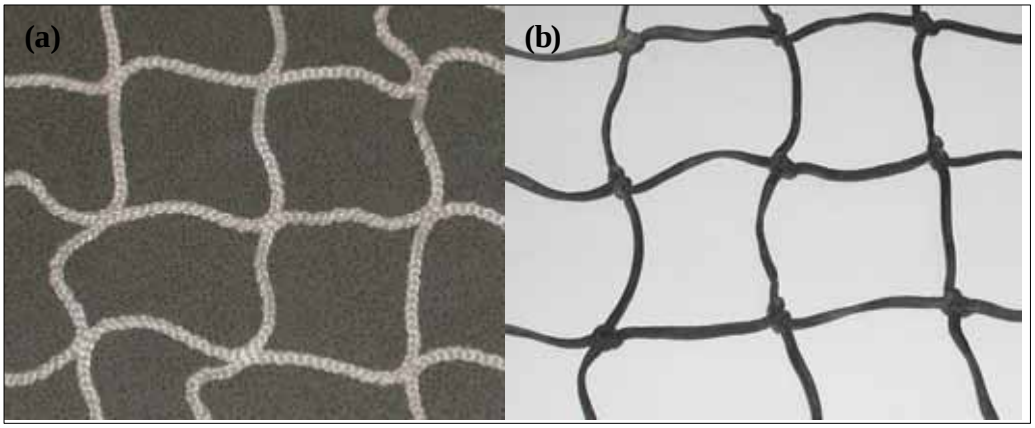


Figure 5.1: Types of netting used on the sea-cage at (a) DI and (b) AF.

[6] Aquafin CRC/FRDC Project 2001/104: Aquafin CRC - Southern Bluefin Tuna Aquaculture Subprogram: Tuna environment subproject - Development of regional environmental sustainability assessments for tuna sea-cage aquaculture.

Multivariate analyses were carried out on the fouling assemblage data for each site following Clarke (1993), using the PRIMER (Plymouth Routines in Multivariate Ecological Research) software package. For each site, differences in fouling composition between depths for each sampling time were tested using Analysis of Similarities (ANOSIM) tests, followed by non-metric Multidimensional Scaling ordination (nMDS) to visualise any patterns. A SIMPER (Similarity Percentages) analysis was also performed to examine the fouling taxa contributing to the similarities and dissimilarities within and between depths and sampling times. The data on percentage cover were arcsine transformed prior to analysis (Zar 1996), and Bray-Curtis similarities were used to eliminate the effects of joint absences of taxa.

As with the fouling assemblage data, net occlusion data were arcsine transformed before each site was analysed separately. A mixed design ANOVA was used with time as a within-subject effect and depth as a between-subject effect. For net occlusion data from AF, only three events (April, June and August) were analysed, as there were missing data for May, otherwise the dataset would be reduced to two replicates for each sampling time and there would be a loss of power to detect differences. For net occlusion data from DI, data for March were excluded for the same reasons. Mauchly's test of sphericity was employed to test for significant differences between the variances of the differences between months. If Mauchly's test was significant ($p < 0.05$), F tests were evaluated using adjusted degrees of freedom based on Greenhouse-Geisser epsilon; otherwise, no adjustments were made. Levene's test was also used to test for homogeneity of variances for each depth of the repeated measures variable (month). Where data were found to be heterogeneous, no further transformation was carried out, as the data were already arcsine transformed and ANOVA is a robust test where the reliability of the results is only affected by severe deviations (Zar 1996). The ANOVA analyses were carried out using the software package SPSS (ver 16). The mean percentage cover of fouling for each transect at each sampling time was also plotted against net occlusion to look at correlations. Water quality data for the inside of the sea-cage were plotted against data from the outside to examine differences.

RESULTS

Fouling assemblages at DI

The mean percentage cover of fouling was variable with time and across depths for the sea cage at DI (Figure 5.2). Only at the 10 m depth was there an obvious increase in the cover of fouling with time, from 20% (SE±12%) in March to 61% (SE±7%) in September. The captured images for the seasonal development of fouling assemblages for the sea-cage at DI are given in Appendix 5.1.

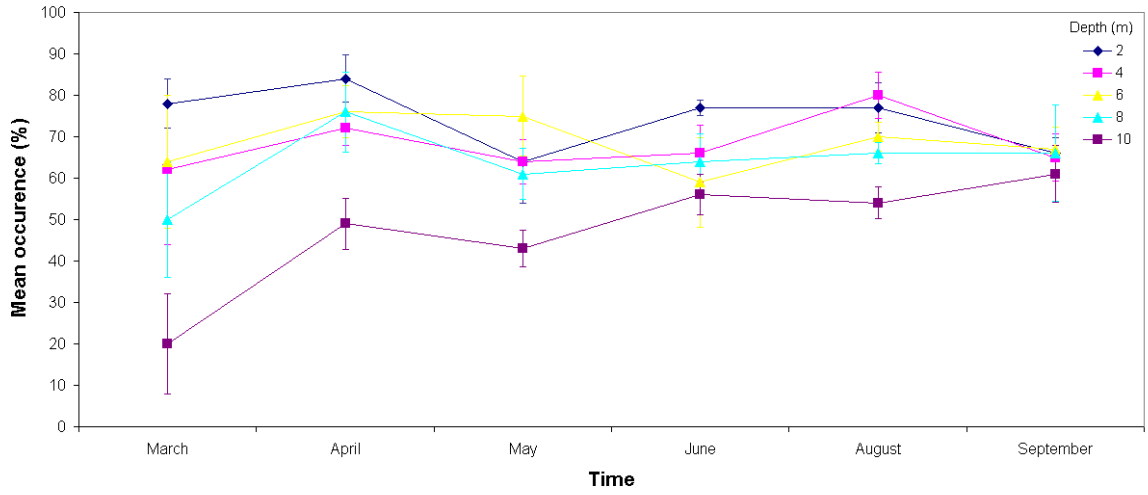


Figure 5.2: Cover of fouling for the sea-cage at DI from March to September 2005 (except July) for all depths (2, 4, 6, 8, 10m).

Multivariate analyses of the fouling assemblages at DI indicated that there were significant differences between depths and across months. The nMDS ordination plots only showed slight separation between depths, in particular between 2 m and 10 m (Figure 5.3a). Differences between sampling times were more obvious (Figure 5.3b). Regardless of depth, the samples for March grouped together, as did the samples from April and May. Samples from June were spread across the ordination plot while samples from August and September were grouped together to the left of the ordination plot (Figure 5.3b). The analysis of similarities confirmed significant differences between depths (global R = 0.14, p = 0.1%) and months (global R = 0.635, p = 0.1%). Pairwise comparisons (with Bonferroni correction for multiple comparisons) indicated significant differences between 10 m and 2 m, 10 m and 4 m, and 10 m and 6 m (Table 5.2), while there were significant differences for all months except between August and September (Table 5.3).

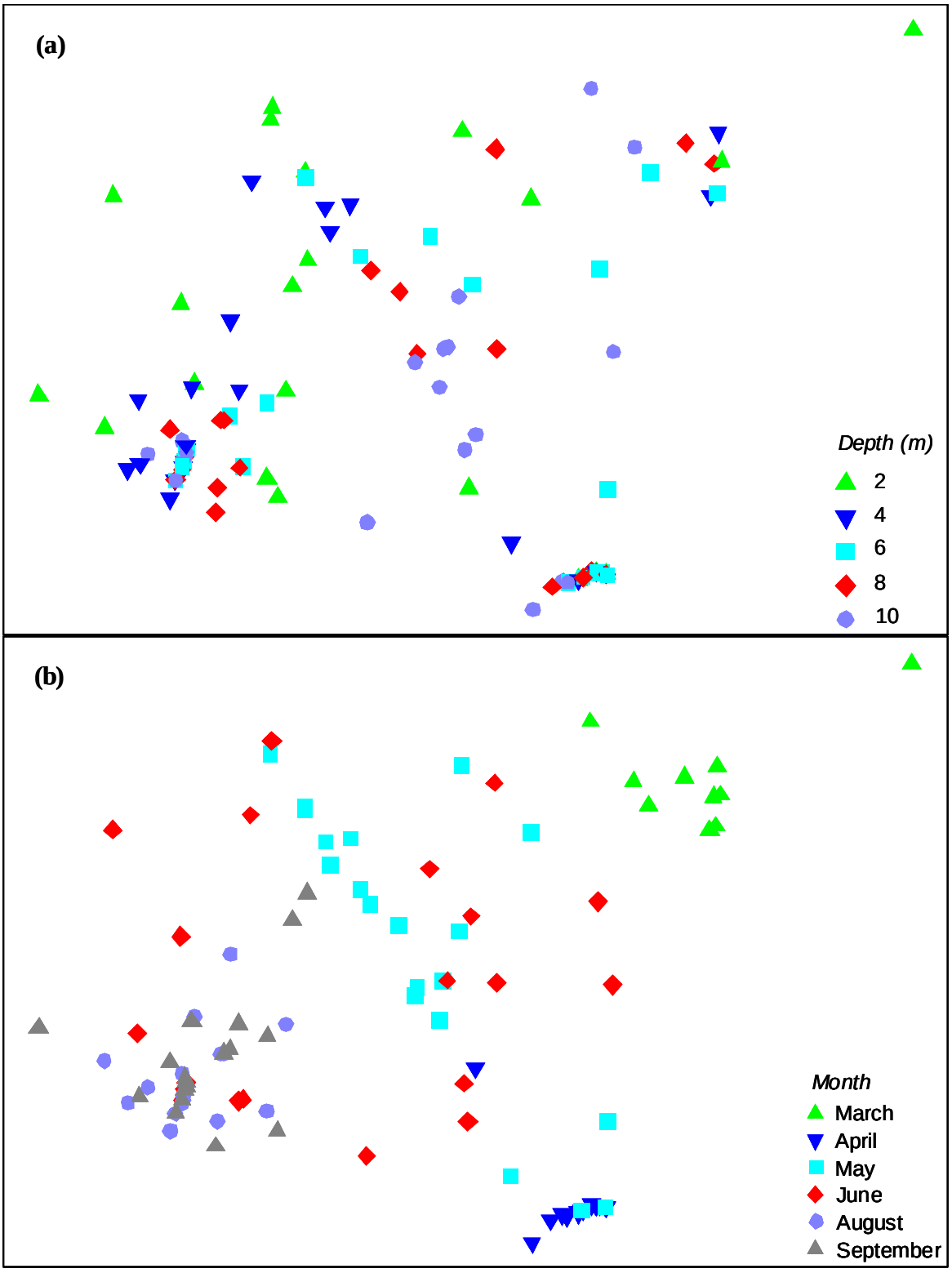


Figure 5.3: Two-dimensional nMDS ordination plots (stress=0.06) of arcsine transformed percentage cover of fouling assemblages for the sea-cage at DI with (a) depth superimposed and (b) month superimposed.

Table 5.2: Analysis of similarities (ANOSIM) for the five depths (across all months) for the sea-cage at DI with the R statistic (bold**) and the significance level (*italic*) between depths. The global R-value was 0.14 at a significant level of 0.1%. * indicates significant difference between depths.**

	2 m	4 m	6 m	8 m	10 m
2 m		**0.068**	**0.113**	**0.154**	**0.423**
4 m	*21.5%*		**0.050**	**0.037**	**0.288**
6 m	*7.6%*	*23.8%*		**-0.050**	**0.206**
8 m	*3.8%*	*27.8%*	*68.8%*		**0.075**
10 m	*0.1%**	*0.1%**	*0.5%*	*16.9%*	

Table 5.3: Analysis of similarities (ANOSIM) for the six sampling months (across all depths) for the sea-cage at DI with the R statistic (bold**) and the significance level (*italic*) between months. The global R-value was 0.635 at a significant level of 0.1%. All months were significantly different except between May and June and between August and September.**

	March	April	May	June	August	September
March		**1.000**	**0.643**	**0.436**	**1.000**	**1.000**
April	*0.1%*		**0.633**	**0.713**	**1.000**	**0.983**
May	*0.1%*	*0.1%*		**0.190**	**0.777**	**0.674**
June	*0.2%*	*0.1%*	*1.6%*		**0.354**	**0.258**
August	*0.1%*	*0.1%*	*0.1%*	*0.1%*		**-0.017**
September	*0.1%*	*0.1%*	*0.1%*	*0.2%*	*55.3%*	

The SIMPER analysis involved the calculation of the average similarity ($\overline{S_i}$) within each depth with all months (and within each month with all depths), and average dissimilarity ($\overline{\delta_i}$) between depths with all months (and between months with all depths). The results of this procedure give a breakdown of the contribution of each fouling taxon to the average overall similarity/dissimilarity. The ratio of this average term ($\overline{S_i}$ or $\overline{\delta_i}$) and the standard deviation give a useful measure of how consistently a taxon contributes to the average similarity or dissimilarity. For within depth or month similarities, a high ratio will indicate that the taxon typify that depth or month while for between group dissimilarities, a high ratio will indicate that the taxon is a good discriminating taxon.

The SIMPER analysis showed that within-depth similarities were greater than 60%, except for the 2 m depth, indicating higher variability at this depth (Table 5.4). Colonial ascidians and mixed algae were the typical fouling taxonomic groups, being recorded consistently across the samples for each depth. Both these taxonomic groups contributed to almost 80% of all within-depth similarities except for the 2m and 10m depths. Colonial ascidians contributed 14% and mixed algae contributed 18% to the overall similarity of 51% at 2m, while at 10 m, colonial ascidian contributed 35% and mixed algae contributed 14% to the overall 67%. Contributions by colonial ascidians for all the other depths were greater than 30% and mixed algae contributed more than 13% (Table 5.4). Rhodophyta (red foliaceous algae) and mixed encrusting were the two additional taxonomic groups, which contributed to the within-depth similarities at 2m and 10m respectively.

The between-depth SIMPER analysis showed that dissimilarities were less than 60% with the greatest dissimilarity between depths 2 and 10 m (58.9%, Table 5.5). Rhodophyta and mixed algae dominated at the shallower depths (2 and 4 m) while mixed encrusting and colonial ascidians dominated at 10 m (Table 5.6). However, none of the taxonomic groups were good discriminators of between-depth differences as the ratio of average overall similarity and the standard deviation were all low (< 1.0).

Table 5.4: Mean similarity of fouling taxonomic groups contributing to a cumulative percentage of ~80% of overall within-depth similarities for each depth at the sea-cage at DI (blank cells indicate that the taxon was not important or absent for that depth).

Overall within-depth similarity	***51.12***	***62.37***	***64.01***	***60.11***	***67.08***
Taxonomic group	**Mean similarity**				
	2 m	**4 m**	**6 m**	**8 m**	**10 m**
Colonial ascidian	14.15	32.99	33.11	31.04	35.17
Mixed algae	18.15	18.66	20.71	17.27	13.73
Rhodophyta	8.98				
Mixed encrusting					10.90

Table 5.5: Average dissimilarities between depths for the sea-cage at DI.

	4 m	**6 m**	**8 m**	**10 m**
2 m	46.83	50.38	52.38	58.90
4 m		38.63	37.79	44.79
6 m			36.72	40.68
8 m				37.27

Table 5.6: Principal taxonomic groups contributing to the dissimilarity between significantly different depths for the sea-cage at DI, listed in order of their contribution ($\overline{\delta}_i$) to the average Bray-Curtis dissimilarity between depths.

Taxonomic group	**Mean percentage cover***		$\overline{\delta}_i$	**Ratio**	**Cumulative %**
	2 m	**10 m**			
Rhodophyta	16.36	0.00	11.98	0.77	20.34
Mixed encrusting	5.45	9.09	10.53	0.88	38.21
Mixed algae	17.82	8.55	6.21	0.62	48.75
Colonial ascidian	14.91	21.27	5.29	0.61	57.73
Herdmania momus	0.91	3.27	4.38	0.55	65.17
	4 m	**10 m**			
Rhodophyta	9.27	0.00	7.82	0.64	17.46
Colonial ascidian	9.22	21.27	7.63	0.63	34.49
Mixed encrusting	2.73	9.09	6.17	0.59	48.28
Mixed algae	15.45	8.55	5.48	0.53	60.51
Herdmania momus	2.18	3.27	4.55	0.56	70.67

*arcsine transformed

SIMPER analysis for sampling months showed that all within-month similarities were greater than 65%, except for May and June, due to higher variability for these two months (Table 5.7). The fouling assemblages for each month were characterised by a particular taxon, hence the high within-month similarities. Seven taxonomic groups variously contributed to approximately 80% of all within-month similarities. Hydroids contributed 47% of the overall similarity of 68% in March while mixed algae contributed 89% in April. Rhodophyta contributed 12% and mixed encrusting contributed 20% to the overall similarity of 41% in May (Table 5.7). The fouling assemblages for both August and September were characterised by

colonial ascidians, while June had fouling assemblages found in all the other months (Table 5.7).

The lowest dissimilarity was between August and September (27%) while all other between-month dissimilarities were greater than 65% (Table 5.8). Seven principal taxonomic groups with average contribution to dissimilarity ($\bar{\delta}_i > 10$) variously contributed to the dissimilarities between sampling months (Table 5.9). Of these seven, mixed algae and colonial ascidians consistently contributed to dissimilarities for between month comparisons except for between March and May and between March and June (Table 5.9). Mixed algae had the highest average contribution to the overall dissimilarity for all comparisons ($\bar{\delta}_i > 49.70$). All other discriminating taxonomic groups (ratio > 1.00) contributed between 15% and 44% to the dissimilarities between each month comparison.

Table 5.7: Mean similarity of fouling taxonomic groups contributing to a cumulative percentage of ~80% of overall within-month similarities for each month at the sea-cage at DI (blank cells indicate that the taxa was not important or absent for that month).

Overall within-month similarity	***68.29***	***88.61***	***41.12***	***27.35***	***75.56***	***70.82***
Taxonomic group	**Mean similarity**					
	March	**April**	**May**	**June**	**August**	**September**
Rhodophyta			11.80	3.07		
Mixed algae		88.61				
Colonial ascidian				10.50	74.65	**66.86**
Hydroid	47.46			2.52		
Mixed encrusting			20.36	2.64		

Table 5.8: Average dissimilarities between months for the sea-cage at DI.

	April	**May**	**June**	**August**	**September**
March	100.00	95.26	90.53	100.00	100.00
April		80.23	93.79	100.00	98.60
May			77.94	93.96	90.49
June				70.51	67.96
August					26.68

Table 5.9: Principal taxonomic groups contributing to dissimilarity between significantly different sampling months for the sea cage at DI, listed in order of their contribution ($\bar{\delta}_i$) to the average Bray-Curtis dissimilarity between months.

Taxonomic group	**Average percentage cover***		$\bar{\delta}_i$	**Ratio**	**Cumulative %**
	March	**April**			
Mixed algae	0.00	0.88	49.70	5.15	49.70
Hydroid	0.57	0.00	31.51	2.45	81.21
	March	**May**			
Hydroid	0.57	0.07	27.34	2.47	28.70
Mixed encrusting	0.00	0.33	18.54	1.17	48.17
Giffordia species	0.34	0.00	15.46	1.13	64.40
Rhodophyta	0.00	0.31	14.13	0.88	79.23
	March	**June**			
Hydroid	0.57	0.10	23.00	1.74	25.40
Giffordia species	0.34	0.00	15.33	1.17	42.11
Colonial ascidian	0.00	0.27	13.94	0.76	57.51
Mixed encrusting	0.00	0.16	8.52	0.59	66.92

Herdmania momus	0.00	0.13	6.65	0.65	74.27
Rhodophyta	0.00	0.15	6.39	0.58	81.32
	March	**August**			
Colonial ascidian	0.00	0.80	44.21	3.31	44.21
Hydroid	0.57	0.00	30.13	2.36	74.35
Giffordia species	0.34	0.00	15.28	1.18	89.62
	March	**September**			
Colonial ascidian	0.00	0.73	41.11	2.44	41.11
Hydroid	0.57	0.00	30.21	2.38	71.32
Giffordia species	0.34	0.00	15.77	1.16	87.10
	April	**May**			
Mixed algae	0.88	0.18	36.17	2.24	45.08
Mixed encrusting	0.03	0.33	15.98	1.20	65.00
Rhodophyta	0.00	0.31	14.70	0.89	83.33
	April	**June**			
Mixed algae	0.88	0.06	40.38	3.23	43.05
Colonial ascidian	0.00	0.27	14.38	0.76	58.39
Mixed encrusting	0.03	0.16	8.02	0.63	66.94
Rhodophyta	0.00	0.15	6.63	0.58	74.01
Herdmania momus	0.00	0.13	6.23	0.67	80.65
	April	**August**			
Mixed algae	0.88	0.00	45.53	6.63	45.53
Colonial ascidian	0.00	0.80	42.32	4.56	87.85
	April	**September**			
Mixed algae	0.88	0.01	45.41	5.59	46.05
Colonial ascidian	0.00	0.73	39.00	3.21	85.61
	May	**August**			
Colonial ascidian	0.00	0.80	38.63	4.69	41.12
Mixed encrusting	0.33	0.06	16.54	1.37	58.72
Rhodophyta	0.31	0.04	12.66	0.87	72.20
Mixed algae	0.18	0.00	9.06	0.49	81.85
	May	**September**			
Colonial ascidian	0.00	0.73	35.53	3.22	39.26
Mixed encrusting	0.33	0.12	17.24	1.44	58.31
Rhodophyta	0.31	0.09	12.42	0.88	72.03
Mixed algae	0.18	0.01	9.63	0.52	82.68
	June	**August**			
Colonial ascidian	0.27	0.80	25.14	1.71	35.66
Mixed encrusting	0.16	0.06	9.34	0.73	48.90
Herdmania momus	0.13	0.04	7.41	0.79	59.42
Rhodophyta	0.15	0.04	6.24	0.63	68.26
Hiatella australis	0.12	0.00	5.64	0.49	76.26
Hydroid	0.10	0.00	4.64	0.53	82.84
	June	**September**			
Colonial ascidian	0.27	0.73	22.53	1.50	33.15
Mixed encrusting	0.16	0.12	9.53	0.81	47.18
Rhodophyta	0.15	0.09	6.35	0.67	56.53
Herdmania momus	0.13	0.00	6.08	0.67	65.47
Hiatella australis	0.12	0.00	5.68	0.49	73.83
Hydroid	0.10	0.00	4.51	0.53	80.47

* arcsine transformed

Fouling assemblages at AF

For the sea-cage at AF, there were increases in mean percentage cover of fouling assemblages with time for all depths (Figure 5.4). The mean percentage cover ranged from 18-22% (SE ± 2-4%) in March to 67-89% (SE ± 4-15%) in August across all depths. The captured

images for the seasonal development of fouling assemblages for the sea-cage at AF are given in Appendix 5.2.

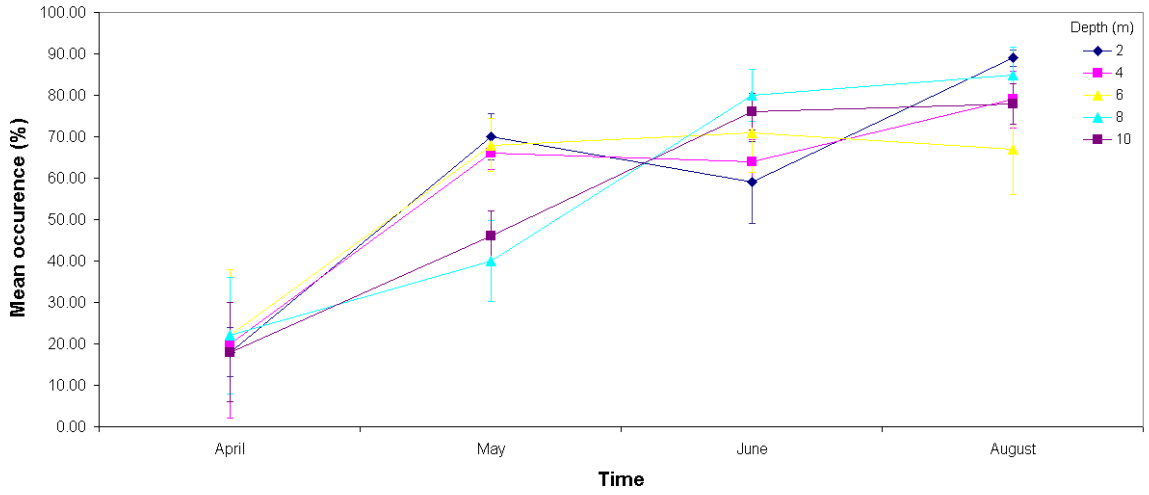


Figure 5.4: Cover of fouling assemblages for the sea cage at AF from April to August 2005 (except July) for all depths (2, 4, 6, 8, 10m).

Multivariate analyses of the fouling assemblage at AF indicated that there were significant differences between depths and across months. However, the nMDS ordination plots showed no distinct separation between depths (Figure 5.5a). Differences between sampling times were more obvious (Figure 5.5b). Samples for April and May were grouped to the right of the ordination plot but separated, while August samples occupied the left with samples from June spread across the ordination space (Figure 5.5b). The analysis of similarities confirmed significant differences between depths (global R = 0.154, p = 0.8%) and between months (global R = 0.591, p = 0.1%). Pairwise comparison (with Bonferroni correction for multiple comparisons) indicated significant differences between 2 m and 10 m (Table 5.10), while there were significant differences for all months except between May and June (Table 5.11).

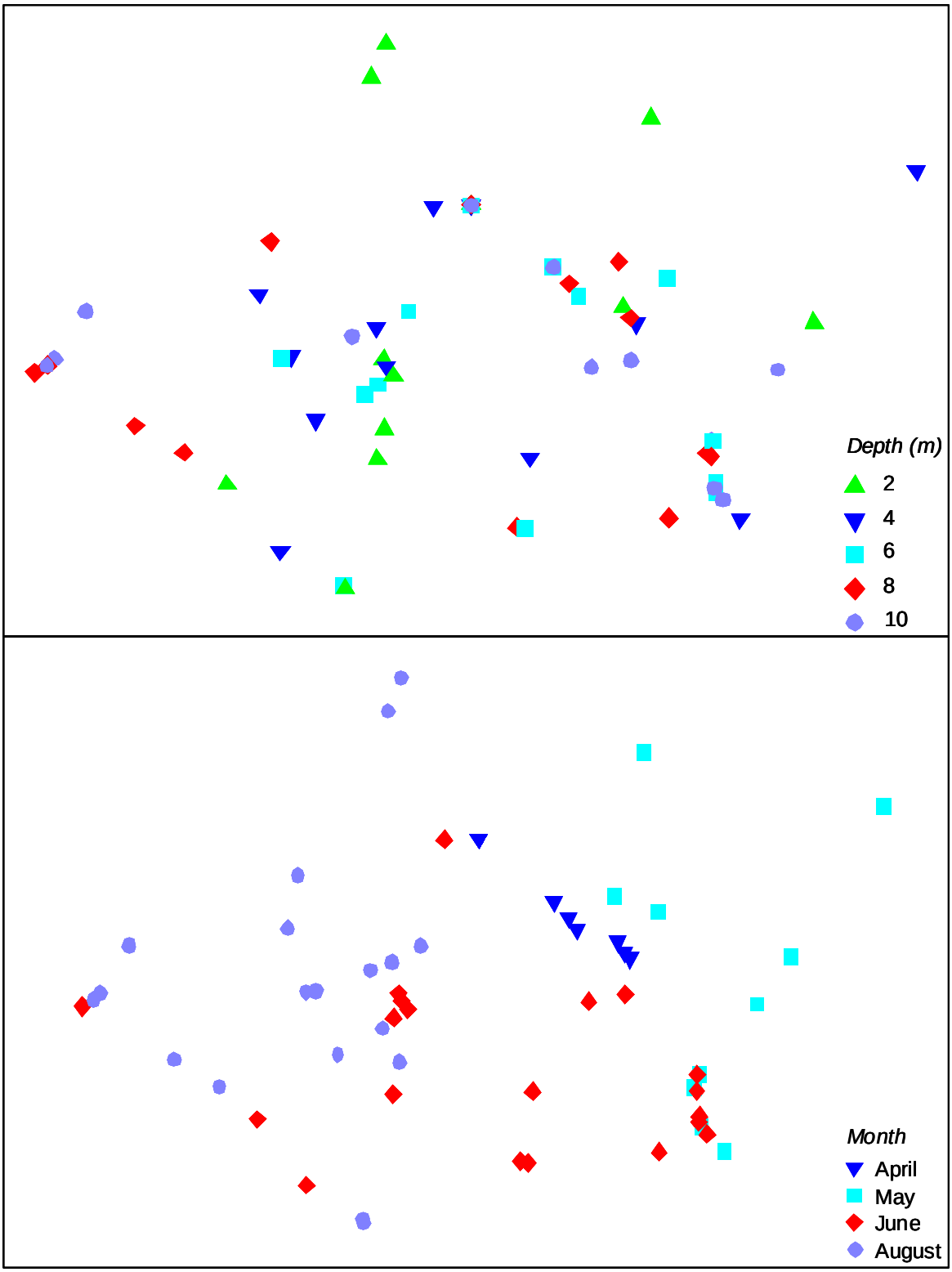


Figure 5.5: Two-dimensional nMDS ordination plots (stress=0.08) of arcsine transformed percentage cover of fouling assemblages for the sea-cage at AF with (a) depth superimposed and (b) month superimposed.

Table 5.10: Analysis of similarities (ANOSIM) for the five depths (across all months) for the sea-cage at AF with the R statistic (bold**) and the significance level (*italic*) between depths. The global R-value was 0.154 at a significant level of 0.8%. * indicates significant difference between depths.**

	2 m	4 m	6 m	8 m	10 m
2 m		**0.033**	**0.111**	**0.361**	**0.457**
4 m	*33.1%*		**-0.028**	**0.213**	**0.301**
6 m	*15.1%*	*54.3%*		**0.058**	**0.097**
8 m	*0.5%*	*3.7%*	*25.0%*		**-0.039**
10 m	*0.2%**	*1.8%*	*18.8%*	*67.1%*	

Table 5.11: Analysis of similarities (ANOSIM) for the four sampling months for the sea cage at AF with the R statistic (bold**) and the significance level (*italic*) between months. The global R-value was 0.591 at a significant level of 0.1%. All between-month comparisons were significant except between May and June (*).**

	April	May	June	August
April		**0.768**	**0.644**	**0.758**
May	*0.1%*		**0.321**	**0.836**
June	*0.1%*	*1.7%**		**0.352**
August	*0.1%*	*0.1%*	*0.2%*	

The SIMPER analysis showed that all within-depth similarities were greater than 55% (Table 5.12). The fouling assemblages for shallower depths (2, 4, and 6 m) were characterised by relative abundances of two taxonomic groups; mixed encrusting and mixed algae. Mixed encrusting contributed 33% and mixed algae contributed 20% to the overall similarity of 59% for 2 m. At 4 m with an overall similarity of 56%, mixed encrusting and mixed algae contributed 38% and 14% respectively. At 6 m with an overall similarity of 57%, contributions by the same two taxonomic groups were 28% and 14% (Table 5.12). Mixed encrusting were also observed in the deeper depths, however, contribution of colonial ascidians to within-depth similarities was obvious at the 8 and 10 m (Table 5.12).

The between-depth SIMPER analysis showed that dissimilarities ranged from 36% to 64% (Table 5.13). Depths 2 m and 10 m had the highest dissimilarity and were the only pair with significant difference (Table 5.10). These depths were characterised by higher dominance of mixed algae and mixed encrusting at 2 m and higher dominance of hydroids and colonial ascidians at 10 m (Table 5.14).

Table 5.12: Mean similarity of fouling taxonomic groups contributing to a cumulative percentage of ~80% of overall within-depth similarities for each depth at the sea-cage at AF (blank cells indicate that the taxa was not important or absent for that depth).

Overall within-depth similarity	***59.12***	***56.04***	***56.90***	***55.39***	***68.21***
Taxonomic group	**Mean similarity**				
	2 m	**4 m**	**6 m**	**8 m**	**10 m**
Mixed encrusting	33.26	37.73	28.09	19.88	28.93
Mixed algae	19.62	13.52	13.76		
Colonial ascidian				19.93	13.27

Table 5.13: Average dissimilarities between depths for the sea-cage at AF.

	4 m	6 m	8 m	10 m
2 m	42.49	49.21	63.53	64.15
4 m		38.63	52.22	51.60
6 m			46.07	43.44
8 m				35.68

Table 5.14: Principal taxonomic groups contributing to dissimilarity between significantly different depths (2 and 10 m) for the sea-cage at AF, listed in order of their contribution ($\bar{\delta}_i$) to the average Bray-Curtis dissimilarity between depths.

Taxonomic group	Average percentage cover*		$\bar{\delta}_i$	Ratio	Cumulative %
	2 m	10 m			
Hydroid	0.07	0.36	17.58	0.90	27.41
Mixed algae	0.32	0.03	14.98	0.89	50.77
Colonial ascidian	0.04	0.20	10.74	0.63	67.52
Mixed encrusting	0.30	0.21	8.48	1.02	80.74

* arcsine transformed

SIMPER analysis for sampling months showed that the highest within-month similarity was for April (77%) and all other months were below 50% (Table 5.15). April and May were characterised by a single taxonomic group. For April, mixed encrusting contributed 75% to the overall similarity of 77%, while for May, hydroids contributed 32% to the overall similarity of 45%. June and August had a mix of three taxonomic groups with hydroids typical in June (29%), and colonial ascidians in August (24%, Table 5.15).

The highest dissimilarity was between May and August and the lowest was between May and June (Table 5.16). Five principal taxonomic groups (average contribution to dissimilarity, $\bar{\delta}_i > 10$) variously contributed to the dissimilarities between sampling months (Table 5.16). Hydroids had the highest average contribution to the overall dissimilarity for all comparisons ($\bar{\delta}_i > 18.00$) except between April and August. All other taxonomic groups contributed between 12% and 33% to the dissimilarities between each month comparison. The ratio (~1.00 or greater) indicated that the principal taxa were generally good discriminating taxa.

Table 5.15: Mean similarity of fouling taxonomic groups contributing to a cumulative percentage of ~80% of overall within-month similarities for each month at the sea-cage at AF (blank cells indicate that the taxa was not important or absent for that month).

Overall within-month similarity	***77.35***	***44.53***	***49.35***	***53.15***
Taxonomic groups	**April**	**May**	**June**	**August**
Mixed encrusting	74.52		7.44	11.71
Hydroid		32.20	25.81	
Mixed algae			16.10	14.58
Colonial ascidian				24.06

Table 5.16: Average dissimilarities between months for the sea-cage at AF.

	May	June	August
April	**75.02**	**70.61**	**75.19**
May		**65.32**	**95.31**
June			**69.09**

Table 5.17: Principal taxonomic groups contributing to the dissimilarity between significantly different sampling months for the sea-cage at AF, listed in order of their contribution ($\bar{\delta}_i$) to the average Bray-Curtis dissimilarity between months.

Taxonomic group	Mean percentage cover*		$\bar{\delta}_i$	Ratio	Cumulative %
	April	**May**			
Hydroid	0.13	0.56	33.16	1.32	44.20
Mixed encrusting	0.38	0.10	21.09	1.64	72.32
	April	**June**			
Hydroid	0.13	0.42	26.21	1.08	36.11
Mixed algae	0.00	0.33	19.69	0.95	65.00
Mixed encrusting	0.38	0.19	17.09	1.67	89.20
	April	**August**			
Colonial ascidian	0.00	0.42	24.66	0.96	32.79
Mixed algae	0.00	0.38	21.27	1.02	61.08
Mixed encrusting	0.38	0.28	12.86	1.25	78.18
	May	**August**			
Hydroid	0.56	0.00	25.45	1.51	26.71
Colonial ascidian	0.00	0.42	20.70	0.96	48.43
Mixed algae	0.00	0.38	16.62	1.07	65.87
Giffordia species	0.30	0.00	13.13	1.04	79.64
Mixed encrusting	0.10	0.28	11.94	1.13	92.17
	June	**August**			
Hydroid	0.42	0.00	18.99	1.00	27.48
Colonial ascidian	0.10	0.42	17.87	1.00	53.34
Mixed algae	0.33	0.38	14.33	1.13	74.09
Mixed encrusting	0.19	0.28	9.89	1.08	88.40

*arcsine transformed

Percentage net occlusion at DI

As biofouling developed, the mean percentage net occlusion generally increased through time for all depths (Figure 5.6). In March, mean occlusion was 9% at 10 m, 28% at 8 m, 31% at 6 m, 41% at 4 m and 46% at 2 m. By September, the mean occlusion had increased to 63% at 10 m and 8 m, 61% at 6 m, 71% at 4 m and 62% at 2 m.

The ANOVA indicated that there were significant differences in net occlusion between months ($F_{(2.4,\ 35.7)} = 16.91$, $p < 0.001$) but no significant differences between depths ($F_{(4,15)} = 1.888$, $p = 0.165$). There was no interaction effect between month and depth ($F_{(9.5,\ 35.7)} = 0.660$, $p = 0.746$). Mauchly's test indicated that the assumption of sphericity was violated for the effect of month ($\chi^2(9) = 17.064$, $p = 0.049$), therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.51$). Levene's test for homogeneity was not significant for any month ($p = 0.112$ for April, $p = 0.719$ for June, $p = 0.139$ for August and $p = 0.851$ for September), except for May ($p = 0.008$). The plot of mean cover of fouling against mean percentage net occlusion had a moderate correlation ($r^2 = 0.55$, Figure 5.7). The correlation is not high because of some data points having high fouling cover but low net

occlusion. This is a consequence of the method used for scoring cover where fouling is scored if its cover is > 50%.

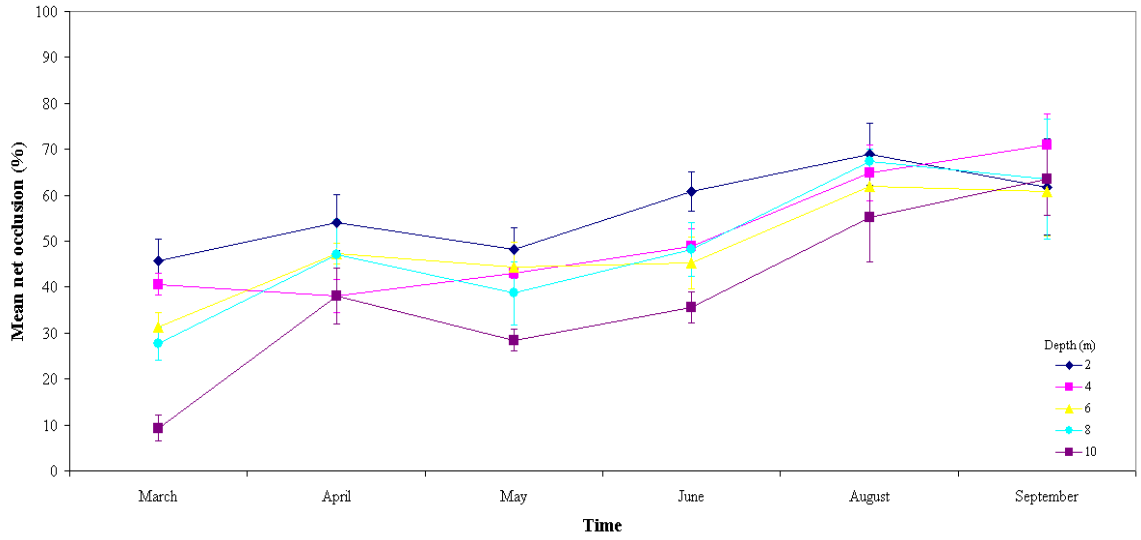


Figure 5.6: Change in net occlusion at each depth due to development of fouling organisms from March to September for the sea-cage at DI.

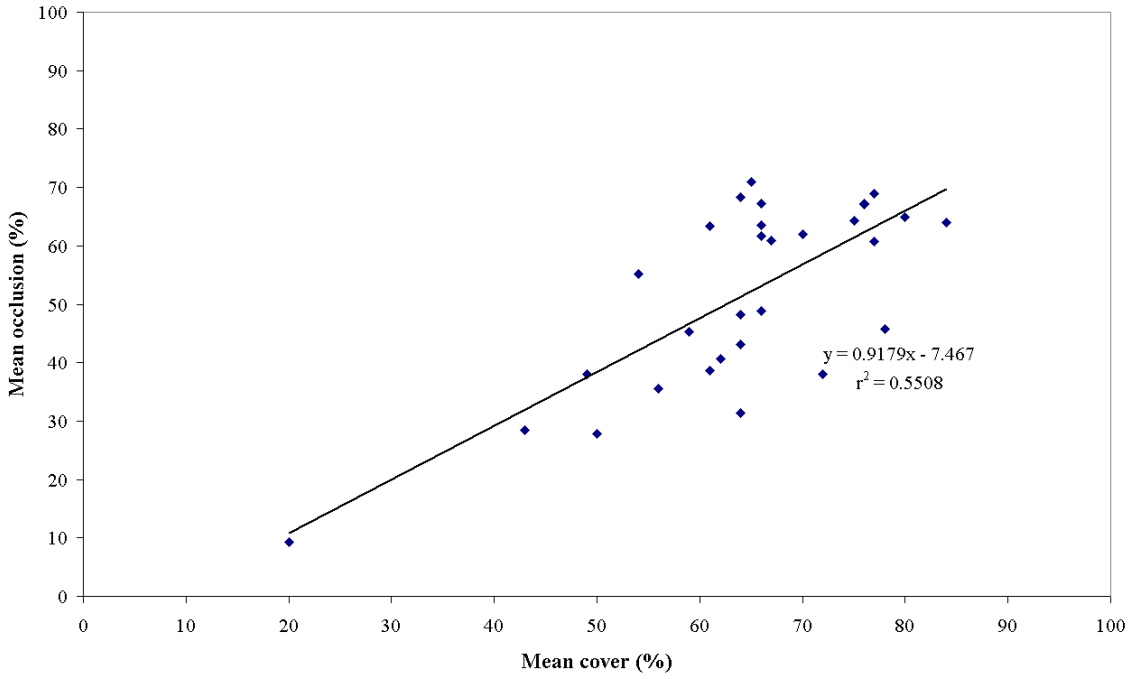


Figure 5.7: Correlation between mean occlusion and mean cover of fouling (r^2 = 0.55) for the sea-cage at DI.

Percentage net occlusion at AF

Development of biofouling on the net at AF occurred later than the net at DI. Video recordings of the net in April showed minimal fouling. The mean occlusions in April ranged from 4% to 8% for all depths and by August, mean occlusions had increased to 79% at 2 m,

51% at 4 m, 56% at 6 m, 70% at 8 m and 79% at 10 m (Figure 5.8). However, there was a decrease in mean occlusion from May (51 – 69%) to June (26 – 56%).

ANOVA indicated that there were significant differences in net occlusion between months ($F_{(2, 30)}$ = 167.56, p = 0.001) but not depths ($F_{(4,15)}$ = 2.681, p = 0.72). Again there was no interaction between month and depth ($F_{(8, 30)}$ = 1.385, p = 0.243). Mauchly's test indicated that the assumption of sphericity was not violated for the effect of month ($\chi^2(2)$ = 0.905, p = 0.497). The plot of mean percentage occurrence of fouling assemblages against mean percentage of net occlusion indicated a high correlation (r^2 = 0.79, Figure 5.9).

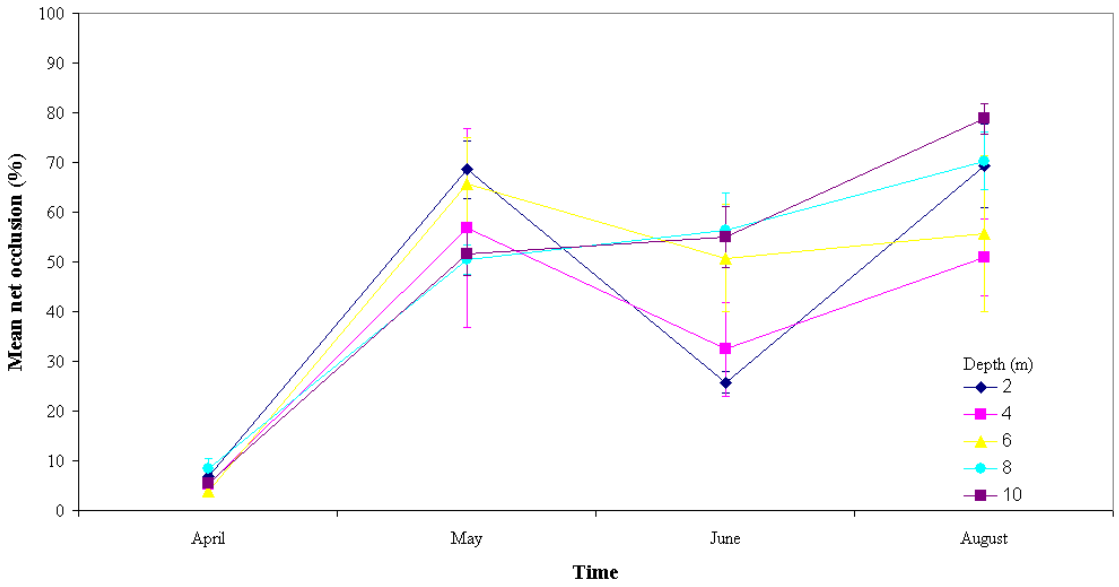


Figure 5.8: Change in mean net occlusion due to development of fouling organisms over time for the sea-cage at AF.

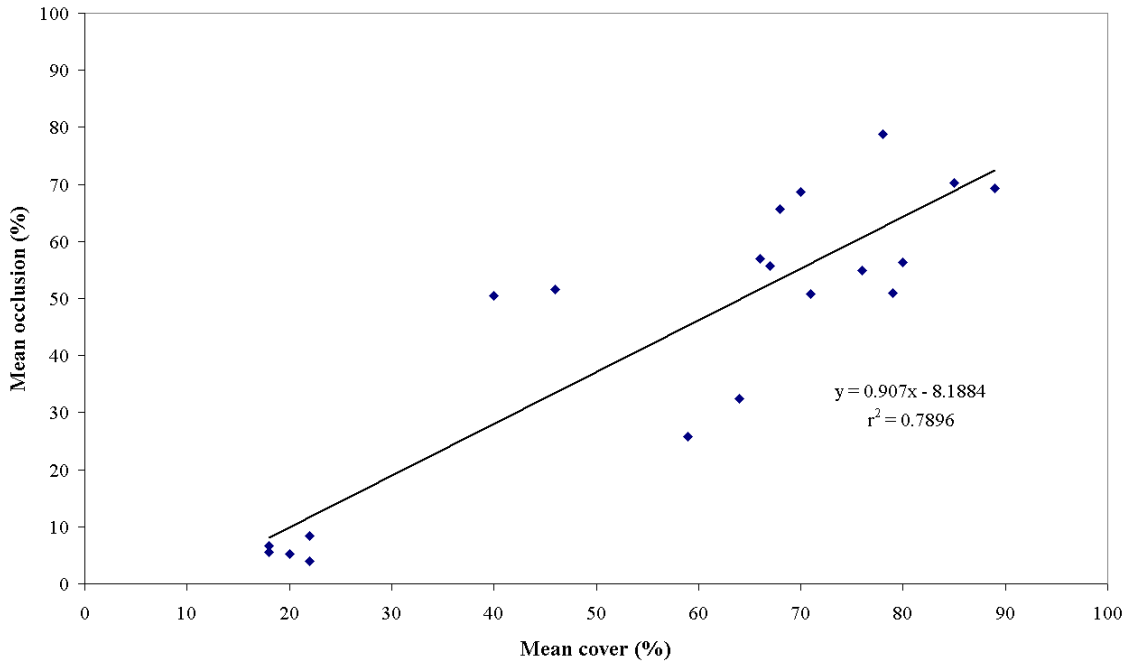


Figure 5.9: Correlation between mean occlusion and mean cover of fouling assemblages (r^2 = 0.79) for the sea-cage at AF.

Water quality data

The SBT telemetry-based environmental monitoring system deployed at the DI pontoon indicated that there were differences between water quality parameters recorded inside and outside the sea-cage. The mean daily salinity showed some higher values inside the sea-cage (Figure 5.10) while there were no differences in mean daily temperature (Figure 5.11). On the other hand, dissolved oxygen (in % saturation) was always different between the inside and outside of the sea-cage, and these differences changed through time. In March and April, there were some differences between the % saturation of dissolved oxygen inside and outside of the sea-cage (Figure 5.12). However, in May, dissolved oxygen started to be higher on the outside than the inside and from June to August, dissolved oxygen was always higher on the outside of the sea-cage (Figure 5.12).

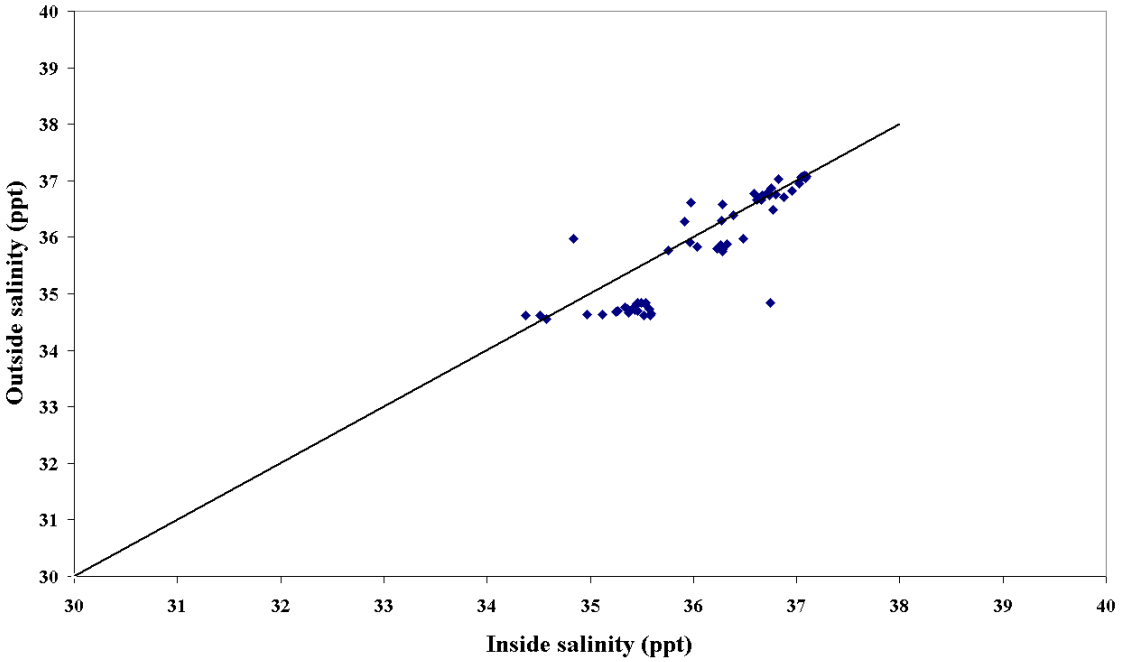


Figure 5.10: Some higher mean daily salinity (ppt) recorded at DI on the inside as compared to the outside as shown by more points lying below the black line, which indicates equal salinity on the inside and outside of the sea-cage.

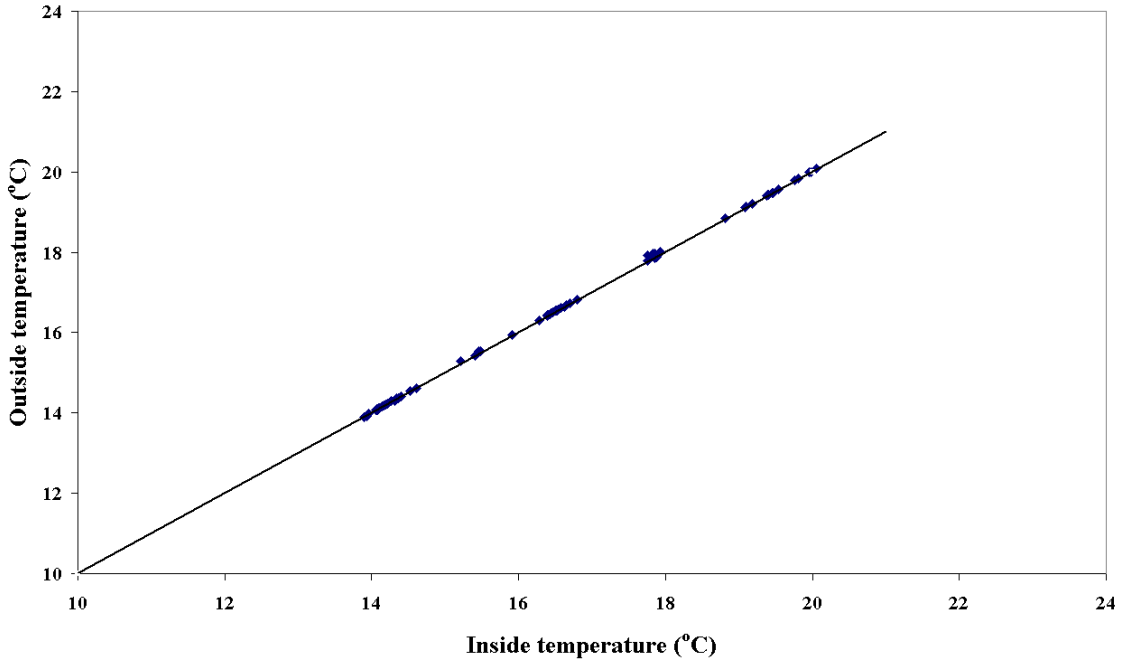


Figure 5.11: No differences in mean daily temperature (°C) recorded on the inside and outside of the sea-cage at DI as shown by most points lying along the black line, which indicates equal temperature between the inside and outside of the sea-cage.

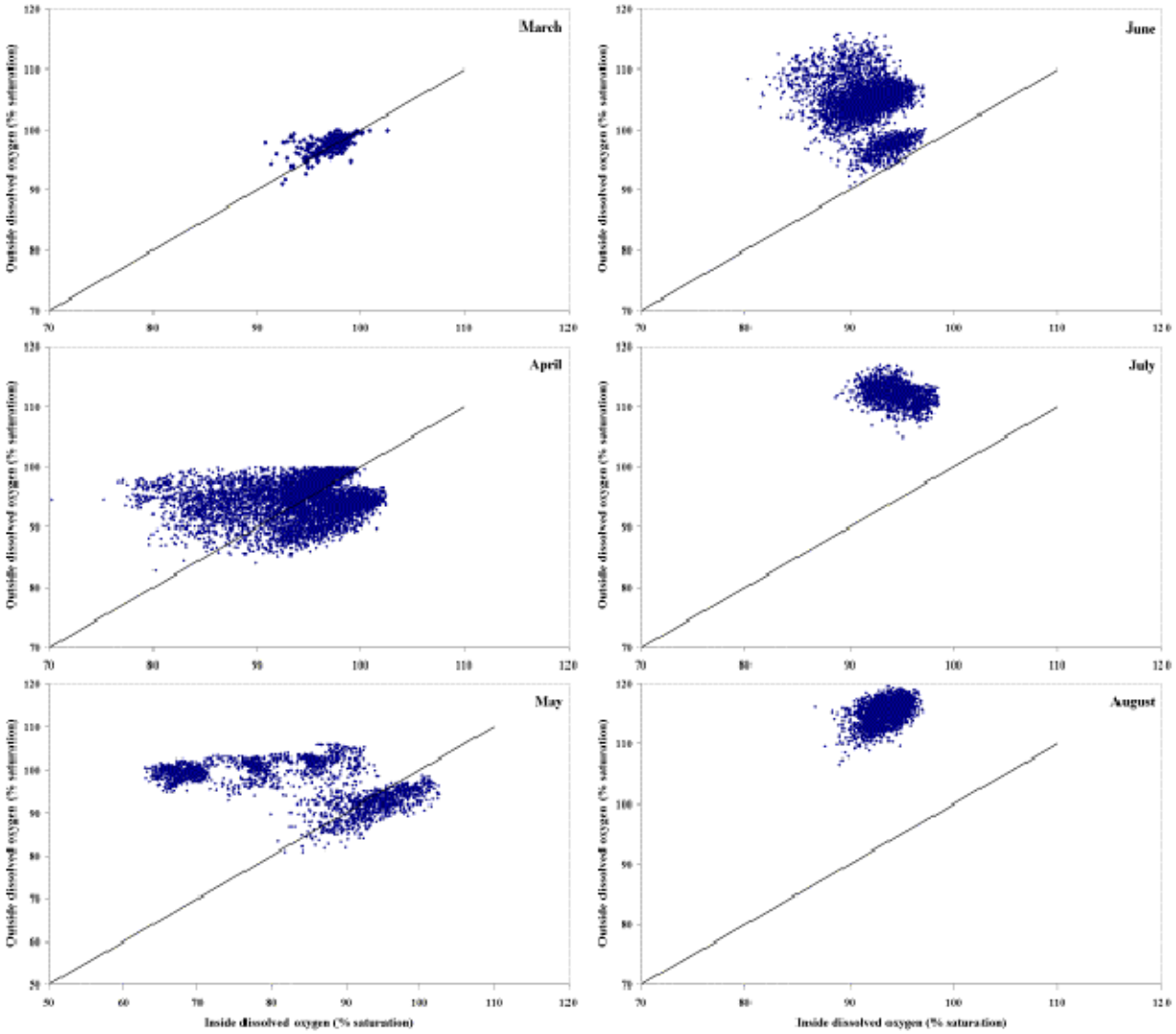


Figure 5.12: Change in dissolved oxygen (% saturation) recorded on the inside (x-axis) and outside (y-axis) of the sea-cage at DI from March to August 2005. Dissolved oxygen became consistently higher on the outside of the sea-cage by June as shown by more points above the black line, which indicates equal dissolved oxygen between the inside and outside of the sea-cage.

DISCUSSION AND CONCLUSION

Net fouling assemblages can be diverse and are predominantly composed of algae and sessile invertebrates (Cheah and Chua 1979, Claereboudt *et al.* 1994, Cronin *et al.* 1999). The fouling assemblages on the sea-cage at DI across all depths comprised a range of 14 taxonomic groups (four animal phyla and three algal divisions), being dominated by colonial ascidians mostly from the family Dideminidae and mixed algae from the divisions Rhodophyta, Chlorophyta and Phaeophyta. However, the occurrence of Rhodophyta at 2 m and mixed encrusting at 10 m significantly separated these two depths, probably a consequence of reduced light availability with depth. The significant differences between months were driven by the seasonal variation in the development of fouling assemblages. Hydroids dominated early in the season (March), followed by mixed algae and encrusting organisms in April and May. The fouling assemblages were dominated by colonial ascidians in August/September. Therefore the dissimilarities between months were characterised by the dominance of algae early in the season and colonial ascidians later in the season. Additionally, the bivalves *Electroma*

georgiana and *Hiatella australis* were recorded from June onwards, although not in high cover. The fouling assemblages on the sea-cage at AF were less diverse with nine taxonomic groups recorded (also from four animal phyla and three algal divisions). Mixed algae and encrusting organisms characterised the shallower depths while colonial ascidians again dominated the deeper depths (8 and 10 m). Similar to the sea-cage at DI, the seasonal development of fouling assemblages on the sea-cage at AF moved from hydroid dominated assemblage in April to ascidian dominated assemblage in August. The bivalve *Electroma georgiana* was also recorded from June onwards.

The dominant fouling assemblages recorded in this study were different from those in previous studies of fouling in the Port Lincoln region (Cronin *et al.* 1999, Svane *et al.* 2006). Cronin *et al.* (1999) recorded the bivalve *Electroma georgiana* as the most dominant taxon, while Svane *et al.* (2006) recorded the green alga *Enteromopha sp.* and sponges as dominant taxa. These differences may be attributed to the different locations of the sea-cages used in the various studies. The two previous works were carried out within Boston Bay (west of Boston Island) while the present study was carried out outside the bay (east of Boston Island) in the tuna aquaculture zone. However, the seasonal development of fouling assemblages on the two sea-cages in this study was generally in agreement with the previous studies. Furthermore, the seasonal and depth-related development of fouling assemblages was in accordance with other studies elsewhere. Algae tend to dominate fouling assemblages at shallower depths (Braithwaite *et al.* 2007), while ascidians and mussels tend to dominate "climax communities" that develop on nets in temperate mariculture (Claereboudt *et al.* 1994, Hodson *et al.* 2000, Braithwaite and McEvoy 2005, Romo *et al.* 2001).

One of the main effects of net fouling on the management and operation of sea-cage systems is to change patterns of water flow through the nets and thereby affect the supply of oxygen and removal of wastes from cages. Macroalgae on nets of sea-cages are not considered to be significant in fouling assemblages, being restricted in their growth by availability of light, although more often they are out-competed by faster growing heterotrophs responding to increased organic input from the farming operations (Cronin *et al.* 1999). Oxygen production by the algal component can be relatively high compared to the respiration rates of the fouling assemblages, but the total oxygen flux of the fouling community was considered to be negligible when compared to that consumed by the fish and underlying sediments (Cronin *et al.* 1999). On the other hand, ascidians and mussels can potentially pose a particular threat to the aquaculture industry because of their relative size and weight, where exchange of materials through the net may be disrupted and structural stress may be caused by the increased weight (Braithwaite and McEvoy 2005). This disruption of exchange through the net was demonstrated in this study from the water quality data collected. In particular, dissolved oxygen concentration within the sea-cage was shown to become increasingly lower through time as net occlusion increased with increased fouling growth.

When SBT farming was first conducted in Boston Bay, nets showed obvious fouling after only 4 weeks (Bond 1993), quickly becoming heavily covered by fouling organisms. In another study, typical fouling rates of between 2 and 4 kg wet weight/m^2 were observed on sea-cages (Cronin *et al.* 1999) and more recent work, also conducted on experimental sea-cages in Boston Bay, largely confirmed these results and has shown that nets, even when treated with anti-foulant, may accumulate substantial fouling loads over relatively short periods (Svane *et al.* 2006). Fouling load can potentially reach levels of 81% in untreated nets compared with 66% for treated nets (Svane *et al.* 2006). Further work carried out in Subproject 2 of this project, where a range of anti-foulants were tested on nets in the Boston Island East farming zone, largely confirmed the overall trend with fouling on untreated nets after 155 days (~5 months) resulting in occlusion of 68% whilst treated nets were typically around 41% (Rough and Ellis 2007).

Managing net fouling is important in any aquaculture operation. Net fouling resulting in occlusion of the net can lead to a reduction in water flow and therefore disruption of exchange through the net. This in turn can limit oxygen supply to the sea-cage, as well as accumulation of undesirable fish farm wastes (e.g. nutrients) within the cage. Consequently, understanding the development of fouling assemblages and its effect on oxygen supply to the sea-cage as demonstrated in this subproject, together with the other components of this project, will assist farm managers to make better decisions about the management of net fouling.

Chapter 6 : SUB-PROJECT 4: DETERMINATION OF THE RELATIONSHIP BETWEEN THE LEVEL OF FOULING AND ITS IMPACT ON NET WEIGHT, NET DRAG AND WATER EXCHANGE USING A FLUME TANK

This chapter was authored by Kirsten Rough (Australian Southern Bluefin Tuna Industry Association) and may be cited as:

Rough KM, Fitzgibbon Q, and Loo MGK (2008) Determination of the relationship between the level of fouling and its impact on net weight, net drag and water exchange using a flume tank. In Rough KM, de Nys R, Loo MGK, and Ellis DC (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp.

SUMMARY

Segments of ten new clean net types made from different materials, with stretched-mesh sizes ranging from 57 to 200mm were contributed by companies for the project. Each was weighed, had its internal mesh area calculated and occlusion value determined by photography and image analysis with computer software. Panels were mounted within a flume tank to evaluate their influence on water flow (both passage through, and dynamics after the net); then deployed in the marine environment to assess weight increase from biofouling growth.

The weight of the clean dry net segments was influenced by the construction material, cord thickness and mesh size. So that the heaviest net included 8 strands of steel and the lightest was Dyneema. Internal mesh area ranged from 8cm² for small kingfish containment net to 100cm² for tuna containment and tow nets. Occlusion value, the amount of the image that was blocked by the presence of net material, was highest (23.6%) for the small meshed kingfish net and lowest (3.9%) for the 190mm (stretch) Dyneema net that had a cord thickness of only 1.5mm.

In the flume tank, water behaviour through the new nets was measured at velocities of 0 to 60cm/sec (0 to 1.18 knots). Resistance to water flow through the net was related more strongly to the internal mesh area (compared to occlusion value) at a velocity setting of 40cm/sec. But at a higher velocity setting of 60cm/sec both decreasing internal mesh area and increasing occlusion value resulted in increased resistance to water flow through the net. The velocity of water after it had passed through the net surface, therefore its ability to penetrate beyond the net wall was reduced for the net with a thick, 7mm cord despite this net having a large stretched-mesh size of 180mm. There were also slight indications that water flow was developing turbulent motion after passage through the nets with thick cord. Increased turbulence can decrease water penetration beyond the net surface.

Biofouling growth occurred on all net segments regardless of the material, the inclusion of metal strands, the colour, the cord thickness and the surface topography. There appeared to be more biofouling growth on the kingfish nets, that had the smallest mesh sizes (smallest internal area and highest occlusion value), but this relationship was not consistent across the larger meshed nets. There were no replicates available for this trial to test if there was delayed colonisation by biofouling assemblages with time, net colour, manufacture material, surface topography, occlusion value, or internal area.

Panels of white nylon 150mm stretched-mesh Badinotti tuna net were deployed sequentially at 2 and 9m depth in the marine environment to determine the influence that biofouling growth has on water flow within a flume tank. Due to a combination of fish grazing panels and strong winds dislodging heavy aggregates of biofouling, only two types and densities of fouling were recovered in the first season. The experiment was repeated for a second season to increase the diversity of biofouling assemblages, and densities recovered.

Biofouling growth was greater at the shallower depth, but due to the diversity of biofouling types there was not a clear pattern of increased weight with time. Dry weight gains of 0 to 877% were recorded across different deployment times and depths. Shell growth made the greatest contribution to weight gain, especially on a dry weight basis. Sparse shell growth was found to give a similar dry weight increase to heavy algal and soft-bodied invertebrate growth.

At increasing water velocities in the flume tank, image analysis of individual panels with light to heavy biofouling growth was repeated. This demonstrated that occlusion values decreased with increasing water velocity, and the magnitude of difference between speeds of 7 to 45cm/sec was greatest where panels had dense fouling of a soft and flexible nature (algal growth).

In the flume tank it was found that light fouling growth (occlusion value approximately 40%) did not reduce water velocity through the net at current speeds up to 50cm/sec. However, the presence of shell growth at an occlusion value of approximately 40% did appear to induce turbulence after water passage through the net and this increased with increasing current speed.

The influence that heavy fouling (occlusion value 70 – 80%) had on water flow varied with the type of biofouling assemblages present. Heavy shell growth reduced water velocity through the net at a current speed setting of 30cm/sec and induced turbulence from a setting of only 10cm/sec. Heavy weed growth did restrict water flow through the net at a low water velocity setting (15cm/sec). But this tended to lessen at higher current speeds, when algal growth was pushed through the mesh, and tended to compress and align behind the net cord. This improvement in water flow was decreased if shell growth was present amongst the algae; the presence of even low density hard shelled organisms induced turbulence that can effectively deflect water back away from the net surface (rather than allowing it to pass through the net).

The presence of biofouling can influence oxygen levels within a net in two main ways, by altering water exchange (see above) and through respiration, whereby the fouling communities extract and use oxygen as water is adjacent to and passes through the net surface. Oxygen consumption by different fouling communities was tested when panels were suspended in the flume tank. Dense fouling growth of exclusively hard shelled invertebrates was found to consume approximately 860mg of oxygen per square metre per hour ($mgO_2/m^2/hr$). This respiration rate is equivalent to a resting 1kg kingfish. Moderate algal growth with a light inclusion of hard shelled organisms had a respiration rate of $37mgO_2/m^2/hr$. Heavy algal growth that contained an abundant population of mobile invertebrates (eg skeleton shrimp and amphipods) consumed approximately $169mgO_2/m^2/hr$. Light fouling of soft invertebrates (hydroids) consumed approximately $107mgO_2/m^2/hr$.

INTRODUCTION

The problems associated with the growth of biofouling on nets within sea-cage finfish aquaculture systems are numerous, and include impacts on both the cultured species and on associated infrastructure; as outlined in the ‘background’ for the entire project and reviewed by de Nys et al (2005a), Chapter 1 this document. The main aims of the ‘Net Fouling

Management to Enhance Water Quality and SBT Performance' project were to better understand the impact of net fouling within the South Australian tuna farming industry, and to investigate antifouling treatment as an option to mitigate these. The purpose of this subproject was primarily to provide data to enhance the Oxytuna model, subproject 5, Chapter 7 (Cheshire and Loo 2008) by establishing the relationship between percentage cover of the fouling community with parameters such as water flow and net drag. However, there was a lot of interesting additional information obtained on the influence of different net types and biofouling loads on water flow, and the respiration rates of fouling communities; and these will be reported here.

METHODS

New Clean Net

A number of tuna companies contributed segments of new net to test within the flume tank and/or deploy in the marine environment to determine weight gain due to biofouling growth. Each net panel was photographed prior to flume tank experiments or deployment, had their internal mesh area calculated and the occlusion value determined by image analysis, as described Chapter 4, this document (Rough and Ellis 2007).

The internal mesh area is a physical measure of the amount of the open space between the net cords. Occlusion value is the amount of an image (photograph) that is occupied by netting, and is influenced by both the thickness of the cord used to make the net, and the number of cords in a given area.

Net With Biofouling

New white, Badinotti 150mm (stretch mesh) nylon tuna net was cut into panels of 400 x 400mm, individually weighed, labelled, mounted into steel frames and then reweighed. These nets were sequentially deployed at an operating tuna lease site on rope frames at 2m and 9m depths at fortnightly intervals from 4th May to 10th September 2004. All panels were retrieved on the 8th November, photographed, individually packed, and then transported chilled overnight to the flume tank laboratory at the University of Adelaide for testing on the following day. Each net panel was photographed prior to the flume tank trials and the occlusion value was determined by image analysis, as described in Chapter 4 of this document (Rough and Ellis 2007).

Panels were mounted in the flume tank, perpendicular to the water flow and tested at a range of water velocities typical of those experienced in the tuna zones (5 to 60 cm/sec). Wet (drip free) and dry weights of clean and fouled panels was determined after the flume tank experiments. Panels of differing fouling levels and types were also deployed in the dark in a closed water chamber to determine oxygen consumption by the biofouling communities.

Flume Tank Experiments

The mesh of a net placed perpendicular to water flow can influence water flow behaviour in two ways; by reducing the amount of water passing through the mesh and altering the velocity and direction of water movement after passing through the mesh. In the flume tank water behaviour was measured as velocity in the directions of X, Y and Z (Figure 6.1); although only X direction (horizontal flow through the net) will be discussed here.

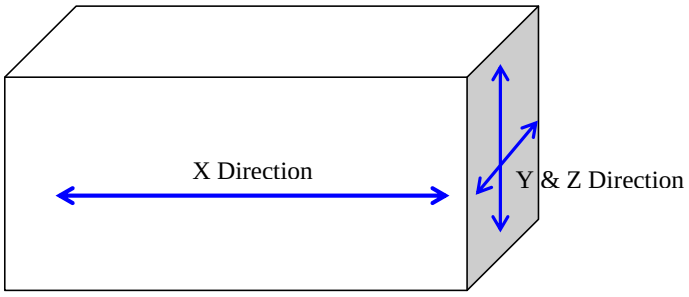


Figure 6.1: Schematic diagram of water flow in a flume tank

An example of a net that has not interfered with water flow is shown in Figure 6.2, whereby the water level and arrows indicating direction and volume of water flow are equal on either side of the net panel.

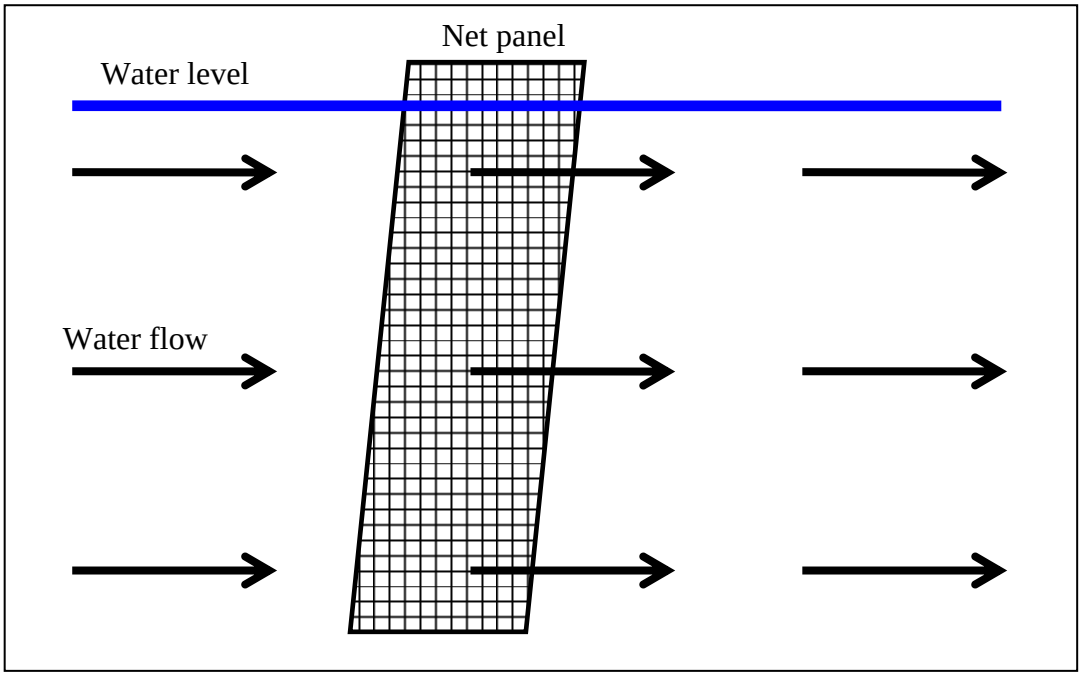


Figure 6.2: Schematic diagram of water behaviour through a clean net panel in a flume tank under low velocity water flow conditions (arrows indicate the direction and relative volume of water flow)

An example of a net impeding water flow is shown in Figure 6.3. Here the water level is elevated immediately before the net panel because water is deflected back away from the mesh; this in turn creates a relative depression in water height behind the panel. This difference between the water heights on either side of the panel is referred to as the pressure gradient in this report; it is a proxy measure of the resistance that the net has on free water flow. The arrows that indicate the direction and volume of water flow show the initiation of turbulence which reduces the velocity and distance that water travels after passing through the mesh. Turbulence after the net panel (i.e. inside a net) can further act to push water away from the mesh and effectively block new water flow into a net.

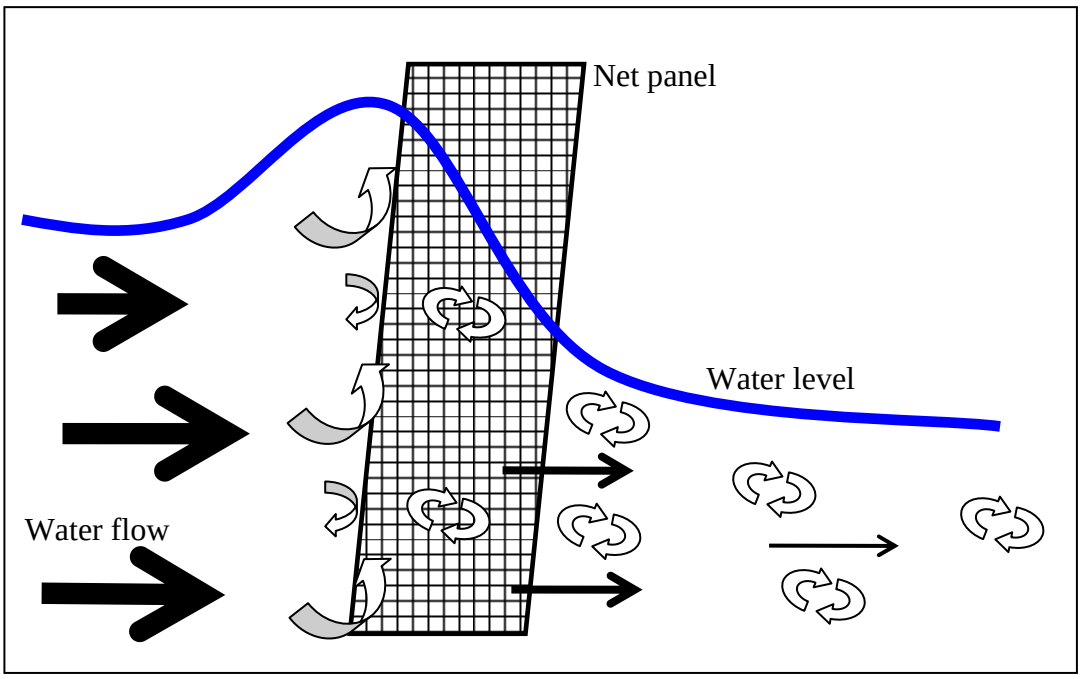


Figure 6.3: Schematic diagram of water behaviour through a very fouled or a small meshed or a thick cord net panel in a flume tank under high velocity water flow conditions (arrows indicate direction and relative volumes of water flow)

RESULTS AND DISCUSSION

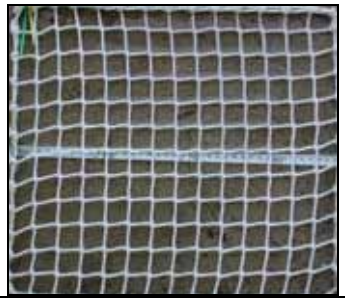
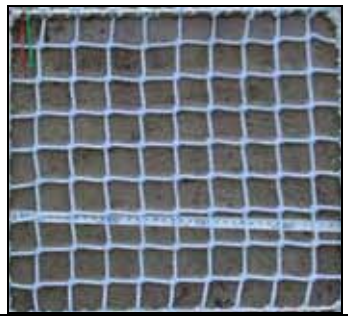
New Clean Net

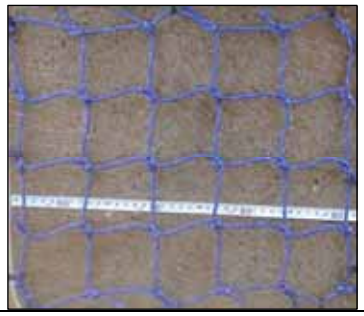
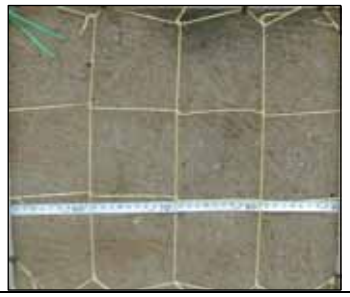
Net Types

Photographs, internal mesh area and occlusion values of new nets contributed by tuna companies can be seen in Table 6.1. The smallest internal mesh area was 8cm² for the 25mm kingfish net, and the greatest was 100cm² for the "8 inch" black tuna net (Table 6.1). The lowest occlusion value of (3.9%) was obtained for the Dyneema net that has a large mesh size (190mm) and thin cord (1.5mm) (190 dyneema kn 1.5; Table 6.1). Of the net types currently used by the industry, the "6 and 8 inch" sized black knotted tuna nets (150 bl kn 3.5, and 200 bl kn 3.5; Table 6.1) gave low occlusion values of 10.7% and 8.5% respectively. The highest occlusion values of 23.6% and 21.7% were obtained from the small (57 wh ro 3) and large (78 wh ro 3.5) meshed kingfish nets respectively (Table 6.1).

It was found that the occlusion values of these 400 by 400mm nets were correlated to the internal mesh area ($R^2 = 0.6173$).

Table 6.1: Net types, photographs, details and occlusion values for new clean netting tested within the flume tank and/or deployed to determine resistance to biofouling growth

Appearance	Description	Internal (open) Area / mesh	Occlusion Value
	Kingfish growout Nylon rochelle Mesh size (stretch): 57mm Cord diameter: 3mm **(57 wh ro 3)***	8.13 cm²	23.6%
	Kingfish growout Nylon rochelle Mesh size (stretch): 78mm Cord diameter: 3.5mm **(78 wh ro 3.5)***	15.21 cm²	21.7%
	Tuna growout and tow Nylon knotted Resin treated Mesh size (stretch): 150mm Cord diameter: 3.5mm **(150 bl kn 3.5)***	56.25 cm²	10.7%
	Tuna growout and tow Nylon rochelle Mesh size (stretch): 150mm Cord diameter: 6mm **(150 wh ro 6)***	56.25 cm²	17.8%
	Not in use Polypropylene knotted With 1 strand steel, 8 copper Mesh size (stretch): 150mm Cord diameter: 5mm **(150 gr kn 5)***	56.25 cm²	16.0%

	Not in use Polypropylene knotted Mesh size (stretch): 150mm Cord diameter: 4mm **(150 blu kn 4)***	56.25 cm²	12.1%
	Not in use Polypropylene knotted With 8 strand steel Mesh size (stretch): 180mm Cord diameter: 6mm **(180 wh kn 6)***	81.00 cm²	15.5%
	Tuna growout and tow Nylon rochelle Mesh size (stretch): 180mm Cord diameter: 7mm **(180 wh ro 7)***	81.00 cm²	17.9%
	Not in use Dyneema knotted Mesh size (stretch): 190mm Cord diameter: 1.5mm **(190 dyneema kn 1.5)***	90.25 cm²	3.9%
	Tuna growout and tow Nylon knotted Resin treated Mesh size (stretch): 200mm Cord diameter: 3.5mm **(200 bl kn 3.5)***	100.00 cm²	8.5%

* id code used in some figures and tables

The weight of each 400 by 400mm net segment and their weight gain due to biofouling growth are given in Table 6.2. The inclusion of metal strands (steel and copper) within the net cord increases the weight of the net. All panels had biofouling growth when retrieved in August. The two kingfish nets (57 wh ro 3, and 78 wh ro 3.5) with the smallest mesh sizes,

hence smallest internal mesh area and highest occlusion values, had the greatest mass of biofouling growth.

Of the four net types with 150mm stretch mesh, equivalent internal mesh area, the black netting with the thinnest cord (150 bl kn 3.5) had the least fouling. The green netting with steel and copper strands (150 gr kn 5) had the most biofouling (Table 6.2). There did not appear to be a trend of increasing fouling growth with increasing occlusion value. Other differences between these net types, such as net material, colour and surface topography could all potentially influence the amount of biofouling growth. This could not be determined in this experiment without replication. The inclusion of copper strands within the net cord (150 gr kn 5) did not appear to deter biofouling growth. However, there may have been a delay in colonisation, but there were no examinations throughout the deployment to confirm this.

There were differences in the dry weight of biofouling growth on the larger meshed nets, but due to no replication it is unclear whether the differences are due to net material, colour, surface topography, cord thickness or a combination of these. The net panel with the steel strands and the net with the finest cord (Dyneema) had the least fouling, but there did not appear to be a trend with internal mesh area or occlusion value.

Table 6.2: Dry weight of each 400 x 400mm net segment prior to deployment, and the dry weight and percentage weight gain due to biofouling growth after 5 months submerged at sea

Identification code (see Table 6.1)	Dry weight New Net (grams)	Dry weight Fouling Growth (grams)	% weight gain (foul wt / net wt)
57 wh ro 3	70.9	230.6	325 %
78 wh ro 3.5	75.2	325.5	248 %
150 bl kn 3.5	52.6	75.0	143 %
150 wh ro 6	81.4	134.9	135 %
150 gr kn 5	97.1	171.7	177 %
150 blue kn 4	54.4	149.6	275 %
180 wh kn 6	135.8	93.9	69 %
180 wh ro 7	69.4	110.8	160 %
190 dyneema kn 1.5	13.0	54.8	422 %
200 bl kn 3.5	47.9	105.2	220 %

Flume Tank Experiments

The net types displayed in Table 6.1, deployed in the flume tank and tested at water velocities ranging from 0 to 60cm/sec[7] (i.e. 0 to 1.18 knots), demonstrated varying degrees of influence on water flow through the panels. Most obvious was in measuring differences in the pressure gradient, the water level on either side of the net panel (Figure 6.4). There was no difference in water height before and after the net panel for all net types tested at water velocities less than 15cm/sec (0.3 knots). At a water velocity of 40 cm/sec (approximately 0.8 knots) a pattern emerged whereby the nets with smaller mesh sizes were creating a greater resistance to free water flow. However, the relationship between the pressure gradient differences and the occlusion value of the net panels was only weak ($R^2 = 0.402$). At greater water velocity (60cm/sec or 1.18 knots), the relationship between the occlusion value and resistance to water flow is much stronger with an R^2 of 0.793. At both of these water velocities, resistance to water flow appeared to be more related to the internal mesh area of the net, whereby the R^2 values were 0.8194 and 0.8772 for 40 and 60cm/sec currents respectively.

[7] Note that 60cm/sec was the maximum velocity attainable with the flume tank used at Adelaide University

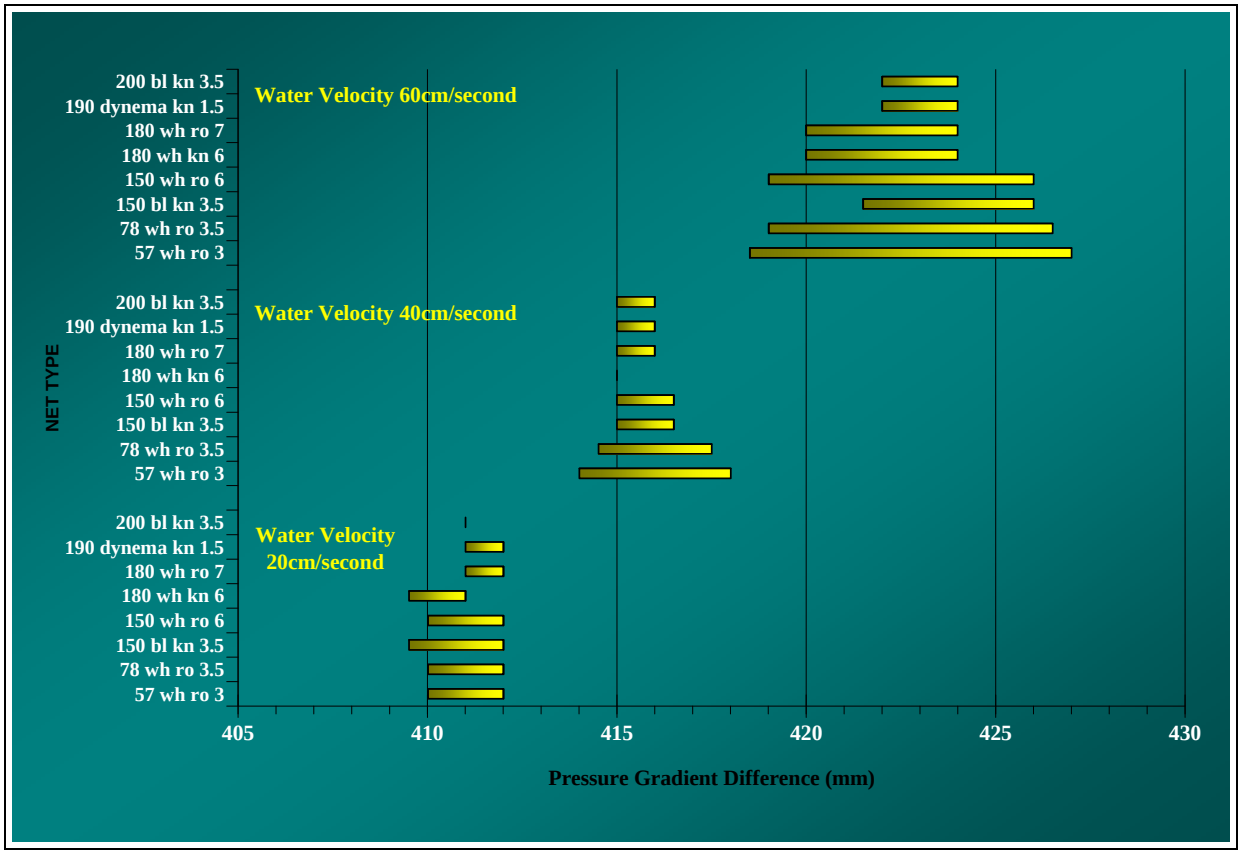


Figure 6.4: Resistance to free water flow incurred by different net types

The speed (velocity) of the water after it has passed through the net relative to the speed of the water in the flume tank is another measure of a nets influence on water flow. A reduction in water velocity as it passes through the net may reduce the penetration of new water into the cage.

Figure 6.5 shows the velocity of water (in the X direction) after passing through the net panel (y axis) relative to the water speed in the flume tank (x axis). There were indications that at the higher water velocities (>40cm/sec), tuna netting with greater cord thickness (eg Figure 6.5e), had decreased the speed of water after it passed through the net, despite this net having a relatively large mesh size (180mm stretch). To a lesser extent the panels of 78mm kingfish net, and 180mm knotted tuna net (Figure 6.5b and f) also reduced the speed of water after it passed through the mesh when the flume tank water speed was 60cm/sec (1.18 knots).

Turbulence is another force that can act to reduce water flow into the net where water movement within the net actually deflects new water away from the net surface. Turbulence created by the panel of netting after water has passed through is measured as directional deviation and plotted (in the X direction) in Figure 6.6. There were indications that at water velocities of 60cm/sec (1.18 knots), the net panels as shown in Figure 6.6 c, e and f, were initiating turbulence.

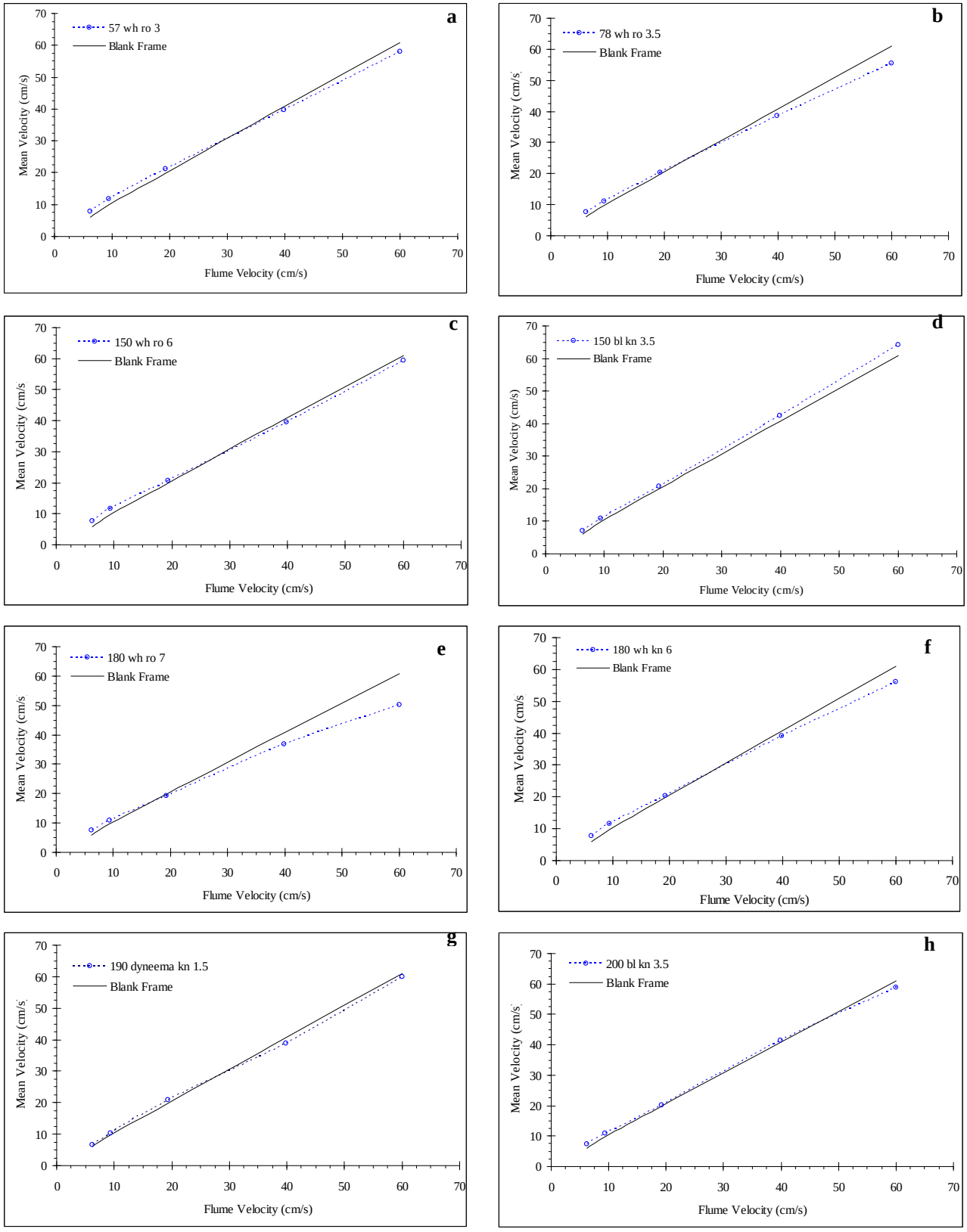


Figure 6.5: Influence of panels of new netting on water velocity in the X direction, compared to an empty frame in a flume tank.

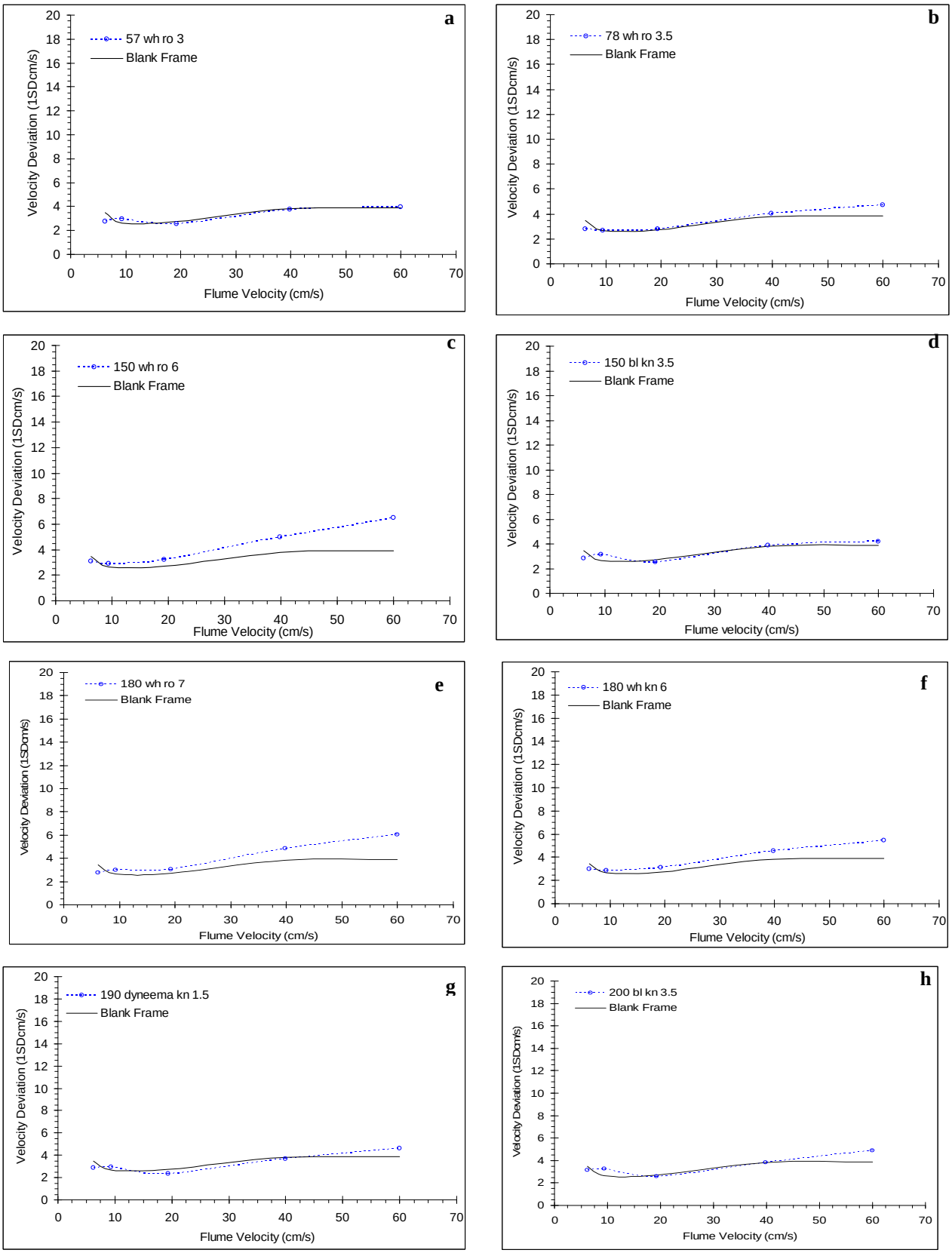


Figure 6.6: Turbulence (X direction deviation) created by panels of new netting, compared to an empty frame in a flume tank.

Net With Biofouling

Biofouling Growth

2004

Despite sequential deployment, the majority of the panels had a similar light covering of biofouling at the completion of the time submerged at sea (Plate 6.1, Plate 6.2). Consequently at the end of the trial, only two types of biofouling were present, very heavy growth of hard shelled invertebrates (*Mytilus edulis*) or light covering of soft bodied invertebrates (mostly hydroids) that showed indications of having been grazed by fish. The panels retrieved at the end of the trial had less fouling than mid way through the trial, as shown in Plate 6.3 and Plate 6.4. This was probably due to a combination of migrating leather jackets consuming the greater majority of fouling after the last panels' deployment in September; and heavy aggregates of loosely attached fouling dislodging with panel movement in strong wind events.

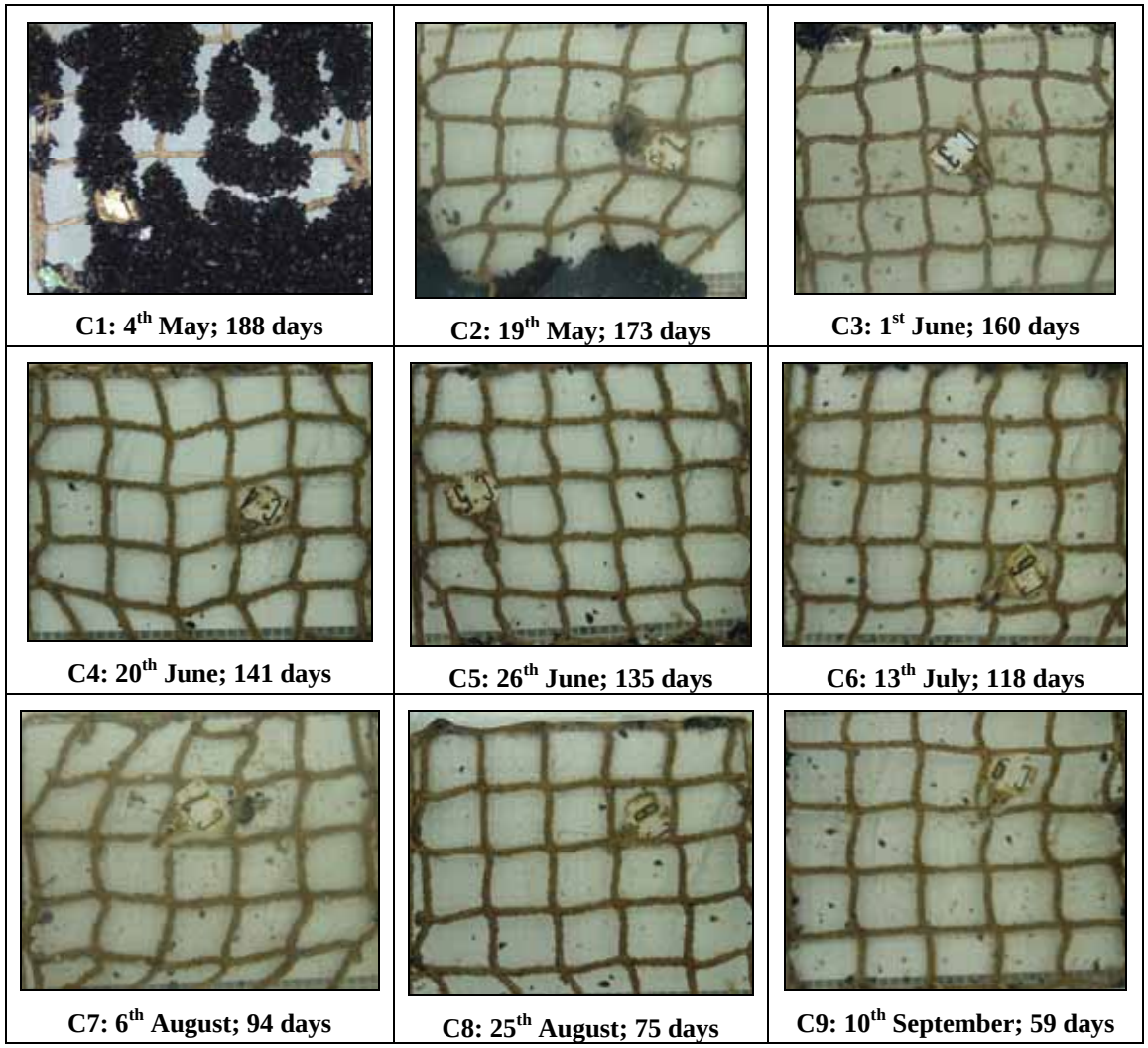


Plate 6.1: Biofouling growth on all panels deployed sequentially at 2m depth from May to September 2004

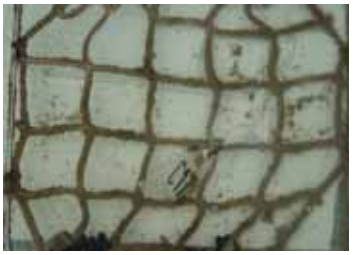
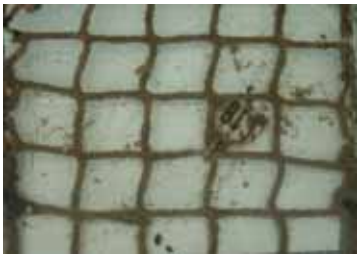
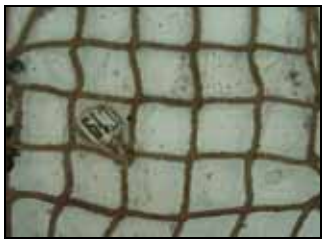
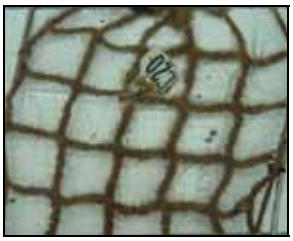
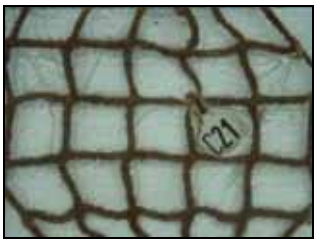
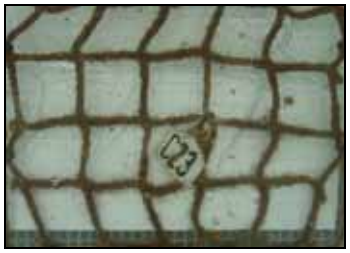
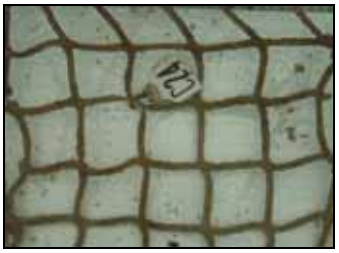
Panel not recovered **C16: 4th May; 188 days**	 **C17: 20th May; 172 days**	 **C18: 3rd June; 158 days**
 **C19: 17th June; 144 days**	 **C20: 7th July; 124 days**	 **C21: 13th July; 118 days**
 **C22: 6th August; 94 days**	 **C23: 25th August; 75 days**	 **C24: 10th September; 59 days**

Plate 6.2: Biofouling growth on all panels sequentially deployed at 9m depth from May to September 2004

Plate 6.3: A photograph of the 2m panel line taken on 27th August 2004, showing heavy biofouling growth on all panels sequentially deployed fortnightly from May.

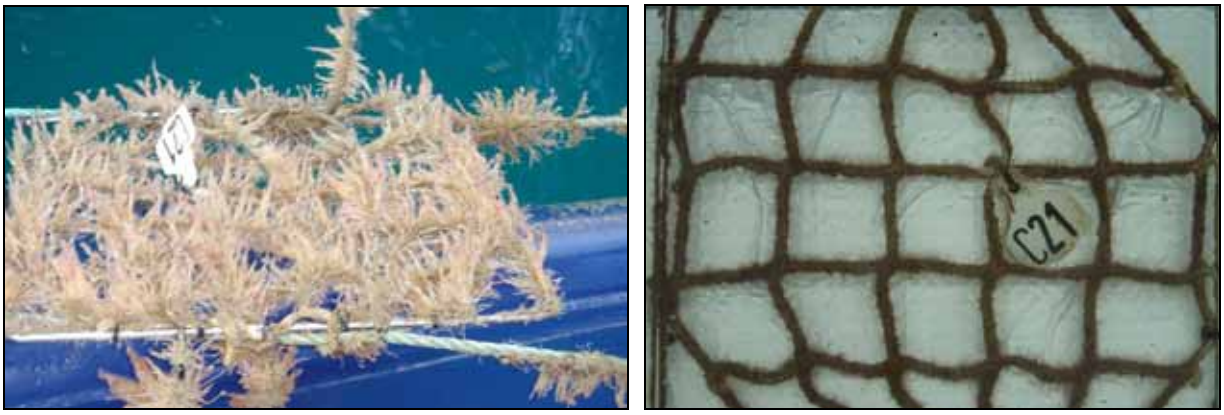
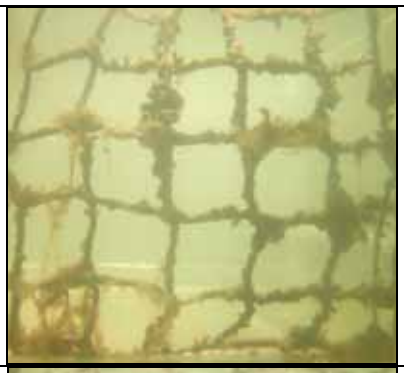
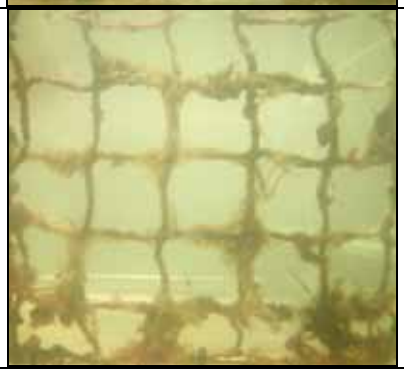


Plate 6.4: Panel C21, deployed at 9m on 13th July 2004; photographed on the 27th August (left) and at the trial completion 8th November (right)

2005

Due to the loss of fouling from predation and/or strong wind events in 2004, the sequential deployment net panels for the flume tank experiment was repeated in 2005. These were retrieved in August prior to the migration of scavenging leather jackets through spring and summer. At retrieval, these panels included more variety of biofouling types and densities compared with those deployed in 2004 (Figure 6.7 and Figure 6.8).

	**A125** **Deployed March; 144 days** **<u>Fouling Load</u>: light to medium** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp. / Hiatella australis*** **20 *Electroma georgiana*** **Minimal red algae / hydroids / bryozoans**
	**A127** **Deployed April; 120 days** **<u>Fouling Load</u>: light to medium** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.*** **8 *Electroma georgiana,* 9 *Hiatella australis,* numerous small Mytilidae sp.** **Minimal hydroids / red algae / *Serpulidae sp. / Colpomenia sp.***

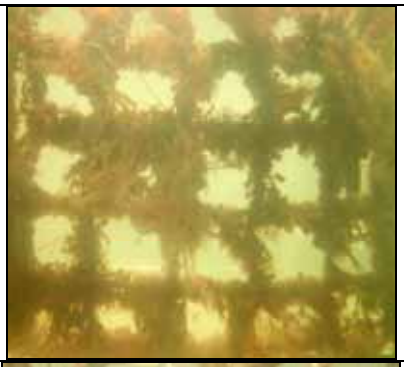
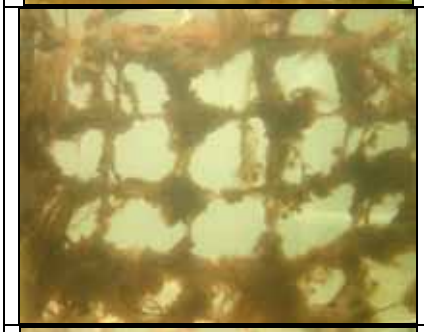
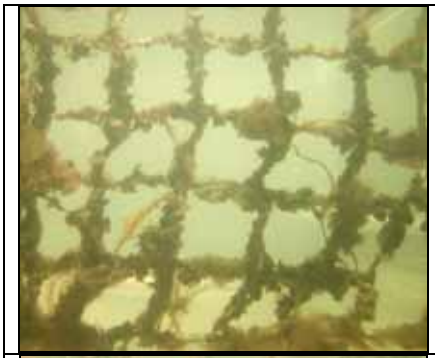
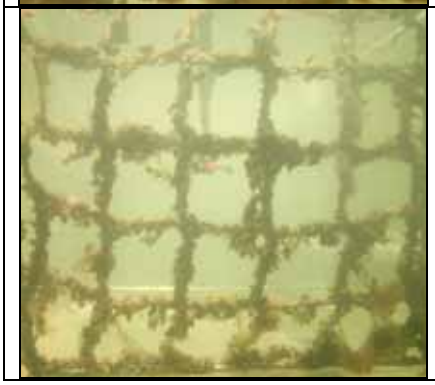
	**A129** **Deployed May; 91 days** **<u>Fouling Load</u>: heavy** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.* / red algae / strap-like brown algae / polychaete tubes / *Hiatella australis* / hydroids** **4 *Electroma georgiana*** **Minimal *Serpulidae sp. / Colpomenia sp.* / green algae**
	**B125** **Deployed June; 57 days** **<u>Fouling Load</u>: medium to heavy** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.*** **2 *Electroma georgiana*, 1 Mytilidae sp.** **Minimal *Serpulidae sp.* and polychaete tubes**
	**B127** **Deployed July; 29 days** **<u>Fouling Load</u>: medium to heavy** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.*** **Minimal red algae and polychaete tubes**

Figure 6.7: Biofouling growth on panels sequentially deployed at 2m depth from March to July 2005, (note photographs taken within the flume tank at a water velocity of 7cm/sec)

	**A126** **Deployed March; 144 days** **<u>Fouling Load</u>: medium** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.* / polychaete tubes / bryozoans / *Hiatella australis*** **2 *Electroma georgiana*** **Minimal *Serpulidae sp.* / strap-like brown algae**
	**A128** **Deployed April; 120 days** **<u>Fouling Load</u>: medium** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.* / polychaete tubes / bryozoans / *Hiatella australis*** **1 *H. momus*** **Minimal *Serpulidae sp.* / red algae**

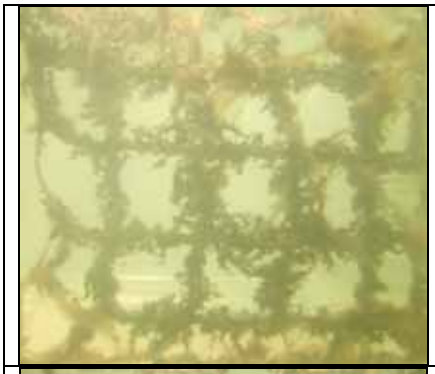
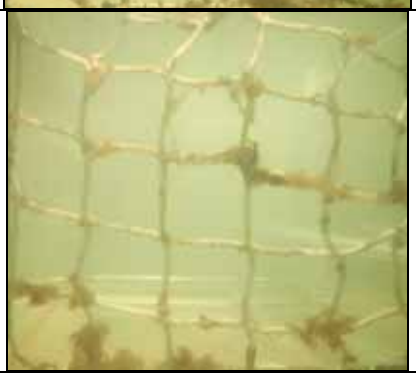
	**A130** **Deployed May; 91 days** **<u>Fouling Load</u>: medium to heavy** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.* / polychaete tubes** **6 *Electroma georgiana*** **Minimal *Serpulidae sp.* / red algae / bryozoans**
	**B126** **Deployed June; 57 days** **<u>Fouling Load</u>: light to medium** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.* / numerous hydroids and polychaete tubes** **2 *Electroma georgiana*** **Minimal red algae**
	**B128** **Deployed July; 29 days** **<u>Fouling Load</u>: light** **<u>Fouling Description:</u>** **Dominant: *Giffordia sp.*** **3 *Electroma georgiana*** **Minimal red algae**

Figure 6.8: Biofouling growth on panels sequentially deployed at 9m depth from March to July 2005, (note photographs taken within the flume tank at a water velocity of 7cm/sec)

Biofouling Weight

2004

As previously discussed in the section on biofouling growth, the majority of panels recovered in 2004 had very little fouling. Consequently the weight increase due to biofouling growth was around 100 grams (wet weight); and <25 grams (dry weight) for most panels (Figure 6.9, Figure 6.10). The exceptions being panel C1 with the heavy growth of mussels that increased in weight by 1782 grams (wet); and 626 grams (dry), and panel C2 which had wet and dry weight increases of 588 grams and 216 grams respectively.

For the net panels deployed through 2004 these weight gains equated to increases of 39% to 1569% wet or 5% to 877% dry (Table 6.3).

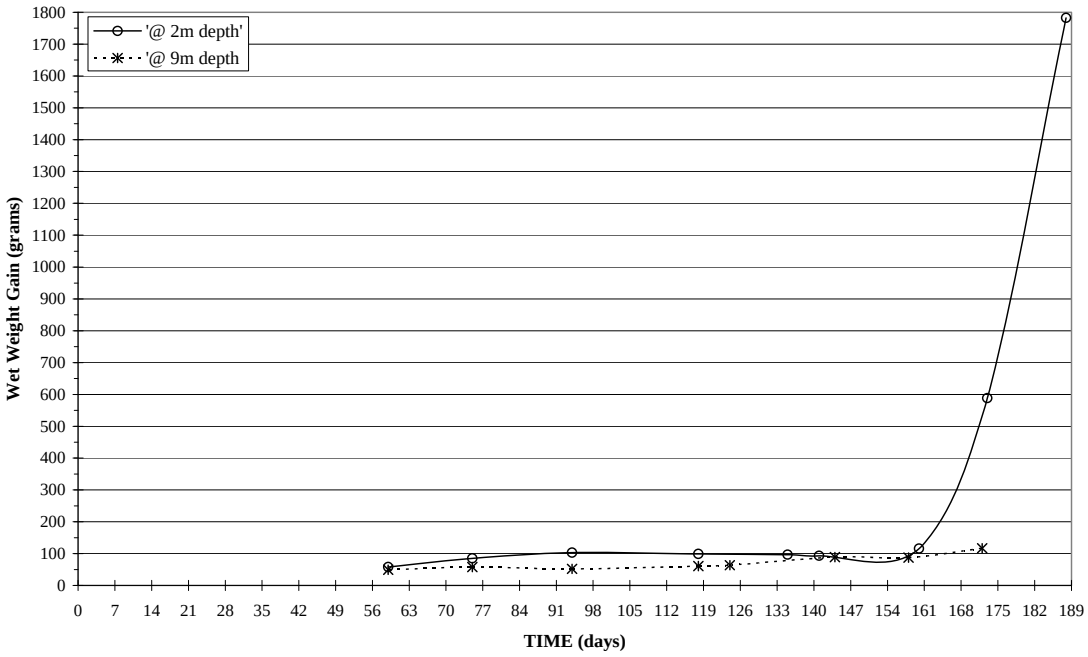


Figure 6.9: Wet weight gain of panels deployed sequentially from May to September 2004

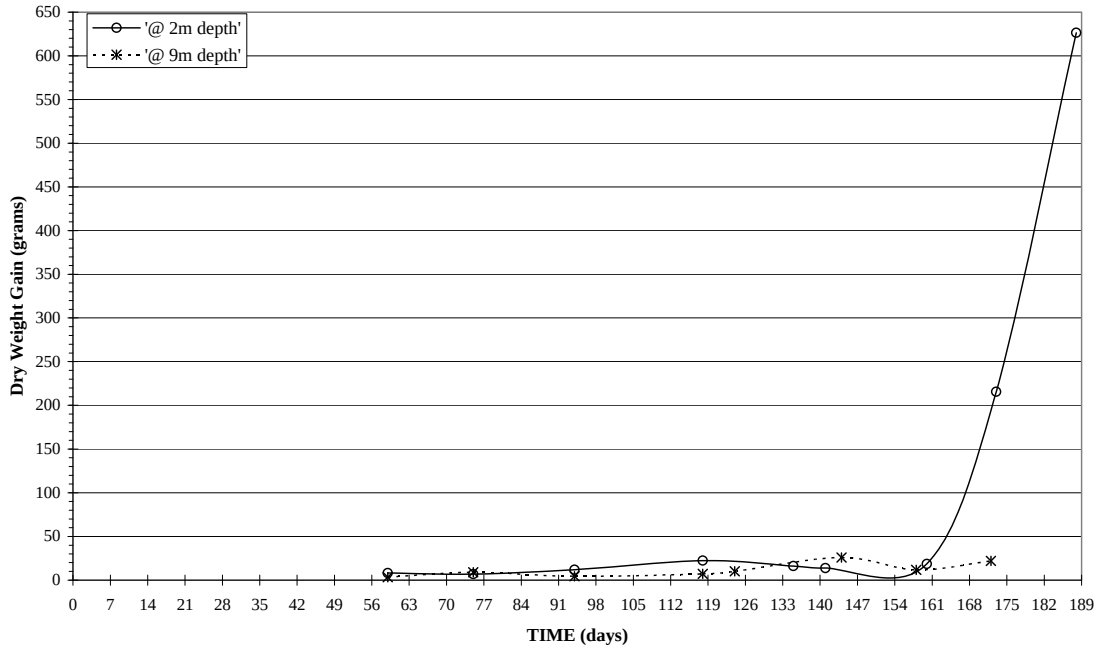


Figure 6.10: Dry weight gain of panels deployed sequentially from May to September 2004

Table 6.3: Wet and Dry weight increases for panels sequentially deployed from May to September 2004

Panel ID. 2m depth	Wet Weight Increase	Dry Weight Increase
C1	1569 %	877 %
C2	491 %	302 %
C3	98 %	26 %
C4	79 %	20 %
C5	83 %	23 %
C6	83 %	31 %
C7	88 %	17 %
C8	70 %	10 %
C9	48 %	11 %

Panel ID. 9m depth	Wet Weight Increase	Dry Weight Increase
C17	102 %	31 %
C18	77 %	17 %
C19	77 %	36 %
C20	55 %	14 %
C21	51 %	10 %
C22	45 %	7 %
C23	50 %	13 %
C24	39 %	5 %

2005

Due to the greater diversity of biofouling types, panels deployed over 21 weeks through 2005, showed a different pattern of biomass gain with time (Figure 6.11 and Figure 6.12). Maximum weight gains, both wet and dry, at 2m and 9m depth, were observed for panels deployed in May, 91 days prior to retrieval. On a wet weight basis, the increase was 235.4 grams at 2m and 185.2 grams at 9m depth. Panels that had been submerged the longest, (144 days) had wet weight gains of 106.8 grams at 2m and 146.8 grams at 9m.

From the dry weight, the panels deployed at 2m depth had only 2 grams difference in weight between 91 and 144 days, despite more than 100g difference in wet weight. This is probably due to the increasing abundance of hard shelled fouling organisms on the panel deployed the longest (see section on biofouling growth). Effectively on a dry weight basis, a light fouling load of bivalves is equivalent to a dense growth of algae and soft invertebrates (see panels A125 and A129 in Figure 6.7).

For the net panels deployed in 2005 the gains equate to increases of 1% to 200% wet weight, or 0% to 55% dry weight (Table 6.4).

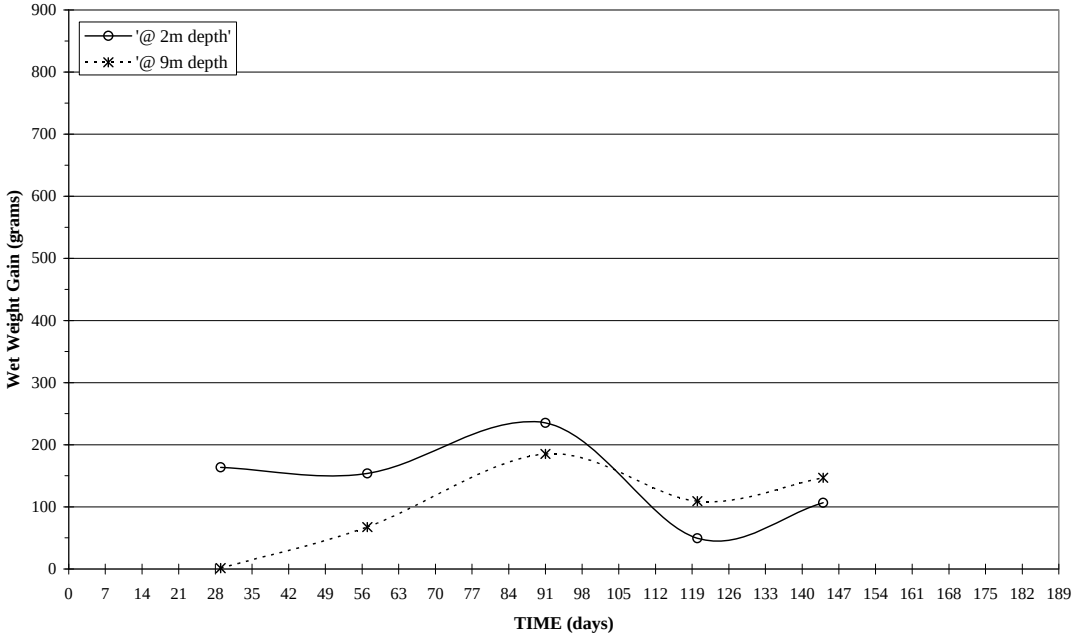


Figure 6.11: Wet weight gain of panels deployed sequentially from March to July 2005

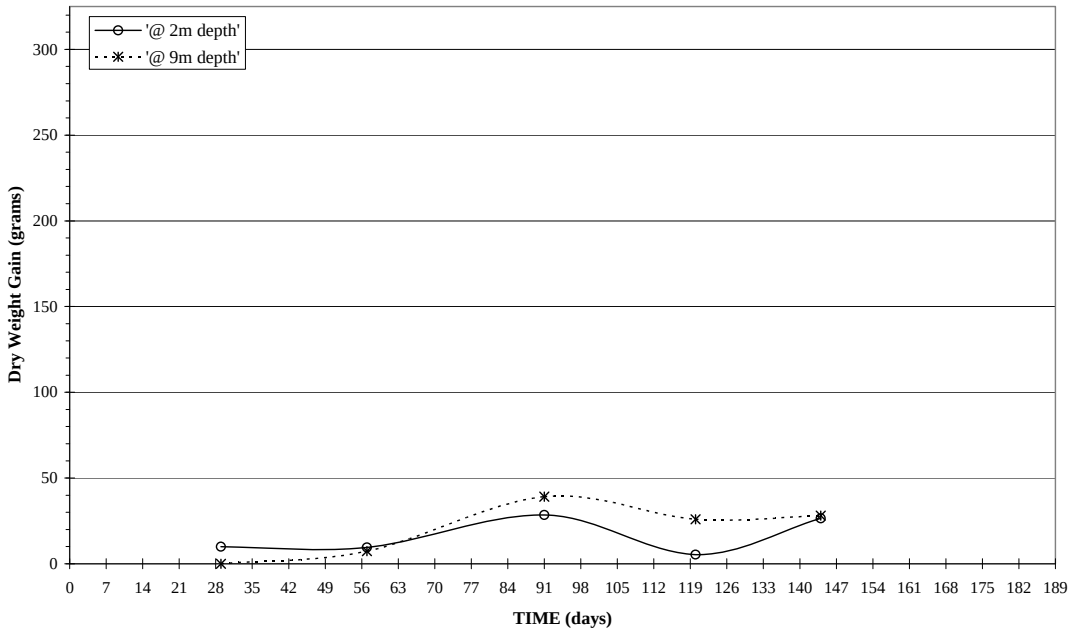


Figure 6.12: Dry weight gain of panels deployed sequentially from March to July 2005

Table 6.4: Wet and Dry weight increases for panels deployed sequentially from March to July 2005

Panel ID. 2m depth	Wet Weight Increase	Dry Weight Increase
A125	91 %	37 %
A127	42 %	7 %
A129	200 %	40 %
B125	131 %	13 %
B127	140 %	14 %

Panel ID. 9m depth	Wet Weight Increase	Dry Weight Increase
A126	125 %	39 %
A128	93 %	36 %
A130	158 %	55 %
B126	57 %	10 %
B128	1 %	0 %

When the fouling biomass values obtained from these small 400 x 400mm panels (5 by 5 meshes) are extrapolated across an entire 40m diameter tuna net (including net walls and base), the weight gains due to biofouling growth can be considerable. The most extreme example of this is panel C1, which was densely covered with mussels and had a wet weight increase of 1.78kg. If this level of fouling occurred throughout the entire net area under the water; the weight increase due to biofouling would be 27.6 tonnes wet weight, or 9.7 tonnes dry weight. Whilst this degree of fouling is non existent in the commercial tuna industry these days, some of the other panels are representative of sections of nets observed within the industry.

Assuming uniform biofouling growth over the entire net wall and base, Table 6.5 gives the projected weight increase for an entire tuna net from the various levels and types of biofouling obtained in the 2004 and 2005 trial.

Table 6.5: Projections of weight increase across an entire tuna net, from various levels and types of biofouling growth encountered on panels deployed through 2004 and 2005, see Plate 6.1, Plate 6.2, Figure 6.7 and Figure 6.8 for panel photographs and details

Panel ID	Wet Weight (kg)	Dry Weight (kg)
C20	986	155
B127	2534	153
A129	3645	441
A125	1653	410

Panel ID	Wet Weight (kg)	Dry Weight (kg)
A130	2867	605
B126	1043	113
A128	1690	401
A126	2273	435

Image Analysis

2004

Due to the fact that only two types and densities of biofouling growth occurred on these panels, mussels or grazed hydroids (Plate 6.1, Plate 6.2); occlusion values obtained through image analysis were limited.

Panel C1, that was heavily colonised by blue mussels, *Mytilus edulis*, had an occlusion value of 80.01%. This means that the net and mussels combined physically occupied 80% of the area in the image/photograph. These are both solid objects; therefore only 20% of the area remains open for free water flow, thereby limiting water exchange across the net surface.

The remaining panels had occlusion values of between 39 to 40%, which includes the net and hydroid growth combined. The occlusion value of just the netting used in this experiment is approximately 18% (see the section on 'net types'). Therefore the open area of the mesh for free water flow is reduced from 82% to 60%. This means that what would be considered "insignificant growth" by operators within the tuna industry, can influence water flow in the same manner as increasing the cord thickness of the net material. This was discussed in the sections on water flow and net types.

2005

Unfortunately the photographs taken at the time the net panels were retrieved from the sea were on a flat dry surface and were therefore not suitable for image analysis. Ideally panels should be suspended in water to allow the fouling communities to be supported in a natural situation, this is particularly important for macrophytes and soft invertebrates (Plate 6.5).

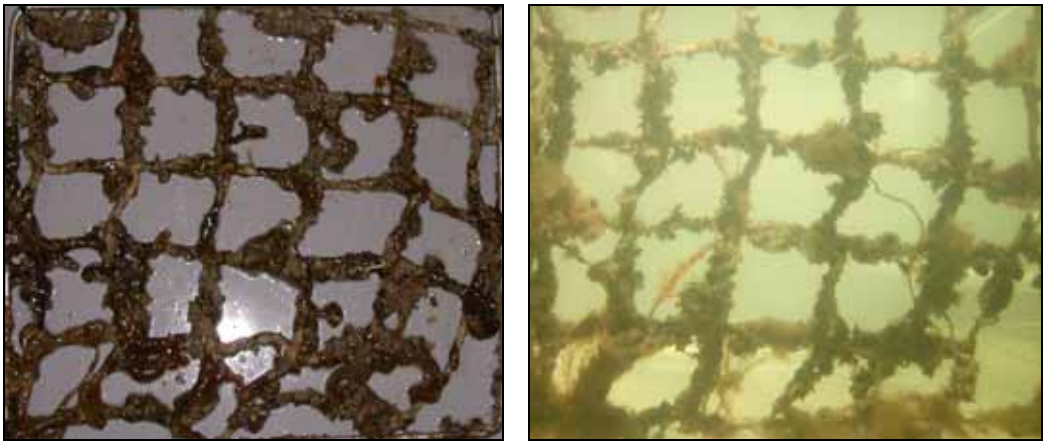


Plate 6.5: Photographs of panel A126 taken out of the water (left) and submerged in the flume tank at low water velocity (7cm/sec) (right)

However, photographs were taken when the panels were mounted in-situ within the flume tank at water velocities of 7, 20 and 45cm/sec. Although the clarity and quality of these photographs were variable (deteriorating with increasing water velocity as seen in Plate 6.6 and Plate 6.7), they could be enhanced to ensure a suitable image for analysis.

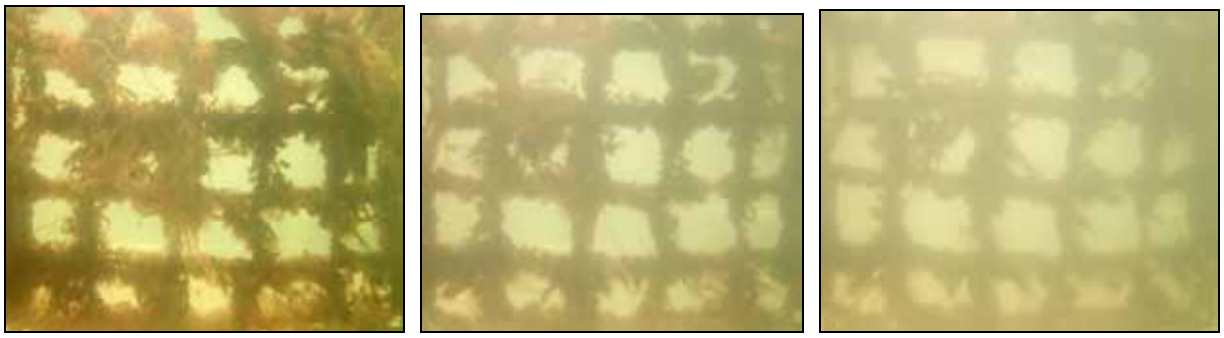


Plate 6.6: Panel A129, photographed within the flume tank at water velocities of 7, 20 and 45cm/sec (left to right), showing the reduced image quality with increased water velocity; and demonstrating how macrophytic growth aligns with the current thereby decreasing the occlusion value.

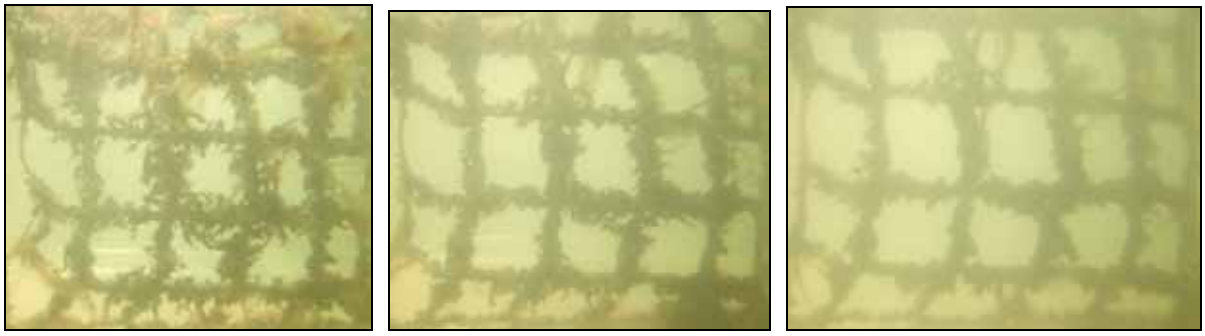


Plate 6.7: Panel A130, photographed within the flume tank at water velocities of 7, 20 and 45cm/sec (left to right), showing the reduced image clarity with increased water velocity; and demonstrating how macrophytic growth aligns and compresses with the current thereby decreasing the occlusion value.

Occlusion values of net panels deployed through 2005 (derived from the low flow photographs) tended to be greater at the shallower depth of 2m (Table 6.6). Maximum occlusion for both depths occurred with panels deployed in May, (approximately 3 months post deployment). Occlusion values were not strongly correlated to the time deployed at sea with $R^2 = 0.5968$ (for panels at 2m depth) and $R^2 = 0.3814$ (for panels at 9m depth). Panels at the 2m depth tended to have decreasing occlusion values from biofouling growth with increasing time deployed. Whereas those at the 9m depth tended to have increasing occlusion values with increasing time. The lower occlusion values for panels deployed for longer times at the 2m depth may be due to the fouling growth getting dislodged because of its weight (Chapter 4 (Rough and Ellis 2007) and the section on biofouling weight). And also community succession, where space occupying macrophytes dominate in the earlier stages of fouling development, and invertebrates, including molluscs dominating at the later stage. Community succession on tuna nets was examined and discussed by Loo 2008, (Chapter 3 of this document).

Occlusion values of individual panels tested at different water velocities tended to decrease with increasing water speed (Table 6.6). This was expected as the biofouling types present were typically of a soft and flexible nature (predominately fluffy red and brown algae,

see section on biofouling growth), that can align and compress with the prevailing water current.

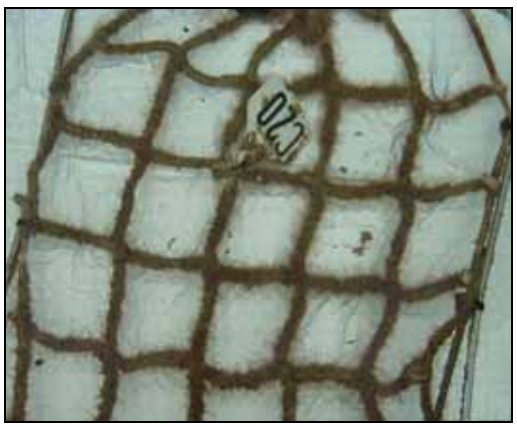
Table 6.6: Occlusion values determined by image analysis, for netting and biofouling deployed at different times and depths; and tested in the flume tank at different water velocities.

Panel Identification and Information	Water Speed 7cm/sec	Water Speed 20cm/sec	Water Speed 45cm/sec
Deployed at 2m depth			
A125 (March)	40.59 %	29.73 %	28.58 %
A127 (April)	35.79 %	30.33 %	26.04 %
A129 (May)	80.07 %	68.87 %	47.69 %
B125 (June)	68.59 %	57.53 %	44.77 %
B127 (July)	73.29 %	63.87 %	45.18 %
Deployed at 9m depth			
A126 (March)	47.64 %	40.79 %	38.10 %
A128 (April)	48.72 %	42.82 %	31.01 %
A130 (May)	71.61 %	62.80 %	45.11%
B126 (June)	27.18 %	30.60 %	19.00 %
B128 (July)	17.03 %	17.99 %	18.00 %

Water Flow

The influence of fouling growth on water movement (flow and behaviour) was determined in the flume tank. As with the section on new clean net types, only the X direction (horizontal flow through the net) and X deviation plots (indicator of turbulence) are discussed here.

Figure 6.13 demonstrates two scenarios of light to medium biofouling growth with similar occlusion values of approximately 40% derived through image analysis, but due to different fouling organisms. These panels had minimal algal growth with C20 having predominately soft flexible invertebrates (hydroids), and A125 having predominately hard shelled invertebrates (paper oysters and other small bivalves). Neither of these panels reduced the velocity of water flow through the net (at the speeds tested). However the shell growth on panel A125 appeared to induce increasing turbulence at higher water velocities, as compared to panel C20.



Panel C20

Deployed July 2004; 124 days at 9m depth

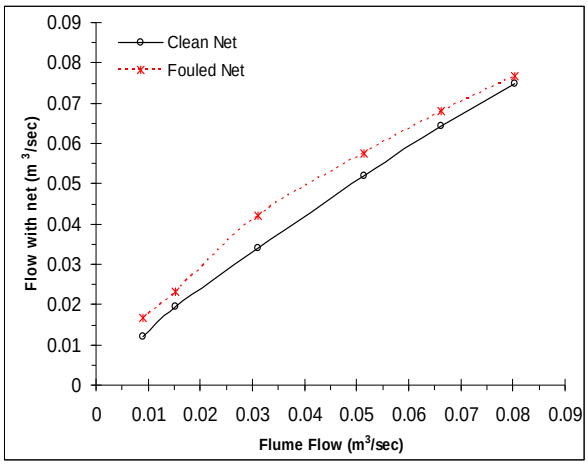
Predominately hydroid growth, grazed

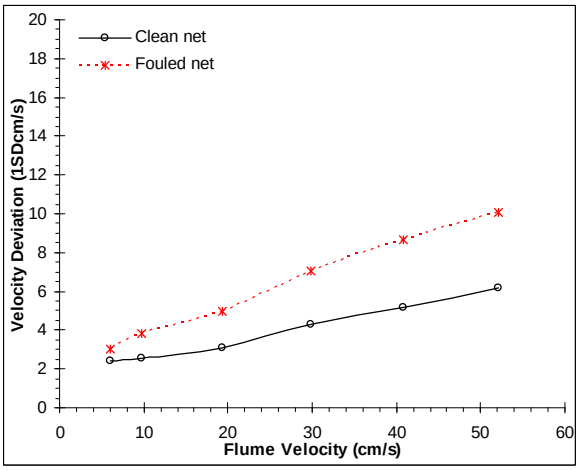
Occlusion value 39.5%

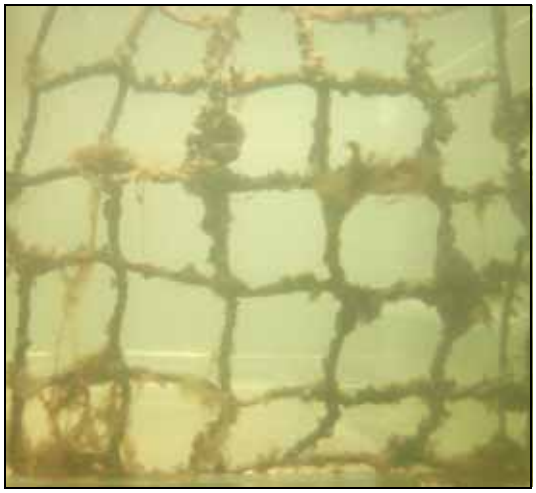
Impact on water flow:

- No influence on velocity through the net

- Slightly increased turbulence after net







Panel A125

Deployed March 2005; 5 months at 2m depth

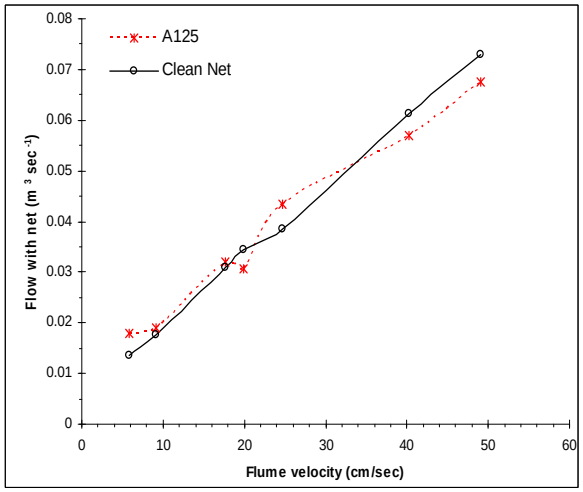
Minimal algal growth, numerous paper oysters and small shell growth

Occlusion value 40.6%

Impact on water flow:

- No influence on velocity through the net

- Turbulence after net increases with increasing current speed



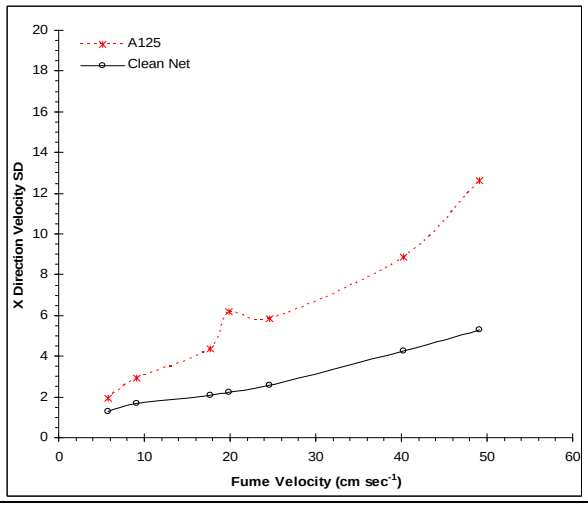


Figure 6.13: Influence that light to medium fouling growth on panels has on water behaviour

Figure 6.14 demonstrates three scenarios of heavy biofouling growth with occlusion values of 70-80% derived through image analysis, but due to different biofouling community combinations. Panel C1 that had dense growth of exclusively hard shelled invertebrates (mussels) reduced the velocity of water flow through the net at speeds exceeding 30cm/sec (0.5 knots). Turbulence after water passage through the net was apparent and increased from a water speed of only 10cm/sec (0.2 knots). The combination of physical obstruction of the net

surface restricting water flow and turbulence within the net (after water passage through the mesh) blocking water flow would result in reduced water exchange.

Both the panels B127 and A129 had high occlusion values due to heavy algal growth, but growth on A129 also included hard and solid invertebrates (polychaete tubes, paper oysters and other small bivalves). Both of these panels showed restriction on water flow through the net but this tended to decrease with increasing water velocity (Figure 6.14). This may be due to the algal growth being pushed parallel to the water current, effectively aligning behind the net cord and thereby decreasing the occlusion value (Plate 6.6, Plate 6.7, and Table 6.6). This was more pronounced with panel B127 that did not have hard fouling. The added influence that the presence of this 'relatively small amount' of hard fouling has is probably best seen with the rapid increase in turbulence at higher water velocities. The hard fouling and turbulence it generates effectively deflects 'new water' away and reduces the velocity and distance that water can travel beyond the net surface.

Panel C1

Deployed May 2004; 188 days at 2m depth

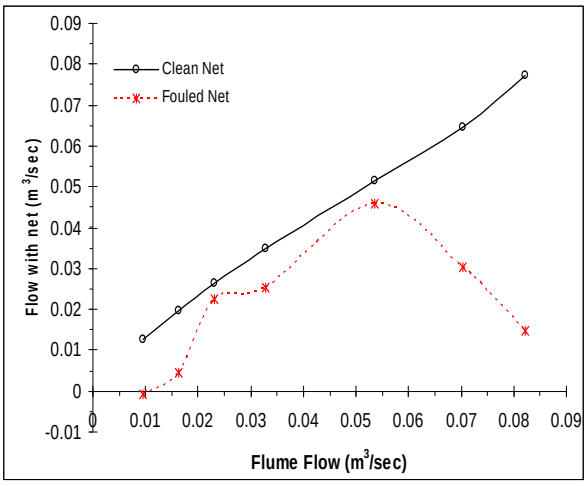
Exclusively heavy mussel growth

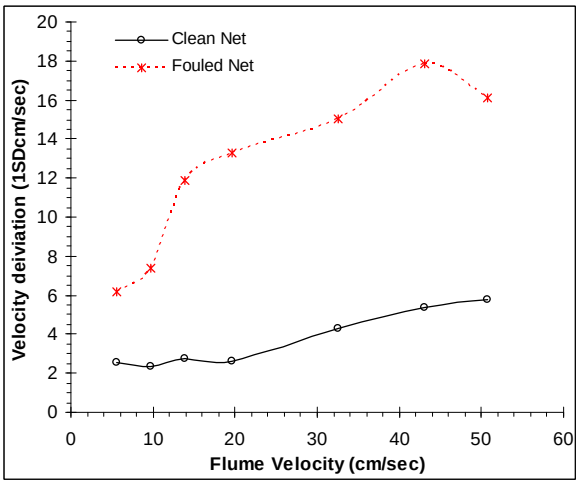
Occlusion value 80%

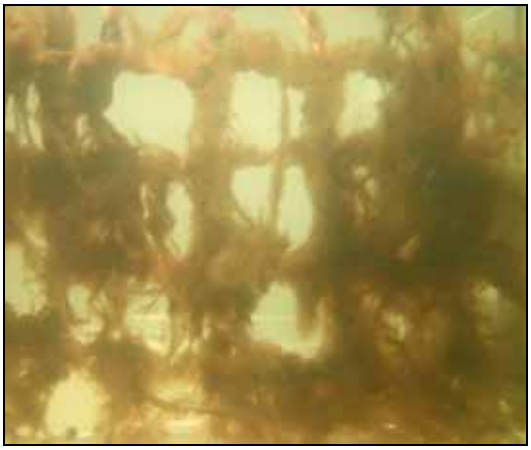
Impact on water flow:

- Decreased water velocity through the net at current speeds exceeding 30cm/sec (0.6kn)

- Increased turbulence after net from 10cm/sec







Panel B127

Deployed July 2005; 1 month at 2m depth

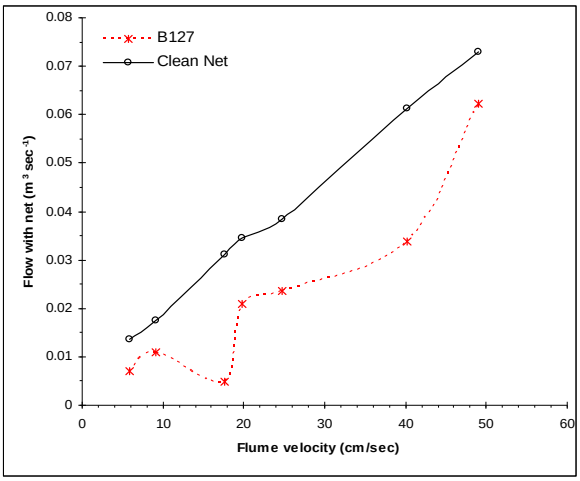
Predominately tufted algal growth, no shell

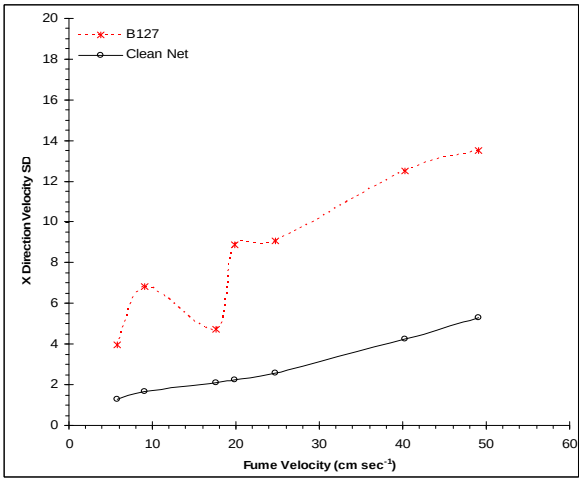
Occlusion value 73.3%

Impact on water flow:

- Reduced velocity through the net at low current speeds, 18cm/sec, flow improves at higher speeds

- Increased turbulence after net





Panel A129

Deployed May 2005; 3 months at 2m depth

Heavy tufted algal growth, with polychaete tubes, shell and hydroid growth

Occlusion value 80%

Impact on water flow:

- Severely reduced velocity through the net at current speeds from 20cm/sec, flow improves at higher speed, 50cm/sec

- Increasing turbulence after net with increasing current speed

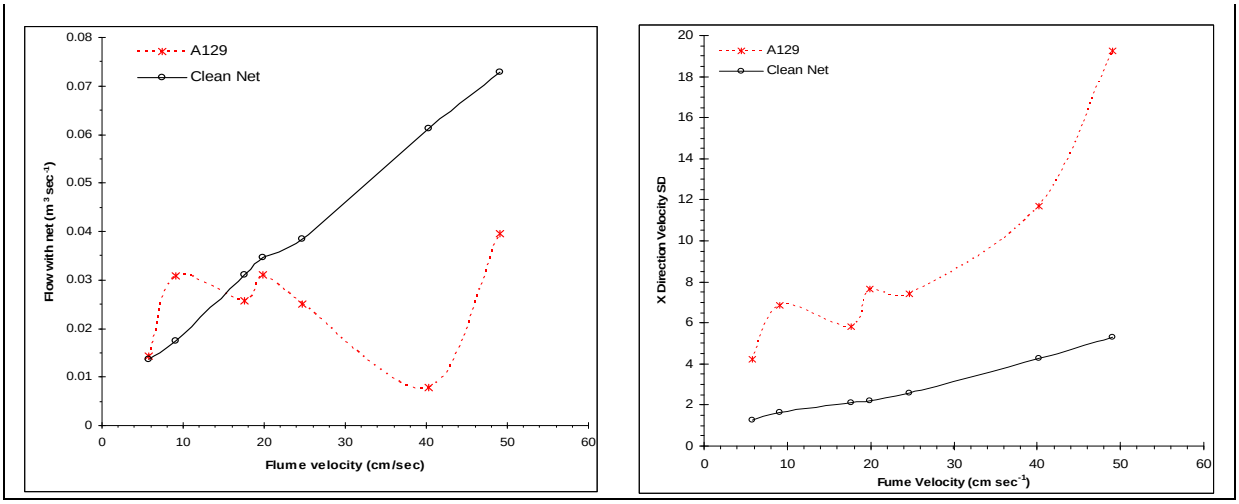


Figure 6.14: Influence that heavy fouling growth on net panels has on water behaviour

Therefore from these experiments it appears that dense algal growth can reduce water exchange at relatively low water velocities; and turbulence as induced by the presence of hard shelled organisms can reduce water exchange at higher water velocities, even when the hard fouling are present at very low densities.

Oxygen Consumption

Biofouling can influence oxygen levels within a net in two main ways, by altering water exchange (as discussed previously) and through respiration; whereby the fouling communities actually extract and consume oxygen from water adjacent to and passing through the net surface. The amount of oxygen consumed by net fouling organisms can potentially affect the amount of oxygen available to tuna held within the net. Oxygen consumption or the respiration rate of different fouling communities was tested by suspending panels in the flume tank.

In 2004, only two types of biofouling were present on the retrieved panels, and both of these were invertebrates, hence consumers of oxygen. Deployment of panel C1 (a 0.16m² segment of net) that was heavily colonised with blue mussels (Plate 6.1) showed that ambient dissolved oxygen in the dark closed water chamber decreased with increasing time (Figure 6.15 left). Oxygen consumption for this panel was calculated at 143.4mg of oxygen per hour. This respiration rate is similar to the amount used by a 1kg kingfish in a resting state (Fitzgibbon pers.comm.)

Although hydroids densely covered the net surface of the remaining panels, the fouling load was considered light. Therefore to measure oxygen use, five panels equating to a net area of 0.8m², were combined for the experiment. Decreased oxygen levels were also obtained with increasing time (Figure 6.15 right); but the rate of consumption was lower at 89.4mg oxygen per hour.

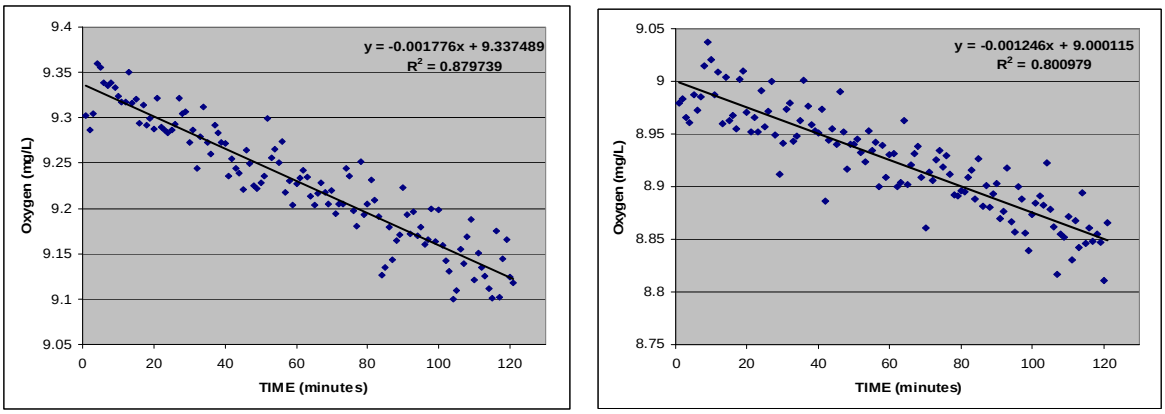


Figure 6.15: Oxygen consumption with time for panels deployed and tested in 2004. Panel C1 (left), heavily colonised by mussels; consumed 143mg/hr. Panels C2, C19, C20, C21 and C23 combined (right), colonised by hydroids; consumed 89mg/hr.

Biofouling communities on panels deployed through 2005 were more diverse than those recovered in 2004. Therefore panels could be grouped so that oxygen consumption of differing fouling communities and densities could be determined. Panels A125 and A127 with a combined area of 0.32m², had the highest density of paper oysters and other small bivalves but also included a light to medium covering of tuft brown algae. The respiration rate of these panels was 12.4mg oxygen per hour. This suggests that the 28 paper oysters and the small mussels and hiatellids present were not in sufficient densities to be dramatically decreasing oxygen levels in the water passing through the net (Figure 6.16).

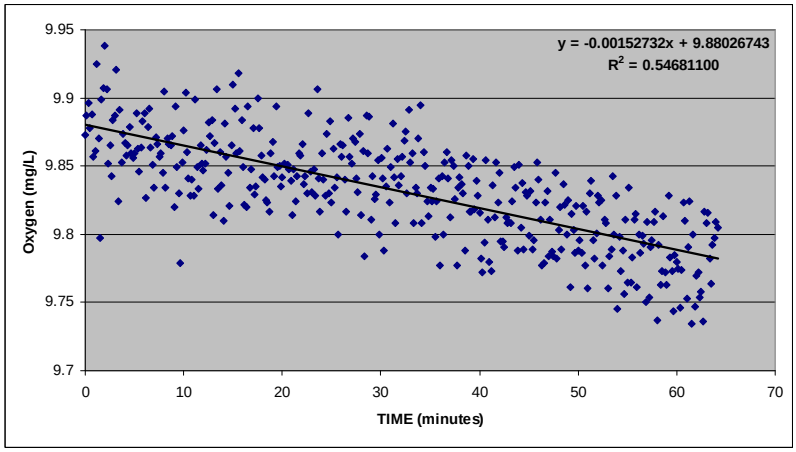


Figure 6.16: Oxygen consumption with time for panels A125 and A127 combined. These panels had a light to medium fouling load that contained numerous small bivalves and tuft brown algae. Oxygen consumption was 12mg/hr.

The oxygen consumption of panels A126, A128 and A130 (combined area of 0.48m²) was 111mg oxygen per hour (Figure 6.17 left). These had similar fouling levels with high density hydroids and polychaete tubes; but also included tuft brown algae and hiatellid bivalves. Another set of panels, A129, B125 and B127 with the combined area of 0.48m², had heavy algal fouling of tuft brown and red varieties and also colonised by caprellids (skeleton shrimp), had an oxygen consumption of 84.5mg oxygen per hour (Figure 6.17 right).

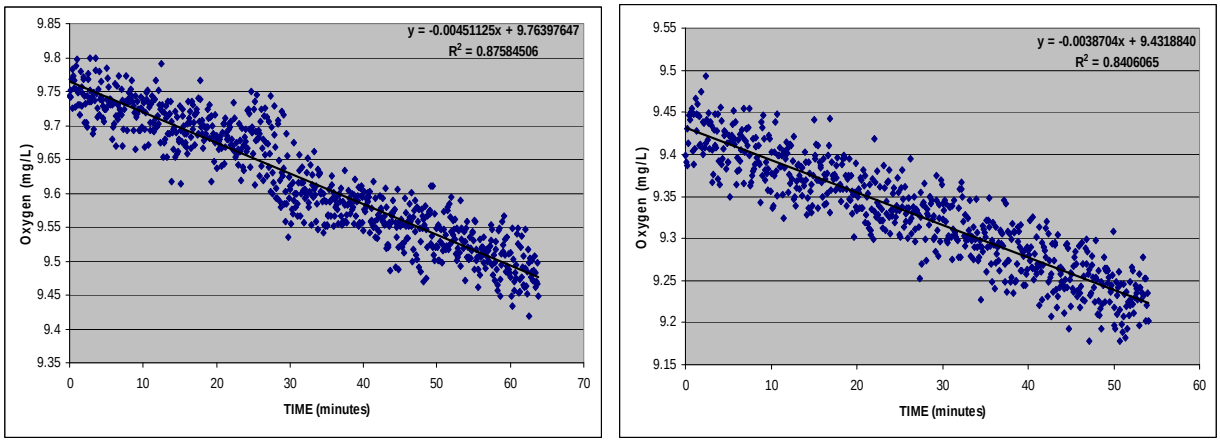


Figure 6.17: Oxygen consumption with time for panels A126, A128 and A130 combined (left) with medium densities of tuft brown algae, numerous hydroids and polychaete tubes was 111mg/hr. Panels A129, B125 and B127 combined (right) had heavy algal growth that contained an abundance of caprellids, had an oxygen consumption of 85mg/hr.

The comparison of different fouling types and oxygen consumption calculated per square metre of netting is given in Table 6.7. From the 2005 data, there is some indication that the presence of algal growth may offset the oxygen consumption by invertebrates that were the only fouling types present in 2004. However, further work needs to be undertaken to verify this potential offset.

Table 6.7: Comparison of hourly oxygen consumption for different biofouling types per square metre of 150mm (stretch mesh) white Badinotti netting

Panel Id.	Major Biofouling Type	Oxygen Consumption ($mgO_2/m^2/hr$)
C1	**Invertebrates, hard** – dense bivalves	860.4
C2, C19, C20, C21, C23	**Invertebrates, soft** – dense hydroids	107.3
A125, A127	**Algae** – light to medium density **Invertebrates, hard** – light bivalves	37.2
A126, A128, A130	**Algae** – light to medium density **Invertebrates, soft** – dense hydroids **Invertebrates, hard** – light bivalves	220.0
A129, B125, B127	**Algae** – dense and containing a high density of mobile soft bodied invertebrates	169.0

CONCLUSIONS

The weight of new net was influenced by construction material, cord thickness and mesh size. The latter two of these physical properties influenced water flow in the flume tank, both as the passage of water through the net meshes, and as the dynamics of water flow after the net. Water flow through new net was influenced by the internal area of the mesh at a velocity setting of 40cm/sec (approximately 0.8knots); and by both the internal area and occlusion value at a higher velocity setting of 60cm/sec (approximately 1.2knots). Biofouling growth occurred on all net types and materials tested, however sequential observation was not undertaken to assess if any types delayed the initial colonisation of biofouling communities.

Biofouling growth on knotless nylon netting influenced water flow in the flume tank. Light fouling growth (occlusion value 40%) did not reduce water velocity through the net, but did induce turbulent motion after passage through the meshes if shell growth was present. The influence that heavy fouling growth (occlusion value 70 to 80%) had on water flow varied with the biofouling assemblages present. Heavy shell growth reduced water velocity through the net at a current speed setting of 30cm/sec (0.6kn) and induced turbulence from a setting of only 10cm/sec (0.2kn). Heavy weed growth does restrict water flow through the net at a low water velocity setting (15cm/sec). But this tended to lessen at higher current speeds, when algal growth was pushed through the mesh, and tended to compress and align behind the net cord. This improvement in water flow was decreased if shell growth was present amongst the algae; the presence of even low density hard shelled organisms induced turbulence that effectively deflects water back away from the net surface (rather than passing through the net).

Both the amount of biofouling growth and the types of biofouling assemblages present influenced oxygen consumption rates.

Chapter 7 : SUB-PROJECT 5: OXYTUNA – A MODEL FOR THE OXYGEN DYNAMICS IN A SOUTHERN BLUEFIN TUNA SEA-CAGE SYSTEM

This chapter was authored by Professor Anthony C Cheshire and Dr. Maylene GK Loo (South Australian Research and Development Institute, Aquatic Sciences, 2 Hamra Avenue, West Beach SA 5024 http://www.sardi.sa.gov.au) and may be cited as:

Cheshire, AC and Loo, MGK (2008). OxyTuna – A model for the oxygen dynamics in a southern bluefin sea-cage system. In Rough KM, deNys R, Loo, MGK, and Ellis DC, (Eds.) Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp.

This sub-project has been released as a stand alone report including the computer model: Cheshire, AC and Loo, MGK (2008). OxyTuna – A model for the oxygen dynamics in a southern bluefin sea-cage system. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 37 pp. SARDI Publication No. F2007/001137. Research Report Series No. 278.

EXECUTIVE SUMMARY

The aim of this subproject ("Enhancement of a dissolved oxygen diffusion model to provide a predictive capacity to industry to evaluate fouling management systems") was to develop a model to illustrate and predict changes in the oxygen concentration in a tuna aquaculture sea-cage. The model provides a platform to investigate the oxygen dynamics of alternative cage configurations and stocking levels, in response to seasonally varying tidal currents, water quality and fouling loads.

The specific objectives of this subproject were:

1. To calibrate the model using the results obtained from Subprojects 3 and 4 to provide a basis for cost:benefit[8] analyses of alternative fouling management systems.

2. To provide farmers and managers with an educational tool that enables them to better visualise the relationship between the level of net fouling, water flow, stocking rate and environmental dissolved oxygen levels.

The OxyTuna© model has been developed to assist farm managers in making better decisions about the management of finfish sea-cage systems. In particular it will help them to better understand the relationship between net fouling and oxygen concentration in cages and how this responds to various management interventions including changes in cage configuration and fouling management (e.g. net cleaning).

The model provides a quantitative prediction of the changes in oxygen concentration through time for different sea-cage configurations (cage size, net type, stocking density, fish species) in response to changes in ambient conditions (temperature, salinity, ambient oxygen concentration and current speed). The dynamic nature of the model allows users to better

[8] Where the improvement in oxygenation of the water inside the cage is a quantitative measure of benefit.

understand the interplay of factors that control oxygen concentration in a sea-cage, and it can therefore be used not only as a management tool but also as a teaching tool.

By implementing OxyTuna© as a Visual Basic for Applications (VBA™) program developed to run within Microsoft EXCEL™, the model outputs can be easily captured and incorporated into other programs by anyone with a basic understanding of EXCEL™. This feature is expected to improve the utility of the model and the opportunity for individual users to develop their own enhancements.

INTRODUCTION

Background

This is the final report of the work carried out for "Subproject 5 – Enhancement of a dissolved oxygen diffusion model to provide a predictive capacity to industry to evaluate fouling management systems" of the "Aquafin CRC SBT Aquaculture Subprogram: Net fouling management to enhance water quality and southern bluefin tuna performance" (Aquafin CRC 4.5/FRDC 2003/226).

The aim of the Net Fouling Management project was to better understand the impact of net fouling on the management of tuna sea-cage systems. A build-up of fouling biota is likely to have numerous effects on the environment within a sea-cage and therefore impacts on the management and operation of the system. Typically the effects of net fouling include:

- Changing patterns of water flow through the nets and thereby the supply of oxygen and removal of wastes from cages.
- Changing the weight (and therefore buoyancy) of farming structures.
- Changing the surface area of cages, which in turn affects the potential for growth and attachment of pathogens.
- Reducing the longevity of nets.

The Net Fouling Management project comprised a total of six sub-projects including the work presented in this report which provides an overview of the development and implementation of the OxyTuna© model. OxyTuna© was developed to address the requirements of Subproject 5 of the Net Fouling Management project, the aim of which was to understand how net fouling influences oxygen supply to cages (cage ventilation).

Importance of oxygen supply to sea-cages

Oxygen supply is a fundamental condition for intensive aquaculture, and sea-cage systems are no exception (Edwards and Edelsten 1976, Madenjian 1990, Silvert 1992). A number of factors, including the oxygen demands of the fish, the plankton, fouling biota on cage nets, and sediment-associated flora and fauna, all interact to deplete oxygen concentrations to levels below that required to optimise fish growth. In sea-cages, 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 water flow (Inoue 1972, Lee *et al.* 1985, Sliskovic and Jelic 2002, Yokoyama *et al.* 2004), which can lead to a reduction in oxygen concentrations to levels that negatively impact upon the cultured organism. This depletion may in turn lead to increased stress on the fish and susceptibility to disease.

Need

Historically, when farming was conducted in Boston Bay, nets showed obvious fouling after only 4 weeks (Bond 1993), quickly becoming heavily covered by fouling organisms. In a previous study (Cronin *et al.* 1999), typical fouling rates of between 2 and 4 kg wet weight/m^2 were observed on sea-cages. More recent work (Svane *et al.* 2006), also conducted on experimental sea-cages in Boston Bay, largely confirmed these results and has shown that nets, even when treated with anti-foulant, may accumulate substantial fouling loads over relatively short periods[9]. Svane *et al.* (2006) showed that fouling load increased on both treated and untreated nets over the course of their five-month study, reaching levels of 81% in untreated nets compared with only 66% for treated nets[10]. Work carried out in Subproject 2, Chapter 4 of this project, where a range of anti-foulants were tested on nets in the Boston Island East farming zone, largely confirmed the overall trend (i.e. less fouling on treated nets). Fouling on untreated nets after 155 days (~5 months) resulted in occlusion of 68% whilst treated nets were typically around 41% (Rough and Ellis 2007).

It is important to manage net fouling because, by occluding the net, it causes a reduction in water flow and therefore limits oxygen supply to cages. This subproject was developed in order to assist farm managers to make better decisions about the management of net fouling. The work detailed in the following focuses on quantifying the relationship between net fouling and the dissolved oxygen concentration in sea-cages, and understanding how this responds to various management interventions including changes in cage configuration (e.g. stocking density) and fouling management (e.g. cage cleaning).

More specifically, the OxyTuna© model that has been developed in this subproject provides a quantitative prediction of the changes in oxygen concentration through time for different sea-cage configurations (cage size, net type, stocking density, fish species) in response to changes in ambient conditions (temperature, salinity, ambient oxygen concentration and current speed).

The specific objectives of this subproject were:

1. To calibrate the model using the results obtained from Subprojects 3 and 4 to provide a basis for cost:benefit[11] analyses of alternative fouling management systems.

2. To provide farmers and managers with an educational tool that enables them to better visualise the relationship between the level of net fouling, water flow, stocking rate and environmental dissolved oxygen levels.

The OxyTuna© Model

This chapter will detail the conceptual schema, model construction and the workings of the model.

[9] Note that prior to the work undertaken in the Net Fouling Management project there have been no quantitative studies of net fouling on cages outside of Boston Bay.

[10] In this study 81% is the amount of space occluded by the net and the associated fouling organisms. This implies that the area of open space in the net is reduced from (typically) 92% in clean nets down to only 19% in these fouled nets. The treated nets (with 66% occlusion) therefore had 34% open space, which provides close to double the area for water to move through the treated net compared to the untreated (heavily fouled) net.

[11] Where the improvement in oxygenation of the water inside the cage is a quantitative measure of benefit.

Overview of model construction

OxyTuna© is a dynamical model that illustrates the changes in dissolved oxygen concentration in a sea-cage through time. The model is based upon a previously published model developed by Emma Cronin (Cronin 1995) that has been substantially modified and upgraded in this subproject. Cronin's model used a relatively simple algorithm to describe changes in the dissolved oxygen concentration in a sea-cage (Equation 1).

Equation 1 - General model for oxygen dynamics in a sea-cage.

$$[\text{Mass Oxygen In Cage}]_{(t+\delta t)} = [\text{Mass Oxygen In Cage}]_{(t)} + [\text{Mass Oxygen Transported Into Cage}]_{(\delta t)} - [\text{Mass Oxygen Transported Out From Cage}]_{(\delta t)} - [\text{Mass Oxygen Respired By Fish In Cage}]_{(\delta t)} - [\text{Mass Oxygen Respired By Fouling Or Other Biota}]_{(\delta t)}$$

The model calculates a mass-balance for oxygen by which the amount of oxygen in a sea-cage at a time δt from now will be equal to the mass of oxygen currently in the cage, plus any extra oxygen that is transported into the cage over the time period (δt), minus any oxygen that is either transported out of the cage or that is consumed through respiration by fouling or other biota over that time period.

In developing the new model the aim was to incorporate a number of necessary improvements including:

- An enhanced user interface that provided:
  - a simple method for constructing scenarios specifically including changes in cage configuration;
  - graphical illustrations of the model outputs;
- An improved model engine that overcame a serious limitation with the time-stepping algorithms in the earlier model (which resulted in aberrant behaviour under moderate-high current flow rates);
- Incorporation of a sub-model that quantified the change in fouling load through time and which can be used to illustrate the merits of anti-foulant treatment of nets;
- Incorporation of a sub-model that quantified ventilation rate (dissolved oxygen exchange for sea-cage) based on tidally induced current flow and variable fouling load;
- Incorporation of a fish respiration sub-model parameterised using recent results from research on tuna respiration;
- Incorporation of a sub-model that characterises seasonal changes in water quality specifically including changes in temperature, salinity and ambient oxygen concentrations.

The revised OxyTuna© model has been implemented as a Microsoft EXCEL™ add-in using Visual Basic for Applications (VBA™). This strategy means that model outputs can be easily captured by anyone with a basic understanding of EXCEL™, thereby improving the utility of the model and the opportunity for individual users to develop their own enhancements.

Model description

OxyTuna© provides a prediction of the concentration of dissolved oxygen in the water, inside a tuna sea-cage over time. The model achieves this by calculating the mass of oxygen inside the cage and then representing this as a concentration (mass/volume; mg/L). The volume of the cage is calculated from a simple sub-model using the physical cage dimensions (diameter and depth).

A generalised schema for the model is provided in Figure 7.1. This figure uses a modified set of Forrester symbols (Forrester 1961; Appendix 7.1) to represent the relationship between state and forcing variables. A state variable describes the state of the system; in this case the concentration of dissolved oxygen within the sea-cage. A forcing variable refers to factors controlling the state of the system (e.g. current speed, ambient temperature or salinity). Arrows represent the material flows (e.g. oxygen moving into cage, thick lines; ➞) and control flows (e.g. temperature/salinity, thin lines; →) that have been used to construct the algorithms and subroutines used in developing the visual basic code for the EXCEL™ implementation of the model.

A number of terms used in the model proposed by Cronin (1995), atmospheric diffusion and respiration by the fouling biota, have been excluded from the present model. In general, the mass transport of oxygen and the amount of oxygen consumed through fish respiration are orders of magnitude greater than that consumed through diffusion or fouling consumption (Cronin *et al.* 1999) and so these terms can be ignored[12].

[12] For land-based ponds, where the only source of oxygen is either via passive diffusion or active oxygenation, these terms would still be required. However, in a marine sea-cage the effect of oxygen transport by water flow is so great that diffusion becomes irrelevant over the time scales that the model runs.

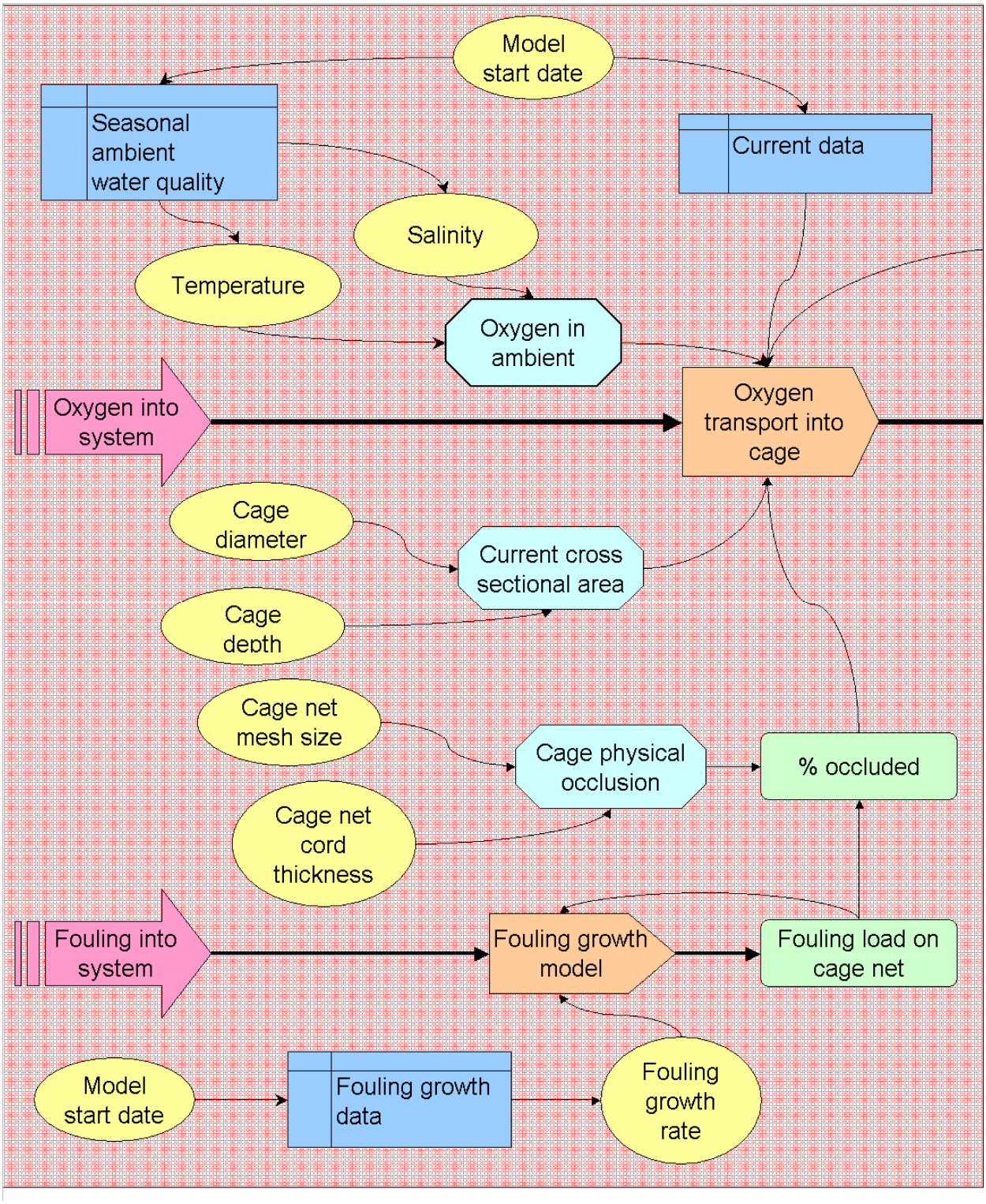


Figure 7.1a: Schematic representation of the OxyTuna© model illustrating transport of oxygen into the sea-cage. See Appendix 7.1 for explanation of the symbols (note arrows leaving to the right of the diagram are picked up in Figure 7.1b).

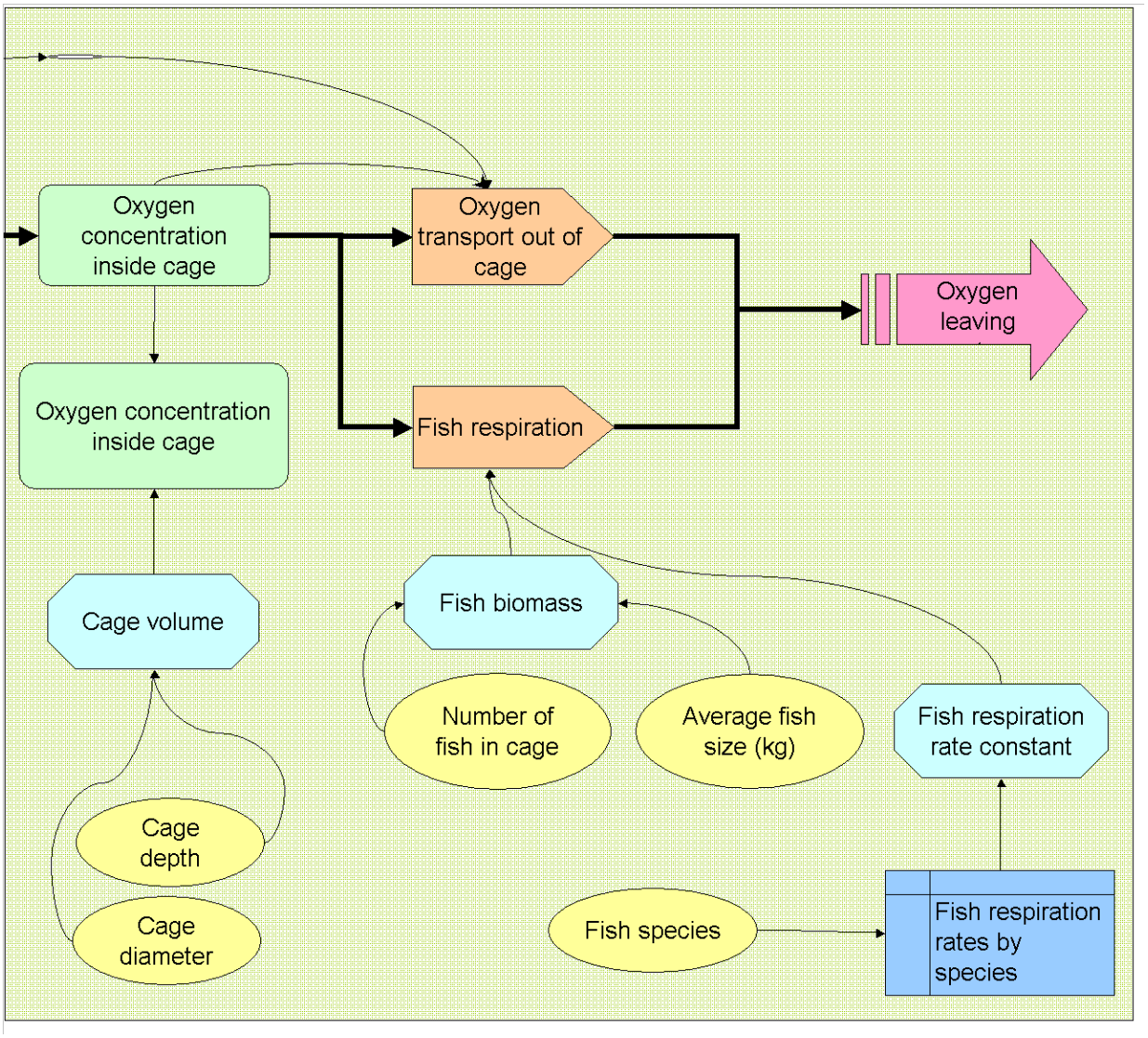


Figure 7.1b: Schematic representation showing oxygen concentration inside the sea-cage and transport processes out of the sea-cage.

Model construction

OxyTuna© uses a simple press-button interface that allows the user to navigate through the process for setting the various run-time parameters (see below) and then executing and saving alternative scenarios. The Command screen provides a pictorial representation of the information required to run the model as well as a single graphical display that shows a time series of modelled oxygen concentration based on the user selected parameter values (Figure 7.2).

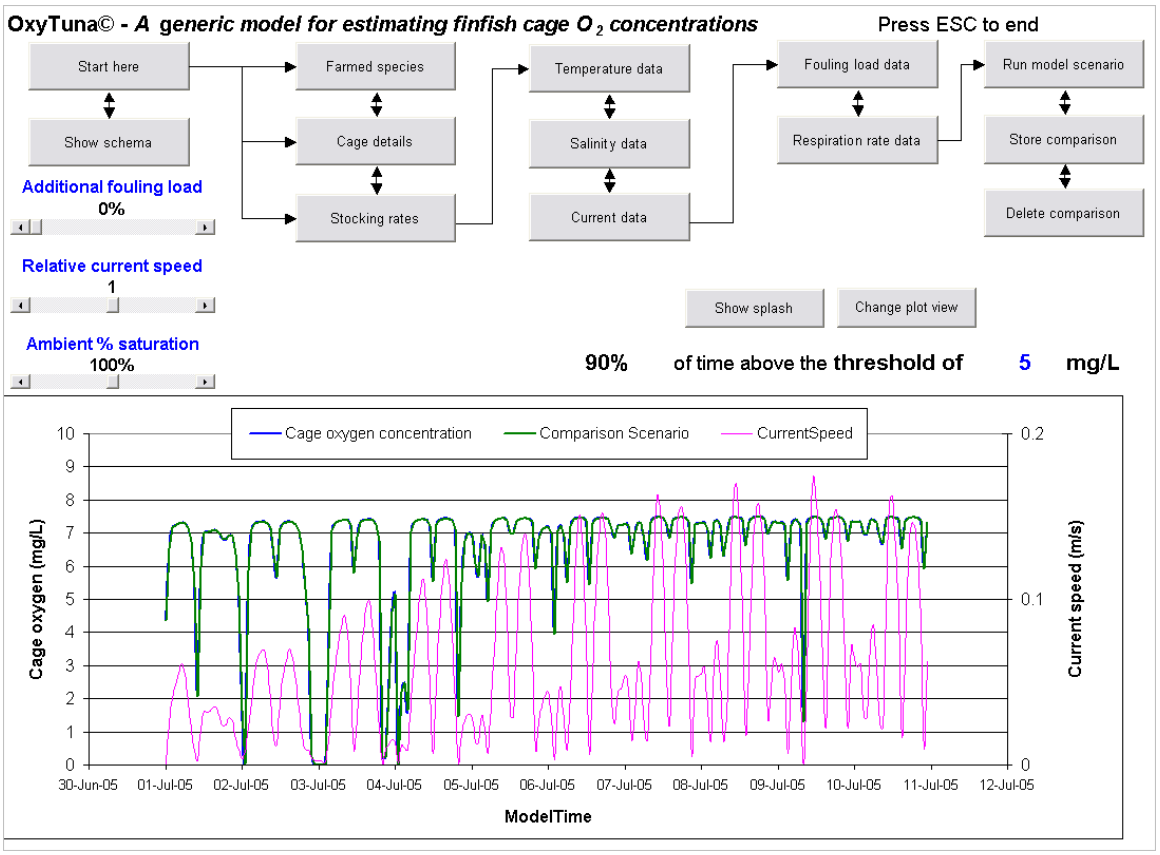


Figure 7.2: Command screen for OxyTuna© showing the buttons that guide the user through the data entry process that is required to set up the parameter sets for a model run. The graphical display in the bottom half of the screen illustrates a time series showing current speed and modelled dissolved oxygen concentration for the sea-cage.

The user navigates through the model by moving the mouse over and pressing any given button (generally starting with "Start here"; Figure 7.3).

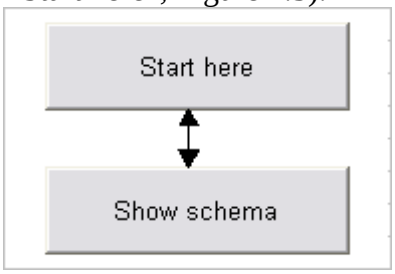


Figure 7.3: Illustration of typical command buttons. Pressing these buttons allows the user to interact with the model, change or check model parameters and execute functions (such as Run the model).

Left clicking the mouse activates the function programmed into that button while right clicking the mouse provides help information to the user (see for example Figure 7.4). Generally, when pressing any button, a default value will be shown. The default value is simply a re-iteration of the current value stored for any given parameter.

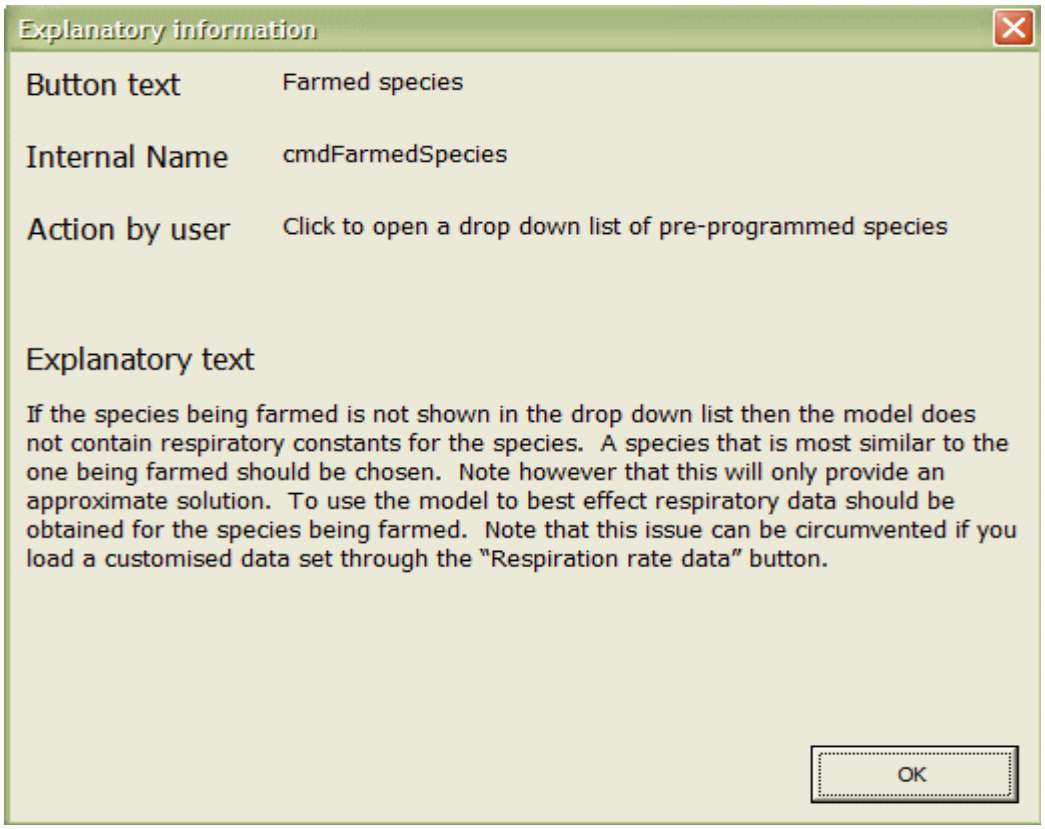


Figure 7.4: Illustration of typical help screen. This information is accessed for any given button by clicking the right-mouse button.

Additional functions include the slider bars that can be used to manipulate data on fouling load (to review the effect of lower or higher fouling load), current speed (to quickly review the effect of lower or higher water flow) and ambient dissolved oxygen concentration (to review the effects of periods of depressed ambient oxygen) (Figure 7.5).

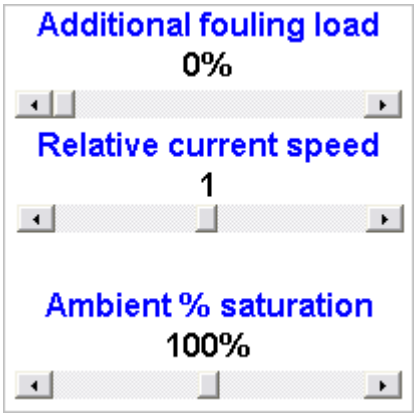


Figure 7.5: Slider bars for additional fouling load, relative current speed and ambient dissolved oxygen (% saturation).

The text showing the percent time above a user-defined dissolved oxygen threshold value (set at 5 $mg.L^{-1}$ - in the illustration; Figure 7.2) provides a simple way of evaluating

whether the settings used for any given run are appropriately bounded. The user can change the threshold value simply by overtyping the value currently shown.

The graphical display (bottom of Figure 7.2) shows the dissolved oxygen concentration in ppm or $mg.L^{-1}$ through time for the latest model run (blue line; scale on left hand y-axis), the green line provides a view of the oxygen concentration for a previously saved run (typically using different parameter values) and the magenta line represents current speed in $m.s^{-1}$ through time for the latest model run (scale on right hand y-axis).

The following is a detailed description of the role of each button and how these link to the underlying data requirements for the model to run.

Model initialise – Start Here

Click to enter run time data - start date, deltaT and period of model run.

The user will be asked to provide three values; the start date for the model run, the value for deltaT (the time interval at which to report the results) and the number of days for the model to run. These data will be used to align the model time with sub-models or data sets for water temperature, salinity, current (tidal) flow, fouling load and respiration rates. Note that if these subsidiary datasets do not provide data to cover the period entered via this button then the model run will fail. At some time in the future we may incorporate an additional input for *Location* to allow the user to access different built-in sub-models for temperature, salinity etc. This will allow users from other industry sectors (e.g. salmon) to use the model more easily[13].

Show Schema

Click to review the model "Schema".

The schema allows the user to review the way in which the model is constructed (shown in Figure 7.1a and b). It provides a representation of the material flows (oxygen) in the model and details how the movement of dissolved oxygen into and out of sea-cages is controlled by the setting of the other model parameters (cage and net configuration, ambient water quality and fouling load).

Farmed species

Click to open a drop down list of pre-programmed species.

If the species being farmed is not shown in the drop down list (see illustration in Figure 7.6) then the model does not contain a respiratory constant for that species. A species that is most similar to the one being farmed should be chosen. Note however that this will only provide an approximate solution. To use the model to best effect, respiratory data should be obtained for the species being farmed. This issue can be circumvented by loading a customised data set through the “Respiration rate data” button (see below). If the user enters respiration rate data, then this overrides the default data in the model for the farmed species selected.

[13] Note however that the model can still be used for other farming systems but users need to provide their own data for changes in temperature, salinity, current flow, etc rather than using the built in model functions.

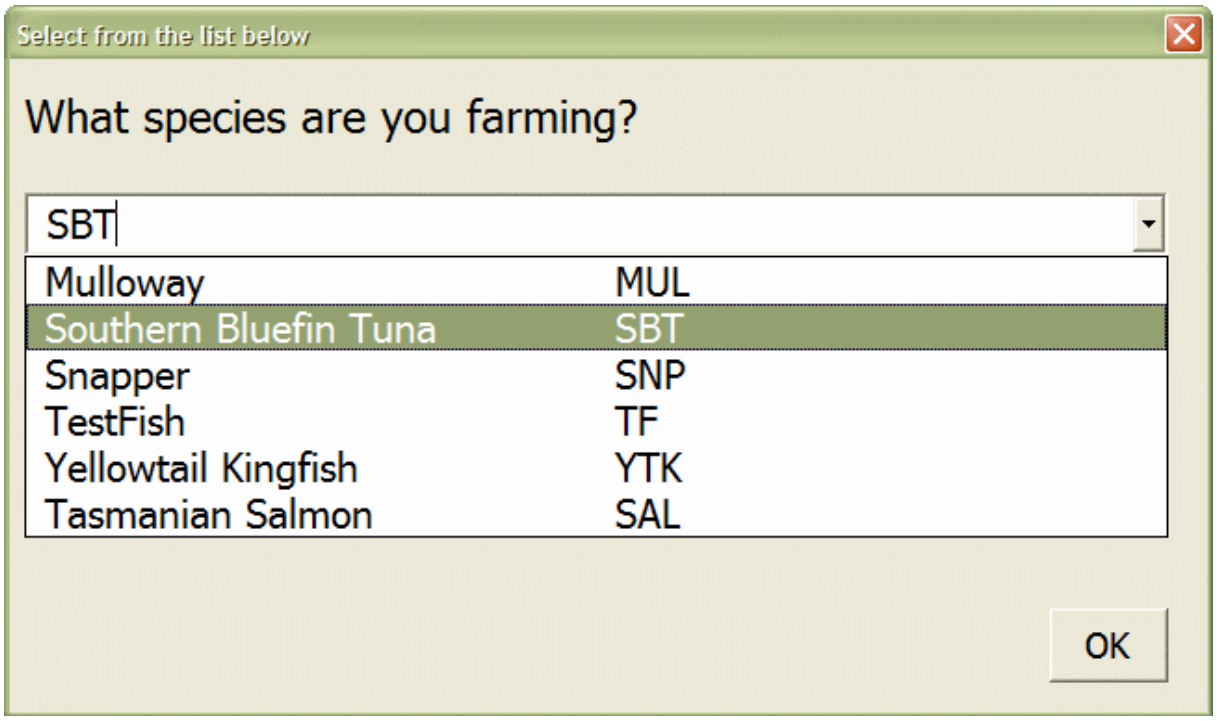


Figure 7.6: Example of a typical dialogue box where the user is asked to provide information. In this example the user has selected SBT.

Cage details

Click to open a series of dialogue boxes that will ask the user for information about the size of the sea-cage and the type of net being used.

The user is asked to provide data for four parameters; the Cage diameter (inside distance across the top of the sea-cage measured from one side of the pontoon to the other running through the middle - measured in m); the Cage depth (distance from the pontoon down through the water column to the base of the cage in the middle - measured in m); the mesh size of the net (length of one side measured in cm) and the cord thickness of the net (measured in mm). Note that the cage details are used to calculate the CageVolume parameter, which is used in the "Stocking rates" function (see below). It is assumed that cages are circular in cross-section.

Stocking rates

Click to open a series of dialogue boxes that will ask the user for information about stocking rate of the sea-cage.

The user will be asked to provide data for the average fish size (measured in kg) and the number of fish in the sea-cage (total count). These data will be used to compute the biomass of fish in the cage (Biomass = AvgSize*Number) and the stocking density (StockDens=Biomass/CageVolume). If these values are not known then the user should provide their best estimate, as the model is very sensitive to these data.

Temperature data

Click to open an option box where you can indicate the source of temperature data for the model (Figure 7.7).

This value will determine whether the model will calculate a value for temperature based on a model of Port Lincoln seasonal sea surface temperatures or utilise a lookup table, showing temperature through time, provided by the user (Figure 7.8). Novice users should use the internal modelled temperature values that are based on data for Lower Spencer Gulf (Port Lincoln region) provided by the Directorate of Oceanography and Meteorology, Australian Government Department of Defence Online data service[14].

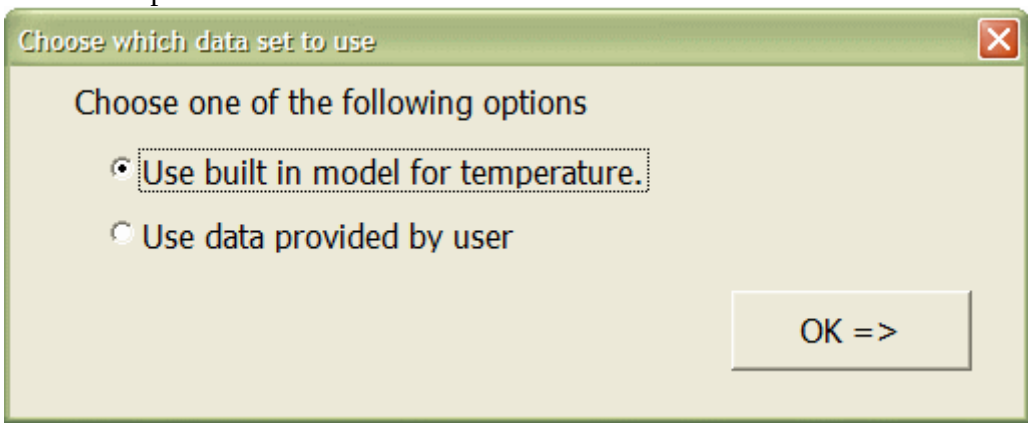
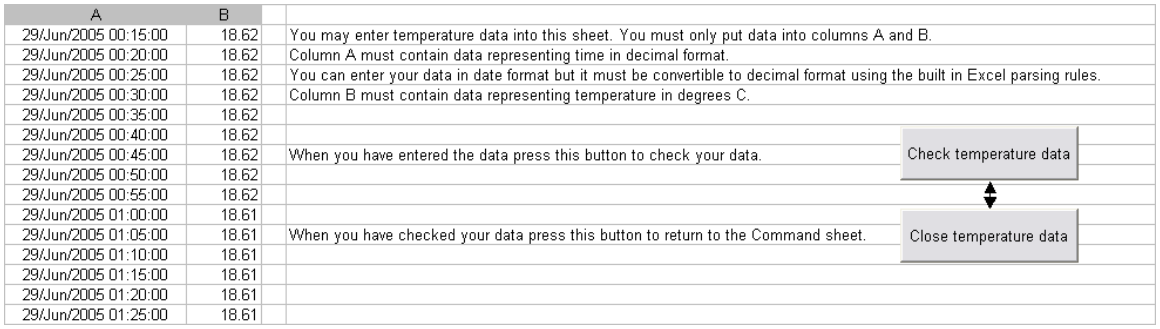


Figure 7.7: Typical dialogue box for options where the user can either use the built in sub-model (in this case for ambient temperature) or alternatively provide their own data.

(a)



(b)

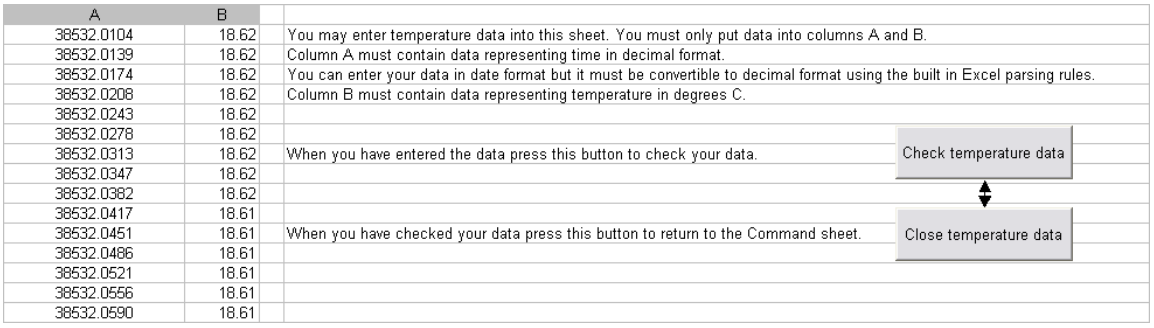


Figure 7.8: EXCEL™ worksheet where the user can provide their own temperature data (similar sheets are available for salinity, current (tidal) flow, fouling load and respiration rate). (a) data in date format, (b) date converted to decimal format.

[14] Source – http://www.metoc.gov.au/products/data.html

Salinity data

Click to open an option box where you can indicate the source of salinity data for the model.

This value will determine whether the model will calculate a value for salinity based on a model of Port Lincoln seasonal salinities or utilise a lookup table, showing salinity through time, provided by the user (similar to the Temperature data button). Novice users should use the internal modelled salinity values that are based on data for Lower Spencer Gulf (Port Lincoln region) provided by the Directorate of Oceanography and Meteorology, Australian Government Department of Defence Online data service[15].

Current data

Click to open an option box where you can indicate the source of current (tidal) flow data for the model.

This value will determine whether the model will calculate a value for tidal flow based on a model of Port Lincoln seasonal tidal flows or utilise a lookup table, showing tidal flow through time, provided by the user (similar to Temperature and Salinity data buttons)[16]. Novice users should use the internal modelled tidal flow values that are based on tidal height data for Port Lincoln in the year 2005 provided by the National Tidal Centre[17].

Fouling load data

Click to open an option box where you can indicate the source of fouling load data for the model.

This value will determine whether the model will calculate a value for fouling load based on a model of Port Lincoln seasonal changes in fouling growth rates or utilise a lookup table, showing fouling load through time, provided by the user (similar to Temperature, Salinity and Current data buttons). Novice users should use the internal modelled fouling load values that are based on empirical data obtained from the Boston Island East Farming Zone (now Lincoln Offshore Aquaculture Zone).

Respiration rate data

Click to open an option box where you can indicate the source of data on respiration rates used in the model.

This value will determine whether the model will calculate a value for respiration rate based on a model for the various fish species selected (via the Farmed species button) or utilise a lookup table, showing respiration rates through time, provided by the user (similar to the above buttons). Novice users should use the internal modelled respiration rate values for the fish species they have chosen.

[15] Source – http://www.metoc.gov.au/products/data.html

[16] OxyTuna© has been developed using a modularised series of sub-routines for the various sub-models. This means that future developments can be easily incorporated (for example the inclusion of tidal flow models from other SBT projects such as Risk and Response).

[17] Source – http://www.bom.gov.au/oceanography/tides/MAPS/lincoln.shtml#form

Run model scenario

Press this button to run the model using the values you have provided at the preceding steps.

The model will be run. Firstly the user will be provided with an estimate of the likely time to complete the run, which can be halted if the estimated time is too long for the user's purposes. If this is the case, increase the time-step in the model or reduce the number of days over which the model is run. Once the user has chosen to continue, the model will begin execution. The results will be presented in graphical form on the chart at the bottom of the screen. Observe the blue line, this is the estimate of oxygen concentration at any point in time over the modelled period. The magenta line represents the tidal current over the same period of time.

Users should note that under a typical run the oxygen concentration (blue line) is more likely to fall during times when current flow (magenta line) is also low. This illustrates the simple fact that when sea-cage ventilation (which is driven by current flow) is low, respiration by fish will draw down the dissolved oxygen level in the sea-cage. When cage ventilation rates are higher (higher current flow), oxygen level stays close to the ambient value (largely determined by saturation percent). This behaviour is strongly influenced by fouling level (higher draw down when fouling is high) and by fish respiration rates (less draw down for lower respiration rates).

Store comparison

Click to store the results from the last model run.

The data from the last run of the model will be stored. You will now have a green line on the chart that represents the results from the stored run. You can use this to compare the effect of changing the model parameters. Typical scenarios that can be used to illustrate the utility of this function could include changing parameters about cage configuration (e.g. reducing the size of the cage) or by changing parameters associated with stocking density (e.g. by increasing the number of fish in the cage). Having done this the model can be re-run. The blue line will represent the prediction based on the new set of parameters while the green line represents the results from the previous scenario. All model results are stored in a separate worksheet and experienced users may export them for use in other programs.

Delete comparison

Click to remove the data stored for a previous scenario.

You do not have to remove the data to store the results from a new run. You can just press the "Store comparison" button to over-write a set of previously stored results. Pressing this button just removes the green line from the graphical display.

Built-in sub-models

Seasonal water quality sub-model

Data on temperature and salinity can be modelled using the equations provided with the model. These data are shown in Table 7.1 and have been obtained from the Directorate of Oceanography and Meteorology[18].

Table 7.1: Average temperature and salinity data predicted for Lower Spencer Gulf (Port Lincoln region) by month, as provided by the Directorate of Oceanography and Meteorology.

Month	Temperature (^{o}C)	Salinity (ppt)
January	20.5	35.6
February	20.7	35.6
March	20.8	35.7
April	20.7	35.7
May	19.4	35.9
June	18.4	35.7
July	17.8	35.6
August	17.8	35.8
September	18.4	35.3
October	17.9	35.5
November	20.2	35.3
December	20.2	35.6

Using these data, an empirical model of temperature (T_{day}) or salinity (S_{day}) for any date during the year can be interpolated from a simple Cosine function using the formulae shown in Equations 2 and 3.

$$T_{day} = R \times \left\{ Cos\left(\frac{2 \times \pi * [day + lag]}{365} \right) \right\} + T_{Base} \qquad \text{Equation 2}$$

The model for salinity is more or less identical in form (Equation 3) to the temperature model but the values for the various model constants (Table 7.2) are different.

$$S_{day} = R \times \left\{ Cos\left(\frac{2 \times \pi * [day + lag]}{365} \right) \right\} + S_{Base} \qquad \text{Equation 3}$$

An explanation for each of the constants and the values applied for Port Lincoln are provided in Table 7.2.

[18] Source – http://www.metoc.gov.au/products/data.html

Table 7.2: Parameter values used for the built-in ambient temperature and ambient salinity sub-models. Parameter values refer to values used in Equations 2 and 3.

Parameter	Temperature sub-model	Salinity sub-model	Explanation of the parameter
T_{day} S_{day}			Temperature or salinity on any given day of the year; *day* is a number between 1 and 365 (or 366 for leap years) corresponding to January 1 through December 31.
R	1.6028	-0.1797	A simple cosine function varies between -1 and +1. The value for R changes the scale of this variation. For temperature the value of R provides for an annual variation of 3.2 degrees (between maximum and minimum values i.e. ± 1.6).
Lag	-60.33	41.06	A simple cosine (scaled over 365 days) would have a maximum value on days 0 and 365 with a minimum value on day 183. The lag value moves the curve to the left or right so that the maximum and minimum values can occur earlier or later in the cycle. For the temperature model the value provides for a maximum 60 days after the start of the year (i.e. in early March).
T_{Base} S_{Base}	19.4	35.61	The base value is the annual mean value and, by definition, the annual fluctuation will increase or decrease relative to this value (determined by the value for R – see above).

These models provide a good fit to the original data (Figure 7.9). The scale of variation in salinity at Port Lincoln is very small, and changes over the course of the year will have no real effect on oxygen concentrations. Nevertheless, incorporation of these sub-models provides for changes in the OxyTuna© model if applied to other regions (e.g. Upper Spencer Gulf), where salinity values undergo large annual fluctuations.

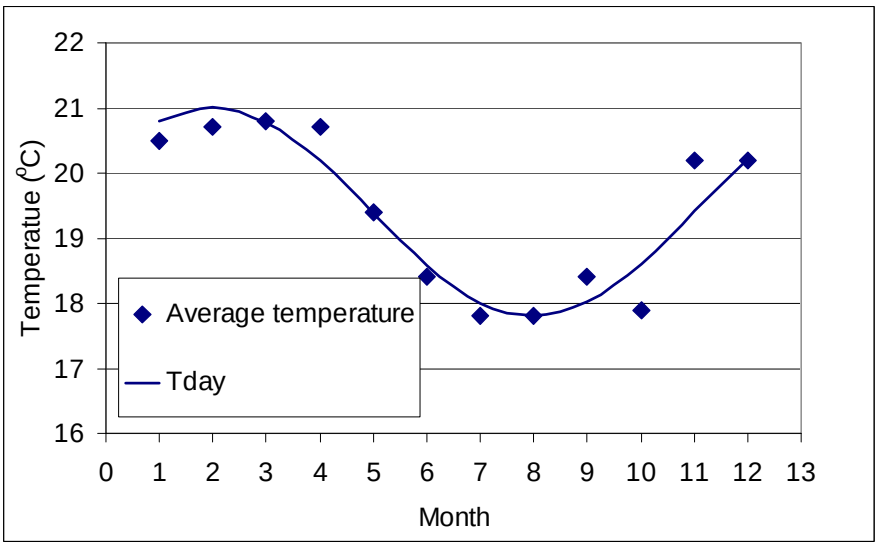


Figure 7.9: Illustration of the goodness of fit between temperature data and temperature sub-model parameterised for Port Lincoln.

Current speed sub-model

The current speed sub-model actually uses a lookup table for current speed at Port Lincoln in the calendar year 2005. The values in this lookup table have been calculated using the published tide tables for Port Lincoln (National Tidal Centre[19]). The current speed in the lookup table was modelled using an empirical equation (Equation 4) calibrated against data from the Tuna Farming Zone under the Aquafin CRC Regional Environmental Sustainability (RESA) project[20] (Bierman *et al.* 2007) over the period 29th June 2005 through 9th August 2005. The goodness of fit between the modelled and actual currents for this period is reasonable (r^2 = 0.585; Figure 7.10).

$$C_t = \left| \frac{H_{t+\partial t} - H_t}{\partial t} \right| \times Fh \qquad \text{Equation 4}$$

In this model C_t represents the Current Speed at model time t, H_t is the tidal height (obtained from published tide tables) at model time t, $H_{t+\delta t}$ is the tidal height at a time δt after the current model time. Fh is a constant that relates current speed to time dependent tidal height differences. The model takes no account of wind-forced currents and has only been calibrated against data (as detailed above) for part of the year. Alternative configurations of the equation that incorporate data on ambient wind conditions have been evaluated in developing the model. While this provides a better fit to the data, it also increases the overall complexity of the model and therefore has not been included into this implementation.

[19] Source – http://www.bom.gov.au/oceanography/tides/MAPS/lincoln.shtml#form

[20] Aquafin CRC/FRDC Project 2001/104: Aquafin CRC - Southern Bluefin Tuna Aquaculture Subprogram: Tuna environment subproject - Development of regional environmental sustainability assessments for tuna sea-cage aquaculture.

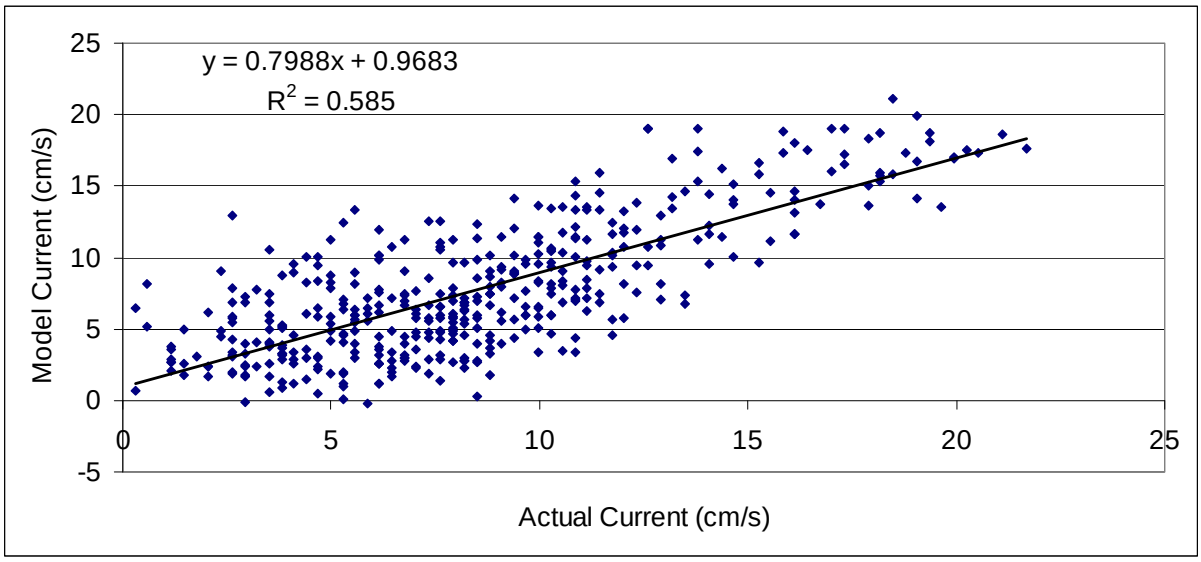


Figure 7.10: Goodness of fit between actual and modelled current using the model specified in Equation 4.

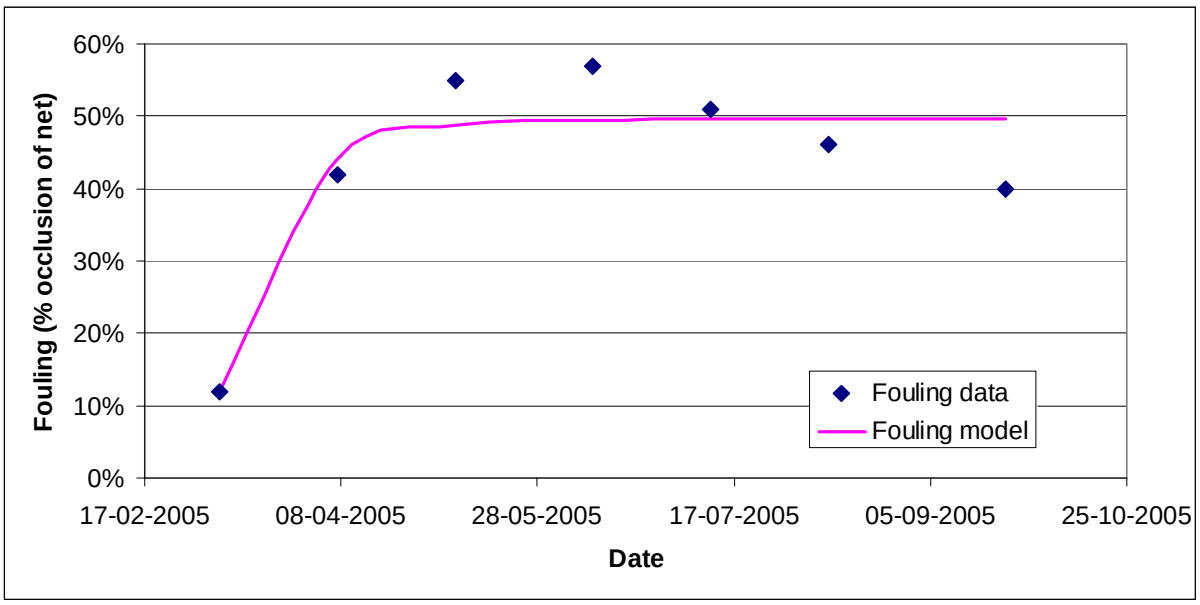
Fouling load sub-model

Sea-cage net fouling changes through time as organisms recruit onto the surface and grow. The data available on changes in fouling load through time are limited to a single set of experimental observations undertaken as part of this project. A simple sub-model for the development of a fouling community has been implemented based on these data (Equation 5). The sub-model provides for an initial rapid phase of colonisation of the net followed by a period where the level remains constant (Figure 7.11). Although the data show an apparent decline in fouling load we have chosen not to incorporate this into the model because we have no basis for extrapolating this behaviour beyond the bounds of the available data.

$$F_t = BaseLevel + \left\{F_{\max} \times \left(1 - e^{[-t/t_k]}\right)\right\} + FoulAdd \qquad \text{Equation 5}$$

F_t is the occlusion of the net (due to fouling and the presence of the net) at time t. At time zero the model assumes a base level of occlusion (*BaseLevel*), which is simply a measure of the obstruction to water flow presented by the physical structure of the net (lines and knots). This parameter will change depending on the type of net used and can be derived directly from the measurements of the net rope thickness and the mesh size (see above section on ***Cage details***). F_{max} is the maximal level of net fouling, t is the model time and t_k is a constant that determines the rate at which fouling will develop on the net. Smaller values for t_k give faster rates for the development of the fouling community and larger values provide for a longer period for fouling to develop. *FoulAdd* is an arbitary constant added to the value derived by the setting on the "Additional fouling load" slider bar (see above). *FoulAdd* allows the user to look at the effect of increased levels of fouling for the purposes of simple scenario analyses. F_t is bounded to ensure that occlusion of the net associated with the fouling community and any arbitrary additional amount from the user setting of *FoulAdd* cannot exceed 100%. While different forms of fouling (hard e.g. mussels versus soft e.g. algae) may have differing effects on water flow no attempt has been made to account for this.

Figure 7.11: Model (magenta line) of fouling load through time based on empirical data from a sea-cage



system in the Boston Island East Farming Zone (now Lincoln Offshore Aquaculture Zone) fitted to Equation 5.

Cage ventilation sub-model

Mass transport of dissolved oxygen into the sea-cage (ventilation) is fundamentally linked to the volume of water moving through the net over any given time. Volume flow into a sea-cage is a function of three key variables:

1. Current speed (Current – measured in metres per second);
2. Extent to which fouling and the physical structure of the net obstructs the flow (Occlusion – measured as a percent of the cage area);
3. Cross-sectional area of the cage measured perpendicular to the direction of water flow.

Theoretically, if we take a 1 m^2 area of net hanging vertically and oriented perpendicular (across) the current then the calculation of mass transport through the net is relatively simple; multiply the current speed ($m.s^{-1}$) by the cross-sectional area (m^2) and this provides us with the volume moving through the net ($m^3.s^{-1}$). In reality the calculation is slightly more complex. Firstly we need to determine the cross-sectional area of the cage that is perpendicular to the current and secondly we need to calculate the extent to which the net and any attached fouling organisms will impede the flow of water.

Area of cage net perpendicular to current flow

The cross-sectional area of the sea-cage can be calculated by taking the projected area of the cage along the perpendicular plane at right angles to the direction of current flow. In effect the cross-sectional area of the net perpendicular to the flow is therefore the diameter of the cage (m) multiplied by the depth of the cage depth (m). This calculation also accounts for the change in cross-sectional area of the net (perpendicular to current flow) associated with curvature of the cage.

Effect of net and fouling on water flow

The presence of the net and any associated fouling results in an effective reduction of the cross-sectional area through which water can flow into and out of the cage. However, the effect of occlusion is not a simple linear reduction in water flow. Rather a complex process of turbulence in and around the net modifies the rate at which water flows through the net. Models of turbulent flow are beyond the scope of this study so an alternative strategy was to develop a simple mathematical model of the relationship between measurements of current speed and the flow rate through nets. Volume flow rates were obtained from the flume tank experiment carried out in Subproject 4 (Rough et al 2008).

Measurements of the flow through net panels with different levels of fouling were made and these data were then used to develop an empirical model of the effect of fouling on water flow (Equation 6). No data are available for nets with an occlusion greater than 72% so the model has been constructed in two parts:

1. A goodness of fit analysis for the flow rate through a net with up to 72% occlusion (Equation 6).

2. A simple linear reduction model for flow rate through a net with more than 72% occlusion (Equation 7).

In this way the model produces data that are consistent with the experimental data for the range of panels used for measurement.

$$FR_t = C_t \times (Flow_A \times [1 - F_t]^{Flow_B}) - (Flow_C \times C_t) - Flow_D \qquad \text{Equation 6}$$

Where FR_t is the flow rate through 1 m^2 of the net ($m^3.s^{-1}$) at time t, C_t is the Current Speed at time t, F_t is the fouling (% of the net occluded) at time t, $Flow_A$, $Flow_B$, $Flow_C$ and $Flow_D$ are constants.

For fouling loads greater than 72% the model assumes a linear decline in flow rate (Equation 7) from the rate achieved at a fouling load of 72% (defined as $FR72_C$ calculated from equation 6 where $F_t = 0.72$) to a value of 0 at a fouling load of 100%. The slope of this line is defined as $FR72_m = -FR72_C / (1-0.72)$.

$$FR_t = FR72_m \times [F_t - 0.72] + FR72_c \qquad \text{Equation 7}$$

Application of the formulae in Equations 6 and 7 over a fouling range (0-100%) and current speeds of 0-1 $m.s^{-1}$ yields the plot shown in Figure 7.12. For any given current speed low levels of fouling (<20%) have little effect on volume flow rate through the net (volume is limited only by current speed). As fouling level increases the volume flow rate decreases until at a loading of 100% volume flow is reduced to zero (the net is effectively impermeable). This model provides a good fit to the experimental data ($r^2 = 0.87$; Figure 7.13).

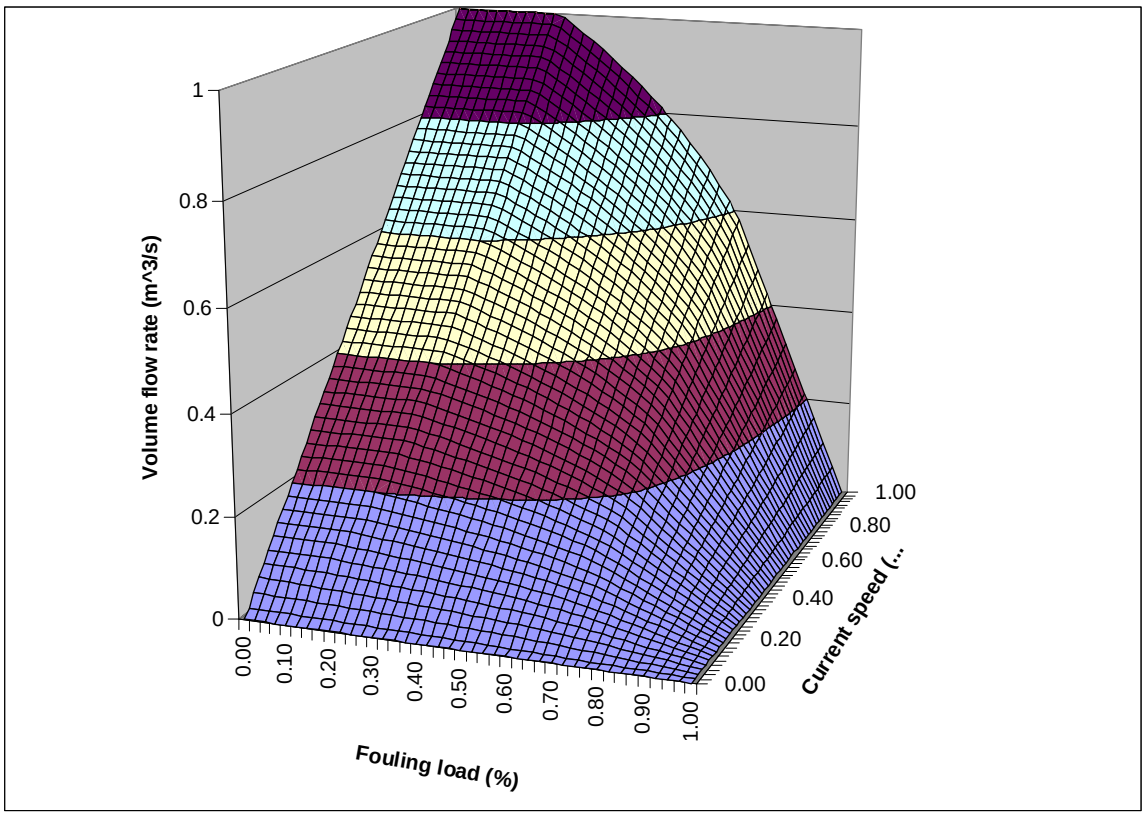


Figure 7.12: Effect of sea-cage net fouling load (x-axis) and current speed (y-axis) on volume flow rate through 1 m^2 cage panel (z-axis). In general terms flow rate increases with increases in current speed and/or decreases in fouling load.

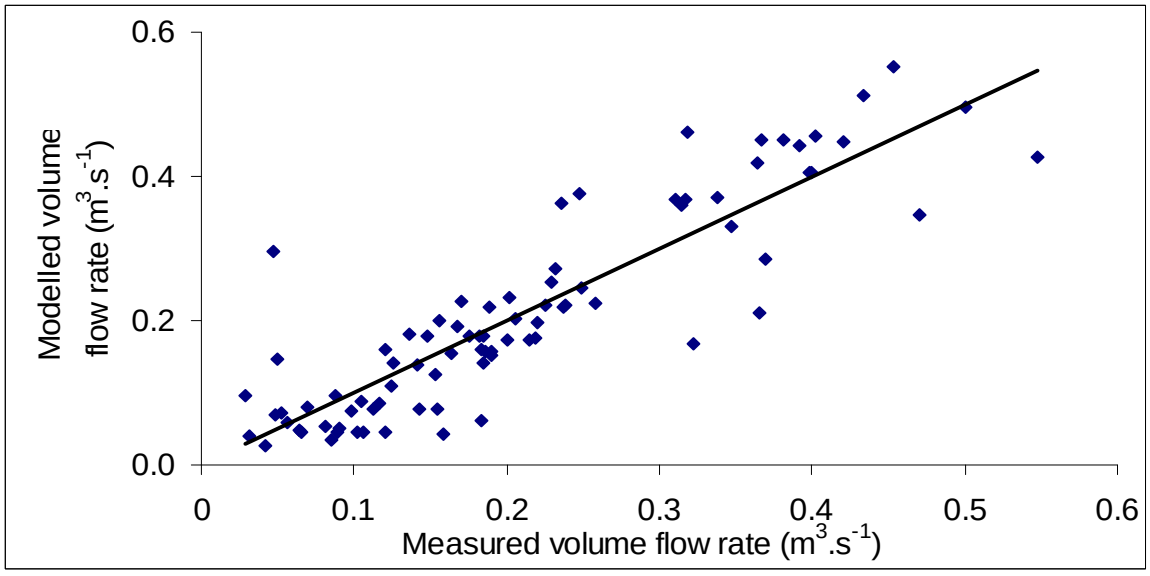


Figure 7.13: Goodness of fit between actual volume flow rates through the net (x-axis) as measured in the flume tank versus the modelled flow rate through the net (using the model developed above; y-axis).

Fish respiration sub-model

The fish respiration sub-model currently uses a simple respiration constant for each species of fish (chosen via the "Farmed species" button). Values have been abstracted from the literature or estimated based on comparisons with similar species. Implicitly the model assumes that respiration rate is constant through time. For southern bluefin tuna, this assumption is not correct (Musgrove and Fitzgibbon 2005) (and this is probably the case for other species) but until more highly resolved data are available on changes relative to feeding rates, water temperature, ambient oxygen, etc, it is the best assumption that can be made. Notwithstanding, the user can still provide their own data on respiration rates via the "Respiration rate data" button. This allows advanced users to build their own models for respiration through time and feed it to the model directly (see Scenario 4 below for an applied example).

Scenario Analysis

This chapter is intended to illustrate the utility of the model through a number of simple scenarios. These scenarios comprise an illustration of the effects of:

1. A 20% reduction in ambient dissolved oxygen concentration with all other parameters being held constant.
2. A 40% increase in stocking density with all other parameters being held constant.
3. A 30% increase in net fouling load combined with a 30% drop in current speed and all other parameters being held constant.
4. A run in which the fish respiration rate increases with current flow (assuming for example that the farmer feeds only during periods of high flow) compared with a run in which the respiration rate is assumed to be constant through time.

The first 3 scenarios can be run quite easily using only the buttons and slider bars provided on the command interface.

Scenario 4 requires the user to enter their own data for respiration rate, which can be modelled based on current flow (see below for an applied example).

<u>Background scenario – basis for comparison</u>

Scenarios 1 to 4 were run against a standard model run. The standard model run had model parameters set as detailed in Table 7.3. With these values the time series for oxygen concentration, as predicted by the model, is shown in Figure 7.14.

Table 7.3: Model parameters against which scenarios 1 to 4 were compared.

Parameter	Value	Units	Explanation of variables
CageDepth	12.0	m	Depth from surface to bottom of side net
CageDiameter	40.0	m	Diameter measured at the surface
MeshSize (bar)	10.0	cm	Distance measured from inside of net rope across mesh to outside of next rope
RopeDiameter	6.0	mm	Net rope thickness
DeltaT	1	hours	Model time step in hours
ModelTimeStep	0.042	days	Model time step in days
RunFor	10	days	Total period to run model over
StartDate	01-Jul-2005 00:00:00	date	Determined by user
StartDay	181.000	day	Day of the year 1-Jan-05 = day 1
EndDay	191.000	day	Day of the year
EndDate	11-Jul-2005 00:00:00	date	End date of model run
TimeEst	0.2	minutes	Estimated time to run model given parameter choices
FoulAdd	0%	%	Obtained from slide bar on command sheet
AveFishSize	20.0	kg	Average size of fish
FishNumber	1000	fish	Number of fish in cage
FishBiomass	20000	kg	Calculated from AveFishSize X FishNumber
FishResp	650	$mgO_2.kg^{-1}.h^{-1}$	Literature derived value (Clarke and Johnston 1999)
FishSpecies	SBT		Species being farmed
StockDens	1.3	$kg.m^{-3}$	Calculated from FishBiomass/CageVolume

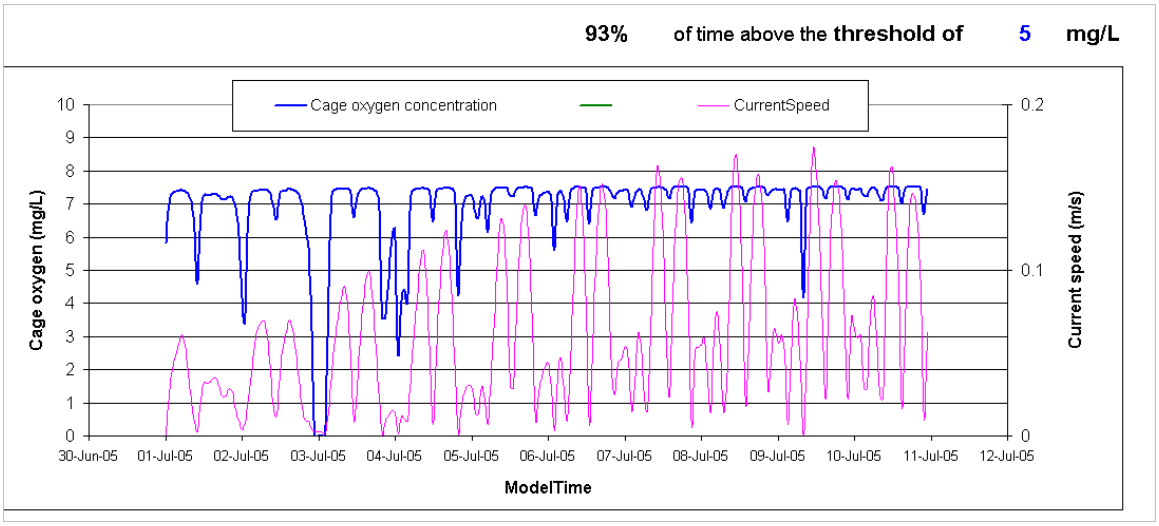


Figure 7.14: Time series prediction using parameter values shown in Table 7.3. The blue line shows dissolved oxygen concentration ($mg.L^{-1}$ or ppm) and the magenta line current speed ($m.s^{-1}$) due to tidal flow.

Scenario 1 – 20% reduction in ambient oxygen

Scenario 1 is enacted with the user dragging the "Ambient % saturation" slider to the left to set the saturation value at 80%. All other parameters remain the same as the background scenario as detailed above.

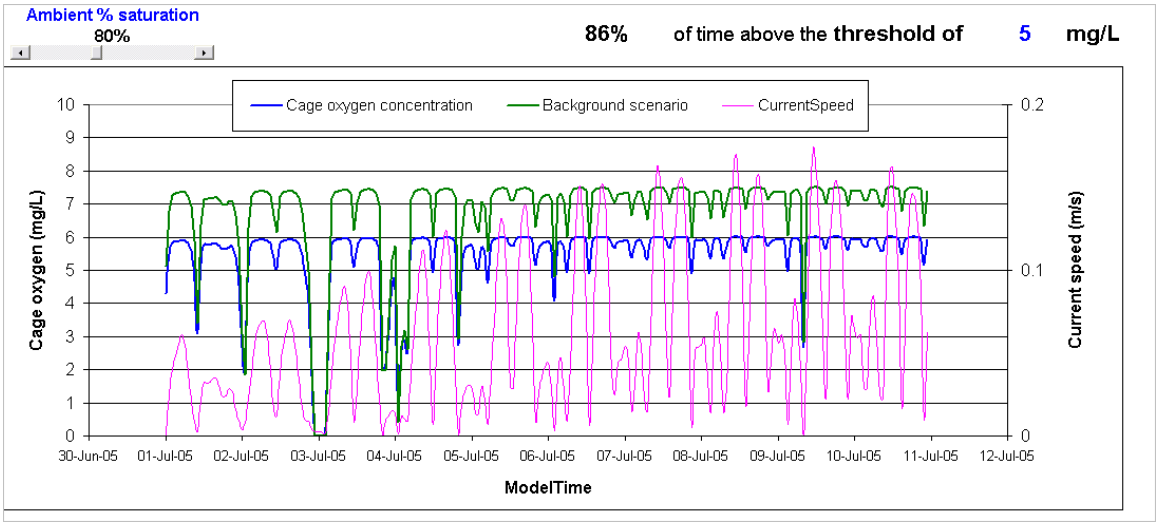


Figure 7.15: Scenario 1 – the plot shows the results with the ambient % saturation set at 80%. The green line on the plot is the background scenario as detailed above and the blue line is the results of Scenario 1.

The scenario predicts a simple downward shift in the oxygen level of around 1.5 $mg.L^{-1}$ (or ppm) over the entire period of the model run. Both this scenario and the background scenario show a precipitous drop in dissolved oxygen in the sea-cage (around 3rd July 2005) associated with a low current flow event when tidal flow was reduced to around zero for a period of 2 hours.

The utility of the "% of time above the threshold" calculator is illustrated by the comparison between the background scenario (Figure 7.14) where dissolved oxygen levels in the sea-cage were above 5 $mg.L^{-1}$ for 93% of the time compared to this scenario (Figure 7.15) where oxygen values were above the threshold for only 86% of the time.

Scenario 2 – 40% increase in stocking density

Scenario 2 is enacted by pressing the "Stocking rates" button and changing either the value for the average size of fish or the number of fish to a value 40% higher. In the background scenario run, the stocking density was set at 1.3 $kg.m^{-3}$ while in Scenario 2 the stocking density was set at 1.9 $kg.m^{-3}$.

The results of this scenario (Figure 7.16) shows that stocking density only has a significant effect during periods of low current flow when mass water exchange is limited relative to the rate of oxygen consumption by the fish.

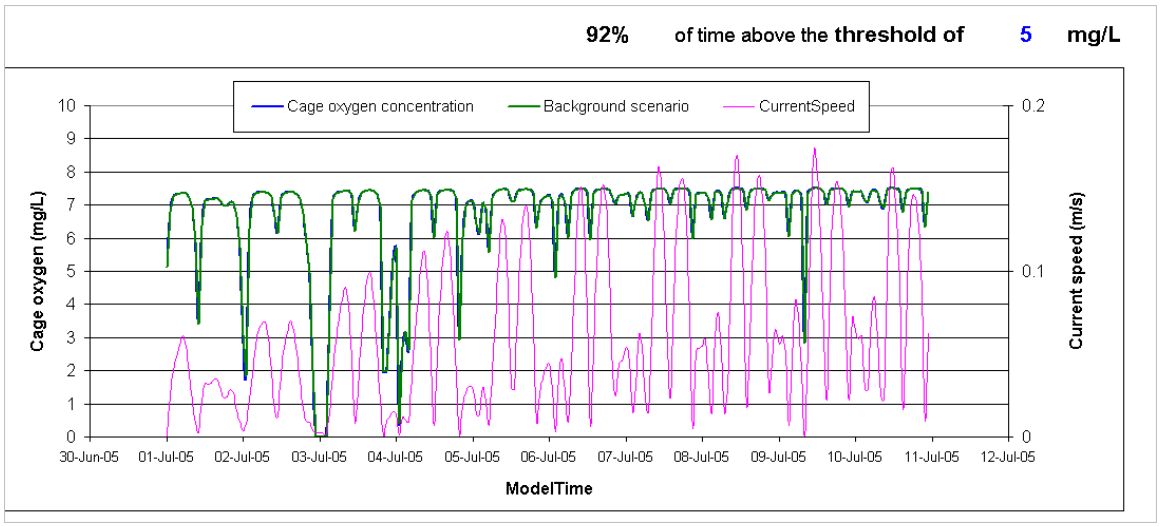


Figure 7.16: Scenario 2 – the plot shows that an increase in stocking density has little impact on sea-cage dissolved oxygen level except during the periods of low flow (note the period around 4-Jul-2005).

Scenario 3 – 30% increase in fouling load combined with a 30% drop in current speed

Scenario 3 is enacted by setting the "Additional fouling load" slider to 30% and the "Relative current speed" slider to 0.7.

The scenario shows that water flow rates (determined by current speed and the level of fouling on the cage) have a substantial influence on dissolved oxygen status inside the sea-cage. During periods of high tidal flow oxygen levels are maintained close to the ambient conditions (Figure 7.17) but oxygen levels fall substantially (relative to the control situation) during periods of low flow. The period of time spent below the 5 $mg.L^{-1}$ threshold is 87% which is only slightly less than that for the control run (93%; Figure 7.14).

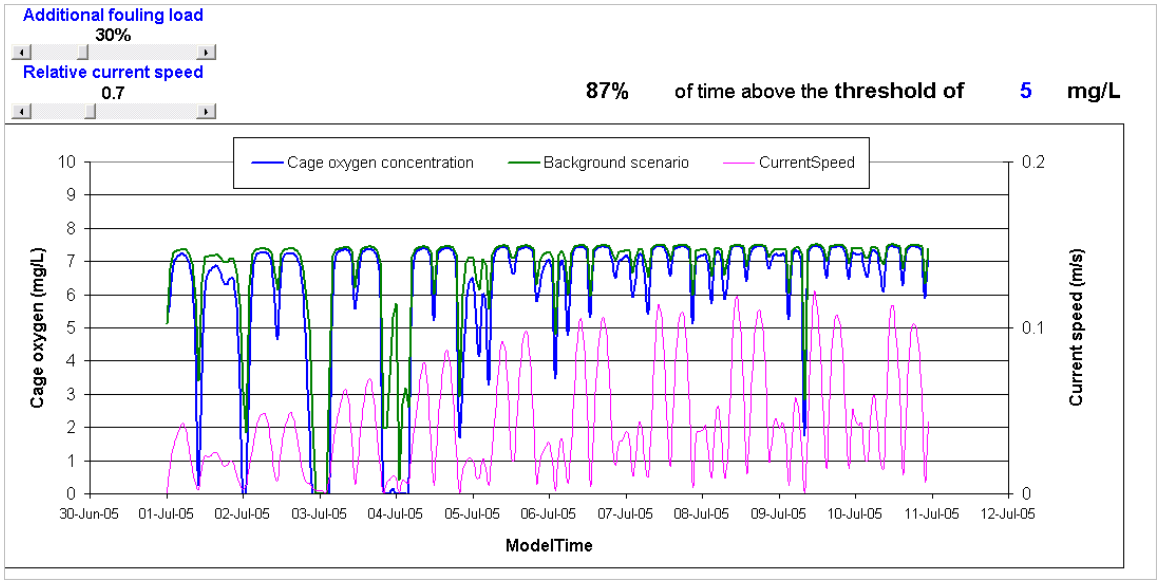


Figure 7.17: Scenario 3 – the plot shows that changes in flow rate and fouling load have a substantial effect on sea-cage dissolved oxygen level during periods of low flow but relatively little effect during periods of moderate-high flow.

Scenario 4 – Comparison of respiration rates linked to current flow

Finfish respiration rates are known to change through time, and particularly in relation to feeding activity by the fish (Musgrove and Fitzgibbon 2005, Seymour *et al.* 2007). Scenario 4 provides a comparison of the effect on cage oxygen when fish respiration rate is assumed to vary in response to feeding. This can be compared to the background scenario in which the respiration rate is assumed to be constant through time.

As detailed above, OxyTuna© was developed to allow scenarios that make use of the built-in sub-models for selected parameters (temperature, salinity, current flow, fouling load and fish respiration rates). Alternatively users may provide their own data for these parameters. Importantly, user supplied data may be derived either from field based observations (i.e. empirical observations) or from new models developed by the user. This scenario has used the latter approach.

Research under Aquafin CRC project 1A.7 (Phase 1 and 2) has shown that respiration rates are unlikely to be constant through time (as assumed in the basic OxyTuna© sub-model); rather, respiration rates are maximised after feeding and then fall through time to the base level (Musgrove and Fitzgibbon 2005, Seymour *et al.* 2007). A simple time-series model was developed in EXCEL™ to illustrate this behaviour. The model assumed that immediately after feeding the respiration rate increased to 1200 $mgO_2.kg^{-1}.h^{-1}$ and then fell, over a period of 12 hours, back to the base rate of 600 $mgO_2.kg^{-1}.h^{-1}$ (this is somewhat faster than the empirical data suggests). Feeding times were selected to coincide with the period of maximum current flow, once every day and during daylight hours. Application of this model provides a time-series for respiration rate as shown in Figure 7.18.

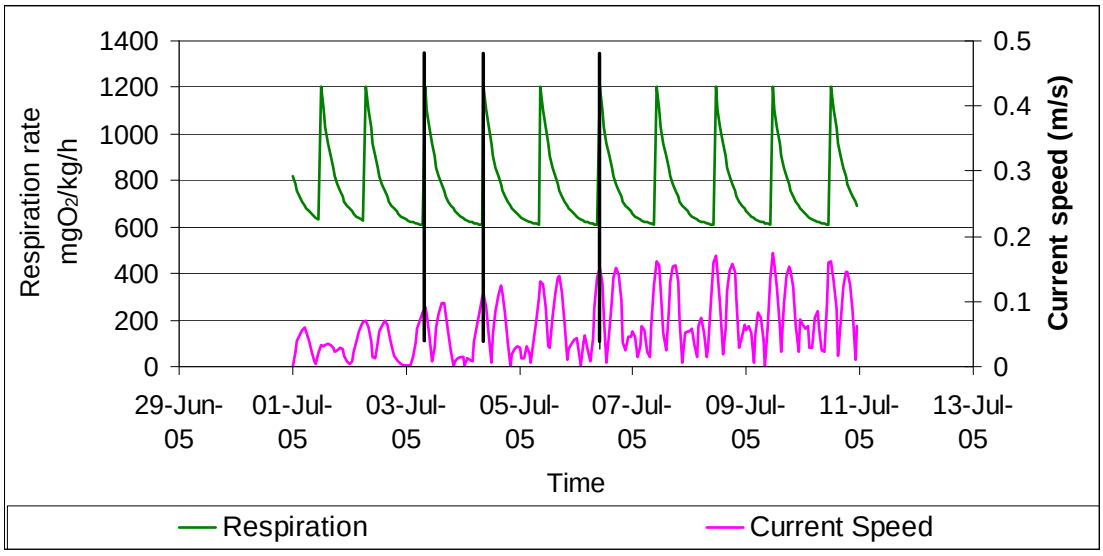


Figure 7.18: Time series plot showing current flow (lower magenta line) and respiration rate (upper green line) used for Scenario 4. Vertical black lines illustrate selected examples of the linkage between periods of higher current flow and the increase in respiration due to feeding.

When OxyTuna© was run using this user-defined model for respiration there was almost no effect on sea-cage dissolved oxygen dynamics (Figure 7.19). This provides a good demonstration of the very low sensitivity of the model to the value for the respiration rate parameter. This result contrasts strongly with those from Scenario 3, which demonstrates a high

sensitivity to current flow and fouling (both of which relate to volume transport of water through the net).

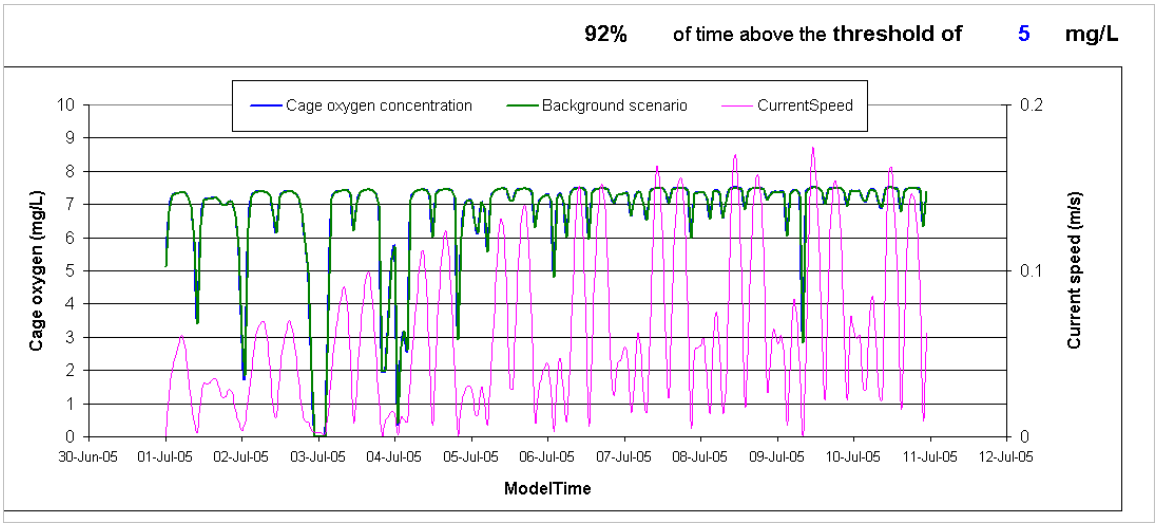


Figure 7.19: Scenario 4 – the plot shows that a periodic doubling of respiration rate has almost no perceptible effect on overall sea-cage dissolved oxygen concentration. The blue line (scenario 4) is almost perfectly superimposed over the green line (background scenario) demonstrating a very low sensitivity to respiration rate.

CONCLUSIONS

The OxyTuna© model has been developed in order to assist farm managers to make better decisions about the management of finfish sea-cage systems and in particular to better understand the relationship between net fouling and dissolved oxygen concentration in cages and how this responds to various management interventions including changes in cage configuration, stocking density and fouling management (e.g. cage cleaning).

The model provides a quantitative prediction of the changes in dissolved oxygen concentration through time for different sea-cage configurations (cage size, net type, stocking density, fish species) in response to changes in ambient conditions (temperature, salinity, ambient oxygen concentration and current speed).

The dynamical nature of the model allows users to better understand the interplay of factors that control dissolved oxygen concentration in a sea-cage, and it can therefore be used not only as a management tool but also as a teaching tool.

The model provides a number of sophisticated features including:

- An enhanced interface that allows the user to quickly and simply develop and analyse simple scenarios relating to changes in stocking density and sea-cage configuration.
- A set of simple sub-models that simulate changes in fouling load, sea-cage ventilation rates (based on tidally induced current flow and fouling load), fish respiration rates for different species and seasonal changes in water quality (including temperature, salinity and ambient oxygen concentration).

- An advanced facility that allows the user to incorporate more sophisticated time series data (or user developed models) that quantify changes in ambient water quality, tidal flow, fouling load and fish respiration rates.

- A simple graphical output that provides a clear representation of the predicted time series.

By implementing OxyTuna© as a Visual Basic for Applications (VBA)™ program developed to run within Microsoft EXCEL™ the model outputs can be easily captured and incorporated into other programs by anyone with a basic understanding of EXCEL™. This feature is expected to improve the utility of the model and the opportunity for individual users to develop their own enhancements.

Chapter 8 : SUB-PROJECT 6: COMMERCIAL PILOT SCALE EVALUATION OF AN ANTIFOULANT WITH A STOCKED TUNA CAGE; INCLUDING ANALYSIS OF FISH HEALTH, RESIDUES, WATER QUALITY AND THE TREATMENTS EFFICACY IN INHIBITING NET FOULING.

This chapter was authored by Kirsten Rough (Australian Southern Bluefin Industry Association Inc.) and may be cited as:

Rough KM and Ellis DC (2008). Evaluation of an antifouling treatment with a commercially stocked cage of southern bluefin tuna (*Thunnus maccoyii*) in South Australia. In Rough KM, deNys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp.

ABSTRACT

The antifouling product, Wattyl Net Clear ZPT was tested on an entire net in a commercial tuna ranching situation from February to September 2006 to determine efficacy, chemical residue accumulation and any influence on tuna health. Net Clear ZPT is a water-based coating that contains the heavy metal zinc as its oxide and the booster biocide zinc pyrithione as active antifouling agents.

The application of this antifoulant did not totally prevent the colonisation and growth of biofouling on the treated net; but there was a marked reduction in the density of the growth. In addition, the composition of fouling assemblages on the treated net was altered so that shell growth was not present at any time or depth. Efficacy was most apparent with increasing water depth; occlusion of meshes due to net cord and fouling growth on the base of the coated net was less than 50% compared to 85% on untreated net.

Zinc based antifoulant on the net did not result in elevated levels of zinc within tuna muscle, tuna skin, bivalve shellfish *Mytilus edulis* suspended in contact with the net or in sediments under and around the site of this trial.

The tuna within the treated net demonstrated no adverse behaviour and fewer mortalities were counted than in the untreated control net. In particular less mortality was observed for those that occur as a result of capture and towing practices and from the disease organism *Uronema nigricans*. Antifoul treatment appeared to have no effect on the burden of gill parasites, gill histopathology, or haematology of tuna sampled from this trial.

INTRODUCTION

Biofouling of aquaculture nets is the settlement and establishment of various biological organisms (bacteria, hydroids, micro and macro alga, invertebrates, ascidians, molluscs etc). It is a sequential event in that once the primary film of microscopic organisms is established; macroscopic organisms can colonise and flourish (Cheah and Chua 1979; Hodson and Burke 1994; Wahl 1989). The growth of the macroscopic organisms can become so dense that the mesh size of the net is effectively reduced or totally occluded (Hodson et al 1995; Svane et al 2006). This can impact fish farming in the following ways: the significant impediment to water flow reduces the supply of dissolved oxygen to the caged fish; the increased weight of the net and resistance to clear water flow increase structural fatigue of cage and anchor

infrastructure; and fouling assemblages may harbour disease-causing organisms (Aarsnes et al 1990; Kent 2000; Braithwaite and McEvoy 2005; de Nys et al 2005a).

Tuna mariculture is different from most finfish farming industries as large (>10kg) sub-adult fish are captured from the wild and stocked into sea-cages for a typically short time of only 3-6 months. The initial large size of fish means that the nets used to enclose the tuna can have a greater internal mesh size (56.25cm² to 100cm²) compared with those used to retain other fish species (typically <10cm²). The short farm cycle, large mesh size nets and low stocking densities have enabled this industry to prosper in the absence of antifouling products to control biofouling. However, experience from other finfish farming operations suggests that a reduction in biofouling growth could be beneficial to tuna productivity and management operating strategies.

This subproject aims to determine the efficacy of an antifoul treatment on a net fully stocked with tuna in a commercial situation, including the assessment of fish health, analysis of chemical residues, water quality and the development pattern of net fouling communities. It addresses objectives 2, 8, 9 and 10 of the overall project 2003/226.

METHODS

Net Management

Two new white, Badinotti 150mm (stretch mesh) knotless nylon nets were manufactured in Tasmania at Nets Pty Ltd in October 2005. Each net had a 126m headline and 9m walls with a 10% taper. One of these nets, for cage number 4, was treated on site (at Kingston, Tasmania) with Net Clear ZPT™ (active ingredients zinc oxide and zinc pyrithione at composition levels at 10-30% and 1-5% respectively (appendix 8.1; Chem Watch MSDS for Wattyl Net Clear ZPT 2002)); and the other was left untreated as a control, for cage number 5. The treatment process involved the total immersion of the entire net in a large trough containing 7200L of antifoulant for 30 minutes. The wet net was lifted to drain excess drips over the trough; then moved under cover to air dry for 3 days (see Plate 8.1). The treated net was deployed at sea on the 15th February 2006 and the control net within 7 days of this. Both nets were set with twenty 100kg net weights.

Plate 8.1a-d: Applying copper antifoulant to a 126m circumference salmon net. It is the same procedure and similar equipment for a tuna net; however the product is different (photos provided by Nets Pty Ltd).

Farm Management

Transfers from a single tow cage into grow out cages occurred on the 17th and 19th of March, where 2018 and 2330 tuna went into the second and third transfers (ie the first control (cage 5) and treated cage (cage 4)) respectively. Insufficient SBT numbers in the tow cage meant that the third transfer required a top up of 1131 fish (from another tow cage caught at a similar time and location) and that there was no fourth transfer, second control (see appendix 8.2 for proposed experimental design). Observation of this species of tuna and management data over the previous 12 years has shown marked differences in mortality rates between cages; therefore to ensure that results would remain relevant to the commercial industry, mortality data were only collected from cages 4 and 5. Cages were anchored near each other and were aligned perpendicular to the direction of the water flow, the lease site and cage layout can be seen in Figure 8.1.

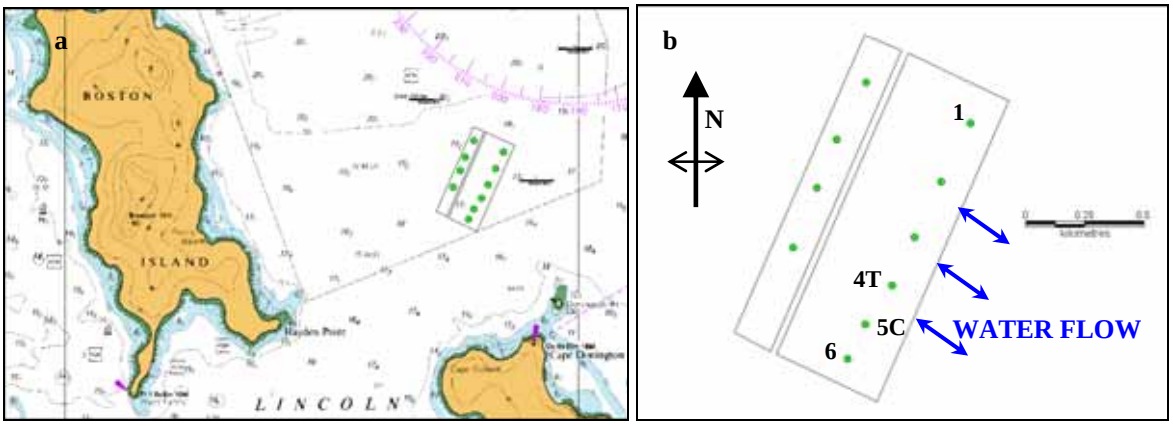


Figure 8.1: Map (a) and site plan (b) of the tuna lease showing the direction of water flow and the relative locations of all cages (green circles). Those relevant to this trial are numbered; cages 4 – 6 were derived from one tow, and cage 1 held tuna from the previous season as part of the long term holding trial.

SBT in each of the experimental cages were fed the same feed types and the same amounts per tuna daily; and feed crews recorded any differences in feeding behaviour between cages. Mortalities were removed and recorded daily, or as frequently as divers could access cages through periods of bad weather.

Feeding crews and divers were to immediately report any aberrations in fish colour or behaviour, especially in the treated cage. Any farm management issues arising that were relevant to this project, ie net treatment, net integrity, net maintenance requirements both through and after the season were recorded and reported.

Water Quality

Water quality was assessed by SARDI as described in Loo (2008) (Chapter 5, subproject 3); using their telemetry systems. Water temperature at 5m depth was recorded at one hour increments throughout the nets' deployment with a Vemco data logger, for consistency with previous trials.

Net Fouling Analysis

Fouling growth on the nets was monitored by a contract dive company (Eyre Diving) undertaking a vertical transect (headline to base) on the northwest side of the cage, on the inside of the net. In this commercial situation, this section of the net would be subject to the least disturbance through the daily activities of feed and dive vessels, and by the harvest net once harvest was occurring. Also due to prevailing currents of the area, this location would be subject to maximum enrichment through farming activities. Transects were undertaken approximately monthly after fish transfers, on the 7th April, 10th May, 23rd June, 8th July and 11th August. These were recorded on a VHS video by a remotely operated vehicle (ROV). The VHS video was transferred onto a DVD and frames extracted at 2m increments from the surface to the base of the net. Photos derived from the DVD were assessed, where possible, for percentage cover of fouling and relative abundance of each fouling type (soft macrophyte and invertebrates, and hard shell) as per Rough and Ellis (2007), Chapter 4, subproject 2.

Chemical residues

SBT and environmental samples (shellfish and sediment) were tested for residue levels of total zinc through the South Australian Research and Development Institute Food Safety Research Program at the VPS laboratory, Glenside, SA.

To collect tuna samples a commercial net harvest (ie 1 or more AV's) was undertaken on two occasions from each cage. Netting a commercial quantity of tuna at each interval was to ensure that the tuna sampled were more likely to be representative of those in the cage at the time and to enable the company to send the product to market. Samples were randomly collected from the processing line so that 30 tuna were sampled from harvests on the 27th June (treated cage), 11th July (control cage), 18th August (control cage) and 26th August (treated cage); so that a total of 60 tuna were sampled from each cage. The gilled and gutted (dressed) weight, length and condition index were recorded for all tuna sampled.

SBT flesh and skin samples were collected as a crescent shaped segment up to 10mm thick from between the 6th and 7th finlets; and therefore included flesh from red and white (akami and chutoro) muscle blocks and skin from dorsal and ventral surfaces. SBT samples were frozen individually in plastic bags with the skin on, and transported with gel paks to the laboratory in Adelaide. Skin and flesh were dissected and analysed separately, as tuna skin is always removed from muscle prior to consumption, with standards, IAEA 407 Fish Flesh NRC DORM-2 Dogfish Muscle, and quality assurance controls. For muscle samples, approximately 0.5g of flesh was dissected from the sample and digested at 340^{o}C with 2ml of nitric/perchloric/sulphuric acids 10:1:1. The digests were made up to 5ml with distilled water

and measured by Flame Atomic Absorption. About 0.2g of skin was analysed for zinc in the same way as for muscle.

Small (<50mm shell length) blue mussels (*Mytilus edulis*) were placed in plastic open mesh oyster bags and hung directly on the tuna nets on the 21st April 2006 and removed for analysis on the 22nd August 2006 (deployed 123 days). Samples were frozen in their shells within plastic bags prior to transport to the laboratory in Adelaide where mussels were shucked and the total tissue content of individual mussels (0.6g – 0.9g) was analysed for zinc in the same way as for muscle with standards, BCR 278 Mussel Tissue NIST 1566a Oyster Tissue, and quality assurance controls.

Sediment samples were collected 100m up current (ie to the South East of the cage) and 100m down current (ie to the North West of the cage) on the 9th August; and directly underneath each net on the 30th September. Samples away from cages were collected remotely from the boat by SARDI using a Happs corer device (5 replicates per site, cores 75mm diameter by 50mm depth); those under cages were collected by contract divers (3 replicates per cage, cores 75mm diameter by 50mm depth). All sediment cores were individually frozen in plastic bags prior to transport to the laboratory in Adelaide where samples were dried at 60°C and about 0.5g of each sample was assayed for zinc in the same way as for muscle with a standard, NRC BCSS-1 Marine Sediment, and quality assurance controls.

A sample of the paint product was diluted with distilled water and a 0.5ml aliquot assayed for zinc. However, due to the large dilution involved (50,000x) the result can only be considered approximate.

Fish health

Fish health was assessed primarily at the 'whole cage' level. Farm and dive staff observed SBT behaviour, and retrieved and recorded mortalities daily. Specific health tests were undertaken on groups of 10 tuna from each of cage numbers 1 (long-term holding trial), 4 (treated with antifoulant), 5 (untreated control) and 6 (untreated control) (Figure 8.1). These tests included examining SBT for the presence of parasites; analysing tissue samples (gills and vital organs) for difference by histopathology, and blood collected for routine haematology.

Parasite checks were undertaken by personnel from the CRC SBT health project (FRDC Project No. 2003/225). SBT were screened specifically for the eye and skin copepod *Caligus*, gill copepods *Psuedocycnus*, and *Eurphorus*, gill fluke *Hexastoma* and blood fluke *Cardicola*. Skin and gill parasites were checked by macroscopic examination and blood fluke by flushing the lumen of the heart ventricle and examination of the settled fluid under the microscope (Cribb et al 2000; Nowak et al 2007). Parasite loads were expressed as prevalence (ie. the percentage of the total number of fish sampled that were infected) and intensity of infection (ie. average number of that organism per infected individual tuna).

Tissue samples were collected at harvest from the gills, liver and spleen of SBT and preserved in 10% neutral buffered formal saline on the vessel. Samples were processed for routine histology including paraffin embedding, sectioning and then staining with haematoxylin-eosin, at the University of Tasmania. Slides were examined and reported on by Professor B. Nowak.

Blood samples were obtained and analysed as described Rough (1998); Rough et al (2005). Samples were collected via the lateral cut (during exsanguination) using 5 or 10mL syringes with no needle attached. Blood was gently expelled from the syringe into pre-treated EDTA tubes and gently mixed by inverting tubes several times. Tubes were kept cool in an insulated container on ice. Well mixed blood was drawn into 75mm x 1.2mm microhaematocrit

tubes and sealed at one end with haematocrit sealing compound. Tubes were centrifuged at 13000RPM for 10 minutes and read manually using a haematocrit reader. Haematocrit was expressed as the percentage of packed red cells (erythrocytes) to the total blood volume. The leucocyte volume (leucocrit) was determined by measuring the height of the buffy layer as a percentage of the total blood volume. Blood smears were prepared using the two-slide technique and allowed to air dry prior to being stained with the Diff Quik technique. Differential leucocyte counts were conducted by categorising 150-200 white cells as neutrophilic, eosinophilic or basophilic granulocytes, monocytes, lymphocytes or thrombocytes. Leucocyte differentiation was determined at 1000x magnification under oil immersion. Counts were expressed as a percentage of the total number of white cells examined.

Data analysis

Data sets for fish health and chemical analyses were assessed for normality using the Shapiro-Wild W test and homogeneity of variance by Cochrans test. Differences between treatments, depth and time were tested by analysis of variance (ANOVA). If the results of ANOVA were statistically significant, Fischer LSD test was used to assess which means were different. Percentage data were arcsine transformed prior to analyses. The results were considered statistically significant if $P<0.05$. Statview software was used for statistical analysis.

RESULTS AND DISCUSSION

Water Quality

The telemetry systems were deployed on the 1st May 2006 but did not have functional oxygen probes until June 2006. By this time harvesting had commenced in the control cage and biofouling growth was very apparent on both cages (see net fouling analyses, below). Therefore the information on dissolved oxygen on either side of the nets from these systems is of very little value for the time period that is critical for tuna health (see fish health section below). The water temperature data derived from the telemetry system is displayed in Figure 8.2. The water temperature through this trial was comparable to the same time period for the panel trial (Rough and Ellis (2007), Chapter 4). This suggests that any differences in product performance would be due to other factors such as the introduction of fish and feed and altered water flow, rather than water temperature.

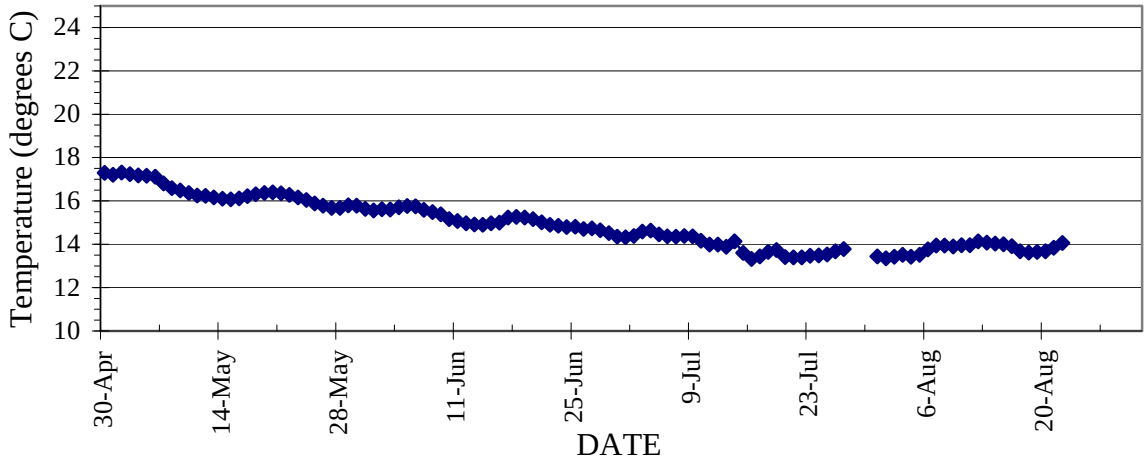


Figure 8.2: Daily water temperature from telemetry system 2006, data supplied by Maylene Loo, SARDI.

Vemco data loggers deployed on nets to monitor water temperature were destroyed in the process of net retrieval; hence no data was available from these.

Net fouling analysis

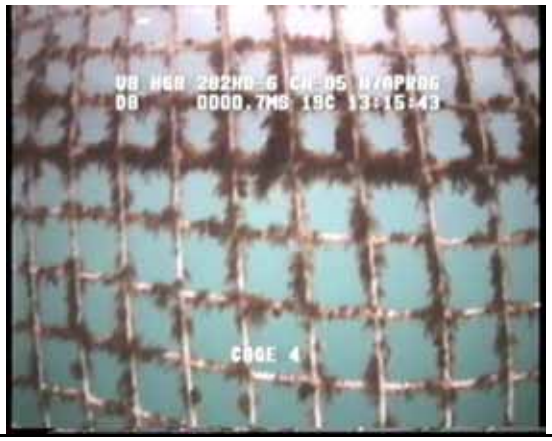
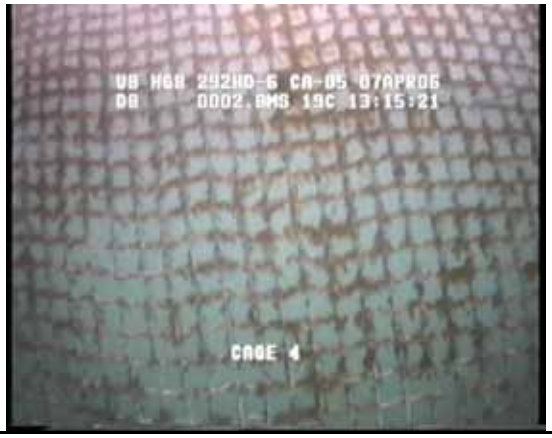
The application of the antifouling paint Net Clear ZPT did not totally prevent the growth of biofouling on the tuna net in this trial. However, it did alter the density and type of growth, especially with increasing water depth. It appeared that the typical community succession was delayed and altered so that hard shelled organisms were not present at any time or depth.

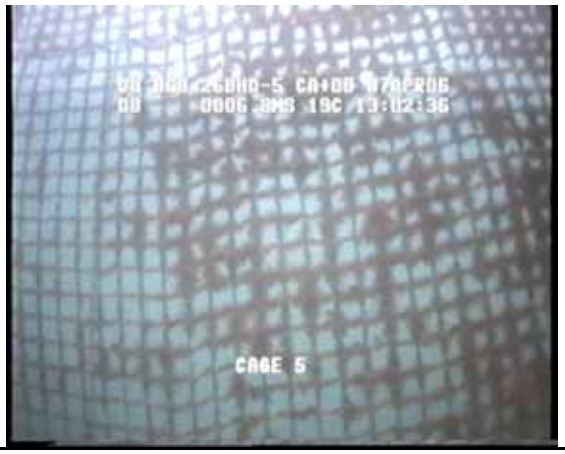
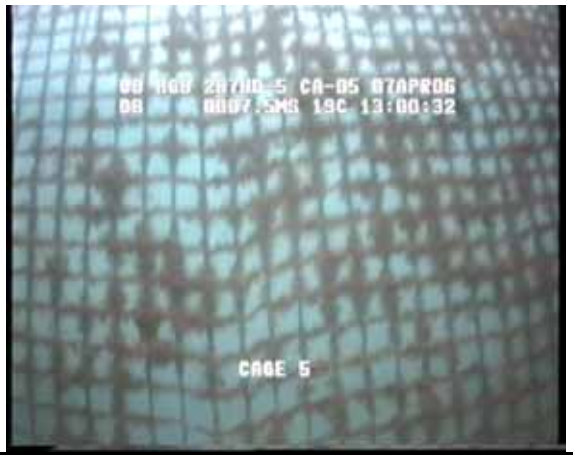
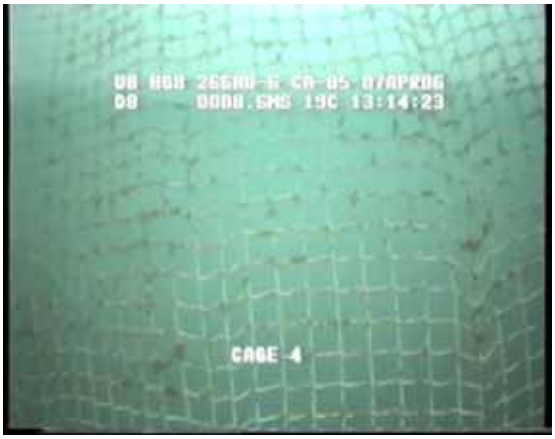
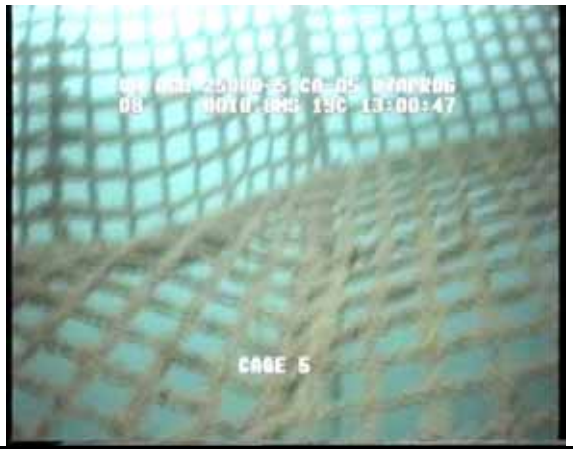
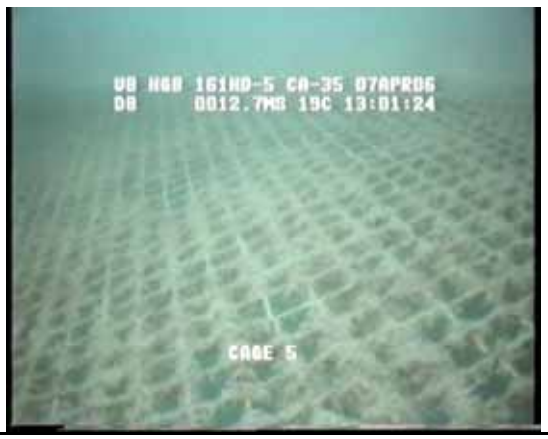
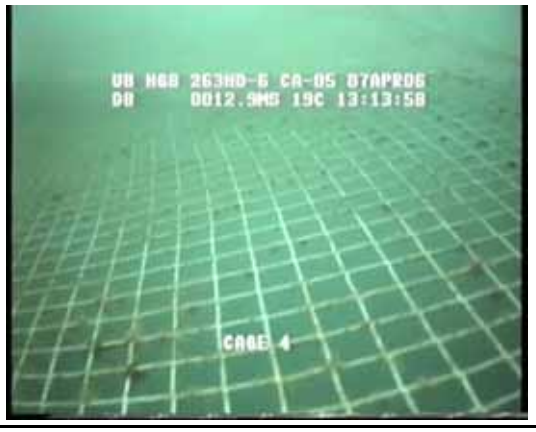
A problem encountered with the use of the Remotely Operated Vehicle (ROV) to record transects down the net was that the video was not at a fixed distance or angle from the net surface. This made it impossible to extract photographs of comparable mesh numbers and at set depths across treatments and time, so as to undertake image analysis. Therefore fouling density will be discussed in relation to the guide of percentage occlusion made as part of the panel trial (Rough and Ellis (2007), Appendix 4.3). In all these comparisons it is important to keep in mind that a new, clean Badinotti 150mm mesh net treated with Net Clear ZPT has an occlusion value of 20-24% (ie a maximum of 76 to 80% free water space), dependant on the thickness of the coating (Rough and Ellis 2007). A new, clean Badinotti 150mm mesh untreated net has an occlusion value of 18-22% (Rough and Ellis 2007, Rough et al 2008).

Growth was present on both nets at the first underwater video survey undertaken on the 7th of April, 51 days after net deployment and 21 days after the addition of SBT (Plate 8.2). At this time the antifoul treated net had light patchy weed growth at the surface, the density of which decreased with depth. From 4m depth onwards the combination of net and biofouling growth amounted to less than 30% occlusion and the cord of the net mesh was clearly visible between the areas of weed colonisation. Towards the lead line and on the base of the net, visible biofouling was confined to isolated meshes; most likely cracks in the coating, and clumps of drift algae passively caught by the net (e.g. Plate 8.3).

By comparison, weed growth on the untreated net in April was much denser and present at all depths. Areas of lighter weed growth were apparent, but all meshes were covered so that the cord of the netting was not visible at any depth. From 4m depth to the lead line, the combination of net and growth amounted to an occlusion range of 40% in the lighter growth areas to 75% in denser patches (Figure 8.3).

In April, both cages' weed growth to around 8m depth was dominated by a type of rhodophyte (red algae), and the untreated net also had species of Chlorophyta (green algae), *Enteromorpha* present. Towards the centre of the base of the net the dominant type was brown algae; all of these algae were quite fluffy in form and readily swayed with the movement of water and the net (Plate 8.4). Around the lead line of the untreated net, deposition of particulate matter on the net meshes was apparent, this was probably a combination of fine silt, organic detritus and tuna faeces, as described by Svane et al (2006). Shell growth was not evident from the video footage on either net at this evaluation.

DEPTH	UNTREATED CONTROL	ANTIFOUL TREATED
Surface		
≈2m		
≈4m		

≈6m		
≈8m		
Lead Line		
Base		

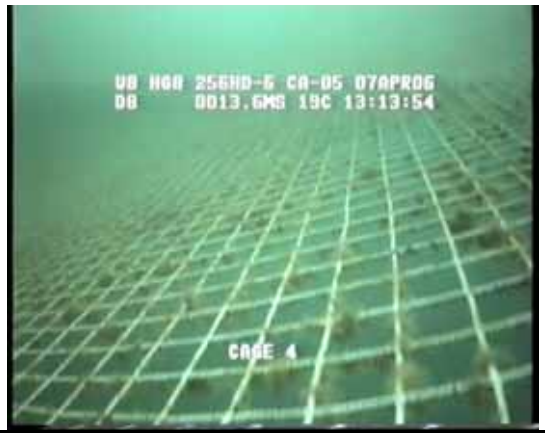
Base		

Plate 8.2: Photographs derived from the video transects of the untreated and antifoul treated nets undertaken in April, 51 days post net deployment

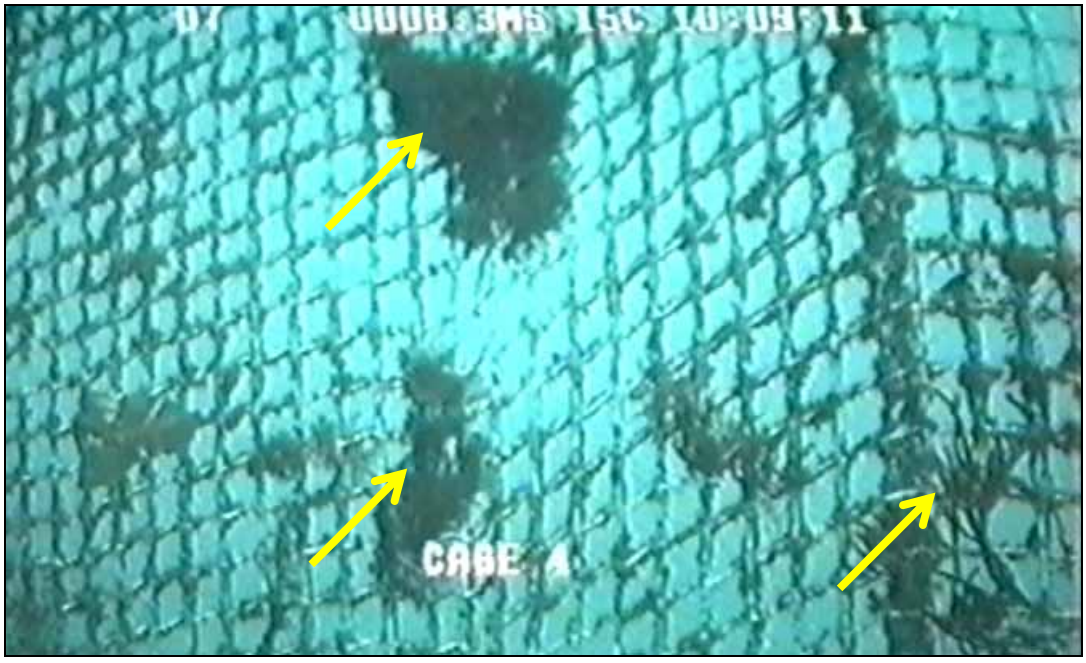


Plate 8.3: Drift macroalgae (*Scaberia, Cystophora and Sargassum spp.*) arrows, caught on the net treated with antifoulant

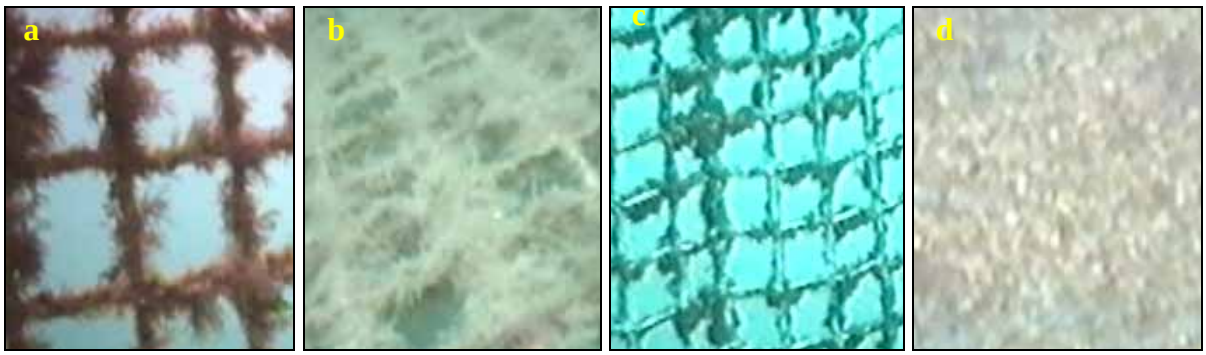


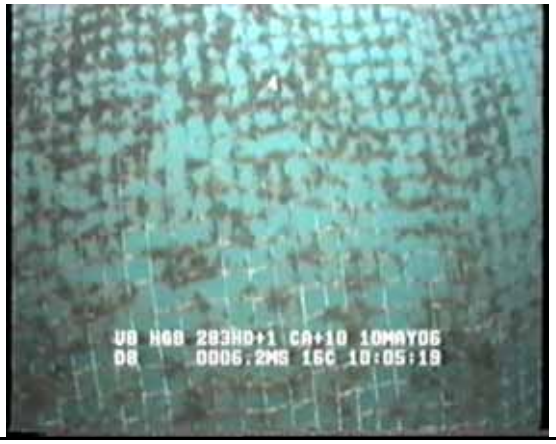
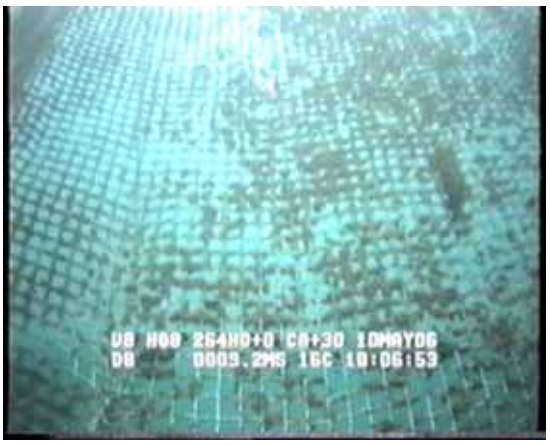
Plate 8.4: Fouling types; a - red algae; b – brown algae; c – sponge; d – shell, sponge, and weed combined

By the 10th of May, 84 days after net deployment and 54 days after the addition of SBT, sponge and shell growth (*Electroma georgiana* and *Hiatella australis*) were becoming apparent on the untreated net. Differences between treatments were most apparent at depths greater than 4m, and remarkably so on the bases of the nets (Plate 8.5, Figure 8.3 and Figure 8.4). At all depths (including the surface 2m) the cord of net meshes was still visible between the weed colonies on the net treated with antifoulant; net cord was not visible anywhere on the untreated net.

Combined, the net and biofouling growth on the sides of the untreated net equated to between 50 and 80% occlusion of meshes, with some smaller areas closer to 100% occluded. While the treated net treated with antifoulant had comparatively lighter and patchier weed growth from the surface to 5m depth, and biofouling growth was reduced at depths greater than 5m. The combined net and biofouling growth resulted in occlusion levels of up to 50% in areas of algal growth, with some isolated meshes being totally occluded by drift algae.

The biofouling growth in May, to a depth of around 8m, appeared to be predominately red algae on the treated net; but a combination of red and brown weed types, ascidians, sponges and molluscs were present on the untreated net. Towards the lead line and on the base of the treated net, visible biofouling (brown fluffy algae) was confined to isolated areas of individual meshes (most likely associated with cracks in the coating) and clumps of broken-off surface algae sitting on the net. By contrast the lead line and base of the untreated net had a uniform coverage of brown algae, sponge-like material trapping particulate matter and obvious shell growth of *Hiatella australis.*

DEPTH	UNTREATED CONTROL	ANTIFOUL TREATED
Surface		
≈2m		

≈4m		
≈6m		
≈8m		
Lead Line		

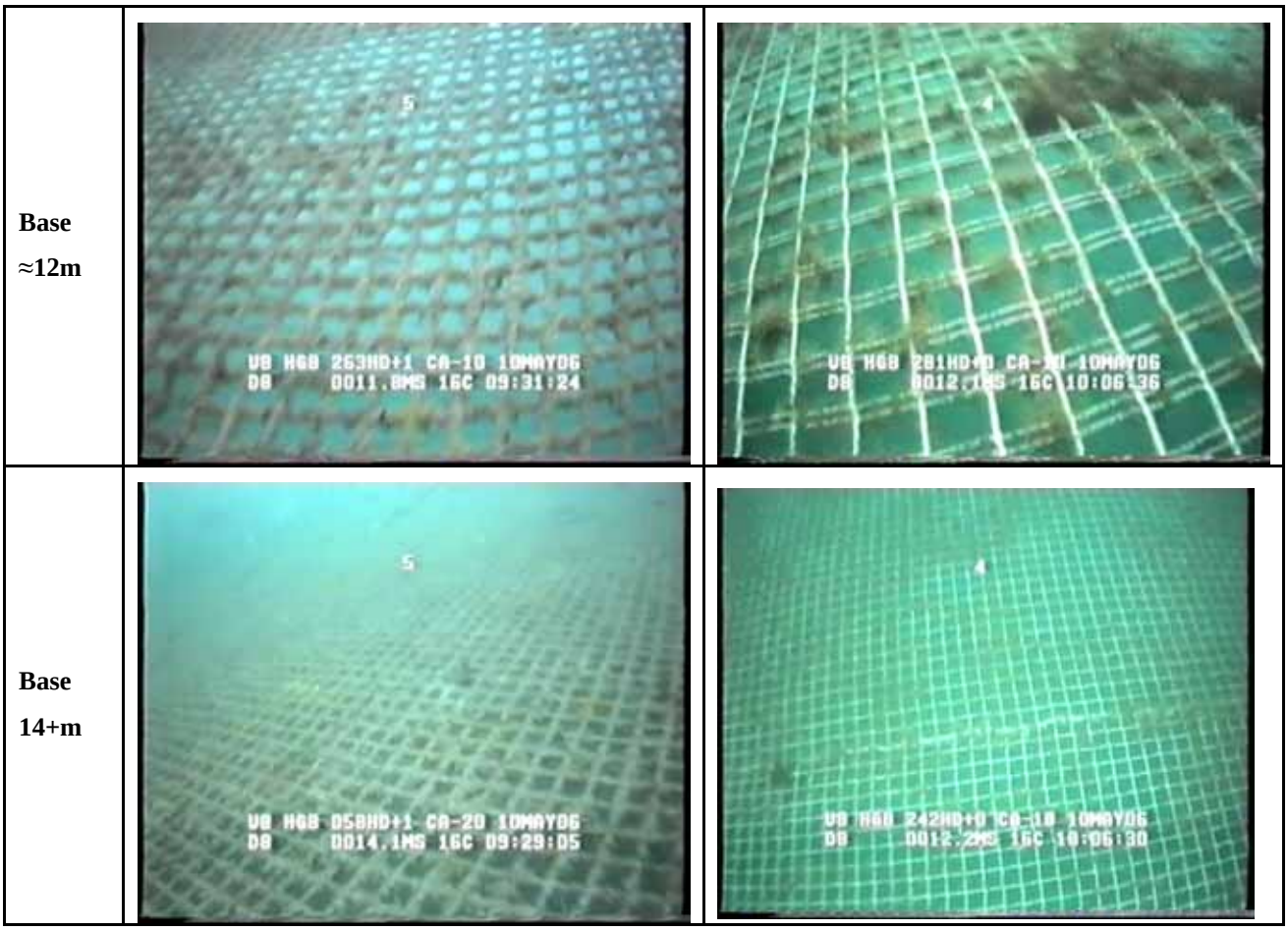


Plate 8.5: Photographs derived from the video transects of the untreated and antifoul treated nets undertaken in May, 84 days post net deployment

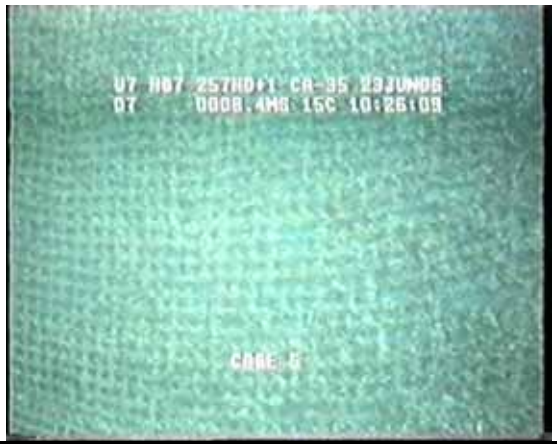
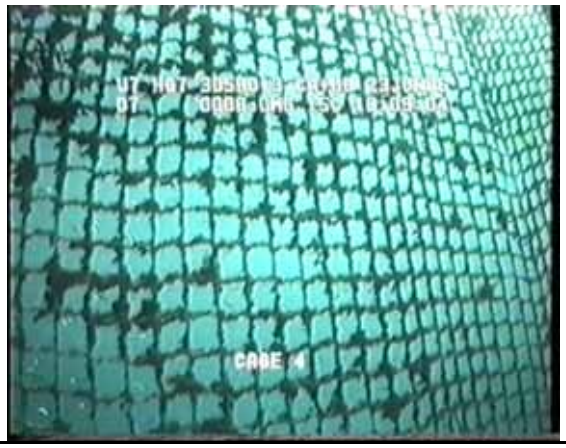
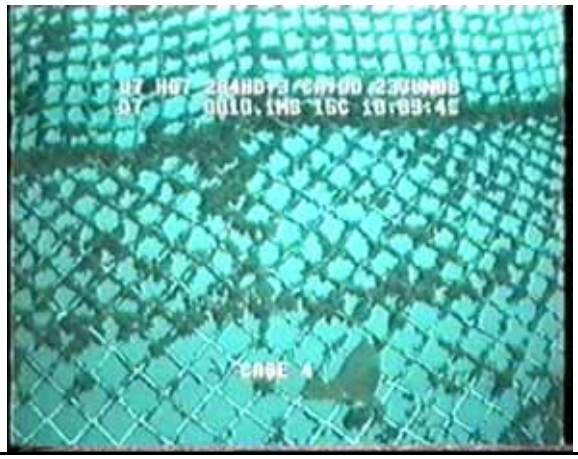
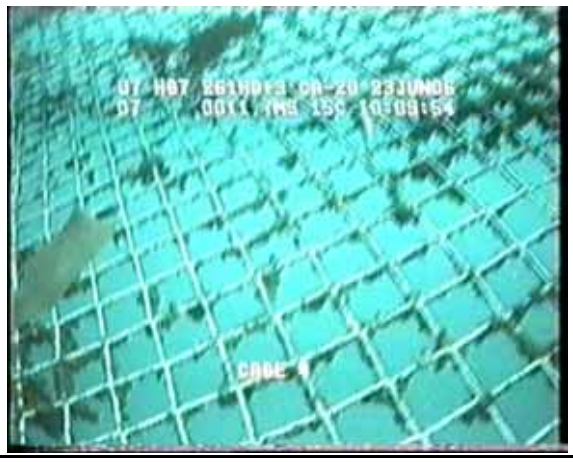
At the June 23rd video transects, 128 days post deployment, biofouling growth continued to increase on the untreated net so that the fluffy red algae were mainly present to 6m depth and heavier more solid growth types dominated at the lower depths. Shell growth was evident at depths greater than 4m, and particularly prevalent on the base of the net (Plate 8.6). From around 3m there were obvious areas where biofouling had been dislodged (rubbed or fallen off) from the net, the contrast in visibility through the net at these points highlights the density of growth generally on the net. It was no longer possible to discern the lead line on the untreated cage, so a photograph was extracted from the DVD at the approximate depth at which the lead line was apparent on the antifoul treated net (Plate 8.6). Occlusion values range from 35% in the areas where growth was removed, to 90% near the surface; and 65-80% generally at all depths below 5m, including on the base (Figure 8.3 and Figure 8.4).

The fluffy red algae continued to be the main type of fouling present on the net treated with antifoulant, and extended to the lead line, a depth of approximately 10m. In the area from 3m depth to the surface density ranged up to 70% occlusion, but throughout the remainder of the net wall and base occlusion ranged from less than 30 to 50%.

When considering occlusion values at this stage of the trial it is important to keep in mind that the growth occurring on the treated net is a soft type that 'floats and undulates' with the movement of water; and while it may temper the velocity of water flow through the net it is unlikely to impede it. The growth on the untreated net, particularly below 5m depth does not move with water flow and is therefore a physical obstruction to water flow and effectively

reduces the internal mesh size, as well as deflecting water away from the net (Rough et al 2008, Chapter 6).

DEPTH	UNTREATED CONTROL	ANTIFOUL TREATED
Surface		
≈2m		
≈4m		

≈6m		
≈8m		
Lead Line		
Base ≈12m		

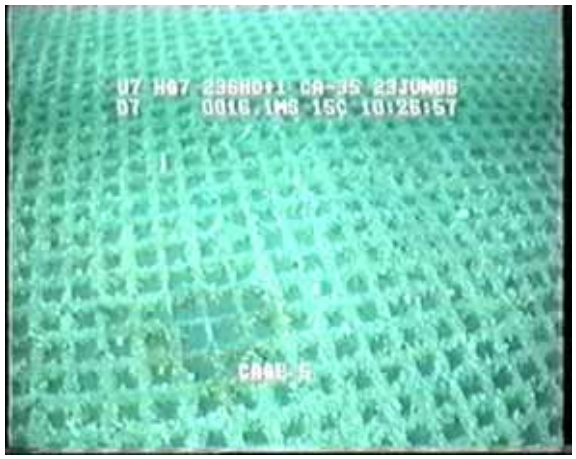
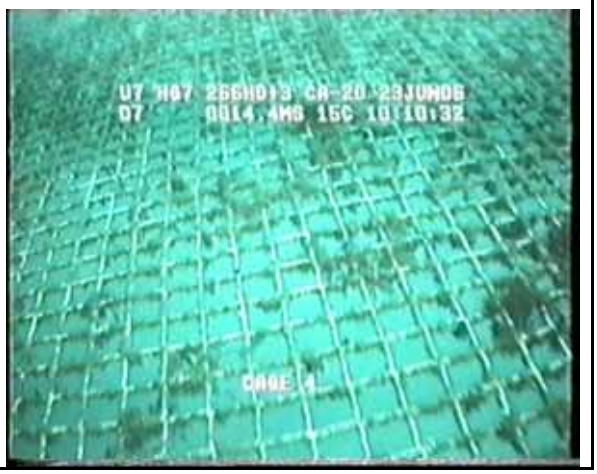
Base **14+m**		

Plate 8.6: Photographs derived from the video transects of the untreated and antifoul treated nets undertaken in June, 128 days post net deployment

By the 8th July, 143 days post net deployment, biofouling growth had continued to increase at all depths on the untreated net, and between the surface and 4m depth on the net treated with antifoulant (Plate 8.7). At depths less than 4m, the green algae, *Enteromorpha sp.*, had increased in prevalence on the untreated net; and the antifoul treated net started showing the long filamentous growth that characteristically appears in the first few weeks of an untreated net being deployed (Plate 8.8). When present, this alga extends up to 3m horizontally from the net meshes, depending on tidal conditions. It tends to be broken off by the movement of water that occurs when tuna feed vigorously at the start of the season, and does not reappear for the remainder of the farm cycle. Its presence in the top 3 metres of the antifoul treated net at this stage of the farm cycle is interesting and may indicate that the activity of the antifouling ingredients at these depths was depleted, or degraded by UV light.

In July, occlusion values in the top 5m of the untreated net ranged from 40% in the isolated areas where the growth was dislodged and removed, to 65-80% generally elsewhere, but with patches of 90-100% (Figure 8.3). Below this depth growth on the untreated net was a combination of soft and hard fouling with densities of 40% in isolated patches where fouling was dislodged and removed and up to 90% elsewhere. The base of the net had an abundance of weed, invertebrate, sponge and shell growth with occlusion values between 65-85% (Figure 8.4). As with the June evaluation, it was difficult to discern the lead line so a photograph was extracted from the DVD footage at a comparable depth to that at which the lead line was visible on the antifoul treated net. On the net treated with antifoulant, the cord of the net mesh was clearly visible at all depths below 4m and occlusion values were comparable to those of the previous survey.

DEPTH	UNTREATED CONTROL	ANTIFOUL TREATED
Surface		
≈2m		
≈4m		
≈6m		

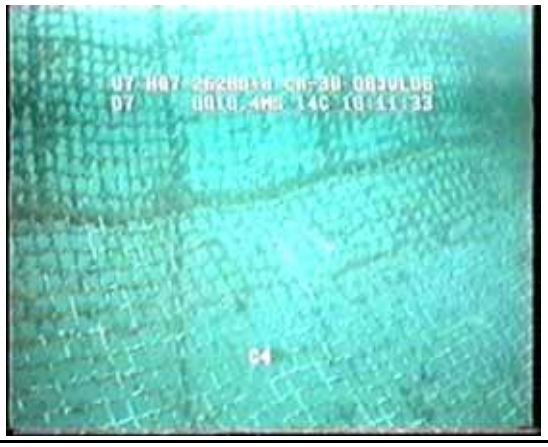
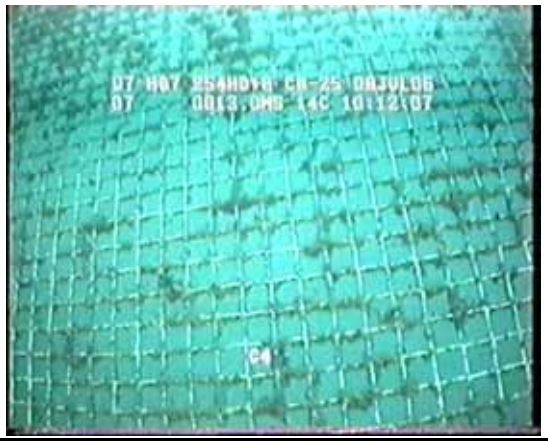
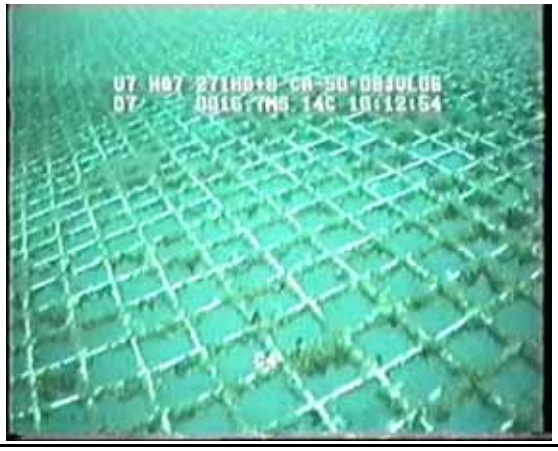
≈8m		
Lead Line		
Base ≈12m		
Base 14+m		

Plate 8.7: Photographs derived from the video transects of the untreated and antifoul treated nets undertaken in July, 143 days post net deployment

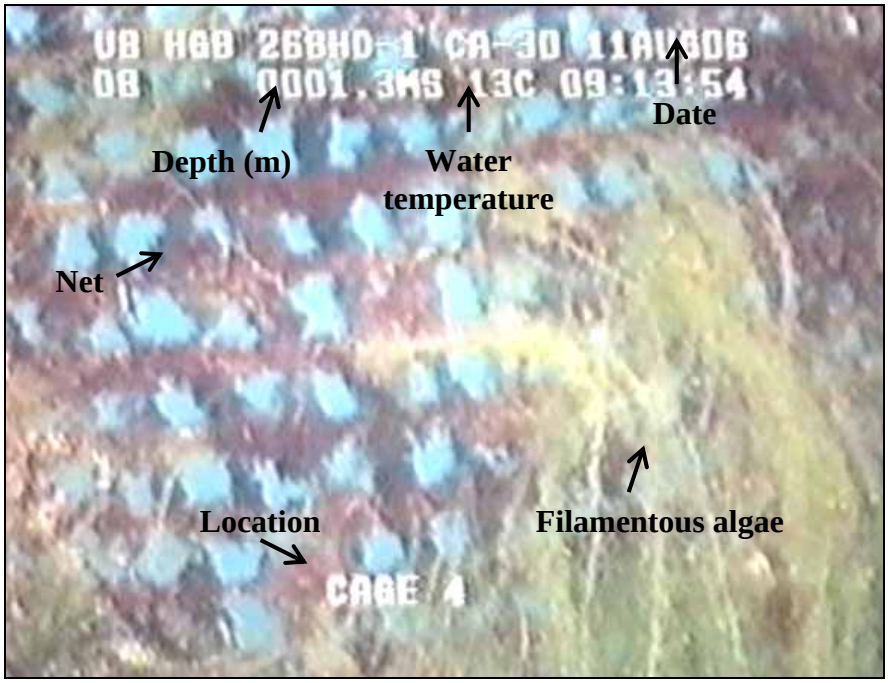


Plate 8.8: Demonstrates the filamentous algae present at 1.3m depth during August, on the net treated with antifoulant

In August, 177 days post deployment, *Enteromorpha* sp and the long filamentous growth remained prominent features in the top few metres of the untreated and antifoul coated nets respectively (Plate 8.9). Below 4m the untreated net had a dense cover of soft and hard fouling that extended throughout including the base; shell and sponge growth being dominant at depth. By comparison the cord of the meshes was still visible at all depths in the net treated with antifoulant. The dominant fouling types on the treated net were soft red algae growth on the sides and brown algae and sponges on the base.

DEPTH	UNTREATED CONTROL	ANTIFOUL TREATED
Surface		

≈2m	 CASE 5 VB H&B 260HD+B CA-10 11AV6DB DB 0002.2MS 13C 09:01:35	 VB H&B 266HD-1 CA-30 11AV6DB DB 0002.5MS 13C 09:14:17 CASE 4
≈4m	 CASE 5 VB H&B 264HD+B CA-10 11AV6DB DB 0004.2MS 13C 09:01:41	 VB H&B 281HD-1 CA-30 11AV6DB DB 0004.5MS 13C 09:14:27 CASE 4
≈6m	 CASE 5 VB H&B 254HD+B CA-10 11AV6DB DB 0006.2MS 13C 09:01:46	 VB H&B 270HD-1 CA-30 11AV6DB DB 0006.0MS 13C 09:14:33 CASE 4
≈8m	 CASE 5 VB H&B 240HD+B CA-10 11AV6DB DB 0008.0MS 13C 09:01:52	 VB H&B 271HD-1 CA-30 11AV6DB DB 0007.2MS 13C 09:14:38 CASE 4

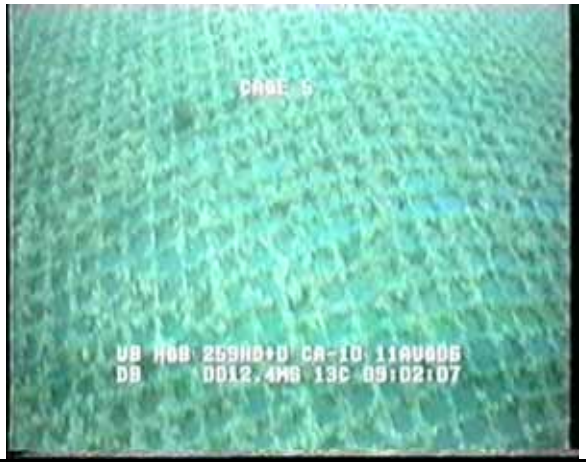
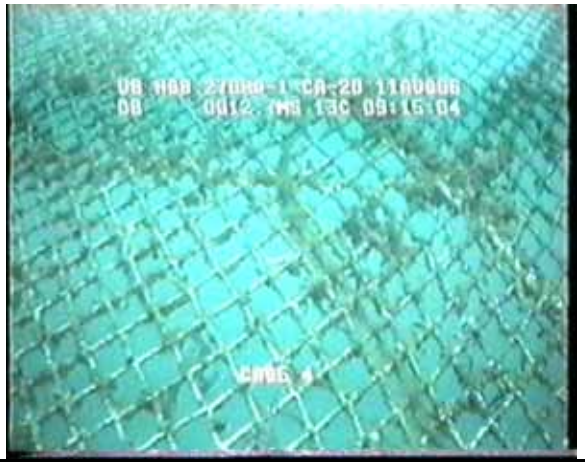
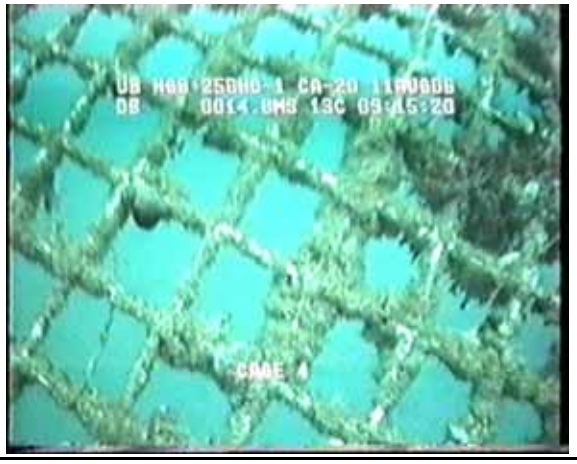
Lead Line		
Base ≈12m		
Base 14+m		

Plate 8.9: Photographs derived from the video transects of the untreated and antifoul coated nets in August, 177 days post deployment

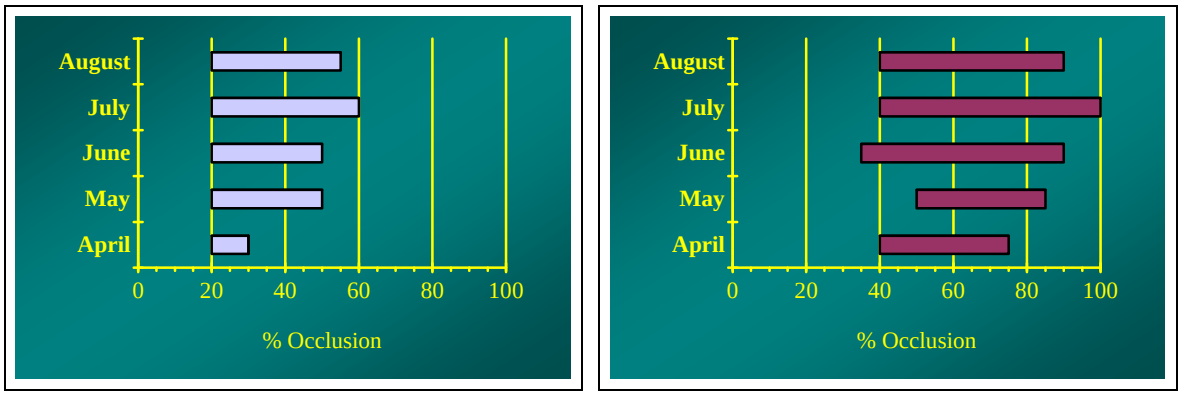


Figure 8.3: Range of occlusion values on the sides (4-9m depth) of the antifoul treated (left) and untreated (right) nets. These plots include areas where fouling was removed but do not include the isolated meshes totally occluded by drift algae.

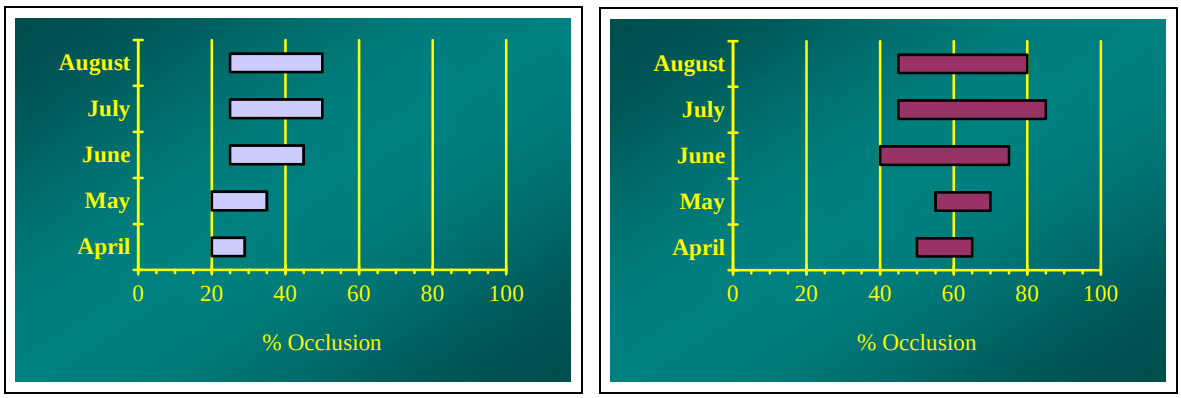


Figure 8.4: Range of occlusion values on the bases of the antifoul treated (left) and untreated (right) nets. These plots include areas where fouling was removed but do not include the isolated meshes totally occluded by drift algae.

Net management

Management issues relevant to this project included net pre-treatment, net integrity and maintenance requirements through and after the season. A problem encountered with the treatment of the net for this season was a prolonged period of rain and high humidity in Tasmania through the summer of 2005 / 2006. This delayed the dipping for a period of 6 weeks, until mid January 2006. This would be less of an issue if large fans were set up within the sheds in Tasmania or if dipping could occur at Port Lincoln or Eyre Peninsula in South Australia, where only 7 significant rainfall events were recorded for that entire summer (Kingston experienced rain every 3 days). A net washing and dipping facility at Port Lincoln would also reduce the substantial time delays and costs associated with transporting nets to and from Tasmania. A further consideration for transport is that once this antifoulant is applied, the net physically occupied nearly twice the freight space as an untreated net (note that freight is charged on both weight and space).

The treated net, which was weighed before and after dipping, was calculated to have 900L of the paint adhere to the net surface. The treated net was much less flexible after treatment, but could be rolled appropriately for transport back to SA and immediate loading onto the vessel for deployment at sea (i.e. no double handling).

At deployment, the treated net did not require any additional or special handling to set up in the water (Plate 8.10). However, it must be noted that it is absolutely essential that a

treated net be deployed at sea for a minimum of 72 hours prior to transferring fish into it (Wattyl product specification, appendix 8.3). The reason for this precaution is that the ingredients within the product are activated by water and will initially release in a large pulse, which settles to the constant low rate of leaching within 24 hours (Wattyl pers comm.). In addition to this, there may be semi-dried product or liquid still present on the internal surfaces of canvas or plastic tubing if these are used to protect down-ropes, as was the case with this net (Plate 8.10b and e).

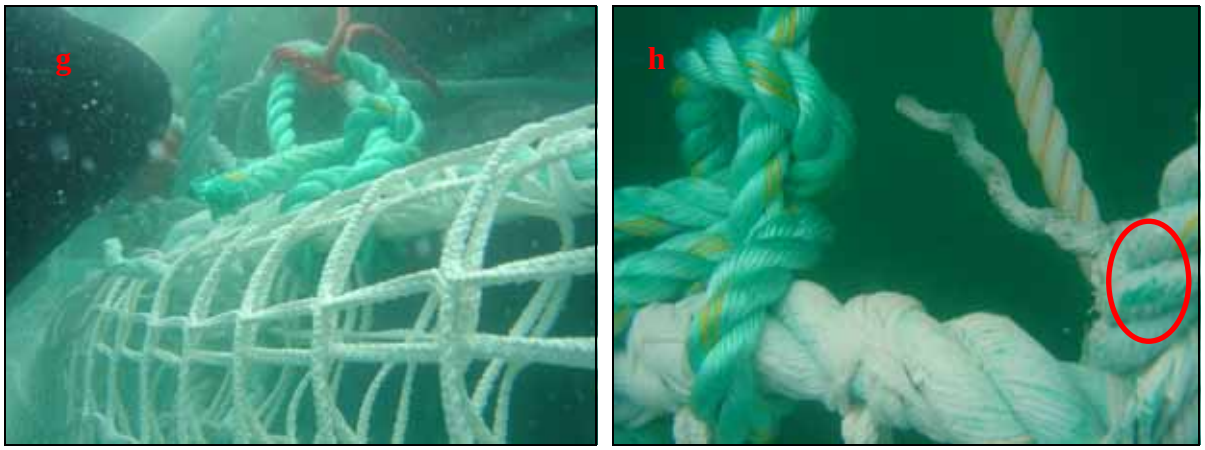


Plate 8.10: Loading and deployment of the antifoul treated net; a) the rolled net on the truck, b) plastic sleeve on down-rope (arrowed), c) net on the vessel, d) close-up of an area where the antifoul paint has cracked, e) deployment of net showing some leakage of liquid from plastic sleeves, f) pulling net across pontoon, g) treated net under water, h) an area of abrasion with no paint left.

Throughout the season the only additional maintenance that was required for the treated net was that an extra 100kg of weight needed to be attached to the net on the incoming tide side after the first large tide of May. During this time the south-eastern section of net was observed billowing inward, but this was not sufficient to mesh fish.

After the final harvest the control net required in-situ cleaning to reduce its weight to enable it to be lifted from the water and onto the vessel. The treated net did not require any pre-cleaning prior to its removal from the water. Once removed from the water and spread on land for cleaning and mending, the control net required four weeks work and the treated net only one week. The main reasons for not requiring in-situ cleaning and for the reduced on-land cleaning and maintenance are the lack of hard shell growth and the reduced soft growth on the nets.

Despite reductions in maintenance requirements throughout the season, additional planning and management may be needed to determine how the treated net is used in the subsequent season(s). As was found with the panel trial (subproject 2, Chapter 4), the ingredients of the Net Clear ZPT were only effective for one net deployment, for a maximum time of 10months (Wattyl pers.comm.). This period is dependent on water temperatures and fouling communities. It was further noted from the treated panels that after the product was dried in the sun, it tended to crack and would readily flake from the net surface when rubbed or when the net was folded or manipulated. Panels from subproject 2 that had been stored in the sun were suspended in a water bath to observe the fate of the cracked paint. When the water was slightly agitated (as with wave action), some of the paint detached from the net surface, with the majority sinking within minutes to the bottom of the container. Paint that was crumbled from the net into your hands and then placed in the water mostly tended to either sink to the bottom or float on the surface, but up to 20% initially remained in suspension through the water column. By one hour after paint flakes were added to the water 100% of the visible particles were settled on the bottom of the water bath.

The implications of the cracking coating are that nets would either need to be thoroughly stripped of the now ineffective (as antifoulant) paint; or recoated every season, even though the application of the antifoulant improved net strength for at least 2 seasons. Options for the ongoing use of a coated net include

-Leaving it in the water after season 1 even though the active ingredients of the antifoulant would be used up. This would negate the paint cracking and flaking, but

would necessitate extensive net cleaning prior to the addition of fish in the following season.

-Removing it from the water, drying and manipulating net as much as possible to try and remove all cracked paint and redeploy early to ensure that all particulates are off the net and away from the vicinity of where the new seasons' tuna will be held.

-Removing from water, drying and mending net as usual, then run the net through a net washer to remove loose cracked paint prior to re-dipping, in the hope that a reapplication of the paint would stick all of the previous season's paint to the net whilst it is in the water for the second season.

At the completion of the trial, the treated net and 3 others were sent to Tasmania for washing and treatment with the Net Clear ZPT antifoulant. However, due to the product being withdrawn from the market, these nets were returned to Port Lincoln without any cleaning or coating. The company's choices were either to dump the net or deploy it and be ready to transfer tuna out immediately if required. The latter option was chosen, and the net treated in the previous season was deployed two weeks prior to the tuna's arrival. Once the new tuna were transferred into the net there were no indications of gill irritation, as evidenced by tuna behaviour or mortality.

Chemical residues

Tuna

Zinc was detected in the skin and flesh of all individuals tested. Zinc levels were much higher in the skin of SBT compared with the levels in the flesh, however there was no relationship between the skin and flesh levels of individuals in either treatment group (treated net $r^2 = 0.0509$; control net $r^2 = 0.0191$).

There was no significant difference in zinc levels in the skin for tuna between the treated or control groups at the first sample interval in June/July; or between the groups tested in August (Table 8.1). However there appeared to be an increase in levels with time for the control group, where results from the second sample interval were significantly higher compared to the first, (*P=0.001*). There was no statistical difference between sample intervals for the tuna in the treated net; however more variation between tuna was evident with this group, especially at the first sample interval (Figure 8.5).

Table 8.1: Zinc residue results for tuna at each sample interval, expressed as mean ± standard deviation witb the number of samples tested shown in brackets (within rows, nsd denotes no significant difference P>0.05; * P<0.05; **P<0.01; *** P<0.001)

SAMPLE TYPE	**TREATED NET** Total Zinc (mg/kg)	**CONTROL NET** Total Zinc (mg/kg)	Statistics
All Tuna Flesh Samples	**4.933 ± 0.412 (n = 60)**	**5.344 ± 0.565 (n = 60)**	*******
Tuna Flesh 1st sampling	4.948 ± 0.309 (n = 30)	5.154 ± 0.445 (n = 30)	*
Tuna Flesh 2nd sampling	4.918 ± 0.499 (n = 30)	5.535 ± 0.613 (n = 30)	***
All Tuna Skin Samples	**60.616 ± 23.605 (n = 60)**	**54.984 ± 14.552 (n = 60)**	**nsd**
Tuna Skin 1st sampling	59.143 ± 26.930 (n = 30)	49.069 ± 13.094 (n = 30)	nsd
Tuna Skin 2nd sampling	62.090 ± 20.096 (n = 30)	60.899 ± 13.673 (n = 30)	nsd

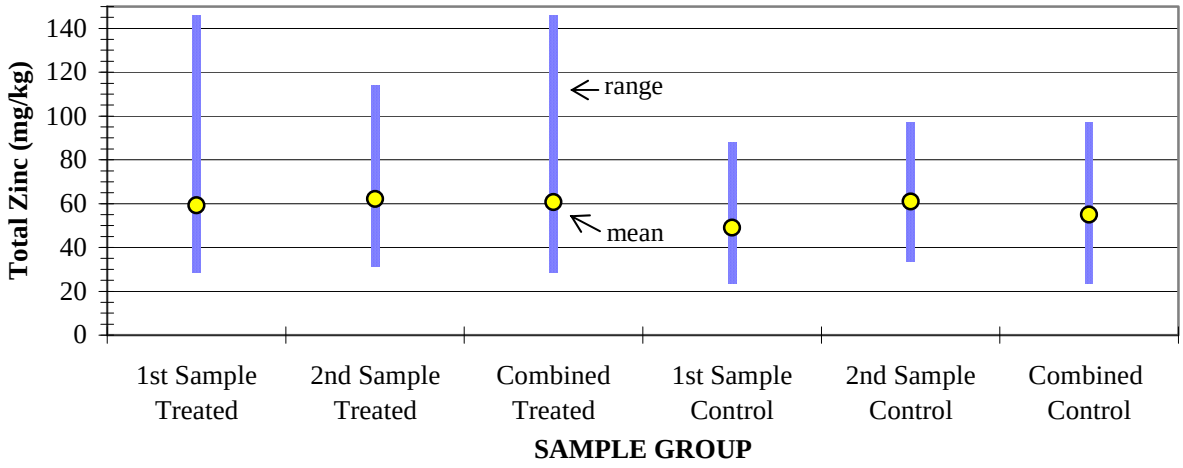


Figure 8.5: Zinc levels in tuna skin sampled from the treated and control nets through 2006 (mean and range of values); 1st sample in June/July n = 30 per group; second sample in August n = 30 per group.

There was a significant difference in the total amount of zinc in the SBT muscle tissue between the treated and control groups at each sample interval, both June/July and August (Table 8.1). The results were contrary to what was expected if the treated net was the source of zinc; with the levels being higher for SBT kept in the untreated control net. As with skin, there appeared to be an increase in flesh zinc levels with time for the control group, where results from the second sample interval were significantly higher compared to the first, (*P=0.008*). The control group appeared to have more variation between individuals, especially at the second sample interval (Figure 8.6). There was no statistical difference between sample intervals for the SBT in the treated net.

However the results in flesh samples of all individuals within each treatment and sample group were comparable to levels of zinc detected in SBT flesh from previous surveys undertaken in South Australia (Table 8.2); and within the range of values found naturally in SBT of this age in the wild (Figure 8.7). Results were also comparable to those of wild yellowfin tuna reported by Food Standards Australia and New Zealand, 0.5mg/100g (i.e. 5mg/kg) (www.foodstandards.gov.au/).

The apparent differences, particularly with the fish in the untreated control cage, suggest that zinc levels in farm SBT are probably more a reflection of physiological processes within the tuna rather than environmental exposure.

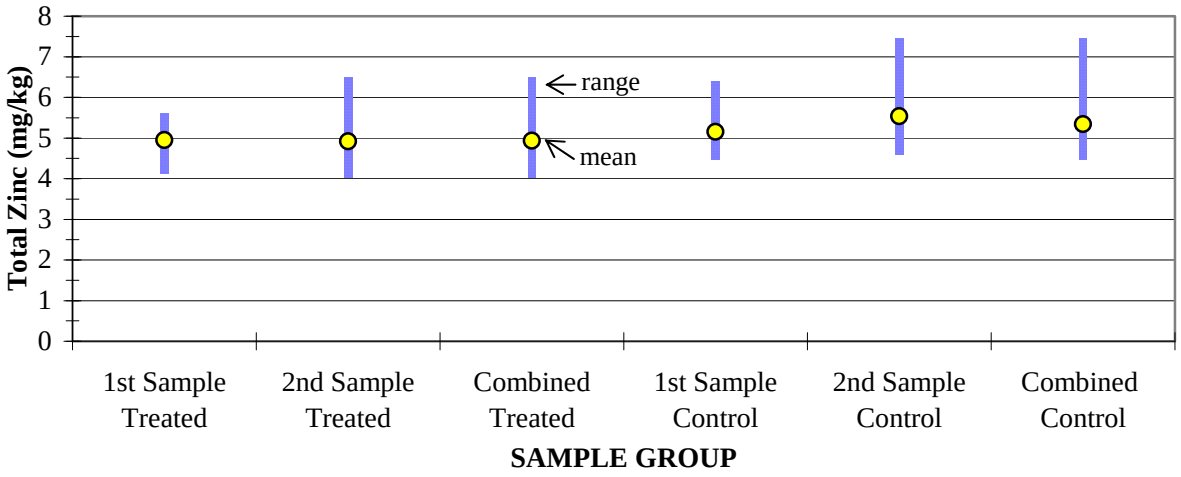


Figure 8.6: Zinc levels in tuna muscle flesh sampled from the treated and control nets through 2006 (mean and range of values); 1st sample in June/July n = 30 per group; second sample in August n = 30 per group.

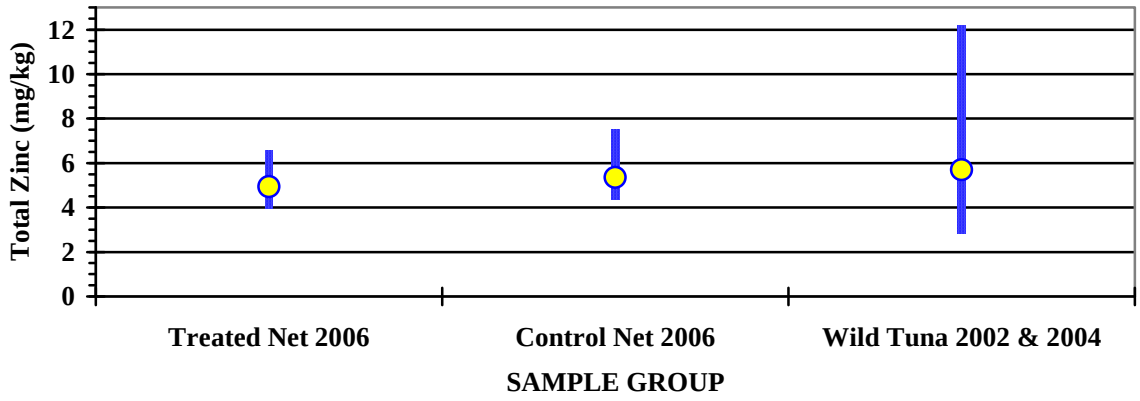


Figure 8.7: Zinc residues in tuna flesh sampled in this project compared with those previously sampled from the fishing grounds. Mean and range of values. Wild tuna data plotted with permission Padula et al 2003, 2005

Table 8.2: Flesh zinc residues in wild tuna sampled at the fishing grounds in the Great Australian Bight and tuna from farms in previous years (ie prior to the use of any antifouling treatments); mean ± standard deviation, sample numbers in brackets. Data reproduced with permission Padula et al 2003, 2005.

YEAR	WILD TUNA Total Zinc (mg/kg)	FARM TUNA Total Zinc (mg/kg)
2002	5.810 ± 1.941 (n = 30)	5.735 ± 4.968 (n = 52)
2003	Not tested	4.270 ± 0.974 (n = 10)
2004	5.040 ± 0.152 (n = 5)	5.000 ± 0.954 (n = 20)

Environment

Bivalve shellfish, the blue mussel *Mytilus edulis* suspended directly in contact with the net treated with antifoulant or with the untreated control net for 123 days, showed no significant difference in total zinc content (Table 8.3). The mean value of those in the treated net was very slightly higher (0.12mg/kg) compared to that of the untreated control net, however given the

range of values obtained from both groups this is probably just a reflection of natural individual variability rather than due to the net treatments (Figure 8.8).

The results obtained from this study are consistent with studies investigating seafood as a beneficial nutritional supplement for human health and well-being; and also where shellfish are used as bio-monitoring agents in environmental studies (Goldberg et al 1978). The Food Standards Australia and New Zealand web page lists raw oysters *Crassostrea gigas* as containing 47.9mg zinc /100g (ie. 479mg/kg); and steamed green mussels *Perna canaliculus* as containing 3.1mg/100g (www.foodstandards.gov.au/). A study of green lip mussels *Perna viridis* off the Malayan Peninsula found zinc levels ranged from 10.5 – 29.4 mg/kg (wet weight) in wild samples and 10.7 – 24.7 mg/kg (wet weight) in cultured mussels (Yap et al 2004).

If antifouling treatments with a heavy metal base are to be used on an ongoing basis within the industry, it may be worthwhile to deploy several samples and monitor sub-samples over multiple farming seasons rather than within a single season, as the tuna farm cycle is so short.

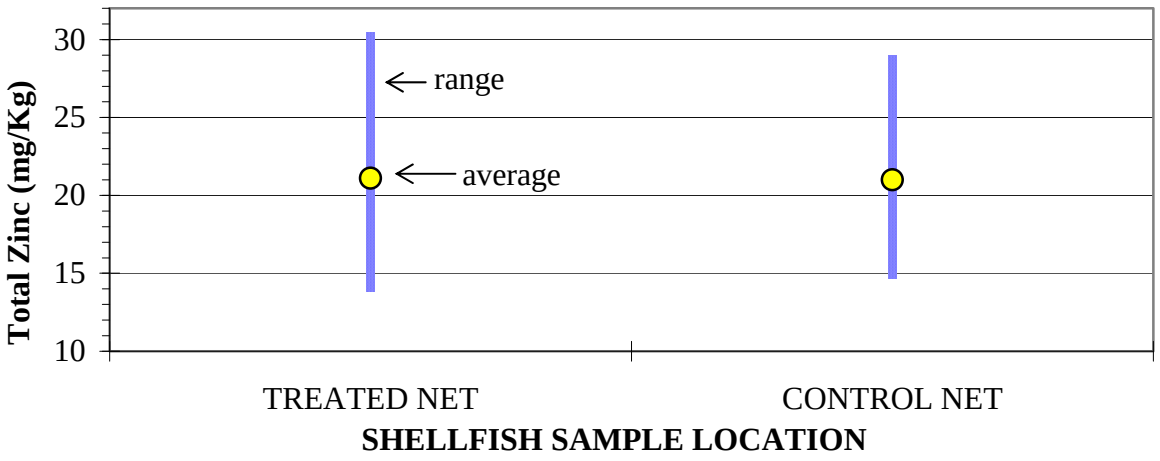


Figure 8.8: Zinc levels in bivalve shellfish attached to the treated and control tuna nets for 123 days (mean and range of values).

Analysis of the sediment sampled up and down current from the experimental cages showed no significant differences in zinc content between treatments (Table 8.3). Nor were there any statistical differences within treatments, i.e. between the northwest and southeast samples of the treated cage etc. There was no significant difference between results for samples taken directly beneath the net of the treated and the control cages, although this may be due to the low number of samples and high variability of results particularly under the treated net (Figure 8.9). One of the samples collected under the treated net contained mussel shell, this sample had a higher reading than all other samples; and it may be that the decomposition of mussels (known to have higher zinc levels naturally) had influenced the result.

Table 8.3: Environmental zinc residue results for the tuna farm trial 2006, expressed as the mean ± standard deviation, with the number of samples tested in brackets (nsd denotes no significant difference *P>0.05*; * *P<0.05*; ** *P<0.01*; * *P<0.001*)**

SAMPLE TYPE	**TREATED NET** Total Zinc (mg/kg)	**CONTROL NET** Total Zinc (mg/kg)	Statistics
Mussels hung on net	21.089 ± 4.891 (n = 9)	20.967 ± 4.998 (n = 9)	nsd
Sediment directly under cage	5.227 ± 4.047 (n = 3)	3.933 ± 1.521 (n = 3)	nsd
Sediment 100m SE of cage	2.364 ± 0.449 (n = 5)	1.816 ± 0.301 (n = 5)	nsd
Sediment 100m NW of cage	1.858 ± 0.348 (n = 5)	2.232 ± 0.792 (n = 5)	nsd
Sediment values from a survey of Boston Bay, Thorney Passage and the tuna farming zone undertaken in 2002 ranged from 1.1 to 34.0 mg/kg, mean 6.0 mg/kg, n = 15 (Padula et al 2003)			

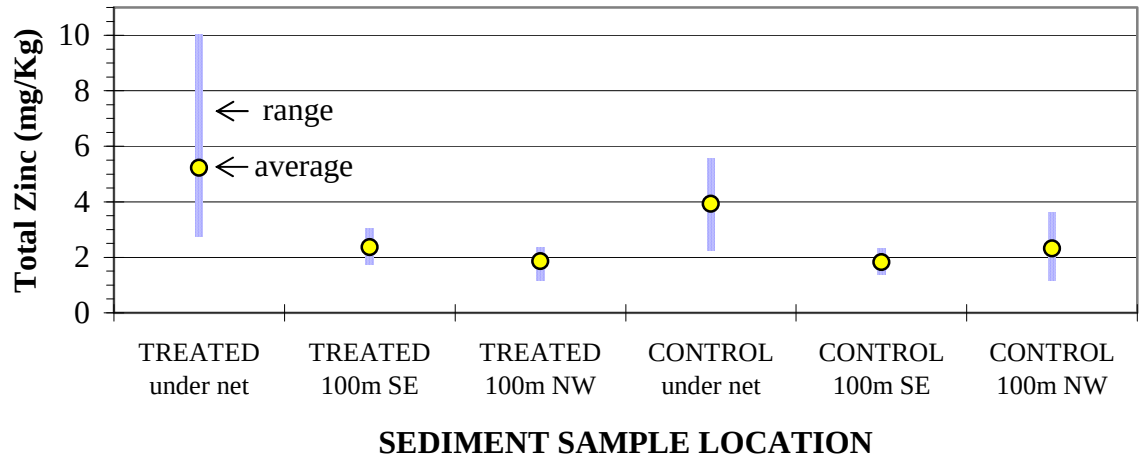


Figure 8.9: Zinc levels in sediment samples collected around and beneath the treated and control tuna nets (mean and range of values).

Results obtained from this trial were comparable to those found previously from a survey of sediment undertaken in 2002 (Figure 8.10). All sites with the exception of the main grain loading terminal and the Porter Bay Marina entrance had values below 6.4mg/kg (dry weight) (Padula et al 2003).

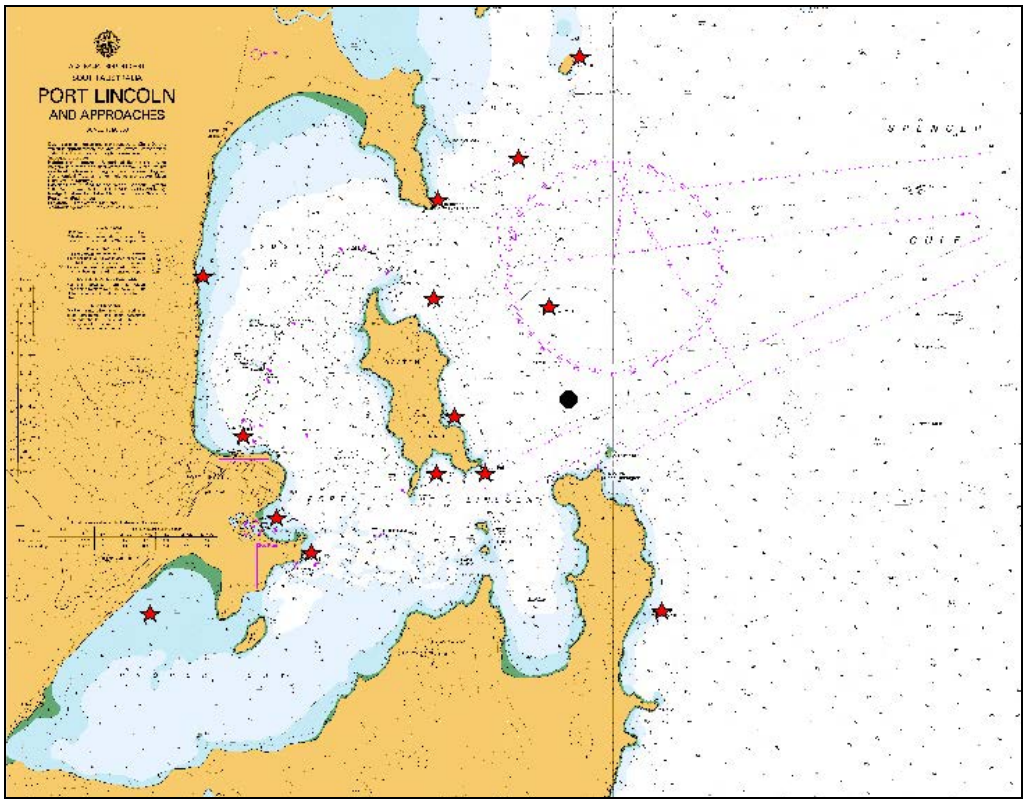


Figure 8.10: Approximate locations for sediment sample collection for residue testing in 2002 (★) and for this project in 2006 (●).

It was not surprising that zinc was detected in all samples tested as part of this project. Zinc is ubiquitous in the environment and is present in most foodstuffs, water and air. It is an essential element and a constituent of more than 200 enzymes; and plays an important role in nucleic acid synthesis and metabolism, cell replication and tissue repair and growth through its function in nucleic acid polymerases (Moffat and Whittle 1999).

Historically within the industry, SBT are exposed to additional sources of zinc (i.e. above what is naturally present in the local water and environment), through the use of bait fish as tuna feed (levels range from 1.0 - 4.0 mg/kg (wet weight) depended on type and species (pers. obs.)) and by the use of sacrificial anodes on vessels and farm equipment (such as feeder cages). Previous analyses undertaken by Padula et al (2003; 2005) showed that these sources had not resulted in elevated zinc levels in tuna or the sediment. Testing undertaken in this project show that the use of a zinc based antifouling paint on a tuna net does not lead to elevated levels in tuna or the environment.

Fish health

Behaviour:

There were no adverse behavioural differences observed by feeding or dive staff throughout the season. Feeding staff did note that during the second and third dodge/neap tides after transfer, SBT in cage 4 maintained their appetite where other cages on the lease site were very slow or went off their food. However, as fish were not fed to satiation throughout this trial, the differences were not evident from the records of daily feed amounts.

Mortality:

Results for the entire farming season show that of the combined total number of dead tuna (192) from these two cages, 38.02% occurred in the cage treated with antifoulant, and 61.98% in the control cage. This is despite the treated cage being stocked with 53.64% of the total tuna held in these two cages (Figure 8.11). A 23.96% reduction in mortality would be a positive result as higher stocking rates often increase the number of mortalities and/or depress the growth rates of SBT under adverse environmental and/or management conditions (pers. obs. 1994-2004).

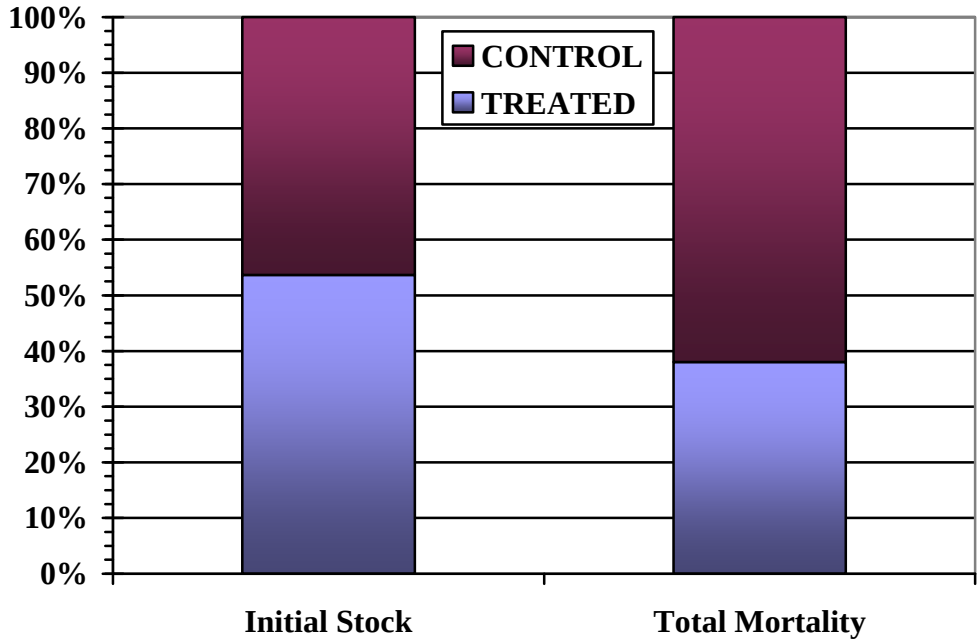


Figure 8.11: Relative proportions of tuna held and mortality received for the two cages in this trial.

Fish deaths within the tuna industry occur for a variety of reasons including 'post capture/tow stress and damage' (which includes both the immediate and the delayed mortality), the 'swimmer syndrome', 'harvesting practices', 'net cleaning procedures and the presence of poachers and predators (grouped as unknown in this report). The relative significance of each of these mortality types for the cages in this trial can be seen in Figure 8.12. This pattern of mortality would be considered typical for the farm location and management strategies of the collaborating company. Generally within the industry, fish deaths as a result of stress incurred through the capture and tow processes account for up to 95% of the total mortalities experienced (industry and personal observation 1994-2004).

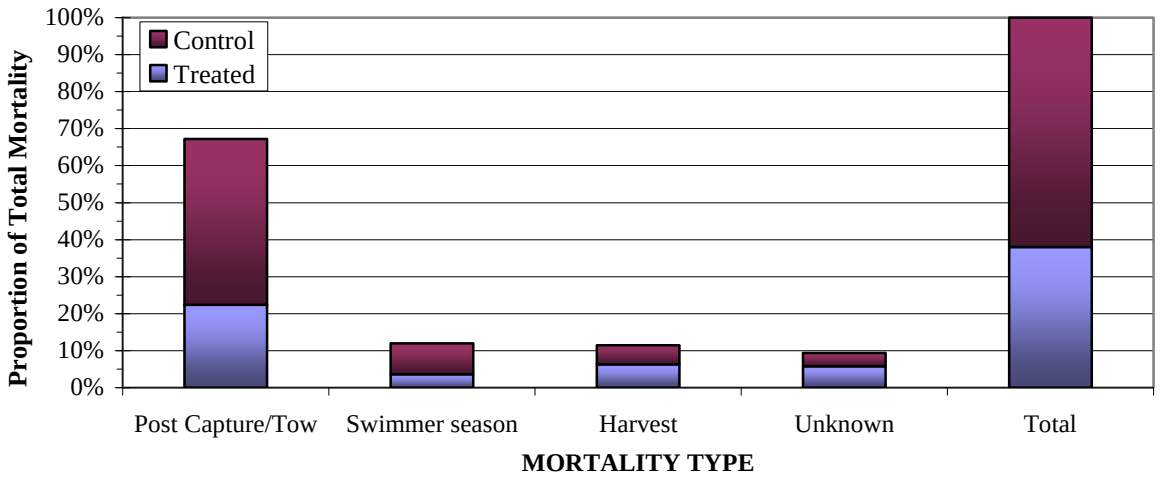


Figure 8.12: Relative proportion that each mortality type contributed to the total number of mortalities that occurred for the entire farming season

The results from the two cages in this trial suggest that the use of an antifouling treatment may have been beneficial in reducing mortality through the post capture tow period and through the swimmer season (Figure 8.13).

Through the post capture / tow period 66.67% of the total number of fish deaths occurred in the untreated control cage. The immediate and delayed tuna deaths as a result of capture and towing conditions and procedures mainly occur during the summer and autumn period. For the majority of tuna farmed in South Australia, the post capture and tow period is characterised by higher water temperatures, and less tidal exchange than during winter and spring. It can be subject to wide variations in levels of phytoplankton, suspended sediment and suspended organic material. These prevailing environmental conditions, in addition to the tuna's voracious eating for this part of the farming cycle are thought to lead to fluctuations in both oxygen availability and tuna's demand for oxygen. Tuna deaths are thought to involve individuals that are sub-lethally impacted by capture/tow conditions and in this compromised condition have trouble adjusting to the prevailing captive environment. The delayed phase (second peak) of these mortalities can occur into the winter months if the tuna are caught late in the catching season and arrive at the grow-out site in late March or April; and in this situation the total number of mortalities tends to be lower (pers. obs. 1994 to 2004). Tuna in this trial arrived on site reasonably late in the season; therefore these results need to be verified with further trials; as trials earlier in the season may result in a greater magnitude of mortality reduction. Less biofouling growth on the net has probably improved water exchange (hence oxygen availability) and the resultant mortality from this cause was decreased.

Through the swimmer season 69.57% of the total number of fish deaths occurred in the untreated control cage. Deaths during the swimmer season occur primarily in winter and are due to a microscopic environmental organism (the ciliate, *Uronema nigricans*) that opportunistically and fatally parasitises the tuna (Munday et al 1997; Rough 2000). Historically, farms with healthy tuna in a clean environment have very few deaths due to this condition (pers. obs. 1994-2004). Less biofouling growth on the net probably reduced both the habitat and food available for the *Uronema* (thereby not promoting their proliferation) and enabled the tuna to be in a healthier condition to fight infection in the early stages, and therefore the resultant mortality was decreased.

Obviously, fish deaths as a result of net cleaning activities should also be reduced if an antifouling agent is used, but net cleaning was not undertaken in cages 4 and 5 whilst fish were

held so these types of mortalities were not monitored. Net cleaning however was performed in other cages on the lease site, and other companies nets were towed past this lease site whilst the trial was in progress. An uncharacteristic spike of mortalities that occurred between the 4th and 14th of September in the treated cage may be a result of these events. There are no clinical signs that are characteristic to this type of mortality, so for this report that spike in mortalities has been included as 'unknown' in Figure 8.13.

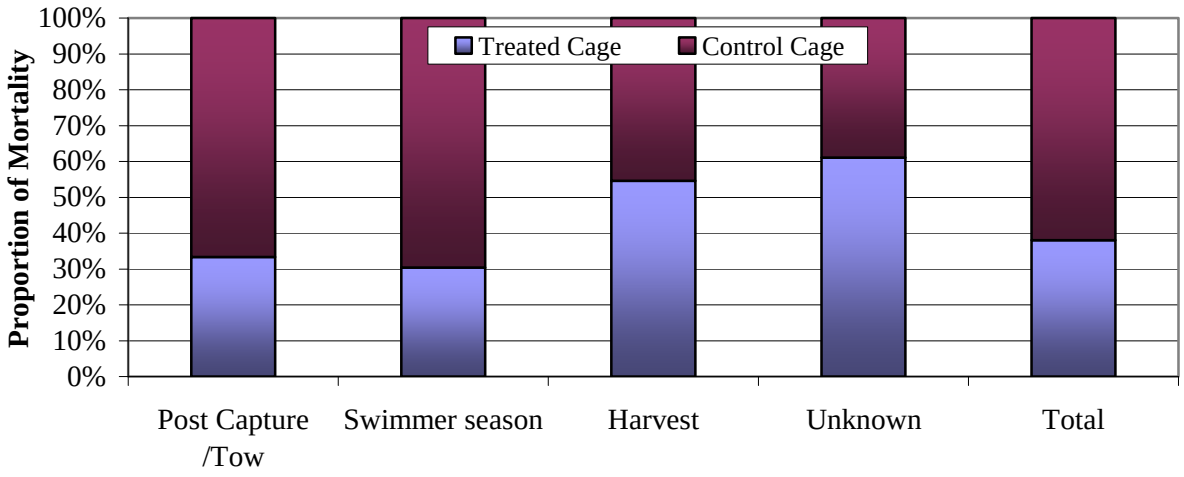


Figure 8.13: Relative proportion that each cage contributed to the various mortality types that occurred through this trial.

Parasite loads

Tuna were screened specifically for parasites that indicate stress, are potentially lethal, or compromise tuna in situations of sub-optimal environmental conditions and/or husbandry practices. Although *Uronema* is known to be a lethal parasite to tuna, it was not included in the sample screen as it has never been found in harvested tuna except where clinical signs are exhibited at the time of harvest (pers. obs. 1994 to 2004; Munday et al 1997; Rough 2000; Nowak et al 2007).

Of the types of parasites screened for, the following were found on the tuna in all cages: gill fluke *Hexostoma thynni*, gill copepods *Pseudocycnus appendiculatus* and *Euryphorus brachypterus*, and blood fluke *Cardicola forsteri.* Skin copepods *Caligus* sp. were not found on any of the fish examined. Parasite loads were highly variable and mostly of low intensity (Table 8.4). There appeared to be no effect of antifouling paint treatment on parasite loads at harvest in August; however it is interesting to note that the treated cage had a higher prevalence of blood fluke. As blood flukes tend to peak then decline by this stage of the farm cycle (Nowak et al 2007), it may be that by delaying fouling growth this peak was deferred until later in the season. Sequential sampling through the season is needed to clarify this.

Table 8.4: Prevalence (% of fish infected) and intensity of infection (average number of parasites per infected fish) for Southern Bluefin Tuna parasites. Each column represents information for one pontoon.

Pontoon	**Cage 4:** Treated (n = 10)	**Cage 5:** Control (n = 10)	**Cage 6:** Control (n = 10)	**Cage 1:** Control (n = 20)
Hexostoma thynni				
PREVALENCE	10	30	0	25
INTENSITY	1	2	0	4.4
Pseudocycnus appendiculatus				
PREVALENCE	20	10	40	80
INTENSITY	2	11	9.75	4.1

Euryphorus brachypterus				
PREVALENCE	0	0	10	5
INTENSITY	0	0	1.7	2
Cardicola forsteri				
PREVALENCE	70	40	30	55
INTENSITY	3.4	4.25	3.7	2.8

Histology

Histology of gills, liver and spleen indicated that the antifouling treatment had no adverse effect on tuna health. There were no significant differences in the structure of gills, liver or spleen between fish from different pontoons. The main gill pathology was due to the presence of blood flukes. Granulomas, inflammation and hyperplastic changes were present in 33% of the fish in each cage. These changes appeared to be most extensive in the gills of fish from the treated pontoon. Inflammatory changes were present in most livers. A few individuals appeared to have bile duct fibrosis, but these were isolated cases present in all pontoons. Both spleen and liver contained melanomacrophage centres, they were very prominent in the spleen. Melanomacrophage centres contained golden pigment in the liver and golden, dark brown and black pigment in the spleen.

Haematology

Results for each parameter and all treatment groups were within the normal range of values for this species, with the exception of neutrophilic granulocytes in one of the controls, cage 5, (Appendix 8.5). Despite generally being within the normal range, differences were apparent between sample groups. SBT sampled from the treated cage had a significantly lower volume of red blood cells in circulation compared to the control cages (Table 8.5). However these results should not cause concern as the erythrocyte population observed in the smears consisted of all stages of red cell development and the haematocrit values of the treated cage more closely resembled values obtained from wild tuna in previous years (Pers. Obs. 1995-2004). Slight haemoconcentration has been typically observed every season (1994-2004) in the farmed SBT population when tuna are fed previously frozen bait fish of reasonable to good quality. What is interesting here is that these 3 cages received the same feed type and quality, therefore the differences in haematocrit may indicate that with reduced biofouling on the net, these tuna did not need to retain older cells or recruit a greater number of new cells to supply their oxygen needs.

There were no apparent differences in the structure or diversity of cells in the circulating leucocyte population. There were however significant differences between sample groups for the relative percentage of neutrophils and lymphocytes (Table 8.5). Generally in fish a peripheral blood neutrophilia indicates either the early phase of acute inflammation resulting from infection or a non specific response to stressors (Satchell 1991; Hine 1992). In SBT, neutrophils have proven to be a reliable indicator of short to medium term stress incurred through husbandry practices and/or prevailing environmental/culture conditions (pers. obs. 1994-2004). The elevation of values in the control cage 5 most likely reflects the fact that these samples were collected towards the end of the harvesting of this cage, and as such these fish had been subjected to multiple netting events. The other two cages were sampled within the first few harvesting events and therefore had not experienced repeated exposure to the harvest net. Although the differences in relative monocyte abundance were not significant between groups, it is interesting to note that the tuna in the treated net had the highest prevalence of blood fluke infection and the reduced levels in circulation may be reflecting redistribution to inflammatory sites. There was a significant difference in the relative abundance of

lymphocytes between the two control cages, but neither was significantly different from the treated cage. The levels in all groups were within the normal range for this parameter and the apparent elevation here is probably due to an immune response by some individuals.

Table 8.5: Haematology of tuna sampled from each cage (mean ± standard deviation). The same superscripts within each row denote no significant difference (*P>0.05*) between treatments

PARAMETER	**Cage 4:** TREATED (n=10)	**Cage 5:** CONTROL (n=10)	**Cage 6:** CONTROL (n=10)
Haematocrit (%)	48.7 ± 5.1 [a]	53.1 ± 2.5 [b]	52.5 ± 1.3 [b]
Leucocrit (%)	1.2 ± 0.6 [a]	1.3 ± 0.3 [a]	1.3 ± 0.4 [a]
Neutrophils (%)	4.90 ± 1.97 [a]	8.16 ± 2.82 [b]	4.99 ± 4.19 [ab]
Eosinophils (%)	2.94 ± 2.04 [a]	4.40 ± 4.62 [a]	5.36 ± 3.80 [a]
Basophils (%)	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Monocytes (%)	0.83 ± 0.94 [a]	2.06 ± 1.56 [a]	2.01 ± 1.57 [a]
Lymphocytes (%)	55.68 ± 12.56 [ab]	54.18 ± 6.71 [a]	61.70 ± 6.34 [b]
Thrombocytes (%)	35.66 ± 11.52 [a]	31.21 ± 7.52 [a]	25.94 ± 5.11 [a]

Fish productivity

The productivity of SBT within a cage can be considered in terms of the stock performance (growth, mortalities etc) and the feed measures such as rates and conversion efficiencies. Mortalities were discussed under fish health in the section analysed at a whole cage level. For the remainder of the productivity measures there were a number of commercial realities that complicated analyses in this trial, in particular, differences in initial stock numbers, feed rates and harvest dates. Therefore for this project a general assessment between the two cages will be discussed here, but specific comparisons on performance will only be made for the 120 tuna harvested as part of the residue survey as these were removed after comparable times in the cages.

The tow cage designated for this trial ran out of SBT mid way through the third transfer (the net treated with antifoulant). Therefore to ensure a commercial quantity of stock in this cage, an additional 800 SBT were sourced from a different tow cage that was caught in the same area and on a similar date. At the time of transfer 1131 SBT swam through the transfer gates, therefore the initial number of SBT in the treated cage was 15% higher than that of the control cage and there was no fourth transfer (second control cage) for mortality and productivity comparisons.

At the completion of harvest the difference in whole cage calculated growth (i.e. biomass harvested minus biomass stocked) between the treated and control cages was less than one tonne; being higher in the treated cage. This is despite the fact that there were a higher number of fish stocked into that cage and that a greater proportion of these were retrieved at harvest (due to the lower number of mortalities). This indicates that the growth performance per individual fish was higher in the control cage. However before too much can be drawn from this there are two confounding factors that need to be considered, the difference in feed rates and time in captivity.

When considering feed amounts, the treated cage performance was better, as the FCR was 11.7% lower than that of the control cage (i.e. less food consumed for the marginally higher increase in biomass achieved). Across the entire season, the fish in the treated cage were generally fed at a lower or the same rate as those of the control cage (an unfortunate reality of doing a trial on a commercial scale). This situation can occur due to differences in pallet weights when feeding frozen bait; but in this case probably arose because of the lower than expected number of mortalities in the treated cage. This meant that on the days tuna were fed, the individuals in the treated cage were offered on average 1.27kg each and those in the control cage 1.35kg per fish (i.e. SBT in treated cage received 6% less food daily). It is a good result to achieve equivalent growth despite less food and the higher stocking density in the treated cage throughout the season. However, the productivity of SBT in the treated cage may have been improved with more food per tuna, and / or a lower rate of stocking.

The other complicating factor when comparing the whole cage productivity in this trial is that the tuna in the treated cage were harvested later (25th September to 14th October) than those in the control cage (2nd June to 29th August). The tuna in the control cage were held for a maximum of 165 days, and those in the treated cage for a maximum of 210 days (treated held 20% longer). For most aquaculture operations a longer time in captivity would be deemed beneficial. However, within the tuna industry the optimum time of harvest is based on condition index and not on fish weight, and the optimum condition index for market is mid to late winter (industry pers. com.). After this time tuna appear to lose weight (industry obs.) and condition index decreases at harvest. However, it is unclear whether this is actual weight loss, or if fish appear skinny due to an increase in length without putting on fat.

Despite these complications, there were 4 occasions where a tuna harvest was undertaken specifically for collecting samples of flesh and skin for the chemical residue tests. So for the purpose of this project, fish weights lengths and condition indices will only be compared for those tuna harvested specifically for the residue testing, as these occasions give a snapshot of the fish's performance for similar times in cages and are discussed below.

Productivity of Tuna Sampled for Chemical Analysis

At the first sample interval there was no significant difference in the weight or length of tuna within each treatment (Table 8.6). However, there was a significant difference in the tuna's condition index ('fat content'), the tuna in the treated cage being higher/fatter than those in the control cage. This was also the case with the second sample interval, tuna in the treated net having higher condition index than those of the control cage. These results indicate that for similar times in cages, the fish in the treated net were performing better than those in the untreated control net. The significance of this becomes more apparent when considering the feed data. From the time of transfer to the time of this harvest, tuna in both cages had been offered the same ration, an average of 1.31kg of food per tuna per day fed. By the time of the second sampling the tuna in the treated cage had been offered an average of 1.33kg food/tuna/day fed and those in the untreated control 1.35kg food/tuna/day fed. Therefore the higher condition index is apparent despite equivalent or less food being offered, and this suggests that tuna are converting food to growth more efficiently with reduced biofouling on the net. Obviously these are preliminary results and need to be verified in further trials.

Table 8.6: Dressed (Gilled and Gutted, (GG)) weight, length and condition indices of tuna sampled for residue tests; mean ± standard deviation, nsd denotes no significant difference *P>0.05*; * *P<0.05*; ** *P<0.01*; * *P<0.001***

FISH DATA	TREATED NET	CONTROL NET	Statistics
1st SAMPLING: JUNE / JULY (n = 60 {30 per cage})			
GG Weight (kg)	27.607 ± 5.987	26.690 ± 4.883	nsd
Fork Length (m)	1.104 ± 0.073	1.109 ± 0.065	nsd
Condition Index (%)	23.289 ± 1.354	22.364 ± 1.505	*
2nd SAMPLING: AUGUST (n = 60 {30 per cage})			
GG Weight (kg)	30.797 ± 7.608	29.100 ± 7.526	nsd
Fork Length (m)	1.123 ± 0.089	1.111 ± 0.104	nsd
Condition Index (%)	24.583 ± 1.180	23.936 ± 1.255	*
COMBINED (n = 120 {60 per cage})			
GG Weight (kg)	29.202 ± 6.975	27.895 ± 6.406	nsd
Fork Length (m)	1.114 ± 0.081	1.110 ± 0.086	nsd
Condition Index (%)	23.936 ± 1.418	23.150 ± 1.586	**

Economic analysis

A common belief amongst owners and managers within the tuna industry is that the added cost to production incurred by treating nets with antifouling compounds would make the practice uneconomical. To assess this an analysis was undertaken utilising net treatment (antifoul coating / cleaning / maintenance) and SBT mortalities as the two major variables influencing cost outputs and loss of income (through decreased pieces for sale). Differences in initial stocking, sale price and yen exchange were also factored in, in various combinations. For these expenditure comparisons the following variables were kept the same, an initial weight sample of 16kg, an average harvest size of 28kg GG and bait cost of $0.70 /kg (assuming 60 days feeding at a rate of 2kg/SBT/day).

In the example with an initial stocking of 2000 SBT, average sale price of ¥1900/kg and a yen exchange of $AUD1 = ¥80 (scenario 1, appendix 8.6): a 3% mortality rate (ie a loss of 60 fish) would result in a $65 140 outlay in direct costs (ie catch and tow and feed for the now dead tuna; and net cleaning and maintenance) and forgone income (1680kg lost as mortality) in the current situation where nets are not treated with antifoulant. In the same scenario where a white net is coated with antifoulant (leading to a 20% reduction in mortality, ie a loss of 48 fish), the direct costs (catch and tow and feed for the now dead tuna; antifoul product, application and freight, net maintenance) and forgone income (1344kg lost as mortality), the expenditure and loss of income is $54 572. Essentially the added costs associated with antifoulant treatment are negated by the lack of necessity for net cleaning and reduced onshore maintenance; and with more product to sell (through reduced mortality) the farmer in this scenario is $10 568 better off per pontoon. The benefit is more pronounced in the case of a

black net as these absorb 33% less antifoulant product, because they have thinner cord and therefore less surface area of netting. In this case the farmer is $14 168 better off per pontoon.

If the mortality rate is increased to 5%, expenditure and loss of income is $96 700 for an untreated net; $79 820 for a white antifoul treated net and $76 220 for a black antifoul treated net. So the relative savings are $16 880 and $20 480 for white and black nets respectively.

If the mortality rate is decreased to 1% expenditure and loss of income is $33 580 for an untreated net; $29 324 for a white antifoul treated net and $25 724 for a black antifoul treated net. So the relative savings are $4 256 and $7 856 for white and black nets respectively.

This pattern remains consistent if the yen exchange is $AUD1 = ¥100 or average sale price reduced to ¥1800, or initial stocking increased to 2200 SBT (appendix 8.6, scenarios 1-8).

These analyses include only the obvious differences (nets and mortalities); the reality is that tuna growth and feed consumption in surviving stock should also be variables, but changes to this projects structure (especially harvest dates) did not allow for reliable collection of these type of data at a whole cage level.

CONCLUSIONS

The application of the antifouling paint Net Clear ZPT™ in this trial did not totally prevent the growth of biofouling on the tuna net that was coated. However it did reduce biofouling density and delayed the typical community succession so that hard shelled organisms were not present at any time or depth. The product was most effective at depths below 5m suggesting that the activity of the antifouling ingredients may be reduced by UV light at the surface. But even in the top 5m, growth was restricted to soft types of macroalgae and the cord of the net meshes was visible between patches of growth, even at the final video transect, 177 days post net deployment. The efficacy of this antifoulant was most apparent on the lower walls and on the base of the net where the total occlusion levels did not exceed 50% at any time, and the fouling growth comprised soft algae and sponges. By contrast the untreated net had mesh occlusion levels of 85% on the base and this growth was a combination of shell, sponge, invertebrates and algae. The occlusion of new untreated and treated netting ranges from 20-24%.

Constraints with antifoul use encountered in this project such as high humidity delaying paint application and the expense and time delays associated with transporting nets to Tasmania for dipping, would be resolved if a dipping site could be established on Eyre Peninsula. Once the coated net was in Port Lincoln, it did not require any alteration to normal industry practice, except that a treated net must be deployed at sea at least 72 hours prior to the addition of tuna. The treated net did not require cleaning at any time through the farm cycle and was deployed for more than 220 days in total. After harvests were completed the treated net could be lifted directly onto a vessel without any form of pre-cleaning. The untreated net was too heavy and required in-situ cleaning before removal from the water. Once removed from the water, the time involved with on land cleaning and mending was reduced by 75%, mainly due to the lack of shell and minimal soft growth. Despite concerns about paint becoming brittle and flaking at the time of subsequent deployment, new tuna introduced to this net in 2007 showed no indications of gill irritation through their behaviour or mortality. However, as a precautionary measure, the net in this case was deployed 14 days prior to the arrival of tuna. The second season deployment of the treated net has required cleaning in the same way as nets never previously treated.

The use of a zinc based antifouling paint on the tuna net did not result in elevated levels of zinc within tuna muscle or skin tissue. All zinc results of the flesh samples in this trial were

within the range of values found naturally in SBT sampled from wild stocks in the Great Australian Bight in 2002 and 2004; and were comparable to results of previous surveys of farmed stocks in 2002, 2003 and 2004. There were no statistical differences between the zinc levels of blue mussels *Mytilus edulis* suspended directly in contact with both the treated and the untreated nets for 123 days. Zinc levels within the surface sediment sampled 100m up and 100m down current from the treated and untreated nets were not significantly different from each other; nor were there differences up and down current within treatments. Samples collected directly beneath the treated net had an elevated average value, probably due to the decomposition of mussels in one replicate, but the results were not statistically different from samples beneath the untreated net. Results from all sediment sample groups in this trial were comparable to sediment values obtained from a survey of the tuna farming zone undertaken in 2002.

SBT in the treated net showed no adverse behavioural differences, and feed staff indicated that they maintained appetite when that of others on the lease site was depressed. Overall 24% fewer mortalities were observed from the treated net compared with the untreated control. The difference between nets was most apparent for the immediate and delayed post/capture tow type mortalities where 67% occurred in untreated net; and also with swimmer type mortalities where 70% occurred in untreated net. Combined these causes of fish deaths accounted for around 80% of the total mortalities occurring in the cages of this trial. Antifoul treatment appeared to have little effect on the parasite loads, gill structure (histology) or haematology of tuna sampled from harvests in this trial.

Analysis of fish productivity at a whole cage level was complicated by differences in initial stock numbers, daily feed rates per tuna and non sequential harvest dates. But even so it appears that equivalent growth was achieved by tuna in the treated net with an FCR that was 12% lower than that of the control cage. The difference in FCR is most likely due to the fact that SBT in the treated cage were offered on average 6% less food daily. Specific comparisons could be made between SBT groups harvested at the same time, for residue testing. These showed that tuna in the treated net had a significantly higher condition index at a time when both cages had been offered an average of 1.31kg food/tuna/day fed. By the second sampling of tuna for residue testing, those in the treated net had been offered an average of 1.33kg food/tuna/day fed and the control net 1.35kg food/tuna/day fed; but despite this the condition index of fish in the treated net was still significantly higher. These results suggest that SBT convert food to growth more efficiently with reduced biofouling on the net, but this needs to be verified in further trials.

This was the first time that an antifoulant of any type has been used on a tuna net in this industry. Unfortunately, due to a number of factors beyond the project team's control, the data obtained in this trial were not always as robust as anticipated; this can be a reality of doing research on a commercial scale. However despite its limitations there are indications that the use of antifouling compounds could be beneficial to the productivity and hence the economics of the tuna industry. This was evident from an industry perspective at the completion of the trial when the collaborating company saw sufficient benefits to commit four nets to an antifouling trial in the following season. It was further indicated through economic analysis incorporating the variables net treatment and maintenance and tuna mortality, showing that expenditure and lost income through these factors are potentially reduced with the use of an antifoulant by between $3 612 and $22 058 per pontoon (dependent on net type, initial stocking, general mortality rate, yen exchange and sale price).

BENEFITS AND ADOPTION

Economic analyses undertaken as an extension to the commercial pilot scale evaluation suggest that the benefit of reducing biofouling growth through the use of an antifouling treatment can excede $22 000 per net (dependant on net type, initial stocking, general mortality rate, yen exchange, and sale price).

The commercial company that undertook the pilot scale project saw sufficient merit in the product to coat 4 nets (50% of their operation) in the following season. Nets were sent to Tasmania for coating, but in the meantime due to an unfortunate set of circumstances Wattyl withdrew the product from the market and nets were returned to Port Lincoln untreated.

Through the life of this project operators within the tuna industry have adopted the concept behind this project. This can be seen through the increase in active biofouling management. In 2004, industry surveys revealed that biofouling management through net cleaning ranged from not at all; to once or maybe twice (of one or two net sections then later the entire net) within a season. In 2007 entire nets were cleaned up to 3 times through the farm season.

FURTHER DEVELOPMENTS

Results from the pilot scale project indicate that there are potential benefits of reducing biofouling growth in the tuna industry. A larger scale project is needed validate benefits seen with this trial (reduced mortality, improved feed conversion, increased condition index etc) and to assess efficacy under differing environmental conditions and management regimes.

Other means of reducing biofouling warrant investigation (eg net materials, colours, types; mechanical cleaners; antifoul coatings etc). Unreplicated panels of various net types deployed through 2006 indicate that, net containing 8 strand steel, 1 strand copper with 6 strand steel, Dyneema, black, white, green, blue, brass, cord thickness and mesh diameter have varying influence on biofouling growth (Rough et al 2008, Chapter 6 this document).

Through 2006, another product, Ultraglide that is silicon based attained registration exemption by APVMA for use on aquaculture nets. Small panels deployed through 2006 showed that this was effective at delaying biofouling growth, and that fouling growth was not well attached (making cleaning easier). It could be applied to new and previously used nets, and would probably work well in combination with a mechanical cleaner.

The product tested in a commercial situation in this project was removed from market at the completion of the trial. The new formulation of Wattyl Net Clear, containing Sea-nine 211, which was tested in the panel trial (Rough and Ellis 2007, Chapter 4 this document); needs to be tested in a commercial pilot scale situation to evaluate tuna health, and tuna and environmental residue accumulation[21]. An earlier formulation of this product was trialled in 2002, reported in Svane et al (2006) with limited success (15% reduction in biofouling measured by occlusion); however personal observation of tuna through this trial demonstrated an atypically high incidence of Caligid copepods (these can be an indicator of stress in tuna).

[21] As an extension to this project the improved formulation of Wattyl Net Clear was applied to an entire tuna net through 2008; the results of this project extension are to be published later in 2009

PLANNED OUTCOMES

Optimum use of antifoulants, to reduce environmental impacts and facilitate management of deep water sites with the longer term holding of tuna.

From this research there is a better understanding of the growth of biofouling communities on tuna netting and the way they impact water flow and water quality (primarily dissolved oxygen levels within the sea cage). By improving the Oxy-Tuna computer model farm operators can test and implement strategies to manage biofouling to improve water flow through sea-cages.

Other benefits from this project are likely to include:

Improved growth rates and improved feed conversion rates

Decreased mortality

Longer life of infrastructure

A reduction in the time required for net cleaning and net maintenance

PROJECT CONCLUSIONS

The objectives and the extent that each was met are as follows (note that more detailed conclusions can be found at the end of each chapter through the document):

Objective 1. Document current industry knowledge and methods used to control bio-fouling on nets and associated structures (both physical and chemical means) for various marine finfish species cultured in Australia and overseas

Successful, achieved by literature review undertaken by the Biofouling Research Group, School of Marine Biology and Aquaculture at James Cook University (de Nys et al 2005a; de Nys et al 2005b; Chapters 1 & 2).

Objective 2. Co-ordinate the tuna industries approach in antifoul treatments

Successfully achieved.

Objective 3. Review currently available commercial antifoulant products, including the mechanisms by which they reduce fouling and the regulations involved in their use.

Successful, achieved by literature review undertaken by the Biofouling Research Group, School of Marine Biology and Aquaculture at James Cook University (de Nys et al 2005b; Houlden and de Nys 2005; Chapters 2 & 3)

Objective 4. Determine efficacy (through reduction in fouling growth and impact on net integrity) of antifoulant products identified by objective 3 with net panels in the local environment where tuna are currently ranched

The project was successful in testing 3 types of antifouling agents Lanolin (Lanotec™), latex with booster biocide Sea-nine 211 (Net Clear™), and a paint containing the heavy metal zinc oxide with booster biocide zinc pyrithione (Net Clear ZPT™) in the local environment. None of these totally prevented fouling growth, but both the latex and paint, Net Clear and Net Clear ZPT were significantly effective at delaying the onset of and at reducing the overall amount of biofouling growth at depths of 2 and 9m compared with untreated net panels through all sample times.

Coating nets with Net Clear and Net Clear ZPT was found to maintain or improve the tensile strength of the netting irrespective of deployment time or depth. Lanotec significantly reduced the breaking strain of meshes compared with new and untreated net. (Rough and Ellis 2007; Chapter 4)

Objective 5. Identify the development pattern of fouling communities on commercial tuna cages that are subject to the current standard industry practices, and relate this to oxygen levels monitored on the outside and inside of these nets.

Net fouling assemblages were studied at two localities and on both net types currently used by industry. At the inshore site, white netting had more diverse fouling assemblages comprised of 14 taxonomic groups (4 animal phyla and 3 algal divisions) across all depths. The fouling assemblages were dominated by hydroids in autumn March/April, moving to mixed algae and encrusting organisms through winter May/June, climaxing with colonial ascidians at the end of the farm season in August/September. At the

offshore site, black netting had less diversity, 9 taxonomic groups (4 animal phyla and 3 algal divisions), but followed the same developmental pattern through time. Depth differences were associated with dominance of algae in the shallower depths and encrusting organisms (including bryozoans and ascidians) in the deeper depths of both sea-cages, bivalves were recorded from mid season (June) but were not in high density.

A disruption to water exchange through the net as a result of increased biofouling was demonstrated using the water quality data collected. The dissolved oxygen concentration within the sea-cage decreased as net occlusion increased concurrently with fouling growth. (Loo 2008; Chapter 5)

Objective 6. Establish relationship between the percentage cover of fouling communities with water flow, net weight and net drag

For the fouling types represented, the flume tank technique was very effective at establishing these relationships, and determining oxygen consumption by biofouling communities. Biofouling assemblages and densities both influenced water flow and oxygen consumption. Light fouling growth (occlusion 40%) was capable of inducing turbulence if it contained hard shelled invertebrates. Dense fouling (70-80% occlusion) that was entirely weed growth restricted water flow at low current speed. Dense fouling (70-80% occlusion) of shell growth restricted water flow at low and high current speeds. Shell growth has the most influence on dry weight gain due to biofouling. Oxygen consumption rates were influenced by the amount of biofouling growth and the types of organism's present. (Rough et al 2008; Chapter 6)

Objective 7. Enhance the dissolved oxygen diffusion model to provide predictive capacity for industry to evaluate fouling management systems.

The OxyTuna model has been developed to assist farm managers in making better decisions about fouling management of finfish cage systems, particularly the relationship between net fouling and oxygen concentration in the cage and its response to intervention (eg net cleaning). The model provides a quantitative prediction of changes in oxygen concentration through time for different sea-cage configurations (cage size, net type stocking density, fish species) in response to changes in ambient conditions (temperature, salinity, ambient oxygen concentration and current speed). The dynamic nature of the model allows users to better understand the interplay of factors that control oxygen concentration in a sea-cage, and is therefore useful as both a management and teaching tool. (Cheshire and Loo 2008; Chapter 7)

Objective 8. Field test the most effective anti-foul treatment identified by objective 4 on a commercial tuna cage with the typical industry regime of tuna stocking density, feeding and net maintenance. Effectiveness of the antifoulant will be assessed utilising methods developed and used in objectives 4 and 5

This was logistically challenging and mostly successfully achieved, except that the experimental design to monitor mortality was compromised through insufficient tuna numbers in the tow cage supplying fish for this trial. Limitations were found in the scientific application of data collected with ROV's, but the video footage was suitable for general visual assessment of product performance. (Rough and Ellis 2008; Chapter 8)

Objective 9. ***Test the chemical residue status of tuna and shellfish within the cage and the sediment beneath the net for the treated cage and compare these to tuna, shellfish and sediment of an untreated control.***

The project was successful in testing the residue status of tuna and the local environment. The use of zinc based antifouling paint on the tuna net did not result in elevated levels of zinc within tuna muscle or skin tissue, nor in shellfish or the surrounding sediment. All zinc results from tuna flesh were within the range of values found naturally in wild southern bluefin and those farmed in years prior to the use of any antifouling treatment. Results from environmental samples collected from the treated net were not significantly different from those of the untreated control net, and sediment values in the vicinity of both nets were comparable to those of a survey of the tuna farming zone in 2002 (prior to the use of antifoulant) . (Rough and Ellis 2008; Chapter 8)

Objective 10. ***Assess the health status of tuna in the treated cage by comparing it with that of two control/untreated cages (health status incorporates behaviour, mortality and histopathology)***

Experimental design to monitor mortality was compromised through insufficient tuna numbers in the tow cage supplying fish for this trial. Specific health tests including parasite checks for prevalence and infection intensity; histopathology; and haematology were achievable and demonstrated no detrimental effect due to treating net with antifoulant. (Rough and Ellis 2008; Chapter 8)

Objective 11. ***Disseminate results to industry on a regular basis through verbal, written and electronic communication***

An overview of the entire project and preliminary results on biofouling influence on water flow and oxygen consumption was relased in 2005. Panel trial results were presented at industry workshops.

The Oxytuna model was presented at industry workshops, in tuna briefs, and also released as a stand alone document including the model.

The pilot scale trial was disseminated through the management of the collaborating tuna company and industry was welcomed to dive and inspect net through the trial.

REFERENCES

Aarsnes JV, Rudi H, Loland G (1990) Current forces on cages, net deflection. Editor In: *Engineering for Offshore Fish Farming.* Thomas Telford, London, pp 137- 152

Ackefors H (2000) Review of Swedish regulation and monitoring of aquaculture. *J Appl Ichthyol* **16:** 214-223

Ahlgren MO (1998) Consumption and assimilation of salmon net pen fouling debris by the red sea cucumber *Parastichopus californicus*: implications for polyculture. *J World Aquaculture Soc* **29**: 133-139

Alderman DJ, Rosenthal H, Smith P, Stewart J, Weston D (1994) Chemicals Used in Aquaculture. ICES Cooperative Research Report No. 202, Copenhagen, Denmark: ICES Working Group Environmental Interactions of Mariculture. 100 pp

Alzieu C (1991) Environmental problems cause by TBT in France: assessment, regulations, prospects. *Mar Environ Res* **32**: 7-17

Alzieu C (1998) Tributyltin: case study of a chronic contaminant in the coastal environment. *Ocean Coast Manage* **40**: 23-36

Alzieu C, Heral M, Thibaud Y, Dardignac M J, Feuillet M (1981) Influence des peintures antisalissures à base d`organostanniques sure lat calcification de la coquille de l`huître *Crassostrea gigas*. *Revue Trav Inst Pêches Marit* **45**: 101- 116

Alzieu C, Sanjuan J, Deltreil JP, Borel M (1986) Tin contamination in Arcachon Bay: effects on oyster shell anomalies. *Mar Pollut Bull* **17**: 494-498

Andersen RJ, Luu HA, Chen DZX, Homes CFB, Kent ML, Le Blanc F, Taylor FJR, Williams DE (1993) Chemical and biological evidence links microcystins to salmon netpen liver disease. *Toxicon* **31**:1315-1325

Angel DL, Eden N, Breitstein S, Yurman A, Katz T, Spanier E (2002) *In situ* biofiltration: a means to limit the dispersal of effluents from marine finfish cage aquaculture. *Hydrobiologia* **469**: 1-10

Anon (1979) Rotating cage in Scottish trials. *Fish-FarmerInt* **3**: 14

Anon (1993) How Ocean Spar solves problems of currents. *Fish Farm Int* **21**: 20

Anon (1994) New machine cleans nets on the cages. *Fish Farm Int* **21**: 22

ANZECC (Australian and New Zealand Environment and Conservation Council) (1995) *Maritime Accidents and Pollution: Impacts on the Marine Environment from Shipping Operations.* Paper for Public Comment, Commonwealth of Australia, 50 pp

APVMA **(**2005**)** website*: http://www.apvma.gov.au/*. Australian Pesticides & Veterinary Medicines Authority.

Balls PW (1987) Tributyltin (TBT) in the waters of a Scottlish sea loch arising from the use of antifoulant treated netting by salmon farms. *Aquaculture* **65**: 227- 237

Barton JR (1997) Environment, sustainability and regulation in commercial aquaculture: the case of Chilean salmonid production. *Geoforum* **28**: 313-328

Bell GM, Chadwick J (1994) Regulatory controls on biocides in the United Kingdom and restrictions on the use of triorganotin-containing antifouling products. *International Biodeterioration & Biodegradation* **34**: 375-386

Berkelmans R (1992) Video photography: a quantitative sampling method. *Reef Research* **2**(1): 10-11

Bernardino FNV (2000) Review of aquaculture development in Portugal. *J Appl Ichthyol* **16**: 196-199

Bers V, Wahl M (2004) The influence of natural surface microtopographies on fouling. *Biofouling* **20**: 43-51

Beveridge M (2004) Cage Aquaculture, Blackwell Publishing Ltd., Oxford, UK pp. 1- 368

Bhadury P, Wright PC (2004) Exploitation of marine algae: biogenic compounds for potential antifouling applications. *Planta* **219**: 561-578

Bierman P, Kaempf J, Fernandes M (2007) Chapter 6 Oceanographic conditions in the offshore southern bluefin tuna farming zone, near Port Lincoln SA. In J. E. Tanner, (Ed.) *Aquafin CRC – Southern Bluefin Tuna Subprogram: Tuna environment subproject: Development of regional environmental sustainability assessments for tuna sea-cage aquaculture Technical Report, Aquafin CRC Project 433, FRDC Project 2001/104*. (pp. 207-237). Adelaide: Aquafin CRC, Fisheries Research & Development Corporation and South Australian Research & Development Institute (Aquatic Sciences). SARDI Publication No F2007/000803-1 SARDI Research Report Series 235.

Bingaman WW, Willingham GL (1994) The changing regulatory environment: EPA registration of a new marine antifoulant active ingredient. *Int Biodeter Biodegr* **34**: 387-399

Blair A, Burgess WH (1979) Rotating, floating cage for fish culture simplifies cleaning and replacement of panels. *Prog Fish-Cult* **41**: 74-76

Blair A, Campbell R, Grant PT (1982) A submersible fish cage that can be rotated on the surface to remove biofouling and for other purposes. *Aquaculture* **29**: 177- 184

Bond T (1992) Port Lincoln Aquaculture Management Plan. Dept. Environment and Land Management; Dept. Primary Industries: Fisheries 75pp

Bond T (1993) *Port Lincoln aquaculture management plan 1993.* Resource Management Division, Department of Environment and Land Management, Adelaide, South Australia. 93 pp

Boxall ABA, Comber SD, Conrad AU, Howcroft J, Zaman N (2000) Inputs, monitoring and fate modeling of antifouling biocides in UK estuaries. *Mar Pollut Bull* **40**: 898-905

Boyd CE (1982) *Water quality management for pond fish culture. Developments in Aquaculture and Fisheries Science*, Vol 9, Elsevier, Amsterdam, 318 pp

Brady RF Jr, Aronson CL (2003) Elastomeric fluorinated polyurethane coatings for nontoxic fouling control. *Biofouling* **19** (Suppl): 59-62

Braithwaite RA, Carrascosa MCC, McEvoy LA (2007) Biofouling of salmon cage netting and the efficacy of a typical copper-based antifoulant. *Aquaculture* **262**: 219-226

Braithwaite RA, McEvoy LA (2005) Marine Biofouling on Fish Farms and Its Remediation. *Advances Marine Biol* **47**: 215-252

Brooks KM (2000) Determination of copper loss rates from Flexgard XI™ treated nets in marine environments and evaluation of the resulting environmental risks. *Report to the Ministry of Environment for the BC Salmon Farmers Association*, 1200 West Pender Street, Vancouver, BC, 24 pp

Brooks KM, Mahnken CVW (2003) Interactions of Atlantic salmon in the Pacific Northwest environment. III. Accumulation of zinc and copper. *Fisheries Research* **62**: 295-305

Bruno DW, Ellis AE (1988) Histopathological effects in Atlantic salmon, *Salmo salar* L., attributed to the use of tributyltin antifoulant. *Aquaculture* **72**: 15-20

Buchanan L (1977) Ranching Atlantic bluefin. *Sea Front* **23**: 172-180

Burgess JG, Boyd KG, Armstrong E, Jiang Z, Yan L, Berggren M, May U, Pisacane T, Granmo A, Adams DR (2003) The development of a marine natural product-based antifouling paint. *Biofouling* **19** (Suppl): 197-205

Bwathondi POJ & Ngoile MAK. (1982) Bivalves fouling floating cages used in aquaculture. *Indian J Mar Sci* **11**: 283-285

Caillouet CW (1972) Rotatable cage for high-density aquaculture. *Prog Fish-Cult* **34**: np

Call DK, Brooke LT, Kent, RJ, Knuth ML, Poirier SH, Huot JM, Lima AR (1987) *Arch Environ Contamin Toxicol* **16**: 607

Callow ME (1999) The status and future of biocides in marine biofouling prevention. In: Fingerman M, Nagabhushanam R, Thompson M-F (eds) *Recent Advances in Marine Biotechnology, Vol 3, Biofilms, Bioadhesion, Corrosion, and Biofouling.* Enfield, NH, Science Publishers, pp 109-126

Callow ME, Fletcher RL (1994) The influence of low surface energy materials on bioadhersion – a review. *Int Biodeter Biodegr* **34**:333-348

Callow ME, Willingham GL (1996) Degradation of antifouling biocides. *Biofouling* **10**: 239-249

Caux PY, Scrimshaw MD, Emmerson RHC, Lester JN (1996) Environmental fate and effects of chlorothalonil – a Canadian perspective. *Crit Rev Environ Sci Technol* **26**: 45-93

Chapman, G. A. (1978). Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. *Trans American Fish Soc* **107**: 841- 847

Chapman GA, Stevens DG (1978) Acutely lethal levels of cadmium, copper, and zinc to adult male coho salmon and steelhead. *Trans American Fish Soc* **107**: 837- 840

Charleton, JHD (1995) Quantitative video sampling of coral reef benthos: large-scale application. *Coral Reef* **14**: 35-46

Cheah SH, Chua TE (1979) A preliminary study of the tropical marine fouling organisms on floating net cages. *Malaysian Nat J* **33**: 39-48

Cheah SH, Chua TE (1983) The rate of encrustation of biofouling organisms on experimental floating net-cages in tropical coastal waters. *Proc Symp on Coastal Aquaculture*, Cochin, India, 1980. Part 2: Molluscan Culture. *Mar Biol Assoc India* **6:** 677-683

Chen DZX, Boland MP, Ptak C, Smillie MA, Klix H, Andersen RJ, Holmes CFB (1993) Identification of protein phosphatase inhibitors of the microcystin class in the marine environment. *Toxicon* **31**: 1407-1414

Chengxing Z (1990) Ecology of ascidians in Daya Bay. *Collections of Papers on Marine Ecology in Daya Bay, 2*. pp 397-403.

Cheshire AC, Loo MGK (2008) Oxytuna – A model for the oxygen dynamics in a southern bluefin tuna sea-cage system. In Rough KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development

Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp. Chapter 7 this document

Cheshire AC, Miller DJ (1999) The impact of sand dredging on benthic community structure at Pt Stanvac Dredge Site: Final report on the results of surveys 1992 to 1999. A report to the Coast and Marine Section, Environment Protection Authority of South Australia. Department of Environmental Biology, University of Adelaide, Adelaide. 38 pp

Cheshire AC, Westphalen G, Kildea TN, Smart A, Clarke S (1996) Investigating the environmental effect of sea-cage tuna farming. II. The effects of sea-cages. Aquaculture Program, South Australian Research and Development Institute, Adelaide. 43 pp. A report to the FRDC and TBOA

Chopin T, Buschmann AH, Halling C, Troel M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *J Phycol* **37**: 975-986

Chou CL, Paon LA, Moffat JD, Zwicker (2000) Copper contamination and cadmium, silver, and zinc concentrations in the digestive glands of American lobster (*Homarus americanus*) from the Inner Bay of Fundy, Atlantic Canada. *Bull - Environ Contam Toxico* **65**: 470-477

Chou CL, Haya K, Paon LA, Moffat JD (2003) Metals in the green sea urchin (*Strongylocentrotus droebachiensis*) as an indicator for the near-field effects of chemical wastes from salon aquaculture sites in New Brunswick, Canada. *Bull Environ Contam Toxicol* **70**: 948-956

Chua TE, Teng SK (1977) Floating fish pens for rearing fishes in coastal waters, reservoirs and mining pools in Malaysia. *Fish. Bull. Minist. Agric. Rural Dev. (Malays)*, No. 20, 36 pp.

Chua TE, Teng SK (1980) Economic production of esturary grouper, *Epinephelus salmoides* Maxwell, reared in floating net cages. *Aquaculture* **20**: 187-228

Ciba-Geigy (1995) Irgarol 1051 in antifouling paints. In *Technical Information Bulletin*. Ciba-Geigy Ltd, Basel, Switzerland.

Claereboudt MR, Bureau D, Côté J, Himmelman JH (1994) Fouling development and its effect on the growth of juvenile giant scallops (Placopecten magellanicus) in suspended culture. *Aquaculture* **121**(4): 327-342

Claisse D, Alzieu Cl (1993) Copper contamination as a result of antifouling paint regulations? *Mar Pollut Bull* **26**: 395-397

Clare AS (1996) Marine natural product antifoulants: status and potential. *Biofouling* **9**: 211-229

Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**(5): 893-905

Clark A, Nowak BF (1999) Field investigations of amoebic gill disease in Atlantic salmon, *Salmo salar* L., in Tasmania. *J Fish Dis* **22**: 433-443

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**(1): 117-143

Clarkson N, Evans LV (1995) Further studies investigating a potential non-leaching biocide using the marine fouling diatom *Amphora coffeaeformis*. *Biofouling* **9**: 17-30

Clarkson N, Evans LV (1993) Evaluation of a potential non-leaching biocide using the marine fouling diatom *Amphora coffeaeformis*. *Biofouling* **7**: 187-195

Conand C, Sloan NA (1989) World fisheries for echinoderms. In: Cadey JF (ed) Marine invertebrate fisheries: Their assessment and management. John Wiley & Sons, NY, USA pp 647-663

Costello MJ, Grant A, Davies IM, Cecchini S, Papoutsoglou S, Quigley D, Saroglia M (2001) The control of chemicals used in aquaculture in Europe. *J Appl Ichthyol* **17**: 173-180

Crawford CM, Mitchell IM, Macleod CKA (2001) Video assessment of environmental impacts of salmon farms. *ICES Journal of Marine Science* **58**(2): 445-452

Cribb TH, Daintith M, Munday BL, (2000) A new blood fluke, *Cardicola forsteri*, (Digenea: Sanguinicolidae) of southern bluefin tuna (*Thunnus maccoyii*) in aquaculture. Records of South Australian Museum **124**: 117-120

Crisp DJ (1984) Overview of research on marine invertebrate larvae, 1940-1980. In: Costlow JD, Tipper RC (eds), *Marine Biodeterioration: An Interdisciplinary Study*, Naval Institute Press, Maryland, pp 103-126

Cronin E (1995) An investigation into the effects of net fouling on the oxygen budget of southern bluefin tuna (*Thunnus maccoyii*) farms. *Honours Thesis*, University of Adelaide, South Australia, 88 pp

Cronin ER, Cheshire AC, Clarke SM, Melville AJ (1999) An investigation into the composition, biomass and oxygen budget of the fouling community on a tuna aquaculture farm. *Biofouling* **13**(4): 279-299

Dahl B, Blanck H (1996) Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosm. *Mar Pollut Bull* **32**: 342- 350

Dahlem C, Moran PJ, Grant TR (1984) Larval settlement of marine sessile invertebrates on surfaces of different colours and position. *Ocean Science and Engineering* **9**: 225-236

Davies IM, McKie JC (1987) Accumulation of total tin and tirbutyltin in muscle tissue of farmed Atlantic salmon. *Mar Pollut Bull* **18**: 405-407

de Nys R, Steinberg PD (2002) Linking marine biology and biotechnology. *Curr Opin Biotechnol* **13**: 244-248

de Nys R, Houlden BA, Hodson SL, (2005a) Impacts of biofouling on marine finfish aquaculture. In Rough KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp. Chapter 1 this document

de Nys R, Houlden BA, Hodson SL, (2005b) Methods and efficacy of biofouling control in sea cage aquaculture. In Rough KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp. Chapter 2 this document

Doedens R (1992) Mechanical cleaning of nets – Preliminary report. In: *Proc 1992 Research and Development Review Seminar*, 1992, pp 203-206

Dosdat A, De la Pomelie C (2000) Regulation and monitoring of marine aquaculture in France. *J Appl Ichthyol* **16**: 157-162

Douglas-Helders, GM, Tan C, Carson J, Nowak BF (2003) Effects of copper-based antifouling treatment on the presence of *Neoparamoeba pemaquidensis* Page, 1987 on nets and gills of reared Atlantic salmon (*Salmo salar*). *Aquaculture* **221**: 13-22

Dubost N, Masson G & Moreteau J (1996) Temperate freshwater fouling on floating net cages: Method of evaluation, model and composition. *Aquaculture* **143**: 303-318

Edwards A, Edelston DJ (1976) Marine fish cages – the physical environment. *Proc Royal Society Edinburgh* (B) **75**: 207-221

Edwards DP, Nevell TG, Plunkett BA, Ochiltree BC (1994) Resistance to marine fouling of elastomeric coatings of some poly(dimethylsiloxanes) and poly (dimethyldiphenylsiloxanes). *Int Biodeter Biodegrad* **34**: 349-359

EEC (1991) European Commission, DG XIV: Report 1991 of the Parliament and Council Commission Concerning the Common Fisheries Policy. 2288 Final. 8 January 1992. Brussels: EC

Ellis D (1991) New dangerous chemicals in the environment: Lessons from TBT. *Mar Pollut Bull* **22**: 8-10

Ernst W, Doe K, Jonah P, Young J, Julien G, Hennigar P (1991) The toxicity of chlorothalonil to aquatic fauna and the impact of its operational use on a pond ecosystem. *Arch Environ Contam Toxicol* **21**: 1-9

European Agency for the Evaluation of Medicinal Products (EMEA), (2000): www.eudra.org/vetdocs/vet/mrl.htm (accessed April (2000))

Evans LV (1981) Marine algae and fouling – a review, with particular reference to ship-fouling. *Botanica Mar* **24**: 167-171

Evans SM (1999) Tributyltin pollution: the catastrophe that never happened. *Mar Pollut Bull* **38**: 629-636

Evans SM, Birhenough AC, Brancato MS (2000) The TBT ban: Out of the frying pan into the fire? *Mar Pollut Bull* **40**: 204-211

Fairhead A, Tanner JE, Miller DJ (2002) Environmental assessment of the biological assemblages at the Port Giles jetty dredging site. Report prepared for Egis Consulting SA. South Australian Research and Development Institute, Aquatic Sciences, Adelaide. 66 pp.

FAO (2004). The State of world fisheries and aquaculture, Food and Agriculture Organization of the United Nations. Data for 2002 accessed April 2005

FAR (1993). Workshop on fish farm effluents and their control in EC countries. Congress Center Hamburg, November 23-25, 1992. Kiel, Germany: Department of Fishery Biology, Institute for Marine Science at the Christian-Albrechts- University of Kiel

Fernandes TF, Miller KL, Read PA (2000) Monitoring and regulation of marine aquaculture in Europe. *J Appl Ichthyol* **16**:138-143

Ferrer I, Barcelo D (1999) Simultaneous determination of antifouing herbicides in marina water samples by on-line SPE followed by LC/MS. *J Chromatog A* **854**: 197-206

Fitzgibbon Q, Frappell P, Clark T, Buchanan J, Ellis D, Carter C, Musgrove R, Carragher J, Seymour R, (2008). Final Report - Aquafin CRC - SBT Aquaculture Subprogram: Activity metabolism in live-held southern bluefin tuna (Thunnus maccoyii) Phase 2. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 123 pp.

Forrester J W (1961) *Industrial Dynamics.* Cambridge: MIT Press. 464 pp

French MS, Evans LV, Dalley R (1984) Raft trial experiment on leaching from antifouling paints. *Trans Ins Mar Eng* **97**: 127-130

Fukuda H (1965) *Report of an investigation on environmental parameters in relation to fish farming.* Tokushima Prefectural Fisheries Experimental Station, pp 1- 32

Fusetani N (2004) Biofouling and antifouling. *Nat Prod Rep* **21**: 94-104

Geffen A (1979) Rotating fish cage to prevent fouling. *Aquaculture* **16**: 83-85

GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) (1991) Reducing the environmental impacts of coastal aquaculture. *Rep Stud GESAMP* **47**, 35 pp

Giacomazzi S, Cochet N (2004) Environmental impact of diuron transformation: a review. *Chemosphere* **56**: 1021-1032

Gibbs PE, Pascoe PL, Bryan GW (1991) Tributyltin-induced imposex in stenoglossan gastropods: pathological effects on the female reproductive system. *Comp Biochem Physiol* **100C**: 231-235

Goldberg ED, Bowen VT, Farrington JW, Harvey G, Martin JH, Parker PL, Risebrough RW, Robertson W, Sneider W, Gamble E, (1978), The Mussel Watch. *Environmental Conservation* **5**: 101-125

Gormican SJ (1989). Water circulation, dissolved oxygen, and ammonia concentrations in fish net-cages. *MSc Thesis*, University of British Columbia, Canada, 62 pp

Gough MA, Fothergill J, Hendrie JD (1994) A survey of southern England coastal waters for the *s*-triazine antifouling compound Irgarol 1051. *Mar Pollut Bull* **28**: 613-620

Gowen RJ, Bradbury NB (1987) The ecological impact of salmonid farming in coastal waters: a review. *Oceanogr Mar Biol Ann Rev* **25**: 563-575

Greene JK, Grizzle RE (2007) Successional development of fouling communities on open ocean aquaculture fish cages in the western Gulf of Maine, USA. *Aquaculture* **262**(2-4): 289-301

Greenland DC, Newton SH & Faucette RF Jr (1988) Effects of cage encrustation by the bryozoan *Plumatella casmiana* on production of channel catfish. *Prog Fish-Cult* **50**: 42-45

Guide to Drug, Vaccine, and Pesticide Use in Aquaculture. 2004 revision, http://ag.ansc.purdue.edu/aquanic/jsa/wgqaap/drugguide/drugguide.htm . Accessed (March 14, 2005)

Gularte RC, Huguenin JE (1984) Mesh hydrodynamic data. *Design guide for use of copper alloy expanded metal mesh in marine aquaculture.* INCRA Project 268B, Woods Hole Engineering Associates Inc, pp 54-58

Guldberg B, Kittelsen A, Rye M, Åsgard T (1993) Improved salmon production in large cage systems (Abstract). In Reinertsen H, Dahle LA, Jørgensen, Tvinnereim K (eds), *Fish Farming Technology*, A. A. Balkema, Rotterdam, p 241

Haegele CW, Miller DC, Brown T J, McCarter PB, Hay DE (1991) Availability of wild feed to salmon in aquaculture cages. *Can Manuscr Rep Fish Aquat Sci* no. 2117, 38 pp

Harris LG, Irons KP (1982) Substrate angle and predation as determinants in fouling community succession. In Cairns J (ed) *Artificial Substrates*. Ann Arbour Science, Michigan, pp 131-174

Hasse J (1974) Utilisation of rabbit fishes (Siganidae) in tropical oyster culture. *Prog Fish-Cult* **36**: 160-162

Henderson AR, Davies IM (2000) Review of aquaculture, its regulation and monitoring in Scotland. *J Appl Ichthyol* **16**: 200-208

Hine PM, (1992), The granulocytes of fish a review. *Fish and Shellfish Immunology* **2**: 79-98

Hisaoka M, Nogami K, Takeuchi O, Suzuki M, Sugimoto H (1966) Studies on sea water exchange in fish farms – II. Exchange of sea water in floating net. *Bull Naiki Regional Fisheries Research Laboratory [Japan]* **23**: 21-43 (in Japanese)

Hodson SL, Burke C (1994) Microfouling of salmon-cage netting: A preliminary investigation. *Biofouling* **8**: 93-105.

Hodson SL, Burke CM, Bissett AP (2000) Biofouling of fish-cage netting: The efficacy of a silicone coating and the effect of netting colour. *Aquaculture* **184**: 277-290

Hodson, SL, Burke, C, Lewis, TE, (1995), In situ quantification of fish- cage fouling by underwater photography and image analysis. Biofouling **9**: 145-151

Hodson S L, Lewis TE, Burke CM (1997) Biofouling of fish-cage netting: Efficacy and problems of in situ cleaning. *Aquaculture* **152**: 77-90

Holmström C, Kjelleberg S (1999) Factors influencing the settlement of macrofoulers. In: Fingerman M, Nagabhushanam R, Thompson M-F (eds) *Recent Advances in Marine Biotechnology, Vol 3, Biofilms, Bioadhesion, Corrosion, and Biofouling.* Enfield, NH, Science Publishers, pp 147-171

Houlden BA, de Nys R (2005) Legislation and regulation of chemicals in aquaculture in Australia. In Rough KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp., Chapter 3 this document

Huguenin JE (1997) The design, operations and economics of cage culture systems. *Aquaculture Engineering* **16**: 167-203

Huguenin J, Ansuini F (1978) A review of the technology and economics of marine fish cage systems. *Aquaculture* **15**:151-170

Huguenin JE, Ansuini F (1975) The advantages and limitations of using copper materials in marine aquaculture. *Ocean '75*, Marine Technology Society, Institute of Electrical and Electronic Engineers, pp. 444-453

Hunter CJ, Farr WE (1970) Large floating structure for holding adult Pacific salmon (*Oncorhynchus* sp.) *J Fish Res Bd Canada* **27**: 947-950

Hunter JA, Evans LV (1990) The toxicity of the biocides zineb, nabam and their derivatives to the ship-fouling diatom *Amphora coffeaeformis*. *Biofouling* **2**: 267-287

Hunter JA, Evans LV (1991a) Degradation of the biocide zineb in marine culture medium. *Biofouling* **3**: 61-68

Hunter JA, Evans LV (1991b) Raft trial experiments on antifouling paints containing zineb and copper. *Biofouling* **3**: 113-137

Inoue H (1972) On water exchange in a net cage stocked with the fish, Hamachi. *Bull Jap Soc Sci Fish* **38**: 167-176 (Abstracts and illustrations in English)

Isles M (1998) Boats follow industry in Tasmania. *Fish Farm Int* **25(5)**: 36-37

Jacobson AH, Willingham GL (2000) Sea-nine antifoulants: an environmentally acceptable alternative to organotin antifoulants. *Sci Total Environ* **258**: 103- 110

Jonsson GS (2000) Licensing, monitoring and regulation of aquaculture in Iceland. *J Appl Ichthyol* **16**: 172-176

Julian MH (1999) The roles and responsibilities of the International Maritime Organisation's Marine Environment Protection Committee. In: *10th International Congress on Marine Corrosion and Fouling*. Melbourne, Australia, 1999

Kadowaki S, Kasedo T, Nakazono T (1978) Continuous records of DO contents by cruising in the coastal culture farms. 1. Relation between DO content and fish density in cages. *Mem Fac Fish Kagoshima Univ* **27**: 273-289. (translated manuscript from Can Transl Fish Aquat Sci, 1985, no. 5189)

Karlsson J, Eklund B (2004) New biocide-free anti-fouling paints are toxic. *Mar Pollut Bull* **49**: 456-464

Kennedy WA, Griffioen W, Solmie A (1977) The 1976 crop of salmon reared on the Pacific Biological Station experimental fish farm. *Mar Fish Serv Tech Rep 726*: 21 pp

Kent ML (1990) Netpen liver disease (NLD) of salmonid fishes reared in sea water: species susceptibility, recovery and probable cause. *Dis Aquat Org* **8**: 21-28

Kent ML (2000) Marine netpen farming leads to infections with some unusual parasites. *Int J Parasitol* **30**: 321-326

Kent ML, Dawe SC, St. Hilaire S, Andersen RJ (1996) Effects of feeding rate, seawater entry and exposure to natural biota on the severity of netpen liver disease among pen-reared Atlantic salmon. *Prog Fish-Cult* **58**:43-46

Kent ML, Margolis L, Fournie JW (1991) A new eye disease in pen-reared Chinook salmon causes by metacestodes of *Gilquinia squali* (Trypanorhyncha). *J Aquatic Animal Health* **3**: 134-140

Kent ML, Sawyer TK, Hedrick RP (1988) *Paramoeba pemaquidensis* (Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea water. *Dis Aquat Org* **5**: 163-169

Kent ML, Whyte JNC, LaTrace C (1995) Gill lesions and mortality in seawater penreared Atlantic salmon *Salmo salar* associated with a dense bloom of *Skeletonema costatum* and *Thalassiosirea* species. *Dis Aquat Org* **22**: 77-81

Kobayashi N, Okamura H (2002) Effects of new antifouling compounds on the development of sea urchin. *Mar Pollut Bull* **44**: 748-751

Konstantinou IK, Albanis TA (2004) Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environment International* **30**: 235-248

Kon-Ya K, Shimidzu N, Miki W, Endo M (1994). Indole derivatives as potent inhibitors of larval settlement by the barnacle, *Balanus amphitrite*. *Biosci Biotechnol Biochem* **58**: 2178-2181

Koops H (1971) Contribution to fouling-prevention of net cages in the Western Baltic Sea. *Arch Fischereiwiss* **22**: 65-67 (in German)

Kuwa, M. (1984) Fouling organisms on floating cage of wire netting and the removal by *Oplegnathus* sp. cultured with other marine fish. *Bull Jap Soc Sci Fish* **50**: 1635-1640 (in Japanese)

Kuwa M (1984) Fouling organisms on floating cage of wire netting and the removal by *Oplegnathus* sp. cultured with other marine fish. *Bull Jap Soc Sci Fish* **50**: 1635-1640 (in Japanese)

Lai HC, Kessler AO, Khoo LE (1993) Biofouling and its possible modes of control at fish farms in Penang, Malaysia. *Asian Fisheries Science* **6**: 93-116

Larsen DK, Wagner I, Gustavson K, Forbes VE, Lund T (2003) Long-term effect of sea-nine on natural coastal phytoplankton communities assessed by pollution induced community tolerance. *Aquat Toxicol* **62**: 35-44

Lee HB, Lim LC, Cheong L (1985) Observations on the use of antifouling paint in netcage fish farming in Singapore. *Singapore J Primary Ind* **13**: 1-12

Leonard GH, Clarke RP (1993) Point quadrat versus video transect estimates of the cover of benthic red algae. *Marine Ecology Progress Series* **101**: 203-208

Leong J, Turner S (1979) Isolation of waterborne infectious hematopoietic necrosis virus. *Fish News* **8**: vi-viii

Lewin R (1984) Microbial adhesion is a sticky problem. *Science* **224**: 375-377

Lewis AG, Metaxas A (1991) Concentrations of total dissolved copper in and near a copper-treated salmon net pen. *Aquaculture* **99**:269-276

Lewis T (1994a) Impact of biofouling on the aquaculture industry. In: Kjelleberg S, Steinberg P (eds) *Biofouling: problems and solutions – Proceedings of an international workshop*. University of New South Wales, Sydney, pp 32-38

Lewis JA (1994b) Biofouling and fouling protection: a defense perspective. In: Kjelleberg S, Steinberg P (eds) *Biofouling: problems and solutions – Proceedings of an international workshop*. University of New South Wales, Sydney, pp 39-43

Li S (1994) Fish culture in cages and pens. In: Li S, Mathias J (eds) *Freshwater Fish Culture in China: Principles and Practice*, Elsevier, Amsterdam, pp 305-346

Little BJ (1984) Succession in microfouling In: Costlow J D, Tipper R C (eds) *Marine deterioration: an interdisciplinary study*. E & F N Spon Ltd, London, pp 63-67

Littlewood DTJ, Marsbe LA (1990) Predation on cultivated oysters, *Crassostrea rhizophorae* (Guilding), by the polyclad turbellarian flatworm, *Stylochus* (Stylochus) *frontalis* Verrill. *Aquaculture* **88**: 145-150

Liu D, Pacepavicius G J, Maguire R J, Lau Y L, Okamura H, Aoyama I (1999) Survey for the occurrence of the new antifouling compound irgarol 1051 in the aquatic environment. *Water Res* **33***:* 2833-2843

Lodeiros C, García N (2004) The use of sea urchins to control fouling during suspended culture of bivalves. *Aquaculture* **231**: 293-298

Loeb G J, Neihof R A (1975) Marine conditioning films. *Adv Chem Ser* **145**: 319-335

Løland G (1993) Current forces on, and water flow through and around, floating fish farms. *Aquaculture International* **1**: 72-89

Loo, M. G. K (2008). Evaluation of the seasonal development pattern of net fouling and the effects on key water parameters in the local environment of South Australia. In Rough

KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.) Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp., Chapter 5 this document

Lumsden JS, Wright P, Derksen J, Byrne PJ, Ferguson HW (1993) Paralysis in farmed Arctic char (*Salevlinus aplinus*) associated with ammonia toxicity. *Veterinary Record* **133**: 422-423

Madenjian CP (1990) Patterns of oxygen production and consumption in intensively managed marine shrimp ponds. *Aquaculture and Fisheries Management* **21**(4): 407-417

Mak PMS (1982) Biofouling of mariculture cages in Hong Kong. *Ph.D thesis*, University of Hong Kong, 534 pp

Maki JS (1999) The influence of marine microbes on biofouling. In: Fingerman M, Nagabhushanam R, Thompson M-F (eds) *Recent Advances in Marine Biotechnology, Vol 3, Biofilms, Bioadhesion, Corrosion, and Biofouling.* Enfield, NH, Science Publishers, pp 147-171

Mance G (1987) Pollution Threat of Heavy Metals in Aquatic Environments. Elsevier Applied Science, New York, 372 pp

Maroni K (2000) Monitoring and regulation of marine aquaculture in Norway. *J J Appl Ichthyol* **16**: 192-195

Matthiessen P, Reed J, Johnson M (1999) Sources and potential effects of copper and zinc concentrations in the estuarine waters of Essex and Suffolk, United Kingdom. *Mar Pollut Bull* **38**: 908-920

McMahon T (2000) Regulation and monitoring of marine aqua-culture in Ireland. *J Appl Ichthyol* **16**: 177-181

Menton DJ, Allen JH (1991) Spherical (Kiel) and square steel cages: first year of comparative evaluations at St. Andrews, NB. *Bull Aquacult Assoc Canada* **91**: 111-113

Meyers TR (1984) Marine bivalve molluscs as reservoirs of viral finfish pathogens: significance to marine and anadromous finfish culture. *Mar Fish Rev* **46(3)**: 14-17

Miller B (1998) An assessment of sediment copper and zinc concentrations in marine caged fish farms in SEPA West region. Report from the Scottish Environmental Protection Authority, March 1998, 77 pp

Miller D (1997) Remote video monitoring as a method of surveying marine soft-bottom epibenthic communities. Unpublished Masters, 85 pp. Department of Botany, University of Adelaide, Adelaide.

Milne PH (1969) Fish farm enclosures. 1. Marine growths on netting. *World Fish* **18**: 26-28

Milne PH (1970) Fish farming: A guide to the design and construction of net enclosures. Marine Research, No. 1, Department of Agriculture and Fisheries for Scotland, HMSO, Edinburgh, 31 pp

Milne PH (1975a) Fouling of marine cages – Part One. *Fish Farming Int* **2(3)**: 15-19

Milne PH (1975b) Fouling of marine cages - Part Two. *Fish Farming Int* **2(4)**: 18-21

Milne PH (1976) Engineering and economics of aquaculture. *J Fisheries Research Board Canada* **33(4)**: 888-898

Milne PH (1979) Selection of sites and design of cages, fishpens and net enclosures for aquaculture. In: Pillay TVR, Dill WA (eds) *Advances in Aquaculture*, Fishing News Books Ltd, England, pp 416-423

Milne PH & Powell HT (1967) Preliminary report of marine fouling of fish netting test panels at Millport. *Department of Civil Engineering Report*, University of Strathclyde and SMBA, 14 pp

Milne PH & Powell HT (1970) Final report of marine fouling of fish netting test panels at Millport, Loch Sween, Loch Creran, Dunstaffnage Bay. *Department of Civil Engineering Report*, University of Strathclyde and SMBA, 45 pp

Moffat CF, Whittle, KJ, (1999), Environemental contaminants in food. Sheffield Academic Press, England. 584pp

Moller D (1979) Recent developments in cage and enclosure aquaculture in Norway. In: Pillay TVR, Dill WA (eds), *Advances in Aquaculture*, Fishing News Books, Oxford, pp 447-53

Moring JR (1973) Aspects of growth, and the effects of some environmental factors on pen-related Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in Puget Sound, Washington. *Ph.D Thesis*, University of Washington, 225 pp

Moring JR & Moring KA (1975) Succession of net biofouling material and its role in the diet of pen-cultured Chinook salmon. *Prog Fish-Cult* **37**: 27-30

Moss B, Marsland A (1976) The effects of underwater scrubbing on marine fouling algae. In: Proceedings, International Marine and Shipping Congress, pp 39-43

Munday BL, Odonoghue PJ, Watts M, Rough K, and Hawkesford T, (1997), Fatal encephalitis due to the scuticociliate *Uronema nigricans* in sea-caged southern bluefin tuna *Thunnus maccoyii*. *Diseases of Aquatic Organisms* **30**: 17-25

Musgrove, R. and Fitzgibbon, Q. (2005). *Final Report - Aquafin CRC - SBT Aquaculture Subprogram: Activity metabolism in live-held southern bluefin tuna (Thunnus maccoyii).* Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 57 pp. SARDI Publication No. RD03/0104-4

Nebeker AV, Schuytema GS (1998) Chronic effects of the herbicide diuron on freshwater cladocerans, amphipods, midges, minnows, worms, and snails. *Arch Environ Contam Toxicol* **35**: 441-446

Needham T (1988) Sea water cage culture of salmonids. Salmon and Trout Farming, pp 117-154

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* **231**: 361-391

Norberg J, (1999), Periphyton fouling as a marginal energy source in tropical tilapia cage farming. *Aquaculture Research* **30**: 427-430

Nowak B, Aiken H, Bott N, Deveney M, Johnston C, McGowan T, Hayward C, (2007), Aquafin CRC – SBT Aquaculture Subprogram: investigations of the relationship between farming practices and SBT health. Aquafin CRC Project 3.5/FRDC Project No. 2003/225. 142pp

Nyström B, Becker-Van Slooten K, Berard A, Grandjean D, Druart JC, Leboulanger C (2002) Toxic effects of Irgarol 1051 on phytoplankton and macrophytes in Lake Geneva. *Water Res* **36**: 2020-2028

Okamura H, Aoyama I, Liu D, Maquire RJ, Pacepavicius GJ, Lau YL (2000a) Fate and ecotoxicity of the new antifouling compound Irgarol 1051 in the aquatic environment. *Water Res* **34**:3523-3530

Okamura H, Aoyama I, Takami T, Maruyama T, Suzuki Y, Matumoto M, Katsuyama I, Hamada, J. Beppu T, Tanaka O, Maquire RJ, Liu D, Lau YL, Pacepavicius GJ (2000b) Phytotoxicity of the new antifouing compound Irgarol 1051 and a major degradation product. *Mar Pollut Bull* **40**: 754-763

Okamura J, Watanabe T, Aoyama I, Hasobe M (2002) Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells. *Chemosphere* **46**: 945-951

Osawa Y, Tawara Y, Taketomi H (1985) Studies on behaviour of fish cages against flow of water. On relationship between volume of fish cages and current velocity. *Bulletin Natural Research Institute for Fisheries Engineering (Japan)* **6**: 297-321 (in Japanese)

O'Sullivan D, Savage J (2005) Status of Australian Aquaculture in 2002/2003. In Walker T (ed) *Austasia Aquaculture 2005 Trade Directory*, Turtle Press, Hobart, Tasmania pp 5-23

Owen R, Knap A, Toaspern M, Carbery K (2002) Inhibition of coral photosynthesis by the antifouling herbicide Irgarol 1051. *Mar Pollut Bull* **44**: 623-632

Paclibare JO, Evelyn TPT, Albright LJ, Prosperi-Porta L (1994). Clearing of the kidney disease bacterium *Renibacterium salmoninarum* from seawater by the blue mussel *Mytilus edulis*, and the status of the mussel as a reservoir of the bacterium. *Dis Aquat Org* **18**:129-133

Padula DJ, Kiermeier A, Madigan TL, Pointon AM, (2003), Review of residues in southern bluefin tuna (*Thunnus maccoyii*) in 2002/03. South Australian Research and Development Institute Food Safety Research Program. Report Nov 2003

Padula DJ, Madigan TL, Kiermeier A, Daughtry BJ, Pointon AM, (2005), A review of residues in Australian commercially farmed and wild caught southern bluefin tuna (*Thunnus maccoyii*) in 2004. South Australian Research and Development Institute Food Safety Research Program. Report Feb 2005

Pantastico JB, Baldia JP (1981) An assessment of algal growth in net cages in Laguna lake. *Fish Res J Phillip* **6**:19-25

Papoutsoglou SE (2000) Monitoring and regulation of marine aquaculture in Greece: licensing, regulatory control and monitoring guidelines and procedures. *J Appl Ichthyol* **16**: 167-171

Paul JD, Davies IM (1986) Effects of copper and tin-based antifoulant on the growth of cultivated scallops (*Pecten maximus*) and oyster (*Crassostrea gigas*). *Aquaculture* **54**: 191-203

Pedersen PB (2000) Monitoring and regulation of marine aquaculture in Denmark. *J Appl Ichthyol* **16**: 144-147

Peterson LK, D'Auria JM, McKeown BA, Moore K, Shum M (1991) Copper levels in the muscle and liver tissue of farmed chinook salmon, *Oncorhynchus tshawytscha*. *Aquaculture* **99**:105-115

Pettitt ME, Henry SL, Callow ME, Callow JA, Clare AS (2004) Activity of commercial enzymes on settlement and adhesion of cypris larvae of the barnacle *Ballanus amhitrite*,

spores of the green alga *Ulva linza*, and the diatom *Navicula perimuta*. *Biofouling* **20**: 299-311

Price RR, Patchan M, Clare A, Rittschof D, Bonaventurea J (1992) Performance enhancement of natural antifouling compounds and their analogs through microencapsulation and controlled release. *Biofouling* **6**: 207-216

Read PA, Fernandes TF, Miller K L (2001) The derivation of scientific guidelines for best environmental practice for the monitoring and regulation of marine aquaculture in Europe. *J Appl Ichthyol* **17**: 146-152

Readman JW, Kwong LL, Grondin D, Bartocci J, Villeneuve JP, Mee LD (1993) Coastal water contamination from a triazine herbicide used in antifouling paints. *Environ Sci Technol* **27**: 1940-1942

Rittschof D, Clare AS, Gerhert DJ, Bonaventura J, Smith C, Hadfield MG (1992) Rapid field assessemtn of antifouling and foul-release coatings. *Biofouling* **6**: 181-192

Rittschof D (2000) Natural product antofoulants: one perspective on the challenges related to coatings development. *Biofouling* **15**: 119-127

Rittschof D, Lai C-H, Kok L-M, Teo SL-M (2003) Pharmaceuticals as antifoulants: concepts and principles. *Biofouling* **19** (Suppl): 207-212

Rodger HD, McArdle JF (1996) An outbreak of amoebic gill disease in Ireland. *Vet Rec* **139**: 348-349

Romo H, Alveal K, Werlinger C (2001) Growth of the commercial carrageenophyte Sarcothalia crispata (Rhodophyta, Gigartinales) on suspended culture in central Chile. *Journal of Applied Phycology* **13**: 229–234

Rosenthal H, Hilge V (2000) Aquaculture production and environ-mental regulations in Germany. *J Appl Ichthyol* **16**: 163-166

Ross K A, Thorpe J P, Brand A R (2004) Biological control of fouling in suspended scallop cultivation. *Aquaculture* **229**: 99-116

Rothwell GN, Nash CE (1977) Aquaculture in Tropical Oceans, Year 01. The Oceanic Institute, Hawaii, 82pp

Rough KM, (1998), Haematology of farmed and wild southern bluefin tuna, *Thunnus maccoyii*. MSc. Thesis, University of Tasmania, 113pp

Rough KM, (2000), An illustrated guide to the parasites of southern bluefin tuna, *Thunnus maccoyii*. Tuna Boat Owners Association of SA. 74pp

Rough KM, Ellis DC, (2007). Evaluation of antifouling treatments utilising panels in the local tuna ranching environment of South Australia. In Rough KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp., Chapter 4 this document

Rough KM, Ellis DC, (2008). Evaluation of antifouling treatment with a commercially stocked cage of tuna. In Rough KM, de Nys R, Loo, MGK, and Ellis DC, (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp., Chapter 8 this document

Rough KM, Fitzgibbon Q, Loo MGK (2008) Determination of the relationship between the level of fouling and its impact on net weight, net drag and water exchange using a flume tank. In Rough KM, de Nys R, Loo MGK, and Ellis DC (Eds.). Net fouling management to enhance water quality and southern bluefin tuna (*Thunnus maccoyii*) performance. Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 292pp., Chapter 6 this document

Rough KM, Nowak B, Reuter RE, (2005), Haematology of southern bluefin tuna, *Thunnus maccoyii*. *Journal of Fish Biology* **66**: 1649-1659

Ruokalahi C (1988) Effects of fish farming on growth and chlorophyll *a* content of *Cladophora*. *Mar Pollut Bull* **19**: 166-169

Sanchez-Mata A, Mora J (2000) A review of marine aquaculture in Spain: production, regulations and environmental monitoring. *J Appl Ichthyol* **16**: 209-213

Santhanam R, Srikrishnadhas B, Natarajan P (1983) Fouling problems in cages and pens. In: *Proc Natl Seminar on Cage and Pen Culture, Fisheries College, Tamil Nadu Agricultural University, Tuticorin.* Tamil Nadu Agricultural University, pp 143-147

Saroglia M, Cecchini S, Saroglia-Terova G (2000) Review of regulations and monitoring of Italian marine aquaculture. *J Appl Ichthyol* **16**: 182-186

Satchell GH, (1991), Physiology and form of fish circulation. Cambridge University Press

Scardino A, de Nys R, Ison O, O'Connor W, Steinberg PD (2003) Microtopography and antifouling properties of sell surface of the bivalve mollusks *Mytilus galloprovincialis* and *Pinctata imbricata*. *Biofouling* **19** (Suppl): 221-230

Scarlett A, Donkin P, Fileman TW, Morris RJ (1999) Occurrence of the antifouling herbicide Irgarol 1051, within coastal-water seagrasses from Queensland, Australia. *Mar Pollut Bull* **38**: 687-691

Seymour R, Fitzgibbon Q, Buchanan J, Ellis D, Musgrove R, Frappell P, Clark T Carragher J (2007) *Final Report - Activity metabolism in live-held southern bluefin tuna (Thunnus maccoyii), Phase 2.* Aquafin CRC, Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic Sciences), Adelaide. 100 pp. SARDI Publication No.

Sharma RN (1979) Cage fish culture in Nepal. *Proc IDRC/SEAFDEC Int Workshop on Cage and Pen Culture of Fish*, Tigbauan, *Iloilo, Philippines*, SEAFDEC pp 93-96

Short JW, Thrower FP (1986) Accumulation of butyltin in mussel of Chinook salmon reared in sea pens treated with tri-*n*-butyltin. *Mar Pollut Bull* **17**: 542-545

Silvert W (1992) Assessing environmental impacts of finfish aquaculture in marine waters. *Aquaculture* **107**: 67-79

Silvert W (1994) Simulation models of finfish farms. *J App Ichthyology* **10**: 349-352

Sliskovic M, Jelic G (2002) Problems of biofouling on fish-cage nets in aquaculture. *Ribarstvo* **60**: 105-115

Smaal AC, Lucas L (2000) Regulation and monitoring of marine aquaculture in The Netherlands. *J Appl Ichthyol* **16**: 187-191

Solberg CB, Saethre L, Julshamn K (2002) The effect of copper-treated net pens on farmed salmon (Salmo salar) and other marine organisms and sediments. *Marine Pollution Bulletin* **45**: 126-132

Sorensen, EM (1991) Metal Poisoning in Fish. CRC Press, Boca Raton, Florida Sutterlin AM, Merrill SP (1978) Norwegian salmonid farming. *Tech Report Fisheries Marine Services of Canada*, No. 779, 47 pp

Sutterlin AM, Merrill SP (1978) Norwegian salmonid farming. *Tech Report Fisheries Marine Services of Canada*, No. 779, 47 pp

Svane I, Cheshire A, Barnett J (2006) Test of an antifouling treatment on tuna fish-cages in Boston Bay, Port Lincoln, South Australia. *Biofouling* **22**: 209-219

Svane I, Dolmer P, (1995) Perception of light at settlement: a comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *Journal of Experimental Marine Biology and Ecology* **187**(1): 51-61

Swain GW, Griffith JR, Bultman JD, Vincent HL (1992) The use of barnacle adhesion measurement for the field evaluation of non-toxic foul release surfaces. *Biofouling* **6**: 105-114

Swain G, Herpe S, Ralston E, Tribou M (2006) Short-term testing of antifouling surfaces: The importance of colour. *Biofouling* **22**(6): 425-429

Swift MR, Fredriksson DW, Unrein A, Fullerton B, Patursson O, Baldwin K (2006) Drag force acting on biofouled net panels. *Aquacultural Engineering* **35**(3): 292-299

Swingle WE (1972) Alabama's marine cage culture studies. *Proceedings of the Third Annual Workshop, World Mariculture Society* **3**: 75-81

Tan CK, Nowak BF, Hodson SL (2002) Biofouling as a reservoir of *Neoparamoeba pemaquidensis* (Page, 1970), the causative agent of amoebic gill disease in Atlantic salmon. *Aquaculture* **210**: 49-58

Terlizzi A, Conte E, Zupo V, Mazzella L (2000) Biological succession on silicone fouling release surfaces: long term exposure tests in the harbour of Ischia, Italy. *Biofouling* **15**: 327-342

Terlizzi A, Fraschetti S, Gianguzza P, Faimali M, Boero F (2001) Environmental impact of antifouling technologies: state of the art and perspectives. *Aquatic Conserv Mar Freshw Ecosyst* **11**: 311-317

Thomas KV (1998) determination of selected antifouling paint booster biocides by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Chromatog A* **825**: 153-158

Thomas KV (2001) The environmental fate and behaviour of antifouling paint booster biocides: a review. *Biofouling* **17**: 73-86

Thomas KV, Benstead RE, Thain JE, Waldock MJ (1999) Toxicity characterization of organic contaminants in industrialized UK estuaries and coastal waters. *Mar Pollut Bull* **38**: 925-932

Thomas KV, Blake SJ, Waldock MJ (2000) Antifouling paint booster biocide contamination in UK marine sediments. *Mar Pollut Bull* **40**:739-745

Thomas KV, Fileman TW, Readman JW, Waldock MJ (2001) Antifouling paint booster biocides in the UK coastal environment and potential risks of biological effects. *Mar Pollut Bull **42**:* 677-688

Thomas KV, McHugh M, Hilton M, Waldock M (2003) Increased persistence of antifouling paint biocides when associated with paint particles. *Environ Pollut* **123**:153-161

Thomas KV, McHugh M, Waldock M (2002) Antifouling paint booster biocides in UK coastal waters: Inputs, occurrence and environmental fate. *Sci Total Environ* **293**:117-127

Tolosa I, Readman JW, Blaevoet A, Chilini S, Bartocci J, Horvat M (1996) Contamination of Mediterranean (Cote d'Azur) coastal waters by organotins and Irgarol 1051 used in antifouling paints. *Mar Pollut Bull* **32**: 335-341

Tomi W, Naiki K, Yamada Y (1979) Investigations into technical development of mariculture on commercial scale applied to offshore region. *Proceedings of the Japan-Soviet Joint Symposium on Aquaculture* **7**: 111-120

Tóth S., Becker-van Slooten K, Spack L., de Alencastro LF, Tarradellas J (1996) Irgarol 1051, an antifouling compound in freshwater sediment, and biota of Lake Geneva. *Bull Environ Contam Toxicol* **57**: 426-433

Tseng WY, Yuen KH (1979) Studies on fouling organisms on mariculture nets and cages in Hong Kong. In: *Proc Aquatic Environment in Pacific Region*. Taipei, China pp 151-159

Turley PA, Fenn RJ, Ritter JC (2000) Pyrithiones as antifoulants: Environmental chemistry and preliminary risk assessment. *Biofouling* **15**: 175-182

Turley PA, Fenn RJ, Ritter JC, Callow ME (2005) Pyrithiones as antifoulants: Environmental fate and loss of toxicity. *Biofouling* **21**: 31-40

Varjopuro R, Sahivirta E, Makinen T, Helminen H (2000) Regulation and monitoring of marine aquaculture in Finland. *J Appl Ichthyol* **16**:148-156

Voulvoulis N, Scrimshaw MD, Lester JN (1999a) Alternative antifouling biocides. *Appl Organomet Chem* **13**: 135-143

Voulvoulis N, Scrimshaw MD, Lester JN (1999b) Analytical methods for the determination of 9 antifouling paint booster biocides in estuarine water samples. *Chemosphere* **38**:3503-3516

Voulvoulis N, Scrimshaw MD, Lester JN (2000) Occurrence of four biocides utilized in antifouling paints, as alternatives to organotin compounds, in waters and sediments of a commercial estuary in the UK. *Mar Pollut Bull* **40**: 938-946

Voulvoulis N, Scrimshaw MD, Lester JN (2002a) Comparative environmental assessment of biocides used in antifouling paints. *Chemosphere* **47***:* 789-795

Voulvoulis N, Scrimshaw MD, Lester JN (2002b) Partitioning of selected antifouling biocides in the aquatic environment. *Mar Environ Res* **53**:1-16

Wahl M (1989) Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Mar Ecol Prog Ser* **58**: 175-189

Wahl M, Kröger K, Lenz M (1998) Non-toxic protection against epibiosis. *Biofouling* **12**: 205-226

Waldcock MJ, Miller D (1983) The determination of tributyltin in sea water and oysters in areas of high pleasure craft activity. *Int Coun Expl Sea, Stat Meeting CM 1983/E:12*

Wee KL (1979) Ventilation of floating cages. *M.Sc. Thesis*, University of Stirling, 42pp

WHO (1999) *Food Safety Issues Associaited with Products from Aquaculture.* Report of a Joint FAO/NACA/WHO Study Group, WHO Technical Report Study Series 883, WHO, Geneva

Wildish DJ, Keizer PD, Wilson AJ, Martin JL (1993) Seasonal changes of dissolved oxygen and plant nutrients in seawater near salmonid net pens in the macrotidal Bay of Fundy. *Can J Fish Aquat Sci* **50**: 303-311

Willemsen PR, Ferrari GM (1993) The use of anti-fouling compounds from sponges in anti-fouling paints. *Surface Coat Int* **10**: 423-427

Willinsky MD, Robson DR, Vangool WJ, Fournier RA, Allen JH (1991) Design of a spherical, submersible, self-cleaning aquaculture system for exposed sites. In: Hirata GN (ed) *Proceedings of the National Science Foundation Workshop on Offshore Engineering and Mariculture*, Honolulu, USA, pp 317-336

www.foodstandards.gov.au/monitoringandsurveillance/nuttab2006/onlineversion

Yap, C.K., Ismail, A., and Tan, S.G., 2004, Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. Food Chemistry **84**: 569-575

Yebra DM, Kiil S, Dam-Johansen K (2004) Antifouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Prog Organic Coatings* **50**: 75-104

Yokoyama H, Inoue M, Abo K (2004) Estimation of the assimilative capacity of fish-farm environments based on the current velocity measured by plaster balls. *Aquaculture* **240**(1-4): 233-247

Zar JH (1996) Biostatistical analysis. 3rd. New Jersey: Prentice-Hall. 662 pp.

APPENDICES

APPENDIX 3.1: LEGISLATION, ACTS

Document including links on accompanying CD

The **legislation** comprised the following Acts, which are tabled below:

• the *Agricultural and Veterinary Chemicals (Administration) Act 1992* [Download Act]

• the *Agricultural and Veterinary Chemicals Act 1994* [No. 36 of 1994] AgricVetsChem1994.pdf

• the *Agricultural and Veterinary Chemicals Code Act 1994* [No. 47 of 1994] AgVetChemCode1994.pdf

• the *Agricultural and Veterinary Chemicals (Consequential Amendments) Act 1994* [No. 37 of 1994] text0010.text

• the *Agricultural and Veterinary Chemical Products (Collection of Levy) Act 1994* [No. 41 of 1994] AgriVetChemProdCollLevy1994.pdf

• the *Agricultural and Veterinary Chemical Products Levy Imposition (Customs) Act 1994* [No. 39 of 1994] text0010.text

• the *Agricultural and Veterinary Chemical Products Levy Imposition (Excise) Act 1994* [No. 38 of 1994] AgVetChemProdLevyImpExc94.pdf

• the *Agricultural and Veterinary Chemical Products Levy Imposition (General) Act 1994* [No. 40 of 1994]. text0010.text

• the *Agricultural And Veterinary Chemicals Legislation Amendment Act 2001 No. 83, 2001* [Download Act]

• *Agricultural And Veterinary Chemicals Legislation Amendment Act 2003 No. 13, 2003* [Download Act]

• *Agricultural And Veterinary Chemicals Legislation Amendment (Name Change) Act 2004 No. 79, 2004* [Download Act]

APPENDIX 3.2: LEGISLATION, STATUTORY RULES AND REGULATIONS

Document including links on accompanying CD

Explanatory Statements of statutory rules can be found at http://scaleplus.law.gov.au for:

Agricultural and Veterinary Chemicals Regulations (Amendment) 1992 No.172

Agricultural and Veterinary Chemical Products (Collection of Interim Levy) Regulations 1994 No. 215

Agricultural and Veterinary Chemicals Regulations 1994 No. 216

Agricultural and Veterinary Chemical Products (Collection of Levy) Regulations 1995 No. 120

Agricultural and Veterinary Chemicals (Administration) Regulations 1995 No. 28

Agricultural and Veterinary Chemicals Code Regulations 1995 No. 27

Agricultural and Veterinary Chemicals Code Regulations 1995 No. 59

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1995 No. 137

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1995 No. 187

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1995 No. 54

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1996 No. 111

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1996 No.162

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1996 No.216

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1996 No.83

Agricultural and Veterinary Chemicals (Administration) Regulations (Amendment) 1997 No. 320

Agricultural and Veterinary Chemicals Code Regulations (Amendment) 1997 No.264

Agricultural and Veterinary Chemicals Code Amendment Regulations 1999 (No. 1) 1999 No. 215

Agricultural and Veterinary Chemicals Code Amendment Regulations 1999 (No. 2) 1999 No. 247

Agricultural and Veterinary Chemicals Code Regulations 1999 1999 No. 242

Agricultural and Veterinary Chemicals Regulations 1999 1999 No. 326

Agricultural and Veterinary Chemical Products (Collection of Levy) Amendment Regulations 2000 (No. 1) 2000 No. 91

Agricultural and Veterinary Chemical Code Amendment Regulations 2002 (No. 1) 2002 No. 207

Agricultural and Veterinary Chemical Code Amendment Regulations 2003 (No. 1) 2003 No. 8

Agricultural and Veterinary Chemicals (Administration) Amendment Regulations 2004 (No. 1) 2004 No. 242

Agricultural and Veterinary Chemicals Code Amendment Order 2004 (No. 1) 2004 No. 55

Agricultural and Veterinary Chemicals Code Amendment Order 2004 (No. 2) 2004 No. 406

Agricultural and Veterinary Chemicals Code Amendment Regulations 2004 (No. 1) 2004 No. 224

Agricultural and Veterinary Chemicals Code Amendment Regulations 2004 (No. 2) 2004 No. 225

Agricultural and Veterinary Chemicals Code Amendment Regulations 2004 (No. 3) 2004 No. 251

Agricultural and Veterinary Chemicals Code Amendment Regulations 2004 (No. 4) 2004 No. 353

Agricultural and Veterinary Chemicals Code Amendment Regulations 2004 (No. 5) 2004 No. 354

APPENDIX 3.3: REGISTERED ANTIFOULING PRODUCTS (APVMA)

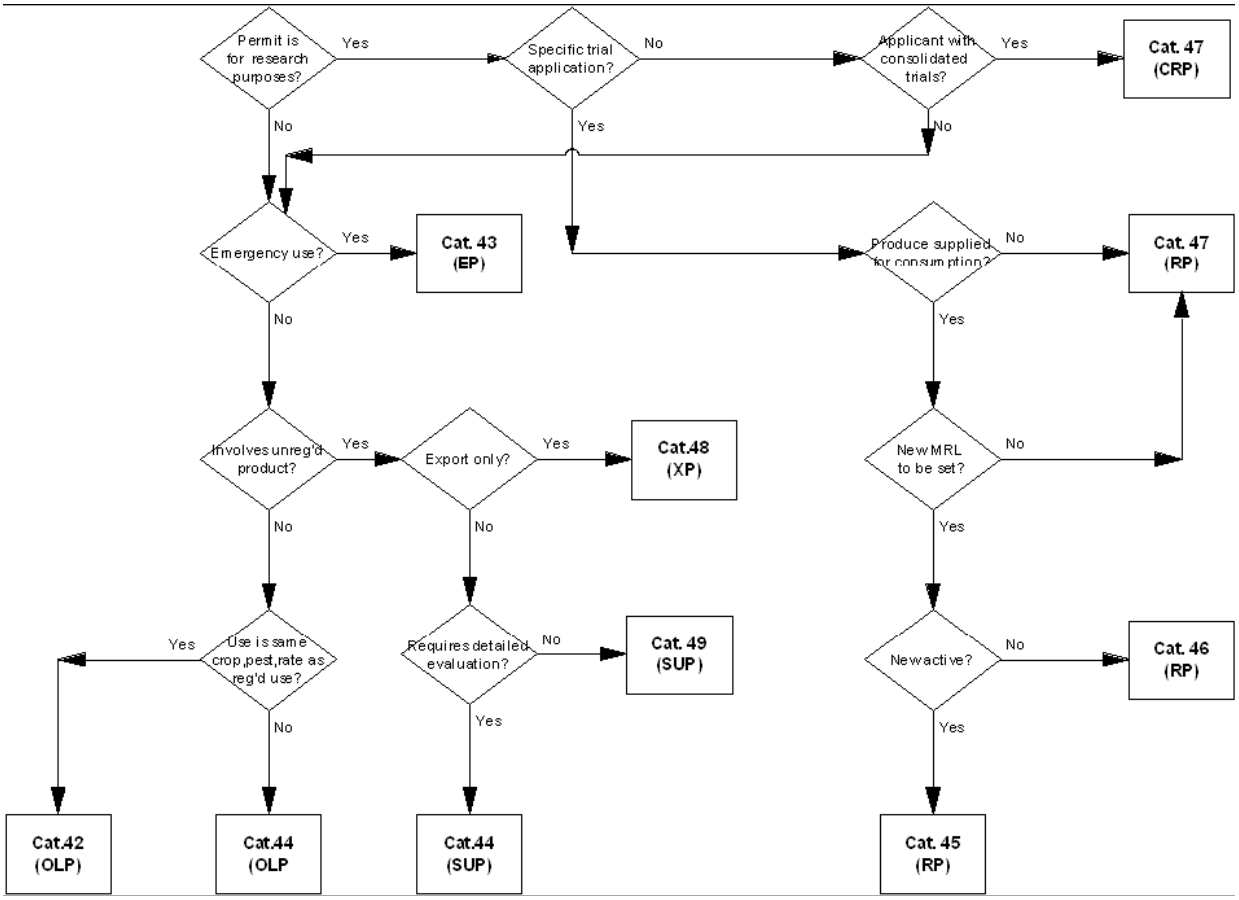
Document including links on accompanying CD

Product Code	Product Name
40163	ANTIFOULING SEAGUARDIAN
40164	ANTIFOULING SUPER TROPIC
40185	WATTYL MARINE COATINGS SEAPRO ANTIFOULING
40186	WATTYL MARINE COATINGS SIGMAPLANE ECOL ANTIFOULING
42439	40 SOUTH MARINE PAINT COPPERTOX LONGLIFE ANTIFOULING
42603	ANTIFOULING OLYMPIC 7154
42708	40 SOUTH MARINE PAINT MEMBRANE CR95 COPPER ANTIFOULING
42709	40 SOUTH MARINE PAINT FISHERMANS ANTIFOULING
42710	TOP QUALITY 40 SOUTH MEMBRANE CR97 CTC ANTIFOULING
46487	ANTIFOULING SEASAFE
46488	ANTIFOULING SEAVICTOR 50
46489	ANTIFOULING SEAVICTOR 40
46918	HEMPELS ANTIFOULING MILLE DYNAMIC ALU
46919	HEMPELS ANTIFOULING MILLE DYNAMIC
46920	HEMPELS ANTIFOULING NAUTIC
46921	HEMPELS ANTIFOULING PACIFIC
47587	INTERNATIONAL INTERVIRON SUPER ANTIFOULING TOPCOAT
47588	INTERNATIONAL INTERVIRON SUPER ANTIFOULING BASECOAT
48675	RABAMARINE AF100 ANTIFOULING
48843	40 SOUTH MARINE PAINT ATLANTIC CONTROLLED SOLUBILITY COPOLYMER ANTIFOULING
48965	MARINE SYSTEMS TRADITIONAL COPPER BASED ANTIFOULING
48969	TRANSOCEAN MARINE PAINT CLEANSHIP ANTIFOULING 2.95
48970	TRANSOCEAN MARINE PAINT LONGLIFE ANTIFOULING 2.77
49606	INTERNATIONAL EPIGLASS LONGLIFE HIGH STRENGTH HARD ANTIFOULING
49607	INTERNATIONAL EPIGLASS INTERSPEED 2000 HARD ANTIFOULING FOR ALUMINIUM
49608	INTERNATIONAL EPIGLASS CRUISER SUPERIOR ABLATIVE ANTIFOULING FOR ALUMINIUM
49609	INTERNATIONAL EPIGLASS VC OFFSHORE EXTRA POLYMER REINFORCED RACING ANTIFOULING
49610	INTERNATIONAL EPIGLASS BOTTOMKOTE ERODING ANTIFOULING
49611	INTERNATIONAL EPIGLASS MICRON CSC HIGH STRENGTH SELF POLISHING ANTIFOULING
49612	INTERNATIONAL EPIGLASS COPPERCOAT ABLATIVE ANTIFOULING
49687	HEMPEL'S SEATECH ANTIFOULING
49871	INTERNATIONAL BIOLUX NEW TECHNOLOGY MICRON OPTIMA WATER BASED ANTIFOULING
49992	INTERNATIONAL EPIGLASS COPPERCOAT EXTRA TRADE ANTIFOULING
51971	INTERNATIONAL INTERSMOOTH 360 ECOLOFLEX ANTIFOULING
52240	NEWPORT 99 COPPER OXIDE FREE ANTIFOULING
52241	NEWPORT 88 HARD RACING ANTIFOULING
52242	WATTYL MARINE COATINGS SIGMAPLANE ECOL HA 120 ANTIFOULING
52243	NEWPORT 77 SELF-POLISHING COPPER ANTIFOULING
52864	WATTYL MARINE COATINGS SIGMA ECOL IV ANTIFOULING BLACK
52961	WATTYL MARINE COATINGS SIGMA ECOL IV ANTIFOULING RED/BROWN
53398	INTERNATIONAL BIOLUX NEW TECHNOLOGY MICRON EXTRA HIGH STRENGTH SELF POLISHING ANTIFOULING
54009	WATTYL MARINE COATINGS TRAWLER ANTIFOULING
54048	NORGLASS TOPFLIGHT ANTIFOULING
54514	HEMPEL'S ANTIFOULING GLOBIC
55875	ABC #3 ANTIFOULING
56205	WATTYL MARINE COATINGS SIGMA ALPHAGEN 20 ANTIFOULING
56303	40 SOUTH MARINE PAINT SUPER STRENGTH XP ANTIFOULING
56524	WATTYL MARINE COATINGS SEAPRO PLUS ANTIFOULING
56562	INTERNATIONAL INTERSMOOTH 460 ECOLOFLEX ANTIFOULING
56582	INTERNATIONAL BIOLUX SELF POLISHING COPOLYMER MICRON 66 ANTIFOULING
58058	ALTEX YACHT & BOAT PAINT NO 5 ANTIFOULING
58059	ALTEX YACHT & BOAT PAINT N05 ANTIFOULING OYSTER WHITE
58268	AWLCRAFT MARINE PAINT AWLCRAFT ANTIFOULING
59136	BOERO SUPERNAVI TRANSOCEANIC YACHT COATINGS SA633 SELF POLISHING ABLATIVE ANTIFOULING

APPENDIX 3.4: REGISTERED ACTIVE CONSTITUENTS IN ANTIFOULING PRODUCTS (APVMA)

Chemical Group	Constituent Name	Australia	Canada	UK
Mineral-copper	COPPER PRESENT AS METTALIC POWDER	X		
Mineral-copper	COPPER PRESENT AS CUPROUS OXIDE	X	X	X
Cyanide	COPPER PRESENT AS CURPOUS THIOCYANATE	X	X	X
Mineral-copper	COPPER PYRITHIONE (COPPER OMADINE)		X	
Mineral-zinc	ZINC AS ZINC OXIDE	X		
Mineral-zinc	ZINC PYRITHIONE (ZINC OMADINE)	X		X
Urea substituted	DIURON [3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA]	X		X
Carbamate-dithiocarbamate	ZINEB (ZINC ETHYLENE-BIS DITHIOCARBAMATE)	X		X
Carbamate-dithiocarbamate	THIRAM	X		
Ungrouped	SEA NINE 211 (4,5-DICHLORO-2-N-OCTYL-4-ISOTHIAZOLIN-3-ONE) aka KATHONE	X		X
Benzene	CHLOROTHALONIL (2,4,5,6-TETRACHLORO-ISOPHTHALONITRILE)	X		X
s-triazine	IRGAROL (2-METHLYTHIO-TERTIARY-BUTYLAMINO-6-CYCLOPROPYLAMINO-STEIAZINE)	X		
	DICHLORFLUANID (N'-DIMETHYL-N-PHENYLSULPHANAMIDE)		X	
	TCMS (2,3,5,6-TETRACHLORA-4-METHYLSULFONYL) PYRIDINE		X	
	TCMTB (2-THIOCYANOMETHYLTHIOBENZOTHIAZOLE)		X	

APPENDIX 3.5: APVMA DECISION TREE FOR MINOR USE PERMITS



APPENDIX 3.6: APVMA SPECIFIC DATA REQUIREMENTS

Document including links on accompanying CD

The full text of the APVMA's requirements and guidelines is available by following the links, and a summary of the data requirements is given below.

Part 1 Application Overview
"A brief overview of the entire application." (APVMA 2005)
part 1 Application overview

Part 2 Chemistry and Manufacture
"Active Constituent: data to identify the active constituent (common name, chemical name, CAS registry number, manufacturer's code number, molecular formula, molecular weight and structural formula/diagram), its manufacturer and manufacturing site address, manufacturing process and quality control, specifications/DoC, batch analysis, analytical methods and validation data.
Product: data to clearly identify the product, formulator, formulation type, composition and manufacturing process, physical and chemical properties, product specifications, batch analysis, stability data, analytical methods and validation data and packaging." (APVMA 2005)
part 2 Chemistry and Manufacture

Part 3 Toxicology
"Results of toxicity studies (acute, short-term and long-term); reproduction studies; developmental studies; genotoxicity studies; and studies of the toxicity of metabolites and impurities, other adverse effects and toxicology of mixtures. Data on human toxicology, the no observable effect level, acceptable daily intake (for humans), and proposed first aid and safety directions." (APVMA 2005)
part 3 Toxicology

Part 4 Metabolism and Toxicokinetics
"Results of metabolic studies in target crops and animals. Metabolic and toxicokinetic studies in laboratory animals. Database of all metabolic studies considered." (APVMA 2005)
part 4 Metabolism and Kinetics

Residues and trade: Part 5
Part 5a Residues
"Complete, detailed proposed use-pattern for the product, including dose rate and regime and proposed withholding period. Data showing the nature, level and safety of residues and metabolites resulting from the proposed use-pattern of the product and the effect of any major variables. Included should be residues in crops, livestock, poultry, eggs, milk and (if applicable) wool. Fate of residues during storage, processing and cooking. A proposed maximum residue limit (MRL) and data on MRLs in Australia, other countries and Codex." (APVMA 2005)

The following chemicals are listed in the MRL Standard Table 5 "Uses of substances where maximum residue limits are **not** necessary":
• copper oxide (antifouling treatment of nets in aquaculture
• sea-nine 211 (antifouling paint on nets in aquaculture)
• zinc oxide (antifouling treatment of nets in aquaculture)
• zinc pyrithione (antifouling treatment of nets in aquaculture)

Part 5b Overseas Trade Aspects of Residues in Food
”Information about the overseas registration status of the product/active constituent, use patterns and MRLs overseas, export intervals, labelling, compliance with overseas MRLs, authorities and growers views on use as proposed, and gazettal/trade advice notices.” (APVMA 2005)
Refer to Residues and Minor crops (factsheet)
part 5A Residues
part 5B Residue Guidelines
part 5B Overseas trade aspects of residues in food commodities

Occupational health and safety: Part 6
“Data on potential occupational exposure of workers to the active constituent, end-use product and residues. Health conditions contraindicating use of the product. Occupational health monitoring, including atmospheric and biological monitoring (as applicable). Safety information to be provided on the label, Material Safety Data Sheets and through education/training.” (APVMA 2005)
part 6 Occupational Health and Safety

Environmental safety: Part 7
“An assessment of the extent of, and potential for, environmental exposure during manufacture, use, disposal and through accident. Results of laboratory studies on the degradation of the chemical in water and by light; the metabolism of the chemical (both aerobic and anaerobic); bioaccumulation in fish, aquatic organisms and other species; and mobility in soil. Results of field studies to determine degradation (persistence) and leachability. Ecotoxicity studies of birds, mammals and other vertebrates; aquatic organisms and non-target invertebrates and native vegetation.” (APVMA 2005)
part 7 Environment

Efficacy and host safety: Part 8
”Comprehensive data from laboratory and field trials which show that the product is effective for the purposes claimed and safe for the intended crops (or species) and non-target crops, plants and animals” (APVMA 2005). **Note that there are specific guidelines on describing antifouling efficacy**
part 8 Efficacy and Crop Safety

Part 9 Other Trade Aspects
”Data on the trade aspects of a product relating to matters other than residues in food; e.g. environmental concerns about residues in wool.”
(APVMA 2005)

Data protection
Applicants need to check for any protected data relevant to their applications (Current Protected Data information 20 January 2005). Where protected data exists, applicants have to demonstrate that the necessary access to the protected data has been granted. There are currently three types of protected data.
1.) Chemical Review (contact on chemrev@apvma.gov.au)
2.) Trade-Related Aspects of Intellectual Property Rights (TRIPS) arising from the World Trade Organisation agreement (contact the Chemistry and Residues Program by phone on 02 6272 3212 or by email at apvma.chemistry@apvma.gov.au.)

3.) Application Protected Data. “On 1 January 2005, the US Free Trade Agreement Implementation Act 2004 commenced. This Act amended both the Agvet Admin Act and the Agvet Code Act to require the APVMA to implement a new data protection and transparency regime to do with applications for active constituents, chemical products and product labels.” (APVMA 2005) For a more detailed explanation of the changes please read The New Data Protection Provisions and the AgVet Chemical Industry Updated 9 February 2005

Applicants should be aware that the APVMA has developed new requirements in order to implement the new legislative provisions (see Data Protection Application Requirements Updated 20 January 2005).

APPENDIX 3.7: USEFUL CONTACTS

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APPENDIX 3.8: REGISTERING A NEW AGRICULTURAL CHEMICAL PRODUCT – PRIMARY APPLICATION (CATEGORY 1)

This category is for applications to register new chemical products which contain at least one new active constituent that has never before been approved or registered in Australia. This applies to all products defined as agricultural chemical products, including domestic situations, home gardens, swimming pools and industrial applications.

Some products based on commodity chemicals and biological products may be considered under the modular fee scale.

The evaluation fee for Category 1 applications includes the cost of evaluation of the new active constituent.

Applicants must supply:

a) a covering letter stating the exact purpose of the application - see Registration Process Manual (update available Dec 2003)

b) the **fee**

c) a fully completed APVMA application form for both the product **(form KP22-2F3)** and the active constituent **(form KP21F8) available from the** forms page.

d) a draft label for each pack size

e) information that addresses each of the following data requirements:

part 1 Application overview

part 2 Chemistry and Manufacture of product

part 3 Toxicology of the product

part 4 Metabolism and Kinetics

part 5A Residues

part 5B Residue Guidelines

part 5B Overseas trade aspects of residues in food commodities

part 6 Occupational Health and Safety

part 7 Environment

part 8 Efficacy and Crop Safety

part 9. Other trade aspects

APPENDIX 3.9: REGISTERING A NEW PRODUCT, APPROVED ACTIVE CONSTITUENT, NEW SITUATION (CATEGORY 14)

Examples of products in this category include a new product intended for commercial use where there is already a registered product with the same active constituent for use in the home garden, or a new product for use in orchards based on an active constituent already approved for use as a seed dressing.

Where the product is intended for use on a new food producing crop, residue data will be required, together with a proposed MRL and withholding period.

All of the data requirements outlined in Agricultural Requirements Series should be addressed. Where an applicant considers that particular data are not required, relevant scientific argument should be provided.

Applicants must supply:

a) a covering letter stating the exact purpose of the application - see Process Manual (new edition due late 2003)

b) the fee

c)a fully completed APVMA application form

d) a draft label for each pack size

e) information that addresses the following data requirements:

part 1 Application overview

part 2 Chemistry and Manufacture of product

part 3 Toxicology of the product

part 4 Metabolism and Kinetics

part 5A Residues

part 5B Residue Guidelines

part 5B Overseas trade aspects of residues in food commodities

part 6 Occupational Health and Safety

part 7 Environment

part 8 Efficacy and Crop Safety

part 9. Other trade aspects

APPENDIX 3.10: VARIATION TO REGISTERED PRODUCT: MAJOR EXTENSION OF USE (CATEGORY 32)

This category includes all extensions to a new host or situation (eg. cotton to apples); (or those involving a higher rate or frequency of use).

Applicants must supply:

a) a covering letter stating the exact purpose of the application and listing all proposed label changes

b) the fee

c) a fully completed APVMA application form

d) a draft labels (two copies, including one highlighting all proposed changes)

e) copy of currently registered label if the product has not previously been assessed by the APVMA

f) information that addresses the following data requirements:

part 1 Application overview

part 5A Residues

part 5B Residue Guidelines

part 5B Overseas trade aspects of residues in food commodities

part 6 Occupational Health and Safety

part 7 Environment

part 8 Efficacy and Crop Safety

part 9. Other trade aspects

APPENDIX 4.1: PANEL EXPERIMENTAL DESIGN

PANEL EXPERIMENTAL DESIGN:

* 5 treatments (fresh zinc; aged zinc; latex; lanolin; untreated control) tested at 2 depths (2-3m and 9-10m)
* 6 sample intervals set 4 to 5 weeks apart
* 4 replicate panels of each treatment to be removed at each sample interval (ie 20 taken off each line and at each depth per sample time)

This statistical design means there are a total of 120 panels per depth

Individual panel size is 8 x 12 meshes (60cm wide x 90cm deep)

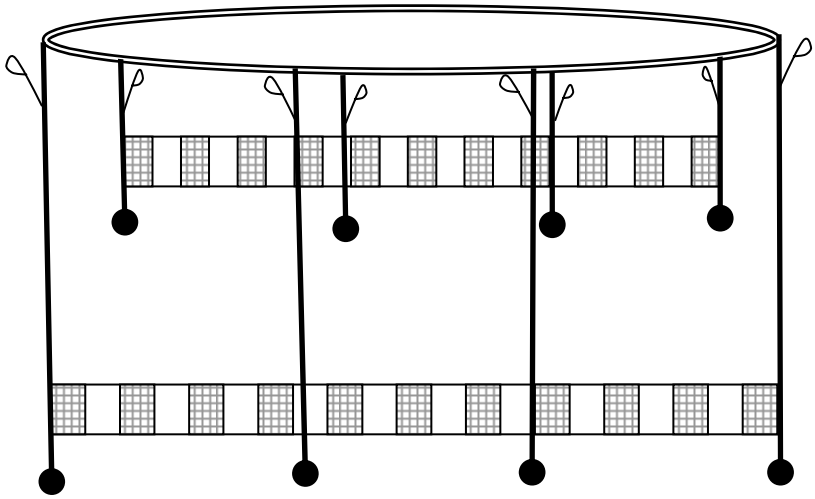
Require 20cm gap / free space between net panels to allow clear water flow

These parameters equate to 72m of netting and 24m of gaps = 96m total.

To ensure panels are approximately cross the current flow and all have equalled and unshaded access to the north; this total length needs to be split into 2 panel lines of 48m each at each depth; ie a total of 4 lines of 60 panels.

BASIC LAYOUT (diagram below not to scale):

* Panel line at 2m depth hung from inside of collar
* Panel line at 9m depth hung from outside of collar
* Two panel lines (1 at 2m and 1 at 9m depth) on north side of cage
* Two panel lines (1 at 2m and 1 at 9m depth) on south side of cage
* Each panel line has 4 down ropes spaced at 16m intervals
* All down ropes have a weight connected and a soft eye spliced near surface for ease of lifting



LEGEND:

Tuna cage collar

Line of net panels and gaps

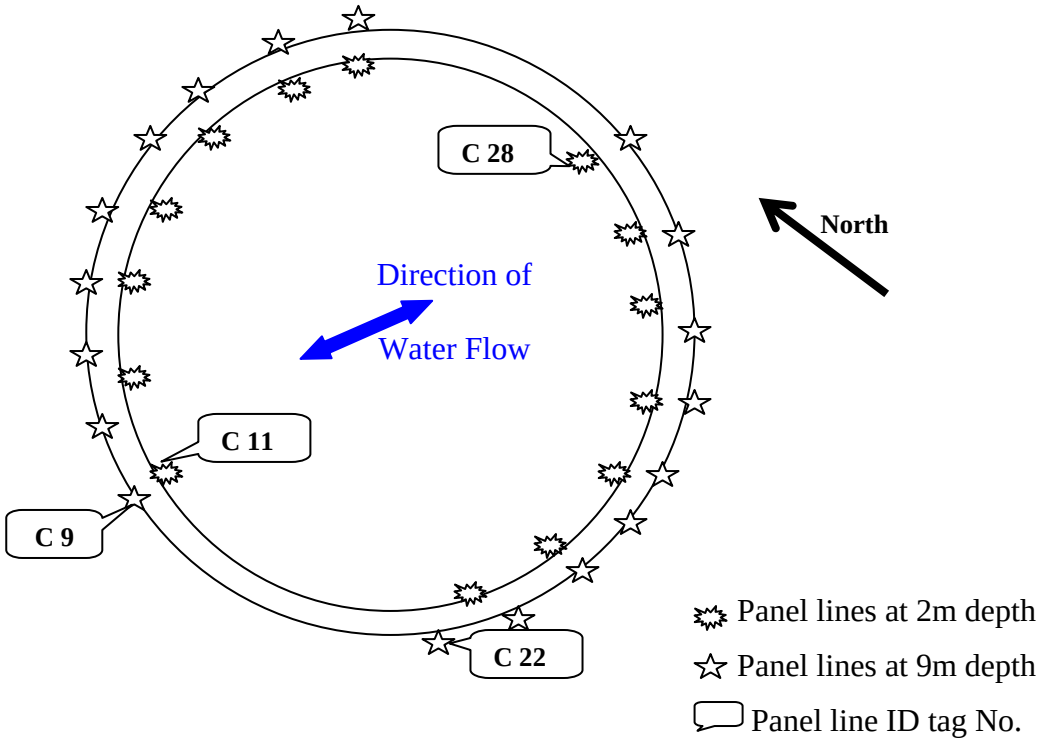
Down rope with weight attached

Soft eye spliced to down rope

SPECIFICATIONS:

- Use 14mm rope throughout (ie all down and cross ropes)
- Colour code or use different colour ropes for the 9m panel lines
- Use 4 x 20kg weights on each 2m panel line
- Use 4 x 100kg weights on each 9m deep panel line
- Leave a minimum 1.5m loose tail at each end of the down ropes (for tying to each of the collar and the weight)
- Splice a 2m length of rope with a soft eye on loose end, into all down ropes (for lifting); so that the splice is approximately 0.5m below tuna cage collar
- Panels to be loosely stitched to cross ropes at 20cm intervals (so that can be easily cut from line without interfering with other panels or damaging main rope line)

DEPLOYED LAYOUT (diagram not to scale):
Approximate Layout of experimental panel lines



Panel number and treatment type:
A 1 to 24 panels treated with Lanolin (LA) and deployed at 2m depth
A 25 to 48 panels treated with Aged Net Clear ZPT (AZ) and deployed at 2m depth
A 49 to 72 panels untreated control (UT) and deployed at 2m depth
A 73 to 96 panels treated with Net Clear (NC) and deployed at 2m depth
A 101 to 124 panels treated with Fresh Net Clear ZPT (FZ) and deployed at 2m depth

B 1 to 24 panels treated with Lanolin (LA) and deployed at 9m depth
B 25 to 48 panels treated with Aged Net Clear ZPT (AZ) and deployed at 92m depth
B 49 to 72 panels untreated control (UT) and deployed at 9m depth
B 73 to 96 panels treated with Net Clear (NC) and deployed at 9m depth
B 101 to 124 panels treated with Fresh Net Clear ZPT (FZ) and deployed at 9m depth

Note: Panels randomly assigned a position along the rope frame to reduce inter treatment interactions. But to minimise disturbance of the majority of the panels at any sample interval, panels are to be retrieved progressively along lines according to the schedule below

Randomised panel location within panel lines, and sample interval that panels retrieved:
1 = 13th April. **2** = 12th May. **3** = 15th June. **4** = 13th July. **5** = 11th August. **6** = 27th September.
TT**** denotes position and number of Vemco data loggers

C 11 @ 2m		
DOWN ROPE		
A 32	AZ	1
A 60	UT	1
A 50	UT	1
A 36	AZ	1
A 104	FZ	1
A 26	AZ	1
A 72	UT	1
A 84	NC	1
A 40	AZ	1
A 22	LA	1
A 24	LA	1
A 112	FZ	1
A 96	NC	2
A 70	UT	1
A 92	NC	1
A 58	UT	2
A 120	FZ	1
A 82	NC	1
A 118	FZ	1
A 54	UT	2
A 90	NC	1
DOWN ROPE TT4632		
A 20	LA	1
A 68	UT	2
A 74	NC	2
A 28	AZ	2
A 86	NC	2
A 38	AZ	2
A 10	LA	1
A 4	LA	2
A 78	NC	2
A 16	LA	2
A 8	LA	2
A 64	UT	2
A 30	AZ	2
A 88	NC	3
A 123	FZ	2
A 114	FZ	2
A 14	LA	2
A 102	FZ	2
A 62	UT	3
A 66	UT	3
DOWN ROPE		
A 18	LA	3
A 76	NC	3
A 48	AZ	2
A 106	FZ	2
A 42	AZ	3
A 34	AZ	3
A 2	LA	3
A 108	FZ	3
A 44	AZ	3
A 110	FZ	3
A 52	UT	3
A 94	NC	3
A 46	AZ	3
A 12	LA	3
A 80	NC	3
A 121	FZ	3
A 6	LA	3
A 56	UT	3
A 116	FZ	3
DOWN ROPE		

C 9 @ 9m		
DOWN ROPE		
B 33	AZ	1
B 65	UT	1
B 11	LA	1
B 77	NC	1
B 105	FZ	1
B 117	FZ	1
B 75	NC	1
B 121	FZ	1
B 5	LA	1
B 9	KA	1
B 79	NC	1
B 37	AZ	1
B 93	NC	1
B 47	AZ	1
B 17	LA	1
B 85	NC	2
B 73	NC	2
B 61	UT	1
B 83	NC	2
B 25	AZ	1
DOWN ROPE		
B 111	FZ	1
B 123	FZ	2
B 49	UT	1
B 81	NC	2
B 119	FZ	2
B 41	AZ	2
B 67	UT	1
B 95	NC	3
B 3	LA	2
B 21	LA	2
B 57	UT	2
B 35	AZ	2
B 39	AZ	2
B 43	AZ	2
B 23	LA	2
B 45	AZ	3
B 51	UT	2
B 113	FZ	2
B 15	LA	2
B 87	NC	3
DOWN ROPE TT 4617		
B 19	LA	3
B 31	AZ	3
B 59	UT	2
B 27	AZ	3
B 109	FZ	2
B 89	NC	3
B 69	UT	2
B 71	UT	3
B 1	LA	3
B 91	NC	3
B 13	LA	3
B 29	AZ	3
B 101	FZ	3
B 63	UT	3
B 107	FZ	3
B 7	LA	3
B 115	FZ	3
B 55	UT	3
B 103	FZ	3
B 53	UT	3
DOWN ROPE		

C 28 @ 2m		
DOWN ROPE		
A 11	LA	4
A 21	LA	4
A 51	UT	4
A 111	FZ	4
A 67	UT	4
A 119	FZ	4
A 25	AZ	4
A 45	AZ	4
A 107	FZ	4
A 55	UT	4
A 109	FZ	4
A 43	AZ	4
A 69	UT	4
A 41	AZ	4
A 5	LA	4
A 63	UT	5
A 49	UT	5
A 122	FZ	5
A 115	FZ	5
DOWN ROPE		
A 113	FZ	5
A 3	LA	4
A 101	FZ	5
A 9	LA	5
A 77	NC	4
A 53	UT	5
A 65	UT	5
A 27	AZ	5
A 7	LA	5
A 117	FZ	6
A 57	UT	6
A 105	FZ	6
A 81	NC	4
A 37	AZ	5
A 73	NC	4
A 39	AZ	5
A 95	NC	4
A 59	UT	6
A 33	AZ	5
A 83	NC	5
A 61	UT	6
DOWN ROPE TT 4599		
A 17	LA	5
A 91	NC	5
A 1	LA	5
A 124	FZ	6
A 29	AZ	6
A 23	LA	6
A 85	NC	5
A 19	LA	6
A 47	AZ	6
A 31	AZ	6
A 71	UT	6
A 15	LA	6
A 89	NC	5
A 93	NC	6
A 75	NC	6
A 35	AZ	6
A 13	LA	6
A 79	NC	6
A 103	FZ	6
A 87	NC	6
DOWN ROPE		

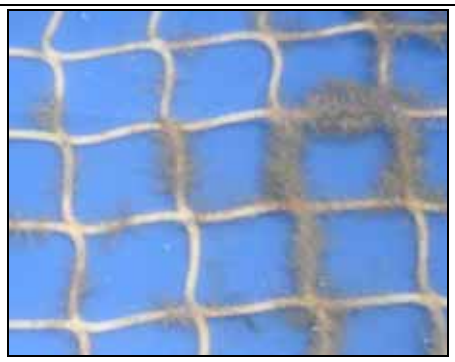
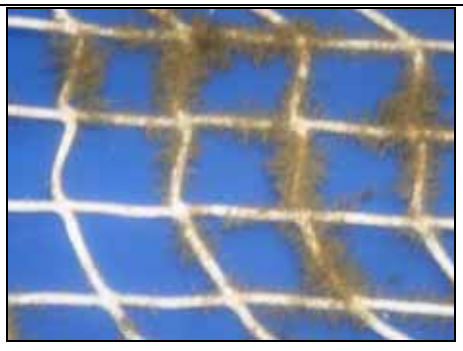
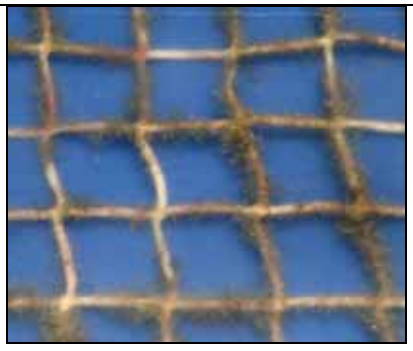
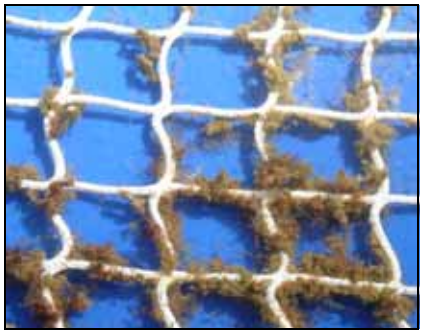
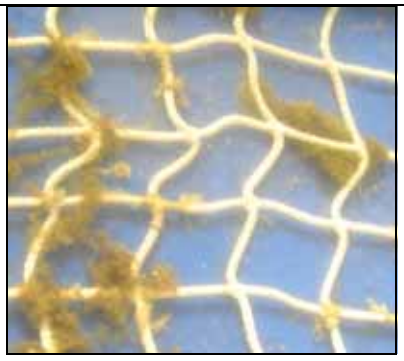
C 22 @ 9m		
DOWN ROPE		
B 36	AZ	4
B 62	UT	4
B 80	NC	4
B 6	LA	4
B 76	NC	4
B 86	NC	4
B 112	FZ	4
B 122	FZ	4
B 30	AZ	4
B 120	FZ	4
B 18	LA	4
B 12	LA	4
B 106	FZ	4
B 58	UT	4
B 74	NC	4
B 78	NC	5
B 114	FZ	5
B 68	UT	4
B 8	LA	4
B 54	UT	4
DOWN ROPE TT 4618		
B 46	AZ	4
B 44	AZ	4
B 88	NC	5
B 42	AZ	5
B 38	AZ	5
B 56	UT	5
B 94	NC	5
B 60	UT	5
B 22	LA	5
B 2	LA	5
B 108	FZ	5
B 66	UT	5
B 40	AZ	5
B 48	AZ	5
B 14	LA	5
B 104	FZ	5
B 32	AZ	6
B 82	NC	5
B 96	NC	6
B 4	LA	5
B 28	AZ	6
DOWN ROPE		
B 24	LA	6
B 90	NC	6
B 92	NC	6
B 10	LA	6
B 110	FZ	5
B 84	NC	6
B 70	UT	5
B 20	LA	6
B 52	UT	6
B 26	AZ	6
B 72	UT	6
B 50	UT	6
B 116	FZ	6
B 124	FZ	6
B 118	FZ	6
B 64	UT	6
B 34	AZ	6
B 102	FZ	6
B 16	LA	6
DOWN ROPE		

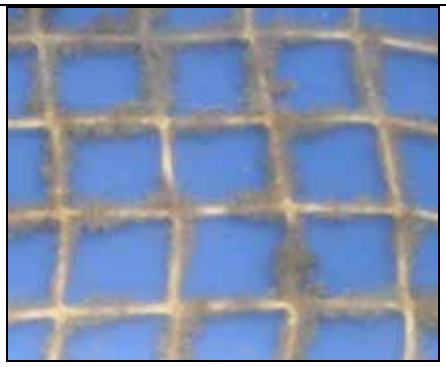
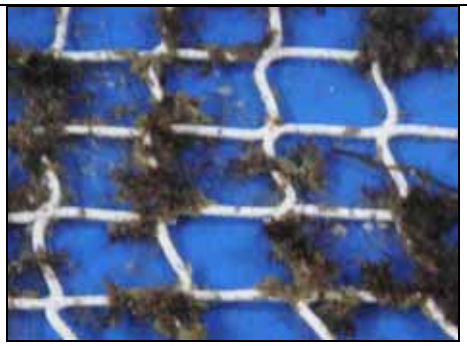
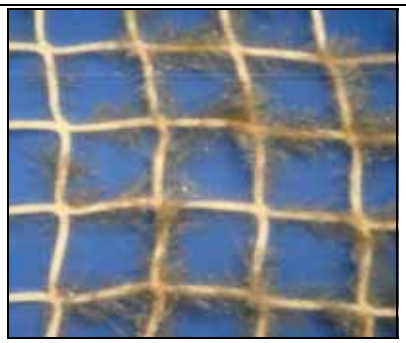
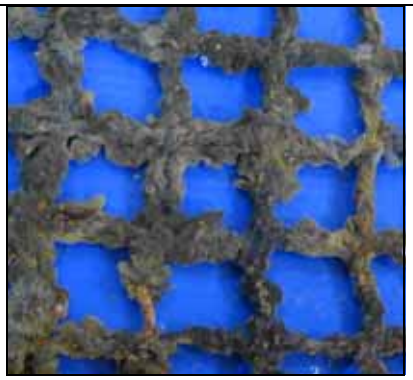
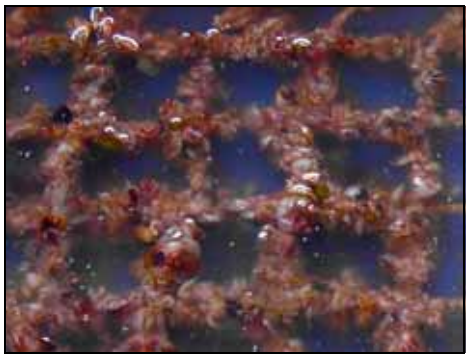
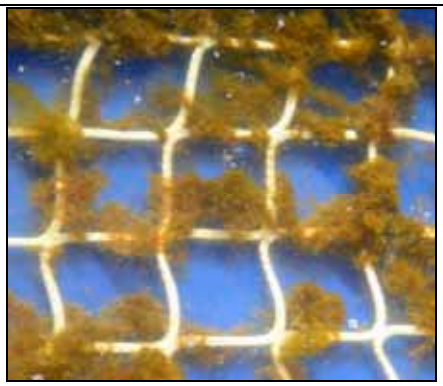
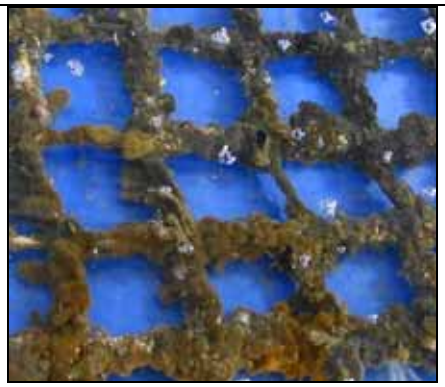
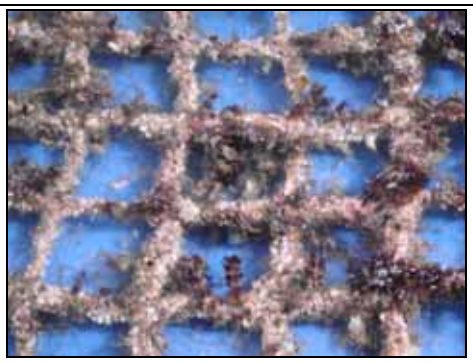
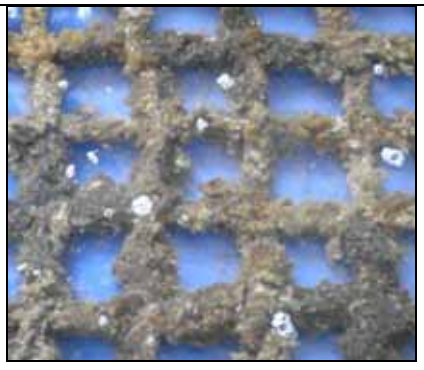
APPENDIX 4.2: PHOTOGRAPHS OF ALL NET PANELS FROM SUBPROJECT 2

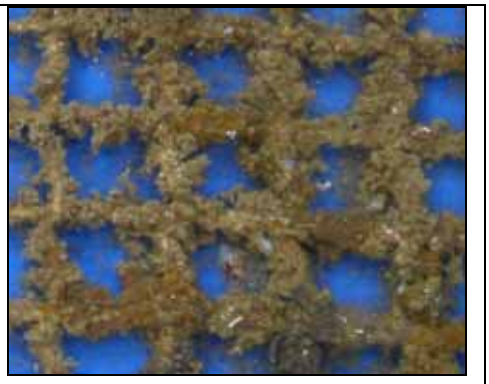
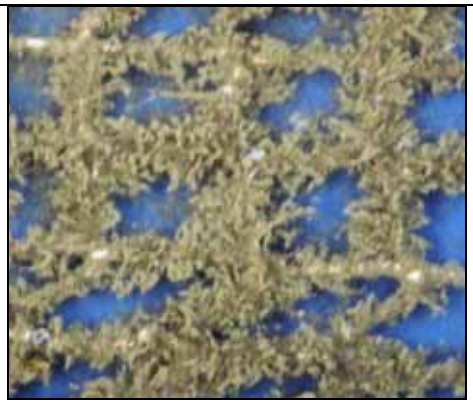
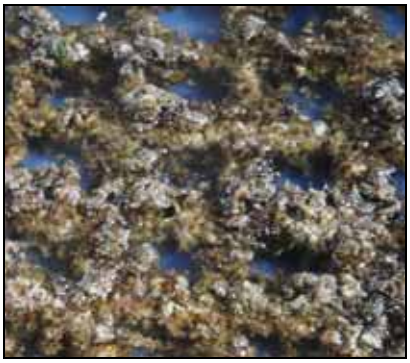
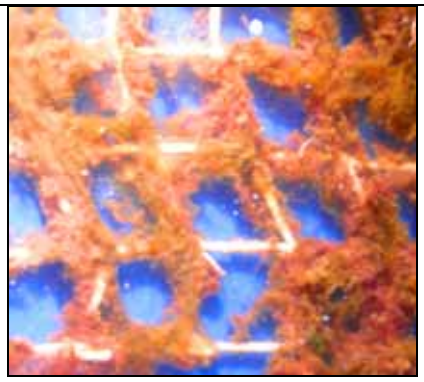
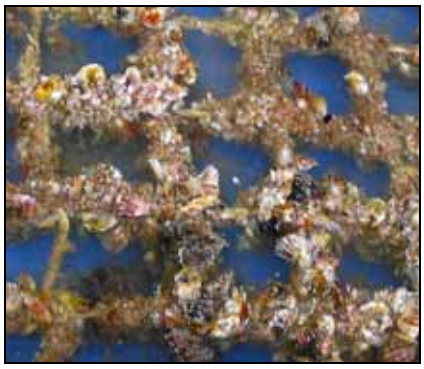
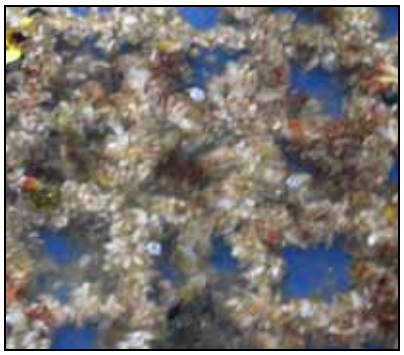
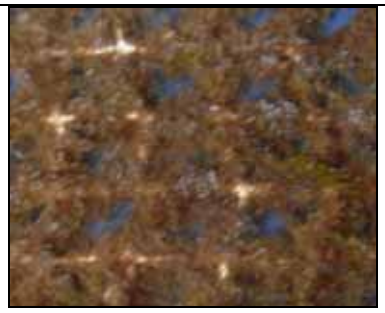
The 240 photographs for this appendix can be found on the enclosed CD.

APPENDIX 4.3: GUIDE TO PERCENTAGE OCCLUSION DUE TO THE COMBINATION OF BIOFOULING GROWTH AND NET CORD *(and percentage open space for free water flow)*

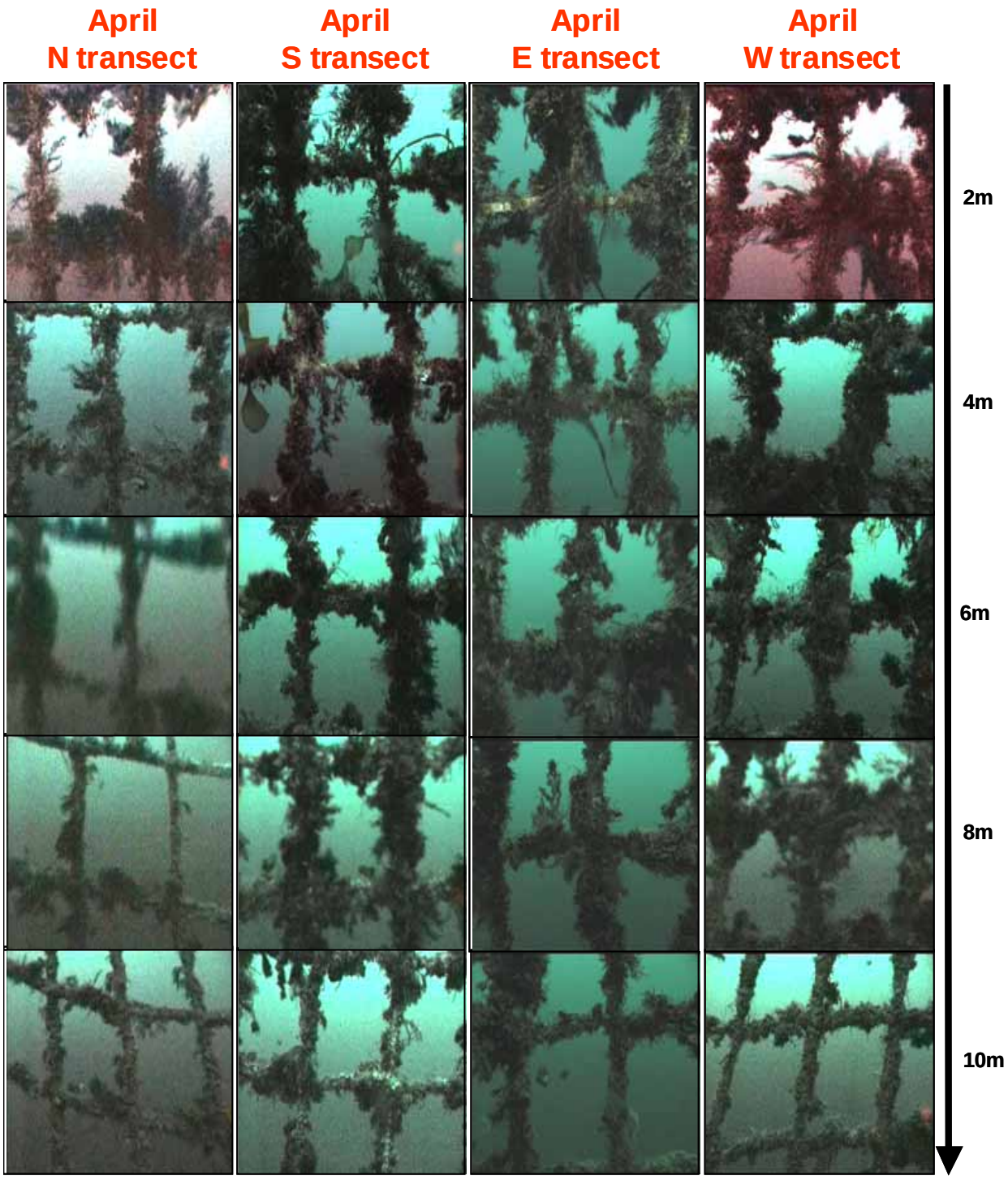
Note that the shell and weed colour in some cases was enhanced to undertake image analysis.

20-24% *(76-80% open)*		
25-29% *(71-75% open)*		
30-34% *(66-70% open)*		
35-39% *(61-65% open)*		
40-44% *(56-60% open)*		

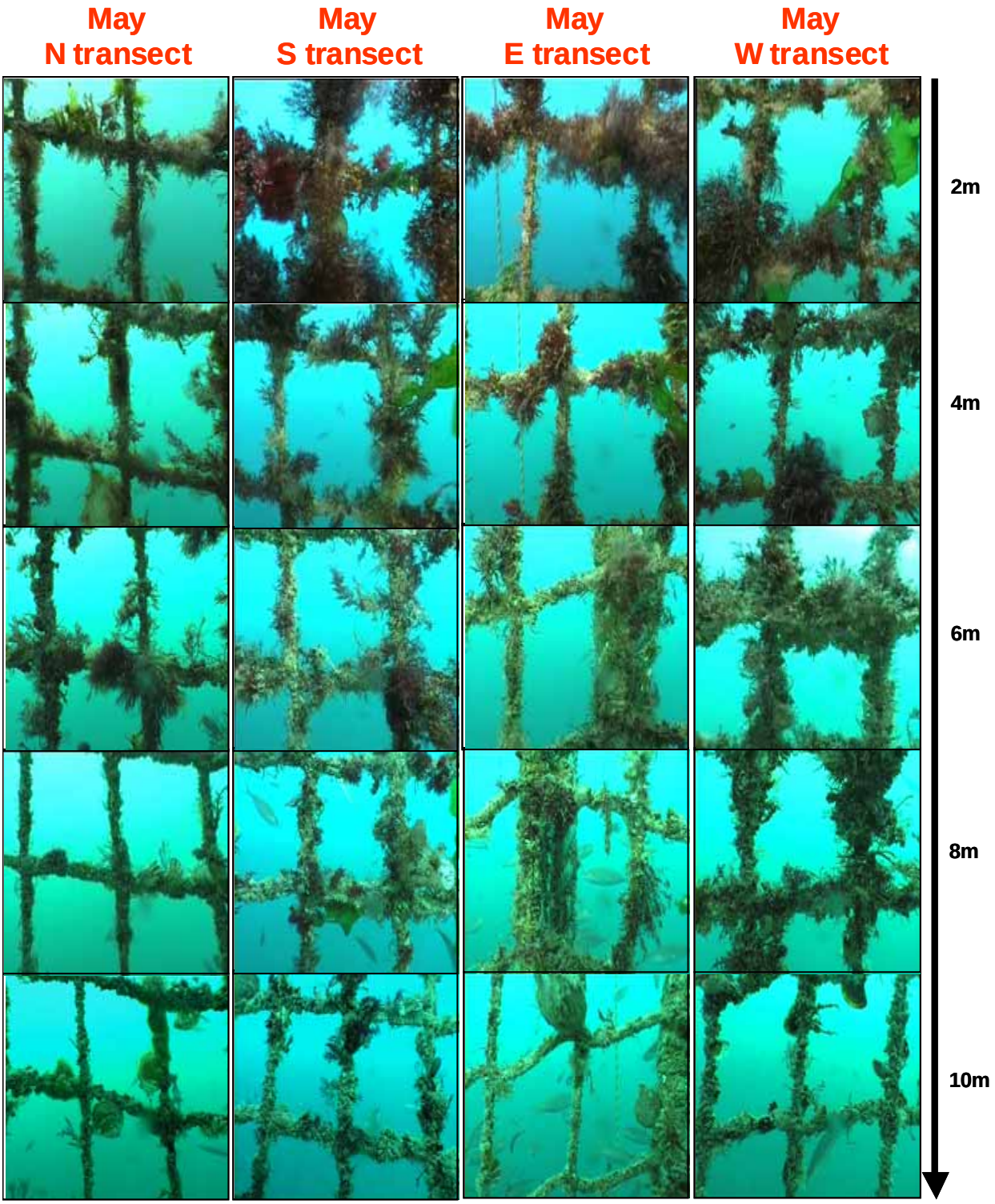
45-49% *(51-55% open)*		
50-54% *(46-50% open)*		
55-59% *(41-45% open)*		
60-64% *(36-40% open)*		
65-69% *(31-35% open)*		

70-74% *(26-30% open)*		
75-79% *(21-25% open)*		
80-84% *(16-20% open)*		
85-89% *(11-15% open)*		
90-100% *(0-9% open)*		

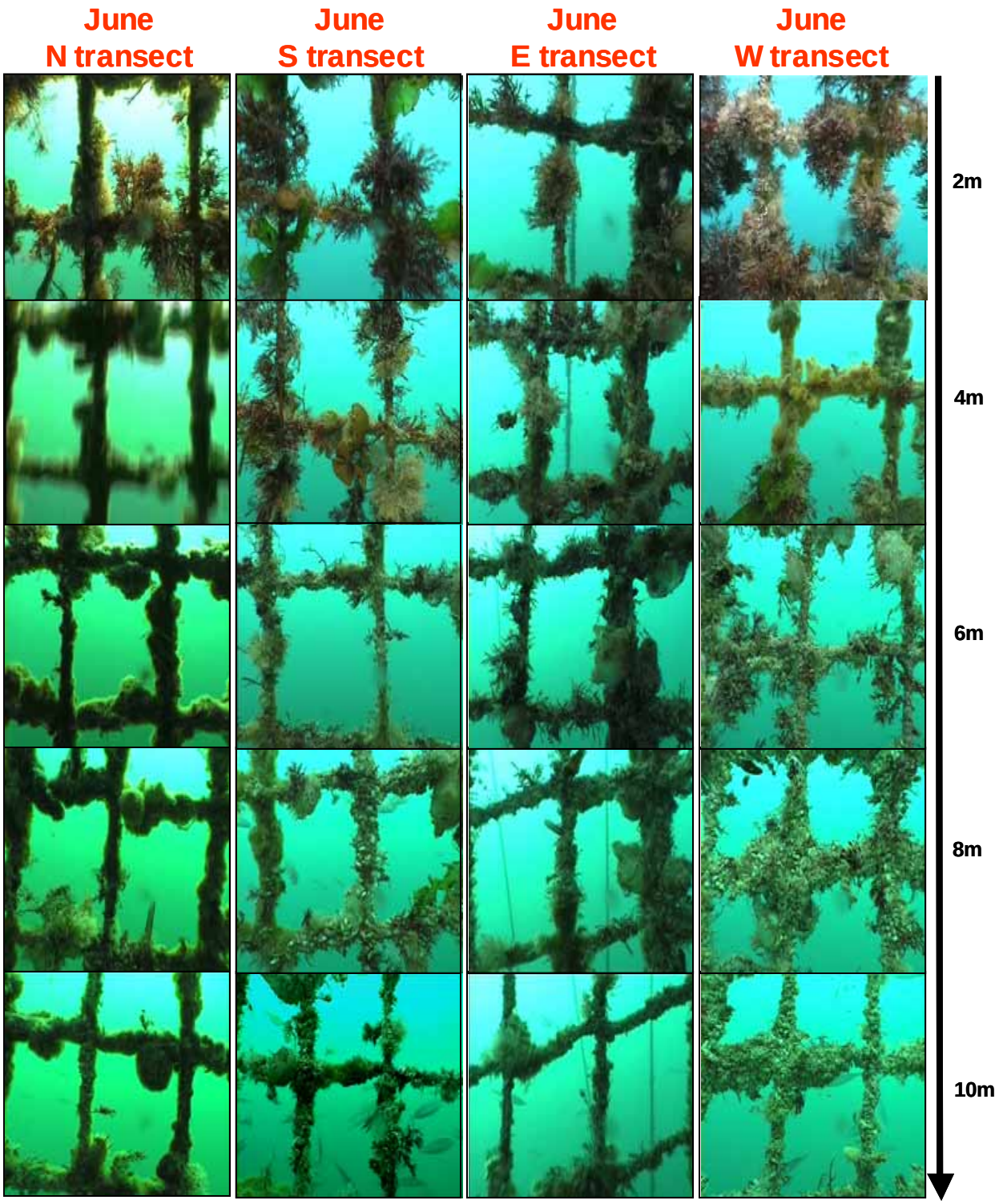
APPENDIX 5.1: SEASONAL DEVELOPMENT OF FOULING ASSEMBLAGES FOR THE SEA-CAGE WITH WHITE NET AT THE DI FISHING INSHORE SITE.



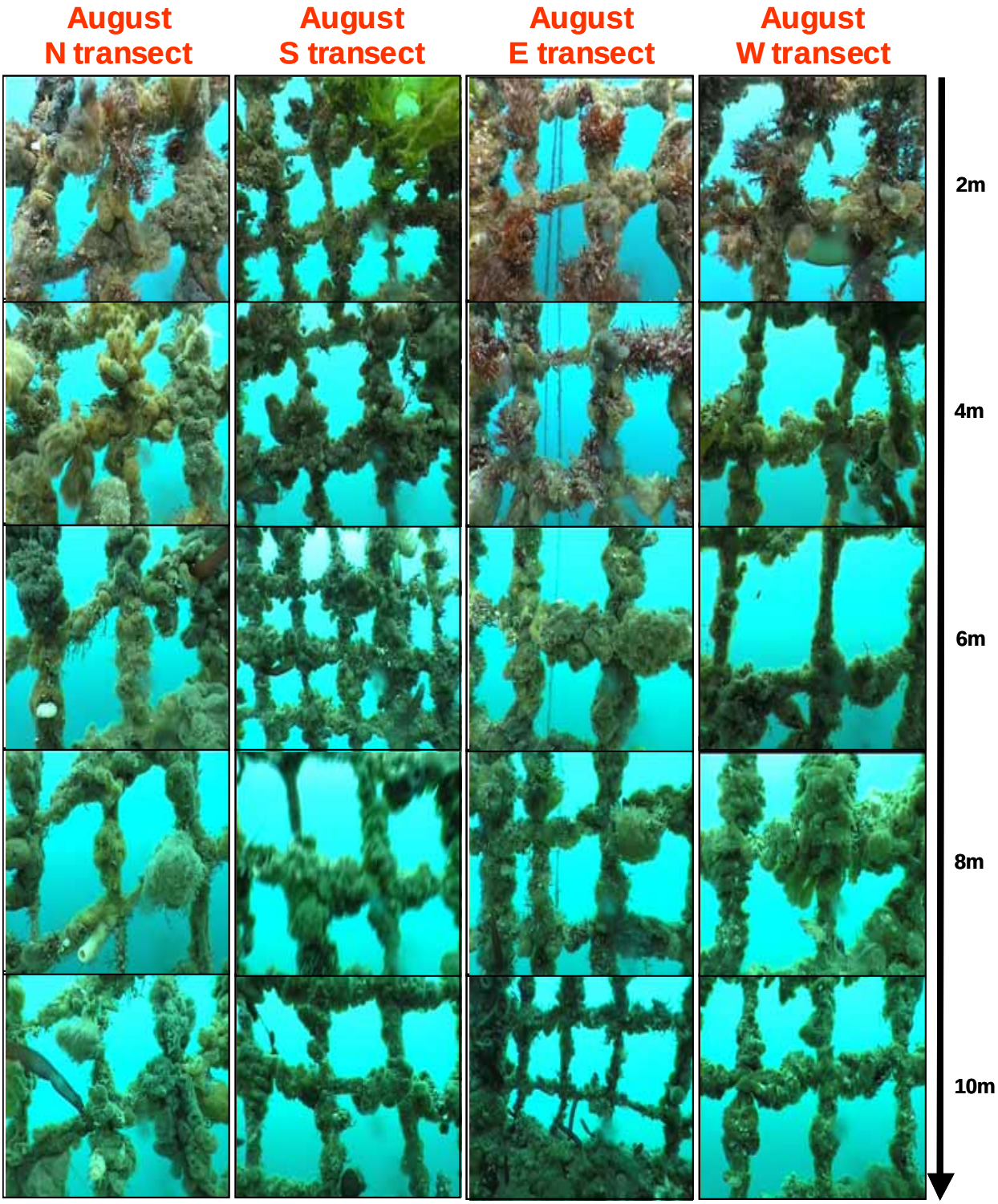
Seasonal development of fouling assemblages for the sea-cage with white net at the DI Fishing inshore site.



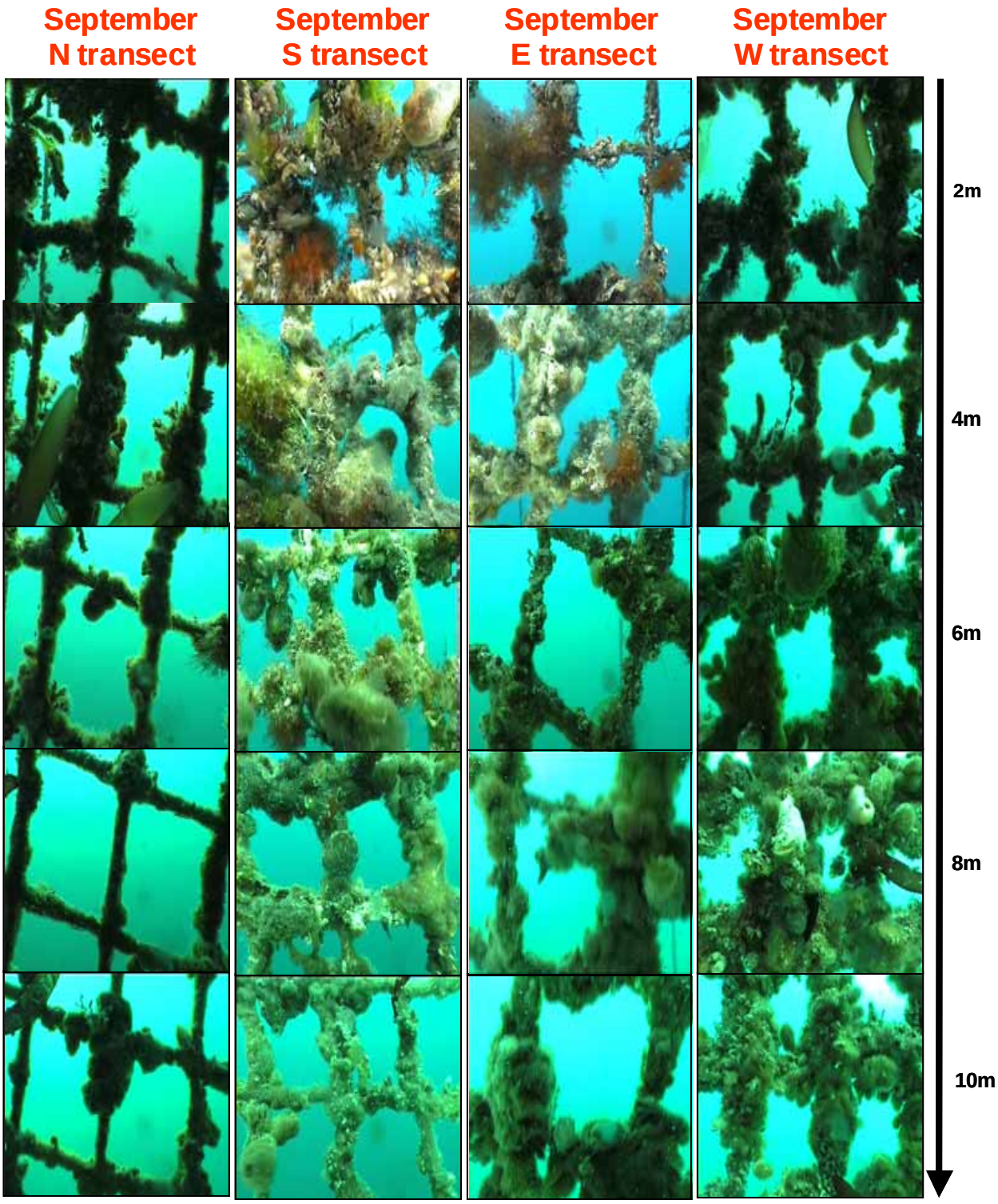
Seasonal development of fouling assemblages for the sea-cage with white net at the DI Fishing inshore site.



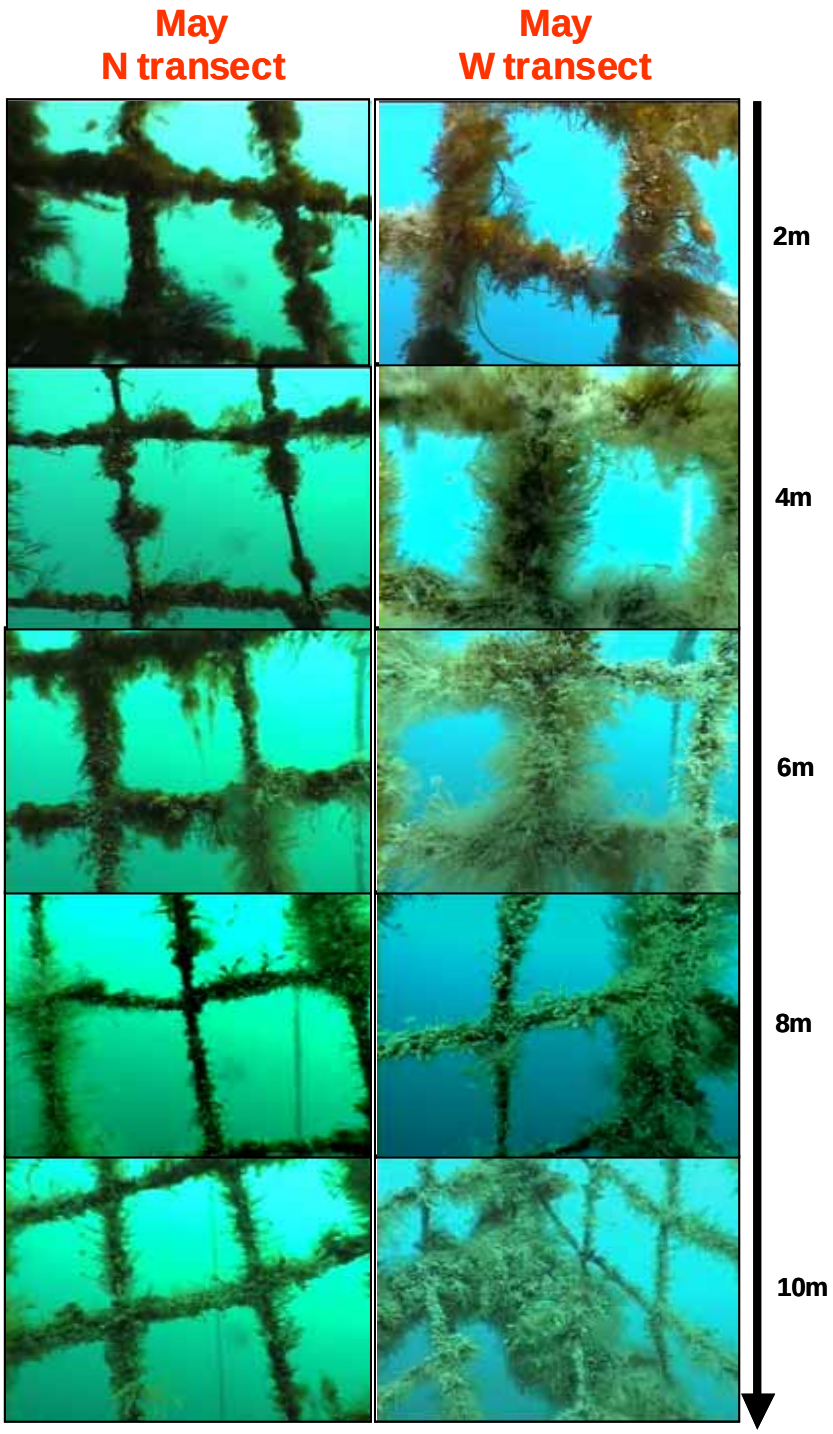
Seasonal development of fouling assemblages for the sea-cage with white net at the DI Fishing inshore site.



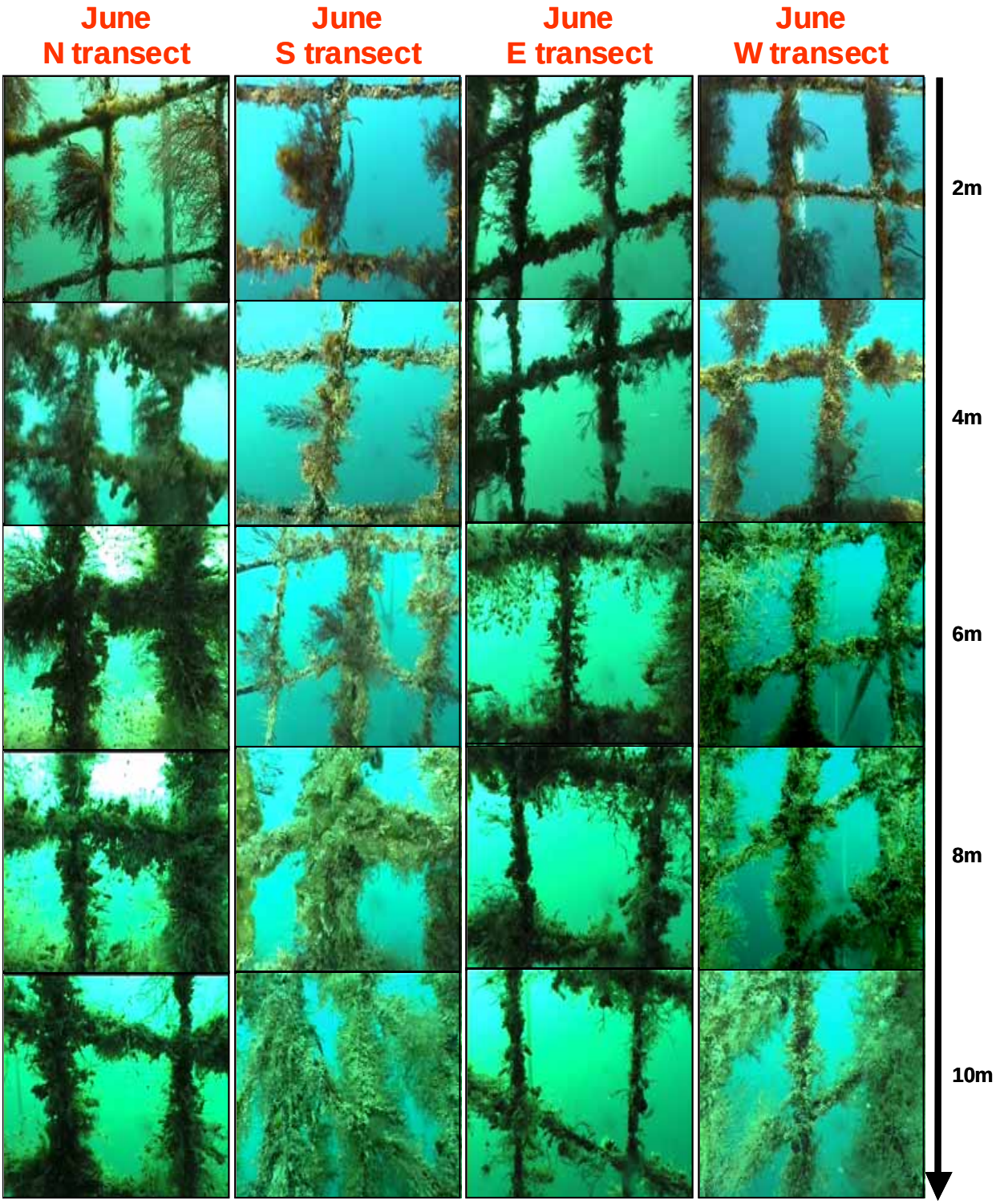
Seasonal development of fouling assemblages for the sea-cage with white net at the DI Fishing inshore site.



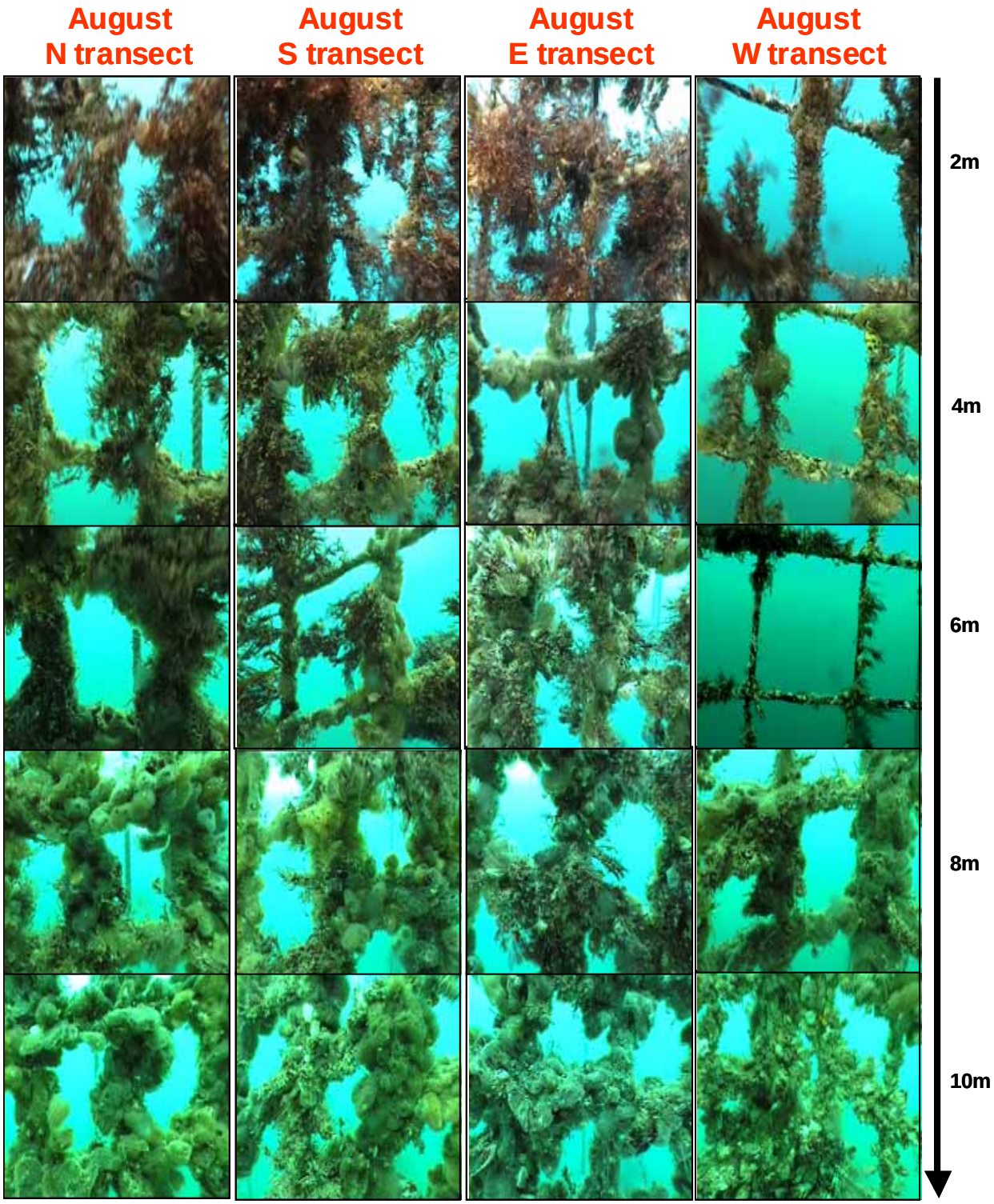
APPENDIX 5.2: SEASONAL DEVELOPMENT OF FOULING ASSEMBLAGES FOR THE SEA-CAGE WITH BLACK NET AT THE AUSTRALIAN FISHING ENTERPRISES OFFSHORE SITE.



Seasonal development of fouling assemblages for the sea-cage with black net at the Australian Fishing Enterprises offshore site.



Seasonal development of fouling assemblages for the sea-cage with black net at the Australian Fishing Enterprises offshore site.

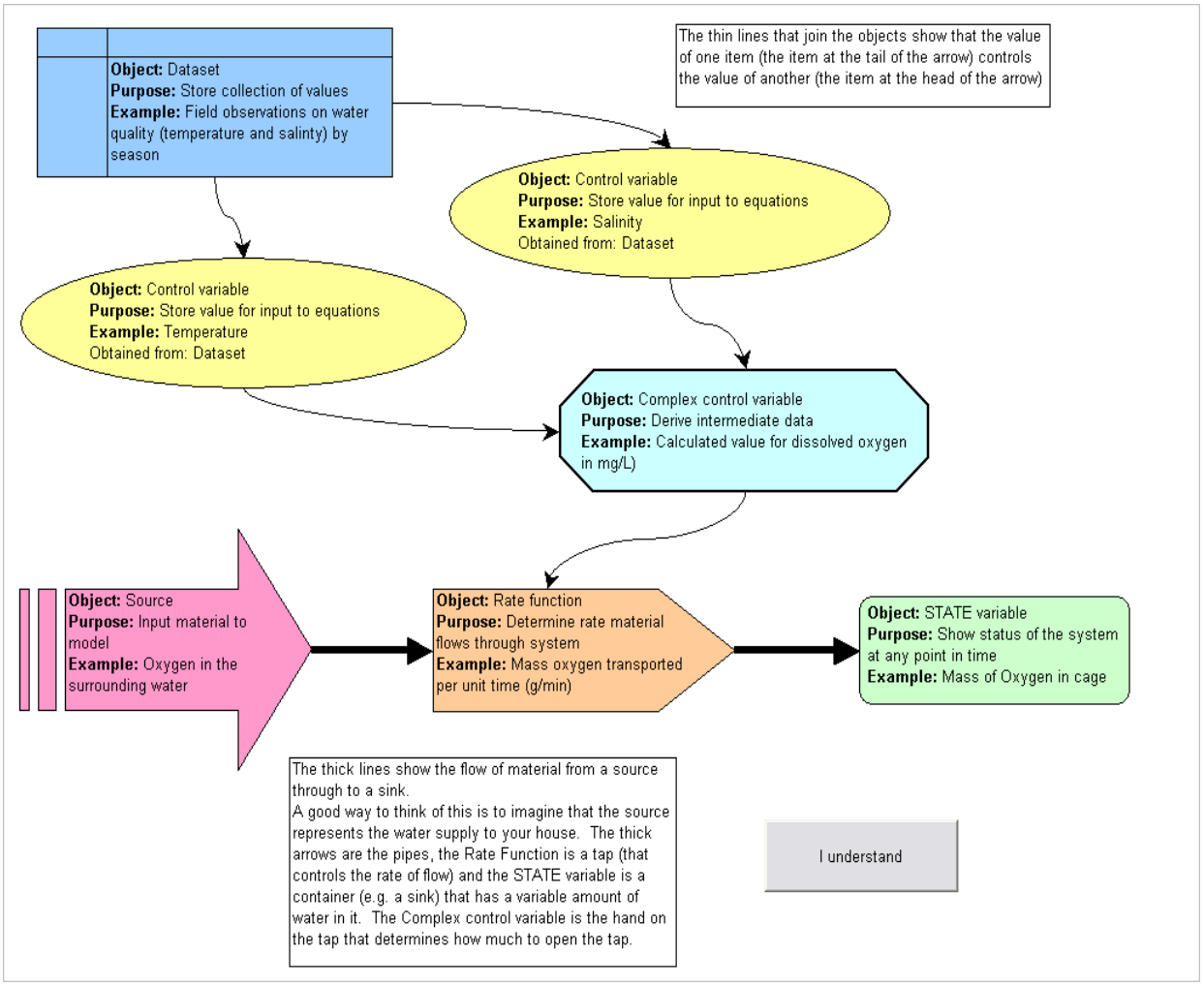


APPENDIX 6.1: WATER VELOCITY CONVERSION TABLE, CENTIMETRES PER SECOND TO KNOTS

cm/second	Knots	cm/second	Knots	cm/second	Knots	cm/second	Knots
5.14	0.1	30.87	0.6	56.59	1.1	82.31	1.6
10.29	0.2	36.01	0.7	61.73	1.2	87.45	1.7
15.43	0.3	41.16	0.8	66.88	1.3	92.6	1.8
20.58	0.4	46.30	0.9	72.02	1.4	97.74	1.9
25.72	0.5	51.44	1.0	77.17	1.5	102.89	2.0

APPENDIX 7.1: EXPLANATION OF THE MODIFIED FORRESTER SYMBOL SYSTEM

The design of the OxyTuna© model has been represented schematically in Figure 7.1. This figure uses a series of symbols that were based on the Forrester symbols (Forrester 1961). Forrester symbols allow the modeller to provide a schematic representation of material flows, control flows, control variables and parameters, rate equations (processes) and state variables. These symbols are summarised below.



APPENDIX 8.1: CHEMWATCH MATERIAL SAFETY DATA SHEET FOR WATTYL NETCLEAR ZPT

WATTYL NETCLEAR ZPT

ChemWatch Material Safety Data Sheet
CHEMWATCH 5076-53
Date of Issue: Thu 10-Oct-2002

Section 1 – CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME
WATTYL NET CLEAR ZPT

SYNONYMS
Aquaculture aqua culture net coating paint

PRODUCT USE
Synthetic coating for aquaculture netting surfaces. Applied by dipping.
Used according to manufacturers directions.

SUPPLIER
Company: Wattyl Australia Pty Limited
Address:
4 Steel St
Blacktown
NSW 2148
Australia
Telephone: 132101
Telephone: (02) 9621 6255
Emergency Tel: 1800 039 008 - 24 hour
Emergency Tel: +61 3 9573 3112
Fax: (02) 9831 2651

HAZARD RATINGS
Flammability: 0
Toxicity: 3
Body Contact: 2
Reactivity: 0
SCALE: Min/Nil=0 Low=1 Moderate=2 High=3 Extreme=4

Section 2 – HAZARDS IDENTIFICATION

STATEMENT OF HAZARDOUS NATURE
HAZARDOUS SUBSTANCE. NON-DANGEROUS GOODS.
According to the Criteria of NOHSC, and the ADG Code.

POISONS SCHEDULE
S6

RISK
Toxic in contact with skin and if swallowed.
Inhalation may produce health damage*.

Cumulative effects may result following exposure*.
May produce discomfort of the eyes, respiratory tract and skin*.
Limited evidence of a carcinogenic effect*.
Possible respiratory and skin sensitiser*.
* (limited evidence)

SAFETY

Keep locked up.
Avoid contact with eyes.
Wear suitable protective clothing.
In case of insufficient ventilation wear suitable respiratory equipment.
Use only in well ventilated areas.
Keep container in a well ventilated place.
To clean the floor and all objects contaminated by this material, use water.
Keep container tightly closed.
This material and its container must be disposed of in a safe way.
Keep away from food, drink and animal feeding stuffs.
Take off immediately all contaminated clothing.
In case of contact with eyes, rinse with plenty of water and contact Doctor or Poisons Information Centre.
If you feel unwell contact Doctor or Poisons Information Centre. (Show the label if possible).

Section 3 – COMPOSITION / INFORMATION ON INGREDIENTS

NAME	CAS RN	%
Synthetic copolymer emulsion		30-60
Zinc oxide	1314-13-2	10-30
Zinc pyrithione	13463-41-7	1-5
ammonium hydroxide	1336-21-6	<0.2
other pigments		1-9
additives unregulated		1-5
water	7732-18-5	10-30

Section 4 – FIRST AID MEASURES

SWALLOWED

Rinse mouth out with plenty of water.
If poisoning occurs, contact a doctor or Poisons Information Centre.

EYE

If this product comes in contact with the eyes:

- Immediately hold the eyes open and wash with fresh running water.
- Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids.
- Continue flushing until advised to stop by the Poisons Information Centre or a Doctor, or for at least 15 minutes.
- Transport to hospital or doctor without delay.
- Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.

SKIN

If skin or hair contact occurs:

- Quickly but gently, wipe material off skin with a dry, clean cloth.
- Immediately remove all contaminated clothing, including footwear.
- Wash skin and hair with running water. Continue flushing with water until advised to stop by the Poisons Information Centre.
- Transport to hospital, or doctor.

INHALED

- If fumes or combustion products are inhaled: Remove from contaminated area.
- Lay patient down. Keep warm and rested.
- Prostheses such as false teeth, which may block airway, should be removed, where possible, prior to initiating first aid procedures.
- Apply artificial respiration if not breathing, preferably with a demand valve resuscitator, bag-valve mask device, or pocket mask as trained. Perform CPR if necessary.
- Transport to hospital, or doctor.

NOTES TO PHYSICIAN

Treat symptomatically.

Section 5 – FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA

Use extinguishing media suitable for surrounding area

FIRE FIGHTING

Alert Fire Brigade and tell them location and nature of hazard.
- Wear breathing apparatus plus protective gloves for fire only.
- Prevent, by any means available, spillage from entering drains or water courses.

Cool fire exposed containers with water spray from a protected location.
Use fire fighting procedures suitable for surrounding area.

FIRE/EXPLOSION HAZARD

- The material is not readily combustible under normal conditions.
- However, it will breakdown under fire conditions and the organic component may burn.
- Not considered to be a significant fire risk.
- Heat may cause expansion or decomposition with violent rupture of containers.
- Decomposes on heating and may produce toxic fumes of carbon monoxide (CO).
- May emit acrid smoke.

Decomposes on heating and produces toxic fumes of zinc oxide.

FIRE INCOMPATIBILITY

None known

HAZCHEM

None

Personal Protective Equipment

Glasses:

Safety glasses.
Chemical goggles.

Gloves:

1. BUTYL
2. NEOPRENE
3. VITON

Respirator:

Type K-P Filter of sufficient capacity

Section 6 – ACCIDENTAL RELEASE MEASURES

EMERGENCY PROCEDURES

MINOR SPILLS

- Clean up all spills immediately.
- Avoid breathing vapours and contact with skin and eyes.
- Control personal contact by using protective equipment.
- Contain and absorb spill with sand, earth, inert material or vermiculite.
- Wipe up.
- Place in a suitable labelled container for waste disposal.

MAJOR SPILLS

Minor hazard.

- Clear area of personnel.
- Alert Fire Brigade and tell them location and nature of hazard.
- Control personal contact by using protective equipment as required.
- Prevent spillage from entering drains or water ways.
- Contain spill with sand, earth or vermiculite.
- Collect recoverable product into labelled containers for recycling.
- Absorb remaining product with sand, earth or vermiculite and place in appropriate containers for disposal.
- Wash area and prevent runoff into drains or waterways.
- If contamination of drains or waterways occurs, advise emergency services.

Personal Protective Equipment advice is contained in Section 8 of the MSDS

Section 7 – HANDLING AND STORAGE

PROCEDURE FOR HANDLING

DO NOT allow clothing wet with material to stay in contact with skin

- Limit all unnecessary personal contact.
- Wear protective clothing when risk of exposure occurs.
- Use in a well-ventilated area.

- Avoid contact with incompatible materials.
- When handling, DO NOT eat, drink or smoke.
- Keep containers securely sealed when not in use.
- Avoid damage to containers.
- Always wash hands with soap and water after handling.
- Work clothes should be laundered separately.
- Use good occupational work practices.
- Observe manufactures storing and handling recommendations.
- Atmosphere should be regularly checked against established exposure standards to ensure safe working conditions are maintained.

SUITABLE CONTAINER
Lined metal can, Lined metal pail/can
Plastic pail
Polyliner drum
Packing as recommended by manufacturer.
Check all containers are clearly labelled and free from leaks.

STORAGE INCOMPATIBILITY
None known.

STORAGE REQUIREMENT
- Store in original containers.
- Keep containers securely sealed.
- Store in a cool, dry, well-ventilated area.
- DO NOT allow to freeze
- Store away from incompatible materials.
- Protect containers against physical damage and check regularly for leaks.
- Observe manufacturer's storing and handling recommendations.

Section 8 – EXPOSURE CONTROLS / PERSONAL PROTECTION

EXPOSURE CONTROLS
None assigned. Refer to individual constituents.

PERSONAL PROTECTION
Short gloves
Goggles
Overalls
Half Face Respirator

EYE

- Safety glasses with side shields; or as required.
- Chemical goggles.
- Contact lenses pose a special hazard; soft lenses may absorb irritants and all lenses concentrate them.

HANDS/FEET

- Barrier cream with polyethylene gloves or
- Wear general protective gloves: i.e. disposable polythene gloves or cotton gloves or Light weight rubber gloves, with barrier cream.

- Preferably safety footwear.

OTHER

- Overalls.
- Eyewash unit.

ENGINEERING CONTROLS

None required when handling small quantities.
OTHERWISE: Use in a well-ventilated area.
General exhaust is adequate under normal operating conditions.
If risk of overexposure exists, wear SAA approved respirator.
Correct fit is essential to obtain adequate protection.
Provide adequate ventilation in warehouse or closed storage areas.

Section 9 – PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE
Milky white liquid coating; mixes with water; mild acrylic paint odour.

PHYSICAL PROPERTIES
Liquid.
Mixes with water.

Molecular weight: Not applicable
Boiling Point (deg C): 100
Melting Point (deg C): Not available.
Specific Gravity (water=1): 1.45-1.49
Solubility in Water (g/L): Miscible
pH (as supplied): 8-10
pH (1% solution): not available
Vapour Pressure (kPa): <1
Volatile component (%vol): 40 approx.
Evaporation rate: slow
Relative vapour density (air=1): not available
Flash Point (deg C): Non Flammable
Lower Explosive Limit (%): Not applicable
Upper Explosive Limit (%): Not applicable
Autoignition temp (deg C): not applicable
Decompostion temp (deg C): not available
State: liquid

Section 10 – CHEMICAL STABILITY, REACTIVITY INFORMATION

CONDITIONS CONTRIBUTING TO INSTABLITY
Product is considered stable and hazardous polymerisation will not occur.

Section 11 – TOXICOLOGICAL INFORMATION

POTENTIAL HEALTH EFFECTS

ACUTE HEALTH EFFECTS

SWALLOWED
Considered an unlikely route of entry in commercial/industrial environments.
The liquid is discomforting and mildly toxic if swallowed.
Ingestion may result in nausea, abdominal irritation, pain and vomiting.

EYE
The liquid may produce eye discomfort and is capable of causing temporary impairment of vision and/or transient eye inflammation, ulceration.
The vapour is mildly discomforting to the eyes.
The material may be irritating to the eye, with prolonged contact causing inflammation. Repeated or prolonged exposure to irritants may produce conjunctivitis.

SKIN
The liquid is highly discomforting to the skin if contact is prolonged and is capable of causing skin reactions which may lead to dermatitis and may rarely cause in some cases, sensitisation.
Sensitisation may result in allergic dermatitis responses including rash, itching, hives or swelling of extremities.
Sensitisation reactions may appear suddenly after repeated symptom free exposures.
The material may accentuate any pre-existing dermatitis condition.
The material may cause skin irritation after prolonged or repeated exposure and may produce on contact skin redness, swelling, the production of vesicles, scaling and thickness.

INHALED
The vapour is discomforting to the upper respiratory tract and lungs.
Inhalation hazard is increased at higher temperatures.
Content of ammonia is low and is not considered a health hazard under good working conditions, however continuous long term working in confined and poorly ventilated areas may cause irritation response, sore eyes/nose.

CHRONIC HEALTH EFFECTS
Principal routes of exposure are usually by skin contact and inhalation of vapour.
Prolonged or repeated skin contact may cause drying with cracking, irritation and possible dermatitis following.
Acrylic polymer emulsions may contain residual traces of odorous acrylic monomers; the amounts remaining in compounded mixtures represents a very low order of exposure, however this may become noticeable with some materials particularly in confined or poorly ventilated spaces.
Sensitisation may give severe responses to very low levels of exposure, i.e. hypersensitivity. Sensitised persons should not be allowed to work in situations where exposure may occur.
As with any chemical product, contact with unprotected bare skin; inhalation of vapour, mist or dust in work place atmosphere; or ingestion in any form, should be avoided by observing good occupational work practice.

Section 12 – ECOLOGICAL INFORMATION

No data for Wattyl Net Clear ZPT.
Refer to data for ingredients, which follows:

ZINC OXIDE:

No data for zinc oxide.

ZINC PYRITHIONE:

The material is classified as an ecotoxin* because the Fish LC50 (96hours) is less than or equal to 0.1mg/L

* Classification of substance as Ecotoxic (dangerous to the environment) Appendix 8, Table 1
Compilers Guide for the Preparation of International Chemical Safety Cards: 1993 Commission of the European Communities

AMMONIUM HYDROXIDE:

Hazardous Air Pollutant: No
Fish LC50 (96hr) (mg/L): 8.2

WATER:

No data for water.

Section 13 – DISPOSAL CONSIDERATIONS

Recycle wherever possible. Consult manufacturer for recycling options.
Consult State Land Waste Management Authority for disposal.
Recycle if possible, otherwise dispose in a chemically secure landfill.
Recycle containers if possible, or dispose of in an authorised landfill.

Section 14 – TRANSPORT INFORMATION

Shipping Name:
NONE
Hazard Class: None
UN/NA Number: None
ADR Number:
Packing Group: None
Lables Required:
Additional Shipping Information:
International Transport Regulations:
IMO: None

HAZCHEM
None

Section 15 – REGULATORY INFORMATION

POISONS SCHEDULE
S6

Section 16 – OTHER INFORMATION

Issue Date: Thu 10-Oct-2002
Print Date: Mon 15-Mar-2004

APPENDIX 8.2: PROPOSAL DISTRIBUTED TO POTENTIAL INDUSTRY COLLABORATORS 2004

FRDC/CRC: NET FOULING MANAGEMENT PROJECT

Whole Project Structure:

The entire project involves 6 distinct subprojects, of which it is subproject 3 and 6 that require collaboration with a commercial company (the exact requirements of the company involvement are detailed later).

Subproject 1: Review of biofouling and antifouling methods for sea-cages, (aims to collect information and document biofouling impacts, antifouling methods, and legislation controlling and driving antifouling technologies of the major sea-cage finfish industries).

Subproject 2: Small scale evaluation using net panels of preferred antifouling treatments identified from subproject 1. Treatments to be tested are currently the most environmentally friendly products available; and include
- Lanolin (a greasy physical deterrent),
- Wattyl Net Clear (a synthetic latex coating that acts as a physical barrier),
- Wattyl Net Clear ZPT (has zinc oxide and zinc pyrithione as active ingredients)

Subproject 3: Evaluation of the seasonal development pattern of net fouling and the effects on key water quality parameters, by video transects and telemetry equipment.

Subproject 4: Determination of the relationship between the level of fouling and its impact on net weight, net drag and water exchange using a flume tank.

Subproject 5: Enhancement of a dissolved oxygen diffusion model to provide a predictive capacity to industry to evaluate fouling management systems.

Subproject 6: Commercial pilot scale evaluation of an antifoulant on a stocked tuna cage, including analysis of fish health, residues in tuna and the environment, water quality and the treatments efficacy in inhibiting net fouling.

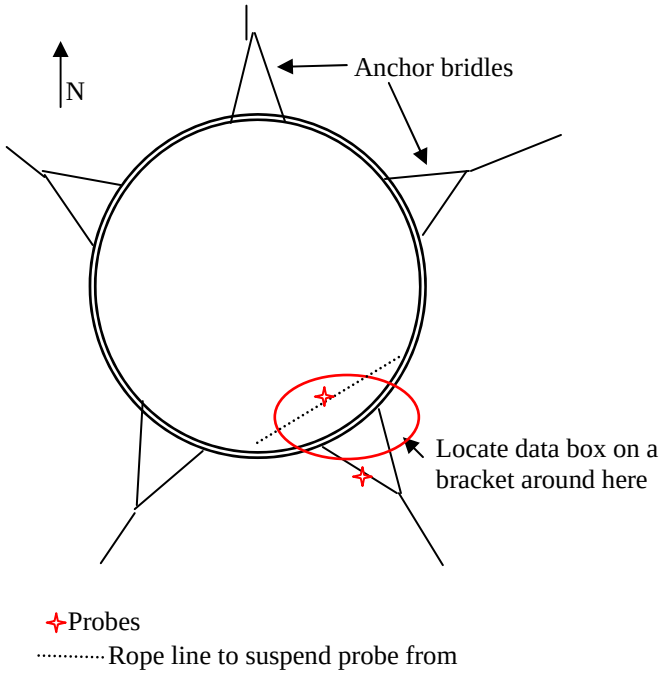
Company project proposal:

The project is currently seeking a second company to collaborate with subproject 3 in 2005 and with subproject 6 in 2006.

2005 project details and requirements:

The seasonal development pattern of net fouling will be determined by SARDI undertaking a video transect of the net from the surface to a depth of 12m at 3 weekly intervals. Transects will be done on the north, south, east and west sides of the cage and analysed for percentage occlusion of the mesh and major species present.

Water quality will be determined by a telemetry system that monitors wind speed and direction, water temperature, dissolved oxygen and conductivity (salinity); that the project will supply. The unit will be maintained throughout the season by SARDI, but may require some company assistance initially with the set up of brackets and deployment. The preferred set up is to mount the data box and equipment on the south eastern side of the cage, so that one probe is suspended outside of the net and one inside the net. Each probe needs to be approximately 5m away from the net and suspended at a depth of 5m. A configuration to achieve this is illustrated below, but this set up is open to modifications based on particular cages or feeder cage set ups.



The information generated from the system can be split so that the wind speed and direction are available to other members of the tuna association via the SARDI website and water quality data are available only by dial-up within the company. The dial-up system is real time and can be accessed 24hours a day.

2006 project details and requirements:

This part of the project involves testing antifoulant on an entire tuna net. It requires one net (can be for 32 or 40m diameter pontoon) that can be sent to Tasmania for coating with an antifoulant treatment (the project covers the cost of the product, treatment and transport to and from Tasmania). Plus 2 cages that can be used as controls, preferably at least one the same size as the cage that is treated. All 3 cages will be subject to regular video transects (fortnightly or 3 weekly by SARDI) and will have telemetry systems deployed (SARDI vessel and personnel to maintain, but may require industry assistance with deployment and final retrieval). Telemetry systems will record wind speed and direction, and have one probe inside and one probe outside of each cage, that will measure oxygen, temperature, conductivity and depth (indicates when tide flowing). Information access will be as for the 2005 project.

Fish health will require 10 fish from each of the 3 cages to have a blood sample and visceral organs collected during a harvest at the end of the trial (30 total). These are non invasive samples and can be done on fish destined for market. Blood samples will be analysed for haematocrit and differential leucocyte counts. Tissue samples from gills and visceral organs will be analysed by histopathology and screened for parasites.

Residue analyses will require the once-off collection of muscle tissue from 60 tuna in each of the treated and one control cage at harvest (ie 120 total) - this will be a muscle section slightly larger than the grading cut. One control and the treated cage will also need to have a bag of mussels suspended in it, and sediment samples collected from underneath and adjacent to the cage. The permit issued by PIRSA may require that the results of the sediment tests be available to EPA (this is not seen as an issue).

This project will require industry vessel, equipment and personnel support. The collaborating company will preferably have someone (can be a technician, diver or skipper) that has appropriate experience working with tuna to be able to detect subtle changes in the fish and surrounds. Cages will preferably be stocked with a January or February intake of tuna, whereby the 2 controls and treated cage are stocked from the same towcage, and only the 2nd, 3rd and 4th transfers used as part of the trial. I will require access to data including daily mortality, feed and feeding behaviour however only summarised/modified mortality data will need to be included in the final report (this may involve redesigning daily data sheets to ensure information is being recorded appropriately). Preferably the company will be amenable to tagging up to 50 tuna in each of the 3 cages to determine growth benefits of clean nets, although these results do not need to appear in the final report.

The antifoulant proposed for use in the trial treatment of the entire tuna net is known commercially as "Net Clear ZPT"; whereby zinc is the active constituent. The product has a valid permit issued from the National Registration Authority for Agricultural and Veterinary Medicines for use as an antifouling coating on aquaculture nets. This process involves assessment of chemistry, toxicology, environmental fate, toxicity and hazard, occupational health and safety, residues in food, efficacy, labelling requirements etc etc. Note that the active ingredient of this product is also used in antidandruff shampoos. The appropriate permit to use the product with tuna in Port Lincoln has also been obtained from PIRSA after discussions with EPA.

If there are any questions regarding either of these proposals please email or phone or arrange a time to meet and discuss in more detail.

REQUIREMENTS FROM COMPANY

Preliminary Antifoul Project Requirements (9th Aug 2004):

- Net panel trial:

Require one anchored unstocked 40m diameter pontoon, will require industry vessel and personnel support – can be located in either tuna zone
Deployment will need to be 2nd week of November 2004 and final retrieval approx mid June 2005
This pontoon will need to have 3 or 4 rope frames strung across the collar that will support the net panels. Rope frames extend to 10m depth (plus weights), with panels suspended between 2-3m and 9-10m. Vessel and/or divers will be required to retrieve subsets of treated panels at 5 weekly intervals, and take an underwater photograph of the seaweed extract panel(s) at the same time.

- Commercial scale trial:

Require one net (can be for 32 or 40m diameter pontoon) that can be sent to Tasmania for coating with an antifoulant treatment. Plus 2 cages that can be used as controls, preferably at least one the same size as the cage that is treated. All 3 cages will be subject to regular video transects (fortnightly or 3 weekly by SARDI) and will have telemetry systems deployed (SARDI vessel and personnel to maintain, but may require industry assistance with deployment

and final retrieval). Telemetry systems will have one sonde inside and one sonde outside of each cage, that will measure oxygen, temperature and depth (indicates when tide flowing). 10 fish from each of the 3 cages will need to have a blood sample and visceral organs collected during a harvest at the end of the trial (30 total). Residue analyses will require the once-off collection of muscle tissue from 60 tuna in each of the treated and one control cage at harvest (ie 120 total) - this will be a muscle section slightly larger than the grading cut. One control and the treated cage will also need to have a bag of mussels suspended in it, and sediment samples collected from underneath and adjacent to the cage. This project will require industry vessel, equipment and personnel support. The collaborating company will preferably have a technician/divers/or skipper that has appropriate experience working with tuna to be able to detect subtle changes in the fish and surrounds. Cages will preferably be stocked with a February intake of tuna, whereby the 2 controls and treated cage are stocked from the same towcage, and only the 2nd, 3rd and 4th transfers used as part of the trial. I will require access to data including daily mortality, feed and feeding behaviour (summarised/modified mortality data will need to be included in the final report) – this may involve redesigning daily data sheets to ensure information is being recorded appropriately. Preferably the company will be amenable to tagging up to 50 tuna in each of the 3 cages, although the results of this need not appear in the final report.

APPENDIX 8.3: APVMA PERMIT FOR NET CLEAR ZPT

Australian Pesticides &
Veterinary Medicines Authority

30 November 2005

David Wheelwright
Registration Consultant for Wattyl Australia
De Groot Technical Services Pty Ltd
256 Formosa Road
GUMDALE QLD 4154

Dear Mr Wheelwright

ISSUE OF PERMIT: PER6009.
EFFECTIVE FROM 14 JULY 2003 TO 30 SEPTEMBER 2007.

Zinc Oxide, Zinc Pyrithione / Fish farm nets / Use as antifouling

As requested I have extended the expiry date of this permit and made some other changes as you requested.

A copy of the revised permit is attached for your information.

Yours sincerely

Colin Byrnes
Manager Fungicides
Pesticides Program

T: (+61) 02 6272 5158 • F: (+61) 02 6272 4753 • E: contact@apvma.gov.au • W: www.apvma.gov.au
John Curtin House, 22 Brisbane Ave, Barton ACT 2600
PO Box E 240, Kingston ACT 2604
ABN: 19 495 043 447

Australian Pesticides &
Veterinary Medicines Authority

WATTYL AUSTRALIA PTY LTD
4 Steel Street
BLACKTOWN NSW 2148

RESEARCH PERMIT (RP)
FOR SUPPLY AND USE OF AN UNREGISTERED AGVET CHEMICAL PRODUCT
FOR THE PURPOSES OF RESEARCH

PERMIT NUMBER - PER6009

This permit is issued under the Agvet Code, of the relevant jurisdictions, to the person stated above. The holder of the permit must comply with all requirements as specified in the Agvet Code. A summary of the key requirements are that the holder must:

- supply any requested information to the APVMA;
- inform the APVMA if they become aware of any relevant information concerning the uses dealt with by this permit;
- comply with a lawful direction or requirement of an inspector; and
- provide copies of the permit to persons who wish to prepare for use and/or use the product for the purpose specified in this permit.

This permit allows the person listed in ***1. Supplier*** to undertake the following actions with the product listed in ***2. Products*** in the jurisdiction listed in ***3. States***:

(i) to have the product in their possession or custody for the purposes of supply;
(ii) to supply, or cause or permit to supply, the product to the persons listed in ***4. Permitted Users***;
(iii) to supply the product in a container that does not have an approved label attached; and
(iv) to claim that the product can be used for the purposes of research as outlined in ***5. Directions for Use.***

This permit also allows persons listed in ***4. Permitted Users*** to have this product in their possession and to use this product for the purposes of conducting research as specified in ***5. Directions for Use***.

If this permit were not issued possession or custody, supply and use of the product, as specified above, would constitute an offence under the Agvet Codes.

The persons listed in ***1. Supplier*** and ***4. Permitted Users*** must comply with all conditions listed in ***CONDITIONS OF PERMIT*** to be effectively covered by this permit.

THIS PERMIT IS IN FORCE FROM 14 JULY 2003 TO 30 SEPTEMBER 2007.
It is in force until it expires or it is cancelled, suspended or surrendered.

DETAILS OF PERMIT

1. Supplier.
Wattyl Australia Pty Ltd
4 Steel Street, Blacktown, NSW 2148

2. Products
WATTYL AQUACULTURE COATINGS NETCLEAR ZPT
Containing: 414.00 g/L ZINC OXIDE
70.00 g/L ZINC PYRITHIONE as the only active constituents.

3. States
SA, TAS

4. Permitted Users
Nets are only to be dipped or treated at the following sites:
1. Nets Pty Ltd, 172 Kermandie Road, Geeveston, TAS 7116
2. Quin Marine, 24 Windsor Avenue, Port Lincoln, SA 5606

Treated nets must only be used by the following trial collaborators:
1. Australian Fishing Enterprises Pty Ltd, Port Lincoln SA 5606
2. DI Fishing Company Pty Ltd, Port Lincoln SA 5606
3. MG Kailis Pty Ltd, Port Lincoln SA 5606
4. The Stehr Group, Port Lincoln SA 5606

5. Directions for Use

Situation	Purpose	Rate
AQUACULTURE NETS.	PREVENTION/INHIBITION OF FOULING ORGANISMS	Apply by dip application methods only. Apply in accordance with the product label (Attachment 1)

Critical Use Comments:
Nets are only to be washed / prepared on shore using appropriate facilities designed to prevent the flow of effluent into streams, rivers or waterways. Effluent / paint particles must be trapped and disposed of in accordance with State regulations and guidelines.

Nets are to be treated / dipped only at the facilities specified in this permit.

For further product specific information refer to the product label (Attachment 1).

Withholding Period:
NOT REQUIRED WHEN USED AS DIRECTED

CONDITIONS OF PERMIT

PERSONS who wish to prepare for use and/or use the products for the purposes specified in this permit must read, or have read to them, the permit particularly the information included in DETAILS OF PERMIT and CONDITIONS OF PERMIT.

Supply:
The supplier must supply the product in a container that complies with the requirements of section 18(1) of the Agricultural and Veterinary Chemicals Code Regulations. Attached to this container must be a label which is identical in content and format to the label in Attachment 1.

The supplier must supply the product in a container which must:
(a) be impervious to, and incapable of chemical reaction with, its contents when under conditions of temperature and pressure that are likely to be encountered in normal service; and
(b) have sufficient strength and impermeability to prevent leakage of its contents during handling, transport and storage under normal handling conditions; and
(c) if its is intended to be opened more than once-be able to be securely and readily closed and reclosed; and
(d) have sufficient excess capacity to prevent it from breaking if its contents expand during handling, transport or storage; and
(e) enable all or any part of its contents to be removed or discharged in such a way that, with the exercise of no more than reasonable care, the contents cannot:
(i) harm any person; or
(ii) have an unintended effect that is harmful to the environment.
Attached to this container must be a label which contains information and instructions on:
- signal heading, product name, active constituent statement;
- directions for use, withholding periods;
- general instructions on preparation, mixing, plus any precautions;
- safety directions, first aid, storage and disposal.
An example of a suitable label is in Attachment 1. For another label to be deemed suitable for use with this permit it must receive prior written consent from the APVMA. The label must not contain any information that contradicts any details or conditions included in this permit.

Trial records
The permit holder must maintain records of the trials performed under this permit. Specifically details must include the date and location where the trials were conducted, commodities treated, rates and frequency of application, total amount of product used and the names and addresses of the persons conducting the trial. These details must be maintained for a minimum period of two years from the date of expiry of this permit and must be made available to the APVMA upon request.

Effluent Disposal
Nets are only to be washed / prepared on shore using appropriate facilities designed to prevent the flow of effluent into streams, rivers or waterways. Effluent / paint particles must be trapped and disposed of in accordance with State regulations and guidelines.

Net Dipping
Nets are only to be treated / dipped at the facilities specified in this permit. Any effluent or waste (drippings etc) must be trapped and disposed of in accordance with State regulations and guidelines.

Area Treated
The maximum number of nets and maximum size of nets to be treated per trial collaborator per annum is set out in Attachment 2.

Amount Treated
The maximum paint consumption per 120m net is expected to be 1,350 litres. Based on this the maximum amount of paint used under this permit is expected to be 10,800L in year 1, 35,100L in year 2 and 35,100L in year 3 of this permit.

Issued by

Delegated Officer

Permit amended on 30 November 2005 to:
1. Replace references to NRA with references to APVMA;
2. Replace Stolt Sea Farm Pty Ltd with DI Fishing Company Pty Ltd as a trial collaborator;
3. Remove Sunaqua as a trial collaborator, and as they were the only user in QLD, remove QLD as a jurisdiction;
4. As nets can be treated in Tasmania, add that state to the jurisdictions where the permit operates;
5. Extend the expiry date to 30 September 2007.

Attachment

POISON
KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING

WATTYL

AQUACULTURE COATINGS

NETCLEAR ZPT

Active constituents: 414 g/L Zinc Oxide
70 g/L Zinc Pyrithione

FOR EXPERIMENTAL USE ONLY. THIS PRODUCT IS NOT REGISTERED.

A water based copper-free antifouling for controlling marine growth on aquaculture nets

CONTENTS: 200 LITRES

{Insert bar code here}

Batch No.
DOM:
{prism code]

Manufactured by WATTYL Australia Pty Limited, 4 Steel Street, Blacktown, NSW 2148
03 9689 9821

WATTYL AQUACULTURE COATINGS NETCLEAR ZPT

DIRECTIONS FOR USE

- This coating is specifically designed to coat growout, predator, and other types of nylon netting used by the fish farming industry. Nets should be thoroughly cleaned and dried before coating.
- The coating must be mixed well prior to use. During application the coating should be maintained at a minimum temperature of 15°C.
- To apply, immerse the net into the coating, contained in a tank of suitable dimensions. Netting should be submerged in the coating for at least 15 minutes.
- The net should be dried for 72 hours in air temperatures of at least 10°C.
- Coated nets should be in the water for 72 hours before fish are added.
- This product must only be applied at the facilities listed and in accordance with the conditions specified under NRA permit PER 6150 and PER 6009.

NOT TO BE USED FOR ANY PURPOSE, OR IN ANY MANNER, CONTRARY TO THIS LABEL UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION.

EQUIPMENT CLEANING: Clean up with freshwater only.

PROTECTION OF WILDLIFE, FISH, CRUSTACEA AND ENVIRONMENT
This product contains an active constituent which, if used incorrectly, can have detrimental effects on marine life, DO NOT contaminate waterways with paint, dust and scrapings, or with used containers.

STORAGE AND DISPOSAL
Store in the original container, tightly closed, in a cool, dry place. Store at temperatures greater than 4°C. Dispose of container by crushing and disposing in a municipal disposal site.

SAFETY DIRECTIONS
Harmful if swallowed. Corrosive. Attacks eyes. May irritate the skin. Avoid contact with eyes and skin. When opening the container and when mixing and using the product wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow length nitrile gloves, goggles water resistant footwear and half facepiece respirator. If product in eyes, wash it out immediately with water. Wash hands after use. After each days use wash gloves, goggles, respirator (if rubber wash with detergent and warm water) and contaminated clothing.

FIRST AID
If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126.
If in eyes, hold eyes open, flood with water for at least 15 minutes and see a doctor.

Material Safety Data Sheet
Additional information is listed in the Material Safety Data Sheet available from the supplier.

Attachment

Site	Year 1		Year 2		Year 3	
	No. of Nets	Size of Nets	No. of Nets	Size of Nets	No. of Nets	Size of Nets
South Australia						
Australian Fishing Enterprises Pty Ltd	2	120 metres	6	120 metres	6	120 metres
~~DJ Fishing Company Stolt Sea Farm~~ Pty Ltd	1	120 metres	6	120 metres	6	120 metres
M G Kailis (tuna) Pty Ltd	1	120 metres	6	120 metres	6	120 metres
The Stehr Group	2	120 metres	6	120 metres	6	120 metres
Queensland						
~~Sunaqua~~	~~1 or 2~~	~~80 metres~~	~~2~~	~~80 metres~~	~~2~~	~~80 metres~~

Page 6 of 6

APPENDIX 8.4: HEALTH REPORT PROVIDED FOR THE EFFECTS OF ANTIFOULING PAINTS ON SBT

Report on the effects of antifouling paints on SBT health

Prepared by Barbara Nowak

Summary
Parasite loads and histology of gills, liver and spleen indicated that the antifouling treatment had no adverse effect on SBT health.

Parasite loads
The following parasites were found on the fish: gill fluke *Hexostoma thynni*, gill copepods *Pseudocycnus appendiculatus* and *Euryphorus brachypterus*, blood fluke *Cardicola forsteri*. Skin copepod *Caligus* sp. was not found on any of the fish examined. Parasite loads were highly variable and mostly low intensity. Parasite loads were expressed as prevalence (percentage of fish infected) and intensity (an average number of parasites in an infected individual). There appeared to be no effect of antifouling paint treatment on parasite loads at harvest in August.

Table 1. Prevalence and intensity of infection for SBT parasites. Each column represents information for one pontoon.

Pontoon	Treated (n=10)	Control 1 (n=10)	Control 2 (n=10)	Control 3 (n=20)
Hexostoma thynni prevalence (%)	10	0	30	25
intensity	1	0	2	4.4
Pseudocycnus appendiculatus prevalence	20	40	10	80
intensity	2	9.75	11	4.1
Euryphorus brachypterus prevalence	0	10	0	5
intensity	0	1.7	0	2
Cardicola forsteri prevalence	70	30	40	55
intensity	3.4	3.7	4.25	2.8

Histology
Gills, liver and spleen samples were taken from fish from three pontoons (treated and two control cages). These samples were processed for routine histology.

There were no significant differences in structure of gills, liver or spleen between fish from different pontoons. The main gill pathology was due to the presence of blood flukes. Granulomas, inflammation and hyperplastic changes were present in 33% of the fish in each cage. These changes appeared to be most extensive in the gills of fish from the treated pontoon. Inflammatory changes were present in most livers. A few individuals appeared to have bile duct fibrosis, but these were isolated cases present in all pontoons. Both spleen and liver contained melanomacrophage centres, they were very prominent in the spleen. Melanomacrophage centres contained golden pigment in the liver and golden, dark brown and black pigment in the spleen.

APPENDIX 8.5: TUNA BRIEF: HAEMATOLOGY OF SOUTHERN BLUEFIN TUNA

Aquafin CRC, Fisheries Research and Development Corporation, Tuna Boat Owners Association of South Australia, South Australian Research & Development Institute, Stehr Group, Barneveld Nutrition Pty Ltd, Flinders University of South Australia, University of Tasmania, CSIRO Division of Marine Research, Australian Animal Health Laboratory - CSIRO Livestock Industries, Institute of Medical and Veterinary Science, Primary Industries and Resources SA, Queensland Department of Primary Industries, South Australian Department of Environment and Heritage, Aquaculture Management Consultants Pty Ltd

Southern Bluefin Tuna Aquaculture Subprogram Newsletter

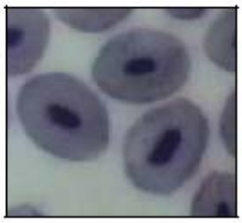
2004 - 9: HAEMATOLOGY OF SOUTHERN BLUEFIN TUNA

Kirsten Rough (TBOAA), Barbara Nowak (University of Tasmania) and Ruth Reuter (Veterinary Pathology Services)

Aquafin CRC southern bluefin tuna (SBT) health research aims to provide background knowledge on fish health to SBT industry and researchers. This includes dissemination of results from previous research on SBT. During our health workshop in May 2004 it was apparent that there was interest in obtaining normal ranges for blood variables. This research area was covered by Kirsten Rough, who completed her Masters thesis on this topic (Rough, 1998) and has a manuscript in press on tuna haematology (Rough *et al*, in press). Kirsten agreed to prepare a Tuna-brief so the results are widely available for the whole SBT industry. The Aquafin SBT health research team is hoping to be able to address this area in more detail in the near future, subject to availability of research students.

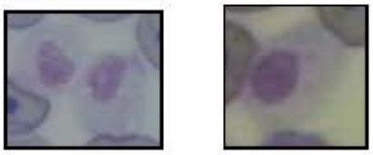
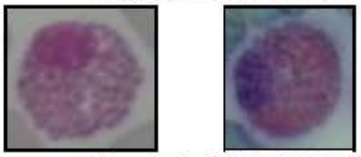
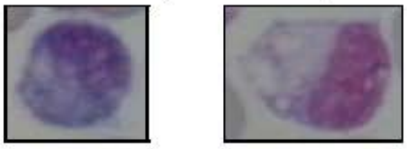
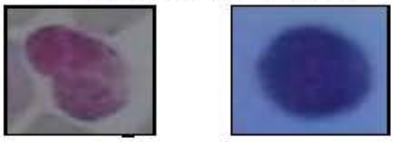
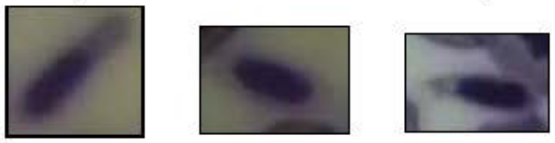
Haematology is the study of blood and involves attributes of the red cells, the white cells and the plasma. Haematology tests can give either a direct or inferential indication of an animal or fishes' functional status and is used for studies of health, physiology and nutritional status. Red blood cells (erythrocytes) are responsible for respiratory gas transport, and carry oxygen from the gills to the organs and tissues; these are the predominant cell type in circulation. White blood cells (leucocytes) are involved in blood clotting and immune responses. The cell types and numbers making up the leucocyte population of fishes vary substantially between species. For this reason it is important that research is conducted for any particular fish (or animal) to establish what is normal for that species. This Tuna-brief illustrates and reports findings of some of this work.

To establish a reference range of haematology parameters for SBT stocked into the farms at Port Lincoln; tuna of the same age class were captured and tested on the fishing grounds in February 1995, April 1996, April 1997 and March 1999. The following table illustrates the actual cells and gives their average dimensions (length by width) and lists the reference range of results for each of these parameters under normal circumstances.

Red Cell Parameters	Whole Cell Size and Cell Appearance	Reference Range
Haematocrit	Erythrocytes 11.46 x 7.70µm; Reticulocytes 9.25 x 8.42µm	41.09 – 55.50 %
Haemoglobin		13.25 – 17.92 g.dL^{-1}
Red Cell Count		2.25 – 3.07 x 10^{12}L^{-1}

When using haematology to assess fish health, it must be noted that some parameters are very sensitive to the physiological state of the fish and also to sample handling techniques. An obvious example of the latter is the significant elevation of haematocrit after only 15 minutes of storage if the sample is not handled appropriately. Therefore, interpretation of results requires experience, whereby both the fish history and the sample handling history are critical factors to consider. Despite this limitation, haematology is a relatively cheap, quick, and effective tool that can assist other testing techniques to diagnose a range of conditions that have occurred in the brief history of tuna farming.

Situations with tuna farming where haematology is useful include assessing the adequacy and quality of the diet, the impact of blood and gill parasites, assessing stress and particularly when assessing fish for dehydration and anaemia. Sometimes when a blood sample is examined, a tuna that is eating and looks perfectly normal swimming in the cage will show a profile characteristic of having undergone short to medium term stress. Whilst this is generally of little consequence when all environmental and other farm conditions are ideal, it is these individuals that are more sensitive and less able to cope if something is not. Tuna attempt to compensate aberrations to maintain stasis and relatively normal function. But often the effort to do so can compromise other functions such as their immune system and growth; and in situations of intense stress (such as predator and poacher harassment) these individuals will rollover rather than recover.

White Cell Parameters	Whole Cell Size and Cell Appearance	Reference Range
Leucocrit		0.62 – 3.00 %
Neutrophilic granulocytes	Neutrophils 9.20 x 8.10µm 	0.00 – 5.45 %
Eosinophilic granulocytes	Eosinophils 9.53 x 9.87µm 	0.69 – 12.06 %
Monocytes	Monocytes 10.50 x 8.88µm 	0.00 – 5.03 %
Lymphocytes	Lymphocytes 7.15 x 5.95µm 	46.97 – 74.32 %
Thrombocytes	Elongate 9.6 x 2.1µm; Oval 8.0 x 2.6µm; Spindle 7.83 x 3.58µm  Spiked 13.50 x 3.25µm 	14.47 – 43.92 %

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References

Rough, K.M (1998). Haematology of wild and captive Southern bluefin tuna *Thunnus maccoyii*. Masters Thesis, University of Tasmania, pp 113.

Rough, K.M., Nowak, B., and Reuter, R.E., (in press). Haematology of southern bluefin tuna, *Thunnus maccoyii*. Journal of Fish Biology (accepted 2002).

APPENDIX 8.6: COST BENEFIT ANALYSES FOR THE USE OF ANTIFOUL TREATMENTS IN SBT INDUSTRY

The following tables summarise the direct cost to farmers:

1) as money outlaid
   - to catch and transport SBT to the farm site (excluding quota and lease costs):
   - to feed SBT until they die
2) and as income forgone due to loss of sales for various rates of mortality.

The tables include values for white and black 150mm stretch-mesh nets with the use of an antifouling treatment on the net; as well as an untreated net for comparison. Note that the difference in cost to coat a white or a black net relate to the cord thickness of these net types, rather than the colour. White nets currently used within the industry have a cord thickness of 5mm, and black nets 3.5mm; this means the white nets have a greater surface area to be covered by antifoulant, and require more product. Note that different surface properties, texture and topography, and different materials may also influence the amount of antifoulant required.

The figure in black in brackets is the difference between the expenditure and income loss for an untreated net and one coated with the antifoulant, THIS FIGURE REPRESENTS THE SAVING TO THE FARMER.

In scenarios 1 to 4 the basic assumptions throughout were that the average **weight sample was 16kg** (at catch and tow costs of $2.50/kg), the **harvest average was 28kg GG** with **average sale price of ¥1900/kg** and a **feed cost of $0.70 /kg** bait fish (assuming 60 days feeding at a rate of 2kg/tuna/day).

SCENARIO 1:

Expenditure and loss of income with initial stock **2000 tuna;** Yen exchange: **$AUD1 = ¥80**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 29 324 ($ 4256)	$ 25 724 ($ 7856)	$ 33 580
3% Mortality [4]	$ 54 572 ($ 10568)	$ 50 972 ($ 14168)	$ 65 140
5% Mortality [4]	$ 79 820 ($ 16880)	$ 76 220 ($ 20480)	$ 96 700

SCENARIO 2:

Expenditure and loss of income with initial stock **2000 tuna;** Yen exchange: **$AUD1 = ¥100**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 27 196 ($3724)	$ 23 596 ($7324)	$ 30 920
3% Mortality [4]	$ 48 138 ($8972)	$ 44 588 ($12572)	$ 57 160
5% Mortality [4]	$ 69 180 ($14220)	$ 65 580 ($17820)	$ 83 400

SCENARIO 3:

Expenditure and loss of income with initial stock **2200 tuna;** Yen exchange: **$AUD1 = ¥80**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 30 902 ($4256)	$ 27 302 ($7856)	$ 35 158
3% Mortality [4]	$ 58 517 ($11357)	$ 54 917 ($14957)	$ 69 874
5% Mortality [4]	$ 86 132 ($18458)	$ 82 532 ($22058)	$ 104 590

SCENARIO 4:

Expenditure and loss of income with initial stock **2200 tuna;** Yen exchange: **$AUD1 = ¥100**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 28 508 ($3724)	$ 24 908 ($7324)	$ 32 232
3% Mortality [4]	$ 51 468 ($9628)	$ 47 868 ($13228)	$ 61 096
5% Mortality [4]	$ 74 428 ($15532)	$ 70 828 ($19132)	$ 89 960

[1] White nets uptake 900L of antifoulant at $12/L + $1500 for application and $2000 for freight; require 4 days maintenance at $600/day

[2] Black nets uptake 600L of antifoulant at $12/L + $1500 for application and $2000 for freight; require 4 days maintenance at $600/day

[3] Untreated nets are cleaned once through cycle and prior to removing from water at $3500 each; require 18 days maintenance at $600/day

[4] Antifoul treated nets have a 20% reduction on untreated mortality number

In scenarios 5 to 8 the basic assumptions throughout were that the average **weight sample was 16kg** (at catch and tow costs of $2.50/kg), the **harvest average was 28kg GG** with **average sale price of ¥1800/kg** and a **feed cost of $0.70 /kg** bait fish (assuming 60 days feeding at a rate of 2kg/tuna/day).

SCENARIO 5:

Expenditure and loss of income with initial stock **2000 tuna;** Yen exchange: **$AUD1 = ¥80**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 28 764 ($4116)	$ 25 164 ($7716)	$ 32 880
3% Mortality [4]	$ 52 892 ($10148)	$ 49 292 ($13748)	$ 63 040
5% Mortality [4]	$ 77 020 ($16180)	$ 73 420 ($19780)	$ 93 200

SCENARIO 6:

Expenditure and loss of income with initial stock **2000 tuna;** Yen exchange: **$AUD1 = ¥100**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 26 748 ($3612)	$ 23 148 ($7212)	$ 30 360
3% Mortality [4]	$ 46 844 ($8636)	$ 43 244 ($12236)	$ 55 480
5% Mortality [4]	$ 66 940 ($13660)	$ 63 340 ($17260)	$ 80 600

SCENARIO 7:

Expenditure and loss of income with initial stock **2200 tuna;** Yen exchange: **$AUD1 = ¥80**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 30 272 ($4116)	$ 26 672 ($7716)	$ 34 388
3% Mortality [4]	$ 56 662 ($10902)	$ 53 062 ($14502)	$ 67 564
5% Mortality [4]	$ 83 052 ($17688)	$ 79 452 ($21288)	$ 100 740

SCENARIO 8:

Expenditure and loss of income with initial stock **2200 tuna;** Yen exchange: **$AUD1 = ¥100**

	White net with antifoulant [1]	Black net with antifoulant [2]	Untreated net [3]
1% Mortality [4]	$ 28 004 ($3612)	$ 24 404 ($7212)	$ 31 616
3% Mortality [4]	$ 49 984 ($9264)	$ 46 384 ($12864)	$ 59 248
5% Mortality [4]	$ 71 964 ($14916)	$ 68 364 ($18516)	$ 86 880

[1] White nets uptake 900L of antifoulant at $12/L + $1500 for application and $2000 for freight; require 4 days maintenance at $600/day

[2] Black nets uptake 600L of antifoulant at $12/L + $1500 for application and $2000 for freight; require 4 days maintenance at $600/day

[3] Untreated nets are cleaned once through cycle and prior to removing from water at $3500 each; require 18 days maintenance at $600/day

[4] Antifoul treated nets have a 20% reduction on untreated mortality number