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Manatū Ahu Matua

Optimising settlement arrays for surveillance of non-indigenous biofouling species

Literature review

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Abbreviations and definitions

MHRSS	Marine High Risk Site Surveillance
NIS	Non-indigenous species
NMS	National Monitoring Strategy of the Australian Commonwealth, State and Territory Governments
OSM manual	Observation Systems Methods Manual (Attachment 2 of the Australian Marine Pest Monitoring Manual Version 1)
Passive sampling	Collection of an environmental sample by exposing a surface or medium to the ambient environment over a fixed period of time
Passive surveillance	The discovery and reporting of suspect risk organism (often by members of the general public, or interested sector groups) from activities that are not purposeful surveys for the organisms
Recruitment	A combination of settlement and post-settlement processes resulting in the settling organism being counted by the observer
Sensitivity (of a sample unit)	The probability that a species will be observed in a sample unit if it has encountered the unit
Settlement	The process whereby the planktonic life stage of an organism makes contact with, and attaches to substratum, which can be reversible. Often (incorrectly) used inter-changeably with "Recruitment"

1 Executive summary

The Ministry for Primary Industries commissioned an evaluation of the utility of passive sampling devices or "settlement arrays" for early detection of marine non-indigenous species (NIS) to complement the sampling methods currently used in the national Marine High Risk Site Surveillance (MHRSS). Passive sampling methods have a long history of use in marine biological research, where they have been used predominantly to study the recruitment of sessile marine organisms from planktonic life stages (larvae, spores, etc.) into a benthic juvenile or adult phase. They may have some advantages (and disadvantages) over current sampling methods used in the MHRSS including:

- the ability to sample species continuously over a period of time ("time integrated sampling"),
- the ability to sample locations that are inaccessible to divers and other sampling methods,
- the ability to sample juvenile (pre-reproductive) stages of NIS, and
- less dependence on specialist expertise to obtain the samples.

This literature review forms the first component of the evaluation. It is intended to inform the design of subsequent field trials of the arrays to optimise their use for detecting a broad range of non-indigenous biofouling organisms.

The review summarises current knowledge on the use of passive sampling methods in marine biosecurity surveillance and aspects of the design and deployment of settlement arrays that are likely to influence the types and abundances of biofouling organisms sampled by them. Published scientific literature, unpublished technical reports and scientific experts were consulted to evaluate the importance of a range of design and environmental influences on the biofouling assemblages sampled by settlement arrays. These included:

- the type and colour of material,
- plate size,
- depth of deployment,
- orientation,
- surface roughness,
- period of deployment (season and duration),
- presence of antifouling coatings (including methods used to prematurely age the coatings),
- broad taxonomic differences in settlement preferences, and
- the number and spatial distribution of arrays.

The review identified a number of key characteristics of settlement arrays that have a strong influence on the types of biofouling organisms that recruit to them. Most important were the timing, duration and depth of deployment, orientation (and shading) of the surfaces, surface rugosity, predation and the presence of antifouling coatings. By comparison, the type of material used to construct the surfaces and its size appear to have relatively minor effects on the composition and richness of the assemblages, but did affect the abundance of individual species.

The presence of light and accumulation of sediment on deployed structures have important influences on biofouling macroalgae and invertebrates. Macroalgal species are more frequently observed on vertically-oriented and upward-facing structures at shallow depths (< 5 m), where there is limited light attenuation. In contrast, the accumulation of sediment on upward-facing surfaces in estuarine environments limits recruitment of invertebrates, many of which exhibit a preference for the shaded undersides of surfaces or on vertically-oriented structures where sediment cannot collect.

Studies have highlighted the importance of fish predation on settlement array assemblages whereby unprotected settlement arrays develop different assemblages compared to protected surfaces, particularly at tropical latitudes.

The presence of biocidal antifouling coatings inhibits recruitment of biofouling organisms, but as the coating degrades the composition of the assemblage is influenced by the tolerance of different species to the residual biocides. Many non-indigenous barnacles, bryozoans, and polychaete worms, in particular, exhibit a high tolerance to common antifouling biocides and occur earlier and in greater abundance on biocidal coated surfaces.

Although settlement arrays are used in a number of marine biosecurity applications overseas, there have been relatively few assessments of their utility for early detection of biofouling NIS. A disadvantage of this method for early detection is that, like other methods of passive sampling, the relationship between the presence and abundance of the target species within the environment and its detection on the settlement surface is complex and difficult to quantify. For biofouling species, this can mean that:

- uncommon (rare) biofouling species, including those that are at an early stage of population establishment, will be under-sampled, and
- absence from an array does not necessarily mean the absence of an established population (because of species-specific variation in settlement preferences).

Although arrays can be deployed and retrieved by people with limited scientific knowledge, another disadvantage associated with the use of settlement arrays is that, identification of accumulated organisms requires specialist taxonomic expertise and can be time-consuming, sometimes requiring up to 4 h processing per plate, and expensive. In Australia, Canada and the USA, where non-specialists have been used to implement surveillance using settlement arrays, the programmes are focussed on a suite of target species, rather than all potential NIS.

Based on the results of the review, and taking into account the constraints of the project budget, it is recommended that the proposed field trials incorporate experimental treatments to determine the best combination of array design features to complement the MHRSS programme:

- orientation of plates (vertical vs. horizontal undersides),
- predation cages (caged and uncaged),
- presence of antifouling coating (three levels: non-biocidal control, thin antifouling top coat and moderate antifouling top coat), and
- surface rugosity (rough only).

The plates should be constructed of polyvinyl chloride and all plates should be deployed at 2 m depth from floating structures. A minimum of ten replicates of each treatment condition should be deployed to allow a robust assessment of their ability to sample NIS richness. The plates should be deployed for a minimum of three months to allow biofouling to reach a size and maturity to enable high taxonomic resolution.

2 Introduction

2.1 BACKGROUND

The Ministry for Primary Industries (MPI) leads and co-ordinates the New Zealand government's biosecurity activities, including providing national leadership for biosecurity surveillance. Biosecurity surveillance can be defined as the collection, collation, analysis, interpretation and timely dissemination of information on the presence, distribution or prevalence of non-indigenous species (NIS) and their adverse effects on New Zealand environments (MAF Biosecurity New Zealand, 2009). Surveillance activities are used as an information base to support MPI's overarching goal of protecting the economy, environment and people of New Zealand from the risks associated with the introduction of NIS, and mitigating the effects of NIS that are already present in New Zealand.

Early detection surveys are an important component of biosecurity surveillance. Lags in the detection of NIS or range extensions by NIS already present in New Zealand provide them with a chance to establish, proliferate, spread and cause harm. Successful eradication and management of NIS and diseases often hinge upon the ability to detect new populations when they are relatively small and easily contained and treated (Tobin et al, 2014).

Since 2002, MPI has funded a nationwide programme of targeted surveillance for high risk marine pest species at a selection of New Zealand's ports and marinas ("Marine High Risk Site Surveillance", MHRSS). The main objectives of the MHRSS are to detect incursions of:

- new to New Zealand NIS listed on the Unwanted Organisms Register at high risk sites throughout New Zealand,
- new to New Zealand non-indigenous or cryptogenic species not listed on the Unwanted Organisms Register at high risk sites throughout New Zealand, and
- established non-indigenous or cryptogenic species that exhibit pest characteristics (e.g., range extensions).

For the MHRSS to be effective for the successful eradication or management of NIS, it must function in such a way that maximises the likelihood of early detection.

Biofouling of vessels is an important vector for the introduction and spread of non-indigenous species (NIS) (Gollasch 2002). A recent analysis of incursions to New Zealand and Australia between 1995 and 2002 recorded 18 and 17 new incursions respectively, with 22 of those species suspected to have arrived via vessel biofouling and five by ballast water (Cranfield et al, 1998, Kospartov et al, 2008, Hewitt et al, 2009). Currently, the MHRSS relies on visual search methods (by SCUBA divers and shoreline observers) to detect high risk sessile fouling species in port environments. In adverse weather conditions or low underwater visibility, the effectiveness of visual surveys can be impaired, with the associated likelihood that target species (particularly juveniles) may be overlooked (Hayes et al, 2005, Gust and Inglis, 2006).

Given the predominance of species arriving in New Zealand by vessel biofouling (Cranfield et al, 1998, Kospartov et al, 2008), methods that are specifically targeted at detecting newly arrived biofouling species may be used to supplement the visual searches currently used in the MHRSS and, thereby, enhance the likelihood that they will be detected early. In the marine environment, representatives of almost every phylum can be found on or adjacent to hard substrata (Maughan and Barnes, 2000). Therefore settlement arrays have the potential to be useful complementary tool for sampling a wide variety of biofouling associated species.

2.2 PURPOSE OF THIS REVIEW

MPI commissioned an evaluation of the utility of passive sampling methods - "settlement arrays" – for the detection of biofouling NIS. The study consists of two components:

1. A review of existing literature on the utility of settlement surfaces for sampling biofouling species to guide their design and deployment for New Zealand marine biosecurity surveillance.
2. A field test of settlement array surfaces and modes of deployment as recommended by the literature review to determine the optimal configuration for detecting the largest number of biofouling NIS.

This literature review forms the first component of the study. It is intended to inform the choice of settlement substrata and method(s) of deployment of the surfaces used in the subsequent field test and to provide recommendations for the design and deployment of settlement arrays within the MHRSS. The selection of relevant resource material for this review was guided by the intended purpose of the settlement arrays, which is to:

- provide an attractive surface for the settlement of biofouling NIS (including, but not limited to, those that are listed on the New Zealand Unwanted Organisms Register), and
- complement the existing MHRSS programme.

Thus, the intention is to optimise a passive sampling method that will capture a variety of non-indigenous biofouling species rather than to optimise the methodology for specific high risk species (e.g., Floerl et al, 2012a). Nevertheless, where literature is available on particular high risk species (i.e., those listed as primary or secondary target species for the MHRSS) summaries of the settlement preferences of those species are provided. In order to complement the MHRSS, any recommended methodology must be timely, efficient, cost-effective and repeatable and should supplement the coverage of the existing programme.

This literature review was compiled by accessing peer-reviewed scientific publications (journal articles, books and book chapters), technical reports and unpublished datasets and through direct contact with relevant specialists in this field. These resources were identified by querying literature databases (e.g., ISI Web of Science; Google Scholar), through personal communication with colleagues and from the author's own collection of relevant publications in this field.

Because there is an extensive collection of literature on marine biofouling, the review focused on publications that dealt with the development of biofouling assemblages on passive sampling devices and those that focus more specifically on broader taxonomic groups of NIS. The groups of organisms for which specific information is provided (where available) are the major taxa identified within MPI's Risk Analysis for Vessel Biofouling (Bell et al, 2011), namely ascidians, macroalgae, molluscs, polychaetes, hydroids, bryozoans, sponges, and crustaceans. Although array design should consider the requirements of these taxa, it should allow for the recruitment of as wide a range of biofouling NIS as possible.

The use of passive sampling methods in environmental monitoring and marine ecological research and their application to biosecurity surveillance are outlined in the following sections. The methods used for settlement arrays in other biosecurity monitoring programmes are described as are the requirements of major biofouling taxa for settlement substrata, surface orientation, depth and the use of antifouling paints. Finally, recommendations are provided for field trials to test the importance of different components of array design for sampling a diverse range of NIS. Recommendations and protocols for use of settlement arrays for on-going surveillance of NIS are provided in the field trial report (Tait et al, 2016).

2.3 GENERAL THEORY AND BENEFITS OF PASSIVE SAMPLING

For the purposes of this review, *passive sampling methods* are defined as those in which an environmental sample is obtained by exposing a surface or medium to the ambient environment over a fixed period of time[1]. The sample accumulates on, or is captured by, the surface as the surrounding environmental medium (e.g., air, water, soil, etc.) moves past it. Passive sampling methods are used routinely in environmental monitoring to measure ambient concentrations of bioavailable pollutants in air, water, and soil (US EPA, 2012). In this context, their advantages over conventional, discrete sampling methods, such as pump or grab samples, are that they:

- can be deployed directly in the environment and accumulate the sample *in situ*,
- provide a sample that is integrated over the deployment period ("time-averaged"), rather than an instantaneous sample, and therefore better reflect average conditions in the environment,
- are often relatively low-cost to purchase, construct and deploy, and
- require little training or expertise to take a sample successfully (Seethapathy et al, 2008, US EPA, 2012).

A key challenge to interpreting the results obtained from passive sampling methods is a need to understand the relationship between the rate at which the sampled entity encounters the collector (as a result of diffusive or directional flow) and its adsorption and retention on the surface (Seethapathy et al, 2008, Floerl et al, 2012a, US EPA, 2012). That is, how representatively the sampling method accumulates the entity from the surrounding environment. This is an important consideration in understanding the utility of passive sampling methods for marine biosecurity surveillance (Floerl et al, 2012a; Section 2.4).

In environmental monitoring, different commercially available passive samplers vary in their efficacy for sampling different pollutants under different environmental conditions. Uptake and sorption of each pollutant by the sampler is a function of, among other things, the sorbent used on the surface, the duration of deployment, flow velocity, and temperature (Kot-Wasik et al, 2007, Seethapathy et al, 2008). Where the objective is to sample a range of pollutants it is often necessary to deploy combinations of samplers ("arrays"). While the physical deployment of the passive samplers is fairly simple, the sampling strategy involved in choosing the number and type of samplers for deployment, their locations, time and duration of exposure, as well as quantification in the laboratory, require careful consideration (Seethapathy et al, 2008). Similar deliberations are required to optimise use of passive sampling for biofouling species, since there may be considerable variability in the attractiveness of different settlement surfaces over the range of biofouling species.

2.4 USE OF PASSIVE SAMPLING IN MARINE ECOLOGICAL RESEARCH

Passive sampling methods have a long history of use in marine biological research, where they have been used predominantly to study the recruitment of sessile marine organisms from planktonic life stages (larvae, spores, etc.) into a benthic juvenile or adult phase (Butler, 1986, Nandakumar et al, 1993, Glasby and Connell, 2001, Johnston et al, 2002). Artificial substrata have been used as research tools because they provide a cheap, convenient and replicable unit of habitat with which to study these life stages. The surfaces present unoccupied habitat to settling planktonic life stages on which they can attach and grow. Although such surfaces do not typically have any specific form of attractant for the target species, they instead rely on the rate at which the organism naturally encounters the surface as it moves through the

[1] Note that this differs from "passive surveillance" which is the discovery and reporting of suspect risk organism (often by members of the general public, or interested sector groups) from activities that are not purposeful surveys for the organisms.

surrounding environment and its attractiveness for settlement. In this sense, they are analogous to the passive sampling methods used in pollution monitoring (described above) and to flight intercept (or barrier) traps used in the study of insects (Southwood, 1978).

As with the aforementioned forms of passive sampling, understanding the relationship between the availability of settling larval stages in the water and what is sampled by the collectors can be problematic. Variation in the rate of recruitment may be influenced by the rate at which larvae encounter the surface (including their abundance, distribution in the water column and transport by advective currents and wave force), the attractiveness of the surface to the larva and post-settlement mortality prior to observation by the researcher (Keough and Downes, 1982).

A wide variety of artificial substrata have been used to sample the settling life stages of marine organisms. These have included mesh pads ("Tuffy" pads; Connolly et al, 2001) and grids (Giangrande et al, 2005), filamentous filters (Moksnes and Wennhage, 2001) and rope (Forrest et al, 2000), and flat plates of glass, asbestos, cement, wood, Perspex, aluminium, polyvinyl chloride (PVC) and polystyrene, among other things (e.g., Schmidt and Warner, 1984, Costello et al, 1986, Johnston and Keough, 2000, Holloway and Keough, 2002, Ramsay et al, 2008, Darbyson et al, 2009). While a range of studies have used the surfaces to study specific groups of organisms or species (e.g., Marshall and Cribb, 2004, Labowitch and Cribb, 2006, Martin et al, 2011, Sephton et al, 2011), other research has been more concerned with the types of assemblages that recruit onto artificial substrata (e.g., Allen and Wood, 1950, Anderson and Underwood, 1994, Knott et al, 2004, DeRivera et al, 2005, Tyrrell and Byers, 2007) and the successional processes of fouling assemblages (e.g., Butler, 1986, Nandakumar et al, 1993, Glasby and Connell, 2001, Johnston et al, 2002).

Biofouling assemblages develop on all submerged surfaces throughout the world's oceans. However, the composition and intensity of biofouling varies widely in space (e.g., between scales of 10's to 100's of metres, climatic regions, different physical environments, depth; Keough, 1983, Davis, 2009) and time (e.g., season, immersion period; Keough, 1983, Butler, 1986, Butler, 1991, Caley et al, 1996, Thomason et al, 2002, Dürr, 2010).

Because of the range of scientific motivations for the use of passive sampling methods in marine ecological research, there is a notable lack of standardisation of the type, size and method of deployment of arrays for most species and processes under study, making cross-study comparisons difficult. Despite this, passive sampling methods have played a key role in advancing our understanding of a large number of ecological patterns and processes in marine environments (Davis, 2009), including a long history of studies on marine biofouling communities (Coe, 1932). For settlement arrays to be useful in early detection of NIS, they must be able to sample NIS when they are still relatively uncommon at the sample location (Sutton and Hewitt, 2004, Hayes et al, 2006, Inglis et al, 2006c, Floerl et al, 2012a).

2.5 USE OF PASSIVE SAMPLING IN MARINE BIOSECURITY SURVEILLANCE

Settlement surfaces have previously been used to study the demography, life history and ecological processes that contribute to the success of NIS (Tyrrell and Byers, 2007, Inglis et al, 2009, Nutsford, 2010, Floerl et al, 2012a). They have also been used to monitor new incursions of NIS for biosecurity management (Marshall and Cribb, 2004, Labowitch and Cribb, 2006, Floerl et al, 2012a) and to document the spread of established NIS (Sephton et al, 2010).

Several national (or regional) monitoring programmes, particularly in Australia, Canada, USA and New Zealand, have utilised settlement surfaces for biosecurity surveillance activities.

2.5.1 Australia

Development of a national management framework for marine NIS in Australia (the 'National System') is coordinated by the Commonwealth Department of Agriculture, Fisheries and Forestry. The National System was developed collaboratively through an introduced marine pests coordination group, consisting of representation from all coastal Australian States and Territories, marine industries, conservation groups and researchers (Hewitt et al, 2009). Underpinning the National System was a series of comprehensive marine biological baseline surveys undertaken in high risk ports and marinas in the 1990's (Campbell et al, 2007, Sliwa et al, 2009).

The Australian State/Territory Governments agreed to an on-going National Monitoring Strategy (NMS) for introduced marine pests that targets 55 NIS (Australian Government Department of Agriculture Fisheries and Forestry, 2010). The design for the settlement plate arrays that was recommended for the NMS was based on protocols described in Sutton and Hewitt (2004; Figure 2-1). Some jurisdictions have subsequently used an alternative design (e.g., Northern Territory and Western Australia).

The protocol recommended in the NMS Observation Systems Methods manual (OSM manual; Australian Government Department of Agriculture Fisheries and Forestry, 2005) used 14.5 x 14.5 cm PVC plates that were abraded by sandblasting on one side. Two holes were drilled in the middle of the each plate and it was secured to a brick with cable ties with the roughened side facing away from the brick. One end of a rope was attached to the brick and the other to a structure in the environment (e.g., wharf or piling) or to a float and marker buoy system (Figure 2-1; Sutton and Hewitt, 2004). Although the NMS OSM manual did not contain specific recommendations for the orientation, depth and duration of deployment of the plates[2], the protocol provided by Sutton and Hewitt (2004) was to secure the plates horizontally at a depth of 2 m below low tide. The timing and duration of deployment depended on the species targeted and the geographical region in which monitoring occurred, but was around 3 months in Tasmania (Sutton and Hewitt, 2004).

[2]Instead, the OMS manual recommended "*Lower(ing) the plates into the water column to a suitable depth for detecting the target species. Plates should be orientated to attract the target organisms, e.g., horizontally or vertically. Leave plates in the environment until encrusting organisms are large enough for taxonomic identification.*"

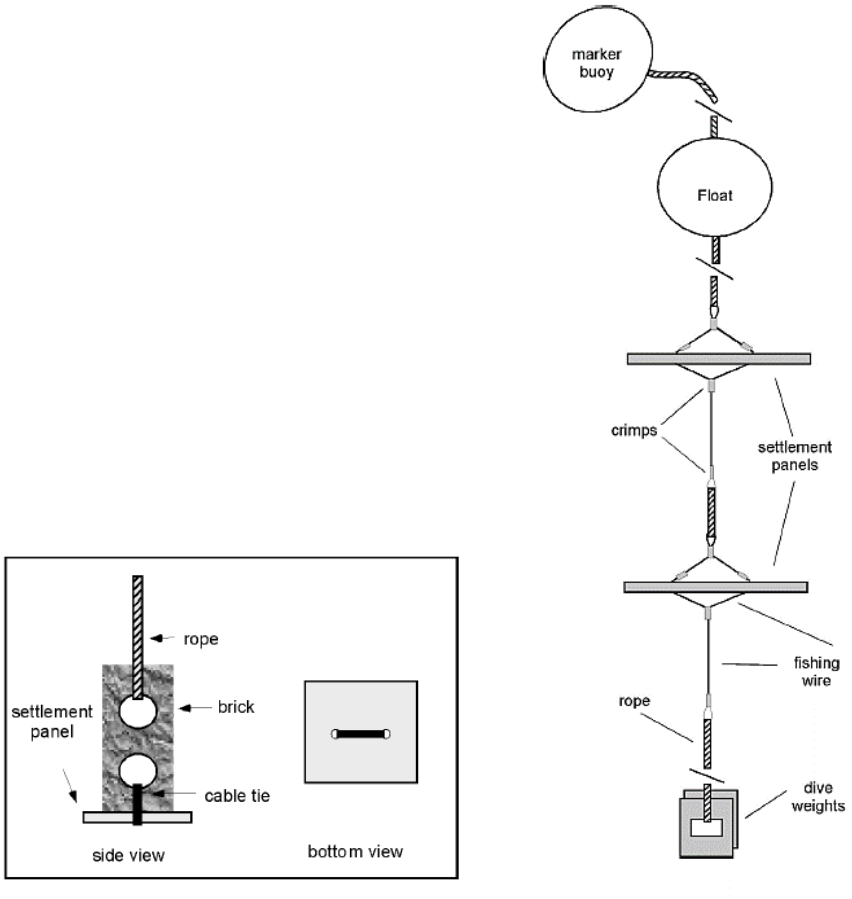

Figure 2-1: Methods for deployment of settlement plates recommended by Sutton and Hewitt (2004).

Although 21 of the 55 Australian target species are listed by the OSM manual as susceptible to sampling by the settlement plates (Australian Government Department of Agriculture Fisheries and Forestry, 2005), no evidence is provided for the sensitivity of the method for detecting these species. For some of the listed species (e.g., *Eriocheir* sp., *Potamocorbula amurensis*, *Charybdis japonica*, etc.) settlement plates are unlikely to be an effective sampling method. Mobile or infaunal species, such as these, are better sampled by baited traps and dredging techniques (Justin McDonald, pers. comm.).

Sutton and Hewitt (2004) compared the range of species detected on these settlement plates that were deployed for up to six months (n = 18 plates analysed per site) with those recorded in scrape samples from wharf piles (n = 9 x 0.1 m^2 scrapes per site) taken from the same five sites. In general, twice as many species were recorded from the wharf pile scrape samples than from the settlement plates. However, each wharf pile scrape sampled an area up to three times that of the settlement plates and the assemblages on wharf piles were mature, likely providing complex habitats for a wider range of species (Sutton and Hewitt, 2004). The study concluded that settlement plates were "*very good at detecting common fouling species* (i.e., species that occurred in more than 40 % of the pile scrape samples), *but had low detection sensitivity for rare species*" (Sutton and Hewitt, 2004). Because many of the common species were non-indigenous, the sampling efficacy for them tended to be greater than for the total species assemblage. A comparable (unpublished) study of biofouling assemblages on settlement arrays and wharf piles in the USA reached a similar conclusion (Gregory Ruiz, pers. comm.). Common NIS tended to be well-dispersed within the sampled environment and were more likely to be sampled by settlement arrays than indigenous species, but uncommon NIS were poorly sampled. These findings are not unexpected as the sampling of uncommon

species is an issue typically encountered in the field of ecological sampling (Cunningham and Lindenmayer, 2005).

To increase the diversity of species detected by settlement plates, Sutton and Hewitt (2004) recommended a sample size of 15-20 plates per site and that the number of sites sampled per port should be up to 16 (depending on the size of the port). They conceded, however, that the effort to do so would be substantial, particularly as their protocol indicated that it could take up to 4 h to process and identify the species on a single plate (Sutton and Hewitt, 2004).

Northern Territory and Western Australia

Following the incursion and subsequent eradication of the black-striped mussel, *Mytilopsis sallei*, in Darwin in 1998, the Northern Territory Government implemented a regular monitoring programme for marine biofouling organisms that began in 2000 (Cribb et al, 2010). The programme principally (but not exclusively) targeted three species of mussel - *M. sallei*, *Perna viridis* (Asian green mussel) and *Arcuatula* (*Musculista*) *senhousia* (Asian bag mussel) – and used a settlement array design that was developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) for surveillance of *M. sallei* following its eradication (Ferguson, 2000).

The original array consisted of a rope backbone to which two PVC pipe T-units were secured (Figure 2-2). The T-units were attached to the rope backbone at two water depths (originally ~1 m below the water surface and ~1 m above the sea floor; Ferguson, 2000). Each T-unit had two horizontal arms comprising 0.7 m lengths of 25 mm diameter PVC pipe. On each T-unit, two 14.5 x 14.5 cm flat sheets of PVC were fixed horizontally and two were fixed in a vertical position to target organisms with different light requirements. A 15 cm length of "hairy" or "Christmas tree" rope mop was suspended midway along each horizontal tube. The arrays were deployed by attaching the rope backbone to the undersides of wharves using a metal eyelet drilled into the wharf. The base of the array was anchored to the seafloor by a single concrete block.

Variations on this design (involving different depths of deployment and numbers and orientations of PVC plates) are currently used in marine pest monitoring programmes in the Northern Territory and Western Australia, respectively (Marshall and Cribb, 2004, Labowitch and Cribb, 2006, McDonald and Travers, 2008, Bridgwood and McDonald, 2010, Muñoz and Bridgwood, 2012, Northern Territory Government, 2014). In the Northern Territory, settlement arrays are suspended from a floating structure (i.e., mooring buoy, pontoon or wharf) so that they move vertically with the tide and the settlement surfaces are maintained at ~2.5 m water depth. The protocol involves deploying three PVC plates and a single rope mop on one arm of the T-unit (the 'tagged' side). The surfaces are inspected monthly for marine pest species of concern and, after two months, a second set of three PVC plates and rope mop are attached to the untagged side of the unit. After four months of deployment the original settlement surfaces are retrieved while the second set of surface is retrieved two months later. This allows for two, overlapping deployments, each of four months duration. In general, two arrays are deployed at each monitoring site within the survey area (seven locations are regularly sampled in Darwin Harbour; Northern Territory Government, 2014). Upon retrieval, the surfaces are inspected for "*known marine pest species*" and the percentage cover of individual taxonomic groups is assessed. Select taxonomic groups of organisms (i.e., ascidians, barnacles, bivalves, bryozoans and polychaetes) are identified to species-level.

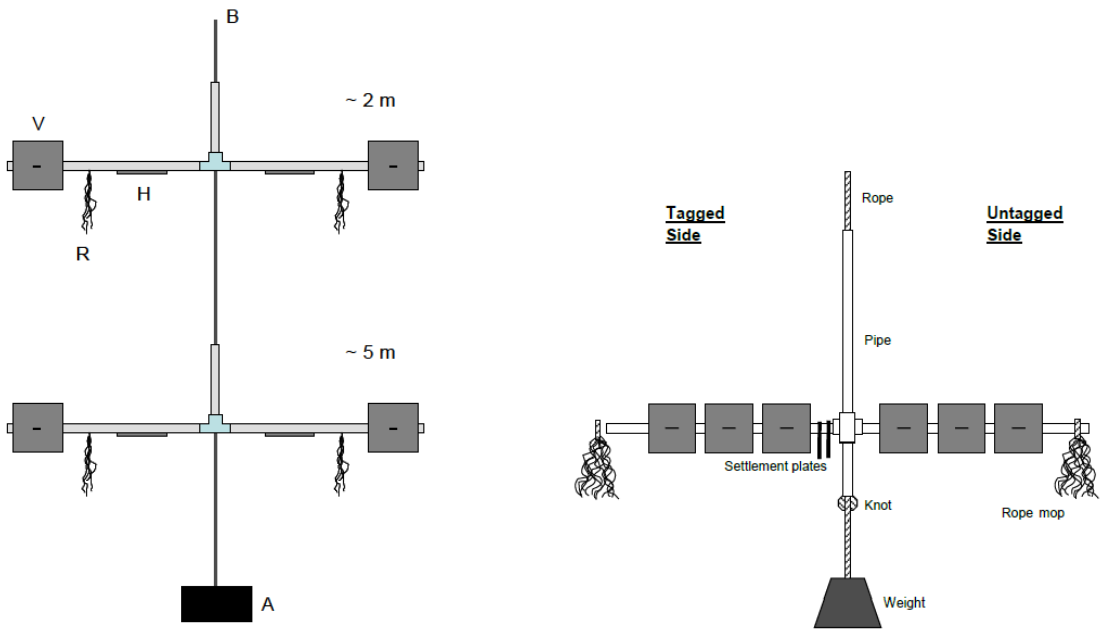

Figure 2-2: Variations on the settlement array designed to monitor black-striped mussels, *Mytilopsis sallei*, in Darwin. (Left) Arrays with a combination of vertical (V) and horizontal (H) PVC plates and rope mops deployed at 2 m and 5 m water depth (Source: Floerl et al, 2012a). (Right) A variation on the design currently used by the Northern Territory Government with multiple vertical plates and a single T-unit (Source: Northern Territory Government, 2014).

In Western Australia, the two-tiered design, containing both vertical and horizontal plates and rope mops is used (Bridgwood and McDonald, 2010). Depending on water depth, a third T-unit may be added to the arrays. The arrays are typically deployed for two to three months before being retrieved. Specimens that may be on the target list of concern are identified to species-level. Other groups are sorted into morpho-species and voucher specimens of each are retained (Bridgwood and McDonald, 2010).

The Australian Navy and Defence Science and Technology Group (DSTG) have also used settlement arrays at a number of its sites in Australia to detect incursions by the Asian green mussel, *P. viridis*. These arrays consisted of a length of commercial mussel spat catching rope connected to a PVC settlement plate (Richard Piola, pers. comm.).

2.5.2 Canada

In Canada, settlement arrays have been used to document the spread of invasive ascidians, such as, *Styela clava* and *Didemnum vexillum* (Sephton et al, 2011). Arrays deployed in Nova Scotia in 2006 consisted of a flowerpot saucer (25 cm diameter) that shaded three Petri dishes attached to the underside of the saucer and six sanded, 10 x 10 cm PVC plates attached to a rope, which passed through the centre of the saucer. From 2007, a modified array was deployed that consisted of a rope with three sanded, 10 x 10 cm square PVC plates spaced 20 to 30 cm apart (Sephton et al, 2011). Similar to the settlement arrays programme in Western Australia, deployment and collection was a collaboration between local agencies and residents.

The arrays deployed proved useful for tracking the spread of ascidians throughout Nova Scotia (Sephton et al, 2011), and a similar programme detected a new incursion of the ascidian *Botrylloides violaceus* in 2009 (Martin et al, 2011). There is, however, no indication of how accurately the detections on the settlement arrays reflected the timing of arrival of the ascidians in other habitats. Unlike the Australian monitoring programmes, the arrays used in

Canada were designed specifically to target ascidians and it is unclear how useful they may be for detecting NIS from other taxonomic groups.

Settlement arrays have also been used to estimate the scale of dispersal of *Ciona intestinalis* in Prince Edward Island and highlighted the importance of situating the arrays in relative proximity to vectors for successful early detection of NIS (Collin et al, 2013). In the case of *C. intestinalis*, peak recruitment occurred 1 km away from the adult population, with time lags between egg release, fertilisation and settlement responsible for the low settlement of recruits in close proximity to adults (Collin et al, 2013).

2.5.3 USA

While the variety of authorities and jurisdictions involved in the management of invasive aquatic species in the USA complicates the development of a national monitoring programme (Hewitt et al. 2009), there is a regional invasive monitoring programme across the Pacific West Coast (DeRivera et al, 2005). The programme had the general goals of:

- detecting biological invasions by NIS, and
- measuring changes in the distribution and abundance of marine invertebrates (Ruiz et al, 2009).

The purpose of the monitoring programme was not to initiate short-term incursion management in response to the detection of new NIS, but to track large scale and long-term change in the composition of the non-indigenous and indigenous species assemblages (Gregory Ruiz, pers. comm.).

The integrated Pacific West Coast programme was collectively named the 'Adopt-a-plate' programme because it incorporated deployment and collection of plates by local residents and agencies. It used settlement plates that had a similar design to Sutton and Hewitt (2004). They consisted of single PVC plate attached to a concrete brick (Figure 2-3; Ruiz et al, 2009). The plates were deployed for three months and were re-deployed quarterly for year round monitoring (Ruiz et al, 2009). They were deployed in 1.5 to 2.0 m of water (from floating docks) with five plates deployed at a central marina/port location and another five plates dispersed among five outlying locations.

Similar to the monitoring programmes in Western Australia (Section 2.5.1) and Nova Scotia (Section 2.5.2), these monitoring systems were intended to engage local residents to contribute to the collection of data and deployment of settlement plates (Ruiz et al, 2009, Doroff et al, 2011).

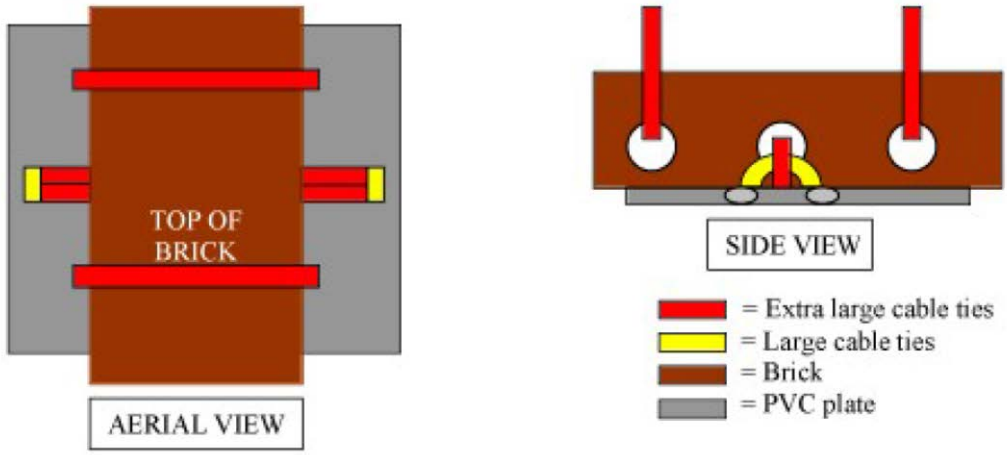

Figure 2-4: Settlement plate deployment design used by Ruiz et al. (2009). Individual plates were deployed attached to a brick, which was secured to floating pontoons.

In the Pacific West Coast settlement plate programme, several authorities and institutions have together developed a large-scale monitoring programme for invasive species in marine protected areas (DeRivera et al. 2005). While this programme includes a range of sampling methods, settlement plates were used at a series of sites to test for the presence of invasive biofouling species (DeRivera et al, 2005). Overall, the programme is designed to detect any invasive organisms and was not restricted to specific target species, as in Canada (Martin et al, 2011). However, given that the arrays used only down-wards facing settlement plates, the approach is unlikely to sample photosynthetic organisms well (see Section 3.3 – Depth and orientation of plates).

The coast-wide study by DeRivera et al, (2005) found 30 NIS on settlement plates as well as five new range expansions. Similar to what has been proposed by MPI, this project highlighted the utility of using multiple survey methods to create more complete lists of NIS within an area (i.e., an inventory-type survey, such as the port biological baseline surveys; Hewitt et al, 2001).

The level of taxonomic resolution used in the USA studies was dependent on the study's purpose and implementation. For example, long-term monitoring at key sites in California (e.g., San Francisco Bay, San Diego, etc.) is undertaken by scientific staff and identification of specimens in the samples is done by trained parataxonomists and expert taxonomists. In more remote field sites, such as in coastal Alaska, the plates are deployed, retrieved and processed by local residents. In this instance, the purpose is to track the northward range expansion of a suite of known invasive species, so the plates are simply photographed and screened for the presence of a few, easily recognised target species. Any organisms that are suspected of being the target species are photographed and, where necessary, the specimen is preserved and sent to an expert for identification (Gregory Ruiz, pers. comm.).

2.5.4 New Zealand

In New Zealand a variety of sampling methods are used in the MHRSS to detect new incursions by NIS. These include baited crab traps, un-baited crab traps, epi-benthic sled tows, diver searches and visual shoreline searches (Inglis et al, 2006c, Morrisey et al, 2007). These sample methods were selected based on their:

- effectiveness at capturing the target species[3] when they are present,
- low cost and ease of use,

[3] Originally, *Asterias amurensis, Carcinus maenas, Caulerpa taxifolia, Eriocheir sinensis, Sabella spallanzanii, Potamocorbula amurensis* and *Undaria pinnaitifida*.

- minimal impact on native marine environments and species, and
- safety for field personnel, the general public and property (Inglis et al, 2006c).

Subsequent to the design of the original programme several biofouling species have been added to the list of target species, including the ascidians *Styela clava* and *Eudistoma elongatum*, and the Asian bag mussel, *Arcuatula* (*Musculista*) *senhousia*. Visual and diver searches are the primary methods used to locate these species currently, although the bag mussel, which typically occupies soft sediments, is sampled mainly by benthic sled.

Settlement arrays were deployed in Lyttelton Harbour to monitor recruitment of the Mediterranean fanworm, *Sabella spallanzanii*, during the incursion response in 2009-10 (Inglis et al, 2009). The arrays used the two T-unit design (Figure 2-2) that included a combination of horizontal and vertical PVC plates and rope mops. Twenty arrays were deployed in six overlapping periods between May 2009 and January 2010. Each deployment lasted ca. eight weeks with a two to three week overlap between deployments. No *S. spallanzanii* were recorded (Inglis et al, 2009).

A diverse range of biofouling organisms was recorded from the settlement surfaces, including both sessile (algae, ascidians, barnacles, bryozoans, bivalves, hydroids, anemones, calcareous worms) and mobile organisms (amphipods, isopods, crabs, brittle stars, nudibranchs, errant worms and a turbellarian worm; Inglis et al, 2009). Within the survey period, the intensity of recruitment was generally greatest in spring and early summer (September to January) and was greater on the undersides of horizontal plates than on vertically-oriented plates. The range of taxonomic groups recorded also tended to be greater on horizontal plates than on vertical plates. Because of the relatively short deployment period (eight weeks) the biofouling assemblages had only limited secondary structure so that relatively few mobile taxa were recorded.

Floerl et al, (2012a) used a combination of field experiments and models to estimate the sensitivity of settlement plates for detecting *S. clava*, *C. intestinalis*, *Undaria pinnatifida* and *S. spallanzanii* at small population sizes. The models combined estimates of reproductive output from populations of different sizes with modelled rates of hydrodynamic delivery of larvae to the surfaces and experiments to determine the relationship between settlement and recruitment onto the plates. In concordance with Hayes et al, (2005), they concluded that, for early detection surveys, the concentration of viable larvae in the water column relative to the size of the adult population (the adult:larvae ratio) is critical in evaluating the efficacy of settlement plates relative to other survey methods that target adult populations (Floerl et al, 2012a). Modelled frequency of larval contact with settlement surfaces (based on estimated larval abundance and measured flow rates) suggests that species with very large per female fecundity and long larval stages may be more suited to early detection in the larval stage by settlement plates or plankton samples than to visual searches for adults (Floerl et al, 2012a). Nevertheless, the overall probability of detection will also be influenced by the location and surface area of settlement plates and their attractiveness as surfaces for settlement of individual species. During the course of their study, Floerl et al, (2012a) observed no settlement of *S. clava*, *U. pinnatifida* or *S. spallanzanii* despite the presence of adults in the surrounding area. They interpreted this as a response to the material used to construct the settlement plates, and suggested that substrate was an important consideration for the sensitivity of the arrays for particular species. However, the small surface area of settlement plates may have been inadequate for sampling small population sizes. Settlement plate material may be an important consideration for *U. pinnatifida*, which is often associated with mature biofouling communities (particularly coralline algae; Thompson and Schiel, 2011) with *S. clava*, *S. spallanzanii* found on plastic settlement plates in studies in other countries (Holloway and Keough, 2002b, Martin et al, 2011). Floerl et al, (2012a) observed that mobile

organisms (e.g., isopods, errant worms, brittle stars and, occasionally, crabs) were detected more effectively by rope mops than plates.

Settlement arrays deployed in New Zealand waters to examine NIS have met with mixed success, with several studies unable to capture prominent non-indigenous biofouling species, such as *S. clava*, *U. pinnatifida* and *S. spallanzanii*, despite the known presence of adult populations in the surrounding environment (McClary et al, 2008, Nutsford, 2010, Floerl et al, 2012a).

2.5.5 Summary of national biosecurity monitoring programmes

While several countries have used settlement arrays for marine biosecurity monitoring, the objectives of the programmes are varied and there have been few evaluations of the suitability of passive sampling for this purpose. That is, how well samples obtained from the settlement arrays achieve the objectives of the monitoring. Of the few evaluations, Floerl et al, (2012a) concluded that the sensitivity of the surfaces they used was poor. Despite sizable populations of several NIS (*C. intestinalis*, *S. clava*, and *U. pinnatifida*) in the surrounding environment, only *C. intestinalis* recruited to the settlement arrays deployed. While the modelling done by Floerl et al, (2012a) estimated high probability of detection, based on hydrodynamic flows and reproductive output of each of the four NIS considered in the study, these estimates appeared *"overly optimistic"* as they did not align with empirical field observations on deployed surfaces (Floerl et al, 2012a). Studies by Sutton and Hewitt (2004) and an unpublished evaluation in the USA (Gregory Ruiz, pers. comm.) reached a similar conclusion about the selectivity of passive sampling devices for uncommon NIS, showing that settlement arrays could usefully detect common biofouling NIS, but were poor at sampling species that were rare or did not disperse effectively.

To increase the chances of detecting NIS during the early stages of an incursion, the number of arrays or the surface area of settlement substrata must be relatively high and the sampling surface must be attractive for settlement of the target organism (Floerl et al, 2012a). Increasing replication of settlement arrays, however, also increases the costs of materials, deployment and, particularly, the time required to process the plates to identify the biofouling organisms. The processing time depends on a range of factors, including the duration, season and location of deployment, all of which affect the species richness of the assemblage, and the objectives of the study. Processing of each sample will ordinarily take much longer where the study requires accurate taxonomic identification of all species and estimates of their abundance. Screening for presence of key target species or broad taxonomic groups (e.g., "morpho-types") can reduce the time taken per sample.

Estimates of the time required to process and identify all organisms on the plates range from between 1.5 to 2 h (Gregory Ruiz, pers. comm.) to up to 4 h per plate (Sutton and Hewitt, 2004). Plate screening to target a small number of high risk species has been employed by several settlement array programmes (Wells et al, 2009, Justin McDonald, pers. comm., Gregory Ruiz, pers. comm.). While this dramatically reduces the total processing time per plate, allowing more plates to be deployed for the same level of resource, it compromises the ability of the programme to detect species that are not on the target list of organisms.

Incorporating passive sampling devices into national marine NIS monitoring programmes may increase the chances of capturing a greater diversity of organisms, but there is limited evidence to support their utility for early detection of incursions by NIS (Hayes et al, 2005, Floerl et al, 2012a). However, the use of settlement arrays provides several potential benefits over many current sampling methods, including the ability to:

- scrutinise juvenile stages of biofouling organisms, and

- sample environments not accessible to other sampling methods (e.g., sampling locations with poor visibility).

The lack of requirement for specialist expertise for deployment of settlement plates make them ideal for community engagement initiatives. Such initiatives increase overall NIS awareness, have the potential to increase sampling intensity for a limited cost (i.e., the cost of the array materials is low compared to the time expense of specialist taxonomists).

3 Array design

A range of factors such as the composition of the substratum, orientation, depth of deployment, and sampling effort (number of experimental surfaces) can affect the recruitment of biofouling species, species diversity/richness, and assemblage composition and, therefore, the ability to detect individual species (Richmond and Seed, 1991, Knott et al, 2004; Sections 2.4, 2.5). Different species within fouling communities will also occur at various stages of succession within the assemblage (Floerl et al, 2012b) and recruit at different times of the year (Inglis et al, 2009). Therefore, the timing and duration of deployment influence the range of species captured.

The objective of this project is to design an array capable of capturing as diverse a range of biofouling NIS as possible. Therefore, finding the right balance between array complexity and overall cost of deployment, retrieval and species identification is vital. To achieve this, short reviews have been conducted on the factors that influence the recruitment of common NIS and biofouling taxa, the methods used to target these groups, and methods that have been successful in sampling them.

3.1 COMPOSITION AND COLOUR OF SETTLEMENT PLATES

Several studies have described differences in the composition of the assemblages recorded on the different settlement plate substrata (Anderson and Underwood, 1994, Marshall and Cribb, 2004, Sephton et al, 2011, Vaz-Pinto et al, 2014). Some of these materials include Perspex and other plastics (e.g., PVC), wood, metal, stone, concrete, rubber, and composite materials (e.g., Hardyflex™; Schmidt and Warner, 1984, Costello et al, 1986, Johnston and Keough, 2000, Holloway and Keough, 2002a, 2002b, Ramsay et al, 2008, Darbyson et al, 2009, Nutsford, 2010). Static water tests of antifouling coatings are typically done using metals (e.g., steel or aluminium) or plastics, with plastics preferred because of its lower cost and weight (Sánchez and Yebra, 2009). Although there are many published recommendations of substrata that may be useful to sample particular groups of organisms, relatively few studies have made direct comparisons of the richness and abundance of NIS sampled by different materials.

Fouling NIS tend to occur in greater richness and abundance on artificial structures in ports and marinas, such as fibreglass and concrete pontoons, and wooden wharf pilings, than on nearby rocky reefs or seawalls (Glasby, 1999a, Glasby, 2000, Glasby et al, 2007, Tyrrell and Byers, 2007, Dafforn et al, 2012, Simkanin et al, 2012). The latter habitats tend to be dominated by indigenous species (Glasby et al, 2007, Dafforn et al, 2012). In part, some of these differences can be explained by the different abiotic environments experienced by the organisms. For example, the abundance of barnacles, sponges and some ascidians, tends to be greater on mobile structures, such as pontoons, than in immobile (fixed) habitats (Glasby, 2001). Shading and proximity to the seafloor also strongly influence the development of biofouling assemblages, with shading greatly increasing the similarity of assemblages on settlement plates to those on shaded wharf pilings. Similarly, assemblages on unshaded plates located close to the seafloor exhibit greater resemblance to the biota of natural reefs than shaded plates or unshaded plates suspended nearer the water surface (Glasby, 1999b).

Several unpublished studies have also compared recruitment of NIS to different substrata (Emma Johnston, pers. comm., Gregory Ruiz, pers. comm.). For example, experiments undertaken by the University of New South Wales showed no clear differences in the species composition of assemblages that recruited to cement, wood and Perspex plates, but found substantially lower recruitment overall on steel plates (Emma Johnston, pers. comm.). Both this and the unpublished USA study observed relatively small differences in species richness on different plate materials, but often recorded large differences in the abundance of individual organisms (Emma Johnston, pers. comm., Gregory Ruiz, pers. comm.).

Nutsford (2010) found no significant difference when comparing recruitment of the solitary ascidian *Ciona intestinalis* to four different types of substratum: rope, PVC, wood and Hardiflex^{TM}.

Darbyson et al, (2009) compared recruitment of the clubbed ascidian, *Styela clava* to settlement plates that were constructed from fibreglass, aluminium or wood and were coated with either black or white antifouling paint or black or white non-biocidal exterior house paint. After 8 weeks, uncoated plates made from aluminium had the largest number of recruits (3.75 cm^{-2}), followed by surfaces coated in black house paint (fibreglass, 2.32 cm^{-2}; wood, 1.55 cm^{-2}). Surfaces coated in black paint or untreated aluminium plates tended to have larger numbers of *S. clava* recruits than any of the white-coloured treatments, but these differences were not statistically significant (Darbyson et al, 2009). Several other non-indigenous ascidians present in the Bay of Fundy, Canada, were found to settle on PVC plates and petri dishes (Martin et al, 2011). In general, however, ascidians have been shown to readily colonise a variety of substrata (Howes et al, 2007, Arsenault et al, 2009).

The attachment and production of byssal threads by mytilid mussels has been shown to vary among concrete, wood and iron surfaces, with concrete offering the most suitable substratum, followed by wood and then iron (Vekhova, 2006). Invasion of the Asian green mussel, *Perna viridis* to South Carolina, USA, occurred on a variety of substrata including plastics (PVC), fibreglass, wood, and steel (Knott et al, 2008). The types of substrata more typically used to sample recruiting mussel larvae are made of rope or other filamentous material (Marshall and Cribb, 2004, Bridgwood and McDonald, 2010). Mussels attach to the filaments of mesh balls (or "Tuffy" pot-scrubbers) that mimic filamentous algae and conspecific mussel byssal threads to which settling veligers ordinarily attach (Paine, 1974). The complex structure also provides protection for this life stage from macro-predators.

Recruitment of filamentous algae in Sydney Harbour was slightly higher on artificial substrata compared to natural rocky reefs, but there were no obvious trends for encrusting coralline algae or foliose algae (Knott et al, 2004). Comparison of algal recruitment between artificial (pilings and pontoons) and natural (sandstone plates) habitat revealed much higher recruitment to natural sandstone habitats (Dafforn et al, 2012). Early settlement and survival of macroalgal germlings has been shown to vary among substrata, with stone or concrete considered the best surface for recruitment (Somsueb et al, 2001).

The invasive seaweed, *U. pinnatifida,* recruits successfully to a wide range of artificial structures such as piles, ropes, tyres, wood, boulders, cobbles, loose gravel, fishing nets, vessel hulls, marine farming equipment, wreckage and the vertical sides of pontoons in ports and marinas (Hay and Luckens, 1987, Hay, 1990, Floc'h et al, 1991, Casas et al, 2008). During a settlement array deployment in Lyttleton Harbour (Floerl et al, 2012a) there was no recorded recruitment of *U. pinnatifida* on PVC plates or rope collectors. *U. pinnatifida* typically recruits into biogenic habitat, such as the articulated coralline alga *Corallina officinalis*, but spores can be settled and grown on scored glass microscope slides in field conditions (Thompson and Schiel, 2011).

Glasby (2000) found that the percentage covers of calcareous serpulid and spirobid polychaetes (*Hydroides elegans* and *H. ezoensis*) were generally lower on wooden plates than

on sandstone or concrete plates, but that the magnitude of these differences were dependent on the orientation of the plate and location of deployment. Qian et al, (2000) recorded greatest settlement by *H. elegans* in Teflon tubes, followed by PVC (two brands) and glass tubes. Lowest settlement occurred on polyurethane and a third brand of PVC (Tygon brand).

Non-indigenous populations of the tubiculous polychaete, *Sabella spallanzanii*, have become established in many subtidal habitats and on a range of artificial substrata including concrete, wood and steel (Currie et al, 2000, Holloway and Keough, 2002a, 2002b, Ross et al, 2013). *S. spallanzanii* is thought to prefer artificial structures for settlement, such as wharf pilings or the vertical sides of pontoons (Currie et al, 2000, Holloway and Keough, 2002a, 2002b). Black Perspex plates attracted considerable (~0.17 cm^{-2}) recruitment of *S. spallanzanii* at two sites within Port Phillip Bay, Victoria, Australia (Johnston and Keough, 2000). Recruitment has also been observed to nylon netting immersed at a Mediterranean location (~0.5 cm^{-2}; Giangrande et al, 2005).

Hydroid species occur on a variety of substrata (Calder, 1991), including as epibiota on other organisms (Gili and Hughes, 1995). Ceramic tiles have proved particularly successful at promoting hydroid colonisation (Migotto et al, 2001). Nevertheless, the ability of hydroids to settle on a variety of substrata, including epiphytic attachment to other species suggests that substratum may have only a relatively minor influence on their recruitment (although epiphytic attachment may be the preferred mode for some species; Gili and Hughes, 1995).

The substratum preferences exhibited by bryozoan larvae range from very specific to very general (Ryland, 1974). A wide variety of materials has been used successfully to study the recruitment of bryozoan species, but other factors such as orientation and surface abrasion may have more influence on colonisation and survival than the substratum type. Most bryozoans require a substratum that provides firm support for attachment, and many appear to also occur in greater abundance on surfaces that have a smooth or glossy finish (Allen and Wood, 1950, Ryland, 1974, Maki et al, 1989). Recruitment also appears to be greater on artificial substrata (concrete) compared to natural surfaces (Knott et al, 2004). However this may be material dependent as Glasby (2000) reported larger abundance of encrusting non-indigenous bryozoans (*Cryptosula pallasiana*, *Watersipora subtorquata* and *Schizoporella errata*) on sandstone and concrete plates than on wood plates. Vail and Tranter (1981) used roughened black Perspex to examine recruitment of bryozoa in Port Hacking, Sydney. They found 10 species of bryozoans over a period of 34 weeks, including *W. subovoidea* and *Bugula neritina*. Petri dishes have also been used successfully in other studies of *Bugula* species (Mihm et al, 1981, Maki et al, 1989, Wieczorek and Todd, 1997).

Sponges are known to attach to a wide range of substrata, including artificial structures such as wooden pilings (Corriero et al, 2006), plastic buoys (Lim et al, 2009) and shipwrecks (Walker et al, 2007). Knott et al, (2004) showed that sponge taxonomic richness was higher on natural rocky reefs than on an artificial substratum (concrete), but there was significant variation in substratum preference between species.

Scyphozoan larvae require hard substrata to develop into benthic polyps before producing pelagic medusae (Holst and Jarms, 2007). Recruitment of scyphozoans was lowest on natural substrata (shells) compared to several artificial substrata (concrete, machined wood, polyethylene and glass; Holst and Jarms, 2007).

Barnacle larvae have also been reported to colonise a range of surfaces (Vedaprakash et al, 2013), including those coated with antifouling paints (Jelic-Mrcelic et al, 2007). Qian et al, (2000) compared larval settlement in tubes made from PVC, polyurethane, Teflon and glass and recorded the highest settlement on glass and PVC. In a field study using multiple substrata, barnacles were found to recruit in greater numbers to concrete and plywood than to fibreglass or aluminium (Anderson and Underwood, 1994). Glasby (2000) found similar

percentage cover of the barnacle *Balanus variegatus* among three substrata: sandstone, concrete and wood.

Settlement plates made of PVC were used to examine the spread of the invasive amphipod, *Caprella mutica*, in British Columbia, Canada (Frey et al, 2009). However, the communities of fouling organisms attached to the settlement plates provided the habitat for *C. mutica*, rather than the plates themselves. This suggests that detection of *C. mutica* would be influenced by the attraction of a fouling community that is able to provide them suitable habitat. Most mobile crustaceans that recruit to settlement plates, with the exception of tube-building amphipods, take advantage of the secondary structure provided by the established biofouling assemblage and are likely to exhibit little preference for the primary substratum (see also the results from Floerl et al, 2012a described in Section 2.5.4).

Relatively few studies have examined the influence of surface colour on recruitment of biofouling species. Swain et al, (2006) investigated the effects of black and white substrata on recruitment of spirorbid tubeworms and the green alga, *Ulva* sp. Both groups of organisms recruited in significantly greater abundance to the black substrata. Darbyson et al, (2009) and Emma Johnston (pers. comm.) have reported similar observations for other fouling species (i.e., *S. clava* and *S. spallanzanii*). Satheesh and Wesley (2010) compared recruitment to plates constructed from red, green, blue, white and yellow coloured acrylic sheets and observed considerably greater recruitment on the red and blue plates than on lighter colours (green, yellow and white). This pattern was strongest for barnacles and sabellid polychaetes and was less pronounced for colonial ascidians (*Didemnum* sp.). Tests of antifouling coatings that have the same formulation, but different dark colour pigments (e.g., black, dark red, blue) also generally show very minor differences in the fouling assemblage. The greatest differences are usually between very dark (i.e., black) and light coloured surfaces (Andrew Scardino, pers. comm.). Satheesh and Wesley (2010) surmised that this may indicate a preference of the larvae for darker surfaces that do not reflect much light.

Although, the type of materials used to construct settlement arrays can have an influence on the types and abundances of organisms that are sampled by them, the effects of surface composition are often inconsistent in space and time and depend on the orientation of the surface. For example, Glasby (2000) showed that there were significant differences in the composition of biofouling assemblages recorded from sandstone, cement and wooden plates, with wooden plates having the most distinctive assemblages. However, the differences were relatively small and were not consistent among sites. Moreover, the assemblages recorded from the plates did not correspond with biofouling assemblages found on nearby artificial structures composed of the same materials. Based on this, Glasby (2000) concluded that the effects of substratum were comparatively minor in explaining why different assemblages develop on natural and artificial substrata and that the composition of the assemblage was more greatly affected by the orientation and location of the plate. Despite small differences in community composition between substrate types in a limited number of cases (Glasby, 2000), there seems to be a number of other factors with a greater influence on community composition, such as orientation (Glasby, 1999b), the environments typical of marinas and harbours (Dafforn et al, 2012), and the prevalence of shallow shaded habitat, such as floating pontoons (Glasby, 2001).

Recent evidence suggests that the substratum composition of artificial and natural structures is of little consequence relative to other factors such as the altered light environment, most notably shading by artificial structures, which is rare in coastal environments, promotes the recruitment of a range of species not typically found in shallow coastal environments (particularly bryozoans and ascidians; Dafforn et al, 2012, Dafforn et al, 2015).

3.1.1 Summary

Biofouling organisms recruit to a wide variety of substrata of both natural and artificial origins. Several studies show that NIS may be more prevalent on artificial structures than indigenous species (e.g., Glasby et al, 2007, Tyrrell and Byers, 2007) and that the species composition on suspended plates differs from those on nearby natural reefs and pier pilings (e.g., Connell et al, 1999, Glasby, 1999a, Dafforn et al, 2009, Dafforn et al, 2012, Floerl et al, 2012b, Vaz-Pinto et al, 2014). However, with a few exceptions (Glasby 2000), most studies have recorded relatively minor effects of plate composition on the species composition and richness of biofouling assemblages. The differences observed in fouling assemblages on natural and artificial habitats appear to be driven more by other environmental influences such as the depth, light availability, mobility and orientation of the plate.

Although individual species may exhibit some preference for particular materials, there are no clear trends among the taxonomic groups reviewed (Table 3-1). Plastics, such as PVC and Perspex, were the most widely used surfaces in the settlement plate studies reviewed, presumably because they are readily available and cheap to construct. Together with cement, plastic plates sample a large range of taxa, including all of the prominent vessel biofouling taxa. Darker coloured surfaces may attract greater recruitment than very light coloured surfaces, but in general, there are few consistent differences between colour treatments (Darbyson et al, 2009).

3.2 PLATE SIZE

The size and number of settlement plate surfaces are important considerations for the design of detection surveys. In general, as the total surface area of the plates increases within the sampled area, the probability that viable larvae will encounter a plate and settle on it also increases (Floerl et al, 2012a). There is, however, a trade-off between plate size and the resources required to process each sample. For these reasons it is important to optimise plate size to ensure high sensitivity, without dramatically increasing processing time.

While plate size and number determine the volume of water sampled by the plates, there have been relatively few studies that have directly examined the effects of plate size on assemblage composition. Most studies that use settlement plates to study the ecology of marine organisms have selected square plates with sides of between 10 to 20 cm length (e.g., Anderson and Underwood, 1994, Glasby, 2000, Marshall and Cribb, 2004, Labowitch and Cribb, 2006, Tyrrell and Byers, 2007, Bridgwood and McDonald, 2010, Martin et al, 2011, Floerl et al, 2012a).

Keough (1983) compared recruitment of biofouling organisms to Hardiflex^{TM} plates that varied in size from 45 to ~180 cm^2. He found no consistent differences in the density of recruitment to plates of different sizes and no evidence that larvae of any taxon were preferentially selecting a particular size of substratum. In a second study in which he used a larger size range of wooden plates (25 to 2500 cm^2), Keough (1984) found that species richness generally increased with plate size. Some groups, such as bryozoans, serpulid and spirorbid worms tended to be more abundant on small plates, while sponges and colonial ascidians recruited preferentially to large surfaces. Overall, however, the effects of plate size were often not evident until one year after the start of the experiment, and therefore, such differences may not be applicable to deployments of shorter durations (Keough, 1984, Butler, 1991).

3.2.1 Summary

Given some species preferences for shaded surfaces, plate size is likely to influence species composition (Keough, 1984, Howes et al, 2007, Martin et al, 2011). Plate size should therefore enable sufficient shade to attract shade seeking species, while not being too large

resulting in increased processing time. Settlement plates used in experiments examining recruitment of marine organisms have typically had a side length of between 10 and 20 cm.

Table 3-1: Settlement substrata for the various taxonomic groups common to biofouling communities. The table includes the number of citations where each material was successfully used to capture the target taxonomic group.

Taxonomic group	Plastics (PVC, Perspex)	Glass	Wood	Cement/ rock	Metal (steel, aluminium)	Fibreglass	Citations
Ascidians	7	-	2	1	1	-	Berntsson and Jonsson 2003, Watson and Barnes 2004, Boyle et al, 2007, Darbyson et al, 2009, Nutsford, 2010, Sephton et al, 2011, Martin et al, 2011, Floerl et al, 2012a, Collin et al, 2013
Macroalgae	3	1	3	5	1	-	Scheer, 1945, Skerman, 1958, Hay and Luckens, 1987, Hay, 1990, Floc'h et al, 1991, Somsueb et al, 2001, Berntsson and Jonsson, 2003, Casas et al, 2008, Holm et al, 2008, Dafforn et al, 2012
Molluscs	2	-	2	3	2	1	Dobretsov and Wahl 2001, Berntsson and Jonsson, 2003, Watson and Barnes, 2004, Vekhova, 2006, Knott et al, 2008
Polychaetes	2	1	2	4	2	-	Scheer, 1945, Nandakumar et al, 1993, Currie et al, 2000, Johnston and Keough, 2000, Holloway and Keough, 2002a, 2002b, Watson and Barnes, 2004, Ross et al, 2013
Hydroids	5	2	-	3	1	-	Scheer, 1945, Skerman, 1958, Calder, 1991, Gili and Hughes, 1995, Migotto et al, 2001, Berntsson and Jonsson, 2003, Watson and Barnes, 2004, Ramadan et al, 2006, Holm et al, 2008
Bryozoans	5	4	-	2	1	-	Scheer, 1945, Allen and Wood, 1950, Skerman, 1958, Ryland, 1974, 1976, Vail and Wass, 1981, Maki et al, 1989, Nandakumar et al, 1993, Johnston and Keough, 2000, Knott et al, 2004, Watson and Barnes, 2004
Sponges and scyphozoans	4	1	2	1	1	-	Otsuka and Dauer, 1982, Johnston and Keough, 2000, Knott et al, 2004, Corriero et al, 2006, Holst and Jarms, 2007, Walker et al, 2007, Lim et al, 2009
Crustaceans	4	1	2	2	3	1	Nandakumar et al, 1993, Anderson and Underwood, 1994, Berntsson et al, 2000, Glasby, 2000, Johnston and Keough, 2000, Qian et al, 2000, Vedaprakash et al, 2013
TOTAL*	19	5	3	12	5	1	

*Total number of studies using each material.

3.3 DEPTH AND ORIENTATION

The orientation of settlement plates has a strong influence on the types of biofouling assemblages that develop on them. In shallow waters, microalgal films, or macroalgal species often dominate upward facing surfaces (i.e., those in direct sunlight), whereas many fouling invertebrates (e.g., sponges, ascidians, etc.) will dominate downward-facing or vertical surfaces (Connell, 1999, Knott et al, 2004, Sánchez and Yebra, 2009). In turbid environments, the accumulation of fine sediment and particulate organic matter on upward facing surfaces can inhibit settlement and recruitment of a range of invertebrates (Dafforn et al, 2012).

Glasby (2000) compared recruitment of biofouling organisms to the vertical and horizontal undersides of sandstone, concrete and wood plates. Although both surface composition and

orientation influenced the covers of sessile epibiota, the effects of orientation tended to be greater. Brown and green filamentous algae were more abundant on vertical surfaces than horizontal undersides, whereas bryozoans, serpulid and spirorbid polychaetes, barnacles and solitary ascidians were most abundant on the horizontal undersides.

Dafforn et al, (2012) looked more explicitly at the effects of plate orientation on non-indigenous and indigenous biofouling species. In contrast to Glasby (2000), they found that invertebrate (indigenous and non-indigenous) richness and abundance tended to be greater on vertical plates than on the horizontal undersides. Upward-facing plates, were dominated by algal growth and the cover of invertebrates on them was negatively related to the sediment load present on the plates (Dafforn et al, 2012).

Ascidians are predominantly associated with the dark undersides of settlement surfaces (Young and Chia, 1985, Howes et al, 2007, Martin et al, 2011) and evidence suggests that ascidian larvae swim into deeper water after becoming photonegative, then swim upward to a dark surface to settle on (Kajiwara and Yoshida, 1985). Depth and orientation are, therefore, likely to be key determinants of ascidian recruitment to the settlement array. Studies of ascidian recruitment in Nova Scotia recorded the highest densities at 4.5 m depth, but juveniles were also found to recruit at 0.5 m depth (Howes et al, 2007).

Costello et al, (1986) compared recruitment of *C. intestinalis* to horizontal and vertically-oriented asbestos and cement roofing slates (30 x 30 cm) in Ireland. Downward facing surfaces were most heavily colonised, with up to 0.4 individuals.cm^{-2}. Recruitment of *C. intestinalis* to the settlement arrays in Lyttelton was also greater on the undersides of horizontal plates than to vertical plates, while there were no significant differences between PVC plates and rope mops (Floerl et al, 2012a).

Recruitment and growth of macroalgae requires sufficient light for photosynthesis. Given the turbid nature of many of New Zealand's ports and harbours (Hayes et al, 2005, Gust and Inglis, 2006, Floerl et al, 2012a), depth of deployment will determine the range of macroalgal species that recruit on settlement plates. Arakawa and Moringa (1994) and Reed et al, (1997) have shown that *U. pinnatifida* has a preference for settling on horizontal (upwards facing) surfaces over vertical surfaces. While some shade tolerant species may occur deeper than 2 m, surfaces hoping to capture macroalgal species should be oriented horizontally (upwards facing) and deployed at shallow depths (1 to 3 m).

Natural recruitment of mytilid mussels in the intertidal zone of rocky reefs occurs predominantly on horizontal surfaces (Menge et al, 2009). However, on artificial surfaces, recruitment of *Mytilus edulis* appears most successful at 0 to 2 m depth on vertically-oriented structures (e.g., Joschko et al, 2008). The settlement of oysters on natural substrata tends to be greatest on vertical surfaces (Glasby and Connell, 2001). Settlement of the bivalve *Cleidothaerus albidus* was much higher on horizontal surfaces than vertical surfaces (Knott et al, 2004).

The tubiculous fanworm, *S. spallanzanii*, colonises both hard and soft subtidal substrata forming dense aggregations (sometimes up to hundreds of individuals.m^{-2}). It recruits heavily to the underside of floating docks (Holloway and Keough, 2002a, 2002b), but is also found on vertical surfaces. Anecdotal observations in New Zealand also suggest the greatest densities of *S. spallanzanii* occur on the undersides of pontoons (Figure 3-1).

Figure 3-1: *Sabella spallanzanii* covering the underside of a pontoon in Waitemata Harbour, Auckland (Image: Crispin Middleton).

There are contrasting reports of the effects of orientation on recruitment of other non-indigenous polychaetes. Knott et al, (2004) examined the role of orientation and substratum type with serpulid worms being found on vertical plates and horizontal undersides in equal abundance. However, Glasby (2000) found that serpulids were more abundant on the undersides of horizontal plates, while Dafforn et al, (2012) recorded greater numbers of the serpulid, *Hydroides elegans*, on vertical plates. Spirorbid polychaetes were consistently more abundant on vertical plates than on horizontal undersides (Glasby, 2000). Dafforn et al, (2012) reported that tubes of an unidentified chaetopterid, *Chaetopterus* sp., were more abundant on the undersides of settlement plates deployed near Sydney. Indigenous New Zealand sabellid worms were found to recruit to vertical and horizontal surfaces during deployment in Lyttelton Harbour (Inglis et al, 2009).

Light is an important factor determining the distributions of many hydroids commonly found in New Zealand, with the swimming planulae larvae initially showing a positive phototactic response, but later becoming negatively phototactic prior to settlement (Gili and Hughes, 1995). There is a general tendency for most hydroids to be less abundant in well-lit situations, possibly as a response to avoid competition with macroalgae (Gili and Hughes, 1995). This suggests that hydroids are more likely to colonise shaded surfaces or in deeper water where competition with macroalgae should be reduced.

Knott et al, (2004) showed that bryozoans preferentially colonise vertical surfaces over horizontal surfaces (facing upwards). Bryozoans, including the antifouling tolerant *Watersipora cucullata,* were a common fouling organism of vertically oriented experimental plates (Allen and Wood, 1950). However, recruitment of the bryozoan *Watersipora subtorquata* occurs extensively on the underside (horizontal) of settlement surfaces (e.g., Floerl et al, 2004).

Recruitment of sponges to vertical and horizontal surfaces composed of natural (rocky reef) substrata showed species-specific responses to surface orientation (Knott et al, 2004).

However, on artificial substrata, overall sponge richness was higher on vertical surfaces. Density of sponges has been shown to increase with depth at sites of moderate flow, but in areas of more turbulent flow, removal of more delicate forms resulted in decreasing sponge diversity (Bell and Barnes, 2000).

Settlement of scyphozoan larvae is strongly associated with shaded habitats, and occurs almost exclusively in areas of low light intensity (Svane and Dolmer, 1995). Unsurprisingly, settlement of five species of scyphozoan larvae was significantly higher on the undersides of plates (Holst and Jarms, 2007). Studies on barnacle recruitment to vertical and horizontal surfaces have shown mixed results, with no consistent preference for surface orientation (Glasby, 2000, Knott et al, 2004). Recruitment of barnacles across a depth gradient indicated a peak at between 1 to 3 m depth (Berntsson et al, 2000).

3.3.1 Summary

The orientation of settlement plates strongly influences the composition of biofouling assemblages that recruit to them, particularly the relative contributions of photosynthetic organisms to invertebrates. Although a range of taxonomic groups show no consistent preference for horizontal or vertical surfaces (Table 3-2), there are many cases of species-specific preferences for surface orientation (e.g., Knott et al, 2004), suggesting that including both vertical and horizontal (downward-facing) plates into the array design may enhance species diversity. Most of the studies reviewed exhibited peak recruitment for a range of taxonomic groups between 0 to 5 m water depth (Table 3-2).

Table 3-2: Substrata orientations and depth distributions for the various taxonomic groups common to biofouling communities. Table includes the number of citations where each orientation was successfully used to capture species from each taxonomic group.

Taxonomic group	Vertical	Horizontal (underside)	Horizontal (topside)	Depth (m)	Citations
Ascidians	1	6	-	0.5 - 5	Gulliksen, 1975, Young and Chia, 1985, Costello et al, 1986, Howes et al, 2007, Martin et al, 2011, Floerl et al, 2012a
Macroalgae	-	-	3	0 - 2	Arakawa and Moringa, 1994, Reed et al, 1997, Knott et al, 2004
Molluscs	2	-	1	0 - 2	Knott et al, 2004, Joschko et al, 2008, Menge et al, 2009
Polychaetes	2	3	-	0 - 4	Holloway and Keough, 2002, Knott et al, 2004, Inglis et al, 2009
Hydroids	1	1	-	> 3	Gili and Hughes, 1995
Bryozoans	2	1	-	0 - 6	Allen and Wood, 1950, Floerl et al, 2004, Knott et al, 2004
Sponges and scyphozoans	2	2	-	4 - 5	Svane and Dolmer, 1995, Bell and Barnes, 2000, Holst and Jarms, 2007
Crustaceans	2	3	1	1 - 3	Berntsson et al, 2000, Glasby, 2000, Knott et al, 2004

3.4 SURFACE ROUGHNESS

Surface topography has also been shown to influence the settlement and attachment of benthic species (Köhler et al, 1999, Bers and Wahl, 2004). Surface rugosity (roughness) can be associated with higher levels of recruitment (Köhler et al, 1999), but different microtopographies can favour different species. For example, Walters and Wethey (1991) showed that fouling invertebrates had predictable settlement into either small crevices or flat surfaces depending on morphology. Settlement of bryozoans onto artificial substrata consisting of "pits" and "flats" showed that sheet-forming species preferred the "flats" while arborescent (tree-like) forms had either greater recruitment or higher survival in "pits" (Walters and Wethey, 1991, Walters and Wethey, 1996).

Experiments performed in the field and in the laboratory show that the barnacle, *Balanus improvisus*, preferentially settles on smooth surfaces (Berntsson et al, 2000). Herbert and Hawkins (2006) reported the opposite effects of surface roughness, with barnacles at two field sites recruiting more effectively on surfaces with greater rugosity. Barnacles may be very specific about the microhabitats which they will recruit to, and show fine scale searching behaviour (Berntsson et al, 2000). Furthermore, Bers and Wahl (2004) showed that microtopographies of several organisms (crab carapace, egg cases and mussels) have fouling prevention properties, suggesting that microtopographies (< 500 µm) can inhibit the recruitment of some larvae. Larger surface abrasions (> 500 µm) may enhance habitat heterogeneity and promote the settlement and survival of recruits.

A large body of evidence from experiments with settlement plates attests to the importance of surface texture in enhancing settlement and survivorship in pits and crevices (e.g., Davis, 2009). In the context of the proposed settlement arrays, including roughened or complex surfaces will increase small-scale habitat heterogeneity and potentially enhance not only species diversity, but also provide small scale refuges for juvenile recruits to survive long enough to be detected.

Reproducing consistent surface rugosity has been done by using sandpaper of a fixed grade (Berntsson et al, 2000), by abrasive blasting (ASTM, 2007, Inglis et al, 2009) and by moulding plates heated in an oven for 3 h at 160 °C, with gridded plankton net pressed between plates (Berntsson et al, 2000). While mouldings produced more consistent profiles (Table 3-3), such treatment methods are likely to work only for plastic substrata.

3.4.1 Summary

Surface texture is important for recruitment of larvae and post-recruitment survival. There is some evidence that very fine surface irregularities will actually inhibit recruitment of some taxa such as barnacles (Bers and Wahl, 2004). However, larger irregularities can provide refuges, enabling recruits to survive beyond vulnerable life-history stages (Walters and Wethey, 1991, Walters and Wethey, 1996). Recruitment of biofouling larvae to substrata of varying roughness is likely to be species specific, but increasing surface heterogeneity is likely to enhance biofouling species richness.

Table 3-3: Roughness profiles of plate treatments from Berntsson et al, (2000). Roughness parameters are average roughness, maximum height of profile and mean width of profile elements (± 95 % CI). Smooth surfaces had the highest rate of *Balanus improvisus* settlement followed by 20 µm and sanded surfaces.

Abrasion treatment	Average roughness (µm)	Maximum height (µm)	Mean width (µm)	Barnacle recruitment (cm^{-2})
Smooth	0.20 ± 0.04	1.22 ± 0.23	112 ± 33.5	1.4 ± 0.5
Molded mesh plate 20 µm	4.38 ± 0.16	20.3 ± 1.18	82.5 ± 8.15	0.4 ± 0.1

Abrasion treatment	Average roughness (µm)	Maximum height (µm)	Mean width (µm)	Barnacle recruitment (cm^{-2})
Molded mesh plate 170 µm	19.9 ± 60.49	74.5 ± 1.03	231 ± 1.55	0.35 ± 0.1
Molded mesh plate 190 µm	30.1 ± 1 1	101 ± 3.28	297 ± 1.67	0.2 ± 0.1
Molded mesh plate 355 µm	28.8 ± 63.41	99.9 ± 13.7	503 ± 3.58	0.3 ± 0.15
Sandpaper	6.13 ± 2.4	32.9 ± 11	204 ± 93.7	0.4 ± 0.1

3.5 ANTIFOULING COATINGS

Fouling of ships is an important historical and enduring transfer mechanism for marine NIS (Davidson et al, 2009). A variety of different types of antifouling coatings have been developed to limit the attachment of biofouling organisms to vessel hulls. Modern coatings include both biocidal paints, which incorporate active substances to kill or prevent attachment of organisms, and non-biocidal coatings that prevent firm adhesion of the organisms (fouling-release coatings) or which are mechanically resistant to regular cleaning (surface treatment coatings, Table 3-4).

A wide range of primary and 'booster' active substances are used in the biocidal coatings, including copper, iron, zinc, Diuron, Irgarol 1051® and others (Floerl et al, 2010). Copper and copper compounds, specifically cuprous oxide, cuprous thiocyanate and copper metal, are the most common primary active substances used in commercially available antifouling coatings (Voulvoulis et al, 2002, Srinivasan and Swain, 2007, Floerl et al, 2010), although zinc oxide is commonly used as a filler or extender. Copper-based coatings typically contain 20 to 76 % cuprous oxide. The three commonly used types of biocidal coating are ablative, contact leaching, and self-polishing. Conventional soluble matrix coatings use natural rosin as the matrix, but newer coatings have additional components to improve the rate and control of dissolution. This class of coating are known as controlled depletion polymer (CDP) coatings (Dafforn et al. 2011). Contact leaching or hard coatings, have an insoluble matrix and biocide release depends on a high concentration of biocide within the coating that enables biocide dissolution though micro-channels created by the dissolving biocide (Dafforn et al. 2011). Early self-polishing copolymer (SPC) coatings were the organotin based coatings in which the paint matrix was based on the copolymer tributyltin methacrylate, which hydrolysed in seawater to release the biocide and a consequent dissolution of the residual polymer base (Lewis 1998; Dafforn et al. 2011). Since the ban on organotin antifouling coatings, copper-based SPC systems have been developed that provide equivalent performance, and some are now specified for docking intervals of up to 90 months (e.g. AkzoNobel 2013, Hempel 2014).

Variability in copper tolerance among different species or populations can mean that copper-tolerant species are more readily transported as biofouling on vessels (Floerl et al, 2004, Piola et al, 2009, Crooks et al, 2011, McKenzie et al, 2012) and may have a competitive advantage over less tolerant species in polluted port environments. Indeed, studies using antifouling paints on settlement plates have found higher proportions of NIS compared to indigenous species (Dafforn et al, 2008, Piola and Johnston, 2008b). Although many of the NIS that recruit to plates treated with antifouling coatings are relatively common in their new environment (Dafforn et al, 2008, Piola and Johnston, 2008b), the selective nature of antifouling paints can enhance the ratio of non-indigenous to indigenous species on the plates, at least while the coating still contains active biocide. 'Booster' biocides (e.g., Irgarol® 1051 to inhibit diatom and macroalgal growth) and broad spectrum biocides (e.g., chlorothalonil and Sea-Nine™ 211) are increasingly used in modern coatings to prevent the settlement and growth of organisms with some degree of copper tolerance (AMOG, 2002 and references therein, Harino, 2004).

Table 3-4: Antifouling coating types currently used on the global market (table modified from Floerl et al. 2010).

Antifouling coating type	Biocidal/non-biocidal	Main biocides	Main use	Service life*
Conventional soluble matrix	Biocidal	Traditionally copper, iron or zinc oxides (previously also arsenic and mercury)	All vessel types	CV**: 48 months, RV: 24 months
Conventional insoluble matrix/contact leaching/hard	Biocidal	Copper compound and booster biocides (usually Diuron, chlorothalonil, Thiram or Zineb)	Commercial and recreational vessels	CV: 12-36 months, RV: 12-24 months
Controlled depletion polymer (CDP)/ ablative	Biocidal	Cuprous compound and booster biocides	Commercial and recreational vessels. Less suitable for high-speed vessels or tropical waters	CV: 36 months, RV: 24 months
Self-polishing copolymer (TBT-free SPC)	Biocidal	Cuprous compound and booster biocides (usually 'new' biocides, including zinc pyrithione, copper pyrithione or Sea-Nine™ 211)	Commercial and recreational vessels. Less suitable for high-speed vessels or tropical waters	CV: 60 months, RV: 24 months
Hybrid coatings	Biocidal	Copper pyrithione is the most commonly used booster biocide for Hybrid SPC-CDP products	Developing technology	
Fouling-release	Non-biocidal; biofouling settlement/ attachment deterrent	Biocide-free; the most successful coatings are based on silicone	High-speed vessels, or regular cleaning required	> 60 months

*Length of service life based on paint being applied and maintained according to manufacturer's specifications

**CV = commercial vessels, RV = recreational vessels

As expected, settlement plates treated with biocidal antifouling coatings have a much slower rate of accumulation of biofouling organisms than non-biocidal control plates. The temporal pattern of recruitment depends on the thickness of the coating and the rate at which it degrades over time, which may vary depending on its exposure to water shear (Sanchez and Yebra, 2009). Early colonists are usually micro- and macroscopic algae, calcareous tubeworms, barnacles and bryozoans (Hall and Baker, 1985, Floerl et al, 2004, Piola and Johnston, 2006, Piola and Johnston, 2009, Sánchez and Yebra, 2009, McKenzie et al, 2011). The encrusting bryozoan, *Watersipora subtorquata,* is one of the few species that is capable of attaching to antifouling coatings at an early stage in the coating's service life (Floerl et al, 2004, Emma Johnston, pers. comm.). Many soft-bodied fouling organisms are unable to colonise the coating until it has colonised by other species, has failed or has been damaged so that little residual biocide remains.

Different measures have been used to simulate aged or failing antifouling coatings in settlement plate studies. Some studies have used full strength coatings (e.g., Floerl et al, 2005, Jelic-Mrcelic et al, 2007). For example, Floerl et al, (2005) deployed freshly painted plates after a brief period (five days) of 'seasoning' in filtered seawater. In two marinas in tropical Australia, some species had recruited to the plates after eight weeks' deployment, but after 16 weeks, total richness on the biocidal surfaces was almost half that on non- biocidal controls.

Dafforn et al, (2008) and Piola and Johnston (2008b) attached Perspex collars that had been treated with a copper-based antifouling coating onto untreated plates. The collars provided a 2 cm wide border of antifouling around the plate edge and, therefore, a gradient in exposure to the biocide across the plate. Floerl et al, (2004) used patches of epoxy putty to create untreated patches on the surface of plates that had been painted with antifouling. Piola and Johnston (2008a) created small disruptions to antifouling coatings (as small as 5 mm wide) by scraping the coatings on the plates.

Manufacturers of antifouling coatings have developed standardised procedures for prematurely ageing the coatings to evaluate their effectiveness and robustness (ASTM, 2002, 2007). This is done by exposing coated plates to a turbulent, high speed flow of seawater that creates shear forces across the surface, thereby simulating the conditions that 'polish' the coatings during a ship's operations (Bishop, 1982). 'Dynamic testing' is done using rotating drum systems that are immersed in seawater or under high powered laminar flow systems (Matias et al, 2003, Swain et al, 2007, Sánchez and Yebra 2009). The drums can be rotated at velocities up to 22 knots to simulate the speeds of different vessels. Periods of dynamic polishing are also often interspersed with periods of static exposure to seawater to simulate the activity cycle of different vessels (ASTM, 2007). Ageing of the coating in this way can be up to 20 times faster than through static immersion (Bishop, 1982). However, the normal testing time for dynamic immersion (i.e., to simulate a normal dry-dock cycle for a vessel with a coating that is 100 to 300 μm thick) can be between six months to over one year (Sánchez and Yebra, 2009, Andrew Scardino, pers. comm.). Inconsistencies associated with using the ASTM methods for estimating biocide release rates are reviewed by Morrisey et al, (2013).

An alternative way to simulate a degraded coating may be to apply a thinner initial coating of the antifouling so that there is less biocide and matrix present on the surface. A single pass by a commercial spray applicator can apply a cover of coating as thin as 15 μm (Richard Piola, pers. comm.), which is ~5 % of a standard application. The erosion rate of the paint matrix can be between 2 to 10 $\mu m.month^{-1}$ in static immersion and between 4 to 31 $\mu m.month^{-1}$ under dynamic immersion, depending on the composition of the matrix (Yonehara et al, 2001, Sánchez and Yebra, 2009), so a much thinner coating of paint is likely to lose its anti-fouling characteristics much sooner.

3.5.1 Summary

The exposure of marine organisms to common antifouling biocides has potentially led to the globalisation of tolerant marine biofouling organisms, particularly bryozoans (Floerl et al, 2004, Dafforn et al, 2008, Piola and Johnston, 2008b, Piola et al, 2009, Crooks et al, 2011, McKenzie et al, 2012). The use of copper-based antifouling paints in settlement plate studies may enhance the relative capture of NIS by reducing the capture of indigenous species unable to recruit these surfaces (Piola and Johnston, 2008b). This may benefit settlement plate-based surveillance by reducing overall biofouling cover and reducing the processing time, while maintaining or enhancing the ability to detect NIS.

3.6 TIMING AND DURATION OF DEPLOYMENT

Timing of array deployment has significant implications for the types of assemblages that will develop and the relative proportions of dominant fouling taxa (Inglis et al, 2009). The timing of reproduction and spawning varies greatly among major biofouling taxa and the first recruits to reach settlement plates have the potential to define the subsequent type of assemblage that develops (Todd and Keough, 1994, Holloway and Keough, 2002). Some species exhibit year-round recruitment with seasonal peaks while others exhibit irregular, intense peaks of recruitment activity (Keough, 1983). In temperate environments, the total

number of recruits tends to be greatest in spring and summer and lowest in winter months (Keough, 1983). The season of deployment will, therefore, be an important factor determining the major taxonomic groups detected by settlement arrays (Watson and Barnes, 2004). However, due to the diverse range of organisms targeted by the proposed settlement array, it is impossible to take into account the spawning or reproductive period of all species using a single deployment.

To achieve high efficiency of species capture, as per the purpose of this project, it is important to optimise the deployment period of the settlement plates. For example, there is evidence which suggests that the longer plates of varied substrata are deployed, the more similar the assemblages on them become (Anderson and Underwood, 1994). This is potentially because of competitive exclusion of early colonisers, which are outcompeted by later arrivals, with superior competitive abilities (Russ, 1982, Nandakumar et al, 1993). Therefore, to optimise taxonomic richness, the settlement plates should be deployed for long enough to accumulate a variety of species with varying life-histories/larval durations, etc., but not so long that all settlement plates develop similar assemblages. For example, in South Australia, Keough (1983) chose an immersion period of two months, based on the findings of Kay (1980), because after this amount of time, recruits did not occupy enough space to inhibit further recruitment, nor did overgrowth interactions occur between growing recruits. Longer deployment times are also likely to be associated with a higher biomass of fouling organisms therefore adding to the cost of analysis due to increased processing time. Furthermore, increased deployment times may limit the options available for responding to an incursion.

Information is provided below on the season of spawning/reproduction for major taxonomic groups (where available) and the duration of deployment required to capture these groups (where possible). In most cases, the patterns of succession are based on recruitment to untreated substrata.

Recruitment of *Ciona intestinalis* to fouling plates was found to peak during late summer to early autumn in San Francisco Bay, USA (Blum et al, 2007). Likewise the recruitment of *Styela clava* was found to be highest in Lyttleton Harbour (Webber, 2010) and the Hauraki Gulf (McClary et al, 2008) during late summer. *C. intestinalis* reproduction in Lyttelton occurs from August to April peaking in output between October and February (Floerl et al, 2012a) and during this period large numbers of *C. intestinalis* recruits were found on settlement plates. Settlement array deployment between May and November 2009 in Lyttelton Port showed peaks of ascidian recruitment between May and July, and again between August and November (Inglis et al, 2009). Accumulation of colonial and solitary ascidians to settlement plates occurred after a minimum of two weeks in one study (Johnston and Keough, 2000). Seventeen of the 19 studies reviewed by Floerl et al, (2012b) had accumulated ascidian species within four weeks of deployment.

Macroalgae represent a taxonomically diverse range of organisms, consequently the range of life-histories is equally as diverse. The invasive laminarian algae, *U. pinnatifida*, releases spores during spring to early summer, with germination of sporophytes occurring during late summer to autumn (Thompson and Schiel, 2012). The highly invasive *Sargassum muticum* has been found to reproduce between autumn and summer along the Pacific North-West coast of USA, with warmer waters resulting in faster onset of reproduction (Norton and Deysher, 1989). Recruitment of macroalgae to settlement arrays in Lyttelton Harbour were limited in the first three weeks of deployment, but at four weeks substrata in a range of studies had accumulated macroalgal species (Floerl et al, 2012b).

On the west coast of USA (Oregon to California) recruitment of mytilid mussels over two decades showed peaks in recruitment from summer (July) till autumn (November) (Menge et al, 2009). Recruitment of indigenous mussels (*Xenostrobus pulex, Mytilus galloprovincialis*) in New Zealand has been shown to peak in winter and summer, but can occur year-round

(Rilov and Schiel, 2011). One of the reasons for the year-round recruitment may be due to secondary settlement, where juvenile mussel larvae move from undesirable locations to more desirable habitat (Rilov and Schiel, 2011). Recruitment of bivalve larvae to settlement arrays occurred after four weeks deployment in 7 out of 19 studies reviewed by Floerl et al, (2012b), suggesting four weeks would be the minimum deployment time to capture bivalve species efficiently.

Studies of reproduction in the invasive tubeworm *S. spallanzanii* (Port Phillip Bay, Victoria, Australia) showed an annual cycle with female mature oocyte density peaking in autumn (March to May; Currie et al, 2000). Spawning occurs when seawater temperature ranges from 11 to 14 °C in both the Ionian Sea (February) and Port Phillip Bay (August) under similar environmental conditions (Giangrande et al, 2000). Three studies have shown accumulation of calcareous tube-forming polychaetes (spirobids and serpulids) after 2 weeks' deployment (Scheer, 1945, Nandakumar et al, 1993, Johnston and Keough, 2000). Eleven of 19 studies reviewed by Floerl et al. (2012b) had calcareous tube-forming polychaetes present after four weeks' deployment.

Hydroids were a common fouling organism on settlement plates in Sydney Harbour and Northern New South Wales, Australia. Large inter-species variability in the timing of recruitment, with some species showing high recruitment during autumn and winter, while others recruited predominantly during spring and summer (Allen and Wood, 1950). Hydroids were present on settlement plates after four weeks in a range of studies (see Floerl et al, 2012b).

In Northern New South Wales, Australia, the bryozoan *Watersipora cucullata* was found to recruit to settlement plates, continuously over 12 months (Allen and Wood, 1950). Although there was a decrease in recruitment during the winter months, new recruits were rarely absent (Allen and Wood, 1950). A range of other bryozoans, including three species of *Bugula* were found to recruit throughout the year, but often at decreased abundance during winter. Recruitment of several bryozoans (both encrusting and arborescent forms) can occur as soon as one week after deployment (Bullard et al, 2004), with most studies showing recruitment within four weeks of deployment (Floerl et al, 2012b).

Settlement of barnacle larvae has been shown to occur during the summer and autumn months in several regions (Menge, 2000, Connolly et al, 2001, Herbert and Hawkins, 2006). In the United Kingdom, Herbert and Hawkins, (2006) showed that settlement and recruitment of *Chthamalus montagui* occurred from July to September. Likewise, recruitment of barnacles across a depth gradient in Sweden peaked between July and September (Berntsson et al, 2000). Barnacle recruitment to settlement arrays in Lyttelton Port occurred in small densities during winter (July-September) and peaked in spring (Inglis et al, 2009). Accumulation of barnacles to deployed plates can occur as soon as one week after deployment (Visscher, 1928), but almost every study reviewed by Floerl et al. (2012b) had accumulated barnacles after four weeks' deployment.

3.6.1 Summary

General findings on the season and timing of recruitment of various taxa to settlement plates are summarised in Table 3-5. Most temperate biofouling taxa are detected on the surfaces after 2 to 4 weeks' deployment, but greatest abundance may occur sometime later than this.

Specific taxa vary in the seasonal timing of peak recruitment, but many biofouling species exhibit some recruitment year-round in warm temperate and sub-tropical environments. The rate of biofouling on settlement plates is related to temperature (Matias et al, 2003), and deployment of biosecurity monitoring arrays in tropical ports (e.g., Darwin, Australia) typically last for up to 2 months, whereas more temperate ports may require longer

deployments (3+ months) to capture sufficient fouling communities (Emma Johnston, pers. comm., Justin McDonald, pers. comm.). Substantially longer periods of deployment are likely to result in overgrowth and displacement of some organisms.

Table 3-5: Settlement timing and duration of deployment of settlement plates for various taxonomic groups common to biofouling communities including citations for each taxonomic group.

Taxonomic group	Season of spawning/ reproduction	Deployment duration	Citations
Ascidians	Late summer	2 - 4 weeks	Keough and Johnston, 2000, Blum et al, 2007, McClary et al, 2008, Webber, 2010, Floerl et al, 2012a, Floerl et al, 2012b
Macroalgae	Spring-summer	4 weeks	Norton and Deyscher, 1989, Floerl et al, 2012b, Thompson and Schiel, 2012
Molluscs	Year-round	4 weeks	Menge et al, 2009, Rilov and Schiel, 2011, Floerl et al, 2012b
Polychaetes	Autumn	2 - 4 weeks	Nandakumar et al, 1993, Currie et al, 2000, Giangrande et al, 2000, Johnston and Keough, 2000, Floerl et al, 2012b
Hydroids	Year-round	4 weeks	Allen and Wood, 1950, Floerl et al, 2012b
Bryozoans	Year-round	1 - 4 weeks	Allen and Wood, 1950, Bullard et al, 2004, Floerl et al, 2012b
Sponges and scyphozoans	Summer	2 - 4 weeks	Johnston and Keough, 2000, Watson and Barnes, 2004
Barnacles	Summer-autumn	1 - 4 weeks	Berntsson et al, 2000, Menge, 2000, Connolly et al, 2001, Herbert and Hawkins, 2006, Floerl et al, 2012b

3.7 OTHER CONSIDERATIONS

3.7.1 Predation

Fish predation is an important driver of sessile community structure (Freestone et al, 2013, Lavender et al, 2014, Emma Johnston, pers. comm.). Selective removal of organisms by fishes will potentially compromise the ability of the arrays to sample the preferred prey items (Gregory Ruiz, pers. comm.). Several research groups have highlighted major differences in the species composition of assemblages on settlement plates that have been protected to exclude predators and those that have been unprotected (Freestone et al, 2013, Lavender et al, 2014, Emma Johnston, pers. comm., Gregory Ruiz, pers. comm.). However, the effects of predation vary geographically, depending on the suite of natural predators available and, in North America, are generally much greater at tropical latitudes than in temperate environments (Freestone et al, 2010, 2013). In the tropics, surfaces that were protected from predators had two to over ten times more species than unprotected surfaces (Freestone et al, 2010, 2013). Although the effect of predation was observed at temperate sites, it was more prominent at tropical sites (Freestone et al, 2013). Soft-bodied organisms, such as colonial ascidians, sponges and some algae appear to be particularly prone to fish predation. A recent study undertaken in temperate New South Wales, Australia, also demonstrated consistently strong effects of fish predation on biofouling assemblages. Encrusting bryozoans, barnacles, hydroids, tubiculous amphipods and solitary ascidians were much more abundant when predators were excluded (Lavender et al, 2014).

3.7.2 Shape

The shape of the collection device may also be an important factor for passive sampling. Although most field studies have used flat surfaces to sample the recruitment of biofouling organisms, Rittschof et al, (2007) noted that cylindrical shapes may have some advantages in

dynamic flows as turbulent flow created by the shape of the cylinder forms eddies that return propagules to the downstream side of cylinders. They found that large PVC cylinders (~30 cm diameter x 50 cm long) facing perpendicular and oblique downstream to bulk flow, collected the largest numbers of barnacles, although per surface area, panels collected barnacles just as well as most cylinder diameters (Rittschof et al, 2007).

3.7.3 Spatial dispersion

The confidence of detecting a given species using a passive sampling method is related to the population size of the target organism and its fecundity (the adult:larvae ratio; Hayes et al, 2005, Floerl et al, 2012a). However, season of deployment, larval duration and the volume of water sampled by the arrays (a function of the size and number of settlement surfaces and patterns of water local movement) are also important influences.

Spatial dispersion of adults (and thence larvae or spores) is a key determinant of the types of assemblages that will be sampled by an array, with local hydrodynamics (Floerl and Inglis, 2003, Watson and Barnes, 2004) and proximity to spawning adults (Floerl et al, 2012a) likely to drive recruitment dynamics, particularly for small, aggregated adult populations. Sites with high flow rates have resulted in a greater diversity of species accumulating on passive sampling devices, while sites with low flows appear to have higher overall rates of recruitment (Watson and Barnes, 2004). In port environments, entrainment of water by permanent break-walls can greatly increase recruitment densities within the port or marina (Floerl and Inglis, 2003). While complex patterns of water circulation within port environments may enhance the transfer of NIS between vectors, entrainment of propagules may also increase the success of capturing NIS on settlement arrays.

The spatial pattern of recruitment of individual species can vary from random to strongly aggregated, with aggregated patterns likely to be associated with settlement near conspecifics or with "swarms" of larvae settling together (Keough, 1983). Species with very short larval life spans (i.e., a few hours) or aggregated settlement may not be sampled effectively by passive sampling methods unless at least some of the surfaces are located close to spawning adults or are sufficiently widely dispersed to encounter a settling "swarm" of propagules. Where there is high water flux, the distance of the arrays from the source of propagules may also be important, particularly for small adult populations that might occur during the early stages of an incursion.

Species composition and abundance on replicated plates immersed at the same time can often vary greatly. Keough (1983) explained some of this variation as a function of the distance between plates. Plates that were closer together (cm to m) were generally more similar in community composition than those further apart (10's to 100's m). The greater compositional similarity at small spatial scales could not always be explained by gregarious behaviour of larvae, and was probably due to small-scale patchiness in the distribution of larvae in the plankton (Keough, 1983). Although wider spacing of settlement arrays may increase the overall diversity of the fouling assemblage sampled, the large variability in recruitment inherent in marine ecosystems, makes predicting the ideal spacing and geographic locations for deployment difficult. The placement of arrays in areas of high shipping turnover and areas of high water entrainment at distances of tens of metres (or more) apart should enhance the chances of detecting a broad range of NIS within a given location.

Another important consideration with respect to location is the influence of riverine input, with more brackish environments typically associated with less diverse and compositionally different fouling communities from more saline locations (Matias et al, 2003, Gregory Ruiz, pers. comm.). For example, fouling assemblages within the Town Basin Marina, Whangarei, are dominated by non-indigenous serpulid polychaetes, bivalves and bryozoans that are tolerant of low salinities (Inglis et al, 2006b). Assemblages on wharf pilings at the mouth of

the harbour (i.e. more saline conditions) contain a more diverse assemblage of indigenous and non-indigenous biofouling species (Inglis et al, 2006a).

3.7.4 Summary

There are very few studies comparing the relative recruitment of organisms to plates of various shapes, although Ritschof et al, (2007) show little difference in barnacle recruitment between cylinders and panels. For cost effectiveness, ease of production, processing and standardisation between other studies, flat plates appears the ideal deployment surface.

Biological processes such as predation have the potential to affect biofouling communities through selective feeding (Freestone et al, 2013, Lavender et al, 2014). Predation on settlement plates may affect the relative capture of hard bodied and soft bodied species with consequences for NIS detection. However, biofouling of the cages themselves have the potential to restrict larval recruitment to the plates themselves without continuous maintenance (Lavender et al, 2014).

Spatial separation of settlement plates is likely to result in large differences in community composition for a variety of reasons including, circulation and current dynamics (Floerl and Inglis, 2003), gradients of environmental variables (e.g., temperature, salinity; Inglis et al, 2006b) and proximity to variable biological assemblages (Floerl et al, 2012a). Separation of settlement plates in the order of 10's to 100's of metres will likely result in variation in the capture of biofouling communities (Keough, 1983).

4 Array design

As outlined in Section 2.2, the purpose of this review is to inform the design and field test of settlement arrays for the surveillance of non-indigenous biofouling organisms. The outcomes of the field study will then be used to develop a design for potential incorporation into the MHRSS. In this section, we provide recommendations for the design of the field test.

4.1 SETTLEMENT ARRAY DESIGN AND PROTOCOL

4.1.1 Plate materials

As described in Section 3.1, the materials used to construct the settlement surfaces can have an influence on the types of organisms that recruit to them, but often the magnitude of difference in the species composition of assemblages found on different substrata is small relative to the effects of other environmental influences (e.g., light availability, surface roughness, orientation, depth, predation, etc.). While plate material may be an important consideration for recruitment of some taxonomic groups, most of the groups that we reviewed exhibited only weak preference for particular substrata (Floerl et al, 2012b) and several studies failed to find any statistically significant differences in community richness amongst substrata (Darbyson et al, 2009, Nutsford et al, 2010, Emma Johnston, pers. comm., Gregory Ruiz, pers. comm.). Based on the information reviewed, **it is recommended that the field trial incorporate only a single type of material** and that, instead, priority be given to testing some of the other factors that have been shown to be important influences on assemblage composition (e.g., orientation, antifouling treatments).

The type of material selected for the arrays should sample a range of non-indigenous species and be relatively cheap to construct. Other considerations in selecting plate materials are consistency with previous studies and ability to apply surface abrasion treatments. The indicative costs of five different materials that have been used to construct settlement plates are summarised in Table 4-1.

Table 4-1: Indicative material costs for settlement plates. Costs based on material sheet size of 2400 x 1200 mm from various New Zealand retailers (Placemakers Ltd, Bunnings Ltd, Vulcan Steel Ltd, Mulford Plastics Ltd).

Material	Plastic (PVC)	Plastic (Perspex)	Metal (steel)	Wood (Marine ply)	Cement (Hardiflex™)
Cost	$160	$190	$180	$150	$50
Thickness (mm)	4.5	4.5	4.0	4.5	4.5

Although metal (steel) and fibreglass are the most common construction materials for the hulls of commercial and recreational craft, they are comparatively difficult and expensive materials to work with. Moreover, the (unpublished) studies conducted by the University of New South Wales suggest that steel may sample a much smaller range of species than other commonly available materials (Emma Johnston, pers. comm.).

Wood and cement pilings are arguably the most common submerged substrata in port and marina environments and are the focus for visual search surveys of NIS (Morrisey et al, 2007). Either would be a suitable material to include in the array. Cement composite (Hardiflex™) has been used in several studies as an "artificial" surface for recruitment (Anderson and Underwood, 1994, Knott et al, 2004, Vekhova, 2006) and is cheap (Table 4-1).

Plastics (PVC and Perspex) are the most widely used materials for both settlement plate studies and other biosecurity surveillance programmes. This is likely to be because they are widely available, low-to-moderate cost, durable and are readily manipulated to create dynamic surface topographies compared to other material types (Berntsson et al, 2000).

Based on the information reviewed, **PVC is recommended as the plate material to be tested in the field trial.**

4.1.2 Plate size

While plate size can influence the recruitment of biofouling organisms (Section 3.2), there are practical constraints to using large plates. For example, large plates will require strong support structures to deploy which will increase the costs of deployment and retrieval. Further large plates will have increased costs associated with species identification and processing. Square 10 x 10 cm plates have been used previously in many settlement array experiments (Marshall and Cribb, 2004, Cribb et al, 2008). However, some evidence for increasing species richness with larger plate size over long deployment periods (Keough, 1984) suggests that larger plates may enable the recruitment of a greater range of species. Based on the information reviewed, **a plate size of 14.5 x 14.5 cm is recommended** to provide adequate size to enable the recruitment of diverse assemblages while being mindful of the costs associated with the deployment of large plates.

4.1.3 Depth and orientation

Plate orientation is an important factor in determining the sampled assemblage (Connell, 1999, Glasby, 2000, Knott et al, 2004, Dafforn et al, 2012). Although upward facing horizontal surfaces sample macroalgal species more effectively than shaded surfaces of the underside of horizontal plates, they may be affected by high sediment loads in different port or marina environments. Since macroalgae are difficult to identify morphologically at the small sizes found on settlement plates and can also be sampled by vertically-oriented plates (Emma Johnston, pers. comm.), incorporating **vertical plates and the underside of horizontally-oriented plates in the arrays is recommended** for the field trial.

Array deployment should be at a depth that supports growth of both algae and invertebrates. Dafforn et al, (2009) found higher diversity and more NIS on settlement plates deployed at

shallow depths (0.5 to 2 m) compared to deeper deployments (5 m). The maximum dredged depth in many marinas is between 5 to 6 m, therefore arrays deployed at 3 m or deeper may be too close to the seafloor during periods of low tide and, as a result, subject to greater influence from suspended sediments. Based on the information reviewed, **it is recommended that plates are deployed at a depth of 2 m**.

4.1.4 Surface roughness

A consistent finding in this review is the important role of surface rugosity in enhancing settlement processes or post-settlement survivorship (see Davis, 2009, and references within). To enhance the habitat heterogeneity of settlement plates, any surface abrasions should be greater than 500 µm. This may be achieved by etching deeper abrasions into the plates than can be achieved using sandpaper alone (Emma Johnston, pers. comm.), by abrasive blasting or by using a moulding procedure (Berntsson et al, 2000, Justin McDonald, pers. comm.). Although the mechanisms of enhanced recruitment due to surface abrasion are not clear, some evidence suggests that post-settlement survival in pits or crevices enhances the accumulation of fouling assemblages on roughened surfaces (Davis, 2009). In order to detect NIS on the plates, it is essential that recently settled recruits survive long enough on the plates to be detected by observers. Based on the information reviewed, **it is recommended that a single rough treatment is tested during the field trial.**

4.1.5 Antifouling coatings

Given the selective pressures for copper tolerant NIS to be transported on vessel hulls (Crooks et al, 2011, McKenzie et al, 2012), incorporating copper-based biocides into settlement array design may effectively target NIS and reduce plate processing time. To simulate antifouling coatings regularly encountered by biofouling organisms, trialling **a readily available copper-based paint commonly used on recreational vessels (e.g., Micron Extra (International®)) is recommended**, based on the information reviewed.

There are difficulties in simulating degraded antifouling coatings consistently to reflect the reduction in biocide concentration observed on in-service vessels (Matias et al, 2003, Swain et al, 2007, Section 3.5). Dynamic immersion may provide a relatively consistent ageing of the coatings, however, the availability of suitable facilities in Australasia is limited. Further the length of time required to deplete the coatings and the associated costs make this option unsuitable for the proposed programme. Alternatives to reduce the biocide concentrations include use of thinner coating applications or applying biocidal paints in patches on the plates (e.g., Floerl et al, 2004, Piola and Johnston, 2008b). However, using patches or borders of paint on each plate can prove time-consuming when there are large numbers of plates to prepare (Richard Piola, pers. comm.). Based on the information reviewed, the **trial of a thin coating of antifouling paint on the plates is recommended**. As this approach has not been trialled before, **it is recommended that more than one coating thickness be incorporated into the field trial** to determine the thickness at which the greatest ratio of NIS to indigenous species is observed. Coating thicknesses to be tested will be determined following discussions with paint manufacturers.

The roughening of surfaces needs to be considered if they are to be then coated with antifouling coatings or non-biocidal primers (as a procedural control). Some of the roughness will be masked if the plates are coated after they have been roughened. Alternatively, if the abrasions are applied after the coatings, the biocidal properties will be compromised.

4.1.6 Timing and duration of deployment

While the dominant period for reproduction in temperate environments is between spring and autumn (September to March in New Zealand), the taxonomic groups reviewed had very

diverse reproductive strategies, including some that are reproductive year-round (Table 3-5). In order to sample as wide a range of organisms as possible, deployment covering several seasons will need to be considered. Based on the information reviewed, **it is recommended that at least three deployments are conducted, with two of these deployments during the same season (spring-summer) in successive years, and one deployment during winter.**

A **deployment period of 3 months is recommended**, in accordance with the studies of Keough (1983) and in-line with settlement plates studies undertaken in Australia and the USA (Section 2.5). At this point, the percentage cover of fouling organisms should be approaching 100 %, but overgrowth and exclusion of new recruits has not yet begun (Keough, 1983, Emma Johnston, pers. comm.).

4.1.7 Caging of plates

Findings from other studies suggest that the greatest range of organisms will be sampled by preventing predation on assemblages developing on the settlement surfaces. It is therefore proposed to **incorporate cages as an experimental treatment** in the field trial (i.e., caged and un-caged treatments) to examine its importance in New Zealand environments.

4.1.8 Method of deployment for the field trial

As the objective of the field trials is to evaluate design characteristics for the settlement arrays, the trials will need to incorporate a range of experimental treatments and replicate plates. These will need to be deployed on durable platforms that can be retrieved easily from port or marina locations. Ideally, the experimental arrays would be secured to a floating structure (e.g., pontoon) to ensure deployment at a constant depth and so the plates are constantly immersed. Other, similar experimental investigations have suspended groups of plates on PVC or steel frames or racks to ensure that the different treatments experience similar environmental conditions (e.g., Floerl et al, 2004, 2005, Piola and Johnston, 2008b).

Based on the information above **the following design is recommended, a flat frame (similar to Figure 4-1), to which 12 settlement plates will be attached (three rows of four), with a single replicate of each treatment randomised within each frame[4]. Up to 10 replicate frames will be deployed for each trial period at a single location to obtain robust estimates of species richness for each treatment condition** (Sutton and Hewitt, 2004, Floerl et al, 2012a, Emma Johnston, pers. comm.). **Replicate frames are recommended to be deployed 10's of m apart, with the maximum distance between settlement arrays no more than ~200 m, to effectively sample variation in recruitment processes across the study location** (Keough, 1983).

A different method may be used to deploy the settlement arrays for incorporation into the MHRSS (e.g., similar to those used in the USA or Australia; Section 2.5). However, that method will be informed by the outcomes of these field trials and the information contained in this review. Recommendations for the design of the arrays for on-going surveillance are provided in the final report for this project (Tait et al, 2016).

[4] Replication within each array would be preferable, as it would allow a measure of the spatial variance in treatment effects within and among frames, but doubling of the number of plates to 24 per frame would make each frame very cumbersome and difficult to deploy and retrieve.

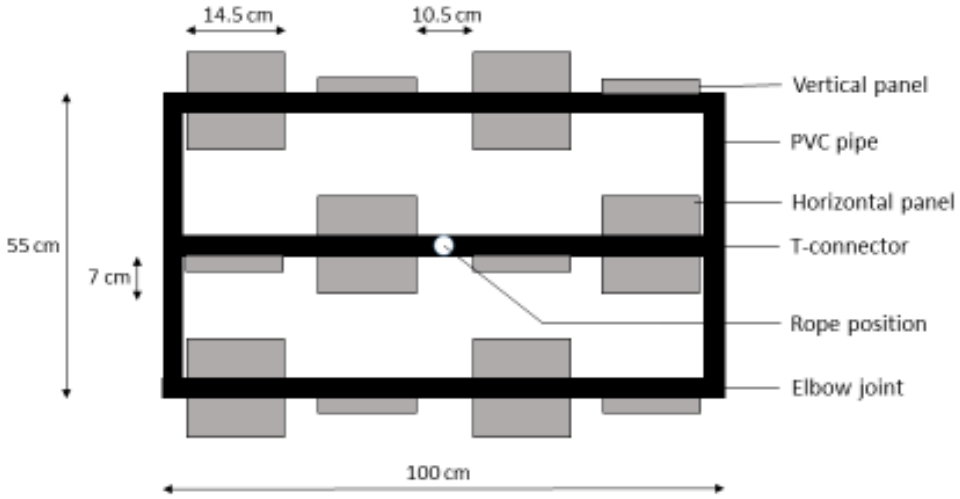

Figure 4-1: Birds-eye view of settlement plate arrangement on settlement array frames.

4.2 SETTLEMENT ARRAY DESIGN AND PROTOCOL

A summary of the proposed experimental design for the trials is presented in Table 4-2, along with the design proposed in the original tender for this project (and modified in the Project Management Plan). The recommended design would use PVC to construct the plates and all plates would be abraded (preferably by abrasive blasting) prior to deployment. The plates would be deployed on PVC or steel frames at a 2 m depth and suspended from a floating structure. The experimental treatments would include:

- orientation (vertical vs. horizontal undersides),
- predation cages (caged and uncaged),
- presence of antifouling coating (non-biocidal control, thin antifouling top coat and moderate antifouling top coat (Micron Extra, International®), and
- single surface texture (rough).

Ten frames containing a single replicate of each treatment condition would be deployed at a single marina location in Waitemata Harbour (Westhaven Marina). It is noted that further consideration of practical constraints may affect the practicality of the caged and un-caged treatment. If caged treatments are deemed practically onerous to set-up and maintain, we propose that this treatment is replaced with two roughness treatments (smooth and rough). Significant surface modifications potentially provide a refuge for recruits to escape predation during vulnerable life-history stages (Walters and Wethey, 1991, Walters and Wethey, 1996), thereby affording similar benefits to cages.

Table 4-2: Proposed experimental scenarios for the settlement array trials.

	Recommended design		Design in Project Management Plan	
Experimental treatment	No. levels	Treatment levels	No. levels	Treatment levels
Material	1	PVC	3	Not specified
Depth	1	~2 m	1	Single depth (not specified)
Orientation	2	Vertical Horizontal (undersides)	1	Single orientation (not specified)
Surface abrasion	1	With surface abrasion Without surface abrasion	2	With surface abrasion Without surface abrasion

Experimental treatment	Recommended design		Design in Project Management Plan	
	No. levels	Treatment levels	No. levels	Treatment levels
Antifouling coating	3	Non-biocidal control ~thin top coat ~moderate top coat	2	- Non-biocidal control ~200-300 µm top coat
Deployments	3	Summer 2014 Winter 2015 Summer 2015	3	Summer 2014 Winter 2015 Summer 2015
Predator exclusion	2	Caged Uncaged		
Plates per array	12		12	
No. of arrays	10		10	
Total No. of plates	120		120	

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