Ministry for Primary Industries
Manatū Ahu Matua

The Efficacy of Settlement Plate Arrays for Marine Surveillance

MPI Technical Paper No: 2012/16

Prepared for Surveillance and Investigation
by Oliver Floerl, Graeme Inglis, Lisa Peacock and David Plew
(NIWA)

ISBN No: 978-0-478-40021-2 (online)
ISSN No: 2253-3923 (online)

August 2012

New Zealand Government

Growing and Protecting New Zealand

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry for Primary Industries (MPI) does not accept any responsibility or liability for error or fact omission, interpretation or opinion which may be present, nor for the consequences of any decisions based on this information. Any view or opinions expressed do not necessarily represent the official view of MPI.

The information in this report and any accompanying documentation is accurate to the best of the knowledge and belief of the National Institute of Water and Atmospheric Research (NIWA) acting on behalf of MPI. While NIWA has exercised all reasonable skill and care in preparation of information in this report, neither NIWA nor MPI accept any liability in contract, tort or otherwise for any loss, damage, injury, or expense, whether direct, indirect or consequential, arising out of the provision of information in this report.

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at
http://www.mpi.govt.nz/news-resources/publications.aspx

Contents

Page

1 Executive Summary

Since 2002, the New Zealand government 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 current programme relies heavily on visual searches (particularly SCUBA) for detecting sessile high-risk species in port environments. However, in adverse weather conditions and/or low underwater visibility the effectiveness of visual surveys can be impaired, with the associated risk that target species (especially juvenile specimens) may be overlooked.

The use of passive detection methods such as settlement arrays has the potential to improve the ability of the MHRSS program to detect founding populations. In 2009-2010, settlement arrays were used as a tool to evaluate the success of the *Sabella spallanzanii* Local Elimination Programme. No *S. spallanzanii* recruits were encountered on any of the array surfaces, but the utility of the arrays for marine pest surveillance could not be determined because no attempts had yet been made to evaluate their efficacy for detecting small, founding populations of marine species.

The objective of this project was to provide an assessment of the efficacy of settlement arrays in detecting non-indigenous species. The project evaluated the efficacy of settlement arrays, using the survey design from the *S. spallanzanii* Local Elimination Programme, for four non-indigenous species − *Styela clava* (Clubbed tunicate); *Undaria pinnatifida* (Asian kelp); *Ciona intestinalis* (Vase tunicate); and *Sabella spallanzanii* (Mediterranean fan-worm) – known to be in Lytellton harbour.

Using a combination of literature review, modelling studies, and field and laboratory experiments, the project evaluated: (i) the capacity of settlement arrays to detect marine pest species at different population densities; (ii) the sensitivity of the arrays for the target species; (iii) the spatial and temporal limitations of the settlement arrays; (iv) efficiency measures to increase the efficacy and sensitivity of the arrays; and (v) the interpretation of negative results.

The rate at which propagules of the target species were likely to encounter the settlement array surfaces was determined by coupling a hydraulic model with know attributes of the target species. The hydraulic model was developed for Lyttelton Port to calculate the volume of water sampled by array surfaces over an 8-week monitoring period. Literature reviews were conducted to determine the reproductive season, fecundity, fertilisation, propagule life-span and settlement preferences of the four target species.

To determine the sensitivity of the array surfaces for detection of target recruits by an observer, *in situ* experiments were conducted in Lyttelton Port during which array surfaces were exposed to controlled "doses" of laboratory-spawned propagules. These experiments were conducted for *C. intestinalis*, *S. clava* and *U. pinnatifida*.

To calculate the confidence of detection stochastic scenario tree models were developed to combine elements of the target species' reproductive biology and the attractiveness of array surfaces for each species at: (i) a range of resident population sizes and (ii) a range of sampling efforts. Similar models were constructed to calculate the confidence of detection of each species using existing surveillance methods and sampling effort (SCUBA and benthic sled tows). However, due to the significant uncertainty in parameterising these models confidence in the outputs was low.

As a consequence of the large uncertainty it was not possible to confidently assert if the use of settlement arrays, along with the existing surveillance program, would significantly alter the likelihood of detecting target species. However, the model has proved to be a robust framework to determine which key parameters require further investigation in order to provide an adequately robust justification for the addition (or not) of settlement arrays to the marine surveillance programme.

2 Definitions

Confidence of the survey – the statistical probability that the pest will be detected by the survey if it is present.

Design prevalence – the level of abundance specified *a priori* for detection (usually expressed as a proportion of the population or as a density).

Propagules – the dispersal stages (larvae or spores) of sessile marine species.

Settlement array – a passive sampling device used to measure the recruitment of planktonic propagules onto artificial habitat surfaces.

Sensitivity of the sample unit – the probability that the pest will be observed in the sample unit if it is present within it.

3 Introduction

3.1 BACKGROUND

MPI leads and co-ordinates the Government's biosecurity activities, providing national leadership for biosecurity surveillance. 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 purpose of this surveillance is to protect the economy, environment and people of New Zealand from the risks associated with, and consequences of, the introduction of damaging risk organisms, and mitigate the effects of risk organisms that are already present.

The main objective of MHRSS is to detect incursions of non-indigenous species that are new to New Zealand early enough to facilitate successful eradication or management (Inglis et al. 2006a). The surveillance is also intended to detect incursions of established non-indigenous species in areas that they were not previously known to occur (i.e. range extensions).

The Biosecurity Surveillance Strategy 2020 was developed by MPI to ensure surveillance systems support biosecurity activities in a planned and effective manner. The strategy requires all surveillance activities to be based upon the best available science and most appropriate technology to achieve the objectives.

The utility of the marine surveillance programme is determined by the confidence with which the field surveys can detect populations of unwanted species at an early stage of invasion (i.e. at small population sizes). Currently, the programme relies heavily on visual searches (on SCUBA and above-water) for detecting sessile high-risk species in port environments, where suitable hard substrata consist primarily of vertical pilings and rock walls, or the undersides of pontoons. In adverse weather conditions and/or low underwater visibility the effectiveness of visual surveys can be impaired, with the associated risk that target species (especially juvenile specimens) may be overlooked (Gust et al. 2006, Hayes et al. 2005).

In 2008, the Mediterranean fan-worm, *Sabella spallanzanii*, was detected by the MHRSS programme in the Port of Lyttelton. MPI launched an incursion response with the purpose of eliminating the fan-worm from the Port by reducing the population to such a low level that it could not successfully reproduce. Settlement arrays, consisting of PVC tiles (vertical and horizontal) and mussel spat collector rope, were used as a tool to evaluate the success of the programme by monitoring recruitment of juvenile *S. spallanzanii* during the elimination programme (Inglis et al. 2009). The arrays used in the programme were developed originally by the CSIRO Centre for Research on Introduced Marine Pests (CRIMP) to monitor recruitment of mussels during the stand-down phase of incursion response to the black-striped mussel (*Mytilopsis sallei*) in Darwin (Ferguson 2000). They are also one of the sample methods incorporated into the Marine Pest Monitoring Programme that is being trialled in several Australian States.

Although settlement plates (and other artificial surfaces) are used widely in marine biological research to study the recruitment of sessile marine organisms (Butler 1986, Glasby & Connell 2001, Johnston et al. 2002, Nandakumar 1996), it is unclear how useful they are for marine pest surveillance, since no attempts have been made to evaluate their efficacy for detecting small, founding populations of marine species.

Settlement arrays may provide benefits for target species surveillance if they are able to reliable detect the presence of small reproductive adult populations in the survey areas. However, the inclusion of settlement arrays in the existing MHRSS would be feasible only if: (i) they enhance the overall ability of the programme to detect target populations, and (ii) their use is cost-effective. The present report describes a research project commissioned by MPI to evaluate the utility and feasibility of using settlement arrays for MHRSS in New Zealand.

3.2 AIMS AND OBJECTIVES

The objective of this project was to provide an assessment of the efficacy of settlement arrays in detecting the following known non-indigenous species in Lyttelton Port:

- *Styela clava* (Clubbed tunicate);
- *Undaria pinnatifida* (Asian kelp);
- *Ciona intestinalis* (Vase tunicate);
- *Sabella spallanzanii* (Mediterranean fan-worm).

The assessment was done using a combination of literature review, modelling studies, and field and laboratory experiments to evaluate:

- the capacity of settlement arrays to detect marine pest species at different population densities;
- the sensitivity of the arrays for the target species;
- the spatial and temporal limitations of the settlement arrays;
- efficiency measures to increase the efficacy and sensitivity of the arrays; and
- the interpretation of negative results.

4 Approach used to evaluate the arrays

4.1 TERMINOLOGY

We follow the terminology for pest surveillance used by (Cannon 2001)and (Cameron 2004). Surveys undertaken to demonstrate freedom from a pest or disease require explicit statements about the overall confidence with which the pest or disease would be detected by the survey if it was present at a specified level of abundance. The statistical probability that the pest will be detected by the survey is equivalent to the power of the survey and is referred to in this report as the *confidence of the survey*. The *a priori* level of abundance specified for detection is referred to as the *design prevalence* and is expressed as a proportion of the population or area surveyed.

We use the term *sensitivity* to refer to the probability that a pest will be detected in lower-level components of the survey design and methodology. In this project, *sensitivity* refers to the probability that the target species is detected by individual settlement array surfaces (plates or rope mops, see descriptions below) or other standard survey units (e.g. SCUBA dive search). Specifically, the *sensitivity* of settlement surfaces is defined as the proportion of individuals that encounter a single surface, recruit and are correctly identified as being present on the surface when it is retrieved from the water. Similarly, the *specificity* of the survey method is the frequency with which the pest is recorded as being absent on the surface when it is truly absent.

4.2 SETTLEMENT ARRAY DESIGN

The settlement arrays evaluated in this study used the same design as those used in the Local Elimination Programme for *S. spallanzanii* (Inglis et al. 2009). Each array consisted of a rope backbone to which two PVC pipe T-units were attached (Figure 1). The T-units were attached to the rope backbone at ~2 m and 5 m below Mean Low Water Spring (MLWS) tide level to sample different depths within the water column. Each T-unit had two horizontal arms comprising 0.7 m lengths of 25 mm diameter PVC pipe. Square settlement plates (sandblasted PVC, grey, 20 cm x 20 cm x 4 mm) were attached to the arms using cable ties in vertical and horizontal orientations. In addition, two 30 cm sections of frayed mussel spat collector rope (from here on referred to as ‘rope mops’) were attached to each T-unit. 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 7 kg concrete block.

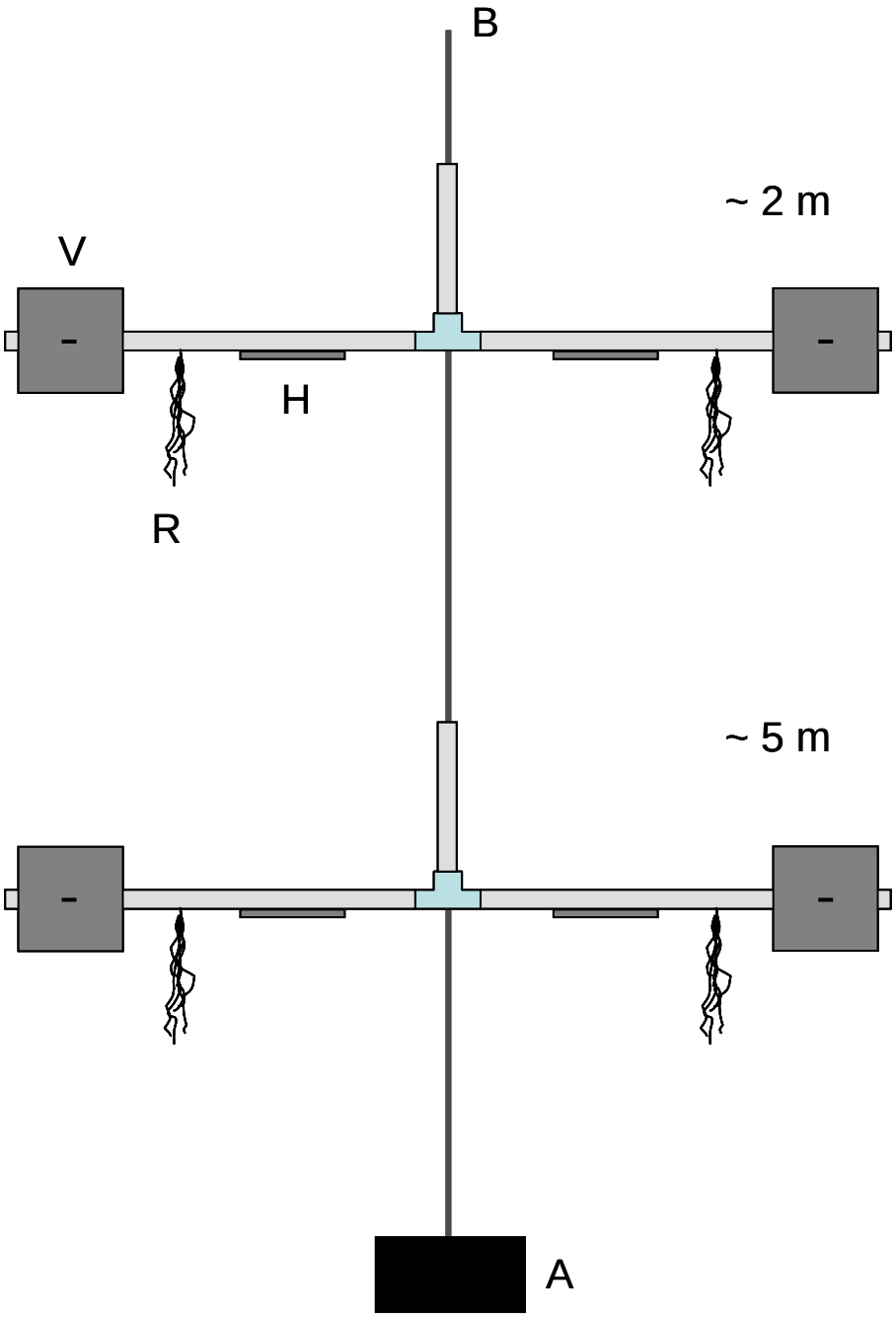

Figure 1: Illustration of a settlement array. Two T-units were attached to a rope backbone (B) at water depths of 2 m and 5 m (MLWS). Each T-unit held two vertical (V) and two horizontal (H) PVC settlement plates, and two Xmas-tree rope collectors (R). The arrays were attached to the undersides of shipping wharves and stabilised using anchoring weights (A).

4.2.1 Estimating the confidence of a pest survey

Hayes et al. (2005) describe the basic formula that can be used to calculate the probability that a pest will be detected by a survey (i.e. confidence of the survey). The level of confidence provided by a single survey (γ) depends on:

- the number of individuals (N) in the survey area or volume (A) (usually expressed as a density or prevalence; $\rho^* = \frac{N}{A}$);
- the area or volume sampled by a single unit of the survey method (a);
- the sensitivity of the sample unit (ϕ);
- the number of sampling units (n);
- the spatial and temporal distribution of the target species; and
- the design of the survey.

For surveys in which it can be assumed there is the same, independent probability of detecting the pest in each sample (i.e. the individuals and/or samples are randomly or evenly distributed throughout the survey area), the number of target individuals detected by the survey can be treated as a binomial random variable with the probability of detection in any particular sample being $\rho^* a\phi$. Hence, the confidence of the survey is given by:

$$\gamma = 1 - (1 - \rho^* a\phi)^n \qquad \text{(eq. 1)}$$

Alternate functions, based on the beta-binomial distribution, can be described for situations in which the spatial distribution of the pest is expected to be aggregated, and where the survey design involves higher-order sample units (e.g. clusters or batches; Venette et al. 2002). Clustering or aggregation of individuals will always lower the probability of detection in a simple, random survey.

In the ensuing sections we describe how we derived values to parameterise eq. 1.

5 Methods

5.1 ESTIMATING ENCOUNTER FUNCTION AT DIFFERENT POPULATION SIZES

The per-sample probability of detecting the pest was described by Hayes et al. (2005) as the "encounter function" of the survey method such that:

$$E = \rho^* a\phi \qquad \text{(eq. 2)}$$

The encounter function defines the average probability that the pest will be detected in a single sample, given its expected density (or prevalence) in the survey area (ρ^*), the area or volume sampled by a single survey unit (a) and the sensitivity of the survey method (ϕ).

Settlement plates (and other surfaces) provide artificial, vacant habitat on which the planktonic life-stages (larvae, spores, etc.) of sessile marine organisms may settle and grow into the adult phase. They are passive sampling devices in that they typically do not have any specific form of attractant for the target species, but instead rely on the rate at which the organism naturally encounters the surface as it moves through the surrounding environment. In this sense, they are analogous to flight intercept (or barrier) traps used in the study of insects (Southwood 1978).

Unlike many other sample methods used in marine biological research, which sample a standard area (e.g. quadrat) or volume (e.g. plankton net tows) of habitat that can be readily quantified, the volume of water sampled by a settlement plate will depend on the size of the surface and the rate at which water passes by it. To estimate the rate at which larvae and spores ("propagules") of the four target species are likely to encounter the settlement arrays it is necessary to know the concentration and distribution of larvae in the water column and the volume of water that is sampled by an array during the time it is deployed. The concentration of competent propagules in the water column (i.e. those that are ready to settle from the plankton) at any point in time will depend on the size of the adult population, its fecundity and reproductive phenology, the volume and mixing of water into which the offspring are released and their behaviour.

5.1.1 Estimating propagule number and concentration

To estimate the range of possible concentrations of larvae and spores in the water column we reviewed information on the reproductive biology of the four target species. For each species, we collated (where available) the following information from published literature:

- per-capita production of reproductive propagules;
- gamete fertilisation rate;
- timing of spawning and settlement;
- larval/spore behaviour;
- published estimates of larval concentrations *in situ* and (where available) known relationships between adult population size, density and larval abundance.

Literature sources were identified using online bibliographic databases (Web of Science, Google Scholar) and communication with relevant taxonomists employed by NIWA.

The reviews were augmented by:

- an analysis of data on the seasonality and density of recruitment of the target species on settlement arrays deployed during the *S. spallanzanii* Local Elimination Programme (RFP11199), and
- research on *S. clava* and *C. intestinalis* undertaken in the Port of Lyttelton by NIWA and students at the University of Canterbury (Nutsford 2010, Webber 2010). This included studies of the seasonality of gamete development and spawning in *S. clava* and attempts to sample larvae *in situ* to estimate concentrations.

The full reviews are presented in Appendix 2.

The rate at which propagules encounter the array surfaces is determined by the concentration of propagules within the port water during the monitoring period and the volume of water sampled by the surfaces (see above). The propagule concentration is dependent on the timing and duration of the period during which propagules are released and its relation to the sampling period of the arrays, the daily rate of propagule release by the resident adult population (a function of population size) and the life span of propagules.

We used a simple stirred-tank model for calculating propagule concentrations within the port. The model assumed that:

- The port is rapidly mixed such that propagules are evenly spread throughout the port basin. In the absence of more detailed knowledge of the port hydrodynamics and spatial concentrations of propagules in the basin this assumption is reasonable to make. More complex mixing models were beyond the scope and budget of this project.
- The tidal flows into and out of the port are approximated as a continuous steady flow. This assumption is reasonable over long time periods.
- The production of propagules outside the port is zero and no additional propagules are imported by the incoming tide.
  The objective of our calculations is purely to calculate expected propagule concentrations (ρ^*) in the port on the basis of population size, per-capita fecundity and hydraulic mixing. The results are used to estimate survey confidence in the event of the presence of isolated populations.
- Propagules that leave the port do not return.
  This assumes that currents outside the port rapidly disperse propagules away from the port. Hydrodynamic models used in the design nationwide surveillance programs (Inglis et al. 2006b) suggest this is a reasonable assumption.

The mathematical basis of our model is explained in detail in Appendix 1.

Steady state concentrations of larvae (numbers m^{-3}) in the port water at time T, when the first released propagules settle were calculated as:

$$C_T = \frac{R}{(Q+kV)}\left(1-\exp\left[-T\left(\frac{Q}{V}+k\right)\right]\right) \qquad \text{(eq. 3)}$$

where C_T is the concentration of larval propagules at time T, R is the rate of larval release (number day^{-1}), V is the volume of the port (m^3), Q is the tidal exchange from the port (m^3 day^{-1}), T is the life span of the propagules (days) and k is a decay function due to mortality of the propagules. The total volume of water contained within the Lyttelton Port basin (V) at

different levels of the tide was calculated using bathymetric maps supplied by Lyttelton Port Company.

The model enabled us to estimate the average concentration of propagules (C_T) per cubic metre of port water for a range of sizes of resident populations (i.e. 10, 100, 1,000, 10,000 and 100,000 adults), and for any day during the spawning period, given a specified larval life span. The model incorporates the mortality of larvae that reach the maximum life span. A larger spawning population results in a higher concentration of propagules and, therefore, increased rates of encounter of propagules with the settlement array surfaces. A breakdown of the population sizes and steady state concentrations of propagules calculated from eq. 3 is provided in Appendix 4.

5.1.2 Natural mortality of propagules

Our model also incorporated an estimate of natural propagule mortality other than mortality induced by failure to settle (i.e. see section above). Larval natural mortality is caused by predation or by stochastic processes such as exposure to unsuitable environmental conditions (spatial variation in temperature and salinity or pollution), disease, genetic defects or failure to find a suitable settlement surface. We used a review of larval natural mortality rates compiled by (Rumrill 1990) that was based on an evaluation of natural mortality of a total of 17 species of bivalves, bryozoans, barnacles, sea-urchins, crabs and other crustaceans, and reported from 24 different studies. Rumrill (1990) calculated the instantaneous (day^{-1}) mortality (M) reported for each species and plotted these mortalities against the larval life span reported for each species. The function of the curve can be used to estimate M for other species with a given larval life span:

$$M = 0.6961 \times T^{-0.5507} \qquad \text{(eq. 4)}$$

where T is the life span of the propagules.

We used this function to calculate the approximate instantaneous mortalities for the four target species examined in this project. Because of the uncertainties surrounding the larval life span of all four species in New Zealand waters, we calculated M for the minimum, average and maximum life spans reported in the literature.

5.1.3 Estimating volume of water sampled by arrays

To estimate the volume of water sampled by the settlement plates, we first described the hydrodynamic environment of the Port of Lyttelton. Patterns of current flow were measured within the port using an Acoustic Doppler Current Profiler (Sontek 1500kHz PC-ADP, "ADCP") and two electronic current meters (model Alec Compact-EM) placed in five approximately equidistant locations around the port, including the port entrance (Figure 2). These locations were chosen because together they capture the range of hydrodynamic conditions encountered around the port.

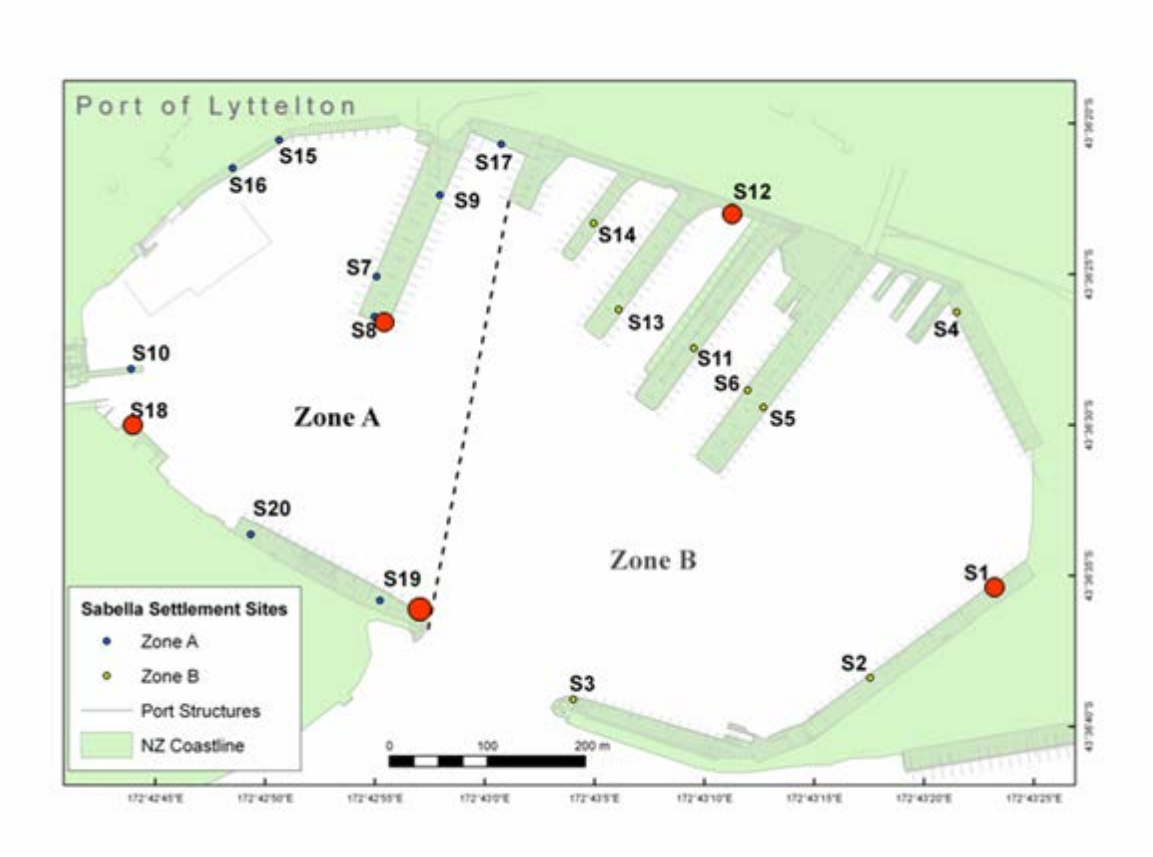

Figure 2: Map of Lyttelton Port showing the locations where settlement arrays were deployed during the *S. spallanzanii* Local Elimination programme (S1 – S20) and the five locations where current meters were deployed in March-April 2010 (red filled circles).

The ADCP was deployed on the sea-floor near the entrance to the port on 2 March 2010. It provided a profile of current flow throughout the water column. The current meters were deployed at a depth of 3 m below the water surface to measure current speed and direction at the general depth at which the settlement arrays were deployed. The instruments recorded flow patterns within the port over a full springs/neaps tidal cycle from 30 March to 15 April 2010. The current meters were re-deployed at an additional two locations in the port for a second springs/neaps cycle (15 – 28 April). All equipment was retrieved on 29 April. We were unable to obtain current data from one of the five locations due to equipment failure.

Data obtained from the current meters and ADCP were used to construct and calibrate a simple, integrated current model for the port that provided information on current flows at different locations, depths and tidal states.

Settlement plates

Settlement arrays will orient themselves to be "in line" with current flow. Horizontal and vertical plates will, therefore, sample the same volume of water and the same calculation can be used for each orientation. When the plates are aligned with the flow, the plate surface directly exposed to the flow is small. However, as water flows over the plate, turbulent eddies form, and these eddies transport entrained particles (e.g. propagules) to the plate surface.

The volume of water sampled by settlement plates was estimated using a scale for the size of the turbulent velocity fluctuations, multiplied by the area of the settlement plates (i.e. 20 cm x 20 cm).

The shear velocity (u_*) is a scale for the size of the turbulent velocity fluctuations resulting from the drag over the plate. The sampled flow rate is $u_* \times A$, where *A* is the area of the plate.

The shear velocity can be estimated from the drag on the plate, which can be determined following the principles described in (Streeter & Wylie 1983). The drag on a flat plate of length L and width W is:

$$F = C_D \frac{\rho U^2}{2} LW \qquad \text{(eq. 5)}$$

Where C_D is a drag coefficient, *U* the velocity of the water in the vicinity of the plate, and ρ is the density of sea water (~1025 kg m^{-3}). The drag coefficient is related to the water velocity, plate length, and viscosity of the sea water ν through:

$$C_D = 1.328\sqrt{\frac{\nu}{UL}} \qquad \text{(eq. 6)}$$

The shear velocity can then be estimated as $u_* = \sqrt{F/\rho A}$. The shear velocity is a scale for the speed of the turbulent velocity fluctuations, but does not represent the actual velocity fluctuations. The turbulent fluctuations will carry material towards the plate for half of the time, and away for the other half, therefore the sampling rate is:

$$Q_s = 0.5 u_* LW = 0.4W\left(\nu L^3 U^3\right)^{\frac{1}{4}} \qquad \text{(eq. 7)}$$

Rope mops

The spat collector rope ("Christmas tree rope mops") we used in this study consisted of filamentous or "frayed" nylon rope of 2.5 cm diameter and 25 cm length. We assumed that all water that flows "into" the frayed structure makes contact with the mop and is sampled. The sampling rate is thus:

$$Q_s = ULD \qquad \text{(eq. 8)}$$

where D is the diameter of the rope mop.

5.1.4 Estimating the sensitivity of settlement surfaces

The sensitivity of surfaces in the settlement array to ascidian (*S. clava* and *C. intestinalis*) larvae and algal (*U. pinnatifida*) zoospores was estimated using a series of *in-situ* "larval dosing experiments". This technique was developed by (Clark & Johnston 2005) to study the relationship between propagule supply and invasion success in marine fouling assemblages. In our experiments (described in the sections below), individual surfaces were isolated and experimentally exposed to known concentrations of propagules harvested from reproductively mature adults. Because *S. clava* and *C. intestinalis* are reproductively mature in late summer, the experiment for these species was conducted between 4 March and 28 April 2010. Experiments on *U. pinnatifida* propagules were conducted between 27 August and 13 October 2010.

At the start of the project, *S. spallanzanii* was present at very small densities (< 1 worm per 100 wharf piles) within the Port of Lyttelton. The collection of reproductive individuals at such small densities was beyond the resources available to this project. For this reason, and because little is known about the reproduction of *S. spallanzanii* within New Zealand, we relied on published studies of the relationships between settlement and post-settlement survival of other species to estimate the sensitivity of settlement surfaces for *S. spallanzanii*.

Production of larvae and zoospores

Styela clava and Ciona intestinalis

Reproductively mature *C. intestinalis* and *S. clava* were collected from pontoon and piling surfaces in Lyttelton Port on 2 March 2010 and transported to the NIWA laboratory in Christchurch. The animals were held overnight in a temperature- and light-controlled aquarium. The gonads of 10 individuals per species were dissected to extract gametes. Eggs and sperm were combined, mixed and left overnight in aerated 1 L containers to fertilise following the methodology described by (Bullard & Whitlach 2004). The following morning, larvae were examined under a binocular microscope for viability (motility) and counted into doses of 60 (*C. intestinalis*) or 50 (*S. clava*) larvae per 25 mL syringe. The differences in dose concentration reflected differences in the total number of larvae that were able to be produced for each species in the time available, which was slightly higher for *C. intestinalis*. Syringes containing the doses of larvae were transported in cooled containers and in the dark to the study site within the port, with intermittent 1 minute exposure to light to delay settlement. Viability was again ascertained at the field site prior to use in the experiment.

Undaria pinnatifida

Zoospores of *U. pinnatifida* were produced using the procedures described in (Forrest et al. 2000). Five mature *U. pinnatifida* sporophytes were collected from Lyttelton Port on 27 August 2010. The sporophyll was removed from each plant, rinsed with filtered sea water, wrapped in tissue paper and left to desiccate in dark conditions for 2 hours. The sporophylls were then re-immersed in fresh filtered seawater (5 μm mesh) and agitated to induce zoospore release. Three sub-samples (1 mL) of the zoospore solution were examined under a compound microscope (100 x magnification) and the number of motile zoospores per sample was counted. The average concentration of the solution was 770 zoospores per mL, resulting in doses of ~19,250 zoospores per 25 mL syringe. The syringes were transported to the field site and checked again for spore motility prior to use in the experiments, as described above for the ascidians.

Design of the dosing experiments

To determine the proportion of propagules that encounter a surface, settle and survive to be observed, we exposed surfaces from the arrays to a known number of propagules and determined the numbers of recruits visible on them after 8 weeks in the port (the same period used in monitoring during the *S. spallanzanii* Local Elimination Programme).

The experiment involved three orthogonal treatments:

- Surface type − horizontal plates, vertical plates, and rope mops;
- Larval dose − dosed with a known number of propagules or not dosed; and
- Enclosure − enclosed in an experimental chamber, enclosed by a partial chamber, or not enclosed.

For each species, we pre-conditioned 18 horizontal plates, 18 vertical plates and 18 rope mops in filtered (5 μm) seawater to allow the development of a biofilm. Biofilms have been shown to increase the attractiveness of artificial surfaces to many sessile marine taxa (Keough &

Raimondi 1995). Delivery of the larval dose treatment necessitated enclosing each surface in a separate experimental chamber (23 x 23 x 10 cm plastic boxes with clip-on sealed lids; Figure 3) so that a controlled number of larvae could be exposed to it for sufficient time to allow settlement (~4 days for *S. clava* and *C. intestinalis* and 7 days for *U. pinnatifida*, Appendix 2). Treatment conditions were randomly allocated to experimental chambers secured on two supporting PVC frames. Circular holes (8 cm diameter) cut in all four side walls of the chambers were covered with 100 μm (*S. clava* and *C. intestinalis*) or 5 μm (*U. pinnatifida*) plankton mesh on 36 of the chambers to allow water flow and small food particles (for the ascidians) to enter while preventing escape of the dosed propagules. On the remaining 18 chambers, the holes were left open to serve as a procedural control for any artefacts the chamber had on survival and recruitment ("partial chambers"). The PVC frames were deployed at a depth of ~2.5 m from the Z-Berth pontoons within Lyttelton Port.

Doses of larvae or spores were applied to the treatments *in situ*. A SCUBA diver injected the contents of a single syringe containing a dose of propagules into each of 27 of the 54 chambers through a small (5 mm diameter) opening in their lid, which was then sealed with a Velcro patch. The remaining 27 chambers were not dosed with larvae to allow an evaluation of the effectiveness of the dosing treatment. The chambers were removed from 9 of the dosed surfaces and 9 of the undosed surfaces after 4 (*S. clava* and *C. intestinalis*) or 7 days (*U. pinnatifida*) so that the surfaces were exposed to natural environmental conditions. Because we anticipated some recruitment to the exposed surfaces from surrounding wild populations of the study species, we left enclosures in place on the remaining 36 surfaces for the duration of the 8 week deployment to allow comparison of the dosing treatment to natural recruitment.

The purpose of the mesh-covered holes in the chamber walls was to allow water and oxygen exchange, but the extremely fine mesh (5 μm) used for the *U. pinnatifida* experiment (spores are 10 μm in size) caused some concern that the mesh would clog and inhibit water flow, resulting in anoxic conditions. To evaluate this possibility, six PVC plates were immersed in aerated aquaria in a laboratory at NIWA Christchurch. The temperature and light regimes of the aquaria reflected those at the field sites. The aquaria were inoculated with a zoospore concentration similar to that of the field chambers and monitored over the same time period. Development of *U. pinnatifida* recruits on laboratory surfaces but not on surfaces deployed in the chambers would provide some indication that conditions within the settlement chambers adversely affected survival.

Figure 3: The experimental chambers. Top row: images of settlement chambers with (left) and without (right) mesh walls enabling water and oxygen exchange. Bottom: PVC settlement frame carrying enclosures with plankton mesh windows (1), without plankton mesh windows (2) and settlement surfaces without enclosures (3).

Analysis of settlement surfaces

After 8 weeks the PVC frames were removed from the water. The plates and rope mops were removed from the frames and transferred to a field laboratory for analysis. Each surface was labelled (to identify the treatment, type and orientation of the substrate), photographed, covered in filtered seawater and examined for juvenile stages of the target species. Examination of settlement surfaces was done using hand-held magnifying lenses (4 x

magnification). Voucher specimens of suspected recruits of the study species were collected, preserved and sent to NIWA's Marine Invasives Taxonomic Service (MITS) for expert identification. Where no target recruits were encountered, a sub-sample of four surfaces was examined under a dissecting microscope to ensure that recruits < 0.5 mm had not been missed. The abundance and size of recruits of the study species were recorded.

Our design included partial enclosure treatments (chambers lacking mesh windows) that had not received a larval dose, but lacked enclosure controls that had received a larval dose. Therefore, two separate models were used to test for artefacts of the enclosures. The first was a 3-factor ANOVA that included the following factors: surface type (horizontal plates; vertical plates; rope mops), larval dose (dose; no dose), and enclosure (present; absent). The partial enclosure treatment was excluded from this analysis. The second model was a 2-factor ANOVA that was limited to treatments that had received no larval dose. The factors were surface type (three levels, as above) and enclosure (present; absent; partial). This model was used particularly to examine differences in *C. intestinalis* recruitment between enclosed, unenclosed and partially enclosed treatments. All tests were performed on log(x+1) transformed abundance data (no. *C. intestinalis* per surface) to stabilise variances. Tukey's pairwise comparisons were used to examine differences between levels of significant effects.

Estimating the relative sensitivity of other types of settlement surfaces

A recent study by (Nutsford 2010) compared recruitment of *S. clava* and *C. intestinalis* within Lyttelton Port on PVC plates and plates made of wood, hardiflex, carpet, concrete, and mussel shell. Replicates of all surface types were immersed at three locations around the port for a period of approximately 7 months. Because the surfaces were deployed at the same time and location as this study they provide a measure of the sensitivity of other surface types relative to the PVC plates used in our study. Our literature review also examined other studies that used some form of artificial surface to monitor recruitment of the target species of this project. Where possible, such information was also used to provide an indication of the relative sensitivity of alternative substrate types and to provide guidance on ways the arrays could be improved to increase sensitivity to the target species.

5.2 ESTIMATING SURVEY CONFIDENCE

We developed stochastic scenario tree models (sensu Martin et al. 2007) to combine the elements of the surveillance systems described above to estimate the confidence of detection for each species.

5.2.1 Settlement arrays

For the settlement arrays, the trees had a very simple structure that consisted of a single risk category node ("Season of survey"), a single detection category node ("Settlement surface type") and a single detection node.

Risk category nodes define factors that divide the sampled population into subsets that represent different risks of infestation (Martin et al. 2007). Each branch in the node has an associated risk that it will be infested (RR_i), which is specified relative to the branch with the lowest risk of infestation (i.e. the lowest risk category is set at 1; Martin et al. 2007). Theoretically, greater confidence of detection is achieved when more sample effort is allocated to components of the population that are at highest risk of infestation, but this will depend on the relative proportion of the population at risk that this component comprises. Thus, the relative risks are adjusted (weighted) according to the proportion of the sampled population that they represent so that the average "adjusted risk" for the population across all risk branches is 1 (Martin et al. 2007).

Deploying settlement arrays in winter and summer will likely have different outcomes depending on the timing of spawning of the species. The probability that reproductive propagules will be present in the water column varies according to the time of the year in which the survey is done, the highest probability occurring during the spawning season of the species. The current Marine High Risk Site Surveillance is structured around semi-annual surveys, with one survey at each high risk site occurring between May and October ("winter") each year and a second survey between the months of November to April ("summer"). The relative proportion of the survey population ($\Pr P_i$) that occurs in winter and summer is, however, the same since, typically, equal numbers of samples are taken on each survey, irrespective of whether it occurs in summer or winter.

For surveys using settlement arrays, we estimated RR_i of summer and winter surveys by examining published literature on the reproductive seasonality of the target species (Appendix 2). RR_i was modelled as random sampled from a beta-Pert distribution using the PopTools function PertDev (min, likely, max), in which we specified the "minimum", "most likely" and "maximum" relative risk levels for larvae to be present in winter and summer (Appendix 2).

For each season, the adjusted risk (AR_i) was calculated as:

$$AR_i = \frac{RR_i}{\sum_i^k RR_i x \Pr P_i} \quad \text{(eq. 9)}$$

where RR_i had a beta-Pert distribution and $\Pr P_i = 0.5$.

Values to parameterise RR_i for each species are summarised in Appendix 3 tables A3-1 and A3-2.

Detection category nodes are factors in the survey structure that influence the probability of detection. In the case of the settlement arrays, different surfaces on the arrays (vertical plates, horizontal plates, rope mops), sampled different volumes of water (a) and had different sensitivities (ϕ) to the propagules. We therefore treated them as separate detection categories in calculating the confidence of detection.

Following eq. 2, the encounter function for an array in Season i with Settlement surface type j is given by:

$$E_{ij} = C_T x AR_i x ba_j x \phi_j \quad \text{(eq. 10)}$$

where C_T is the expected steady-state concentration of larvae in the water, AR_i is the adjusted risk for Season i, a_j is the volume of water sampled by surface type j, b is the number of surfaces of that type on an array and ϕ_j is the sensitivity of the surface type for the target species.

Because there is uncertainty in the literature about the likely planktonic duration of propagules from each of the target species (D_P), C_T was calculated for a range of values of T for each population size. C_T was modelled as a random variable sampled from a population whose distribution was defined by the values of T available in the literature. For *S. clava* and

C. intestinalis, C_T was calculated separately for the minimum and maximum larval durations reported in the literature. Values of C_T used in the scenario tree model were then sampled from a uniform distribution that was bounded by these minimum and maximum values (i.e. the PopTools function RandReal (min., max.)).

For *S. spallanzanii* and *U. pinnatifida*, C_T was sampled from a beta-Pert distribution (PopTools function PertDev (min., likely, max.)) in which values for the minimum, most likely and maximum values of C_T were calculated using the minimum, median and maximum values for T recorded in the literature.

Similarly, because the dosing experiments did not produce measurable results for all species (see Results section), we had to derive values for the sensitivity of the surfaces (ϕ_j) for some target species. These were taken either from values in the literature or our best judgement. For *C. intestinalis*, however, ϕ_j was sampled from a Normal distribution defined by the mean (and standard deviation) proportion of dosed larvae observed on the enclosed plates at the end of the dosing experiments (PopTools function NormalDev (mean, stdev)).

The confidence of a survey in Season i using n arrays each with b surfaces of type j was then calculated as:

$$\gamma_{ij} = 1-(1-Eij)^n \qquad \text{(eq. 11)}$$

By extension, the overall confidence of a survey in Season i that uses n arrays each containing the three surface types is given by:

$$\gamma_i = 1-(1-\gamma_{iV})(1-\gamma_{iH})(1-\gamma_{iR}) \qquad \text{(eq. 12)}$$

where γ_{iV}, γ_{iH}, and γ_{iR} are the individual values of confidence determined for the vertical plates, horizontal plates and rope mops, respectively.

Values used to parameterise the scenario trees for each species are presented in Appendix 3, Tables A3-2 and A3-3. Separate calculations were done for each season (winter vs. summer) and population size (10, 100, 1,000, 10,000, and 100,000 adults). We also varied the number of arrays over three values (n = 10, 20 and 40 arrays per survey) and compared changes in average confidence of detection for each level of survey effort. Monte Carlo simulations, involving 100 iterations, were used to calculate the mean, variance and 95% confidence intervals around the confidence of detection for each scenario.

5.2.2 Scenario Trees for Existing Survey Methods

Simple scenario trees were constructed for survey methods that are currently used for the target species in the Marine High Risk Site Surveillance. These are principally visual surveys by divers (*S. spallanzanii, U. pinnatifida, S. clava* and *C. intestinalis*) and benthic sled tows (*S. spallanzanii*) (Inglis et al. 2006a). They are techniques that sample sedentary adults or sub-adults most effectively, rather than juvenile stages.

"Season" wasn't incorporated as a risk factor in these trees as we did not expect the probability of infestation or detectability to vary between summer and winter surveys. Mature life stages are present year-round except for *U. pinnatifida*, where the adult sporophyte

senesces over the summer months. However, as the base of the sporophyte stipe often remains intact and is easily distinguishable,

"Habitat type" was included as a risk category node as different benthic habitats vary in the likelihood that they will be infested. For example, intensive surveys for *S. spallanzanii* during the Local Elimination Programme in Lyttelton showed that it is more likely to occur (and be detected) on wharf piles and pontoons than in soft sediment environments, with generally largest densities occurring on wharf piles(Inglis et al. 2009).

Four types of habitats were included as branches in the habitat type category: breakwalls, wharf piles, pontoons and soft-sediments. Values for the relative risk of infestation in each habitat (RRi) were based on existing field observation, data from the Marine High Risk Site Surveillance and published literature. Values specified by the study team for the "minimum", "most likely" and "maximum" relative risk for each habitat and species were used to model RRi as a random sample from beta-Pert distribution using the PertDev (min., likely, max) function in PopTools.

The RRi values used to parameterise the models for each species are presented in Appendix 3 Table A3-3. For example, in the case of *S. spallanzanii*, breakwall habitats were considered at lowest risk of infestation of the four habitat types, since *S. spallanzaynii* was not recorded from breakwalls during the *S. spallanzanii* Local Elimination Programme (Inglis et al. 2009) and it is not as common on breakwalls as in other habitat types (Currie et al. 2000). Soft-sediment habitats were considered between 1x and 2x more likely ("most likely" = 1.33x) to be infested by *S. spallanzanii* than breakwalls (i.e. PertDev (1, 1.33, 2), pontoons between 1x to 5x more likely ("most likely" = 3x) and wharf piles between 2x and 9x more likely ("most likely" = 7x).

RRi values were adjusted relative to the proportions of the total area of the inner harbour of the Port of Lyttelton that the various habitat types occupied. These were derived using GIS from maps of the inner harbour. The area of seafloor habitats, such as soft sediment, was determined in m^2. Because wharves, pontoons and other artificial structures had low levels of detail on the maps, we used GIS to measure the 2-dimensional area of these structures (i.e. length of outer perimeter x depth). We did not formally include the width of wharf structures (i.e. under the wharf) in our estimates of area, since dive searches are done predominantly on the outer row of pilings (Inglis et al. 2006a). However, allowance was made in our estimates of ϕ_j for the dive searches to account for the proportion of the population that was not sampled by the searches (see below).

The adjusted risk (AR_i) for each habitat type was calculated using eq. 9.The encounter function for existing surveillance methods is given by

$$E_{ij} = \rho^* x AR_i x a_j x \phi_j \quad \text{eq. 13}$$

where ρ^* is the expected density of the pest in the survey area, a_j is the area sampled by survey method j and ϕ_j is the sensitivity of survey method j for the target species.

In the current Marine High Risk Site Surveillance, divers search a standard unit of area at each location that is approximately 50 m long x 5 m deep (Inglis et al. 2006a) such that a_j ~ 250 m^2. The length of sled tows can vary, depending on the sediment type. For the purpose of

these models we have assumed an average tow of 100 m x 0.5 m width (i.e. a_j = 50 m^2). As all of the target species for this study are sedentary, we have assumed that the proportion that flee or actively avoid the sample unit is zero.

Empirical estimates of the sensitivity of dive searches (ϕ_j) have been made for *S. clava* and *S. spallanzanii* based on recovery of mimics and independent, repeat searches of infested areas (i.e. "double-counting"; Gust et al. 2006, Inglis et al. 2009). On average, searches by trained divers appear to detect 60% to 100% of *S. spallanzanii* > 10 cm tube length (mean = 79 %, std. error = 5 %), even in comparatively poor water visibility (i.e. < 1 m; Inglis et al. 2009). Around 80 % of *S. spallanzanii* removed from the Port of Lyttelton occurred at depths shallower than 6 m and 62 % of the worms removed were on outer pilings (Inglis et al. 2009). This means that the dive profile used in the Marine High Risk Site Surveillance could expect to encounter ~50% of the population with a recovery rate of ~80%. Thus, ϕ_j for *S. spallanzanii* was set at 0.4, with a margin of error of 0.05.

Similarly, dive searches for adult *S. clava* are very efficient in even relatively turbid conditions (Gust et al. 2006), with 65 % to 100 % of *S. clava* recovered in water visibility of 0.5 m or greater (mean = 82 % std. error = 6 %; Gust et al. 2006). Most (~75 %) *S. clava* occur in the uppermost 5 m of water (Gust et al. 2008) and we have assumed that a similar proportion of the population to *S. spallanzanii* occur on the outer pilings, such that ~47 % of the population is available for observation by divers in the marine high risk site surveillance. Thus, ϕ_j was set at 0.38 (+ 0.05) for *S. clava* and *C. intestinalis* due to the similarity in size and habit of *S. clava* and *C. intestinalis.*

Unlike the invertebrates, the distribution of *U. pinnatifida* is limited by the availability of light. In the turbid waters of the Port of Lyttelton, > 90 % of sporophytes could be expected to occur in the upper 5 m of water and a similar proportion on outer wharf piles and pontoon facings, such that ~81 % of the population is likely to occur in the search profile of the dives. *U. pinnatifida* sporophytes are also large and distinctive increasing their detectability by divers compared to the smaller invertebrates (~0.9). Thus, we estimated ϕ_j for *U. pinnatifida* to be 0.73 (i.e. 0.9 x 0.9 x 0.9) with a margin of error of 0.05.

Of the four species, benthic sled tows are likely to be an effective survey method for only *S. spallanzanii.* In the absence of empirical data on the effectiveness of sled tows for *S. spallanzanii*, we set a conservative "most likely" value for ϕ_j at 0.2, with a minimum value of 0.1 and maximum value of 0.5 (i.e. PertDev (0.1, 0.2, 0.5)). For all other target species, ϕ_j for the sled tows was set at 0.

6 Results

6.1 REPRODUCTION AND PROPAGULES FROM POPULATIONS OF DIFFERENT SIZES

Styela clava

Reproduction of *S. clava* in Lyttelton Port may extend from November to April, with peak reproductive output in December to February. Based on Nutsford's (2010) recommended fertilisation model, we modelled an average fecundity of 77,230 eggs per reproductive female per spawning season. Also based on Nutsford (2010) 90 % of annual reproductive output was assumed to take place between December and February. This resulted in a daily release of between 3,861 to 38,615,000 eggs into the port water for adult population sizes of 10 to 100,000 reproductive individuals.

Ciona intestinalis

The results of the settlement monitoring component of the *S. spallanzanii* Local Elimination Program indicated that *C. intestinalis* reproduction in Lyttelton occurs from August to April peaking in output between October and February (unpubl. data). Carver et al. (2003) reported that individual females can release 500 eggs per day. Consequently, output was estimated as 2,500 to 25,000,000 eggs per day for resident population sizes of 10 to 100,000 reproductive individuals.

Undaria pinnatifida

The peak reproductive season for *U. pinnatifida* in Lyttelton Port is between July and September (Appendix 2). On average, reproductive sporophytes were modelled to release 5 x 10^8 zoospores during this period. This resulted in a daily release of 5.55 x 10^7 to 5.55 x 10^{11} zoospores into the port water for resident population sizes of 10 to 100,000 reproductive plants.

Sabella spallanzanii

The reproductive season for *S. spallanzanii* in Lyttelton was assumed to be between September and October with an output of 50,000 eggs per female (Appendix 2). Thus daily release was modelled at 4464 to 44,642,857 eggs, for resident population sizes of 10 to 100,000 reproductive worms

6.2 FERTILISATION RATES AND PROPAGULE CONCENTRATIONS

Fertilization rates of marine invertebrate species that produce male and female gametes are highly variable due to the influences such as reproductive strategies (e.g. broadcast, brooding or sperm-casting), abundance and density of reproductive adults, and local hydrodynamic conditions. However, other environmental parameters will also influence success with experimental fertilisation rates ranging from 0 – 100 % in replicates of the same experiment (Bishop & Pemberton 2006). Moreover, published fertilisation rates may underestimate actual success because of bias introduced into many manipulative experiments and the evolution of adaptive strategies to prevent sperm limitation not captured in experimental evaluations (Yund 2000). Therefore, in the right conditions, fertilisation may be relatively successful even when adult densities are low (Pemberton et al. 2003, Yund 2000).
In the absence of specific information for this project it was consequently assumed that small population densities can attain high fertilisation rates. Modelled fertilisation rates were

constant for any modelled population size and have not included the possibility of Allee effects. Values of the steady-state concentrations of planktonic propagules (C_T) calculated for different population sizes of the target species are presented in Appendix 4.

Styela clava

Nutsford (2010) developed a fertilisation model from field and laboratory studies of *S. clava* that indicated an average 3.9 % of the eggs produced by *S. clava* are fertilised and metamorphose into viable planktonic larvae. This rate is within the range of those reported by Pemberton et al. (2003) and Yund (2000) from other studies.

The larval life span of *S. clava* is estimated to be between 12 and 24 hours (Davis, M.A. 1997, Minchin et al. 2006, Svane 1984).

Given a fertilisation rate of 3.9 % and a larval life span of 12 hours, the expected steady-state concentration (C_T) of *S. clava* larvae in the port water during peak reproductive activity ranges from 0.000013 m^{-3} to 0.1279 m^{-3} for resident populations of 10 and 100,000 reproductive adults, respectively. For a larval span of 24 hours, C_T ranges from 0.000020 m^{-3} to 0.2049 m^{-3} for the same population sizes (Appendix 4).

Ciona intestinalis

The fertilisation success of *C. intestinalis* was assumed to be similar to *S. clava* in the absence of definitive information. Larval life span ranges from 2 to 10 days (Appendix 2) and we modelled both of these values, as well as a mid-value (5 days). The expected steady-state concentration of *C. intestinalis* larvae in the port water during peak reproductive activity, for resident population sizes of 10 and 100,000 reproductive adults, ranged from 0.000021 to 0.2117 m^{-3} for a larval life span of 2 days, from 0.000033 m^{-3} to 0.3326 m^{-3} for a larval life of 5 days and from 0.000041 to 0.4087 m^{-3} for a larval life of 10 days (Appendix 4).

Undaria pinnatifida

We did not consider fertilisation rates *per se* for *U. pinnatifida*. Zoospores settle and metamorphose into male and female gametophytes and this latter process was captured by our propagule dosing experiments. Given the daily release rate of zoospores from reproducing sporophytes, the expected steady-state concentration of *U. pinnatifida* zoospores in the port water during peak reproductive activity ranges from 0.488 to 4,880 m^{-3} (zoospore life span = 1 hour), 2.56 m^{-3} to 25,600zoospores.m^{-3} (life span = 6 hours) and 8.47 to 84,700 m^{-3} (life span = 2 days) for resident population sizes of 10 to 100,000 reproductive adult plants (Appendix 4).

Sabella spallanzanii

Fertilisation in *S. spallanzanii* appears to occur inside the female tube (Giangrande et al. 2000, Stabili et al. 2009). Only fertilised eggs are released into the plankton and we assumed that all of these will develop into larvae. Our calculated steady-state concentrations of *S. spallanzanii* larvae in the port water during peak reproductive activity range from 0.0010 to 9.696 m^{-3} (larval life span = 2 days), 0.0017 m^{-3} to 17.03 m^{-3} (life span = 7 days) and 0.0020 m^{-3} to 20.10 m^{-3} (life span = 14 days) for resident population sizes of 10 to 100,000 reproductive adult worms (Appendix 4).

6.3 SAMPLING RATE OF ARRAY SURFACES (Q_s)

Our hydrodynamic measurements in the Port of Lyttelton indicate that a single 20 x 20 cm recruitment plate sampled, on average, 1.95 m^3 of water per day, or 109 m^3 over an 8-week

period of immersion. A single rope mop sampled more than twice this volume: 4.85 m^3 per day or 271 m^3 over 8 weeks. A single settlement array consisting of eight plates and four rope mops, therefore, will sample 35 m^3 of water per day, or 1,960 m^3 over the 8-week immersion period used in the *S. spallanzanii* Local Elimination Program.

6.4 SENSITIVITY OF SETTLEMENT SURFACES: "DOSING" EXPERIMENTS

Styela clava

No recruits of *S. clava* were observed on any of the experimental surfaces, including treatments that were open to the ambient environment (i.e. not enclosed). Reference specimens of several solitary ascidian recruits collected from the settlement surfaces were identified as *Corella eumyota*, *Ascidiella aspersa*, *Asterocarpa cerea* and a species of *Styelidae* (suspected *A. cerea*). Other taxa encountered on the surfaces included bryozoa, barnacles, algae, tubeworms, hydroids and mobile crustaceans.

The results of the dosing experiment indicate that the sensitivity of the array surfaces offered to *S. clava* was $<\frac{1}{50}$ (i.e. < 0.02).

Ciona intestinalis

Recruits of *C. intestinalis* were encountered on 34 of the 45 experimental surfaces, at numbers ranging from 1 to 321 recruits per settlement plate or rope mop (Figure 4).The enclosures were effective in preventing natural recruitment of *C. intestinalis*, irrespective of dosing or surface type. On average, unenclosed surfaces had far greater recruitment of *C. intestinalis* (67.8 ± 25.5 recruits) than surfaces that remained within enclosures for the duration of the experiment (2.8 ± 1.8 recruits) (ANOVA model 1: Enclosure effect F = 67.45; $P < 0.001$; Table 1; Figure 4). Importantly, no *C. intestinalis* were encountered on any enclosed surfaces that had not received larval doses (Figure 4). Recruitment to partial enclosures was no different to recruitment to surfaces lacking enclosures, indicating that the enclosures did not hinder recruitment, but did prevent natural recruitment (ANOVA model 2: Enclosure effect: F = 46.93; P < 0.001; Tukeys' pairwise comparison: partial enclosure vs. no enclosure, P = 0.276; Table 2).

Recruitment of *C. intestinalis* to horizontal plates was significantly greater than to vertical plates (ANOVA model 1: significant Surface type effect; Tukey's pairwise test $P < 0.001$; Table 1) while there were no significant differences between plate types and rope mops. These patterns were consistent irrespective of whether recruitment was natural or as a result of larval dosing (ANOVA model 1: no significant interaction terms; Table 1).

Natural recruitment to the experimental surfaces was substantially greater than recruitment to dosed surfaces, indicating that over the duration of the experiment (8 weeks) a far greater number of natural larvae had encountered the surfaces than the concentrations used in the dosing experiment.

Table 1: ANOVA model 1: Comparison of recruitment of *C. intestinalis* to horizontal and vertical plates or rope mops that had (or had not) been exposed to larval dosing and that had (or had not) been enclosed within an experimental chamber.

Factor	df	Sums of Squares	Mean Squares	F-ratio	P	Post-hoc comparison (Tukey's, P < 0.05)
Surface (S)	2	9.871	4.935	5.445	0.011	Horiz. > Vert. plate
Dosing (D)	1	7.841	7.841	8.651	0.007	Dosing > No dosing
Enclosure (E)	1	61.130	61.130	67.445	0.000	No encl. > encl.
S x D	2	2.837	1.419	1.565	0.230	
S x E	2	5.623	2.811	3.102	0.063	
D x E	1	0.914	0.914	1.008	0.325	
S x D x E	2	1.125	0.563	0.621	0.546	
Error	24	21.753	0.906			

Table 2: ANOVA model 2: Comparison of natural recruitment of *C. intestinalis* to horizontal and vertical plates or rope mops that had been subject to no enclosure, full enclosure or partial enclosure.

Source	df	Sums of Squares	Mean Squares	F-ratio	P	Post-hoc comparison (Tukey's, P < 0.05)
Surface (S)	2	8.587	4.293	6.239	0.009	
Enclosure (E)	2	64.549	32.275	46.903	0.000	No encl. > Encl Partial encl. > Encl. No encl. = partial encl.
S x E	4	6.237	1.559	2.266	0.102	
Error	18	12.386	0.688			

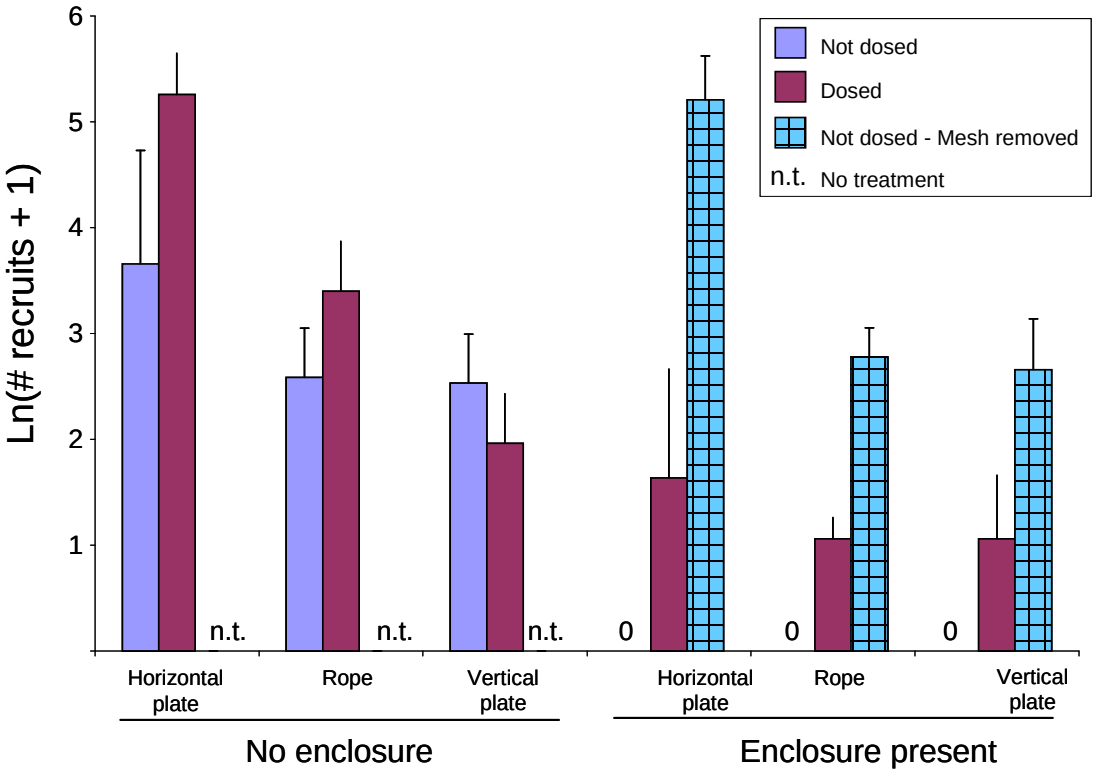

Figure 4: Number of *C. intestinalis* recruits on different types of settlement surfaces (horizontal and vertical plates; rope mops) that were (or were not) subject to larval dosing (n = 60 *C. intestinalis* larvae) and that were (or were not) enclosed within an experimental chamber or a partial enclosure (lacking mesh windows). Note that number of recruits on y-axis are plotted on a logarithmic scale.

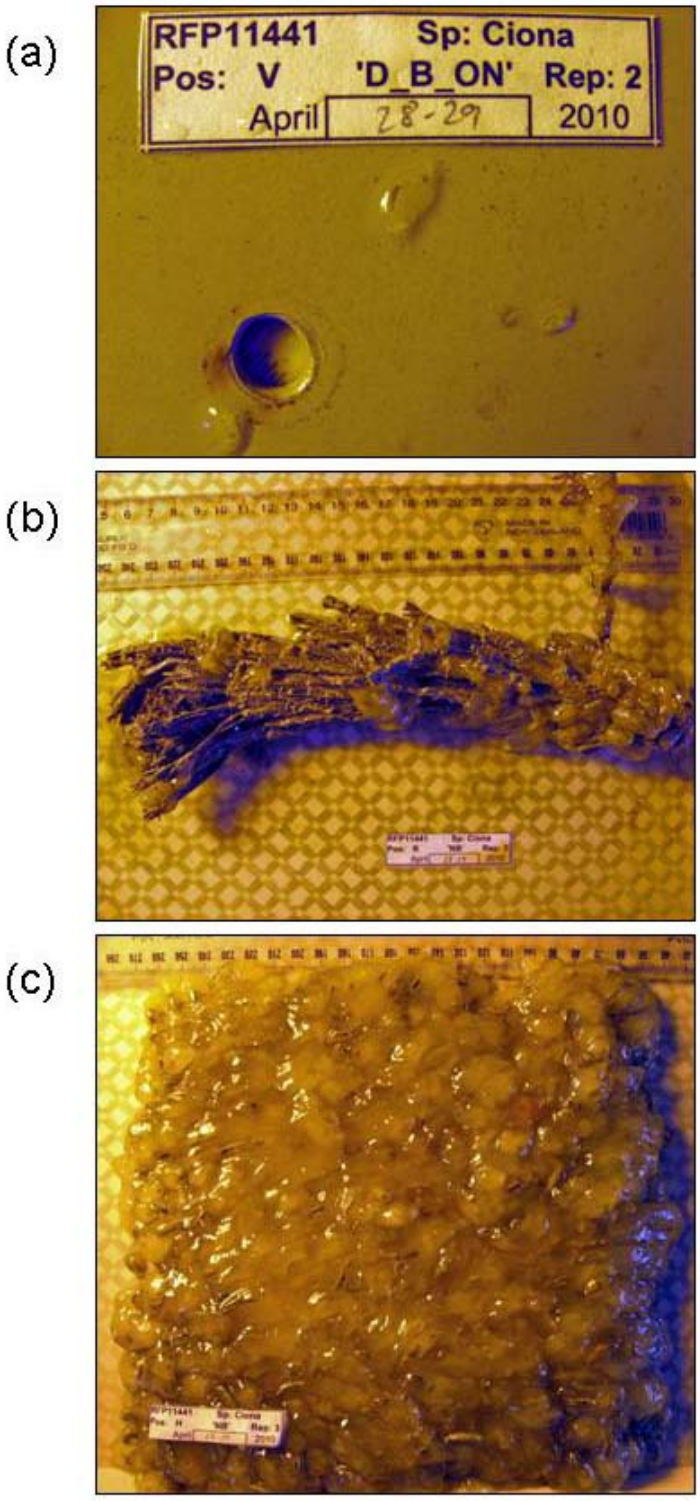

Figure 5: Settlement surfaces colonised by *C. intestinalis*. (a) Vertical plate that had been dosed with 60 *C. intestinalis* larvae and remained enclosed for the duration of the experiment. (b) Rope mop that had been dosed and lacked an enclosure. (c) Undosed and unenclosed plate showing extensive natural recruitment of *C. intestinalis* (approx. 250 *C. intestinalis* on this plate).

Undaria pinnatifida

No *U. pinnatifida* recruits were encountered in the experiment on any of the experimental treatments, including unenclosed controls. Recruits encountered included ascidians, bryozoa, barnacles, algae, tubeworms, hydroids and mobile crustaceans.

Based on the results obtained in the experiment, the sensitivity of the surfaces offered to *U. pinnatifida* in our experiments was $< \frac{1}{19250}$ (i.e. < 0.00005), meaning that an encounter rate of at least > 19250 spores per settlement plate or rope mop is required to result in recruitment of *U. pinnatifida* to the surface.

Sabella spallanzanii

No experimental evaluation of the sensitivity of settlement surfaces was made for *S. spallanzanii*. Instead, we used information contained in the published literature to indirectly estimate the sensitivity of different surface types for *S. spallanzanii*.

S. spallanzanii is thought to prefer vertically oriented artificial structures for settlement, such as wharf pilings or the vertical sides of pontoons (Currie et al. 2000, Holloway & Keough 2002a), although this has not been formally evaluated to date. In a marina near Adelaide, where Holloway and Keough (2002b) encountered ~250 *S. spallanzanii* m^{-2} on pier piles and pontoons, the mean density of recruits detected on vertically orientated settlement panels after a 2-week immersion period during summer was ~0.01 recruits cm^{-2} (Table 3). Average densities of recruits to vacant settlement panels over a 4-week immersion period at two sites within Port Phillip Bay were ~0.17 *S. spallanzanii* cm^{-2} (Johnston & Keough 2000; Table 3). Giangrande et al. (2005) reported an estimated density of ~0.5 recruits cm^{-2} to a net deployed at 10 – 20 m depth over a 3-month period in the Mediterranean. The adult densities at or near the study site are not known.

The average standardised weekly post-settlement mortality rate for juvenile sessile marine invertebrates reported by Gosselin and Qian (1997) ranges from 8.5 % (average of lowest values reported for all taxa) to 23.8 % (average of highest values for all taxa). By combining the range of recruit densities obtained in the studies above, the duration of the various experiments and the standardised post-settlement mortality rates derived by Gosselin and Qian (1997) we back-calculated approximate estimates of sensitivity for these studies. Due to the variation in substrates used, retrospectively determined sensitivities have a wide range (0.04 – 0.84; Table 3). Using the more conservative, higher post-settlement mortality rates, the sensitivity of vertically oriented grey PVC (~0.58) is slightly higher than that of horizontally oriented black Perspex settlement plates (~0.34) (Table 3).

Table 3: Existing data on recruitment of *S. spallanzanii* to different surface types and back-calculation of approximate sensitivities. Sensitivity was calculated using the range of standardised average weekly post-settlement mortality rates derived by Gosselin & Qian (1997), who calculated a minimum average mortality rate of 8.5 % week^{-1} and a maximum average mortality rate of 23.8 % week^{-1}. A higher mortality rate results in lowered sensitivity values.

	Holloway & Keough (2002b)	Johnston & Keough (2000)	Giangrande et al (2005)
Location	Adelaide	Melbourne	Mediterranean
Adult density	~ 250 m^{-2}	Unknown	Unknown
Substrate	Grey PVC plates, 400 cm^2; vertical orientation	Black Perspex plates, 121 cm^2; horizontal orientation	Nylon mesh, 15 mm mesh size
Immersion period	2 weeks	4 weeks	12 weeks
Recruitment density	0.01 cm^{-2}	0.17 cm^{-2}	0.5 cm^{-2}
Substrate sensitivity [a]	0.58 – 0.84	0.34 – 0.70	0.04 – 0.34

[a] Lower sensitivity value derived using weekly mortality rate of 23.8 %; higher value derived using weekly mortality rate of 8.5 % (Gosselin & Qian 1997).

6.5 SENSITIVITY OF SETTLEMENT SURFACES: LITERATURE BASED ESTIMATES

Styela clava

Darbyson et al. (2009) compared *S. clava* recruitment to settlement plate types (10 x 10 cm) that were either constructed from fibreglass, aluminium or wood and that were coated with either black or white antifouling paint or exterior house paint. Following an immersion period of 8 weeks, uncoated plates made from aluminium had the largest number of recruits on them (370.5 ± 87.9, or 3.75 cm^{-2}), followed by surfaces coated in black non-toxic house paint (fibreglass: 232.0 ± 70.9 (2.32 cm^{-2}); and wood (155.8 ± 75.2 (1.55 cm^{-2})). Overall, surfaces coated in black non-toxic paint or untreated aluminium plates were not statistically significant but had significantly larger numbers of *S. clava* recruits than any of the white-coloured treatments (Darbyson et al. 2009).

Bourque et al. (2007) obtained up to 0.95 *S. clava* recruits cm^{-2} using vertically-oriented settlement plates of the same size (10 x 10 cm) made from roughened PVC (colour not specified). Arsenault et al. (2009) used dark grey PVC plates (10 x 10 cm; similar in nature to the 20 x 20 cm plates used in the present study) to monitor *S. clava* recruitment around aquaculture facilities in north-eastern Canada and obtained up to 370.8 ± 42.9 recruits per plate (3.78 cm^{-2}), a density equivalent to that obtained for black or aluminium plates by Darbyson et al. (2009). A direct comparison of the two studies may be confounded, however, by differences in the density of resident reproductive adult *S. clava* in the study locations.

Attempts to monitor recruitment of *S. clava* using artificial surfaces in New Zealand have largely been unsuccessful. McClary et al. (2008) immersed opaque, vertically-orientated PVC settlement plates in Bayswater Marina (Waitemata Harbour) for a period of 2 weeks, but did not observe any recruits. Webber (2010) immersed 15 x 15cm hardiflex panels and 3-m long sections of 3-mm nylon rope at five sites and three depths (0, 0.5, 1 m) in Lyttelton Port and monitored these surfaces for recruitment on a weekly basis between November 2008 and March 2009. No recruits were recorded on any of the surfaces. Nutsford (2010) immersed horizontally-oriented 10 x 10 cm plates of six different surface types (treated wood, sanded grey PVC, rope, mussel shell, concrete, and hardiflex) in Lyttelton Port from November 2009

to March 2010. The surfaces were immersed at three sites, with 10 replicate plates per site, but no *S. clava* recruits were observed on any of the surfaces.

Ciona intestinalis

C. intestinalis recruits prolifically to a wide range of surfaces. Howes et al. (2007) compared the attractiveness of upward and downward facing Petri dishes (opaque surface), but obtained significant recruitment (up to 105 *C. intestinalis* per Petri dish) only on downwards facing surfaces. They also immersed PVC tiles (10 x 10 cm) which yielded lower densities of recruits (17 ± 3 *C. intestinalis*) than Petri dishes (35 ± 3), on average and when standardised for surface area (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 x 0.5 cm) in Ireland. Downward-facing surfaces were also most heavily colonised, with up to 0.4 *C. intestinalis* cm^{-2}.

Nutsford (2010) compared recruitment of *C. intestinalis* on four different substrate types: rope, PVC, wood and hardiflex. Average percentage cover ranged from ~30 ± 19 % (mean ± standard error) on wood to ~58 ± 15 % on PVC surfaces but there were no significant differences between surface types.

Undaria pinnatifida

U. pinnatifida recruits successfully on 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 (Casas et al. 2008, Floc'h et al. 1991, Hay, C. 1990, Hay, & Luckens 1987). Arakawa and Moringa (1994) and Reed et al. (1998) have suggested it has a preference for settling on horizontal (upwards facing) surfaces over vertical surfaces. However, we were unable to locate any studies that compared recruitment of *U. pinnatifida* on a range of surface types.

Recruits of *U .pinnatifida* were not observed in any of the three recent settlement plate studies carried out in Lyttelton Port (Nutsford 2010; Webber 2010; Inglis et al. 2009). The only published study reporting recruitment of *U. pinnatifida* to artificial surfaces is Forrest et al. (2000) involving the use of mussel spat ropes.

In New Zealand, Forrest et al. (2000) released an estimated 2.7×10^{10} zoospores of *U. pinnatifida* into a pre-designed dispersal corridor that had vertical mussel spat ropes (surface to 2 m depth) located from 1 to 200 m from the spore release point. On average, 9.5 sporophytes developed on each rope section located 1 m or 2 m from the point of release. Assuming that all zoospores had an equal chance to encounter each rope, this equates to an estimated sensitivity of 0.35×10^{-10} (0.00000000035) (Forrest et al. 2000). These sensitivities are several orders of magnitude lower than those derived during the present project ($< 0.5 \times 10^{-5}$).

Sabella spallanzanii

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

6.6 MODELLED DETECTION PROBABILITIES USING THE SETTLEMENT ARRAYS

Detection curves for different population sizes of the four target species are presented in Figure 6. As expected, the confidence of detection for each species increased with population size and the number of arrays that was deployed.

According to the scenario tree models, large confidence in detecting *S. clava* (i.e. γ > 90%) was achieved using the arrays once the population had exceeded 1,000 individuals. In summer (November to April), 90% confidence of detection was predicted for a population size of just over 1,000 individuals when 40 arrays were used (Figure 6a). Lower levels of sample effort achieved the same level of confidence when population sizes were in the order of 4,000 (*n* = 20 arrays) and 6,000 individuals (*n* = 10 arrays), respectively (Figure 6a). In the winter surveillance season (May to October), when spawning of *S. clava* is less likely, the same confidence of detection was not achieved until much larger adult populations sizes had been reached (n = 6,000 individuals when 40 arrays were used, n = 9,000 individuals for 20 arrays and n = 35,000 individuals for 10 arrays; Figure 6b).

For *C. intestinalis*, large confidence of detection was predicted at relatively small population sizes (Figure 6c & d). In summer, for example, the model predicted that an average population as small as ~70 individuals could be detected with a relatively high degree of confidence (i.e. γ > 90%) when 40 arrays were deployed. Use of smaller numbers of arrays (n = 10 and n = 20) would, on average, detect populations of ~500 individuals with the same degree of confidence (Figure 6c). In winter, when spawning is less likely, γ > 90 % was predicted only when adult populations exceeded 5,000 (n = 20 or 40 arrays) or 7,000 individuals (Figure 6d). In each season, there was large variance around estimates of the mean confidence of detection for the different population sizes. This is likely to reflect the relative imprecision of the values of sensitivity (ϕ_j) used to parameterize the model for *C. intestinalis*. For example, the mean ϕ_j used in the model for horizontal plate surfaces (ϕ_j = 0.2) had a coefficient of variation (CV) of 1.5, meaning that values selected at random from the distribution for use in the model could vary widely between 0 and ~0.4 (95 % of values). The large CV probably reflects the comparatively low replication in the dosing experiment from which this estimate was obtained. As it was derived empirically, however, it is a realistic estimate of variation in the sensitivity of the surfaces to recruitment by *C. intestinalis*.

High confidence of detection was also predicted for very small adult populations of *S. spallanzanii* and *U. pinnatifida* (Figure 6e to h). Populations of *S. spallanzanii* as small as 70 individuals were predicted to be detected with γ > 90 % when 40 arrays were used, irrespective of the season of deployment (Figure 6e & f). Smaller numbers of arrays achieved γ > 90 % for populations of 150 (*n* = 20 arrays) and 600 individuals (*n* = 10 arrays). For *U. pinnatifida*, the initial model runs predicted that adult populations of just 10 individuals could be detected with γ > 90 % when only 10 arrays were deployed in winter (Figure 6g & h).

Outputs from the scenario tree models for the arrays were strongly influenced by the values of planktonic duration (T) used to calculate steady state concentrations of propagules and by inputs on the sensitivity of the array surfaces (ϕ_j). These were the elements of the species' life-history for which there was the greatest uncertainty in the models. For example, the predictions for *U. pinnatifida* in Figure 6g & h, used ϕ_j = 2.6 x 10^{-5}, which was based on the outcome of the dosing experiment for *U. pinnatifida*. Despite each experimental surface being dosed with an estimated 19,000 zoospores, none recruited to be visible after 8 weeks deployment. The "most likely" value for ϕ_j used to parameterise the scenario tree model was,

therefore, set arbitrarily at a value that was approximately twice the dosing used in the experiment (i.e. 1 recruit for every 38,000 zoospores). In Figures 6i & j we show the effects of using an alternate value for ϕ_j, that was based on the outcome of zoospore dispersal experiments undertaken by Forrest et al. (2000). In that study, recruitment of *U. pinnatifida* to ropes deployed within 1 to 2 m of zoospore release occurred at a rate of ~3/10,000,000,000 zoospores. When this value is used to parameterise the scenario tree model, γ > 90 % is not achieved for any of the population sizes simulated, even in winter when zoospore production is greatest (Figure 6j).

Similarly, a change in ϕ_j of just one order of magnitude (from ϕ_j = 0.001 to ϕ_j = 0.0001) had a significant effect on the confidence of detection of *S. spallanzanii*. Use of the lower value for ϕ_j meant that high confidence of detection (γ > 90 %) was likely to be achieved only for populations > 3,500 (n = 40 arrays), 5,500 (n = 20 arrays) or 7,000 individuals (*n* = 10 arrays; Figure 6k). These were up to 50 x larger than the populations predicted to be detected with γ > 90 % when ϕ_j = 0.001. In the absence of empirical data on ϕ_j for these two species it is difficult to determine which values are the more realistic. However, in each case, our field observations tend to align more closely with predictions made using the much lower values for ϕ_j (see Discussion).

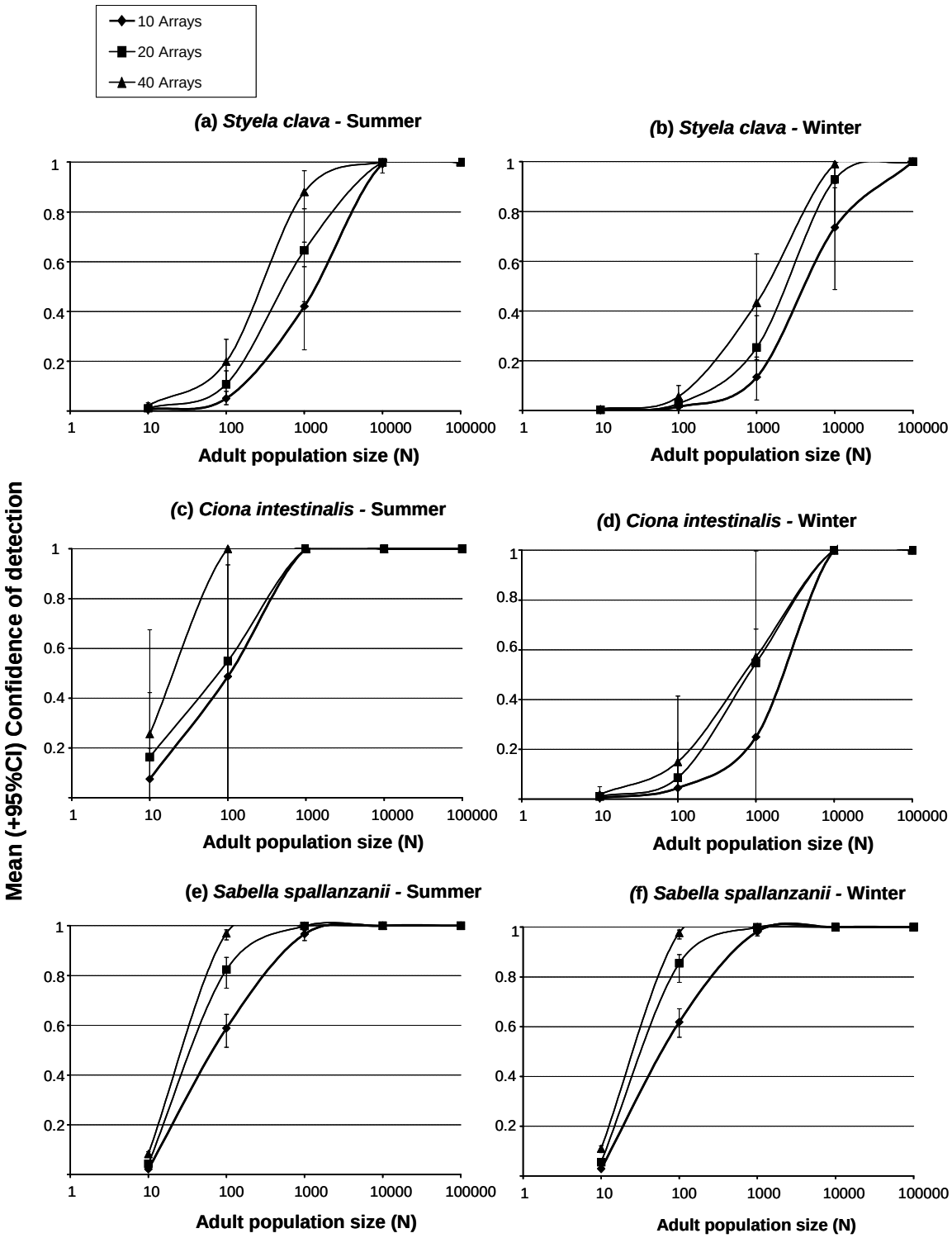

Figure 6. Modelled detection curves (mean + 95% confidence limits of detection) for populations of increasing size of the four target species. Separate curves were constructed for summer and winter deployment of different numbers (*n* = 10, 20 and 40) of the settlement arrays.

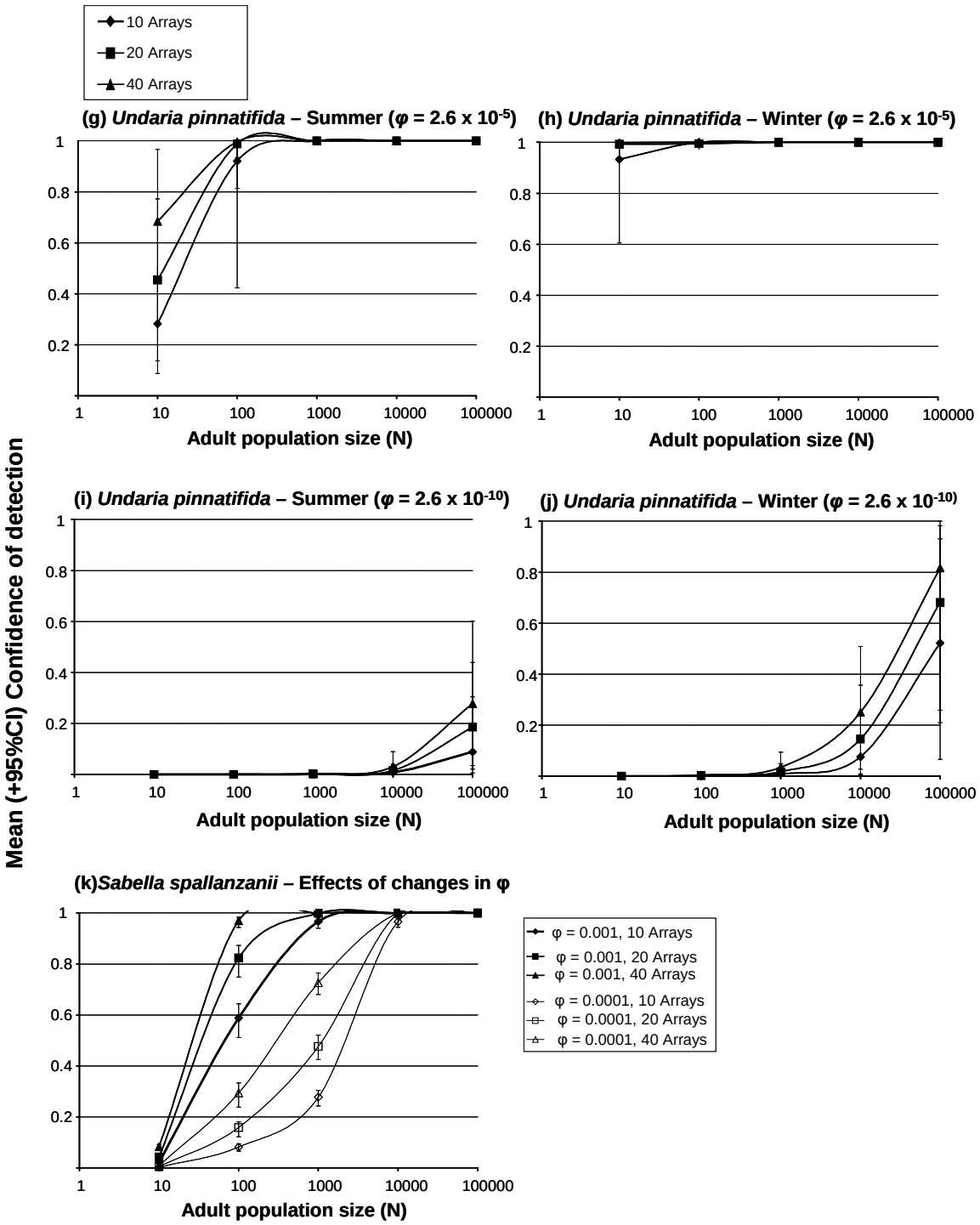

Figure 6 (continued). Curves in (i), (j), and (k) depict the effects of changing the values of surface sensitivity (ϕ_j) used to parameterise the models for *U. pinnatifida* and *S. spallanzanii*.

6.7 MODELLED DETECTION PROBABILITIES FOR EXISTING SURVEY METHODS

Scenario tree models based on the existing surveillance methods were parameterised using the survey effort (~20 dive searches and 50 benthic sled tows in the inner port) and relative allocation of samples between different habitat types that are currently used in each survey. For a standard survey, around $\frac{2}{3}$ of the searches are allocated to areas of wharf piles while the remaining $\frac{1}{3}$ are divided among pontoons and breakwalls.

Detection curves for populations of different sizes of the four target species are presented in Figure7. For *S. clava*, $\gamma > 90$ % is predicted to occur at population sizes of 150 individuals, using the current survey effort ($n = 20$), and 70 individuals when search effort is doubled ($n = 40$; Figure7a). For *C. intestinalis*, $\gamma > 90$ % is, on average, achieved at population sizes of 250 ($n = 20$ searches) and 80 individuals ($n = 40$ searches; Figure7b). Dive searches for *S. spallanzanii* were predicted to detect the worm with $\gamma > 90$ % when populations were > 400 individuals (Figure7c). Doubling the search effort would, on average, reduce this to around 100 individuals (results not shown). Sled tows were much less effective than dive searches for *S. spallanzanii*, with a population > 5,000 individuals required before $\gamma > 90$ %. Consequently, the sled tows contributed relatively little to the joint probability of detection when both dive searches and sled tows were undertaken (< 5 % gain in relative efficiency compared to dive searches alone).

The high visibility of *U. pinnatifida* sporophytes within the search profile of the dives meant that very small populations were predicted to be detected with comparatively high levels of confidence (Figure7). At current levels of search effort, $\gamma > 90$ % was predicted when the population size was ~80 individuals. Doubling survey effort reduced this number to 70 individuals (Figure7).

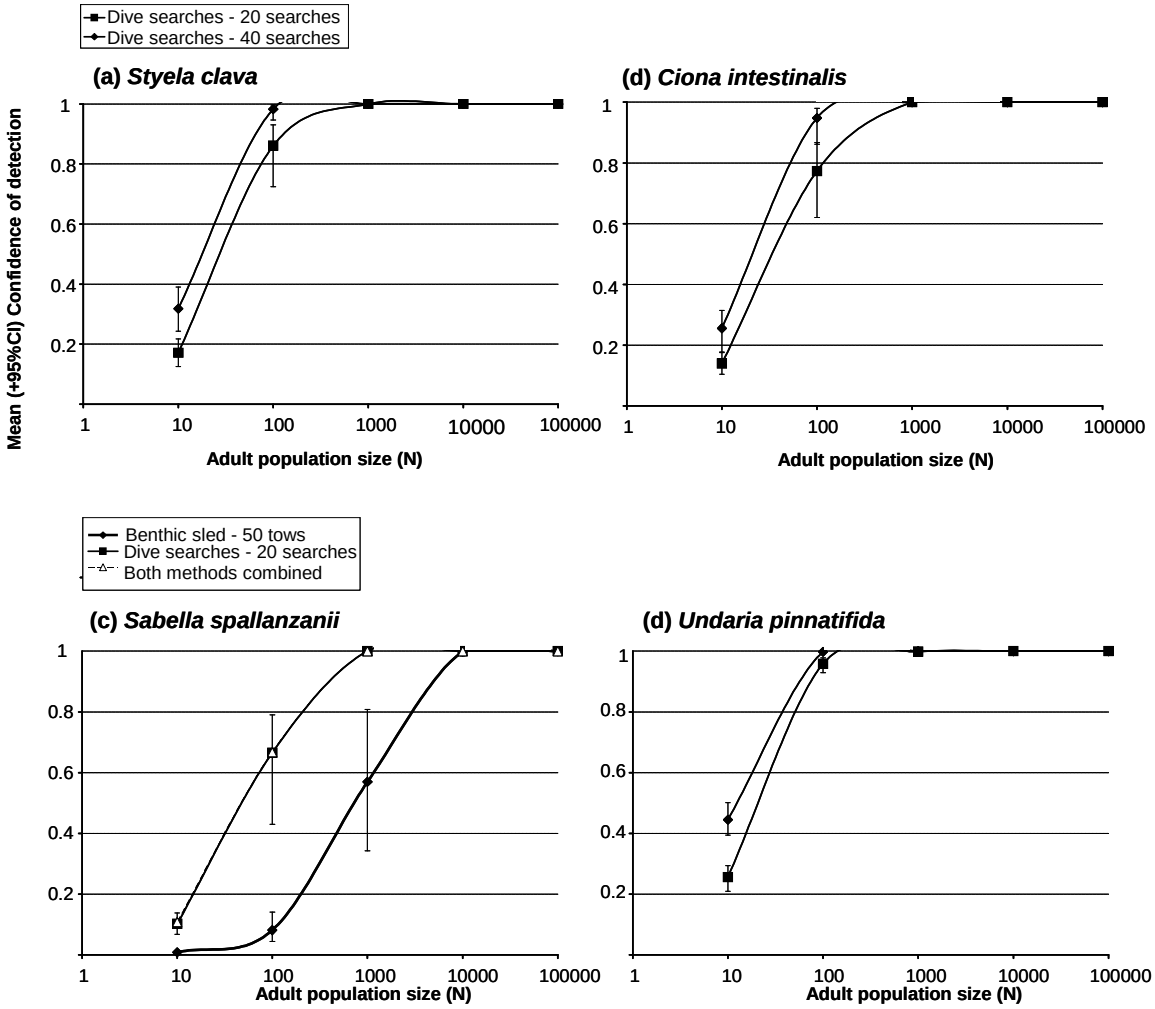

Figure 7. Modelled detection curves (mean + 95% confidence limits of detection) for populations of increasing size of the four target species. Separate curves were constructed for different numbers of dive searches within the inner harbour (n = 20, 40) and, in the case of *S. spallanzanii*, for dive searches and benthic sled tows.

7 Discussion

7.1 CAPACITY TO DETECT MARINE PEST SPECIES

The scenario tree models we created to estimate the confidence of detection of *S. spallanzanii, S. clava* and *U. pinnatifida* using settlement arrays appear overly optimistic and do not align well with field observations of recruitment by these species. For example, the model for *U. pinnatifida* predicted very high confidence of detection of quite small adult populations (~10 individuals) in both winter and summer. Yet, *U. pinnatifida* did not recruit to any of the experimental surfaces used in the dosing experiment, nor to any of the 120 arrays that were deployed during the sentinel monitoring component of the *S. spallanzanii* Local Elimination Project in 2009-10 (Inglis et al. 2009), despite sizeable adult populations being present in the port. Given that young sporophytes have average weekly growth rates of 1 to 14 cm during the first 15 weeks of their life (Thornber et al. 2004), it is likely that any *U. pinnatifida* that had recruited to the arrays would have been visible to the field teams. Similarly, no *S. spallanzanii* or *S. clava* were observed in the dosing experiment or sentinel monitoring, again despite the presence of adult populations in the port.

In developing the models we have had to make some simplifying assumptions about the relationships between population size, reproductive success, larval survival and recruitment. Our models assumed very simple linear relationships between adult population size, fecundity and reproductive success and did not allow for Allee effects at small population sizes. The use of such simple conceptual models was necessitated by a lack of empirical data on the early-life stages of the target species. As Hayes et al. (2005) note, the adult/larvae ratio is critical to evaluating the efficacy of survey methods that target the planktonic life-stages of pests (e.g. plankton tows, settlement surfaces) relative to the efficacy of methods targeting adults, but for most marine populations this ratio is difficult to quantify and is highly variable in space and time (Underwood & Fairweather 1989). Hughes et al. (2000) showed that, in corals, the relationship between fecundity and recruitment was distinctly non-linear. Recruitment increased disproportionately as the proportion of gravid colonies in the populations approached 100 %, suggesting much enhanced fertilisation success during synchronised spawning events. At small population sizes, clustering of adults and the synchronicity of spawning are likely to have particularly strong effects on fertilisation success in species that broadcast their gametes (Gasgoine & Lipcius 2004).

Our calculations are particularly sensitive to uncertainty in three parameters, for which there was very limited empirical data for the target species. These were the duration of planktonic development (T), the instantaneous mortality of planktonic propagules (k) and the sensitivity of the array surfaces to recruitment (ϕ_j). Underwood and Fairweather (1989) used a simple model to demonstrate how relatively small changes in T and k can have dramatic effects on the total number of offspring that survive through to recruitment (in our case, the steady state concentrations of larvae that were calculated for each population size). In Table 4 we have recreated their example using the values of T and k that we derived from the literature to parameterise our model for *C. intestinalis*. Following Underwood and Fairweather (1989), the number of planktonic propagules available for recruitment after a period of T days is given by the equation:

$$N_T = N_0 e^{-kT}$$

where N_0 is the original number of offspring (Underwood & Fairweather 1989). Because this is a negative exponential relationship, small changes in k and T can create large changes in N_T. Our example in Table 4 depicts a population of ~1000 adult *C. intestinalis* with a total reproductive output over the 8 week period of deployment of the arrays of ~550,000 larvae (again, without allowance for Allee effects). Depending on the combination of k and T used in the model, the number of offspring surviving to recruitment could vary from < 1 % to > 67 % of the number produced by the adult population.

Table 4. Number of planktonic propagules available for recruitment after planktonic development of T days and daily mortality of k proportion of propagules. Calculations are based on a starting population of 550,000 larvae and a timeframe of 8 weeks.

		T	
k	2	5	10
0.19	373388	211160	81664
0.29	305704	128075	30042
0.47	213282	52071	4966

Also, in the absence of empirical values of k for the target species, we derived our "best" estimates for use in the models from the relationship between k and T described by Rumrill (1990) (eq. 4). As a negative exponential model, this relationship has its greatest variance (and error) when $T < 10$ days such that, over this range, very small changes in T can result in very large changes in the values used for k. The uncertainty in both k and T for the target species mean that our calculations of steady state concentrations could be in error by several orders of magnitude relative to natural populations, potentially greatly inflating the calculated encounter rate with the array surfaces.

The very high fecundity of many marine organisms is thought be an evolutionary adaptation to the challenges of fertilisation in marine environments and to the large and spatiotemporally highly variable rates of mortality experienced during the planktonic life phase and early recruitment (Underwood & Fairweather 1989). Although *k* is difficult to quantify it is an important determinant of the dynamics of adult populations, since the survival of large concentrations of larvae to settlement is often highly correlated with high densities of consequent adult year-classes (Underwood & Fairweather 1989).

7.2 SENSITIVITY OF THE ARRAYS

In addition to uncertainty about the rate at which larvae are likely to encounter the surfaces, our model outputs were also strongly influenced by the values used to describe the sensitivity of the settlement surfaces (ϕ_j; i.e. the proportion of larvae encountering the surfaces that successfully settle and recruit). For three of the four target species we were unable to gain useful empirical estimates of ϕ_j from the dosing experiment and had to resort to using what little information was available in the literature (or in the absence of any information, "best guesses") to parameterise the models. In some cases, the values used appear to be overly optimistic (see Figure 6I, j, k). Substantially lower values of ϕ_j would significantly reduce the confidence of detection calculated by our models.

The dosing experiments showed that *C. intestinalis* was capable of recruiting to all of the substrata used in the arrays (vertical and horizontal grey PVC plates; rope mops) at relatively low encounter rates. According to our model, the encounter rates simulated during the dosing experiment (60 larvae per surface) are comparable to those expected given a resident population size of 100,000 individuals (~45 larvae per surface over 8 weeks). Despite the

relatively low level of replication used in the experiment (n = 3 replicates per treatment), recruits were detected on dosed and enclosed surfaces, and on all surfaces exposed to natural recruitment.

The absence of any recruitment by *S. clava* or *U. pinnatifida* in the propagule dosing experiments appears to be because the surfaces used in the experiment are unsuitable for these species. There was no natural settlement of these species on the unenclosed surfaces, despite sizeable adult populations in the surrounding port. Similarly, none of the propagules exposed to the surfaces in the dosed treatments recruited successfully. A further possibility is that the dosing process caused larval/spore mortality. We consider this unlikely: syringes were checked prior to dosing to confirm larvae and spores were viable and "active" when injected into the chambers.

While other surface types may be more effective for recruitment of these species (discussed below), recruitment of *S. clava* has been observed on PVC plates in other studies (Arsenault et al. 2009, Howes et al. 2007). Its absence from our field experiment may be a result of relatively low larval encounter rates, both naturally and experimentally. For example, despite sizeable adult populations of *S. clava* in Lyttelton, Nutsford's (2010) attempts to sample larvae during the period of peak reproductive activity have met with limited success. Monthly sampling for larvae between February 2009 and February 2010 returned only very low concentrations of larvae, with maxima of 0.098 and 0.13 larvae per m^3, equivalent in our model to steady-state concentrations achieved by adult populations of between 1,000 and 10,000 individuals. These concentrations are, nevertheless, substantially lower than larval concentrations recorded in Prince Edward Island, Canada, which have been measured at up to 560 larvae m^{-3} (Bourque et al. 2007). In contrast to the results of recruitment studies conducted around Prince Edward Island (Bourque et al. 2007, Darbyson et al. 2009), a 2-year study of recruitment of *S. clava* in Lyttelton Port resulted in nil recruits (Nutsford 2010, Webber 2010).

U. pinnatifida is also abundant within Lyttelton Port and its absence from our experimental surfaces may be the result of potentially unsuitable illumination of the substrates. While *U. pinnatifida* occurs at depth of up to 4 m on pilings throughout the Port, this depth is considerably reduced at low tide, resulting in increased light levels at lower water levels. In dosing our experiment, settlement surfaces were submerged off a pontoon, at a constant depth of 2.5 m in relatively turbid water. Because of the location and orientation of the pontoon, the experimental surfaces were shaded from the sun during most of the day.

7.3 SPATIAL AND TEMPORAL LIMITATIONS

Species with very short larval life spans (e.g. a few hours) and/or gregarious settlement may not be sampled effectively by artificial settlement surfaces unless the surfaces are located close to spawning adults. This may be the case for *S. clava* (larval life span of 12-24 hours) and, potentially, *C. intestinalis* (larval life span may be as short as 2 days). The circulation and mixing of water within Lyttelton Port may help to increase the probability that larvae are delivered to relatively isolated settlement arrays. However, in more open monitoring environments array distance from propagule sources may be of higher importance, particularly for small adult populations, as might occur during the early stages of an incursion. Natural dispersal of *U. pinnatifida* zoospores is also considered to be short-range, typically not exceeding 10 m (Stuart 2004). Consequently, if arrays were to be effective for this species, they would have to be used in large numbers and well-dispersed throughout the port to ensure placement within 10 m of small populations of adult sporophytes.

It is unlikely that a single deployment of settlement arrays can be used effectively to detect all four target species. Even if the surfaces were suitable for recruitment of all four species, our reviews suggest that the best period for monitoring for the presence of *S. clava* and *C. intestinalis* is between December and March, when reproduction and settlement are likely to be greatest. However, this is not a suitable period for recruitment of *U. pinnatifida*, which has its peak reproductive period during the winter months (July – September) in Lyttelton Port. The timing of reproduction in *U. pinnatifida* may vary around New Zealand's coastline, however, and settlement monitoring programmes should take this variation into consideration.

Little is known about the reproduction and recruitment of *S. spallanzanii* in New Zealand. Available literature suggests that it spawns at water temperatures > 11°C. Recruitment of larvae is usually observed during spring and summer although variation in the timing and duration of the reproductive period has been reported for some non-indigenous populations (Appendix 2).Monitoring for *S. spallanzanii* in Lyttelton Port may be most successful in spring and summer. However, further studies on recruitment in extant New Zealand populations are needed to understand its reproductive phenology here.

Our use of a relatively long immersion period for the settlement arrays (e.g. 8 weeks) has both advantages and disadvantages. One potential disadvantage is that, in areas where recruitment and space occupancy is dominated by a single species, surfaces will be crowded after 8 weeks and early colonists – potentially including target species – may become displaced and go undetected (Russ 1982). In our study and the *S. spallanzanii* Local Elimination Sentinel Monitoring Programme (Inglis et al. 2009), *C. intestinalis* recruited heavily to the settlement surfaces. Its dominance of the surfaces may have prevented other potential target species from recruiting. Shorter immersion periods (e.g. 4 weeks) may reduce competition and dominance and could help to avoid the problem described above, but will also reduce the number of larvae that encounter the surfaces, resulting in lower confidence of detection. Another potential disadvantage of shorter immersion times is the difficulty in identifying very young recruits. This is the case with, for example, *S. spallanzanii*. The immersion period of 8 weeks used in the Local Elimination Program Sentinel Monitoring was chosen specifically to enable identification to species. Taxonomic experts will have difficulties identifying species belonging to a wide range of marine invertebrates and algae from juvenile specimens of 4 weeks or younger (Floerl et al. 2010). For example, *C. intestinalis* and *C. savignyi* both occur in Lyttelton Port, but at 4 weeks of age recruits of the two species cannot be distinguished without use of molecular markers. Any uncertainties in identification or the need for more complex techniques may compromise the effectiveness of the monitoring or increase its cost.

7.4 MEASURES TO INCREASE EFFICACY AND SENSITIVITY

7.4.1 Styela clava

S. clava larvae settle preferentially in areas of low light intensity, including both horizontal and vertical surfaces (Davis & Davis 2007). The inclusion of dark-coloured (black or dark grey) painted settlement plates, or unpainted plates made from aluminium into the settlement arrays, would provide a more suitable settlement substratum for *S. clava*. *S. clava* also settles preferentially on surfaces containing an established biofilm (Kashenko 1996) or that are already encrusted with other organisms (Lützen 1999). Studies reporting significant recruitment of *S. clava* (e.g. Arsenault et al. 2009, Darbyson et al. 2009, Ramsay et al. 2008) used immersion periods of 3 to 4 months. Monitoring for *S. clava* in New Zealand may also require extended periods of immersion. Array deployments of 2 weeks (North Island - McClary et al. 2008) and 8 weeks (this study) have so far failed to detect *S. clava*. An

immersion depth of approximately 1-3 m should be suitable for this species, since this is the depth at which most of the adult population seems to occur (Gust et al. 2008).

7.4.2 *Ciona intestinalis*

C. intestinalis also appears to settle preferentially in low-light conditions (Tursi 1980). Results from this study and the sentinel monitoring component of the *S. spallanzanii* Local Elimination Program suggest that *C. intestinalis* settles preferentially to the undersides of horizontal surfaces. Howes et al. (2007) did observed highest recruitment at a depth of 2-4 m, and only very low levels near the water surface or at depth exceeding 4.5 m. Based on available information we conclude that settlement monitoring for *C. intestinalis* may be done using the same surfaces as recommended for *S. clava* and an immersion depth of 2-4 m. An immersion period of 6-8 weeks is suitable.

7.4.3 *Undaria pinnatifida*

During a dispersal experiment conducted in the Marlborough Sounds, aggregations of sporophytes were observed on experimental settlement surfaces (mussel spat collector ropes) deployed near the water surface. No settlement was detected below a depth of 0.75m (Forrest et al. 2000). Based on these results and the absence of *U. pinnatifida* from any surfaces deployed in the Port of Lyttelton at 2-5 m depth (Inglis et al. 2009, Nutsford 2010, Webber 2010; this study), we recommend that settlement substrata for monitoring *U. pinnatifida* should be floating and deployed at a depth of < 1 m. Dense aggregations of *U. pinnatifida* occur on floating car tyres around marina pile moorings and pontoons in Lyttelton Harbour. Monitoring surfaces deployed in a similar manner and made from mussel spat rope would most likely be suitable settlement substrata for this species. Monitoring for *U. pinnatifida* should be carried out during winter. Because there is little other recruitment during this season, immersion periods of 6-12 weeks are unlikely to risk displacement of sporophytes by competing, dominant species. Growth rates of 1 to 14 cm per week (Thompson 2004, Thornber et al. 2004) mean that *U. pinnatifida* sporophytes can be identified if such immersion periods are used.

7.4.4 *Sabella spallanzanii*

Because little is known about the reproductive biology and general ecology of *S. spallanzanii* in New Zealand it is difficult to make strong recommendations for a particular type of surface that could be used to monitor settlement by this species. Based on the literature reviews undertaken for the *S. spallanzanii* Local Elimination program (RFP11199) and the present project, it appears that the PVC plates used on the present arrays are suitable substrates for *S. spallanzanii*, as overseas studies have recorded it from these surfaces. One possible improvement may be the use of dark grey or black PVC plates. Plates deployed in both horizontal and vertical orientation are suitable. Highest densities of recruits have been reported from horizontal plates (Johnston & Keough 2000) but this is based on a relatively small number of studies. None of these specifically evaluated the effect of substratum orientation on settlement. The immersion depths of 2 to 5 m used in the *S. spallanzanii* Local Elimination Program sentinel monitoring are considered suitable. To enable the identification of recruits to species level, an immersion period of at least 8 weeks, as used in the array monitoring so far, appears suitable. However, in locations with heavy natural recruitment of opportunistic species – such as *C. intestinalis* / *C. savignyi* in Lyttelton Port, crowding/displacement may be an issue.

7.5 THE INTERPRETATION OF NEGATIVE RESULTS.

As discussed earlier in the report, without better estimates of ϕ_j for the settlement surfaces, it is not possible to interpret a lack of recruitment to the settlement arrays as indicating the absence of adult populations. The dosing experiments suggest that lack of recruitment to the surfaces is a poor indicator of the presence or size of adult populations of *S. clava* and *U. pinnatifida* in the surrounding area, since sizeable populations of these species were present in the Port of Lyttelton, but no recruitment was recorded on the surfaces. Our calculations of steady-state concentrations of propagules for these species suggest that sufficient numbers of propagules should have been produced by the established populations to result in reasonably high rates of encounter with the surfaces. The absence of any recruitment during this study or the *S. spallanzanii* Local Elimination Programme suggests that the surfaces used in the arrays were relatively poor substrata for settlement of these species (i.e. very low ϕ_j) and, if recruitment were to occur at all, it is likely to occur only when adult populations and consequent larval concentrations were extremely high. In contrast, *C. intestinalis* recruited in relatively high proportions to the settlement plates. Larval dosing suggested that, on average, around 20 % of *C. intestinalis* larvae exposed to the surfaces settled successfully. Nevertheless, the large variation associated with this estimate means that calculations of the confidence of detection (and, therefore, confidence of absence of the species) are also highly variable (Figure 6c&d).

7.6 RELATIVE GAIN IN CONFIDENCE OF DETECTION

Given our doubts about the reliability of our modelled estimates of the confidence of detection of the target species using the arrays, it is not useful to formally calculate the gains made in statistical confidence relative to the existing survey techniques. The models we developed for dive searches and sled tows predicted relatively high confidence of detection for moderate sized populations of the target species (100's to 1000's of individuals). We are more confident in these models because fewer assumptions are involved in calculating the design prevalence (ρ^*) for pest populations of different sizes (they are simply number of adults / available area of habitat) and because studies have already been undertaken to quantify ϕ_j for several of the target species. Indeed, the population size of *S. spallanzanii* that the models predicted would be detected with $\gamma > 90$ % at current levels of survey effort (n = 20 dive searches), aligned well with the size of the population of *S. spallanzanii* detected in Lyttelton in 2008 (2009, Inglis et al. 2008b). Nevertheless, we believe that these models also need refinement to account for likely clustering of individuals during new incursions, as was described for the *S. spallanzanii* population (Inglis et al. 2008b). Significant clustering is likely to reduce γ for the same level of sample effort (Martin et al. 2007).

7.7 LIMITATIONS OF THE STUDY

A limitation of this project is that the evaluation of surface sensitivity (propagule dosing experiments) were carried out only on a single occasion and using minimal replication. This resulted in high variance associated with mean estimates of surface sensitivity (*C. intestinalis*) and complete failure of settlement for the other two species. Should future experimental evaluations follow from this initial assessment we recommend that at least two time series of the dosing experiments are conducted and that the budget allows for (1) the inclusion of several concentrations of dosing per species, and (2) higher replication within each experimental treatment. Furthermore, and as discussed in the sections above, an improved understanding of propagule life-span (T) and natural planktonic mortality (k) of the target

species would serve to reduce the uncertainty associated with the current model estimates. Once this uncertainty is reduced the model framework developed here can be used to achieve the original project objectives.

8 Acknowledgements

We thank Tristan Williams, Tim Oakley and Kim Kelleher (Lyttelton Port Company) for facilitating access to the Port and making available the Z-Berth sampling location and facilities for a field laboratory. Thanks are also due to Matt Enright, Kate Schimanski, Kimberley Seaward, Caroline Williams, Kate Willis and Chris Woods for assistance with collection of reproductive adult organisms, laboratory-based dissection and extraction/production of reproductive propagules, and *in situ* dosing of submerged settlement surfaces. Chris Woods provided helpful comments on a draft of this report.

9 Appendices

APPENDIX 1: CALCULATING PROPAGULE CONCENTRATIONS

An analytical mathematical model was developed to estimate the concentration of propagules (larvae of *C. intestinalis*, *S. spallanzanii* or *S. clava*, or *U. pinnatifida* zoospores) inside the port. The model can be used to estimate how larval concentration increases during spawning, decreases as propagules are carried out of the port by the tide, and decays as propagules die. In this model, the following simplifying assumptions are made:

We first developed the model for when there is a constant release of propagules and no decay or mortality.

The rate of change of propagules in the port is:

$$\frac{d(VC)}{dt} = R - QC$$

where V is the volume of the port (m^3), C the concentration of propagules in the port (number m^{-3}), Q the rate of flow out of the port (m^3 d^{-1}), and R the propagules spawning or release rate (number d^{-1}). The equation states that the rate of change of the number of propagules in the port $\frac{d(VC)}{dt}$ equals the rate at which propagules are released (R), minus the rate at which particles are carried out of the port (QC). Note that throughout the model, volumes are measured in m^3, time in days, concentrations in number of propagules m^{-3}, and flow rates in $m^3.d^{-1}$.

If the volume of the port, the flow rate and release rate are constant over time, then:

$$V\frac{dC}{dt} = R - QC$$

This equation is solved to obtain $C(t)$, the concentration of propagules inside the port at time t. The solution is dependent on the initial concentration $C(t_1)$, which is the concentration at time t_1.

$$C(t) = \frac{R}{Q} + \left[C(t_1) - \frac{R}{Q} \right] \exp\left[-\frac{Q}{V}(t - t_1) \right]$$

If the initial concentration (t_1 =0) is zero, then:

$$C(t) = \frac{R}{Q}\left(1 - e^{-\frac{Qt}{V}} \right)$$

If propagules are released continuously, then eventually the concentration in the port will reach a steady state value. Steady-state occurs when the rate at which propagules are carried out of the port (QC) equals the rate that propagules are produced (R). The steady state concentration (when dC/dt = 0) will be:

$$C = \frac{R}{Q}$$

Propagules with fixed life span

The propagules of the target species we examined have a fixed life span, after which they die. When propagules have a fixed life span, propagule concentrations need to be reduced when they die. If the propagule life span is D_P, the proportion of propagules released at an instant in time that remain in the port after a time T will be:

$$p = \exp\left[-\frac{T}{\theta}\right],$$

where θ = V/Q = hydraulic residence time, which is a measure of the average length of time that fluid remains in the port.

The mass balance is then adjusted by removing propagules at a rate of pR after time T:

$$V\frac{dC}{dt} = R\left(1 - e^{-T/\theta}\right) - QC = R^* - QC \text{ , t > } T.$$

Note that $R^* = R\left(1 - e^{-T/\theta}\right)$. The concentration after the first propagules released begin to die is therefore:

$$C(t) = \frac{R^*}{Q} + \left[C(t_1) - \frac{R^*}{Q}\right]\exp\left[-\frac{Q}{V}(t - t_1)\right]$$

If the port initially has zero propagules ($t_1 = 0$, $C(t_1) = 0$), then when propagules begin dying at time T, the concentration is:

$$C(T) = \frac{R}{Q}\left(1 - \mathrm{e}^{-\frac{T}{\theta}}\right)$$

After this time, $t > T$:

$$C(t) = \frac{R^*}{Q} + \left[C(T) - \frac{R^*}{Q}\right]\exp\left[-\frac{(t - T)}{\theta}\right]$$
$$= \frac{R}{Q}\left(1 - e^{-T/\theta}\right)$$

Thus, after time T, the concentration in the port remains constant. The concentration of propagules during the spawning period can therefore be estimated using two equations. The first equation is the initial growth period before propagules begin to die:

$$C(t) = \frac{R}{Q}\left(1 - \mathrm{e}^{-\frac{t}{\theta}}\right), \text{ t < T}$$

The second equation applies after the first propagules begin to die at time T:

$$C(t) = \frac{R}{Q}\left(1 - e^{-\frac{T}{\theta}}\right), \; t > T$$

If the larval release stops, then the rate of change of propagules in the port is:

$$V\frac{dC}{dt} = -Re^{-T/\theta} - QC$$

The larval concentration is:

$$C(t) = \frac{R}{Q}e^{-\frac{T}{\theta}} + \left[C(t_1) + \frac{R}{Q}e^{-\frac{T}{\theta}}\right]\exp\left[-\frac{(t - t_1)}{\theta}\right]$$

Here, t_1 is the time at which the propagule release or spawning stops. If the port had reached steady-state before the release stopped ($t_1 > T$), then:

$$C(t) = \frac{R}{Q}\left(e^{-\frac{T}{\theta}} + e^{-\frac{(t-t_1)}{\theta}}\right)$$

The concentration will reach $C = 0$ when $t - t_1 = T$.

APPENDIX 2: LITERATURE REVIEWS FOR THE TARGET SPECIES

Styela clava

Reproduction

Reproductive strategy

S. clava is a hermaphroditic solitary ascidian that develops separate male and female gonads (Parker et al. 1999). Self-sterility has been suggested for *S. clava* since gonad maturation is asynchronous (in: Clarke & Therriault 2006, Kott 1985, Wallace 1961; described as S. mammiculata). Gametogenesis is also asynchronous within populations, with several stages of gonad maturation present at any one time in different individuals (Bourque et al. 2007, Parker et al. 1999). Gametes are broadcast into the water column and fertilisation of eggs is external (Svane & Young 1989).

Fecundity

S. clava individuals are reproductively mature at 10 - 12 months of age (Davis et al. 2007, Lützen 1999). Mature females produce approximately 5,000 eggs per spawning season (Davis et al. 2007). Bourque et al. (2007) suggest that *S. clava* spawns continuously, redeveloping their ovaries during the reproductive period. A "Spawning with Replacement" model developed by Nutsford (2010)indicates that reproductive female *S. clava* produce on average 77,230 eggs per spawning season, with the vast majority of reproductive output taking place from January to March.

Seasonality and reproductive period

Studies of the reproductive cycle of non-indigenous populations of *S. clava* indicate a seasonal pattern, with gametogenesis generally beginning in spring, peaking in summer and continuing through to autumn, although some geographic variation in timing and duration is evident. Reported reproductive periods range from 4 to 10 months in different locations.

Histological analysis of the gonad maturation stages of *S. clava* in Cork Harbour (Ireland) detected early gametogenesis in mid-February, continuing through to November 1997, with a peak between August and October. Spawning occurred during September and October and gonad regression was observed in November and December (Parker et al. 1999). In comparison, ripe ovaries were not detected for *S. clava* at Prince Edward Island (Canada) until late June and were present until late October in 2004, with maximum recruitment of larvae in late September (Bourque et al. 2007). The reproductive cycles of populations in Southampton (United Kingdom) and the Limfjord (Denmark) follow a similar pattern; ripe gametes are detected from spring, spawning and settlement peak in late summer / early autumn and continue through to mid-autumn (Holmes 1969, Lutzen & Sorensen 1993). Spawning in Newport Bay (California) occurs from June through September (Kelly 1974). In New England (north-eastern USA), larvae were detected between June and November with peaks between July and October (Osman & Whitlach 1999).

Research conducted by NIWA and the University of Canterbury in 2008 - 2010 indicates that spawning of *S. clava* may extend from November to April, with peak reproductive output in December to February (Willis pers. comm. 2010; Nutsford 2010).

McClary et al. (2008) reported continuous gametogenesis throughout the year for a population of *S. clava* in Bayswater Marina (Waitemata Harbour) and suggested that since the minimum sea surface temperature was 9.9°C at this location, temperature may not be a limiting factor

for reproduction. Replication of histological analyses was comparatively low for this study with gonad index evaluations conducted for only five individuals every two weeks during the assumed reproductive period.

Seawater temperature may contribute to gametogenic control in some ascidian species and it appears to play a role in the timing of gonad maturation for *S. clava*. The water temperature threshold for early gonad development is reported to be as low as 8°C. Spawning is thought to occur at temperatures above 15°C (Bourque et al. 2007, Holmes 1968 in: Davis et al. 2007, Parker et al. 1999). Distribution studies in European waters indicate that no populations exist at locations where water temperatures do not exceed 16°C during summer (Davis & Davis 2007). Peak spawning in populations at Cork Harbour occurs in late summer / early autumn, around when water temperatures reach approximately 18°C (Parker et al. 1999). The first major spawning event of the season for the study population at Bayswater Marina (Waitemata Harbour) coincided with average sea surface temperatures reaching 15°C after winter in late-August to early September (McClary et al. 2008). The average monthly sea surface temperatures at the heads of Lyttelton harbour during the observed reproductive period of *S. clava* in the port were 18.3°C, 18°C and 16.7°C (January, February, March) in 2009 and 17.1°C, 18.7°C and 17.1°C in 2010.

Light appears to be a factor that is correlated with spawning in ascidians, although there is variation among species in the time of day that the event occurs and the latency period (duration of light prior to a spawning event), along with geographic variations in timing (Bourque et al. 2007). The latency period of a related solitary ascidian, *S. plicata*, decreases as the period of darkness before spawning increases (West & Lambert 1976). A laboratory-based evaluation of latency period for *S. clava* demonstrated that spawning did not occur in the absence of a dark period, but that the average latency period for individuals that did spawn remained consistently 12 hours regardless of dark period duration manipulations (Bourque et al. 2007).

Duration of planktonic phase

Free-swimming tadpole larvae hatch from eggs 12 to 15 hours after fertilisation at temperatures between 16 and 20°C (Bourque et al. 2007, Davis et al. 2007, Davis, 1997). Larvae are active in the water column for between 12h (Davis 1997, Minchin et al. 2006) and 24h (Svane 1984) before settling to hard substrata. Laboratory studies have demonstrated the potential for larvae to survive in the plankton for up to 3 days (Kashenko 1996) and the ability to postpone metamorphosis for several days if suitable substrata are not available (Svane 1984). The factors that influence larval duration are not well understood in *S. clava*, but it is thought that habitat selection, presence of conspecifics and light conditions may play a role (Bourque et al. 2005).

At Prince Edward Island, populations of *S. clava* have attained “extremely high abundances” in some regions, especially on mussel aquaculture facilities. The seasonal pattern of larval concentrations has been documented within the Murray River estuary, in the vicinity of many *S. clava*-infested mussel farms (Bourque et al. 2007). Three replicate 180 L water samples were collected weekly from June to November 2003 using a bilge pump raised and lowered within the first 2 m of the water column. Sampling took place early in the afternoon, which was demonstrated to be the optimum time of day to collect *S. clava* larvae. Larvae were first detected in July and were present in samples until late October. Two peaks of larval abundance were observed, with maximum concentrations of 0.24 and 0.56 larvae L^{-1} recorded during late July and early August, respectively. Recruitment of larvae was also monitored for this population (described below).

Attempts to obtain recruits or collect *S. clava* larvae from plankton samples in New Zealand have not been successful. McClary et al. (2008) attempted to investigate the timing of larval release and recruitment of *S. clava* in the Waitemata harbour. Plankton samples were collected weekly during the expected peak reproductive season (mid October 2006 to late April 2007) at sites adjacent to adult populations of *S. clava* within Bayswater Marina. Three replicate samples of 150-200 L were collected via vertical tows of a zooplankton net during the early afternoon. Over the entire 7-month sampling period only 10 ascidian larvae were collected in samples, none of which were identified as *S. clava*. The relatively low adult population density at the study site and the short planktonic phase of *S. clava* were suggested as possible explanations for the lack of success in obtaining larvae.

Monthly sampling for larvae in Lyttelton Port was conducted by Nutsford (2010) between February 2009 and February 2010. Each month, six samples were taken during which approximately 5120 L of water were sampled between 0.5 and 1.5 m below the surface. Only very low concentrations of larvae were encountered, with maxima of 0.098 and 0.13 larvae m^{-3} for 2009 and 2010, respectively. These concentrations are 1840 – 5700 times lower than those reported form Canada (described above).

Settlement and recruitment

Larval behaviour and settlement preferences

S. clava larvae are lecithotrophic (non-feeding) with tadpole-like morphology and the ability to propel themselves over short distances (a few mm to a few cm) during brief bursts of sustained swimming activity (Davis & Davis 2007). Consequently, dispersal is relatively short-ranged and larvae often congregate close to the parent population. It has been suggested that most of the larvae released from an individual settle within a short distance (≤ 10 m) of the parent (Stoner 1990). Transport via water currents is limited by the short duration of the pelagic phase (Stachowicz et al. 2002), but larvae may be dispersed within an area encompassed by two tidal excursions (Minchin et al. 2006).

Ascidian larvae use diffuse light as the primary cue for orientation and settlement (Darbyson et al. 2009). *S. clava* larvae are negatively buoyant, but exhibit negative geotaxis (upward movement) and positive phototaxis (movement toward light) (Davis & Davis 2007). Some ascidian larvae switch from positive to negative phototaxis before settling, accumulating in regions of low light intensity, and this pattern has also been observed in *S. clava*, which may settle on both vertical or the undersides of horizontal surfaces (Bourque et al. 2007, Darbyson et al. 2009). In a study that investigated settlement of *S. clava* larvae to a range of test panels, recruitment was significantly higher on surfaces coated with black paint than on those coated with white paint, and highest on unpainted metal substrates (Darbyson et al. 2009).

Diel variations in the concentration of *S. clava* larvae in the Murray River estuary (Prince Edward Island) were also observed by Bourque et al. (2007, 2005). The number of larvae in water samples peaked around noon then steadily declined to pre-peak concentrations. This pattern could reflect vertical migration of larvae, a common behaviour among zooplankton, which has implications for sampling larval concentrations. Actual larval abundances may be similar throughout the day, but sampling methodologies may be misleading if larvae have migrated outside the range of the sample collections. Further field tests at different depths (0, 2 and 4 m) demonstrated that larval abundance increased with depth (Bourque et al. 2007).

Although adult *S. clava* are relatively robust to fluctuations in salinity and temperature (NIMPIS 2001), larval survival may be negatively affected in low salinities (< 20 psu). Larvae exhibit substrate selectivity, with a preference for surfaces with bacterial films

documented at a salinity range of 24 - 34 psu (Kashenko 1996). *S. clava* is considered a secondary fouling species, preferentially settling on surfaces already encrusted with other organisms (Lützen 1999). However, successful recruitment has been observed on settlement panels that were preconditioned only to a stage where biofilm covered the surfaces and deployed for only one week (Bourque et al. 2007).

Settlement to artificial surfaces

Spatial distribution patterns of *S. clava* indicate a preference for sheltered, low energy habitats, such as inlets, bays, harbours and marinas. Population densities are often much higher on artificial surfaces than on natural substrates. For example, populations growing on natural substrates (e.g. rocks or oyster beds) may reach densities of 50 - 100 m^{-2}, whereas 500 - 1000 *S. clava* m^{-2} have been recorded from artificial substrates like docks, sluice walls or settlement panels (Lützen 1999 and references therein). In ports and marinas, *S. clava* populations are commonly observed growing near the water surface and on floating substrata including pontoons, rafts, buoys and fenders (Davis et al. 2007). It is also frequently found fouling wharf piles, vessel hulls, aquaculture equipment and other artificial substrata (Gust et al. 2008).

In Lyttelton Port, the abundance and distribution of *S. clava* was surveyed by diver and above-water searches, which detected a widely dispersed, low density (1 - 10 m^{-2}) population established on piles, pontoons and ropes, but absent from rock breakwalls (Gust et al. 2008). Higher densities (11 - 100 m^{-2}) were observed on pontoons within the port and on the hull of a vessel at nearby Magazine Bay Marina. *S. clava* was approximately three times more abundant in the shallower depth strata searched by divers (< 5 m) than at greater depths (5 to 13 m).

Settlement of *S. clava* larvae has been quantified in several studies using experimental settlement surfaces, usually PVC panels suspended in the water column. Bourque et al. (2007) used vertically-orientated settlement plates to investigate seasonal patterns of *S. clava* recruitment, in conjunction with water sampling for larvae, in the Murray River at Prince Edward Island. The 10 x 10 cm plates were pre-conditioned to provide a biofilm-covered surface, then deployed at a depth of ~2 m for 1 week. Sampling occurred weekly from June to November 2003. The density of recruits on the settlement plates increased gradually from late June to an initial peak in mid-August (0.68 individuals cm^{-2}), followed by a decline to 0.22 individuals cm^{-2} in early September, and a second, more intense peak in late September (0.95 individuals cm^{-2}).

In a study that investigated the process of invasiveness of exotic tunicates at Prince Edward Island, Ramsay et al. (2008) quantified recruitment of *S. clava* and *C. intestinalis*. Three (during 2006 study) to five (during 2003 and 2005 studies) vertically-orientated PVC settlement plates were suspended at 2 m depth from June to October. An initially high mean abundance of *S. clava* recruits was documented on plates in the Brudenell estuary during 2003 (351 ± 44.5 individuals; mean ± standard error), followed by declining abundances in 2005 (average of 267 ± 28.4 individuals per collector plate) as *C. intestinalis* populations increased. By 2006, no *S. clava* recruits were detected on any plates. No quantification of *S. clava* adult population abundance or density was provided in this study, although it was classified as an "exotic nuisance species" at the beginning of the study and "in decline" by 2006.

In September 2002, a new incursion of *S. clava* was detected in the March Water area of Malpeque Bay, Prince Edward Island. An extensive dive survey of mussel aquaculture facilities in the region detected fewer than 15 individuals concentrated within a small area (100 m diameter) of the bay. Settlement collectors (dark grey PVC plates orientated

vertically), deployed annually at various distances from the "epicentre" from mid-June to early November, documented an initial mean annual recruitment per collector of 0.4 ± 0.2 (mean ± standard error) individuals in 2003. The density increased significantly over the following three years of surveys, to 3.4 ± 1.0, 77.7 ± 10.7 and 370.8 ± 42.9 individuals per collector in 2004, 2005 and 2006, respectively. Information about local adult population abundance or density during this period is not detailed in this study. Recruitment was not significantly different between collectors located at the epicentre or at 0.5 km, 1.0 km and 2 km distances, but was significantly higher within mussel farms compared to sites adjacent to the facility (198.9 ± 30.5 vs. 65.2 ± 14.4 individuals per collector, respectively). This was attributed to more habitat and propagule pressure available within farms, providing a larger inoculant pool and increased chance of gamete encounter and successful fertilisation. Collectors were deployed 1.0, 1.5 and 2m below the water surface, but recruitment was not significantly different between these depths (Arsenault et al. 2009).

Recruitment of *S. clava* larvae to a variety of test panels representing different vessel hull materials was investigated in an assessment of the potential for spread by boating activities at Prince Edward Island (Darbyson et al. 2009). Bare aluminium panels and fibreglass or wooden panels with different paint treatments (coated with black or white antifouling paint or untreated exterior paint) were deployed vertically from near the waters' surface to 3 m depth in June 2003. Four replicates of each treatment were collected and examined after 1, 2, 4, 8 and 12 weeks. No settlement was detected during weeks 1 and 2 and only low numbers had recruited by week 4. Panels collected during weeks 8 and 12 revealed highest recruitment on bare aluminium panels (~400 individuals.100 cm^{-2}), moderate levels (~250 - 280 individuals $100cm^{-2}$) on wooden and fibreglass panels coated with black exterior paint, with minimal recruitment to antifouling-coated surfaces. Recruitment to surfaces coated with black paint was significantly higher than to those coated with white paint, which may reflect larval behaviour and use of diffuse light for orientation and selection of settlement surfaces.

Immersion of colourless vertically-orientated PVC settlement plates in Bayswater Marina (Waitemata Harbour) failed to detect any *S. clava* recruits (McClary et al. 2008). However, the low level of replication (three plates at three sites) and limited sampling period (two weeks only) may not have been sufficient to detect settlement for this population.

Factors influencing density of recruits

The potential relationships between adult population density or abundance, and larval concentrations, fertilisation success and recruitment have not been specifically investigated for *S. clava*. However, some cautious links may be suggested using the patterns of larval concentrations and recruitment described by Bourque et al (2007) for the Murray River estuary on Prince Edward Island, where *S. clava* adult population abundance was not formally quantified but was described as "extremely high". In other highly invaded locations where population density was quantified, maximum densities attained 500 to 1000 individuals m^{-2} (Lützen 1999).

Bourque et al. (2007) found recruitment of *S. clava* to be closely related to the larval concentrations detected in the water column, with the exception of a second peak in recruitment in September which may have been due to misidentification of small recruits of another ascidian, *Mogula* sp., since *S. clava* larvae were not abundant in the water column at this time. *S. clava* larvae were first detected in late June, gradually increasing in concentration to 0.24 larvae L^{-1} in early August, followed by a significant decline and then an increase to a second, larger peak of 0.56 larvae L^{-1} in mid-August. Recruits were first detected at low densities (< 0.05 cm^{-2}) on plates in late June, increasing to an initial peak (0.68 individuals

cm^{-2}) in mid-August and a second peak (0.95 individuals cm^{-2}) in late September. No recruitment was observed after 21 October.

The far lower larval concentrations reported by Nutsford (2010) from Lyttelton Port compared to those reported from Prince Edward Island, Canada, and are mirrored by the far lower adult densities reported from the two locations.

Ciona intestinalis

Reproduction

Reproductive strategy

C. intestinalis is a simultaneous hermaphrodite that produces both eggs and sperm at the same time and broadcasts gametes into the water column (Niermann-Kerkenberg & Hofmann 1989). Protandric hermaphroditism (production of sperm before eggs) may occur during early stages of maturity (Carver et al. 2006). Fertilisation and embryogenesis are external processes, with eggs either released individually into the water column or retained in mucus strings (Petersen & Svane 1995).

C. intestinalis is generally considered to be self-sterile or self-discriminating due to mechanisms allocated to the egg membrane (Morgan 1940, 1945, Pinto et al. 1995). However, some incidences of low-level self-fertilisation have been reported for populations in the Gulf of Naples, Italy (15 %; Rosati & Santis 1978), the Uranouchi Inlet, Japan (21 %; Kawamura et al. 1987) and Newport harbour, California (< 10 %; Morgan 1938). In comparison, high self-fertility has been demonstrated for a population of the closely related ascidian *C. savignyi* in Santa Barbara (Jiang and Smith 2005). In Lyttelton Port, the *Ciona* sp population is comprised of both *C. intestinalis* and *C. savignyi* (pers. comm. K. Smith, Cawthron 2010). Distinguishing between these species, particularly at the newly settled and juvenile ascidian stages, is very difficult. The specimens that were sampled and from which larvae were reared for this study were *C. intestinalis*.

Fecundity

Given suitable conditions, mature *C. intestinalis* individuals can produce gametes continuously during the reproductive season, ceasing when temperatures become unsuitable (Carver et al. 2006). The rate of gamete release is estimated to be approximately 500 eggs day^{-1} $female^{-1}$ for populations in Nova Scotia, Canada (Carver et al. 2003) and a rate of 1000 eggs day^{-1} $female^{-1}$ is reported for a population in Japan (Yamaguchi 1975). Estimates of total egg production by an individual during its lifespan range from > 10,000 (Petersen & Svane 1995) and ~12 000 (Carver et al. 2003) to 100,000 (Yamaguchi 1975). This variation in reproductive output is likely to be a result of environmental differences, particularly water temperature and light, or the relative life spans of the populations (Carver et al. 2006, Svane & Young 1989).

Seasonality and reproductive period

The timing of gamete release for *C. intestinalis* appears to be linked to water temperature and to light conditions (Berrill 1947, Dybern 1965, Lambert. & Brandt 1967, Svane & Havenhand 1993, Svane & Young 1989, Whittingham 1967). Spawning frequency and duration vary significantly, depending on seasonal water temperature fluctuations and differences at a regional scale. Mature individuals produce and release gametes either during distinct periods or continually throughout the year, with varying intensity. Dybern's (1965) summary of reported reproductive seasonality for *C. intestinalis* in Europe indicates that breeding occurs throughout or for most of the year in the Mediterranean Sea and is increasingly restricted to summer months in the northern region of its range. Year-round gamete-release is also

observed for populations in the relatively warm waters of Japan (Yamaguchi 1975), whilst single or multiple peaks of reproductive activity occur in spring and summer in cooler waters (e.g. Sweden, Norway, Canada).

The lower temperature threshold for normal embryonic development of *C. intestinalis* appears to be 8°C, with spawning occurring at water temperatures of between 8 and 12°C (Dybern 1965). Millar (1971) commented that, whilst water temperature is a major controlling factor for reproductive cycles, season cessation may not be closely linked to a particular critical temperature. Dybern's (1965) observations indicate that spawning ceases in autumn due to gonad changes resulting from falling temperatures rather than a response to a critical temperature. Some studies report no evidence of a clear relationship between temperature profiles and the timing of reproductive events, other than a minimum temperature threshold of 8°C (Howes et al. 2007), whilst others have demonstrated a close link between water temperature and recruitment patterns (Ramsay et al. 2009). In regions where the minimum winter water temperature is relatively high (e.g. >11°C in Auckland, New Zealand), ascidian reproduction may occur year-round despite large seasonal temperatures fluctuations (Millar 1971, Skerman 1959).

In cooler waters, large temperature ranges result in more distinct spawning periods (Dybern 1965). Recent research conducted in Lyttelton Port suggests that reproduction of *C. intestinalis* occurs from early spring (late August) to late autumn (May), with peak larval abundance and settlement in October-March (NIWA unpubl data 2009 - 10). Very low levels of *C. intestinalis* recruitment were also detected in June/July but the small size of the recruits encountered made species-level identifications unreliable. During the Lyttelton experiments, water temperatures in July - August dropped to ~ 7°C.

Histological assessments of *C. intestinalis* populations in Nova Scotia, Canada, indicated that gametes develop during spring (March - April) when seawater temperatures increased to > 3°C, continuing through to mid-May and into June (Carver et al. 2003). Spawning occurred from mid-May (mid-spring) to August (mid-summer) at water temperatures of > 8°C. The timing, pattern and amount of reproductive output were variable between two study populations, with offset spawning events (mid-May through June vs. mid-July to mid-August) and two recruitment peaks observed at one site, but only one at the other. A two-season study of a nearby population detected two distinct periods of recruitment (June to late July and early September to mid-November) that did not correlate closely with changes in water temperature (Howes et al. 2007).

At Prince Edward Island, Canada, recruitment was detected when water temperatures exceeded 8°C, increasing in intensity gradually to a single peak as temperature increased, and ceasing as temperatures dropped below 8°C again (Ramsay et al. 2009). Other observations of populations in this region indicate that individuals that settle during May-June are capable of producing and spawning eggs in August of the same year (Carver et al. 2003), which is consistent with Dybern's (1965) observations of two co-occurring breeding generations for populations living close to the water surface in fjords in Sweden. A single spawning period during late spring to summer is reported for *C. intestinalis* populations on the west coast of Ireland, again with a minimum threshold temperature of 8°C (Costello et al. 1986). In San Francisco Bay, a study of the effect of *C. intestinalis* on species richness detected a main recruitment peak during August followed by a smaller peak in October (Blum et al. 2007).

Spawning appears to be synchronous within a population, timed such that settlement occurs at midday. Several studies have demonstrated that in temperate waters, spawning and hatching occur at or just before sunrise (Berrill 1947, Castle 1896, Conklin 1905), and suggest that

settlement is likely to occur during daylight hours when conditions are optimal for site selection and metamorphosis (references in: Bullard et al. 2004, Svane & Havenhand 1993). Other laboratory studies of *C. intestinalis* spawning at locations where water temperatures are warmer (e.g. Japan) report that eggs and sperm are released during the hours of darkness and that larvae hatch within 12 hours and settle by midday (Yamaguchi 1970 in: Svane & Havenhand 1993). Svane & Havenhand (1993) observed spawning throughout the 24 hour cycle. In a study that specifically investigated spawning response to light, 66.8 % of tested *C. intestinalis* adults released gametes in response to a one-hour dark-adaption period followed by light exposure, after an average of 27.3 minutes (Lambert & Brandt 1967), however Whittingham (1967) found that gametes were shed 4.07 minutes (± 2.6) after exposure to light.

Variation in population dynamics is reported for *C. intestinalis*, including irregular and intense recruitment events and erratic population explosions that are apparently not triggered or related to changes in environmental conditions (Cayer et al. 1999 in: Carver et al 2006, Keough 1983). Incidences of rapid decline after reaching "pest-level densities" have been reported for mussel farms in New Zealand and South Africa, and for port habitats in Australia; (see references in: Carver et al. 2006). Recruitment intensity fluctuated widely from year to year for *C. intestinalis* during a long-term (12 year) study of solitary ascidian communities in Sweden (Svane 1983).

Duration of planktonic phase

Sperm and eggs released into the water column by *C. intestinalis* generally remain viable for fertilisation for 1 to 2 days. Sperm, in the absence of eggs, may remain viable for up to 16 hours after release, but duration is reduced to 1.5 hours when eggs are present (Bolton & Havenhand 1996). Eggs are viable for fertilisation for up to 30 hours after release (Carver et al. 2006).

Water temperature influences the duration of both embryogenesis (the period between fertilisation and larval hatching) and the pelagic phase of larvae. Estimates of the duration of embryogenesis for populations of *C. intestinalis* in the Northern Atlantic range from 18 to 63 hours at various water temperatures, whilst a study of embryogenesis for populations in Japan reported a shorter duration of 12 hours (Carver et al. 2006). Laboratory trials observed larval hatching after 25 hours at 16°C water temperature (Berrill 1935) and 27 hours at 15°C, continuing for 5 to 6 hours (Havenhand & Svane 1991).

A range of estimates of larval phase duration are reported in the literature (Table A1). Nakayama et al. (2005) report that newly hatched *C. intestinalis* larvae attain competence for metamorphosis after 4 to 5 hours, a process that is initiated soon after settling and adhering to suitable substrate. Berrill (1935) reported a free-swimming period of 6 to 36 hours, noting that the duration is usually greater than 12 hours. Larval settlement under laboratory conditions can occur within 24 hours after hatching, although some larvae have been observed swimming actively after 5 days (Havenhand & Svane 1991).

Table A1: Summary of information presented in the review by Carver et al. (2006)

Duration of larval phase (hours or days)	Water temp	Location	Reference
6 – 36 h	-	Britain	Millar 1952
24 – 36 h	18-20°C	Scandinavia	Dybern 1965
0 – 6 days*		Laboratory trials, Sweden	Svane and Young 1989
4 – 5 days	10-12°C	Scandinavia	Dybern 1965
2 – 10 days	-	Britain (same region as Millar 1952 study)	Jackson 2005

Svane and Havenhand (1993) estimated that, for populations in the Gullmarsfjorden area of Sweden, the maximum dispersal time for the planktonic stages of *C. intestinalis* is the sum of (1) the time to egg fertilisation (0-30 hours), (2) the larval development period (24 hours) and (3) the free-swimming larval phase for eggs that were not released within mucus strings (0-6 days). The minimum dispersal period is therefore 24 hours, the period of larval development. The maximum could be 8 days, although the 6 day free-swimming phase estimate is a maximum value based on laboratory observations and settlement may occur much sooner (within minutes or hours). Dispersal of larvae that develop from eggs spawned in mucus strings is either prevented altogether (if larvae are retained) or reduced to the duration of the free-swimming phase (for larvae that emerge from the mucus).

Settlement and recruitment

Larval behaviour and settlement preferences

C. intestinalis eggs that are released individually (i.e. not enveloped in mucus) are negatively buoyant and sink in the absence of strong water currents. The egg surface is adhesive and sticks to substrata. Mucus strands containing eggs and/or larvae often become entangled with or adhere to nearby adult *C. intestinalis* and other substrates (Svane & Havenhand 1993).

After hatching, the non-feeding tadpole larvae either swim freely in the water column or are retained in the mucus strands until settlement. Laboratory observations indicate that 60 % of artificially fertilised *C. intestinalis* eggs develop and hatch normally (Havenhand & Svane 1991), and that the proportion of larvae that exit the mucus and are dispersed may be between 40 and 60 % (Petersen & Svane 1995).

Free-swimming larvae are reported to initially exhibit positive phototropism and negative geotropism, swimming upwards (Millar 1971). It is suggested that this behaviour may promote dispersal. Dybern (1963) also reported that *C. intestinalis* larvae pass through both photopositive and photonegative phases. However, Svane and Young (1989) comment that careful behavioural experimentation is required to support these field observations and conclusions based on distribution patterns. Other experiment-based research has demonstrated that larvae display photonegativity throughout the free-swimming phase (Svane 1987 unpublished data in: Svane & Young 1989).

Observations of swimming by newly hatched larvae report an average speed of 14 mm s^{-1} (Nakagawa et al. 1999 in: Carver et al. 2006), although estimates based on tail size suggest that a speed of 4 mm s^{-1} could be achieved (Berrill 1931).

As larvae develop and approach the end of the planktonic phase, a strong photonegative response is demonstrated as they swim or sink downwards towards dark-coloured or shadowed habitats where water movements are reduced (Carver et al. 2006). Shade appears to

be an important selection criterion for *C. intestinalis* larval settlement, with 96 % of larvae selecting shaded over non-shaded habitats in a study by Tursi (1980). This research also demonstrated that surface orientation may be an important factor, with 61 % of larvae selecting obliquely-oriented surfaces compared to results for horizontal and vertical surfaces (28 % and 11 %, respectively).

Negative phototactic behaviour by larvae may explain cryptic distribution patterns of *C. intestinalis* observed in Sweden, based on field studies of recruitment and laboratory experiments (Dybern 1963). It is possible that this pattern may be enhanced by post-settlement mortality on upwards-facing surfaces (Svane & Young 1989), but the hypothesis that distribution patterns are a result of larval behaviours is supported by reports of settlement and survival on such surfaces in deep water / low light environments.

Populations of *C. intestinalis* tend to be aggregated and dispersal is considered to be limited, particularly in sheltered locations such as fjords and inlets (Petersen & Svane 1995). An estimated dispersal distance of 100 - 1000 m is reported by Jackson (2000); however water currents, larval duration and swimming speed will all influence the pattern and maximum distance of dispersal. Larvae do not display gregarious behaviours or use chemical cues to detect and select settlement sites based on the presence of adults (Havenhand & Svane 1991). Localised settlement patterns are more likely to be a result of the hydrodynamic characteristics of the location and retention due to the sticky mucus strings and eggs. Since light has been demonstrated to affect settlement, larval behaviour and preferences may also contribute to aggregated settlement patterns (Svane & Havenhand 1993).

Settlement may be influenced by the presence of a biofilm layer on substrata, however, the type and age of the bacterial community can affect larval response (Keough & Raimondi 1995, Wieczorek & Todd 1997). Szewzyk et al. (1991) did not detect a settlement preference for biofilm-coated vs. clean (hydrophobic or hydrophilic) surfaces, commenting that settlement patterns may be a result of both active habitat selection and passive deposition or entrapment of larvae. Wieczorek and Todd (1997) observed that as biofilm age on surfaces increased, the number of settled larvae increased, with maximum densities on surfaces coated with 12 day old biofilm.

As described above, *C. intestinalis* appears to recruit preferentially in habitats with reduced exposure to currents and wave action, with highest recruitment recorded at the most sheltered study site (Howes et al. 2007). In a study of the effects of caging on epifaunal communities, Schmidt and Warner (1984) observed that *C. intestinalis* larvae exhibited strong preferences for caged structures that provided habitats with reduced light intensity and water movement.

Settlement to artificial surfaces

Settlement and distribution patterns indicate a preference for hard substrates (e.g. rocks, shells) over soft sediments, but *C. intestinalis* may also be found growing on algae, seagrass or on other organisms within fouling assemblages. Non-indigenous populations of *C. intestinalis* mainly colonise artificial substrates within harbours (Carver et al. 2006). A common biofouling species, it is frequently observed growing on wharf pilings, floating pontoons (Cohen 2000, Lambert & Lambert 1998, 2003) and aquaculture equipment (Mazouni et al. 2001). Ropes, chains, buoys and vessel hulls are also colonised by this species (Holland 2002, Kott 1990). It is a particularly effective coloniser of cleared or newly introduced surfaces, which may explain proliferations at aquaculture facilities. In a newly constructed harbour at Lezardrieux (France), *C. intestinalis* rapidly recruited to floating metal tanks, attaining densities of up to 2000 individuals m^{-2} (Carver et al. 2006).

PVC panels of were used by Blum et al. (2007) to collect newly settled *C. intestinalis* in San Francisco Bay. The panels were of similar construction to those of the settlement arrays evaluated by this project (grey PVC, sanded settlement surface), although all 5 replicates were mounted in a face-down orientation and suspended at 1 m water depth for 1-month periods. During peak settlement in August, variable densities of *C. intestinalis* recruits were detected (between ~70 and > 500 per 188 cm^2 panel).

Howes et al. (2007) used stacks of Petri dishes to monitor recruitment of *C. intestinalis* near aquaculture facilities at Indian Point, Nova Scotia. Specially designed shelves held 16 pairs of Petri dishes, 1 facing upwards and 1 facing downwards. The collectors were deployed at 4.5 m water depth for the duration of the season, at sites ranging from sheltered to wave-exposed. Each week, 3 sets of dishes were removed from designated shelf-positions for analysis and replaced by fresh dishes that were pre-conditioned to cultivate a biofilm. This settlement array design successfully detected *C. intestinalis* recruitment at this location, which was the site of a population explosion during the years prior to the experiment. Significant recruitment was only observed on the downwards-facing dishes. Two recruitment events were detected (which did not track with changes to temperature profiles) and fewer larvae were observed settling at exposed sites compared to sheltered sites.

Three replicate ABS (similar to PVC) plastic plates (10 x 10 cm) were also deployed by Howes et al. (2007) on lines at a range of depths (0.5, 4.5, 8.5, 12.5 and 16.5 m) to look for patterns in recruitment depth. At the end of the reproductive season the arrays were retrieved and analysed, revealing that most recruitment occurs at 4.5 m, some occurs at 8.5 m and very little occurs at the surface or below 8.5 m.

To investigate the effects of caging on epifaunal communities, Schmidt and Warner (1984) deployed black perspex panels (0.25 m^2 x 5mm thick) in Langstone harbour, Hampshire, UK. The panels were attached vertically to steel frames suspended from rafts at a depth of 1 m. Some panels were caged (screened with 12.5 x 12.5 mm galvanized mesh) with various cut-outs to manipulate water flow. The effect of caging on the abundance of *C. intestinalis* was significant. Low cover values were recorded for un-caged controls throughout the experiment, but there was high panel occupation of open- and closed-caged surfaces (at least 50 % after 5.5 months) and surface monopolisation on closed-caged surfaces after 8 months due to growth of individuals. The authors concluded that subsequent dominance by *C. intestinalis* was a result of the impact of caging per se on larval behaviour, which showed a strong preference for caged structures with reduced light intensity and water movement.

Asbestos and cement roofing slates (30 x 30 x 0.5 cm) were used by Costello et al. (1986) to investigate settlement and colonisation of epifaunal assemblages at Bird Rock on the west coast of Ireland. The tiles were oriented vertically and horizontally around a large metal frame and deployed at 2 sampling depths (15 m and 25 m) for a period of 14 months, with one to three panels sampled at regular intervals. Percentage covers of species on both sides of the tiles were analysed, indicating dominance by ascidian species on all surfaces except the upwards-facing horizontal tiles. Downwards-facing horizontal tiles were particularly heavily colonised. *C. intestinalis* was the most prolific ascidian species at both study depths, settling during a single period between April and July. Settlement intensity peaked between May and June when densities of between 500 and 1000 individuals 0.25 m^{-2} were recorded on both cleared and undisturbed tiles.

Factors influencing density of recruits

Studies that specifically investigate relationships between adult population density and larval abundance were not encountered in the literature reviewed.

One account reported “relatively low concentrations of *C. intestinalis* larvae in plankton samples collected at locations of high adult population abundance and density”. At Gullmarsfjorden and Strömmarna on the Swedish west coast, the populations studied by Dybern (1965) and later by Svane and Havenhand (1993) are extremely abundant and as dense as several thousand m^{-2}. Despite this, plankton sampling during the breeding season detected surprisingly few larvae and eggs in the adjacent water column. This observation is supported by reports by Thorson (1946) and Öhlund (1977). Localised retention of propagules due to the sticky mucus strings and egg surfaces, or the negative buoyancy observed for larvae early in the planktonic phase, may explain these observations of low concentrations in the water column. Net tows within 3 – 4 m of dense adult populations collected between zero and 139 viable larvae per tow (sample volume not reported) along with eggs at various stages of development. Specialised plankton nets mounted on the rock wall habitat at 2 – 3 m depth collected between 2 and 12 viable larvae in 4 replicate nets, including 9 - 47 pre-hatch stage larvae, and 9 - 61 newly metamorphosed individuals. Diver observations of mucus strings in the field detected retained eggs of various developmental stages, along with 5 and 18 retained larvae in two of the four replicates (Svane & Havenhand 1993).

Undaria pinnatifida

Reproduction

Reproductive strategy

The life cycle of *U. pinnatifida* is heteromorphic, incorporating a microscopic, haploid gametophyte generation, and a diploid sporophyte generation that develops into the macroalga. As *U. pinnatifida* individuals (sporophytes) become reproductively mature, two fluted sporophylls develop along the stipe above the holdfast. Millions of motile zoospores are released into the water column from these structures. The spores swim actively for several hours before settling onto suitable substrata. Following settlement, zoospores germinate to form microscopic male and female gametophytes that, given suitable environmental conditions, mature to produce reproductive propagules. Swimming spermatozoa are released from the male gametophytes, fertilising eggs that remain attached to female gametophytes. Sporelings develop from the fertilised eggs in situ, giving rise to juvenile sporophytes (Floc'h et al. 1991, Parsons 1994).

Fecundity

The fecundity of *U. pinnatifida*, in terms of zoospore production, is reported to be millions of spores per gram of sporophyll tissue, with between 10 million and 100 million released per sporophyte during the reproductive season (NIMPIS 2011).

Schaffelke et al. (2005) calculated a maximum zoospore release rate of 62×10^3 zoospores cm^{-2} sporophyll tissue $hour^{-1}$, corresponding to ~4.3×10^8 zoospores per sporophyte $hour^{-1}$ from *U. pinnatifida* at Tinderbox Marine Reserve, Tasmania. The majority of spores were released from the largest size classes of sporophytes (> 55 cm in length) during spring to late summer (November – February, the latter months of the growth season), although spore release was also recorded during winter and early spring (July – October) and from smaller size classes. Sporophyte maturation took 40 days, followed by a zoospore release period of 3 months. The reproductive output of this study assemblage was estimated to be 2×10^9 zoospores m^{-2} $hour^{-1}$ during the month of January.

A maximum spore release rate of 12.1×10^5 zoospores cm^{-2} sporophyll tissue $hour^{-1}$ is reported for *U. pinnatifida* in Port Philip Bay, Melbourne (Primo et al. 2010), which is 20 times greater than the rate recorded for *U. pinnatifida* in Tasmania. Spore release competency ranged from 2 to 3×10^5 zoospores cm^{-2} sporophyll tissue $hour^{-1}$ throughout most of the growth season, exceeding the rates reported for Tasmanian populations by 3 to 5 times. These two populations have unique origins and different morphologies, so the authors were not surprised to detect differing reproductive phenologies. The morphology of *U. pinnatifida* Port Philip Bay is similar to that of populations in Wellington Harbour (Hay & Luckens 1987).

Propagule release rates ranging from 1×10^8 zoospores to 7×10^8 zoospores per sporophyll (release time unknown; Brown 1999) have been recorded from introduced *U. pinnatifida* populations in New Zealand. These maximum rates were recorded during a 2-month period (August and September), and a lower rate of $< 2 \times 10^8$ observed during the rest of the study period. A demographics study of *U. pinnatifida* populations along the eastern coast of the South Island of New Zealand estimated the potential lifetime reproductive output of an individual plant to be $10^8 – 10^9$ spores, depending on sporophyll size (Thompson 2004).

Seasonality and reproductive period

Strong seasonal developmental patterns are exhibited by *U. pinnatifida* populations within its native range (i.e. temperate Japan, Korea, China and the south-eastern coast of Russia). Microscopic sporophytes are first detected during winter, develop and mature with spring conditions and begin to deteriorate and senesce as water temperatures increase in summer (Thornber et al. 2004). Transition between life history stages corresponds to changes in environmental variables including seawater temperature, light intensity and day length, with temperature regarded as the most important factor influencing growth and reproduction of gametophytes (in: Choi et al. 2005, Morita et al. 2003).

U. pinnatifida sporophyte growth has been reported at a range of water temperatures in different locations (see review by Floc'h et al. 1991), generally occurring between 5 and 20°C. The optimum temperature for zoospore maturity and release is reported by Saito (1975) to be 17 - 22°C in Japan, triggered when the 10-day average for water temperature exceeded 14°C. Published accounts from populations within Asian waters range from 6 to 23°C (see review by Floc'h et al. 1991). Zoospore germination to form gametophytes occurs at temperatures between 13 and 24°C (Saito, Y 1956).

Gametophyte growth and gamete release occurs at temperatures between 8 and 28°C (Campbell & Burridge 1998), with optimal growth at 17 - 20°C (Tamura 1970). At water temperatures of 24 - 30°C gametophytes enter an encysted phase, suspending growth until late summer or early autumn when temperatures cool to ~ 20°C (Saito, Y. 1975, Thornber et al. 2004). Low irradiance has been experimentally demonstrated to retard the performance of gametophytes by Choi et al. (2005), who suggest that day length determines the level of growth, reproduction and sporophyte production. Optimum reproductive performance was observed at a day length of 12 hours.

Introduced *U. pinnatifida* populations, established at locations where the ranges and patterns of environmental variables differ from those of native habitats, may not show such clearly defined seasonality. In New Zealand, sporophyte senescence is observed during summer, but at some locations sporophytes capable of zoospore release may be present year-round (Brown 1999, Hay & Villouta 1993, Stuart 1997), giving rise to several recruitment peaks per year and overlapping sporophyte generations (Thompson 2004). This may be attributed to the relatively small range of annual seawater temperatures in New Zealand, in comparison to the extreme variations within the native range, or the persistence of cool water temperatures of ~15°C (Thornber et al. 2004). Stuart (1997) has suggested that, in New Zealand, *U. pinnatifida* is not a true annual plant, but an a seasonal annual. This lack of distinct seasonality has also been observed in other introduced *U. pinnatifida* populations (Castric-Fey et al. 1999, Fletcher & Farrell 1999).

The growth season of *U. pinnatifida* populations in Otago Harbour (New Zealand) and in Port Philip Bay (Melbourne, Australia) begins in April – June, with a peak in abundance during October and November and senescence in February to March (Primo et al. 2010). Sporophytes are present year-round in populations of *U. pinnatifida* in Argentina, but minimum and maximum abundances are reported for February and June – August, respectively (Casas et al. 2008). The growth season for *U. pinnatifida* in Tasmania starts later (July) and sporophyte numbers increase rapidly during August, followed by a decline to zero by April (Hewitt et al. 2005, Schaffelke et al. 2005).

In Santa Barbara Harbour (California), two distinct recruitment pulses of *U. pinnatifida* were observed in August to September and January to May (Thornber et al. 2004). The pulses were

strongly correlated with a 4°C drop in water temperature to < 15°C two months prior to recruitment, indicating that the development of gametophytes and sporophytes into recruits is stimulated when temperatures exceed this threshold. Warmer water temperatures (~21°C) were experimentally demonstrated to inhibit microscopic sporophyte development and survivorship.

Sporophylls were dissected and examined to assess the reproductive maturity of different size classes of *U. pinnatifida* growing in Port Philip Bay, Melbourne. Developing zoospores were detected for sporophytes with a diameter of 55 mm and approximate thallus length of 400 mm, which corresponds to an age of approximately 50 days (Campbell & Burridge 1998). Zoospore release was experimentally demonstrated to occur at temperatures between 10 – 20°C, with an output of up to 100,000 zoospores mL^{-1}. Zoospores settled onto glass slides, developed into gametophyte rhizoids and grew at a rate of 11.46 ± 0.19 µm day^{-1}. After 10 days of growth, male and female gametophytes were distinguishable on the glass slides. Fertilisation and development of diploid sporophytes were successful under the experimental conditions (10, 15 and 20°C).

Zoospore release from *U. pinnatifida* sporophytes in New Zealand has been reported at temperatures as low as 9°C in New Zealand (Hay 1990), and was observed at 13°C and 17°C in laboratory cultures for Monterey and Santa Barbara (California), respectively (Thornber et al. 2004).

Duration of planktonic phase

There is variation in the duration of the planktonic phase of zoospores reported in the literature. Hay and Luckens (1987) report that zoospores, released from sporophylls, swim actively for 5 - 6 hours before settling onto suitable substrata. Other sources report that settlement occurs 1 - 6 hours after release, with a maximum pelagic phase of 2 days (NIMPIS 2011). Forrest et al. (2000) conducted laboratory trials and demonstrated a zoospore viability of at least 5 days, with some remaining viable after 14 days. However, the ability to attach to settlement surfaces is elsewhere reported to be lost several hours after release (Suto 1950).

Zoospore germination into microscopic male and female gametophytes occurs within a day (Thompson 2004). Depending on environmental conditions, gametophyte development and maturation may take 7 days (NIMPIS 2011). Release of swimming spermatozoa from mature male gametophytes and fertilisation of eggs that remain attached to female gametophytes is reported to occur over another 7 day period. Thornber et al. (2004) report that zoospore settlement and germination occur 3 days post zoospore release, which gametophyte development occurring approximately 8 days post-release. Choi et al. (2005) observed gametophytes bearing eggs 14 days following zoospore release, and young sporophytes started to develop after an additional 4 days. In California, 4-week old sporophytes were on average 17 cm long, with average weekly growth rates of 1 - 14 cm $week^{-1}$ observed during the first 15 weeks of a sporophyte's life (Thornber et al. 2004).

Settlement and recruitment

Larval behaviour and settlement preferences

Descriptions of *U. pinnatifida* propagule behaviour in natural environments are rare in the scientific literature. In a dispersal experiment conducted in the Marlborough Sounds, Forrest et al. (2000) observed aggregations of sporophytes growing near the waters' surface on experimental settlement surfaces (mussel spat collector ropes), with no settlement detected below a depth of 0.75 m. This pattern was not considered to be due to differential sporophyte development and growth (as available light decreases with depth); rather it was suggested to

be the result of limited zoospore sinking over the distance and time between the source and the settlement ropes. Other explanations included potential entrapment in surface tension since the zoospores were released at the waters' surface or active swimming by the zoospores to move towards or remain at the surface, although the authors considered these options less likely.

The maximum depth of *U. pinnatifida* distribution is reported to be 10 – 15 m, depending on ambient light conditions (Floc'h et al. 1991). The MPI targeted surveillance program, implemented by NIWA at 11 major ports in New Zealand, incorporates diver searches of wharf piles, pontoons and breakwalls. Observations of the distribution of *U. pinnatifida* in these often relatively turbid port environments indicate that growth is most prolific at depths of < 4 m. On mussel farms in France, *U. pinnatifida* was found to be distributed mostly within the first 2 m below surface, with 50 % of plants growing within the first 0.5 m (Floc'h et al. 1991).

Natural dispersal of *U. pinnatifida* zoospores is considered to be short-range, typically not exceeding 10 m (Stuart 2004). Valentine and Johnson (2003) describe an effective "spore shadow", wherein settlement is restricted to within a few meters of the parent plant. Forrest et al. (2000) detected sporophytes growing on settlement ropes no further than 10 m from the artificial spore source and suggested that dispersal distance is primarily limited by increased dilution of zoospores as a function of distance from their source. Dilution decreases the likelihood that an individual will settle close enough to another of the opposite sex to achieve successful fertilisation.

Water currents may transport zoospores to more distant locations, provided current speed does not inhibit settlement. *U. pinnatifida* zoospores have been observed swimming at speeds of 3 – 8 mm s^{-1} under laboratory conditions (Suto 1950). At simulated slow current speeds of 8 cm s^{-1} or less, *U. pinnatifida* zoospores swim against the current and creep over the surface of substrates before settlement (Saito 1975). Faster current speeds of more than 14 cm s^{-1} sweep zoospores away from the substrate.

Settlement to artificial surfaces

In natural systems, *U. pinnatifida* recruits to hard substrates including bedrock, boulders and cobbles, with a preference for sparsely covered surfaces. In New Zealand, *U. pinnatifida* does not usually displace native algal species; however, competitive displacement of native algal species has been demonstrated in Nuevo Gulf, Argentina (Casas et al. 2008). *U. pinnatifida* is a very successful opportunistic alga, rapidly colonising disturbed habitats. It is successful in areas where the density of native algae is reduced to low levels by grazing sea urchins ('urchin barrens') and on other disturbed or unstable surfaces such as sand-scoured reef. Valentine and Johnson (2003) demonstrated that disturbance resulting in removal of algal canopy is required for *U. pinnatifida* to successfully establish in habitats dominated by native algae in eastern Tasmania.

U. pinnatifida establishes very successfully on artificial structures such as piles, ropes, tyres, wood, boulders, cobbles, loose gravel, fishing nets, vessel hulls, wreckage and the vertical sides of pontoons in ports and marinas(Casas et al. 2008, Hay 1990, Hay & Luckens 1987). It can be a significant fouling pest on marine farm equipment and stock, weighing down floating structures and necessitating de-fouling (Inglis et al. 2008a). *U. pinnatifida* recruits to, and can form dense populations on, mussel farm longlines, ropes, nets and even to the mussels themselves (Floc'h et al. 1991). *U. pinnatifida* has been cultured on longlines since the early 1960's in Japan, Korea and China, where it is an important commercial species and food source. Forrest et al. (2000) successfully used mussel spat collector ropes to investigate *U.*

pinnatifida spore dispersal and settlement. In laboratory experiments, *U. pinnatifida* zoospores settle successfully onto glass slides (Campbell & Burridge 1998, Choi et al. 2005, Thornber et al. 2004).

A potential settlement preference for horizontally over vertically oriented surfaces has been described for *U. pinnatifida* and other algal species (Arakawa & Moringa 1994, Reed et al. 1998). However, in port, marina and aquaculture facility environments, horizontal, upward-facing surfaces are rare, resulting in the majority of *U. pinnatifida* plants occurring on wharf pilings, the vertical sides of pontoons and vertically suspended mussel spat settlement ropes (Forrest et al. 2000).

Settlement and recruitment of *U. pinnatifida* has been quantified by field observations of the appearance of sporophytes on large floating docks in Santa Barbara harbour, which were cleared of macroscopic sporophytes (Thornber et al. 2004); on experimental mussel spat collector ropes in the Marlborough Sounds (Forrest et al. 2000); in natural habitats in Tasmania (Schaffelke et al. 2005) and in New Zealand (Russell et al. 2008, Thompson 2004) and has been observed under laboratory conditions (Campbell & Burridge 1998, Thornber et al. 2004), but the use of PVC settlement plates to specifically monitor settlement has not been reported in the current literature.

Zoospore production (fecundity) and sporophyte recruitment in laminarian kelps are known to be very high, yet establishment and survival of recruits to reach visible size is very low (Schaffelke et al. 2005). Chapman (1984, 1986) estimates that the chance of a developing sporophyte reaching visible size is less than 1:500 000.

Factors influencing density of recruits

No information in the relationships between resident population size, propagule concentration in the water and recruitment density is available in the scientific literature.

Sabella spallanzanii

Reproduction

Reproductive strategy

S. spallanzanii is a gonochoric species, with separate male and female individuals producing sperm and eggs in the coelom rather than in discrete testes or ovaries (Currie et al. 2000). Early studies of *S. spallanzanii* reproduction within its native range in the Mediterranean Sea found the minimum size of sexual maturity to be 150 mm body length (crown not included). The sex ratio of specimens collected was 1:1 for larger worms ranging in length from 250 – 300 mm, but of the smaller worms collected (<200 mm) only males were detected. These observations lead to the suggestion of protandric hermaphroditism for *S. spallanzanii*, i.e. individuals develop sequentially male then female gonads (Giangrande & Petraroli 1994). Subsequent studies of introduced populations in Port Phillip Bay, Australia (Currie et al. 2000) and native populations in the Ionian Sea (Giangrande et al. 2000), reported equal representation of males and females across the range of sizes sampled, contesting the suggestion of hermaphroditism and providing evidence for gonochorism. Early studies of *S. spallanzanii* within its native range in the Mediterranean Sea reported a minimum size of sexual maturity of 150 mm body length (crown not included). In introduced Australian populations, sexual maturity was detected at considerable smaller sizes (~50 mm body length). In the *S. spallanzanii* population in Lyttelton port, most females > 150 mm tube length were found to be reproductively mature (Inglis et al. 2009).

Until recently, *S. spallanzanii* was thought to be a broadcast spawning species with external fertilisation in the water column (Currie et al. 2000). A form of 'sperm casting' (sensu Pemberton et al. 2003) has now been suggested for *S. spallanzanii*, involving dispersal of sperm by the male, retention of eggs by the female and *in situ* or internal fertilisation. Observations of only unfertilised eggs in the coelomic fluid of females, and already fertilised eggs moving along the faecal groove and being released from the tube (Giangrande et al. 2000, Stabili et al. 2009), have led to the suggestion that fertilisation occurs inside the tube just after egg release (i.e. *in situ*), rather than in the water column (Giangrande et al. 2000). Female *S. spallanzanii* exhibit characteristics commonly associated with *in situ* fertilisation, including the production of relatively few, large eggs (Currie et al. 2000, Giangrande et al. 2000)

The degree to which *S. spallanzanii* reproduction is limited by mate or sperm availability is unclear (Inglis et al. 2009). The sperm casting strategy is assumed to be associated with mechanisms that efficiently capture and concentrate sperm to supply the egg(s) retained by the female, reducing the threat of sperm limitation (Bishop 1998, Pemberton et al. 2003). By effectively increasing the encounter rate of sperm and eggs, some species may be able to achieve high rates of fertilisation even when the ambient concentration of sperm in the water column is low (Pemberton et al. 2003).

Fecundity

Estimates of mature oocyte production for large *S. spallanzanii* (~400 mm body length) indicate that more than 50,000 eggs are produced and released per female during spawning (Currie et al. 2000). The diameter of mature eggs is reported to range between 190 to 250 µm (Currie et al. 2000, Giangrande et al. 2000). Eggs are released embedded in mucus containing antibacterial substances thought to protect the eggs from bacterial attack (Stabili et al. 2009). The strings of mucus can measure up to ~1 m in length (Currie et al. 2000). Only fertilised eggs are contained within mucus strands released by females (Stabili et al. 2009). Laboratory observations indicated a fertilisation success of 100 % (Giangrande et al. 2000) but exact *in situ* fertilisation rates are not currently known.

Quantification of sperm production and output are not available in the literature, although mean percentages of coelom occupancy by mature sperm provide an indication of the timing of spawning events (see Section: Seasonality and reproductive period). The heads of mature sperm measure 2 µm in diameter and the tail flagellum between 8 to 12 µm in length (Currie et al. 2000), with a morphology typical for free-spawning species (Giangrande et al. 2000, Rouse & Fitzhugh 1994).

Field observations of the rate at which gametes or larvae are released during the spawning period and the concentration of larvae present in the water column for given adult population abundances are not available in the literature. Giangrande et al. (2000) reported a high survival rate for laboratory-spawned larvae during the planktonic phase (almost nil mortality over two weeks) but high post-metamorphosis mortality (90 %), although this was possibly due to lack of suitable settlement substrates.

Seasonality and reproductive period

Available literature suggests that *S. spallanzanii* spawning occurs at water temperatures of 11°C or greater, and recruitment of larvae is usually observed during spring and summer although variation in the timing and duration of the reproductive period has been reported for some non-indigenous populations.

Within its native Mediterranean range, *S. spallanzanii* spawns over the period of 1 month during late winter (January – February) when water temperatures range from ~11°C to 14°C (2005, Giangrande et al. 2000). Oocyte development is a relatively long process, beginning soon after spawning ceases in February, with maturation complete by December. Spermatogenesis is not observed until September, but sperm maturation is synchronised with that of the eggs.

In Port Phillip Bay, Australia, episodic spawning has been observed over an extended period during autumn and winter at water temperatures ranging from 11 to 20°C (Currie et al. 2000). The coeloms of male *S. spallanzanii* collected from Queenscliff (Melbourne) were packed with mature sperm by March (early autumn), with large reductions in sperm indicating major spawning events in autumn and winter (July - August in 1996 and May – August in 1997). Spermatogenesis began again immediately after spawning, occurring gradually during spring (September to November) and increasing during summer (December to February). Female spawning, inferred by significant and rapid decrease of mature oocytes in the coelom, occurred in autumn. Some inter-annual variation in the timing of the major female spawning event was evident over the two cycles observed by this study (March in 2006, May in 1997). The numbers of oocytes continued to decline over winter, then began to accumulate again in spring and proliferated during summer.

It has been suggested that spawning for some populations in Australia may occur over an even longer period during a large proportion of the year. Recruitment of *S. spallanzanii* to settlement panels has been observed in Port Phillip Bay during late autumn to late summer at water temperatures as warm as 22°C (Holloway & Keough 2002a, Holloway & Keough 2002b, Johnston & Keough 2000, Johnston & Keough 2002), and spawning during both winter and summer is reported for populations in Western Australia (Clapin & Evans 1995, Pollard & Rankin 2003).

There appears to be a correlation between the gametogenic cycle of *S. spallanzanii* and seawater temperature and/or day-length (Giangrande et al. 2000), however it is not currently clear whether or which of these physical parameters play a role in controlling spawning. The relative importance of seawater temperature and photoperiod is difficult to determine since they are often closely linked (Currie et al. 2000). Studies of the reproductive cycle of *S. spallanzanii* populations at Queenscliff (Melbourne, Australia) indicate that gamete production and maturation coincide with maximum seawater temperatures (20.4°C in February 1996, and 22.8°C in January 1997). Onset of early gametogenesis and the peak of late gametogenesis also coincided with the shortest and longest day-lengths, respectively. Two distinct periods of spermatogenesis were observed, one beginning in late winter (August) and a second, less intense pulse in mid-Autumn (April). Spawning began as seawater temperatures decreased, coinciding with the spring equinox during 1996. During 1997, initiation of spawning was delayed until the photoperiod had decreased to < 650 minutes. Gamete release continued until shortly after minimum seawater temperatures were reached (11.3°C in July 1996, and 12.6°C in August 1997), extending beyond the shortest day, and ceased prior to the autumn equinox (Currie et al. 2000).

In Lyttelton, a local elimination programme was initiated in response to the detection of an incursion of *S. spallanzanii* to the port. Specimens removed by divers from port structures during August and September 2008 were mostly (> 63 %) mature and gravid individuals. This seemed to indicate that a potential mass spawning event was imminent (Inglis et al. 2009). The detection of a new cohort of small specimens in April 2009 corresponded to the estimated timing of this spawning event and subsequent recruitment. In 2009, surface water temperatures in Lyttelton dropped below the 11°C in late May, to a minimum of 7.7°C in late

July. Going by the threshold temperature for spawning reported in the literature (> 11°C), no reproductive activity may take place in Lyttelton's *S. spallanzanii* population during this period.

Duration of planktonic phase

Under laboratory conditions, mucus-enveloped eggs sink rapidly after release and develop into early embryos then lecithotrophic (non-feeding) trochophore larvae after 24 to 36 hours (Giangrande et al. 2000). Larvae are free-swimming, although little is known about swimming ability, speed or dispersal distances.

The duration of pelagic development in natural populations has not yet been determined for *S. spallanzanii* (Currie et al. 2000), although Giangrande et al. (2000) reported a planktonic phase of 2 weeks for laboratory-cultured larvae. This is the longest larval duration reported among sabellid worms. The authors suggest that the potential for a long larval phase was demonstrated by the laboratory results, but that settlement may have been stalled due to lack of suitable settlement surfaces. This suggested ability to modify the duration of the larval phase may increase the capacity for human-mediated dispersal if larvae can survive in ballast water tanks of ships by suspending settlement until suitable habitats become available on discharge (Patti & Gambi 2001). Changes in spatial distribution over time within Port Phillip Bay indicate that "larvae produced during each annual spawning appear to be carried only short distances (< 20 km) from their parent stock prior to settling" (Parry et al. 1996 in: Currie et al. 2000).

Settlement and recruitment

Larval behaviour and settlement preferences

No specific investigations of the behaviour or settlement preferences of *S. spallanzanii* larvae could be found in the literature, although Giangrande et al. (2000) observed gregarious larval behaviour and clusters of new settlers under laboratory conditions. In this experiment, microtopography appeared to be an important requirement for successful recruitment, with jar corners, sand grains and gravel providing attractive surfaces for larvae. Mortality was high for larvae that settled directly onto the glass sides of the jars.

The presence of a microbial film on settlement surfaces has been demonstrated to facilitate the recruitment of many marine invertebrate larvae (Keough & Raimondi 1995, Zardus et al. 2008). No studies have specifically examined the importance of biofilm for settlement of *S. spallanzanii*. Larvae develop a distinct eye spot during the trochophore stage of development (Giangrande et al. 2000), which may indicate that light is used at least to some extent for orientation. Short-term photo adaptation has been observed in laboratory experiments for a variety of marine invertebrate larval forms, including some serpulid polychaete larvae (Young 1995, Young & Chia 1981).

Settlement to artificial surfaces

In Port Phillip Bay, non-indigenous populations of *S. spallanzanii* have become established in most subtidal habitats, including a range of artificial substrates including rocks, concrete, wood and steel, along with vessel hulls; including car ferries, fishing boats and pleasure craft (Currie et al. 2000). Although described as solitary at shallow depths, gregarious settlement patterns are reported at greater depths (> 7 m) in soft sediment habitats where the availability of hard substrates is limited. Distribution patterns in Port Phillip Bay indicate a preference for harbours and embayments sheltered from direct wave action. A possible preference for vertical settlement surfaces may be indicated by higher densities on wharf pilings (Currie et

al. 2000). It is also present on the vertical sides of pontoons in Adelaide, South Australia (Holloway & Keough 2002a).

The local elimination programme in Lyttelton port detected *S. spallanzanii* on pontoons, wharf piles and a recreational vessel. More than 80 % of worms were found at depths shallower than 6 m below the lowest astronomical tide (Inglis et al. 2009). *S. spallanzanii* has also established widespread a population in Waitemata Harbour, Auckland. In 2009, several hundred individuals were removed from a barge moored near the Port of Auckland. *S. spallanzanii* was also found on the hulls of several recreational vessels moored in an upper harbour environment.

Factors influencing density of recruits

Relationships between adult population density or abundance, and larval concentrations in the water column and recruitment have not been specifically investigated for *S. spallanzanii*. However, some useful information is available from a range of studies that have examined the distribution or reproduction of *S. spallanzanii* in different locations.

There are several accounts of recruitment of *S. spallanzanii* to experimental settlement surfaces including PVC panels, rope collectors and netting. Passive collectors deployed in proximity to well established populations of *S. spallanzanii* have provided some information on recruitment densities in situations where the supply of larvae is likely to be relatively high. In a marina located in the Outer Harbour region of Adelaide (South Australia), where ~250 individuals m^{-2} grow on the piles and pontoons, the mean density of recruits detected on vertically orientated settlement panels after a 2 week deployment period during summer was ~0.01 recruits cm^{-2} (Holloway & Keough 2002a). Average recruitment densities on settlement panels at 2 sites within Port Phillip Bay ranged from ~0.17 – 0.32 recruits cm^{-2} over a 2 - 4 week deployment period (Johnston & Keough 2000). The adult population abundance and density were not reported for this study. Settlement of larvae onto fine-mesh netting is reported by Giangrande et al. (2005); about three months after a settlement event an estimated density of ~0.5 recruits cm^{-2} was observed on a net deployed at 10 – 20m depth in the Ionian Sea. Natural annual settlement patterns were reported to be variable and unpredictable, with settlement to the netting "almost absent" in the following year.

During the *S. spallanzanii* Local Elimination Program, between August 2008 and November 2009, divers repeatedly removed visible adult worms from the population throughout Lyttelton Port. Over that time, densities were reduced from a highly clustered state on a few wharves (~ 11 worms per 100 wharf piles searched) to a more evenly distributed, low density (< 1 worm per 100 wharf piles searched) population throughout the inner port. The total population size is likely to have been reduced to fewer than 50 visible (i.e. > 10 cm tube length) worms by November 2009 (Inglis et al. 2009). Between May 2009 and January 2010, an attempt was made to monitor recruitment of *S. spallanzanii* on settlement surfaces deployed at locations throughout the port. Twenty settlement arrays, consisting of eight grey PVC plates (four vertically-orientated, four horizontally orientated) and four segments of mussel spat ropes, were deployed for six overlapping periods of 7 - 8 weeks. The settlement plates resembled those used in other studies where recruitment of *S. spallanzanii* was reported (Holloway & Keough 2002a, 2002b, Johnston & Keough 2000, 2002, McDonald et al. 2009). No *S. spallanzanii* recruits were observed on any of the surfaces immersed in Lyttelton, although native sabellid worms did successfully recruit to all substrate types, including the PVC tubes the various surfaces were attached to. This may be because no spawning occurred in the time that the plates were deployed or because larval concentrations were too low to be detected by the number of arrays (20 units, 240 surfaces in total) that was deployed on each occasion.

APPENDIX 3. MODELLED STEADY-STATE CONCENTRATIONS OF PROPAGULES IN THE PORT OF LYTTELTON

Table A3-1: Information on the parameterisation of the Stochastic Scenario Tree models, obtained via literature reviews and from research conducted by University of Canterbury and NIWA (unpublished work) (Appendix 2)

	S. clava	*C. intestinalis*	*U. pinnatifida*	*S. spallanzanii*
Reproductive strategy	Hermaphrodite, self-sterile. Broadcast spawning, external fertilisation of gametes.	Hermaphrodite, self-sterile. Broadcast spawning, external fertilisation. Eggs either released individually into the water column or aggregated in mucus strings.	Heteromorphic, incorporating a microscopic, haploid gametophyte generation and a diploid sporophyte generation that develops into the macroalga. Zoospores released from sporophyll tissue, settle onto suitable substrate and form male and female gametophytes. Swimming spermatozoa released from male gametophytes, fertilising eggs that remain attached to female gametophytes. These grow into the adult plant.	Gonochoric. *S. spallanzanii* employs a form of 'sperm-casting', involving dispersal of sperm by the male, retention of eggs by the female and internal fertilisation of eggs in the female tube. Fertilised eggs are then released from the tube.
Reproductive season in Lyttelton Port	Variable. Possibly October to March, but majority of reproduction over 90 days in December – February. Continuous release of gametes during spawning period. Assumed here that 90 % of annual reproductive output occurs in December – February (Nutsford; Willis, pers. comm. 2010).	Reproduction may occur from early spring to late autumn, with peak larval abundance and settlement in October-March. Continuous release of gametes during spawning period.	In Lyttelton Harbour and the East coast of the South Island, *U. pinnatifida*'s peak reproduction occurs in July-September.	Introduced populations in Australia reported to spawn late autumn through to late summer in Port Phillip Bay, and year-round in Western Australia. In Lyttelton, spawning is thought to occur around September/October based on examination of adult specimens throughout the year. Our model assumes a spawning period of 8 weeks (September-October).

	S. clava	*C. intestinalis*	*U. pinnatifida*	*S. spallanzanii*
Fecundity	In Lyttelton, Nutsford (2010)estimates mean annual fecundity of 77230 eggs produced per reproductive female.	Gametes can be produced continuously during the reproductive season, with approximately 500 eggs released daily by a mature individual.	Varies geographically. New Zealand studies indicate 10^8 to 10^9 spores per sporophyte (adult plant) per year. We used an average fecundity of $5x10^8$ spores released per adult plant during the reproductive season (July-September).	Large *S. spallanzanii* (~400 mm body length) can release more than 50,000 eggs during spawning. This value was used in our model.
Fertilization	On average, 3.9 % of eggs produced by a female are fertilised and result in a larva (laboratory studies by Nutsford 2010).	No reliable data available. Used estimate obtained for *S. clava* (3.9 % of eggs produced survive to become viable larvae).	In the dosing experiment we released zoospores that settle to a surface to develop into male and female gametophores. We are thus not reliant on an estimate of fertilisation rate.	Assumed that all eggs released by a female (see cell above) are fertilised as fertilisation occurs internally prior to release.
Duration of planktonic phase	12 – 24 hours. We modelled both of these values.	Reported as 2-10 days, with shorter planktonic phase where settlement substrates available. We modelled larval life spans of 2, 5 and 10 days to simulate a range of scenarios.	Zoospores settle to suitable substrates within several hours but can survive for up to 14 days if no substrates available. Our models use zoospore life spans of 2, 7 and 14 days.	Not well understood. Laboratory studies found *S. spallanzanii* larvae can remain viable for 2 weeks but these studies offered no suitable settlement substrates. Our models therefore use a range of larval life spans of 2, 7 and 14 days.
Larval concentrations in situ	Determined by hydrodynamic model of Lyttelton Port. However, actual observed concentrations reported from Lyttelton (0.098 - 0.13 larvae m^{-3}) and Canada (240 - 560 larvae m^{-3}).	Determined by hydrodynamic model of Lyttelton Port. However, actual observed concentrations reported from Lyttelton by Nutsford (2010) range from 0.01 - 0.7 larvae m^{-3}, with peaks of 0.4 and 0.7 larvae m^{-3} observed in 2009 and 2010.	Unknown.	Unknown.
Planktonic mortality	The instantaneous (daily) mortality rate, *k*, was calculated using the equation derived by Rumrill (1990). *k* varies with larval life span: 12 h: $k = 0.787$ 24 h: $k = 0.696$	The instantaneous (daily) mortality rate, *k*, was calculated using the equation derived by Rumrill (1990). *k* varies with larval life span: 2 d: $k = 0.475$ 5 d: $k = 0.286$ 10 d: $k = 0.195$	Zoospore settlement was assumed to occur relatively soon following release (between 1 h and 2 d). No daily mortality rate was included in the models.	The instantaneous (daily) mortality rate, *k*, was calculated using the equation derived by Rumrill (1990). *k* varies with larval life span: 2 d: $k = 0.475$ 7 d: $k = 0.238$ 14 d: $k = 0.163$

Table A3-2: Parameter values used in the Stochastic Scenario Tree calculations for settlement arrays for the four species.

Species	Parameter	Notation	Value[1,2,3]	Source
S. clava	Relative Risk of Spawning (Summer: Winter)	RR_i	PertDev(2,5,7)	K. Willis (unpubl. data);(McClary et al. 2008, Nutsford 2010)
	Proportion of samples per season (Summer: Winter)	$\Pr P_i$	0.5 : 0.5	Summer vs. Winter surveys
	Steady State Concentration of propagules (numbers m^{-3})	C_T	RandReal(T = 12 hrs, T = 24 hrs)	Hydrodynamic models Literature review – min. & max values based on larval durations of 12 hrs and 24 hrs, respectively.
	Adult population = 10		RandReal(0.000013, 0.000020)	
	Adult population = 100		RandReal(0.000128, 0.000205)	
	Adult population = 1,000		RandReal(0.00128, 0.00205)	
	Adult population = 10,000		RandReal(0.0128, 0.0205)	
	Adult population = 100,000		RandReal(0.1279, 0.2045)	
	Method Sensitivity	ϕ_j		
	Horizontal plates		PertDev(0.001, 0.01, 0.02)	Dosing experiment (max. value = $\frac{1}{50}$)
	Vertical plates		PertDev(0.001, 0.01, 0.02)	
	Rope mops		PertDev(0.001, 0.01, 0.02)	Literature review
C. intestinalis	Relative Risk of Spawning (Summer: Winter)	RR_i	PertDev(17,23,29)	*S. spallanzanii* Local Elimination Programme – monitoring
	Proportion of samples per season (Summer: Winter)	$\Pr P_i$	0.5 : 0.5	Summer vs. Winter surveys

Species	Parameter	Notation	Value[1,2,3]	Source
	Steady State Concentration of propagules (numbers.m^{-3})	C_T	RandReal(T = 2 days, T = 10 days)	Hydrodynamic models Literature review – min. & max. values based on larval durations of 2 and 10 days, respectively.
	Adult population = 10		RandReal(0.000021, 0.000041)	
	Adult population = 100		RandReal(0.000211, 0.00041)	
	Adult population = 1,000		RandReal(0.002117, 0.00408)	
	Adult population = 10,000		RandReal(0.021176, 0.04088)	
	Adult population = 100,000		RandReal(0.21176, 0.40878)	
	Method Sensitivity	ϕ_j	NormDev(Mean, s.dev)	Dosing experiment results
	Horizontal plates		NormDev(0.2, 0.3)	
	Vertical plates		NormDev (0.05, 0.06)	
	Rope mops		NormDev (0.033, 0.017)	
S. spallanzanii	Relative Risk of Spawning (Summer: Winter)	RR_i	1:1	Literature review
	Proportion of samples per season (Summer: Winter)	$\Pr P_i$	0.5 : 0.5	Summer vs. Winter surveys
	Steady State Concentration of propagules (numbers.m^{-3})	C_T	PertDev(T = 2 days, T = 7 days, T = 14 days)	Hydrodynamic models Literature review – min., likely & max. values based on larval durations of 2, 7 and 14 days, respectively
	Adult population = 10		PertDev(0.00097, 0.00170, 0.00201)	
	Adult population = 100		PertDev(0.00969, 0.01703, 0.02010)	
	Adult population = 1,000		PertDev(0.09696, 0.17033, 0.20102)	
	Adult population = 10,000		PertDev(0.96959, 1.70331, 2.01023)	
	Adult population = 100,000		PertDev(9.69595, 17.0331, 20.1023)	

Species	Parameter	Notation	Value[1,2,3]	Source
	Method Sensitivity	ϕ_j		Literature review
	Horizontal plates		0.001	
	Vertical plates		0.001	
	Ropemops		0.001	
U. pinnatifida	Relative Risk of Spawning (Summer: Winter)	RR_i	PertDev(2,10,13)	Literature review
	Proportion of samples per season (Summer: Winter)	$\Pr P_i$	0.5 : 0.5	Summer vs. Winter surveys
	Steady State Concentration of propagules (numbers.m^{-3})	C_T	PertDev(T = 1 hr, T = 6 hrs, T = 2 days)	Hydrodynamic models Literature review – min., likely & max. values based on planktonic durations of 1 hr, 6 hrs and 2 days, respectively
	Adult population = 10		PertDev(0.4877, 2.5623, 8.4757)	
	Adult population = 100		PertDev(4.8773, 25.6234, 84.7566)	
	Adult population = 1,000		PertDev(48.773, 256.234, 847.566)	
	Adult population = 10,000		PertDev(487.7, 2562.3, 8475.6)	
	Adult population = 100,000		PertDev(4877, 25623, 84756)	
	Method Sensitivity	ϕ_j		Dosing experiment results (max. value = $\frac{1}{19{,}000}$)
	Horizontal plates		PertDev(2.63 x 10^{-6}, 2.63 x 10^{-5}, 5.26 x 10^{-5})	
	Vertical plates		PertDev(2.63 x 10^{-6}, 2.63 x 10^{-5}, 5.26 x 10^{-5})	
	Ropemops		PertDev(2.63 x 10^{-6}, 2.63 x 10^{-5}, 5.26 x 10^{-5})	Literature review

[1]PertDev (minimum, most likely, maximum) = random sample from a Beta Pert distribution defined by the minimum, most likely and maximum values considered possible by experts
[2]RandReal (minimum, maximum) = random sample from a uniform distribution bounded by the minimum and maximum values considered possible.
[3]NormDev (mean, standard deviation) = random sample from a normal distribution defined by the mean and standard deviation.

Table A3-3: Parameter values used in the Stochastic Scenario Tree calculations for existing surveys for the four species.

Species	Parameter	Notation	Value[1,2]	Source
S. clava	Relative Risk of Infestation in different habitats	RR_i		Gust et al. (2008)
	Soft sediment		1	
	Breakwalls		PertDev(1,2,2.5)	
	Wharf piles		PertDev(5,7,10)	
	Pontoons		PertDev(10,20,30)	
	Proportion of habitat area in inner harbour comprised of each habitat type	$\Pr P_i$		Maps of the inner Port of Lyttelton
	Soft sediment		0.9113	
	Breakwalls		0.0048	
	Wharf piles		0.0816	
	Pontoons		0.0023	
	Method Sensitivity	ϕ_j		Gust et al. (2006), (2008)
	Dive searches		NormDev (0.38, 0.05)	
	Sled tows		0	
C. intestinalis	Relative Risk of Infestation in different habitats	RR_i		Observations by the surveillance dive team, Published literature
	Soft sediment		1	
	Breakwalls		PertDev(1,2,2.5)	
	Wharf piles		PertDev(5,7,10)	
	Pontoons		PertDev(5,7,10)	
	Proportion of habitat area in inner harbour comprised of each habitat type	$\Pr P_i$		Maps of the inner Port of Lyttelton
	Soft sediment		0.9113	
	Breakwalls		0.0048	
	Wharf piles		0.0816	
	Pontoons		0.0023	

Species	Parameter	Notation	Value[1,2]	Source
	Method Sensitivity	ϕ_j		Gust et al. (2006), (2008)
	Dive searches		NormDev (0.38, 0.05)	
	Sled tows		0	
S. spallanzanii	Relative Risk of Infestation in different habitats	RR_i		Inglis et al. (2009) (2008b), (2009)
	Soft sediment		1	
	Breakwalls		PertDev(1,2,2.5)	
	Wharf piles		PertDev(5,7,10)	
	Pontoons		PertDev(10,20,30)	
	Proportion of habitat area in inner harbour comprised of each habitat type	$\Pr P_i$		Maps of the inner Port of Lyttelton
	Soft sediment		0.9113	
	Breakwalls		0.0048	
	Wharf piles		0.0816	
	Pontoons		0.0023	
	Method Sensitivity	ϕ_j		Inglis et al. (2009) (2008b), (2009)
	Dive searches		NormDev (0.40, 0.05)	
	Sled tows		PertDev(0.1,0.2,0.5)	
U. pinnatifida	Relative Risk of Infestation in different habitats	RR_i		Observations by the surveillance dive team, Published literature
	Soft sediment		1	
	Breakwalls		PertDev(2,4,5)	
	Wharf piles		PertDev(5,7,10)	
	Pontoons		PertDev(5,7,10)	

Species	Parameter	Notation	Value[1,2]	Source
	Proportion of habitat area in inner harbour comprised of each habitat type	$\Pr P_i$		Maps of the inner Port of Lyttelton
	Soft sediment		0.9113	
	Breakwalls		0.0048	
	Wharf piles		0.0816	
	Pontoons		0.0023	
	Method Sensitivity	ϕ_j		Observations by the surveillance dive team, Published literature
	Dive searches		NormDev (0.73, 0.05)	
	Sled tows		0	

[1]PertDev (minimum, most likely, maximum) = random sample from a Beta Pert distribution defined by the minimum, most likely and maximum values considered possible by experts
[2]NormDev (mean, standard deviation) = random sample from a normal distribution defined by the mean and standard deviation.

APPENDIX 4. MODELLED STEADY-STATE CONCENTRATIONS OF PROPAGULES IN THE PORT OF LYTTELTON

Values were calculated for five sizes of adult population (10, 100, 1,000, 10,000 and 100,000 individuals) and for different potential periods of planktonic duration (T).

Species	Adult population size	C_T Minimum	C_T Most Likely	C_T Maximum
S. clava		T =12 hrs	n/a	T = 24 hrs
	10	0.00001		0.00002
	100	0.00013		0.00020
	1,000	0.00128		0.00205
	10,000	0.01279		0.02050
	100,000	0.12791		0.20496
C. intestinalis		T = 2 days	T = 5 days	T = 10 days
	10	0.00002	0.00003	0.00004
	100	0.00021	0.00033	0.00041
	1,000	0.00212	0.00332	0.00409
	10,000	0.02118	0.03323	0.04088
	100,000	0.21176	0.33226	0.40878
S. spallanzanii		T = 2 days	T = 7 days	T = 14 days
	10	0.0010	0.0017	0.0020
	100	0.0097	0.0170	0.0201
	1,000	0.0970	0.1703	0.2010
	10,000	0.9696	1.7033	2.0102
	100,000	9.6960	17.0332	20.1023
U. pinnatifida		T = 1 hr	T = 6 hrs	T = 2 days
	10	0.4877	2.5623	8.4757
	100	4.87735	25.6234	84.7566
	1,000	48.7735	256.2341	847.5664
	10,000	487.7349	2562.3409	8475.6639
	100,000	4877.3499	25623.4092	84756.6391

10 References

Arakawa, H.; Moringa, T. (1994). The rate of brown algal zoospores adhered in the seaweed substrate in relation to gradient of the substrate. *Nippon Suisan Gakkaishi (in Japenese with English abstract) 60*: 461-466; in Forrest et al. 2000.

Arsenault, G.; Davidson, J.; Ramsay, A. (2009). Temporal and spatial development of an infestation of *Styela clava* on mussel farms in Malpeque Bay, Prince Edward Island, Canada. *Aquatic Invasions 4(1)*: 189-194.

Berrill, N. (1931). Studies in tunicate development. Part II. Abbreviation of development in the Mogulidae. *Philosophical Transactions of the Royal Society Series B 219*: 281-346.

Berrill, N. (1935). Studies in tunicate development. III. Differential retardation and acceleration. *Philosophical Transactions of the Royal Society of London B 225*: 255-326.

Berrill, N. (1947). The development and growth of *Ciona*. *Journal of the Marine Biological Association of the United Kingdom 26*: 616-625.

Bishop, J.D.D. (1998). Fertilization in the sea: are the hazards of broadcast spawning avoided when free-spawned sperm fertilize retained eggs? *Proceedings of the Royal Society - Biological Sciences (Series B) 265*: 725-731.

Bishop, J.D.D.; Pemberton, A.J. (2006). The third way: spermcast mating in sessile marine invertebrates. *Integrative and Comparative Biology 46*: 398-406.

Blum, J.; Chang, A.; Liljesthrom, M.; Schenk, M.; Steinberg, M.; Ruiz, G. (2007). The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness. *Journal of Experimental Marine Biology and Ecology 342(1)*: 5-14.

Bolton, T.; Havenhand, J. (1996). Chemical mediation of sperm activity and longevity in the solitary ascidians *Ciona intestinalis* and *Ascidiella adspersa*. *Biological Bulletin 190*: 329-335.

Bourque, D.; Davidson, J.; MacNair, N.G.; Arsenault, G.; LeBlanc, A.R.; Landry, T.; Miron, G. (2007). Reproduction and early life history of an invasive ascidian Styela clava Herdman in Prince Edward Island, Canada. *Journal of Experimental Marine Biology and Ecology 342(1)*: 78-84. <http://dx.doi.org/10.1016/j.jembe.2006.10.017>

Bourque, D.; MacNair, N.; LeBlanc, A.; Landry, T.; Miron, G. (2005). Preliminary study of the diel variation of ascidian larvae concentrations in Prince Edward Island. *Canadian Technical Report of Fisheries and Aquatic Sciences 2571*: I, IV-16.

Brown, S. (1999). Dispersal characteristics of the adventive seaweed *Undaria pinnatifida* in New Zealand. MSc Thesis. University of Otago, New Zealand, 103 p.

Bullard, S.G.; Whitlach, R.B. (2004). A guide to the larval and juvenile stages of common Long Island Sound ascidians and bryozoans. . *Connecticut Sea Grant College Program. No.* 18 + Appendices p.

Bullard, S.G.; Whitlatch, R.B.; Osman, R.W. (2004). Checking the landing zone: Do invertebrate larvae avoid settling near superior spatial competitors? *Marine Ecology-Progress Series 280*: 239-247.

Butler, A.J. (1986). Recruitment of sessile invertebrates a five sites in Gulf St. Vincent, South Australia. *Journal of Experimental Marine Biology and Ecology 97*: 13-36.

Cameron, A. (2004). Principles for the design and conduct of surveys to show presence or absence of infectious disease in aquatic animals. . *National Aquatic Animal Health Technical Working Group - Policy Document No.* 37 p.

Campbell, S.J.; Burridge, T.R. (1998). Occurrence of *Undaria pinnatifida* (Phaeophyta: Laminariales) in Port Phillip Bay, Victoria, Australia. *Marine and Freshwater Research 49(5)*: 379-381.

Cannon, R.M. (2001). Sense and sensitivity - Designing surveys based on an imperfect test. *Preventive Veterinary Medicine 49(3-4)*: 141-163.

Carver, C.; Chisholm, A.; Mallet, A.L. (2003). Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. *Journal of Shellfish Research 22(3)*: 621-631.

Carver, C.; Mallet, A.; Vercaemer, B. (2006). Biological Synopsis of the solitary tunicate *Ciona intestinalis*. Canadian manuscript of Fisheries and Aquatic Sciences No. 2746. v + 55 p.

Casas, G.; Piriz, M.; Parodi, E. (2008). Population features of the invasive kelp *Undaria pinnatifida* (Phaeophyceae: Laminariales) in Nuevo Gulf (Patagonia, Argentina). *Journal of the Marine Biological Association of the United Kingdom 88*: 21-28.

Castle, W. (1896). The early embryology of *Ciona intestinalis* Flemming (L.). *Bulletin of the Museum of Comparative Zoology, Harvard 27*: 201-280.

Castric-Fey, A.; Beaupoil, C.; Bouchain, J.; Pradier, E.; L'Hardy-Halos, M. (1999). The introduced alga *Undaria pinnatifida* (Laminariales, Alariaceae) in the rocky shore ecosystem of the St Malo area: growth rate and longevity of the sporophyte *Botanica Marina 42(1)*: 83-96.

Cayer, D.; MacNeil, M.; Bagnall, A. (1999). Tunicate fouling in Nova Scotia aquaculture: a new development. *Journal of Shellfish Research 18*: 327.

Chapman, A. (1984). Reproduction, recruitment and mortality in two species of Laminaria in southwest Nova Scotia. *Journal of Experiemental Marine Biology and Ecology. 78*: 99-109.

Chapman, A. (1986). Population and community ecology of seaweeds. *Advances in Marine Biology 23*: 1-161.

Choi, H.; Kim, Y.; Lee, S.; Park, E.; Nam, K. (2005). Effects of daylength, irradiance and settlement density on the growth and reproduction of *Undaria pinnatifida* gametophytes. *Journal of Applied Phycology 17*: 423–430.

Clapin, G.; Evans, D.R. (1995). The status of the introduced marine fanworm *Sabella spallanzanii* in Western Australia: a preliminary investigation. Centre for Research on Introduced Marine Pests (CRIMP), Tech. Report No.2, Hobart. p.

Clark, G.F.; Johnston, E.L. (2005). Manipulating larval supply in the field: a controlled study of marine invasibility. *Marine Ecology Progress Series 289*: 9-19.

Clarke, C.; Therriault, T. (2006). Biological synopsis of the invasive tunicate *Styela clava* (Herdman 1881). *Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 2807 No.* vi + 23 p.

Cohen, A. (2000). Biological invasions in coastal waters. *Journal of Shellfish Research 19(1)*: 630-631.

Conklin, E. (1905). The organization and cell lineage of the ascidian egg. *Journal of the Academy of Natural Sciences of Philadelphia 13*: 1-119.

Costelloe, J.; Keegan, B.; Könnecker, G. (1986). Rocky subtidal assemblages on the west coast of Ireland. *Hydrobiologica 142*: 97-111.

Currie, D.R.; McArthur, M.A.; Cohen, B.F. (2000). Reproduction and distribution of the invasive European fanworm *Sabella spallanzanii* (Polychaeta: Sabellidae) in Port Phillip Bay, Victoria, Australia. *Marine Biology 136(4)*: 645-654.

Darbyson, E.A.; Hanson, J.M.; Locke, A.; Martin Willison, J.H. (2009). Settlement and potential for transport of clubbed tunicate (Styela clava) on boat hulls. *Aquatic Invasions 4(1)*: 95-103.

Davis, M.; Lützen, J.; Davis, M. (2007). The spread of *Styela clava* Herdman, 1882 (Tunicata, Ascidiacea) in European waters. *Aquatic Invasions 2(4)*: 378-390.

Davis, M.A. (1997). Physical factors influencing larval behaviour in three species of solitary ascidian. Ph.D thesis. Open University, 360 p.

Davis, M.H.; Davis, M.E. (2007). The distribution of *Styela clava* (Tunicata, Ascidiacea) in European waters. *Journal of Experimental Marine Biology and Ecology 342*: 182-184.

Dybern, B. (1963). Biotope choice in *Ciona intestinalis* (L.). Influence of light. *Zool. Bidr. Uppsala 35*: 589-602.

Dybern, B. (1965). The life cycle of *Ciona intestinalis* (L.) f. typica in relation to the environmental temperature. *Oikos 16*: 109-131.

Ferguson, R. (2000). The effectiveness of Australia's response to the black striped mussel incursion in Darwin, Australia. *A report of the marine pest incursion management workshop-27-28 August 1999 No.* 75p p.

Fletcher, R.L.; Farrell, P. (1999). Introduced brown algae in the North East Atlantic, with particular respect to *Undaria pinnatifida* (Harvey) Suringar. *Helgolander Meeresuntersuchungen 52(3-4)*: 259-275.

Floc'h, J.Y.; Pajot, R.; Wallentinus, I. (1991). The Japanese brown alga *Undaria pinnatifida* on the coast of France and its possible establishment in European waters. *Journal du Conseil, Con- seil International pour l'Exploration de la Mer 47(3)*: 379-390.

Floerl, O.; Wilkens, S.; Woods, C. (2010). Temporal development of biofouling assemblages. *NIWA Client Report prepared for Department of Agriculture, Forestry and Fisheries, Australia No. NAU10105*. 46 p.

Forrest, B.M.; Brown, S.N.; Taylor, M.D.; Hurd, C.L.; Hay, C.H. (2000). The role of natural dispersal mechanisms in the spread of *Undaria pinnatifida* (Laminariales, Phaeophyceae). *Phycologia 39(6)*: 547-553.

Gasgoine, J.; Lipcius, R. (2004). Allee effects in marine systems. *Marine Ecology Progress Series 269*: 49-59.

Giangrande, A.; Cavallo, A.; Licciano, M.; Mola, E.; Pierri, C.; Trianni, L. (2005). Utilization of the filter feeder polychaete *Sabella spallanzanii* Gmelin (Sabellidae) as bioremediator in aquaculture. *Aquaculture International 13*: 129-136.

Giangrande, A.; Licciano, M.; Pagliara, P.; Gambi, M.C. (2000). Gametogenesis and larval development in *Sabella spallanzanii* (Polychaeta: Sabellidae) from the Mediterranean Sea. *Marine Biology 136*: 847-861.

Giangrande, A.; Petraroli, A. (1994). Observations on reproduction and growth of *Sabella spallanzanii* (Polychaeta, Sabellidae) in the Mediterranean Sea. *Memoires du Museum National d'Histoire Naturelle 162*: 51-56.

Glasby, T.M.; Connell, S.D. (2001). Orientation and position of substrata have large effects on epibiotic assemblages. *Marine Ecology Progress Series 214*: 127-135.

Gosselin, L.A.; Qian, P.Y. (1997). Juvenile mortality in benthic marine invertebrates. *Marine Ecology Progress Series 146*: 265-282.

Gust, N.; Inglis, G.; Floerl, O.; Peacock, L.; Denny, C.; Forrest, B. (2008). Assessment of population management options for *Styela clava*. *NIWA Client Report Prepared for MPI-Biosecurity NZ, Technical Paper No: 2009/04 No.* 228 p.

Gust, N.; Inglis, G.; Peacock, L.; Miller, S.; Floerl, O.; Hayden, B.J.; Fitridge, I.; Hurren, H.; Johnston, O. (2006). Rapid nationwide delimitation surveys for *Styela clava*. Technical Report prepared for Biosecurity New Zealand Post-clearance Directorate. NIWA Report number CH2006-024. *No.* 81 p.

Havenhand, J.N.; Svane, I. (1991). Roles of hydrodynamics and larval behaviour in determining spatial aggregation in the tunicate *Ciona intestinalis*. *Marine Ecology Progress Series 68*: 271-276.

Hay, C. (1990). The dispersal of sporophytes of *Undaria pinnatifida* by coastal shipping in New Zealand, and implications for further dispersal of *Undaria* in France. *British Phycological Journal 25*: 301-313.

Hay, C.; Luckens, P. (1987). The Asian kelp *Undaria pinnatifida* (Phaeophyta: Laminariales) found in a New Zealand Harbour. . *New Zealand Journal of Botany 25*: 329-332.

Hay, C.H.; Villouta, E. (1993). Seasonality of the adventive Asian kelp *Undaria pinnatifida* in New Zealand. *Botanica Marina 36(5)*: 461-476.

Hayes, K.; Cannon, R.; Neil, K.; Inglis, G. (2005). Sensitivity and cost considerations for the detection and eradication of marine pests in ports. *Marine Pollution Bulletin 50*: 823-834.

Hewitt, C.L.; Campbell, M.L.; McEnnulty, F.; Moore, K.; Murfet, N.B.; Robertson, B.; Schaffelke, B. (2005). Efficacy of physical removal of a marine pest: the introduced kelp *Undaria pinnatifida* in a Tasmanian Marine Reserve. *Biological Invasions 7*: 251-263.

Holland, P. (2002). Ciona. *Current Biology 12(18)*: R609.

Holloway, M.; Keough, M.J. (2002a). Effects of an introduced polychaete, *Sabella spallanzanii*, on the development of epifaunal assemblages *Marine Ecology Progress Series 236*: 137-154.

Holloway, M.G.; Keough, M.J. (2002b). An introduced polychaete affects recruitment and larval abundance of sessile invertebrates. *Ecological Applications 12(6)*: 1803-1823.

Holmes, N. (1969). Aspects of the biology of *Styela clava* Herdman. United Kingdom, Southampton University, PhD thesis: 284 p.

Howes, S.; Herbinger, C.M.; Darnell, P.; Vercaemer, B. (2007). Spatial and temporal patterns of recruitment of the tunicate Ciona intestinalis on a mussel farm in Nova Scotia, Canada. *Journal of Experimental Marine Biology and Ecology 342(1)*: 85-92. <http://dx.doi.org/10.1016/j.jembe.2006.10.018>

Hughes, T.P.; Baird, A.H.; Dinsdale, E.A.; Moltschaniwskyj, N.A.; Pratchett, M.S.; Tanner, J.E.; Willis, B.L. (2000). Supply-side ecology works both ways: the link between benthic adulty, fecundity, and larval recruits. *Ecology 81(8)*: 2241-2249.

Inglis, G.J.; Floerl, O.; Seaward, K.; Woods, C.; Read, G.; Peacock, L. (2009). *Sabella* Local Elimination Programme - Phase 1. *Final Assessment Report Prepared for MPI Biosecurity NZ Research Project MPI11199 No.* 43 p.

Inglis, G.J.; Hurren, H.; Gust, N.; Oldman, J.; Fitridge, I.; Floerl, O., Barbara J (2006a). Surveillance design for early detection of unwanted exotic marine organisms in New Zealand. *Prepared for Biosecurity New Zealand Post-clearance Directorate for Project ZBS2001-01 No. Biosecurity New Zealand Technical Paper No: 2005-17*. 110 pp. p.

Inglis, G.J.; Hurren, H.; Oldman, J.; Haskew, R. (2006b). Using habitat suitability index and particle dispersion models for early detection of marine invaders. *Ecological Applications 16*: 1377-1390.

Inglis, G.J.; Seaward, K.; Lewis, A. (2008a). *Undaria pinnatifida*. Rapid Response Manual Version 1. *Prepared for the Australian Commonwealth Department of Agriculture, Fisheries and Forestry Project NAU07908 No.* 71 pp. p.

Inglis, G.J.; Woods, C.M.C.; Willis, K.J.; Read, G.; Seaward, K. (2008b). Incursion response to the Mediterranean Fanworm, *Sabella spallanzanii* (Gmelin, 1791), in the Port of Lyttelton -

Interim Measures. *Prepared for Biosecurity New Zealand Research Project MPI09501 No. NIWA Client Report: CHC2008-141*. 68 pp. p.

Jackson, A. (2000). Ciona intestinalis: A sea squirt. Marine Biological Association of the United Kingdom: Plymouth. Available from: http://www.marlin.ac.uk/speciesfullreview.php?speciesID=2991, Date accessed: 27 June 2011. p.

Johnston, E.L.; Keough, M.J. (2000). Field assessment of effects of timing and frequency of copper pulses on settlement of sessile marine invertebrates. *Marine Biology 137*: 1017-1029.

Johnston, E.L.; Keough, M.J. (2002). Direct and indirect effects of repeated pollution events on marine hard-substrate assemblages. *Ecological Applications 12(4)*: 1212-1228.

Johnston, E.L.; Keough, M.J.; Qian, P.Y. (2002). Maintenance of species dominance through pulse disturbances to a sessile marine invertebrate assemblage in Port Shelter, Hong Kong. *Marine Ecology Progress Series 226*: 103-114.

Kashenko, S. (1996). Effects of desalination on the larval settlement and metamorphosis of the ascidian *Styela clava*. *Biologiya Morya, Vladivostok 22*: 174-178 (in Russian, with an English summary).

Kawamura, K.; Fujita, H.; Nakauchi, M. (1987). Cytological characterization of self incompatibility in gametes of the ascidian, *Ciona intestinalis*. *Development, Growth & Differentiation 29*: 627-642.

Kelly, D. (1974). Aspects of the reproductive ecology of three solitary ascidians, *Ciona intestinalis* (L.), *Styela plicata* (L.), and *Styela clava* (H.), from Southern California. MA Thesis. California State University, Fullerton. 51 p.

Keough, M.J. (1983). Patterns of recruitment of sessile invertebrates in two subtidal habitats. *Journal of Experimental Marine Biology and Ecology 66*: 213-245.

Keough, M.J.; Raimondi, P.T. (1995). Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. *Journal of Experimental Marine Biology and Ecology 185(2)*: 235-253.

Kott, P. (1985). The Australian Ascidiacea. Part 1: Phlebobranchia and Stolidobranchia. *Memoirs of the Queensland Museum 23*: 1-438.

Kott, P. (1990). The Australian Ascidacea. Part 2, Aplousobranchia. *Memoirs of the Queensland Museum 29(10)*: 1-298.

Lambert, C.; Brandt, C. (1967). The effect of light on the spawning of *Ciona intestinalis*. *Biological Bulletin 132(2)*: 222-228.

Lambert, C.; Lambert, G. (1998). Non-indigenous ascidians in southern California harbours and marinas. *Marine Biology 130*: 675-688.

Lambert, C.C.; Lambert, G. (2003). Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. *Marine Ecology Progress Series 259*: 145-161.

Lützen, J. (1999). Styela clava Herdman (Urochordata, Ascidiacea), a successful immigrant to North West Europe: Ecology, propagation and chronology of spread. *Helgolaender Meeresuntersuchungen 52*: 383-391.

Lutzen, J.; Sorensen, V. (1993). Ecology, reproduction and further spread of the immigrant East-Asiatic ascidian Styela clava Herdman in Danish waters. *Flora og Fauna 99(2)*: 75-79.

Martin, P.A.J.; Cameron, A.R.; Greiner, M. (2007). Demonstrating freedom from disease using multiple complex data sources. 1: A new methodology based on scenario trees. *Preventive Veterinary Medicine 79(2-4)*: 71-97.

Mazouni, N.; Gaertner, J.-C.; Deslous-Paoli, J.-M. (2001). Composition of biofouling communities on suspended oyster cultures: an *in situ* study of their interactions with the water column. *Marine Ecology Progress Series 214*: 93-102.

McClary, D.J.; Phipps, C.; Hinni, S. (2008). Reproductive behaviour of the Clubbed Tunicate, *Styela clava*, in northern New Zealand waters *MPI Biosecurity New Zealand Technical Paper No: 2009/01 No.* 34 p.

McDonald, J.I.; Wells, F.E.; Travers, M.J. (2009). Results of the 2007 survey of the Albany marine area for introduced marine species. Fisheries Research Report No. 188. Department of Fisheries, Western Australia. 30 p.

Millar, R. (1971). The biology of ascidians. *Advances in Marine Biology 9*: 1-100.

Minchin, D.; Davis, M.H.; Davis, M.E. (2006). Spread of the Asian tunicate *Styela clava* Herdman, 1882 to the east and south-west coasts of Ireland. *Aquatic Invasions 1(2)*: 91-96.

Morgan, T. (1938). The genetic and the physiological problems of self-sterility in *Ciona*. I. Data on self- and cross-fertilization. *Journal of Experimental Zoology 78*: 271-318.

Morgan, T. (1940). An interim report on cross and self-fertilization in *Ciona*. *Journal of Experimental Zoology 85*: 1-32.

Morgan, T. (1945). The conditions that lead to normal or abnormal development of *Ciona*. *Biological Bulletin 88*: 50-62.

Morita, T.; Kurashima, A.; Maegawa, M. (2003). Temperature requirements for the growth and maturation of the gametophytes of *Undaria pinnatifida* and *U. undarioides* (Laminariales, Phaeophyceae). *Phycological Research 51*: 154-160; in Choi et al. 2005.

Nakayama, A.; Satoh, N.; Sasakura, Y. (2005). Tissue-specific profile of DNA replication in the swimming larvae of *Ciona intestinalisZoological Science 22*: 301-309.

Nandakumar, K. (1996). Importance of timing of panel exposure on the competitive outcome and succession of sessile organisms. *Marine Ecology Progress Series 131*: 191-203.

Niermann-Kerkenberg, E.; Hofmann, D. (1989). Fertilization and normal development in Ascidiella aspersa (Tunicata) studied with Nomarski-optics. *HelgolanderMeeresuntersuchungen 43*: 245-258; in: Stachowicz et al. 2002

NIMPIS. (2001). *Styela clava* general information. *National Introduced Marine Pest Information System, Viewed: 30 June 2011 <http://www.marinepests.gov.au/nimpis>*p. Retrieved 30 June 2011, from <http://www.marinepests.gov.au/nimpis>.

NIMPIS. (2011). *Undaria pinnatifida* general information. *National Introduced Marine Pest Information System, viewed 30 June 2011 <http://www.marinepests.gov.au/nimpis>*p. Retrieved, from

Nutsford, S.C. (2010). Reproductive dynamics of the invasive ascidian, *Styela clava*. University of Canterbury, Christchurch. p.

Öhlund, E. (1977). A comparative study of three zooplankton communities on the Swedish west coast with respect to compositions, dynamics and function. PhD Thesis. University of Göteborg, Sweden, Göteborg. 181 p.

Osman, R.W.; Whitlach, R.B. (1999). "Ecological interacations of invading ascidians within epifaunal communities of southern New England." Presented at the Marine bioinvasions: proceedings of conference January 24-27 1999, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Parker, L.E.; Culloty, S.; O'Riordan, R.M.; Kelleher, B.; Steele, S.; Van der Velde, G. (1999). Preliminary study on the gonad development of the exotic ascidian *Styela clava* in Cork Harbour, Ireland. *Journal of the Marine Biological Association of the United Kingdom 79(6)*: 1141-1142.

Parsons, M. (1994). Status of the introduced brown seaweed *Undaria* in New Zealand. *Conservation Advisory Science Notes No. 112; Department of Conservation, Wellington No.* 24 p.

Patti, F.P.; Gambi, M.C. (2001). Phylogeography of the invasive polychaete *Sabella spallanzanii* (Sabellidae) based on the nucleotide sequence of internal transcribed spacer 2 (ITS2) of nuclear rDNA. *Marine Ecology Progress Series 215*: 169-177.

Pemberton, A.J.; Hughes, R.N.; Manriquez, P.H.; Bishop, J.D.D. (2003). Efficient utilization of very dilute aquatic sperm: sperm competition may be more likely than sperm limitation when eggs are retained. *Proceedings of the Royal Society - Biological Sciences (Series B) 270*: S223-S226.

Petersen, J.; Svane, I. (1995). Larval dispersal in the ascidian *Ciona intestinalis* (L.). Evidence for a closed population. *Journal of Experimental Marine Biology and Ecology 186*: 89-102.

Pinto, M.; Santis, R.; Marino, R.; Usui, N. (1995). Specific induction of self-discrimination by follicle cells in *Ciona intestinalis* oocytes. *Development, Growth & Differentiation 37(3)*: 287-291.

Pollard, D.A.; Rankin, B.K. (2003). Port of Eden Introduced Marine Pest Species Study. *NSW Fisheries Final Report Series No. 46 No.* 71 p.

Primo, C.; Hewitt, C.; Campbell, M. (2010). Reproductive phenology of the introduced kelp *Undaria pinnatifida* (Phaeophyceae, Laminariales) in Port Phillip Bay (Victoria, Australia). *Biological Invasions 12*: 3081-3092.

Ramsay, A.; Davidson, J.; Bourque, B.J.; Stryhn, H. (2009). Recruitment patterns and population development of the invasive ascidian *Ciona intestinalis* in Prince Edward Island, Canada. *Aquatic Invasions 4*: 169-176.

Ramsay, A.; Davidson, J.; Landry, T.; Arsenault, G. (2008). Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. *Biological Invasions 10*: 1311-1316.

Reed, D.; Amsler, C.; Ebeling, A. (1998). Variation in algal dispersal and recruitment: the importance of episodic events. *Ecological Monographs 58*: 321-335.

Rosati, F.; Santis, R. (1978). Studies on fertilisation in the ascidians. I. Self-sterility and specific recognition between gametes of *Ciona intestinalis*. *Experimental Cell Research 112*: 111-119.

Rouse, G.W.; Fitzhugh, K. (1994). Broadcasting fables: is external fertilization really primitive? Sex, size and larvae in sabellid polychaetes. *Zoologica Scripta 23*: 271-312.

Rumrill, S.S. (1990). Natural mortality of marine invertebrate larvae. *Ophelia 32(1-2))*: 163-198.

Russ, G.R. (1982). Overgrowth in a marine epifaunal community: competitive hierarchies and competitive networks. *Oecologia 53*: 12-19.

Russell, K.; Hepburn, C.D.; Hurd, C.; Stuart, M. (2008). The expanding range of Undaria pinnatifida in southern New Zealand: distribution, dispersal mechanisms and the invasion of wave- exposed environments. *Biological Invasions 10*: 103-115.

Saito, Y. (1956). An ecological study of *Undaria pinnatifida* Sur.-I: On the influence of environmental factors upon the development of gametophytes. *Bulletin of the Japanese Society for Scientific Fisheries 22*: 229-234 (in Japanese), in: Morita et al. 2003.

Saito, Y. (1975). *Undaria*. *In*: Tokida, J.; Hirose, H. (eds). Advances in Phycology in Japan, pp. 304-320. Junk: The Hague,

Schaffelke, B.; Campbell, M.L.; L., H.C. (2005). Reproductive phenology of the introduced kelp *Undaria pinnatifida* (Phaeophyceae, Laminariales) in Tasmania, Australia. *Phycologia 44*: 84-94.

Schmidt, G.; Warner, G. (1984). Effects of caging on the development of a sessile epifaunal community. *Marine Ecology Progress Series 15*: 251-263.

Skerman, T.M. (1959). Marine fouling at the port of Auckland. *New Zealand Journal of Science 2(1)*: 57-94.

Southwood, T.R.E. (1978). Ecological Methods with Particular Reference to the Study of Insect Populations. Chapman and Hall, London. p.

Stabili, L.; Schirosi, R.; Licciano, M.; Giangrande, A. (2009). The mucus of *Sabella spallanzanii* (Annelida, Polychaeta): Its involvement in chemical defence and fertilization success. *Journal of Experiemental Marine Biology and Ecology. 374*: 144-149.

Stachowicz, J.J.; Fried, H.; Osman, R.W.; Whitlatch, R.B. (2002). Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process. *Ecology 83(9)*: 2575-2590.

Stoner, D. (1990). Recruitment of a tropical colonial ascidian: relative importance of pre-settlement vs. post-settlement processes. *Ecology 71*: 1682-1690.

Streeter, V.L.; Wylie, E.B. (1983). Fluid Mechanics. McGraw-Hill, New York. p.

Stuart, M. (1997). Seasonal ecophysiology of *Undaria pinnatifida* in Otago Harbour, New Zealand. PhD Thesis. University of Otago, New Zealand, 193 p.

Stuart, M. (2004). Review of research on *Undaria pinnatifida* in New Zealand and its potential impacts on the eastern coast of the South Island; DOC Science Internal Series 166 *No.* p.

Suto, S. (1950). Studies on shedding, swimming and fixing of the spores of seaweeds. *Bulletin of the Japanese Society for Scientific Fisheries 16*: 1-9.

Svane, I. (1983). Ascidian reproductive patterns realted to long-term population dynamics. *Sarsia 68*: 249-255.

Svane, I. (1984). Observations of the long-term population dynamics of the perennial ascidian *Ascidia mentula* O. F. Mueller, on the Swedish west coast. *Biological Bulletin 167*: 630-646.

Svane, I.; Havenhand, J. (1993). Spawning and dispersal in *Ciona intestinalis* (L.) *Marine Ecology 14(1)*: 53-66.

Svane, I.; Young, C. (1989). The ecology and behaviour of ascidian larvae. *Oceanography and Marine Biology: an Annual Review 27*: 45-90.

Szewzyk, U.; Holmstrom, C.; Wrangstadh, M.; Samueelsson, M.O.; Malci, J.; Kjlleberg, S. (1991). Relevance of the exopolysaccharide of marine Pseudomonas sp. Strain S9 for the attachment of *Ciona intestinalis* larvae. *Marine Ecology Progress Series 75*: 259-265.

Tamura, T. (1970). Section 21.2.2: Propagation of *Undaria pinnatifida*. *In*: Tamura, T. (ed.). Marine Aquaculture. National Technical Information Service, National Science Foundation. Translated from Japanese by Mary I. Watanabe. 2nd rev. ed. NTIS 1970, Washington DC, pp. 14.

Thompson, G.A. (2004). Mechanisms of invasion and persistence of the invasive kelp *Undaria pinnatifida* (Harvey) Suringar within intertidal areas of southern New Zealand. PhD Thesis. University of Canterbury, Christchurch. 166 p.

Thornber, C.S.; Kinlan, B.P.; Graham, M.H.; Stachowicz, J.J. (2004). Population ecology of the invasive kelp *Undaria pinnatifida* in California: environmental and biological controls on demography. *Marine Ecology Progress Series 268*: 69-80.

Thorson, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates. *Meddr. Kommn. Danm. Fisk. – og Havunders., (Plankton Series) 4*: 1-523; in: Svane and Havenhand 1993.

Tursi, A. (1980). Quelques aspects de la fixation de *Ciona intestinalis* (L.) – Tunicata. *Vie et milieu (Life and environment), Paris 30(3-4)*: 243-251.

Underwood, A.J.; Fairweather, P.G. (1989). Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution 4(1)*: 16-20.

Valentine, J.P.; Johnson, C.R. (2003). Establishment of the introduced kelp *Undaria pinnatifida* in Tasmania depends on disturbance to native algal assemblages. *Journal of Experimental Marine Biology and Ecology 295(1)*: 63-90.

Venette, R.C.; Moon, R.D.; Hutchison, W.D. (2002). Strategies and statistics of sampling for rare individuals. *Annual Review of Entomology 47*: 143-174.

Wallace, H. (1961). The breeding and development of *Styela mammiculata* Carlisle. *Journal of the Marine Biological Association of the United Kingdom 41*: 187-190.

Webber, D. (2010). Demography and population projections of the invasive tunicate *Styela clava* in southern New Zealand. University of Canterbury, Christchurch. p.

West, A.; Lambert, C. (1976). Control of spawning in the tunicate *Styela plicata* by variations in a natural light regime. *Journal of Experimental Zoology 195*: 263-270.

Whittingham, D. (1967). Light induction of shedding of gametes in *Ciona intestinalis* and *Mogula manhattensis*. *Biological Bulletin 132*: 292-298.

Wieczorek, S.K.; Todd, C.D. (1997). Inhibition and facilitation of bryozoan and ascidian settlement by natural multi-species biofilms: effects of film age and the roles of active and passive larval attachment. *Marine Biology 128*: 463-473.

Yamaguchi, M. (1970). Spawning periodicity and settling time in ascidians, *Ciona intestinalis* and *Styela plicata*. *Records of Oceanographic Works 10(2)*: 147-155.

Yamaguchi, M. (1975). Growth and reproductive cycles of the marine fouling ascidians *Ciona intestinalis, Styela plicata, Botrylloides violaceus* and *Leptoclinum mitsukurii* at Aburatsubo-Moroiso. *Marine Biology 219*: 253-259.

Young, C.M. (1995). Behaviour and locomotion during the dispersal phase of larval life. *In*: McEdward, L.R. (ed.). Ecology of Marine Invertebrate Larvae, pp. 249-277. CRC Press, Boca Raton.

Young, C.M.; Chia, F.S. (1981). Laboratory evidence for delay of larval settlement in response to a dominant competitor. *International Journal of Invertebrate Reproduction 3*: 221-226.

Yund, P.O. (2000). How severe is sperm limitation? *Trends in Ecology & Evolution 15*: 10-13.

Zardus, J.; Nedved, B.; Huang, Y.; Tran, C.; Hadfield, M. (2008). Microbial biofilms facilitate adhesion in biofouling invertebrates. *Biological Bulletin 214*: 91-98.