

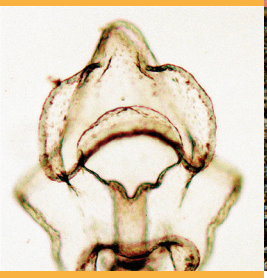


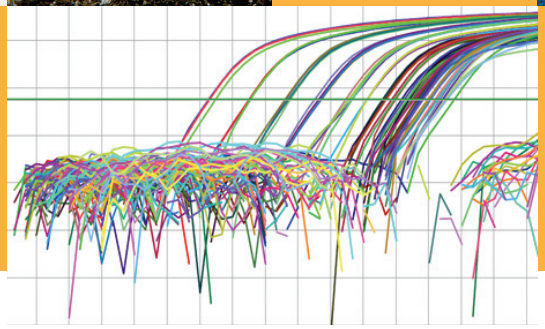
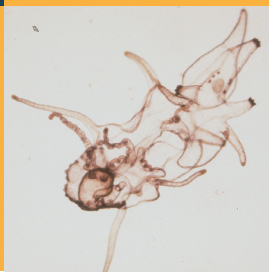

Natural Heritage Trust

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An Australian Government Initiative

April 2006

Evaluation of National Control Plan management options for the North Pacific Seastar *Asterias amurensis*

Prepared by:

Nicholas Bax, Piers Dunstan, Rasanthi Gunasekera, Jawahar Patil and Caroline Sutton

Final Report for the Department of Environment and Heritage by CSIRO Marine Research

CSIRO

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1. EXECUTIVE SUMMARY

National control plans are an integral component of the developing National System for the Prevention and Management of Marine Pests. A control plan has been developed for *Asterias amurensis*, which threatens Australia's southern ocean waters from Sydney to Perth WA out to a depth of at least 100m.

Management strategy evaluation, a procedure developed in the International Whaling Commission in the early 1990s, was used to assess the consequences of a range of management strategies and present the results in a way that clarified their performance against management objectives.

The first project deliverable was to provide "*a forum for environmental managers, industry and NGOs to discuss and prioritise management options for A. amurensis".* Two forums were held as part of this project. Steady progress was made in defining suitable management objectives, strategies and actions. Early discussions with managers (as part of an earlier NHT-funded project) started with "maintaining biodiversity" as the management objective. Since biodiversity is neither clearly defined nor readily measurable it was clear that it did not provide the necessary quantitative rigour against which alternative strategies could be evaluated. Subsequent refinement led to "reducing the spread of invasive species" which provided a measurable objective, but did not differentiate between highly-valued and less-valued habitats, nor between areas where a pest species could be expected to spread anyway (in prevailing currents) and areas where it could only reach on(in) an anthropogenic vector. In the first workshop held as part of this project, management objectives were further honed to "stopping human-mediated dispersal" and "protecting high-value habitats". Progress was also made on defining potential management strategies, with the decision being made to concentrate on ballast water as a vector.

In a second workshop held in April of this year that included government managers, community groups, NGOs and industry, a more structured approach was taken to developing management and operational objectives, actions and performance indicators. After some interpretation, this resulted in 4 core objectives, 8 operational objectives, and 23 actions with associated indicators. The results from this workshop will be reported in August to the National Introduced Marine Pest Coordination Group. One unique contribution of this workshop was to extend the often highly specific scientific and policy objectives associated with control plans to include:

- community beliefs about invasion processes and the relative value of different measures to address invasions
- community values (and approaches to involve communities)
- international responsibilities

- ensuring that invaded areas remain valued for their other attributes
- providing appropriate infrastructure to enable effective user education and vector management, especially in the recreational sectors.

It was also clear that the MSE approach provided an effective framework to involve diverse stakeholder groups in determining desirable management objectives, strategies, actions and performance indicators. For many participants, being informed and being involved was the most valuable aspect of the workshop, although there was some concern over how (or whether) stakeholder input to the workshop would be taken forward in future deliberations. Several participants saw the need to repeat this workshop in different areas and at different times in the future and some improvements were recommended. New ways of presenting the tradeoffs between different management options determined by the MSE modelling will be needed as the stakeholder groups attending these workshops become increasingly informed on the issues and possibilities. There is the potential to repeat this workshop in other southern Australian states affected or likely to be affected by the seastar.

The second project deliverable was to provide: "*A MSE model that can be used to compare management strategies for the control and management of A. amurensis and their performance against agreed performance measures".*

The operational model for the MSE comprises biological and physical components that resemble the dynamics of the natural systems off southern Australia. An anthropogenic layer was added to describe the movement of commercial shipping. Management objectives, strategies and performance measures are still being developed and this constrained evaluation of alternative strategies.

Strategies evaluated were: compulsory ballast water exchange; ballast water exchange following the Decision Support System (DSS) with different monitoring levels; genetic control – daughterless, sonless, and female lethal; and physical removal.

Ninety five percent exchange does not achieve result in a 95% reduction in the probability of invasion. There are complex interplays between larval production, larval retention and the exchange efficiency that will determine the exact reductions in probability from location to location. Thus the results presented here are specific to *A. amurensis.*

Sites that have long retention times (i.e lagoons and estuaries with narrow entrances or very large volumes) and sites where limited space leads to higher larval densities are at greater risk from *A. amurensis* and 95% reduction will be insufficient to prevent establishment. Increasing the number of tanks of flow through exchange further decreases the risks of establishing a population at any density. However, the median probability is never reduced by 95%.

For sites with greater larval exchange and reduced larval densities, 95% reduction in larval supply is sufficient to prevent establishment. Reducing the number of tanks exchanged to one does not have a dramatic impact on establishment probability and is enough to limit growth in many of the sites that are invaded without ballast control. These sites are marginal habitat for *Asterias* (i.e. in terms of habitat size and retention time).

Using the risk tables to determine the reballasting requirements achieved a similar reduction in invasion probability to the best outcomes for mandatory exchange with costs at least 10 times lower than mandatory exchange. The cost of monitoring did not significantly increase the cost of management when the risk tables were used, despite the assumption that monitoring occurred in all 104 ports present in Southern Australia. Using risk tables to manage ballast water limits the number of times ballast water must be exchanged and controls the spread of an invasive species as efficiently as mandatory exchange. However, the effectiveness of risk tables is very dependent on accurate monitoring of ports and prompt management responses when an invasion is detected. Removing ballast water exchange requirements should not occur until it is certain that a self-sustaining population is established.

The three genetic control techniques examined all provide potential for the removal of pest populations. However they vary in the effort needed to achieve the removal of a relatively small population in the Derwent estuary. All three techniques operate by reducing the number of larvae that survive to reproduce in the next generation. The effectiveness of sonless over daughterless is dependent on the dynamics of fertilisation. Daughterless control limits the production of eggs, but in this case increases the fertilisation rate of those remaining. Sonless increases egg production, but decreases the number of eggs fertilised. Sonless is more efficient as it targets the limiting process in the reproductive cycle of *Asterias*. Similar dynamics may exist for many marine species that are broadcast spawners with external fertilisation. This will not be true for species where the supply of eggs limits population growth rate for these species, daughterless or female sterile control would be more effective control options.

At an early stage in the invasion, controlling the source population(s) through genetic sex-ratio distortion is the most effective control method. As the species becomes more established throughout southern Australia, the benefits of controlling the increasing number of source populations are reduced and controlling the spread becomes more important. Compulsory ballast water exchange (3 tank flow thru; no route deviation) and adding transgene seastars to selected estuaries to reduce the probability of further spread achieve similar results. Costs associated with the development and maintenance of genetic techniques appear at first glance to be less than those associated with exchange based on the risk tables, however, this comparison does not account for the role of ballast water exchange in reducing the invasion probability of other invasive species, nor does it take into account the risks associated with the genetic control, in particular that hatchery-reared seastars would not be able to compete with naturally produced seastars.

Improved estimates of costs and effectiveness of genetic techniques will be needed before they can be proposed as a viable management option.

The effect of physical removal is tied very closely to assumptions of density dependence in the model and we cannot estimate its effect without further empirical evidence. However, the intrinsic growth rate of populations in estuaries indicates the proportion of the population that will need to be removed in order to reduce the population. The mean intrinsic growth rates, calculated from MSE simulations, of populations in Westernport, Andersons inlet, Lake Tyers and Mallacoota Inlet were 0.096, -0.234, 0.196 and 0.416 respectively. In order to limit the growth of population in these locations, it is necessary to remove 10% of the population from Westernport per year, 20% of the population from Lake Tyers and 50% of the population from Mallacoota Inlet. Andersons Inlet is the exception as it is unable to sustain a population without continual input from Port Phillip Bay.

We have started validating the predictions of the MSE model with regard to larval spread by developing a quantitative genetic technique that will have broad application to the monitoring of all pest species. A nested PCR approach was developed, where environmental samples were first amplified with non-specific primers in a standard PCR, then amplified with specific primers in real-time PCR, using a TaqMan probe for quantification. Primers previously developed for *Asterias* were refined. A TaqMan probe was developed which is specific to *A. amurensis* unlike the primers that cannot distinguish this species from *A. rubens.*

The real-time PCR technique was used to estimate *A. amurensis* larval densities off eastern Tasmania and southern Victoria. The genetic technique worked well – its was more sensitive than the standard nested PCR approach and provided reliable estimates of DNA. However, the sensitivity of this approach highlighted problems in standard techniques of plankton splitting used to subsample large samples. While the Fulsom plankton splitter does a good job dividing the total biomass (or DNA content) of a sample, it does not evenly divide rare organisms within that sample, i.e. *A. amurensis* larvae. Further work on field sampling techniques is required. Use of high velocity tissue disintegrator is recommended for larger environmental samples.

Based on the DNA content of an individual stage 1 bipinnaria larvae being 1000 pg and given the caveats associated with the non-uniform sample splitting there were between 50 and 1000 larvae per tow off Maria Island in September and 1 larvae or less per tow (i.e. a fragment) in all other areas and times. Of the five areas in Victoria, only one – Ann St pier in Williamstown, Port Phillip Bay – was positive for *A. amurensis* (Table 7). All three samples were positive and larval densities were of the order of 1 or 2 per sample.

Reanalysis of the Port of Hastings data with real-time PCR study (Patil et al 2004), would be informative. While in that study, we were able to show when ballast water considered low-risk by the risk assessment Decision Support System (DSS) contained the target species, we were not able to determine the concentration of target species and this restricted our ability to distinguish

a failure in the ballast water management system from an acceptable reduction in risk. Quantitative DNA estimates would further this analysis.

Finally, as a prelude to developing genetic options for the management of *A. amurensis,* we have begun a systematic characterisation of the molecular pathways involved in sex differentiation and determination in the species. The conserved DNA binding domain (the DM domain) is found in a family of transcription factors important for sexual determination across phyla. In a preliminary study we identified one gene in the DM domain of *A. amurensis* that appears to be sex specific. However this needs to be further evaluated for its expression pattern during early larval ontogeny and in other adult tissues.

The third project deliverable was the production of this report and the fourth development of "*A MSE framework that can be extended to other high profile marine pest species. This framework will be presented at NIMPCG and used to discuss how to address developing National Control Plans for other species.*"

The MSE framework developed for *A. amurensis* is readily transferable to other species. Recent completion of BlueLink allows us to extend the MSE model to all Australian waters. The main model components would remain the same in application to other species, all that is required is to update the biological information for the new species and its uptake by vectors. A statistical module representing recreational boat traffic needs to be developed because these data have not come from other projects.

Once the MSE framework has been extended to cover the entire Australian coast, it will be possible to evaluate management strategies for performance over the range of marine pest species deemed high risk to Australia. This will provide a means to link the management strategies, actions and indicators for all National Control Plans so that marginal benefits for each species and overall benefits for all species can be determined.

Early in the project it became clear that the MSE framework provided more than a mechanism to assist the development of clear management objectives, strategies and performance measures for managing marine pests. The process involved in developing these attributes also identified the values and beliefs of diverse stakeholders. And perhaps as importantly allowed those stakeholders to be included (and to be felt to be included) in the management process.

Further workshops in other affected States will ensure broad input to defining objectives, etc, and a process to include a diverse group of stakeholders from different areas. Ensuring that stakeholders feel included in the process is an important component of developing the National System as many of the vector management measures will be voluntary depending on stakeholder goodwill. Experience from other areas suggests that involving these stakeholders in the process to determine why and which management measures are needed will be an effective approach to achieving high uptake.

Finally, we have started to explore the possibility of alternative presentation of the results that communicate the essential information from MSE in an easy to understand graphical format.

CONTENTS

2. BACKGROUND AND AIMS

National control plans are an integral component of the developing National System for the Prevention and Management of Marine Pests. It is proposed that a National Control Plan be developed for all introduced marine pests in Australia that are seen as likely to cause significant economic and/or environmental harm. Because, management actions can be costly to industry, it is planned that a National Control Plan will be required for any species managed under the domestic ballast water risk assessment process. To date only one national control plan has been developed – the one for the Northern Pacific seastar, *Asterias amurensis*, which threatens Australia's southern ocean waters from Sydney to Perth WA out to a depth of at least 100m.

It is important that control plans provide some prioritisation of options to achieve specified management objectives and that there is an objective framework through which prioritisation can be made. As a first step in the objective evaluation of management options, the National Heritage Trust funded a project for the Department of the Environment and Heritage to compare alternative approaches to restricting the spread of *Asterias amurensis*. A Management Strategy Evaluation (MSE) approach was used to clarify management objectives, establish performance measures, and evaluate the success (or failure) and costs of different management strategies in achieving these objectives (Bax et al. 2003, Bax and Dunstan 2004).

Management strategy evaluation is used to assess the consequences of a range of management strategies or options and present the results in a way which lays bare the tradeoffs in performance across a range of management objectives. The procedure was developed in the International Whaling Commission in the early 1990s, and is consistent with the Environmental Management System (ISO 14001/4: 1996). The approach is mandated in the US for fisheries in recovery mode and in Canada for all fisheries.

The approach was first developed in Australia in 1994 to examine management alternatives for the severely depleted East coast gemfish fishery, has since been used in other Australian fisheries, and is currently being applied to whole of system management for the Northwest shelf.

Management strategy evaluation involves constructing a quasi-realistic model of the system to be managed, including system dynamics, assessment and monitoring capability, management response and implementation uncertainty (Figure 1). Uncertainty can be introduced at any step; for example assessment of system state would have associated uncertainty and potentially bias reflecting the situation faced by managers in the real world.

There are several advantages of this approach over previously other methods that often attempt to define the optimum management response to a given situation:

- the MSE approach does not attempt to predict the single most likely future, but instead attempts to determine the full range of potential futures and then determine which management strategy is most successful over this range

- the approach does not try to determine what might be an optimum management outcome for a given situation, but (ideally) makes it clear in simple terms (or graphics) what are the various likely outcomes of different strategies. This allows the managers to review the options and come to their own decisions

- the approach requires that there are quantitative performance measures, targets, thresholds to measure performance against objectives. This requires stakeholders to determine management objectives, and values

- the approach includes our lack of complete knowledge of a natural system and implementation uncertainty on management intervention

- ideally the approach can be used to evaluate adaptive management strategies where the value of updating management strategies based on acquiring further information can be evaluated.

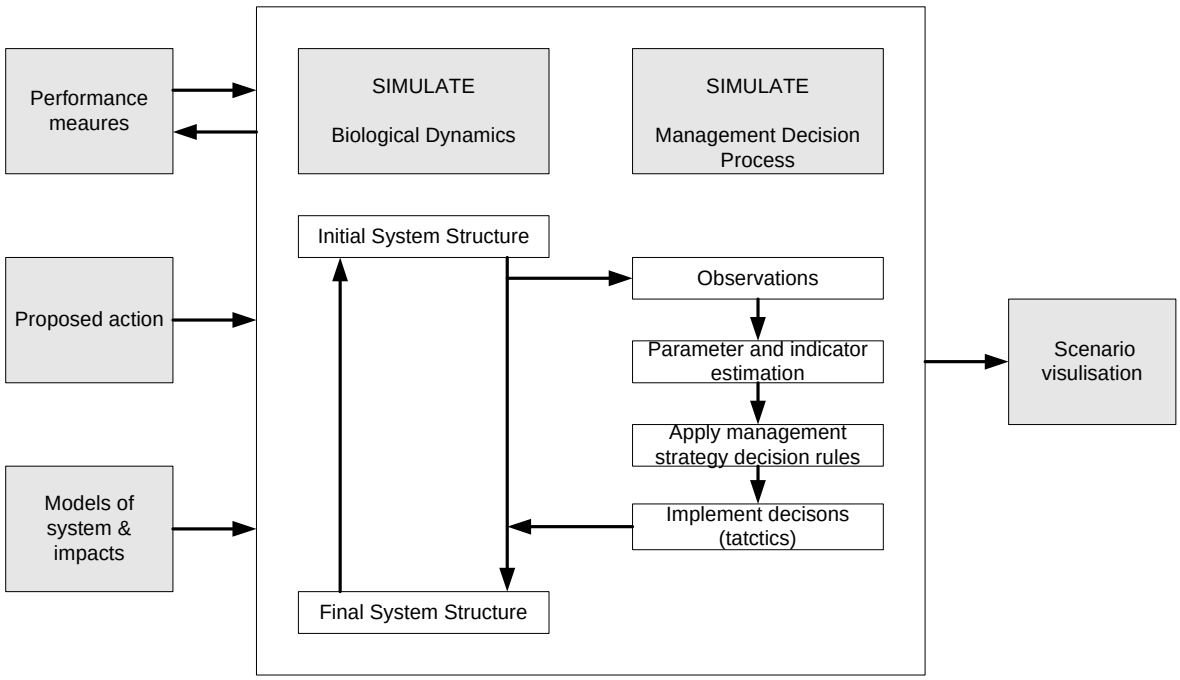

Figure 1. General framework for monitoring/management strategy evaluation (from Sainsbury pers. comm.)

The management strategy evaluation approach was developed to test the efficacy of alternative management strategies in reducing the possible effects of *A. amurensis* (Bax and Dunstan 2004). Workshops were held to determine qualitatively which of 18 potential vectors pose the greatest threat of transporting this species beyond its current major concentrations of the Derwent River estuary and Port Phillip Bay. Ballast water was considered to be the most significant vector by far and was the focus of Bax and Dunstan (2004). While it was clear that reballasting was effective at reducing spread (in the model) we were not able to distinguish between the various exchange options as their efficacy was linked to assumptions of larval mortality which were ill-defined.

Our overall aim in this project was to use the MSE approach as an approach to evaluate the National Control Plan for *A. amurensis*, and more generally to provide a framework that would assist the development and evaluation of any National Control plans for other marine invasive species. In particular, we aimed to:

- improve definition of management objectives, strategies and performance measures
- resolve, to the extent possible, ambiguities over larval mortality and re-evaluate ballast water management options
- extend the MSE approach to include other potential control methods
- extend the MSE approach to include other vectors (as data become available)
- develop quantitative genetic approaches that could be used to test the model predictions with respect to larval distribution and transport.

2.1. Project Objectives and Deliverables

The objectives are:

1. Extend the existing MSE framework so that population level processes of control and management can be evaluated;
2. Examine and prioritise alternative management and control options for *A.* amurensis, in conjunction with State and Commonwealth environmental managers, industry and NGOs;
3. Develop a quantitative gene probe for use in real-time PCR for the quantitative estimation of *A. amurensis* larvae from unsorted plankton samples;

4. Conduct limited field sampling of plankton to test predictive capacity of the model for spread and establishment of *A. amurensis* outside the Derwent estuary;

5. Provide a framework that can be developed to evaluate and prioritise management options for other high priority marine pest species, eg. By NIMPCG; and
6. Integrate the marine pest MSE framework with the SE Australian MSE framework being developed by CSIRO so that management of marine pests can be considered in conjunction with other environmental management programs (eg. Fishing; MPAs; aquaculture). This objective is subject to the progress on other independent projects.

The deliverables are:

1. A forum for environmental managers, industry and NGOs to discuss and prioritise management and control options for *A. amurensis;*

2. A MSE model that can be used to compare management strategies for the control and management of *A. amurensis* and their performance against agreed performance measures;

3. A final report that details the estimated benefits and costs of management and control options in the National Control Plan for *A. amurensis;* and

4. A MSE framework that can be extended to other high profile marine pest species. This framework will be presented at NIMPCG and used to discuss how to address developing National Control Plans for other species.

3. WORKSHOPS

3.1. Commonwealth/State managers, scientists, June 24th 2004

Participants included: Simon Barry (BRS), Nic Bax & Piers Dunstan (CSIRO), Warren Geeves & Sarah Johnstone (DEH), John Gilliland (SA), Don Hough (VIC) Alice Morris (TAS), Naomi Parker & Jacinta Innes (DAFF).The aims of this workshop were to summarise the progress on developing a management strategy evaluation approach for *A. amurensis* and to get feedback on future development of the model (Appendix II). The second aim was to start developing management objectives and performance indicators. The main outputs from this workshop were:

Additional roles identified for the MSE approach:

- mechanism for broader stakeholder consultation
- communication
- helping society make decisions.

Management objectives defined:

- stop human-mediated dispersal
- protect high-value habitats
- concentrate on ballast water in first instance

Technical options and management strategies

- importance of different vectors for *A. amurensis* (and others)
- identification of nodal points
- include costs
- how to measure effectiveness of removal (numbers, density?)
- determining when it would be worthwhile to intervene (likely outcome, contrast with doing nothing)
- value of buying time through intervention
- identifying types and areas of intervention with highest benefit cost ratio
- identifying key areas for monitoring

Uncertainties in modelling approach

- establishment success (field sampling required)
- has full range of uncertainty been captured
- full range of vectors needed

3.2. Forum for managing the North Pacific Seastar, Melbourne, April 14th, 2005, Final workshop report.

Over 100, possibly as many as 400, marine species from around the world have established populations in Australian waters. Some like the North Pacific seastar, which has a population of ~100 million in Port Phillip Bay, are highly visible predators of shellfish as they can be readily seen in the shallow waters they invade. Others like the New Zealand screwshell, which covers an area of the continental shelf the size of Tasmania, are unseen by most of us, but are permanently changing our marine environment. These and other introduced marine species threaten our marine environment, economy and social amenities.

In response to this national threat, The Commonwealth and State governments are working together to develop and implement a National System for the Prevention and Management of Marine Pest Incursions. Each species of concern will have an associated control plan that will focus Government, industry and community activities to provide maximum relief from the impacts of the pest species. CSIRO is assisting in the development of control plans, in particular that for the North Pacific seastar to determine objectives for its management and control (what do we want to achieve), performance standards (how will we know if we are making progress), and an evaluation of alternative management approaches (selecting the best tool for the job).

As part of this development CSIRO, with the cooperation of the Victorian Government, held a public stakeholder workshop. The objective of this workshop was to provide stakeholder input on the objectives, standards and approaches that should be included in the control plan for the North Pacific seastar and, by extension, other species in the future.

Twenty one people attended the workshop, representing a broad range of Commonwealth, State and local agencies, key recreational and industry bodies, scientists, consultants and environmental groups (Appendix 1).

The workshop started with Warren Geeves providing the background for the National System for Prevention and Management of Marine Pest Incursions and the role played by National Control Plans in this process (Appendix 2). Nic Bax provided a brief synopsis of the current status of the Northern Pacific seastar, *Asterias amurensis* and introduced the concept of using a computer model to evaluate the success of alternative management strategies in the face of the uncertainties expected in managing natural systems, stressing the need for clear objectives and performance criteria to proceed. Piers Dunstan demonstrated how the management strategy evaluation (MSE) approach had been applied to the seastar, including visualisation of the underlying biological and oceanographic components, and the evaluation of alternative ballast water management options.

The workshop then divided into three teams to develop sets of objectives for managing the seastar. Objectives (components) from all teams were then amalgamated and regrouped into High level objectives: Overarching Objectives, Prevention, Rapid Response and Control and Management (the option of developing alternative High level objectives to group the components was briefly suggested but it was rapidly decided that the High level objectives used in the National System were most appropriate). Following this grouping process, each team took on the task of developing operational objectives and performance indicators for one of the High level objectives. The time left at the end of the workshop was used for a general question and answer session rather than reporting back. It was agreed that reporting back would occur through this report. Workshop participants were asked to fill out a form evaluating the workshop before they left.

The original objectives and performance indicators, as transcribed from the paper output, are provided in Appendix 3. I summarised and reorganised these notes (Appendix 4), before adding a level of interpretation and simplification to produce a final list of High level objectives, components, operational objectives and performance indicators (Table 1). Note that the names given to objectives and indicators has consolidated since the workshop, and I have reflected those changes here.

Table 1. Interpreted objectives and performance indicators

High level objective	Component	Operational objective (action)	Performance indicator
How are we going to do it → ← What are we trying to achieve			
Overarching Effective management	Effective management	Adaptive approach	Clear management objectives, goals, approach, monitoring and response
		Appropriate cost/benefit	C/B ratio at or below C/B ratio for other environmental management
		No missed opportunities	Number of established populations minimised by action
		Mitigate for any loss in environmental services	Net environmental loss close to zero
		Sufficient knowledge to make appropriate management choices	Understand current distribution, potential distribution, major nodes, vectors and impacts
	Community involvement	Effective community involvement	1. Communities values, knowledge of risk and acceptance of damage measured. 2. Education programs and workshops established (eg. school curricula). 3. Proportion of community (in any sector) aware and using preventative measure, attending workshops, at or above median levels for similar environmental projects AND increasing. 4. Proportion of informative reports (eg. correct ids) increasing. 5. Community understands continuing values of invaded areas.
Prevention	Prevent further spread	Prevent spread overseas	Number of larvae carried overseas reduced
		Prevent self-sustaining populations establishing in areas outside current range of natural spread	Number of populations established in SA or WA, especially high value areas – MPAs, high community value, fisheries
		Prevent self-sustaining populations establishing in current range of natural spread	Number of potentially self-sustaining populations minimised, especially in high value areas – MPAs, high community value, fisheries

Table 1. continued

Objective	Component	Operational objective	Performance indicator
		Prevent wilful introductions	Effective deterrents available
		Receiving environments managed to reduce risk of spread	Identified nodes managed to reduce risk of establishment (eg. estuary openings, vectors) and monitored if early eradication an option
Rapid Response	Early detection	Detect new invasions early enough to enable rapid response where appropriate	Appropriate locations for rapid response identified. Early warning program in place. Proportion of invasions detected in time for rapid response
	Effective response	Reduce risk of population reproducing	Proportion of new populations removed prior to spawning
		Effective eradication techniques available	Number of effective eradication tools evaluated and available
Control and Management	General	Improved knowledge of control measures and their effectiveness	Number of control measures evaluated.
		Improved knowledge of relationship of population density to spread	Models predicting future spread and nodes consistent with current knowledge
		Improved knowledge of relationship of population density to impacts	Robust environmental cost/benefit ratios estimated for key areas. Specific goals and control approaches developed for each area.
		Suitable infrastructure and management processes established	Eg. pest awareness requirement of boating license renewal; number of wash downs for boats/scuba gear, etc
	Impact minimisation	Impacts on biodiversity, ecology, fishing, aquaculture, recreation and other marine uses insignificant	Detectable reduction in impacts. Risk of further spread reduced/unlikely
	Population eradication	Long-term reduction of existing populations to non-sustainable levels	Decrease in abundance over time (eg. 25 years)
		Minimise impact on native taxa	Change in abundance over time (eg. 25 years)
		Minimise impact on commercial resources	Change in catch per unit effort over time (eg. 25 years)
		All possible tools considered for use	Number of approaches evaluated

3.2.1. Workshop evaluation

From my perspective, I thought that the workshop proceeded well with much learnt on all sides. It would have proceeded more smoothly with a professional facilitator present, but that might also have lost some of the creative spontaneity from learning by our own mistakes. The workshop format was developed following input from a professional facilitator. Clearer explanation of objectives, strategies, actions and performance indicators (including examples that could be referred to during the workshop) would have aided participants in developing more consistent objectives and indicators.

One unintended result of showing the predicted future spread of the seastar in the MSE modelling was that some attendees questioned the value of intervening, or whether it was realistic to expect that we could be successful in restricting spread or even reducing current populations. Three points can be made here:

1. Although the model predicts that the seastar will gradually spread along Australia's east coast into NSE, it does not predict that it will spread to the west, against the current, to SA and WA. This will only occur if we transport it. Knowing how to best manage those vectors is something that MSE can assist.
2. While the model predicts spread to the east, this is neither guaranteed nor immediate. If we can buy time by reducing the risk associated with key vectors, for which we can make a conscious choice, then it is possible that we can develop strategic options to reduce the size of source populations (and hence the risk of it spreading), reduce the probability that vectors can carry it, and implement measures to reduce the risk of it establishing in key nodes. Approaches such as sex-ratio distortion are being investigated for long-term control.
3. In some cases intervention will not be worthwhile. The advantage of a strategic approach to managing this (and other) marine pest is that it will indicate when we are likely to be successful and when we are not. . A history of successful interventions will maintain the enthusiasm of stakeholders for future responses.

Twelve participants filled in an evaluation form. Participants found the afternoon positive (OK to very well), informative, provided a good understanding of the seastar and the means available to control it, were glad to be able to participate (not just listen). One respondent commented that it would have been good to have more non-government stakeholders participating. (Note: many more were invited than were able to attend). The three hour duration was considered to be a maximum amount of time for the workshop as there was a lot of information to take in and people would start to fade – introductory remarks on government could be collapsed for a more general audience. Clearer and more accessible explanation of objectives and indicators – what they are, how to generate them, and their relevance to management – was requested for future workshops, preferably in advance.

Several participants saw the need for this kind of workshop to be repeated in other locations and/or in the future. This raised several interesting questions on how we would change the workshop format to accommodate participants increasing knowledge of the situation – changing baseline effect – and how we could provide explicit provisions for reconciling alternative views, based on both values and evidence, and given fixed resources (time or budget). (Note: that reconciling the implications of alternative views, incorporated either as inputs or outcomes is a strength of the MSE approach, but more consideration needs to be given to how results are communicated to facilitate this discussion).

The most significant outcomes for respondents ranged from just knowing that some activity is occurring to having input into the control plan. Others included an improved understanding of the link between ecology, dispersal and establishment and the research being undertaken; realising that controlling the seastar is a more realistic goal than eliminating it; and seeing the level of interest and cooperation on this issue among diverse user groups.

Respondents generally thought that we were on the right track and that a realistic appraisal of options was important to manage stakeholder expectations. One respondent questioned whether the model used to evaluate management strategies was reliable, while another thought it essential that the management strategy evaluation approach be extended to the other 10 key pest species (ranked by potential risk). One respondent stressed the need to look at all vectors, and another respondent emphasised that model outcomes (eg. natural dispersal) are only predictions that may not come to pass, while vector intervention is something tangible that we will choose to do or not do. Three respondents stressed the need for wider community consultation and/or involvement in any solution.

With respect to aspects critically missing from the workshop, three respondents were concerned about how (or whether) the stakeholder input from this workshop would be used to influence future actions (at the national level) and one suggested that a clear process for incorporating stakeholder views needed to be established. One respondent was concerned that we did not recognise the measures already in place, another that there was as yet no national response to match Victoria's ballast water controls. (The Australian Government DEH representative noted that the Seastar was one of the 11 target species for controls on international ballast water that operate under Australia's quarantine laws.) One respondent suggested that more information on technical management options was required including their likelihood of meeting objectives, another that more emphasis should be placed on biological control (or augmentation?). One respondent was concerned that we did not consider managing the spread within PPB.

With regard to what future actions needed to be taken to make participants confident that progress is being made, six respondents wanted to see plans develop into actions (including high level support and targeting of those areas at highest risk of developing self-sustaining populations), four respondents

wanted to see education, feedback and future involvement of the community, one wanted a formal process to implement suggestions made at workshop, and one wanted to see a reduction in seastars and no more spread.

3.2.2. Contribution of workshop outputs to development of National Control Plans

The Northern Pacific seastar is the only marine pest in Australia for which a Control Plan exists, and it is being used as a test case for the development of National Control Plans for other identified marine pests present in Australia. At the present time there are an additional 10 marine pest species for which business cases are being prepared, to determine whether National Control Plans should be developed. All species managed under the domestic ballast water management arrangements will have National Control Plans. Community consultation is already identified as a component of those control plans; the results from this workshop will be used to help specify how that community consultation needs to proceed.

The results from this workshop will be reported in August to the National Introduced Marine Pests Coordination Group which is charged with developing the National System, including control plans. In addition, one attendee of the workshop is leading the group developing business cases (the precursor to the control plans) and we can presume that the workshop outcomes will form part of his process. For me, the unique contribution of this workshop has been to extend the often highly specific scientific and policy objectives associated with control plans to include:

- community beliefs about invasion processes and the relative value of different measures to address invasions
- community values (and approaches to involve communities)
- international responsibilities
- ensuring that invaded areas remain valued for their other attributes
- provision of appropriate infrastructure to enable effective user education and vector management, especially in the recreational sectors.

There is the potential to repeat this workshop in other southern Australian states affected or likely to be affected by the seastar.

4. MANAGEMENT STRATEGY EVALUATION MODEL

The operational model for the MSE comprises biological and physical components that resemble the dynamics of the natural systems off southern Australia. An anthropogenic layer was added to describe the movement of commercial shipping and the various control and monitoring options available. The biological component is described in Appendix 2. Options for the genetic control of *A. amurensis* were included, and the potential of physical removal evaluated.

Management objectives, strategies and performance measures are still being developed (since previous sections) and this constrained evaluation of alternative strategies.

Strategies evaluated were:

- compulsory ballast water exchange (various levels of exchange and distances from shore)
- ballast water exchange associated with a management decision rule activated following a positive result from plankton monitoring with gene probes (2, 5, 10 and 20 samples)
- genetic control – daughterless, sonless, and female lethal
- physical removal

Finally, we have started to explore the possibility of alternative presentation of the results that communicate the essential information in an easy to understand graphical format.

4.1. Methods

4.1.1. Oceanic transport and estuarine exchange

The MECO (Model of Estuaries and Coastal Oceans), a three-dimensional, non-linear hydrodynamic model was used to describe circulation patterns of water across south-eastern Australia between in an area between 118° E and 153° E and between 27° S and 47° S and is a 47 x 136 cell simulation of oceanic conditions through southern Australia. It has previously been used to describe a wide range of coastal and estuarine systems (Bruce et al. 2001, Condie et al. 1999, Walker 1996, 1999). The oceanic dispersal includes coupled estuarine retention so the larvae can be moved into and out of the 97 estuaries included in the model. The data on the retention times for the 97 estuaries were sourced from the SERM II model (Simple Estuarine Response Model, www.per.marine.csiro.au/serm2/index.htm). Estuaries were modelled as either lagoonal (1 estuarine cell) or salt wedge (5 estuarine cells) estuaries.

Estuarine volumes were obtained from the Ozestuaries database (www.ozestuaries.org/).

4.1.2. Calculation of larval mortality and settlement

The biological model was parameterised with data from empirical measurements of adult mortality and density, fecundity and fertilisation dynamics (Bax and Dunstan 2004). The biological model is an age structured constant mortality model for adult populations. Larvae are produced by the adult populations at rates determined by the density of adults and the age structure of the population. Larvae are dispersed from the adult source population into the estuarine or oceanic cells. However, we were unable to obtain information on the rates of larval mortality (L_z) or the probability of settlement success (S). For this reason we estimated these parameters from a simplified model of the population that exists in the Derwent estuary. The population in the Derwent is of necessity self seeding as no other population exists in Tasmania. We estimated the possible rates of larval mortality and settlement success by assuming that the population in the Derwent maintains a static population and used the model with known parameters to estimate the values of L_z and S. We used the simplified model to explore the values of L_z that maintained the Derwent population while varying S between 1 and 0.1 (Figure 2). Two combinations of L_z and S were chosen for the simulations, Lz=0.1 and S=0.5 (Set1) and Lz=0.108 and S=0.9 (Set2). Populations with a settlement rate of less than 0.5 showed an extremely stochastic signal, and were unstable over any time period. We did not use parameters in this range as the variation in population numbers does not match observations for the Derwent.

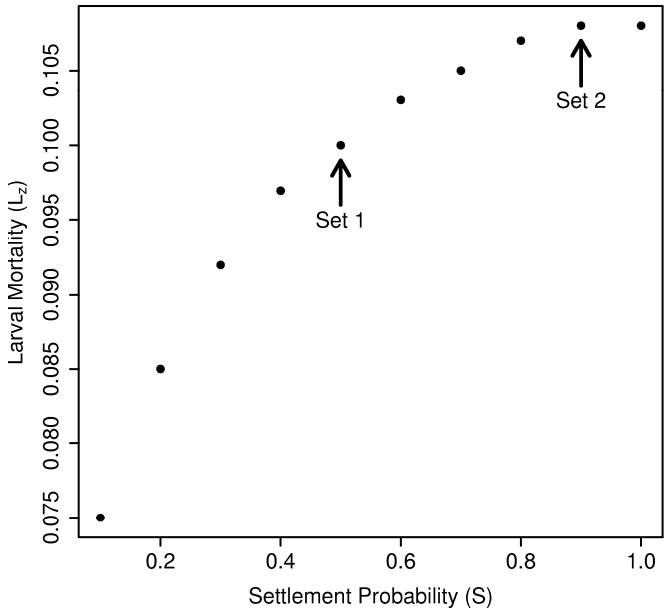

Figure 2. The range of values for Lz and S that reproduce the population densities observed in the Derwent estuary.

4.1.3. Simulation of commercial shipping

The anthropogenic component of the model comprised the movement of commercial shipping around southern Australia, between Esperance in the west and Eden in the east. Shipping movements were estimated from the Client Place Move (CPM) data from Lloyds Maritime Information Utility (LMIU). Vessels leaving ports were classified into one of six groups, Bulk carrier, Container, General Cargo, Roll-on/Roll-off (Ro/ro), Tanker and Woodchip carrier. Each of these vessel types had different movement and ballast characteristics. Five years of CPM data from 1998 through to 2002 were used to estimate the probability of movement between ports, the number of ships leaving each port, and an empirical Gaussian kernel distribution of the dead weight of each class of vessel. This provided information on the rate of movement of vessels between ports and the size of each ship.

To estimate the ballast water discharged from each vessel it was necessary to link ballast water discharge with the dead-weight of each vessel. From 1998 to 2001, the Australian Quarantine Inspection Service (AQIS) collected data on ballast water discharge from international vessels entering Australian Ports (Vessel Management System, AQIS). This information was used to calculate an expected discharge for each of the ship types (Bulk carrier, Container, General Cargo, Roll-on/Roll-off (Ro/ro), Tanker and Woodchip carrier). For Bulk Carriers, General Cargo, Tankers and Woodchip Carriers the relationship was:

$$D = m * DWT^2 + c * DWT + \varepsilon$$

where ε is distributed as a pert distribution (a modified beta distribution), with parameters a,b,c,w; where a is the minimum value, b is the most likely, c is the maximum value and w is the weight, determining the spread of the distribution. For Container carriers and Ro/ro the relationship was:

$$D = (m * DWT + c + \varepsilon)^2$$

with ε distributed as above. For ports with a high number of vessel arrivals, it was possible to calculate values of the parameters *m* and *c* for those particular ports. However, for ports with low volumes of traffic, generalised parameters calculated from information from all ports was used. All ports within the model region were incorporated into the model and their positions fixed in either estuaries or oceanic cells. If the ports were in estuaries, it was assumed that the port would be in the estuarine cell closest to the adjoining oceanic cell.

Larvae in the MSE model in oceanic cells and estuaries are distributed as well-mixed particles and are spatially homogeneous within estuaries and oceanic cells larvae. Larval uptake rate is calculated from the volume of water in the ships ballast tank that will be discharged at the next port relative to the volume of water in the uptake port, either the estuarine (from the OzEstuaries database) or the oceanic cell (to a depth of 10m, $4.84 * 10^9$ m^3) volume. Thus, if an estuary contains 100,000 m^3 of water with 1,000 larvae and a vessel uptakes 1,000 m^3 of water, the ship will contain 10 larvae after

water ballast has been loaded. Upon arrival at the destination port, 80% of the ballast water is discharged along with 80% of the transported larvae, simulating the behaviour of ships that do not completely discharge all water in their ballast tanks.

4.1.4. Ballast water exchange

There are few data available on the retention of *Asterias* larvae (or any other life history stages) by vectors during ballast exchange at sea. It is a commonly accepted (though not generally proven) wisdom that 3 times volumetric ballast water exchange will lead to the loss of 95% of planktonic organisms assuming that the larvae are distributed randomly throughout the ballast tanks. In the current MSE model, ballast exchange at sea occurs as described in Rigby and Hallegraeff (1994) which describes as situation where exchange occurs with perfectly mixed conditions in the ballast tanks. The amount of ballast water exchanged, as a proportion of the total ballast in the tank per cell as the vessel moves through a cell is:

$$proportion\ of\ tank\ exchanged = 1 - e^{\frac{-22 \times 800}{s \times DWT \times 0.34}}$$

where 800 is the expected pumping capacity of the vessel in m^3/hr, *s* is the speed of the vessel in km/hr, DWT is the dead weight of the vessel, and 0.34 is the proportion of the ships DWT carried as ballast and 22 is the distance in km across each cell.

At the beginning of a journey between two ports, a route is selected according to the ballast water management rules. The route selection algorithm chooses the two points closest to each port that satisfy the management rule and then plots a route between them that minimises journey distance but preferentially moves in a direction that satisfies the management rule where possible. In each cell that satisfies the management criteria, a proportion of ballast is exchanged according the DWT of each vessel and the vessel speed. Vessel speed is varied to ensure that the required number of tanks of ballast is exchanged over the duration of the voyage.

Simulations of a particular management option were repeated 1000 times to estimate the probabilities that a particular location (an oceanic cell or estuary) was invaded at a particular density. Comparisons of the probabilities over the range of potential dispersal determined the effectiveness of each management option. To remove the effects of biological dispersal, simulations were run without anthropogenic vectors to determine the limits of natural dispersal. These locations were removed from the analysis, as were those that were never invaded in any simulation.

The management options compared were:

- shipping without any ballast water control,
- shipping with flow through ballast water exchange for three equivalent ballast tanks along the coast,
- shipping with ballast water exchange one cell from the coast,
- shipping with ballast water exchange two cells offshore, and
- shipping with ballast water exchange in cells at least 200m deep.

Simulations were also run comparing the change in the probability of invasion with changes in the number of tanks of ballast water that were exchanged. The numbers of tanks compared were one, three, five and seven tanks of flow-through exchange.

4.1.5. Risk Tables and Monitoring

Risk tables have been proposed as a management option to reduce the expense of having ships exchange between every port when carrying water ballast (Hayes and Sliwa 2003). The risk tables rely on having an assessment of the survival in the new port and the presence or absence of the target species in the port of origin and the destination port. If both ports are uninfected, then no exchange is required. If the target species is in the port of origin and can survive in the uninfected destination port, ships are required to exchange their ballast water. If both ports are infected then no ballast exchange is required. When ships exchange ballast water they must be one cell offshore and exchange 3 tanks via flow-through exchange. This makes the effectiveness of risk tables directly comparable to the performance when ballast is exchanged on all trips.

To properly implement this management option, monitoring of ports is required to detect infection. Recent advances in genetic technologies allow the testing of plankton samples for the presence of larvae, enabling the rapid detection of larvae within a port. Ports are sampled on the 255th day of each year. The parameters used to define the net diameters, tow times (etc) can be found in Hayes et al. (in press). We tested the effectiveness of taking 2, 5, 10 and 20 samples in each port and calculating the probability of detecting a single *Asterias* larva given the number of larvae present in the port on that particular day.

4.1.6. Analysis of Invasion Patterns

The MSE simulation is repeated 1000 times for any particular combination of parameters and management options. For each location, the number of times the adult density reaches a given density is recorded so that the probability that a location will be invaded over the 1000 simulations is calculated. The

results of the MSE were analysed across all locations, both estuaries and coastal cells where seastars could potentially establish a population. The spread of seastars via ballast water and the effectiveness of control options are analysed first by removing all locations where seastars disperse to via oceanic currents. This means that only sites invaded through shipping are considered. The sites are grouped into 10 evenly spaced bins from 0 to 1 according to their probability of invasion. Results are summarised graphically as the percent of sites in the simulation that are invaded with in each bin. The reduction in invasion probability for each site that is achieved with ballast water management compared with unmanaged ballast water is also calculated and grouped in bins from -1 (i.e. a reduction of probability from 1 to 0) to 0 (i.e. no change in invasion probability).

4.1.7. Genetic Control Options

The general population dynamics of the populations of *A. amurensis* are described in Appendix 2. These dynamics are the same as used in the Asterias Management Strategy Evaluation (Bax and Dunstan 2004), with some slight modifications to allow for greater estuarine detail and estimation of the distribution of densities within populations. This general model was modified to explore the dynamics of genetic control within local populations. It was not attempted to expand the genetic model to locations other than the Derwent Estuary and Port Phillip Bay (i.e. those locations with current populations).

We have considered three genetic control options here. Two of the control options modify the sex ratios of populations, and the third introduces a lethal gene into the population.

Sex ratio modification controls change the sex-ratio of the larvae so that there is a predominance of either males or females. Daughterless control reduces the production of female offspring, thereby reducing the numbers of eggs produced in subsequent generations. Sonless control reduces the production of males, thereby reducing the production of sperm in subsequent generations.

4.1.7.1. Daughterless control

Transgene males are introduced into the population with genetic constructs that will produce a reduced number of female offspring, thereby distorting the sex ratio. The transgene males are genotypic females (i.e. contains no male sex chromosome) that contain a number of genetic (RNAi) constructs, each of which will prevent a genotypic female turning into female, but instead remaining a phenotypic male that produces sperm. It is assumed that the constructs are inserted into separate chromosomes and are independent so that the number of constructs in sperm will be distributed according to a binomial distribution with p = 0.5 (i.e. a 50% chance of inheriting a construct from a pair of chromosomes) and G = maximum number of constructs in the individual. For example, a transgene male with 2 constructs will produce sperm with 2 constructs in 25% of the sperm, 1 construct in 50% of the sperm

and 0 constructs in 25% of the sperm. In this case, rather than producing 50% male and 50% female as wild-type males do, transgene males will only produce 25% females and a total to 75% males.

The determination of the number of constructs contained in the seastars is determined by the mixing ratios of sperm in the population. The proportion of sperm containing only wild-type male and female chromosomes (F^W and M^W) and transgene males containing h constructs M^h are

$$F^w = \frac{M_m^w}{2} + \sum_{g=1}^{g=G} \binom{g}{0} 0.5^g \times M_m^g$$

$$M^w = \frac{M_m^0}{2}$$

$$M^h = \sum_{g=1}^{g=G} \binom{g}{h} 0.5^g \times M_m^g$$

where G is the maximum number of constructs in the introduced transgene males, M_m^w is the proportion of wild- type males that are mature and M_m^g is the proportion of mature transgene males of construct number g.

4.1.7.2. Sonless control

Transgene females are introduced into the population with G copies of the genetic construct. The constructs will turn a genetic male into phenotypic female and have no effect on genetic females (other than they are carriers of the constructs). The transgene females are introduced as genotypic males and females in equal proportions (i.e. the genetic sex ratio is 50:50). It is assumed that the constructs are inserted into separate chromosomes and are independent so that the number of constructs in eggs will be distributed according to a binomial distribution with p = 0.5 (i.e. a 50% chance of inheriting a construct from a pair of chromosomes) and G = maximum number of constructs in the individual. Thus, eggs may contain either male or female wildtype sex determination genes plus a number of genetic constructs ranging from either 0 to G. It is further assumed that larvae that result from the fertilisation of eggs with sperm with male wildtype genes result in viable offspring. In human terms this is equivalent to saying that the fitness of XY individuals is the same as YY individuals and both result in functional males.

The determination of the sex and number of constructs of offspring is calculated from the construct numbers in the eggs of females. The proportion of sperm containing only wild-type male and female chromosomes (F^W and M^W) and transgene females containing h constructs F^h is:

$$M^w = \frac{1}{2} \times \sum_{g=0}^{g=G} \binom{g}{0} \times \frac{1}{2}^g \times E^g$$

$$M^h = 0$$

$$F^w = \frac{1}{2} \times \sum_{g=0}^{g=G} \binom{g}{0} \times \frac{1}{2}^g \times E^g$$

$$F^h = \sum_{g=h}^{g=G} \binom{g}{h} \times \frac{1}{2}^g \times E^g$$

where E^g is the proportion of eggs of copy number g and G is the maximum number of copies.

4.1.7.3. Lethal gene control options

We explored the possibility of controlling seastar populations with female lethal genes. Female lethal genes kill all female larvae that have any constructs in their genetic makeup. Males do not show any effects of the constructs and are able to pass on the constructs to the next generation. It is assumed that the genetic constructs are in place in different chromosomes and that the distribution of chromosomes will be binomial as in the case of sonless and daughterless. Transgene animals are input into existing populations as males. We input males into the populations with a maximum of 2, 6 and 10 constructs. The numbers input are varied in the same way as for daughterless and sonless. The proportions of males and females produced in each generation can be written as:

$$F^w = \frac{S^0}{2} + \frac{1}{2} \sum_{g=1}^{g=G} \binom{g}{0} \frac{1}{2}^g \times S^g$$

$$M^w = \frac{S^0}{2} + \frac{1}{2} \sum_{g=1}^{g=G} \binom{g}{0} \frac{1}{2}^g \times S^g$$

$$M^h = \frac{1}{2} \sum_{g=1}^{g=G} \binom{g}{h} \frac{1}{2}^g \times S^g$$

where F^w and M^w are the proportions of wildtype females and males, M^h is the proportion of males of copy number h, G is the maximum copy number and S^g is the proportion of sperm of copy number g.

4.1.7.4. Implementation of genetic control options

The equations outlined in Appendix 2 are retained with two changes. The fertilisation rates of broadcast spawners are determined by the density of the population and the sex ratio (SR). In the standard MSE model the sex ratio is

assumed to be static at 50% male and 50% female. However, the introduction of the daughterless construct manipulates the sex ratio and the ratio is allowed to vary in the genetic model. Thus, the fertilisation rate for sex ratio SR and density D is

$$F_D^{SR} = 0.223 \times \ln(SR) + 0.164 \times \ln(D) + 0.797$$

This equation is derived from Morris (2002). Varying the sex ratio changes the fertilisation dynamics in a fundamental way. If the proportion of males is low, fertilisation remains low, irrespective of the density. However, for populations with more than 50% males the fertilisation rate rapidly increases for a given density (Figure 3).

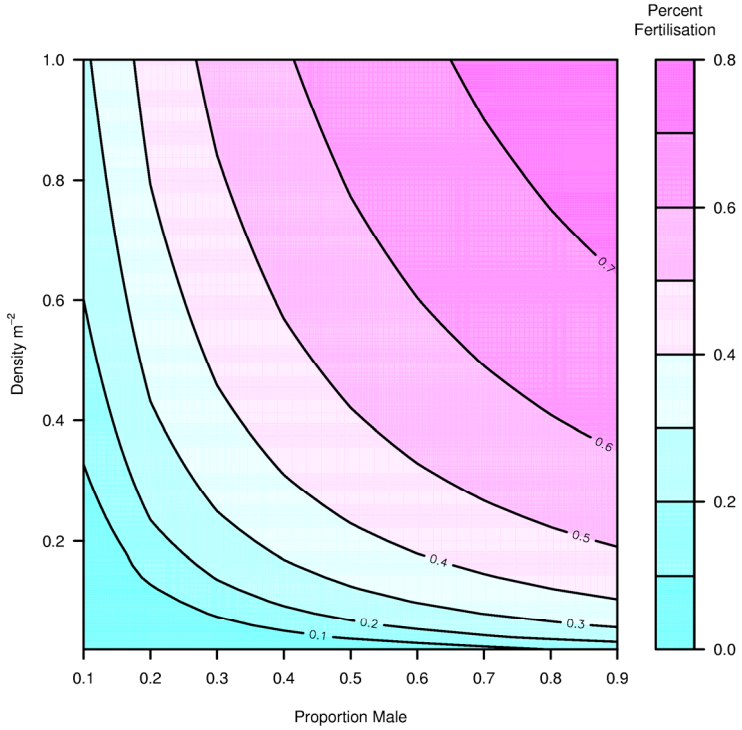

Figure 3. The effect of density and sex ration on fertilisation success.

The number of larvae produced by the population will be:

$$L_f = \sum_{a=0}^{a=96} \sum_{D=D_{min}}^{D=D_{max}} A_f^a \times E_a \times F_D^{SR} \times F \times F^W$$

$$L_m = \sum_{a=0}^{a=96} \sum_{D=D_{min}}^{D=D_{max}} A_f^a \times E_a \times F_D^{SR} \times F \times M^W$$

$$L_h = \sum_{a=0}^{a=96} \sum_{D=D_{min}}^{D=D_{max}} A_f^a \times E_a \times F_D^{SR} \times F \times M^h \quad 1 \le h \le G$$

where L_f,L_m and L_h are the number of female, male and transgene larvae.

The transgene adults (either male or female) were input into the population at one year age. The model is simulated for the Derwent estuary only. The estuary is split into 5 cells with residence time increasing with distance from the estuary mouth. The estuary has an average residence time of 15 days. Once the larvae are moved into the oceanic cell at the mouth of the estuary they can be lost to the system at a rate determined by the current vectors in that cell.

4.1.7.5. Evaluation of Genetic Control within the MSE framework.

The effectiveness of introducing transgene adults into both the current Asterias populations compared with introduction to selected estuaries was tested using the MSE model with application of genetic control technologies. Genetic techniques were compared with the base scenario of no control and the option of exchanging ballast water 1 cell offshore with no genetic control. A total of 10 different control options were compared against the option of no control and ballast exchange.

Son-less Control	Daughterless control
Introduction of 10% of population yearly to PPB and the Derwent	Introduction of 10% of population yearly to PPB and the Derwent
Introduction of 20% of population yearly to PPB and the Derwent	Introduction of 20% of population yearly to PPB and the Derwent
Introduction of 1000 1 year adults to estuaries with residence >15 days	Introduction of 1000 1 year adults to estuaries with residence >15 days
Introduction of 2000 1 year adults to estuaries with residence >15 days	Introduction of 2000 1 year adults to estuaries with residence >15 days
Introduction of 10000 1 year adults to estuaries with residence >15 days	Introduction of 10000 1 year adults to estuaries with residence >15 days

In all simulations with genetic control transgene animals had 8 copies of the construct and the values for Z and K were held constant at 0.57 and 0.29. This was done to reduce the number of simulations needed to capture the variability in the system as each simulation was significantly more CPU intensive than simulations without genetic constructs. All other parameter values were retained from the ballast water MSE simulations. Simulations for each control option were repeated 50 times.

4.1.8. Physical Removal

It has been suggested that the removal of high densities of *Asterias*, particularly those found around wharves and jetties where food is abundant,

can reduce the reproductive output of a population and potentially control the overall abundances. The hypothesis suggests that by removing the dense aggregations, fertilisation success and larval density are reduced. This reduction is reflected in a reduction of recruits to the population and a consequent reduction in the adult abundances through time.

The effectiveness of this technique depends on the link between larval abundance and the number of larvae that recruit to the adult population. If there are no density dependent processes acting in the period between fertilisation and recruitment to the adult population, then this technique should be extremely effective at reducing the numbers. This is how the dynamics are modelled in the MSE framework. As such, the removal of high densities in the Derwent estuary results in an immediate and substantial reduction in the *Asterias* abundance.

However, it is possible that there are density dependent processes acting in the period between fertilisation and recruitment. These may include food limitation for recruits or increased recruit mortality due to predation or sub-optimal habitat. In order to understand how effective removing adults may be at removing a population we modelled the Derwent Estuary using the algorithms in Appendix 2, with the exception that the limit on fecundity was removed and another limit was placed on the population size, so that the total population could never exceed 25 million seastars. This is an extremely simple form of density dependence. Once the population limit has been reached, no new larvae can settle until the population has reduced in size. This number includes animals from age zero. We kept the probability of settlement at 0.5 and reduced larval mortality from 0.1 to 0.09. Seastars of random ages were removed from the population and the final population size after 50 years was recorded. The number of seastars removed was varied from 20,000 to 200,000 per year and the seastars were removed prior to spawning. Simulations were repeated 100 times for each combination of larval mortality and number removed and the mean of the final population size calculated. We then calculated the relative decrease of the population compared to the population size without removal.

4.2. Results

4.2.1. Ballast water management

The distributions of invasion probabilities over the area simulated (less sites never invaded or invaded as a consequence of oceanic dispersal) were calculated. When larval dispersal was parameterised with Set 1 and ships did not exchange their ballast water, the median probability of invasions was 0.671 for a seastar density of 1×10^{-4} per m2 and 0.256 for a seastar density of 0.1 seastars per m-2 (Table2). The lower density would be barely detectable, the higher density a pest population.

The distributions are not symmetric around these medians but are highly skewed (Figure 4). In these simulations, the management of ballast water reduced the probabilities of invasion by up to 80% (Figure 4). However, the median probability was reduced by 10-30% depending on the density achieved. The reductions were greatest at low densities, decreasing as population density increased. Significantly, the performance of all the management options where ballast water was exchanged was the same suggesting that for this species ballast water exchange without ship delay would be not increase invasion risk compared to a requirement delaying the ship until the full 3 ballast waters volumes were exchanged. The reduction in densities shows identical decreases in invasion probability.

Table 2. The median probabilities that a site will be invaded at a particular density for the various management options.

	Density per m^2	***No exchange***	***Coastal***	***1 cell offshore***	***2 cells offshore***	***200m depth***
Set1	1×10^{-4}	0.671	0.364	0.388	0.402	0.416
Set1	1×10^{-2}	0.477	0.206	0.210	0.224	0.229
Set1	1×10^{-1}	0.256	0.111	0.115	0.122	0.124
Set2	1×10^{-4}	0.664	0.388	0.411	0.403	0.421
Set2	1×10^{-2}	0.639	0.355	0.370	0.373	0.370
Set2	1×10^{-1}	0.391	0.205	0.215	0.214	0.216

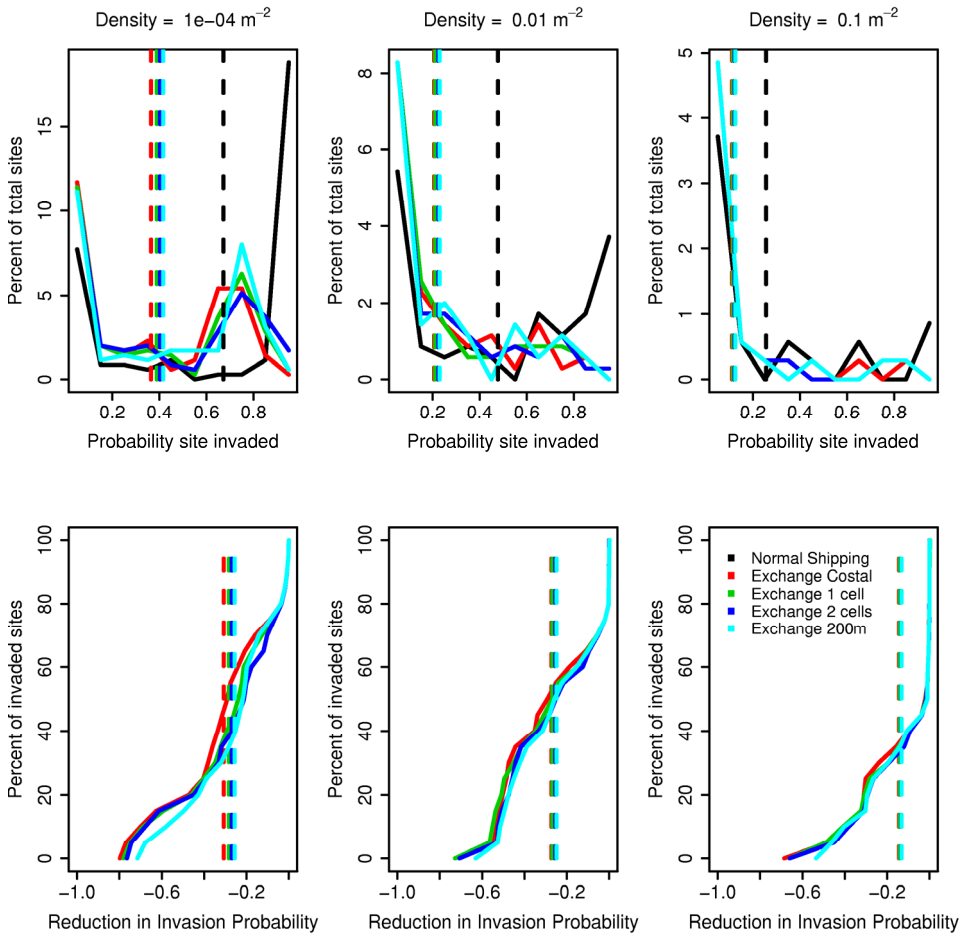

Figure 4. The probability of sites invaded through the transportation of larvae in ballast water (Set 1 parameters). Sites that are invaded via oceanic transport from current populations have been removed. The upper three figures show the percent of sites (y-axis) reaching one of three densities with a given probability (x-axis) for each management option (coloured lines). The lower figures show the reduction in invasion probability for each site. Larvae mortality and settlement are Set1. Dashed lines indicate the median probabilities for each management option.

This pattern is also shown in simulations parameterised with larval dynamics parameterised with Set 2 (Figure 5). Invasion rates are higher in simulations with un-managed ballast water and decrease when ballast water management is applied. All ballast water options produce similar results and the median reductions are comparable with those from Set 1. It is worth noting that the probabilities of invasion are higher than comparable ones from simulations with parameters from Set 1.

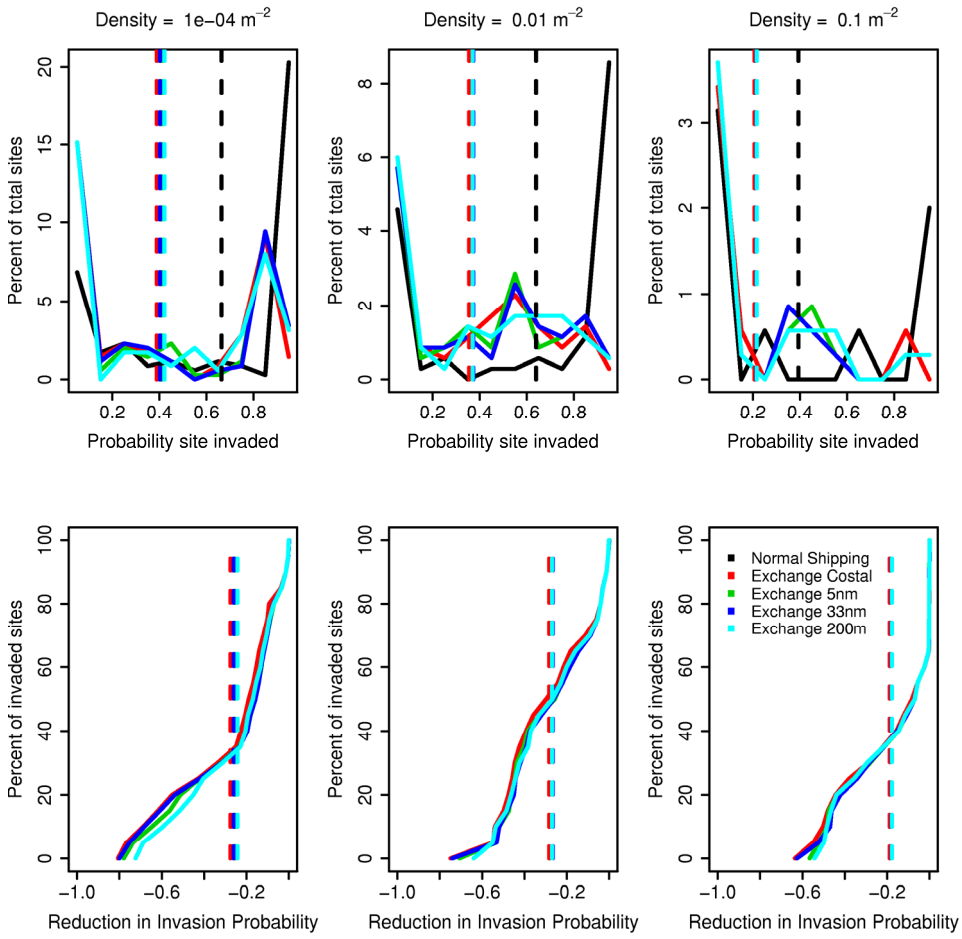

Figure 5. The probability of sites invaded through the transportation of larvae in ballast water (Set 2 parameters) . Sites that are invaded via oceanic transport from current populations have been removed. The upper three figures show the percent of sites (y-axis) reaching one of three densities with a given probability (x-axis) for each management option (coloured lines). The lower figures show the reduction in invasion probability for each site. Larvae mortality and settlement are Set 2. Dashed lines indicate the median probabilities for each management option.

Changing the number of equivalent tanks exchanged also changed the probabilities of invasion. We compared the probabilities of invasion when vessels exchanged either 1, 3, 5 or 7 tanks 1 cell offshore. The distributions of invasions when ships exchanged either one or three tanks were very similar (Figure 6). The reductions in probabilities achieved by exchanging three tanks were also achieved by exchanging only one tank. In contrast, exchanging five or seven tanks resulted in a further significant reduction in the probabilities of invasions, with exchanging seven tanks achieving the largest reduction.

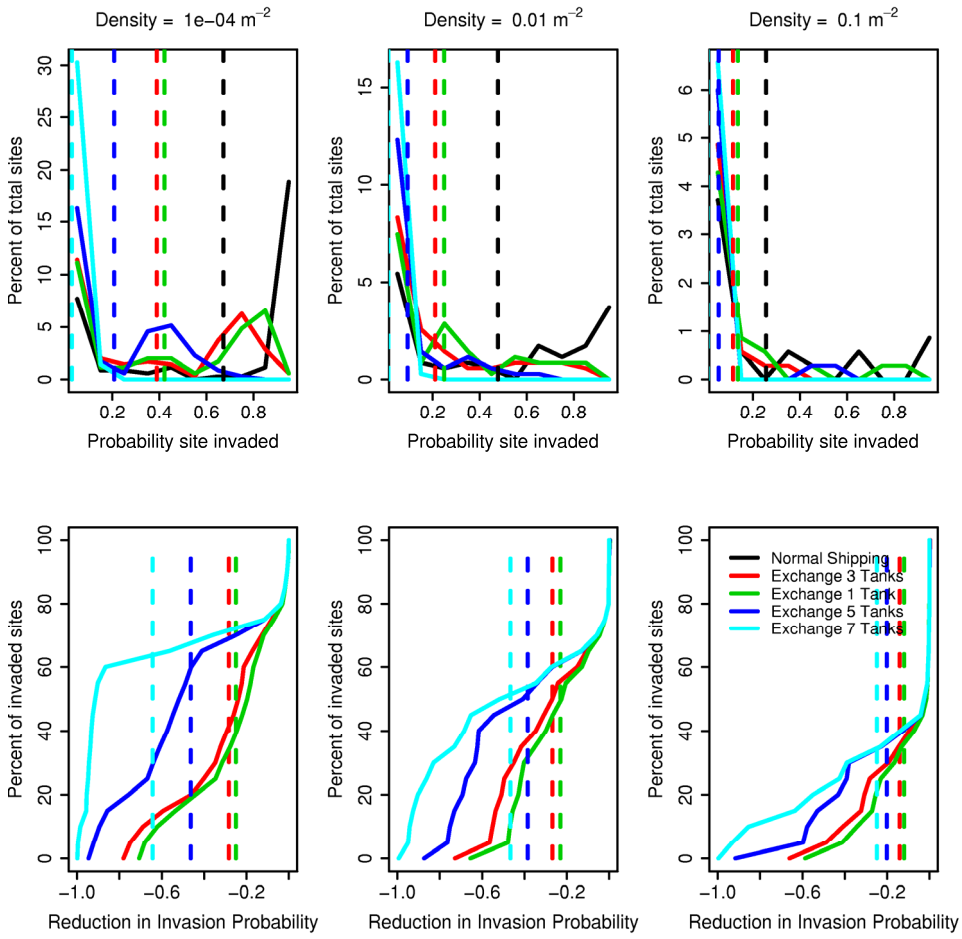

Figure 6. The changes in invasion probability with changes in the number of tanks of flow through exchange from 1 to 7. The upper three figures show the percent of sites (y-axis) reaching one of three densities with a given probability (x-axis) for each management option (coloured lines). The lower figures show the reduction in invasion probability for each site. Larvae mortality and settlement are Set2. Dashed lines indicate the median probabilities for each management option.

The efficiency of ballast water management using the risk tables proposed by Hayes and Sliwa (2003) was also examined. We compared ballast exchange from ports that were surveyed with 1, 3, 5 and 7 plankton samples to detect the presence of *Asterias* larvae. The performance of the risk tables is comparable with the performance of ballast exchange where ballast must be exchanged between all ports (Figure 7). All combinations of the risk tables and sample number significantly reduced the median likelihood of invasion compared with the median probability without ballast control. However, in a small number of sites, using large numbers of samples to inform the application of risk tables increased the probability of invasion (Figure 7). In sites that show a large reduction in invasion probability (i.e. > 0.4 reduction)

taking 10 and 20 samples increased the likelihood of invasion compared with taking 2 samples or mandating ballast exchange over all trips. This pattern was most apparent at low densities but was present at higher densities.

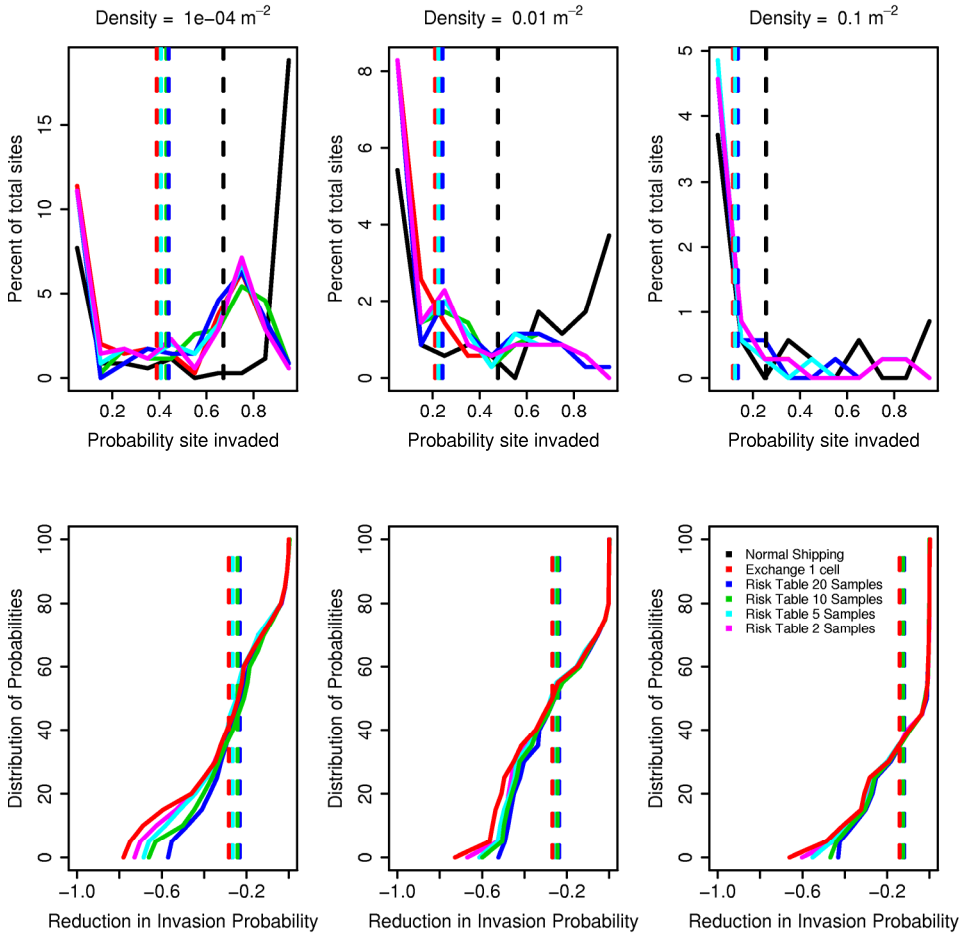

Figure 7. The probability of sites invaded through the transportation of larvae in ballast water where ballast is managed using the risk tables, informed by differing numbers of plankton samples. Sites that are invaded via oceanic transport from current populations have been removed. The upper three figures show the percent of sites (y-axis) reaching one of three densities with a given probability (x-axis) for each management option (coloured lines). The lower figures show the reduction in invasion probability for each site. Larvae mortality and settlement are Set1. Dashed lines indicate the median probabilities for each management option.

4.2.2. Costs associated with Ballast water management

Changing the management requirements also increased the duration of trips between ports. For normal shipping the median time between ports was 14 hours (table 2). However, mandating exchange along the coast increased the

duration to 36.8 hours, 1 cell from the coast increased this time to 38.5 hours, two cells 40 hours and mandating exchange in 200m depth to 55.6 hours. An estimate of the charter costs for shipping is $2080 AU per hour (O'Keeffe 2005). Excluding the direct costs associated with exchange, mandating ballast exchange can increase the cost of shipping substantially. The estimated cost of chartering to shipping from modelling increased from 95 million dollars per year with no exchange to 139, 154, 154 and 231 million dollars per year when exchange was required along the coast, one cell, two cells and 200m depth respectively. However, utilising the risk tables with two samples taken at each port, increased total cost to shipping by only 4 million dollars to 99 million per year. The total cost of sampling and processing to obtain presence/absence information for all ports, sampled once a year, is $171,000 AU, which does not add significantly to the cost of exchange (Hayes et al. 2005). A risk table based approach yields similar reductions in invasion rates to compulsory exchange, but is at least 10 times cheaper, even when the costs of monitoring are added. These figures are similar to those calculated by O'Keeffe (2005) who analysed the costs of ballast water management for 8 of the species to be controlled under the National System when only the costs for *A. amurensis* are considered.

It should be noted that these results only apply to *A. amurensis.* As other species are included in the MSE framework, the savings from using the risk tables will be reduced as more ports become positive for target species and ballast water exchange becomes a more frequent requirement.

Table 3. The median journey duration and cost of ballast water control compared with no control.

	Normal Shipping- no ballast water control	***Exchange coastal***	***Exchange 1 cell***	***Exchange 2 cells***	***Exchange 200m***	***Risk Tables (2 samples)***
Median Time (h)	14	36.8	38.5	40.2	55.6	14
Cost per year ($AU ,000,000)	95	139	154	154	231	99
Exchange cost per year ($AU ,000,000)	-	44	99	99	136	4

4.3. Genetic control options

4.3.1.1. Daughterless

The dynamics of populations with the addition of transgene males with 2, 6 and 10 constructs and an input of between zero and 2 million transgene males of one year age are explored. In all cases the number of wild type males in the population decreases to close to zero with relatively little input (Figures 8, 9, &10). However, while the population of wild-type males declines rapidly, the total population size and the number of wild-type females remains stable across much of the domain explored. The abundance of transgene males with one copy increases beyond the initial number introduced to replace the wild-type males in the population, effectively producing a new sex gene. Only when copy number or transgene input are increased is the total population reduced to close to zero.

When transgene males with two copies are introduced, the total population remains stable, but the numbers of females decline with increasing transgene input. The fertilisation dynamics of *A. amurensis* can explain this dynamic. When the proportions of male and female are equal (i.e. 50% male and 50% female), the fertilisation rates are low, irrespective of density (Figure 8). As transgene males are input into the population, the proportion of males grows beyond 50% and the fertilisation rate increases. This increase in fertilisation rate is sufficient to counter the reduction in eggs released from females. Thus, even when female numbers are reduced by 50% (i.e. 25% of total population), the additional input of sperm from transgene males compensates by raising the fertilisation rate and the population remains at the same level.

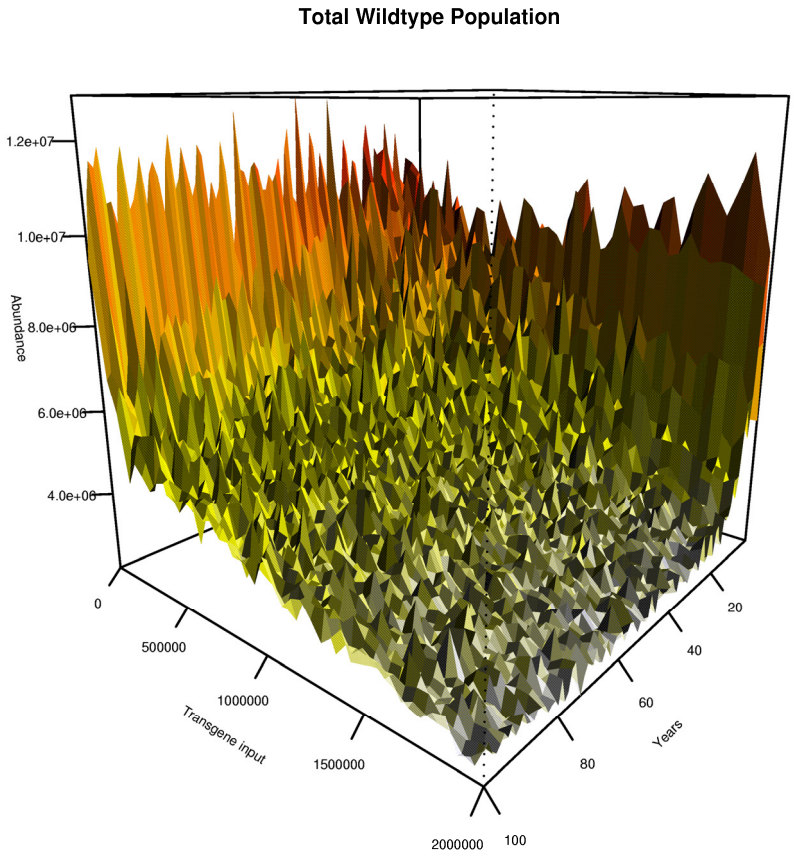

Figure 8. The total wildtype population through time with the addition of transgene males with 2 daughterless constructs.

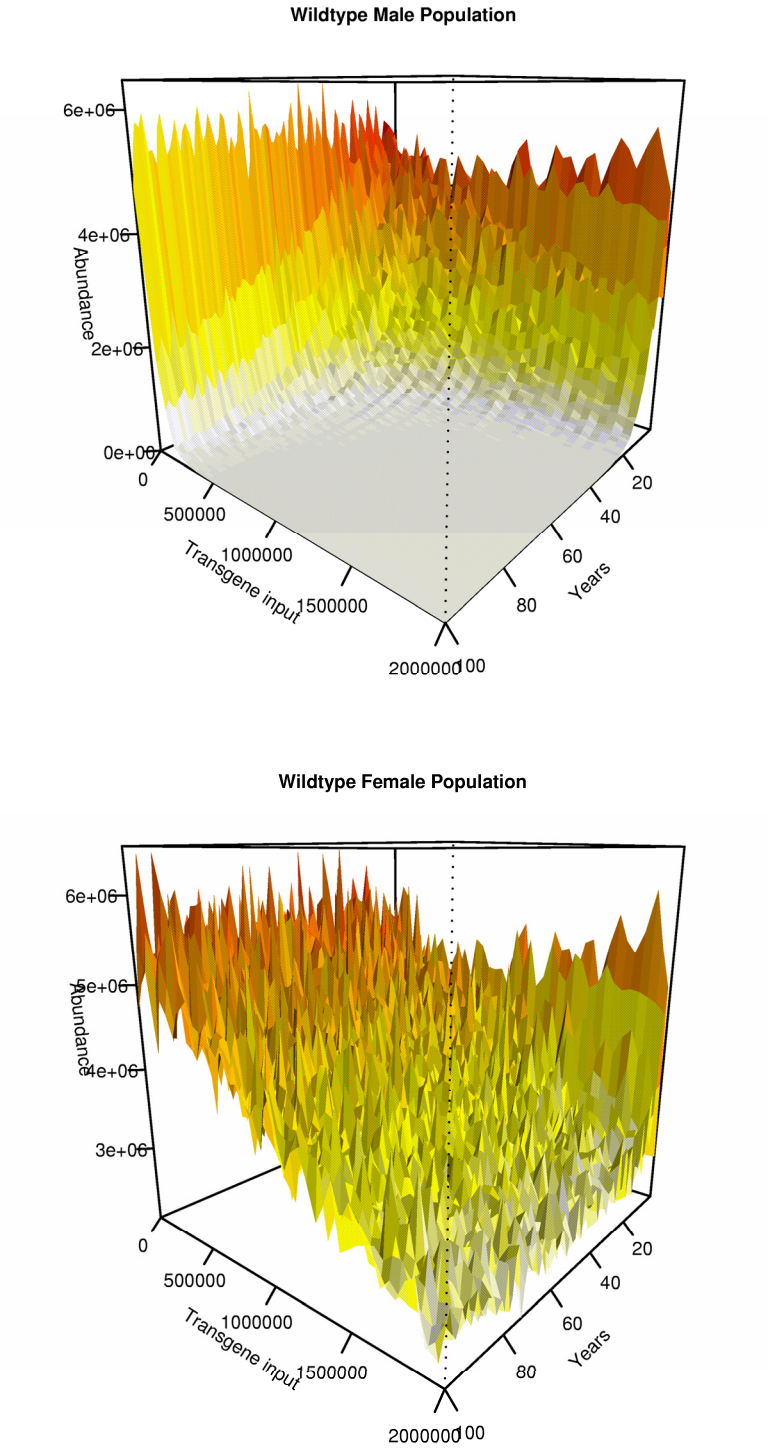

Figure 9. The dynamics of wildtype male and wildtype female populations with the addition of transgene males with two daughterless constructs.

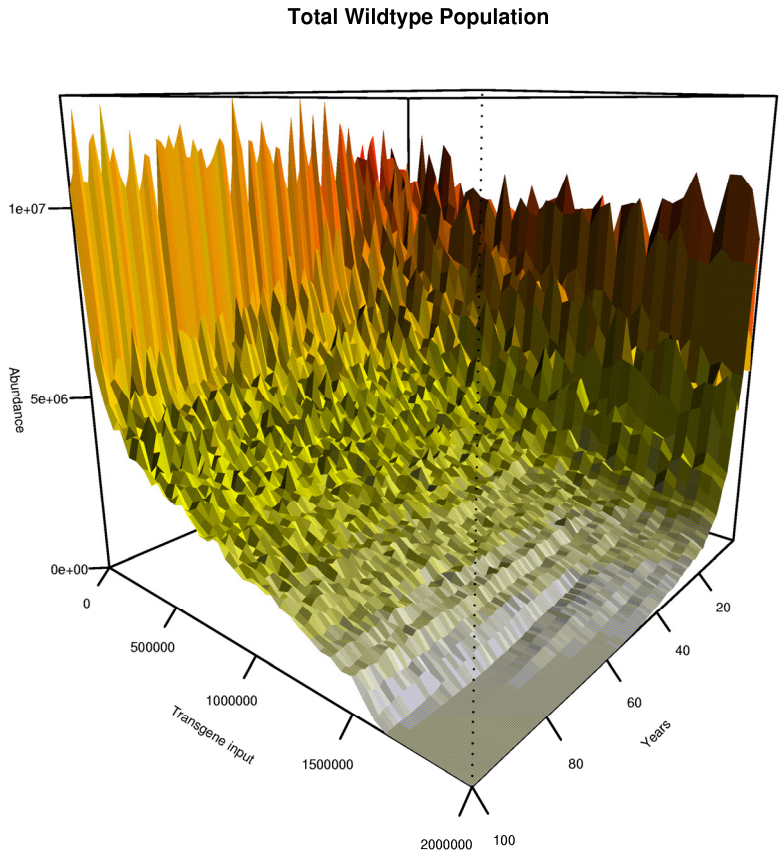

Figure 10. The total wildtype population through time with the addition of transgene males with 6 constructs.

The input of transgene males with more constructs reduces the proportion of females significantly (figures 9 & 10). The higher the number of constructs, the more the sex ratio is distorted and fewer transgene males are required to cause a population collapse. The populations do not collapse until the number of females approaches zero. If transgene males with 6 constructs are input into the population, approximately 1.6 million one year old males are needed each year for 60 years before the population collapses. If transgene males with 10 constructs are input into the population, approximately 1 million transgene males are needed over 40 years.

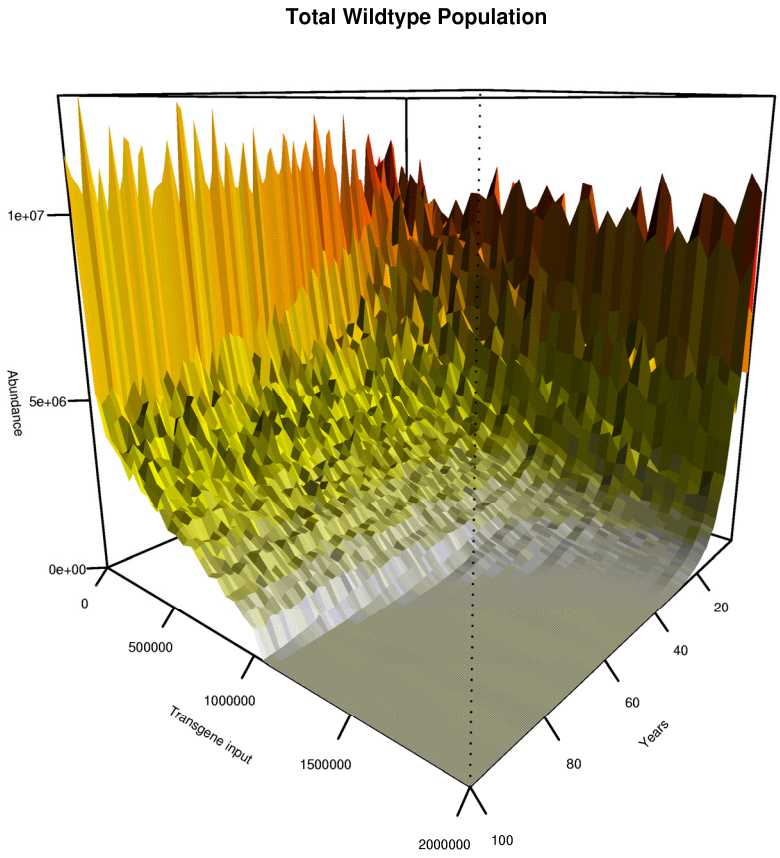

Figure 11. The total wildtype population through time with the addition of daughterless transgene males with 10 constructs.

4.3.1.2. Sonless

We have also explored the Derwent estuary population dynamics with the introduction of transgenic one year old seastars with 2, 6 and 10 genetic constructs causing the development of phenotypic females from genotypic males. The dynamics are significantly different to the dynamics of populations with daughterless inputs. Populations that have transgene animals with two constructs rapidly became extinct after the insertion of more than 250,000 transgene animals (Figure 12). The strength of the insertion is significantly greater than that seen when daughterless constructs are used. Both male and female wildtype populations have similar behaviour (Figure 13). When compared to the behaviours of populations with daughterless constructs, it is apparent that there is no buffering of the female populations. When sufficient transgene animals are input into the simulations the male and female population decrease at the same rate.

The sonless construct limits the production of sperm, reducing the fertilisation rate of eggs. Once the proportion of males drops below 50% the fertilisation rate drops rapidly across all densities (Figure 3). While more eggs are produced, an increasing proportion are not fertilised and the overall production of larvae drops.

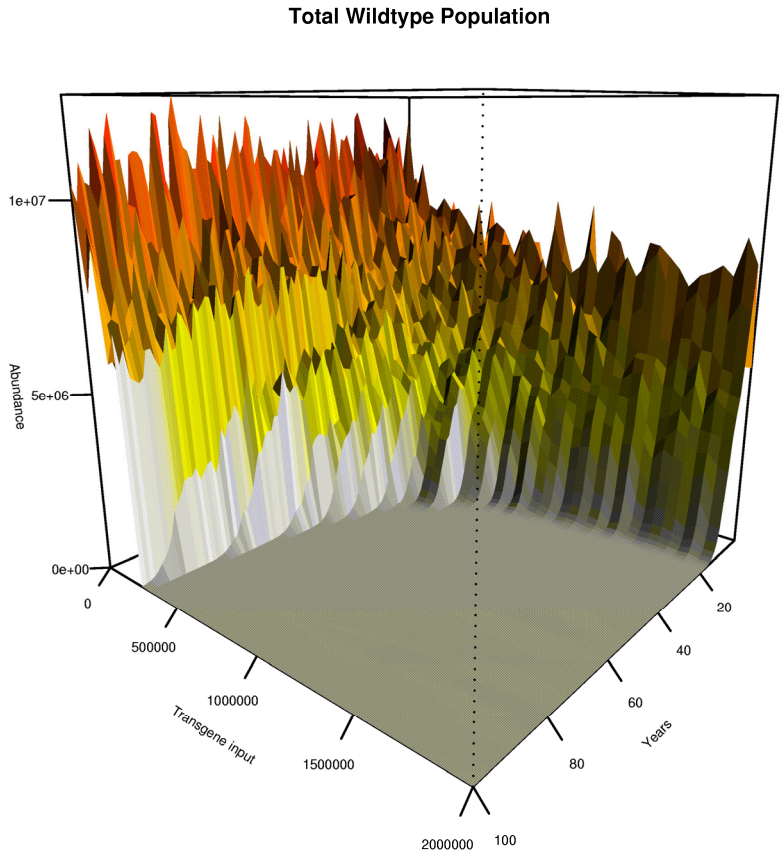

Figure 12. The total wildtype population through time with the addition of sonless transgene males with 2 constructs.

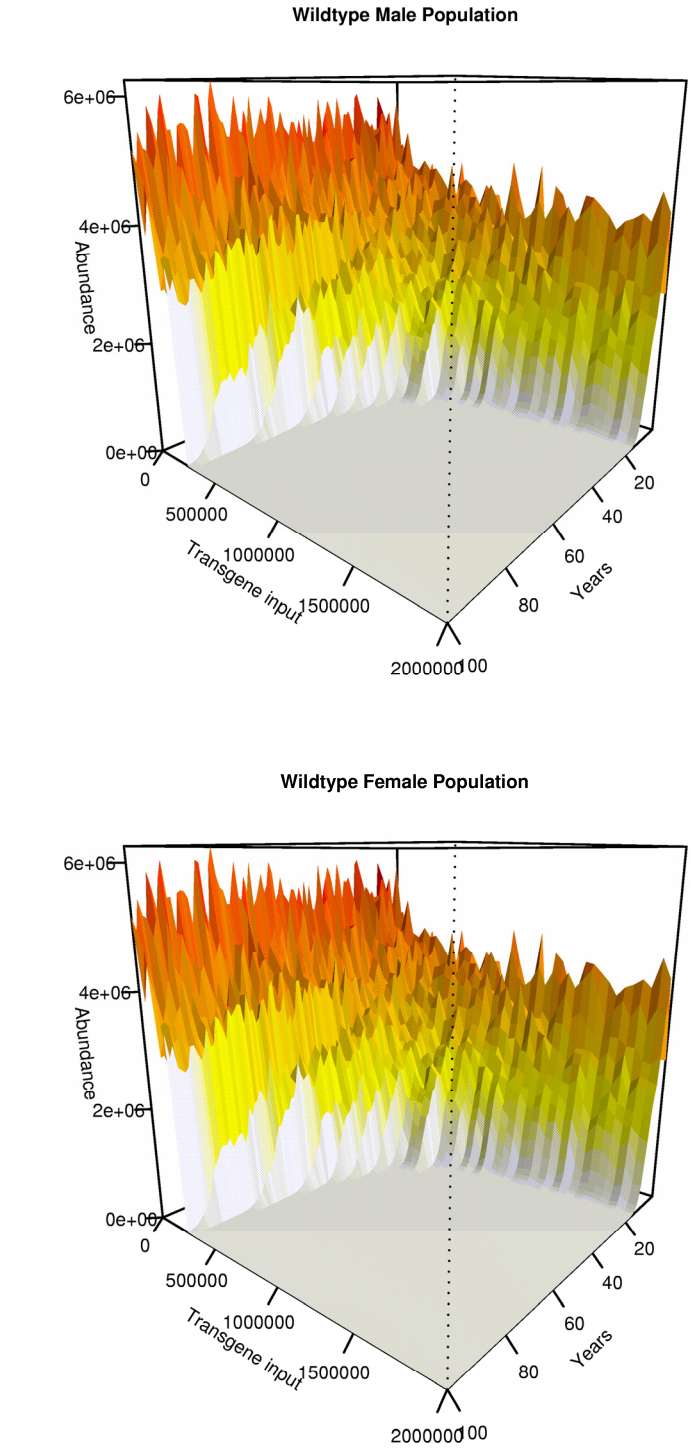

Figure 13. The dynamics of wildtype male and wildtype female populations with the addition of transgenic animals with two sonless constructs.

The dynamics of populations with additional construct numbers are identical to population with two constructs. Sonless constructs require smaller numbers of animals to remove the existing population than daughterless constructs for all numbers of constructs. As the number of constructs increases the minimum number of animals input into the system needed to achieve a reduction in wildtype population decreases. Increasing the number of sonless constructs beyond 8 does not appear to yield any more reductions in the number of animals needed. In contrast, the numbers of animal introduced with daughterless constructs are still decreasing with 12 constructs.

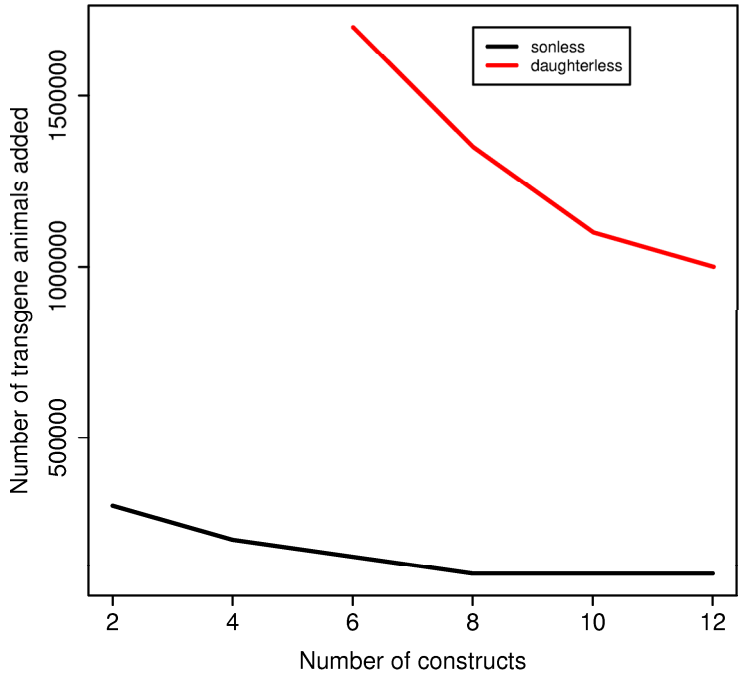

Figure 14. The number of transgenic animals input into the Derwent needed to reduce the population to below 1 within 100 years for different numbers of genetic constructs.

4.3.1.3. Female lethal

We found that when males with two constructs were inserted into the population the population decreased with increasing input, but did not ever go extinct (Figure 15). The dynamics of the total wildtype population were very similar to the dynamics of populations with daughterless control with the same number of constructs. The key difference between the two control types is the replacement of wildtype males with copy 1 transgenic males in daughterless populations. In populations with female lethal control, wildtype

male are retained at the same numbers as the copy 1 males in the daughterless populations.

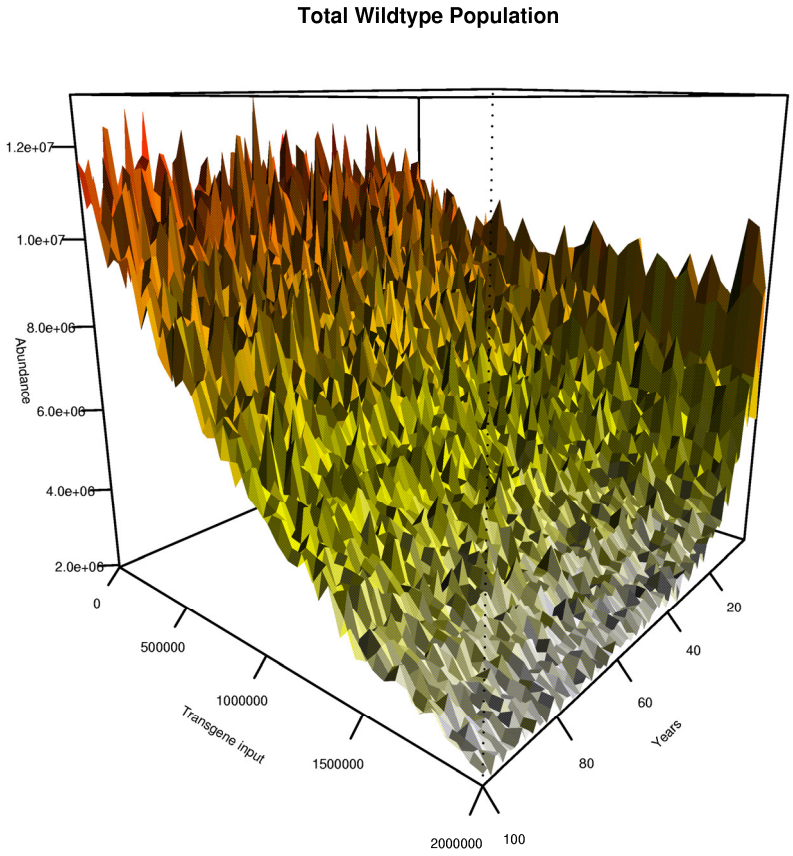

Figure 15. The total wildtype population through time with the addition of lethal female transgene males with 2 constructs.

As has been seen with the other control options, increasing the copy number increases the rate at which the population will go extinct. The dynamics of population with increased copy numbers is identical to the dynamics of the daughterless populations with the same maximum number of copies.

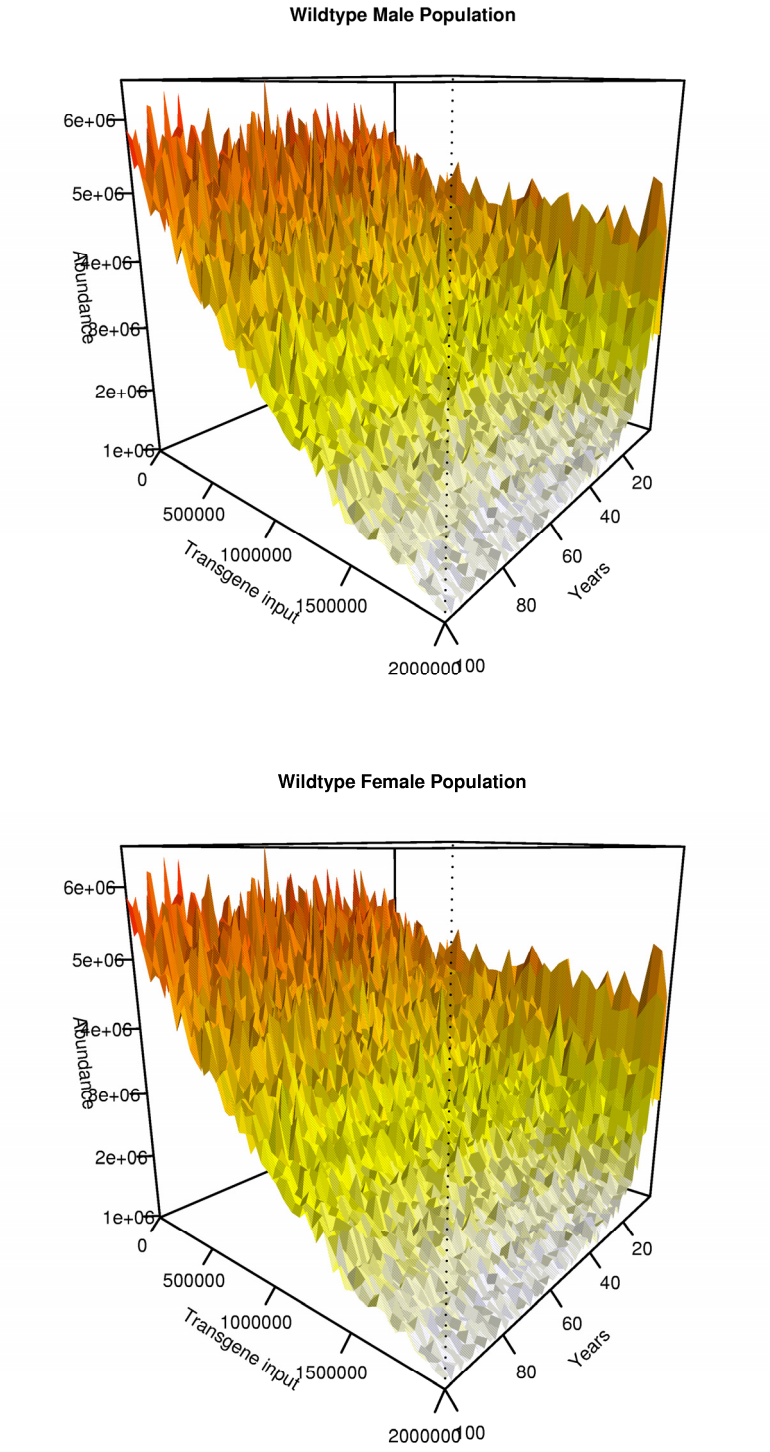

Figure 16. The dynamics of wildtype male and wildtype female populations with the addition of transgenic animals with 2 lethal female constructs

4.3.1.4. Evaluation of Genetic control with the MSE framework

The effectiveness of genetic control options in preventing the spread of Asterias was considered across its potential range using the MSE model that had been modified to include genetic techniques. The performance of genetic control was compared with no control (the present situation) and exchanging ballast water of vessels 1 cell offshore.

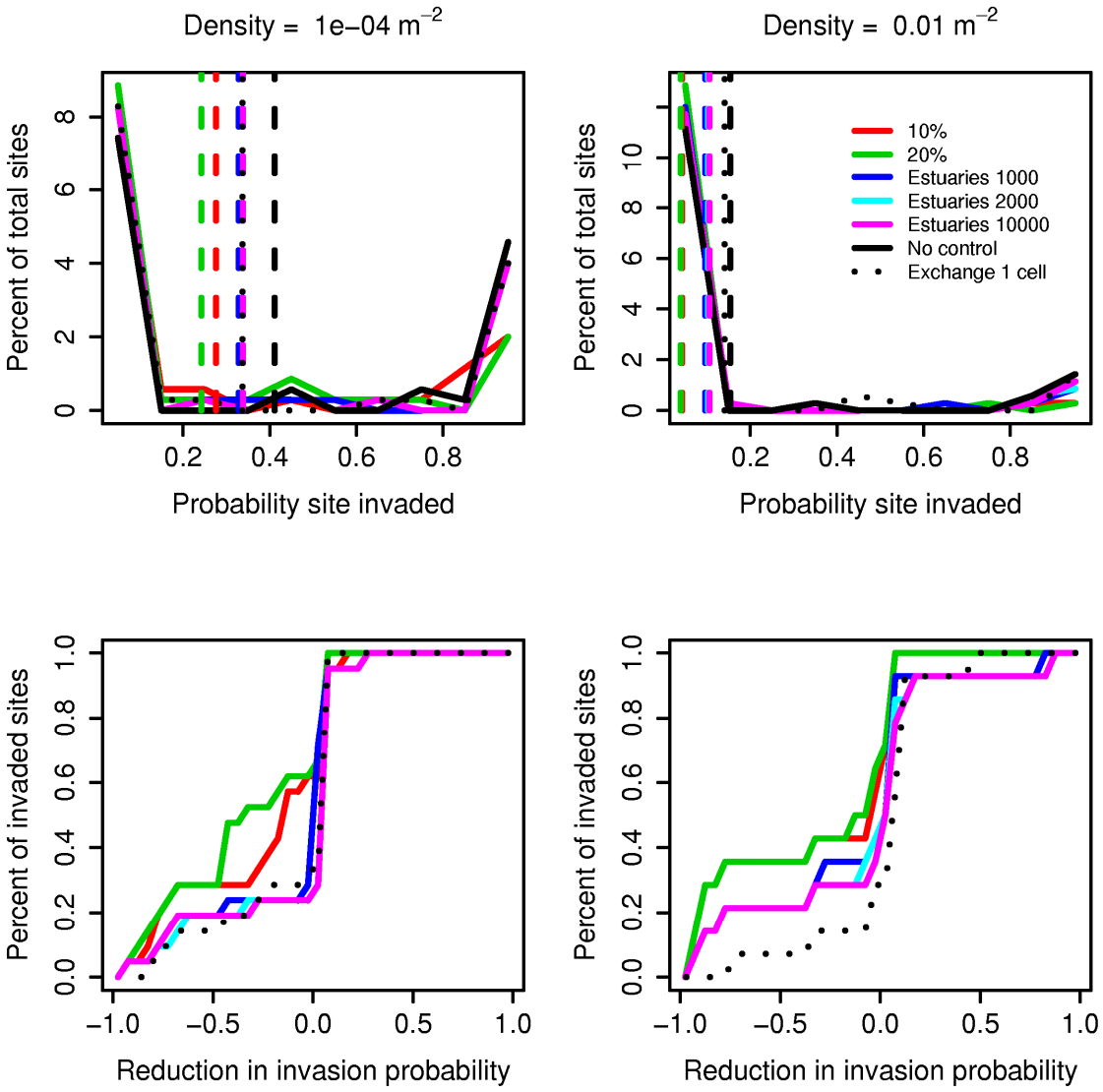

Figure 17. The effectiveness of daughterless control technologies in preventing the spread of Asterias amurensis across southern Australia.

The simulations for exchange did not include genetic control options. Using genetic control on the source populations (i.e. PPB and the Derwent Estuary) was the most efficient option for preventing the spread of Asterias (Figure 17) at both high and low densities. There was little difference between the options of adding 10% (starting at 2,000,000 seastars) of the populations or 20%

(starting at 4,000,000), however the difference in raw number was significant. Adding seastars to estuaries had approximately the same effect as exchanging ballast water, but was not as efficient at reducing spread as reducing the source populations.

Predictably, using sonless constructs was more efficient than daughterless at reducing the establishment of new populations. Adding sonless to estuaries was as efficient as ballast exchange as preventing establishment at low levels (1×10^{-4} seastars m^{-2}), but significantly more efficient at preventing the establishment of higher densities. Control of the source populations was the most effective option, however as with daughterless, the number inserted was significantly higher compared to insertion into estuaries. Adding more than 1000 transgene males did not significantly improve the performance of this control option.

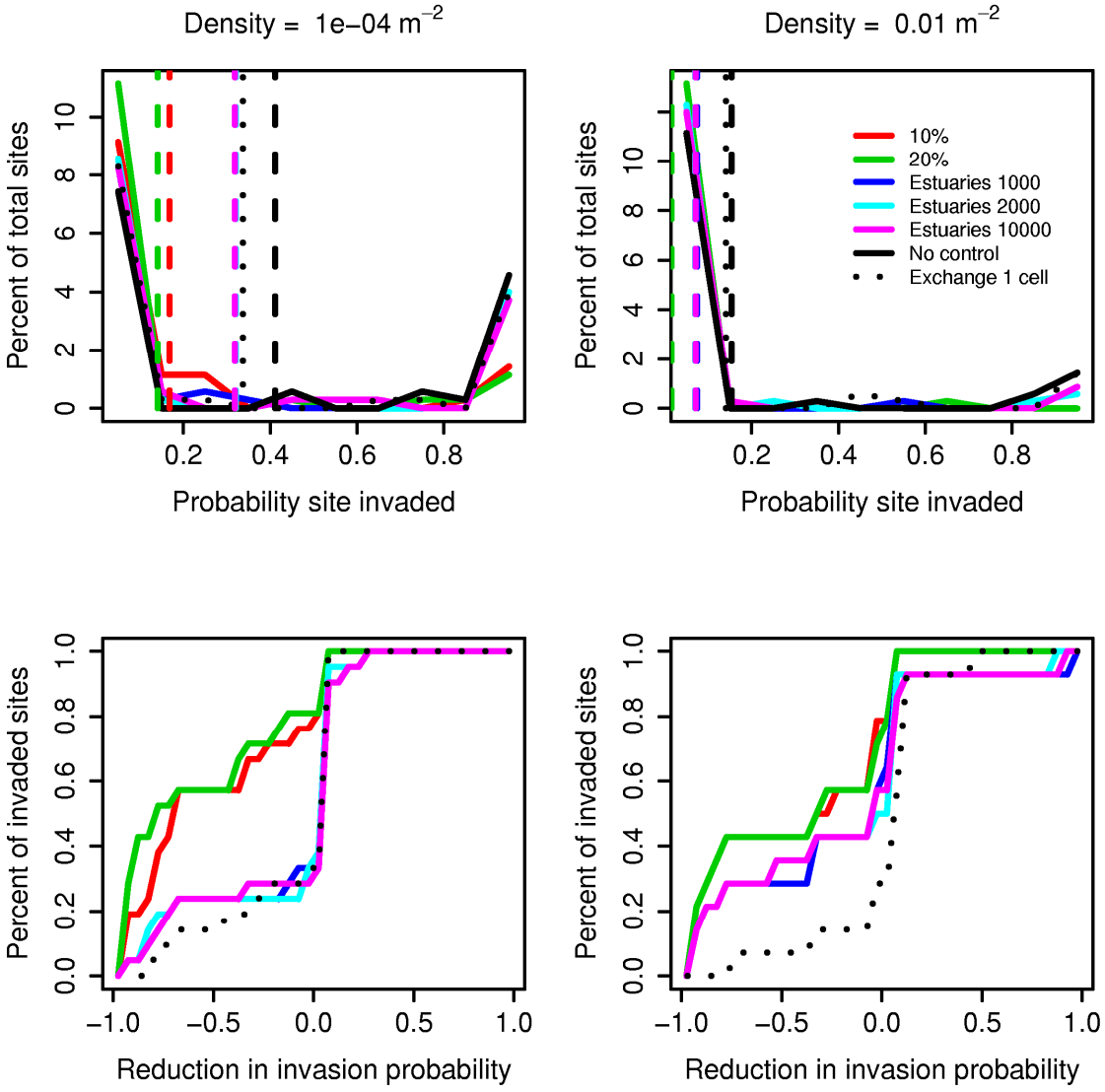

Figure 18. The effectiveness of sonless control technologies in preventing the spread of Asterias amurensis across southern Australia.

4.3.2. Physical removal

Under baseline conditions, the MSE model is run with larval mortality equal to 0.1. Under these conditions, removal of adults is a very effective way of reducing the population size (Figure 17). However, as larval mortality reduces, the effectiveness of adult removal as a control technique rapidly diminishes. As larval mortality drops, more larvae survive to settle. The removal of additional adults merely makes room for additional recruits, and has little effect on the total population size.

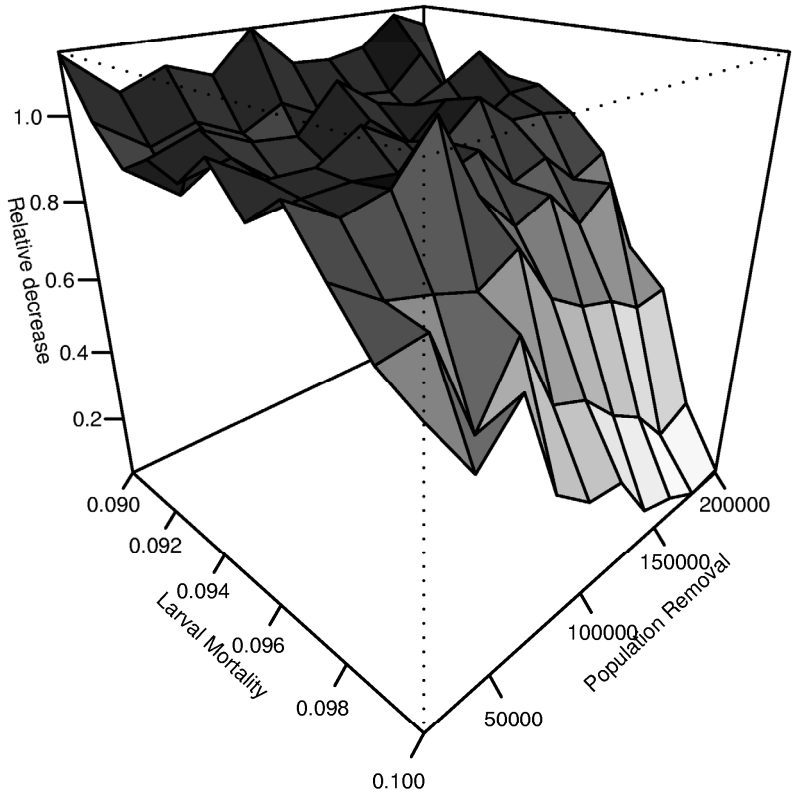

Figure 19. The relative efficiency of removing the adults from the population to reduce the adult population size. Values of larval mortality range from 0.09 to 0.1 and between 20000 and 200000 adults were removed.

4.4. Discussion

4.4.1. Ballast water management

There are two main points to draw from this first application of a Management Strategy Evaluation approach for a marine invasive species. First, requiring 95% exchange does not achieve a median 95% reduction in probability of invasion. There are complex interplays between larval production, larval retention and the exchange efficiency that will determine the exact reductions in probability from location to location. However, there are some broad patterns that emerge. Sites that have long retention times (i.e lagoons and estuaries with narrow entrances or very large volumes) are more likely to retain larvae that will, given time, develop into a high density population. Sites where space is limited, so that higher densities are achieved with fewer adults are also more at risk. These sites will produce more larvae than sites with the same numbers but spread over a larger area. The exchange efficiency also affects the final outcome. Because the population dynamics of a site will be to some degree influenced by the retention and physical size, the input of the same number of larvae from ballast water into two different estuaries may result in two different outcomes. Thus, for some sites the reduction of larval input will decrease larval supply to the point that establishment will be prevented. In other sites, a 95% reduction will be insufficient to prevent establishment. For these sites, further ballast water exchange is necessary.

Increasing the number of tanks of flow through exchange further decreases the risks of establishing a population at any density. Increasing the number of tanks increases the proportion exchanged, further reducing the percent remaining in the ballast tanks. This has the expected consequence of further reducing the establishment of populations. However, the median probability is never reduced by 95%.

Reducing the number of tanks to one tank does not have a dramatic impact on the median probability or the distribution of probabilities compared with exchanging three tanks. Exchanging one tank reduces ballast risk by the greatest proportion. This reduces larval input by enough to limit growth in many of the sites that are invaded without ballast control. These sites are marginal habitat for *Asterias* (i.e. in terms of habitat size and retention time).

The use of risk tables to manage the exchange of ballast water is as effective as mandating ballast exchange on all trips in most cases. The exception arises in the situation where the port of origin is infected and the destination port is not. Because the monitoring method used is a plankton survey, it can never detect adults present in the port, only the larvae which are assumed to come from the adults. However, if larvae are released from ballast water in the absence of an adult population, it will be these larvae that will be detected rather than local reproductive output. When both origin and destination ports are infected, the risk tables do not require exchange between ports. If this happens and the destination does not have an adult population, the port will receive the full 100% of the ballast water, increasing the likelihood of invasion.

This indicates that an improved management strategy would be to classify a port as infected for ballast water uptake, but uninfected for ballast water discharge until further surveys have confirmed a self-sustaining population has established.

Taking 20 plankton samples in a port ensures a very high level of detection, so high that the presence of small numbers, irrespective of their origin can be detected. Thus, some ports are declared infected and ballast exchange is not required, increasing the probability of infection by an adult population. Taking only 2 samples significantly reduces the ability to detect larvae, so that the inputs of ballast water are not detected and ballast exchange is required. If a population establishes in the port and produces sufficient larvae, the larvae will be detected with 2 samples. This problem has been referred to a type 1 error, the probability of a false positive. False positives are not a large problem for designing monitoring strategies with the specific purpose of detecting a species, they become a problem when the results of the monitoring are applied to management rules and the decisions are made on false premises, with resulting unintended consequences.

For normal shipping the median time between ports was 14 hours. Mandatory exchange increased this time to between 37 hours 56 hours depending on the depth of exchange. This increased the operating cost of shipping from a normal 95 million dollars per year with no exchange to between 139 and 231 million dollars per year with mandatory exchange (excluding comparatively minor direct costs of the exchange and using estimated charter cost of $2080 Au per hour – O'Keefe 2005). By comparison, using the risk tables with two samples taken at each port, increased cost to shipping by only 4 million dollars to 99 million per year (sampling costs to obtain presence/absence information for all ports, sampled once a year, would add an additional $171,000 AU - Hayes et al. 2005). Thus a risk table based approach yields similar reductions in invasion rates to compulsory exchange, but is at least 10 times cheaper, even when the costs of monitoring are added.

It should be noted that these results only apply to *A. amurensis.* As other species are included in the MSE framework, the savings from using the risk tables will be reduced as more ports become positive for target species and ballast water exchange becomes a more frequent requirement.

It would have been preferable to test the entire monitoring framework with the MSE, however the framework has not been finalised and so we could test only one aspect of it. It is not clear what implications of all the differing monitoring designs would have when applied to a single species. When applied to a multi-species framework the implications are obscure to say the least. There will be interactions between multi-species population dynamics, transport vectors, monitoring and management that are completely unexplored.

These results cannot be applied to other species without consideration of species specific demography (e.g. fertilisation dynamics, mortality rates, larval duration). For example, because *Asterias* has a long larval phase, distance offshore has no effect on the efficiency of ballast exchange. It is conceivable that for a species with a much shorter larvae phase, (i.e. days to weeks

instead of months), distance offshore would have a considerable impact on the exchange efficiency. Shorter lived larvae would not disperse as far after exchange and would be delivered at higher concentrations to suitable habitat if ballast exchange was close to shore. Likewise, shorter lived larvae will be retained closer to their source population than longer lived larvae, with the result that more sites along the coast will be vulnerable to invasion. Changing the species demography may also have unpredictable effect on the applications of monitoring and management decisions, and application to multispecies system further compounds the uncertainties.

4.4.2. Genetic control

The three techniques examined here all provide potential for the removal of pest populations. However they vary in the effort needed to achieve the removal of a relatively small population in the Derwent estuary. In the simulations here, daughterless control does not appear to be an efficient method of controlling *A. amurensis*. Distortion of the sex ratio has the unintended consequence of increasing the fertilisation rates and compensating for the reduced egg production. Additionally, transgene males with one construct act in an identical way to wild-type males. The sex of larvae will be 50% with one construct and 50% female. When transgene males with two constructs are inserted into the population, transgene males with one construct completely replace wild-type males, resulting in a new stable dynamic where the transgene males are essentially a new sex. Only when the number of constructs is increased and both the number of wild-type males and transgene males with one construct are reduced does the population become unstable and collapse.

While daughterless control does not reduce a population easily, it does offer another potential control option if an inducible lethal gene were linked to the genetic construct. Transgene males could be introduced into a population and the males with one construct could be allowed to replace wildtype males. At this point, the inducible lethal gene could be triggered, resulting in the complete elimination of the male population, and subsequently the collapse of the total population.

The effectiveness of the female lethal control is similar to daughterless. The principle difference is that instead of creating a new “male” sex of transgene males with copy number one, the wildtype male population is retained. As the equations for male and females wildtypes are identical, if follows that the dynamics of each sex are identical and wildtype males decrease in unison with the wildtype female population. Female lethal control essentially generates wasted reproductive effort. A proportion of the eggs are fertilised with sperm from transgene males that will kill ½ of the eggs. Given sufficient time and input of transgene males, this will eventually lead to population decline and extinction. However, it does not do so any more efficiently than daughterless control.

In contrast, the sonless control is significantly more effective than either of the other two options. Sonless control exploits an aspect of the population

dynamics of *Asterias* to reduce population numbers. Because the fertilisation rate is so strongly tied to the sex ratio, the effect of removing males results in significant and immediate reductions in the population. Despite the fact that there are initially more eggs produced with the input of transgene females, a steadily decreasing proportion of them are fertilised, leading to an eventual decline in the population.

All three techniques operate by reducing the number of larvae that survive to reproduce in the next generation. The effectiveness of sonless over daughterless is dependent on the dynamics of fertilisation. Daughterless control limits the production of eggs, but in this case increases the fertilisation rate of those remaining. Sonless actually increases egg production, but decreases the number of eggs fertilised. Sonless is more efficient as it targets the limiting process in the reproductive cycle of *Asterias*. Similar dynamics may exist for many marine species that are broadcast spawners with external fertilisation. This will not be true for all species. In many species, it is the supply of eggs that limits the population growth rate, and sperm is produced in abundance, sufficient to fertilise all eggs. For species with this reproductive strategy, daughterless or female sterile control would be more effective control options than sonless and would not suffer the same inefficiencies as with *Asterias*.

Using sonless genetic control was as effective at preventing the establishment of new populations as ballast exchange options where 3 tanks were exchanged through flowthrough ballast exchange. This would require the addition of approximately 1000 transgene seastars into between 20 -30 estuaries across southern Australia. However, this option is not as effective as removing the source populations. Since *Asterias* is at an early stage of its invasion into southern Australia, removing the sources is probably the most efficient option, since this would also prevent the establishment of populations throughout Bass Strait. However, as *Asterias* spreads, adding transgene seastars to estuaries may become a more efficient option, particularly at controlling small population that establish.

Removing the source populations was the most efficient techniques, but also required the greatest input of seastars. The cost of rearing 2,000,000 seastars yearly (although this number would drop with time) would be significantly more than the cost of rearing 30,000 seastars to add to specific estuaries. Estuarine control may be a useful alternative or complement to ballast control, at a significantly reduced price compared with controlling the source populations.

4.4.3. Physical removal

These results reflect the uncertainty in our estimates of larval mortality and the processes that occur between fertilisation and recruitment to the adult population. In the MSE model, we have made the assumption that all larvae that can settle do and that newly settled recruits are subject to adult levels of

mortality. We do not have enough empirical evidence to test these assumptions and it is likely that in some situations they may fail. Since the minutia of the settlement phase is so important to the population dynamics, without this information it is not possible to estimate the efficiency of adult removal (or increasing predator densities) as a method of control..

The intrinsic growth rate of population in estuaries will also give an indication of the proportion of the population that will need to be removed in order to reduce the population. The mean intrinsic growth rates, calculated from MSE simulations, of populations in Westernport, Andersons inlet, Lake Tyers and Mallacoota Inlet were 0.096, -0.234, 0.196 and 0.416 respectively. In order to limit the growth of population in these locations, it is necessary to remove 10% of the population from Westernport per year, 20% of the population from Lake Tyers per year and 50% of the population from Mallacoota Inlet per year. Andersons Inlet is the exception as it is unable to sustain a population without continual input from Port Phillip Bay.

5. DEVELOPMENT OF A QUANTITATIVE (REAL-TIME) PCR APPROACH TO QUANTIFY *ASTERIAS AMURENSIS* LARVAE IN MIXED PLANKTON SAMPLES AND VALIDATE MSE RESULTS

5.1. Introduction

An important component of the MSE model is how it represents the dispersal of *Asterias* larvae in natural currents from the Derwent Estuary and Port Phillip Bay. To test the compatibility of model results with natural spread we sampled *Asterias* larvae off the east coast of Tasmania and along Victoria's southern coast. A genetic approach was developed to quantify the number of *Asterias* larvae present, as they are indistinguishable from the larvae of native seastars.

We have previously shown that *Asterias* larvae can be detected in mixed plankton samples using specifically designed genetic probes and a nested PCR approach to improve sensitivity (Deagle et al. 2003). While this approach was sensitive – it could reliably detect 10 bipinnaria larvae in a spiked test sample of 200mg plankton, which equates to a sensitivity of 3-80 larvae/m^3 of water for plankton densities typically observed in the Derwent estuary – the approach was not quantitative. Thus while we could determine the presence of larvae in ships' low risk ballast water during the trial of the domestic ballast water management system at the Port of Hastings, we could not determine the significance of their presence (Patil et al. 2004).

One of the continuing challenges in studying the larval ecology of invasive species, to abate and manage their further spread is to estimate larval abundance. Quantitative understanding of larval dispersal either by natural advection or through human vectored translocation is particularly important to the dynamics and management of benthic invertebrates such as *A. amurensis*. Because benthic invertebrates have relatively sedentary juvenile and adult stages, the larval dispersal essentially represents their lifetime migration. While tremendous advances have been made since quantitative methods for analysis of nucleic acids via various hybridisation techniques became available, applications have generally been limited by the sensitivity of the procedures. Such methods for quantification of gene expression have traditionally involved the use of flurogenic or radiolabelled probes for detection of a particular DNA. These protocols are time-consuming and costly. PCR technology has greatly increased the sensitivity of methods for gene detection, but it is inherently nonquantitative. Quantitative PCR combines the sensitivity of PCR with real-time measurement of amplification and thus allows quantification of the original target concentration.

A PCR-based quantitative assay, first described by Holland et al. (1991) and referred to as a real-time PCR (RT-PCR), has recently emerged as a powerful tool for quantification of larval and microbial abundance. While this technique

offers all the advantages of conventional PCR, such as high sensitivity and specificity, it also allows quantification of PCR product formation during the exponential phase of the reaction. PCR product formation is monitored by determining the increase in fluorescence either due to binding of the amplicon to a fluorescent DNA stain, such as SYBR green, or due to the release of a fluorescent moiety from an oligonucleotide probe (i.e., a TaqMan probe) specific for the amplicon. TaqMan probes are short oligonucleotides, which are labelled with a fluorescent reporter dye and a quencher at the 5' and 3' ends, respectively. During template elongation the probe is cleaved by the 5' exonuclease activity of Taq DNA polymerase, which releases the 5'-linked reporter dye from the 3'-linked quencher, resulting in an increase in fluorescence with product formation. The probe is also removed from the target strand which allows primer extension to continue to the end of the template strand. Therefore inclusion of the probe does not inhibit the overall PCR. Even though real-time PCR was originally developed for clinical applications, it has recently been applied to larval and microbial ecology (Bowers et al 2000; Guy et al 2003).

Taking advantage of the specific primers developed previously (Deagle et al 2003) we have designed new primers and a TaqMan probe for quantification of the Asterias larvae. The work was directed at the mitochondrial COI gene locus, as a surrogate for detection and enumeration of the seastar larvae in plankton samples. To maintain the sensitivity of the nested PCR approach, we first amplified the product with conserved primers in a standard PCR, before amplifying with the new primers.

5.2. Materials and Methods

5.2.1. Genetics

5.2.1.1. Sample Collection

DNA samples from twelve seastar species (Table 4) broadly representing Australian taxa were obtained from a previous study (Evans et al 1998). These samples comprise 6 families from 3 orders including two species (*Coscinasteris muricata* and *Uniophora granifera*) from the family Asteriidae. Adult *A.amurensis* specimens from six Japanese populations and one Russian population were collected by Ward and Andrew (1995). Australian *A. amurensis* and some additional *Petricia vernicina* samples were collected by Deagle et al (2003).

Adult *A. amurensis* (Tasmanian) were bred in the laboratory to produce the larva used in the study. Larva were reared to the bipinnaria stage and samples at various developmental stages including unfertilised eggs were collected and then fixed and stored in SET (0.75 M NaCl, 5mM EDTA, 80mM Tris HCl, pH 7.8) buffered 80% ethanol.

Environmental plankton samples were collected at several locations along the south eastern Tasmania (see sampling strategy and collection section for more details) using a plankton net (100 µm mesh), plankton were rinsed with ethanol and stored in SET buffered 80% ethanol, until required.

Table 4. Seastar species used in this study

Species name	Number of specimens
Asterias amurensis	9
Asterias rubens	2
Asterias. forbesi	3
Cocsinasterias muricata	2
Uniophora granifera	2
Patiriella calcar	1
Tosia magnifica	1
Tosia australis	1
Nectria ocellata	1
Echinaster arcystasus	2
Plectaster decanus	1
Petricia vernicina	2

5.2.1.2. Sample processing and DNA extraction

Fixed eggs and larvae were rinsed in distilled water, isolated and counted under a dissection microscope, known numbers transferred to Eppendorf tubes, frozen and stored at -80ºC. Genomic DNA was extracted from pre counted eggs, early gastrula/late bipinnaria and stage 1 bipinnaria samples as follows. The samples were freeze dried to remove excess water and DNA was extracted using DNeasy plant mini kit (QIAGEN) following supplier's instructions. DNA was retrieved in 100 µl elution buffer quantified using UV spectrophotometer (Beckman) and stored at 4 °C.

All pre-processed and fixed off-shore plankton samples were concentrated by vacuum filtration through a 5 µm pore-sized hydrophilic Durapore Filter (Millipore). The residue was briefly air-dried, weight measured, transferred to a 50 ml tube and DNA extracted using the DNeasy Plant maxi Kit (QIAGEN) following suppliers instructions. DNA was retrieved in 1000 - 1500 µl elution buffer quantified using UV spectrophotometer (Beckman) and stored at 4 °C. All the plankton samples were diluted to get < 15 ng DNA before PCR amplification.

5.2.1.3. Design of primers and TaqMan probe

Sequence data from the COI region of 12 species (Table 4) of seastar were aligned using CLUSTAL_X (Thompson et al., 1997) and regions that discriminate *Asterias* from other species were identified. This region was used to design TaqMan primers and Probe. Primer Express software (version 2.0) provided with an ABI 7500 Real Time PCR System (Applied Biosystems) was used to design primers in accordance with guidelines therein.

Primers and probes were designed in a 122bp region of COI locus with a GC content of 30-50% and having the probe Tm 8-10 ºC higher than primers. The probe was designed to have a polymorphic site in the central third of the sequence and labelled with the reporter dye 6-FAM at the 5' end and a Minor Groove Binder (MGB) /non fluorescent quencher at the 3' end. TaqMan (MGB) probes were chosen to enhance the melting temperature (Tm) of the probe and also allow measuring reporter dye contributions more precisely because the quencher does not fluoresce. The details of the TaqMan primers and probes are given in Table 5. Both the primers were synthesised locally (Proligo, Lismore, Australia) and the TaqMan MGB probe was synthesised by Applied Biosystems (USA).

Table 5. TaqMan primers and probes designed in this study.

Primer/Probe	Type	Sequence (5'-3')	Length bp	T_m (ºC)	GC (%)
Ast_TaqF	Forward	GCACAACCGGGATCTTTACTTC	22	59	50
Ast_TaqR	Reverse	AGTCATTTACCAAATCCTCCTATCATAATA	30	58	30
Ast_TaqMGB	Probe	6FAM- TCATGCTCTTGTAATGATAT- NFQ	20	68	30

NFQ – non fluorescent quencher

5.2.1.4. Primer specificity

The designed forward (Ast_TaqF) and reverse (Ast_TaqR) primers were quickly tested against DNA samples of all the species of seastars listed in Table 3, in a standard PCR reaction. Briefly, standard PCR reactions were done in a 25 µl volume containing 0.2 µM of each primer, 0.2 mM dNTPs, 2 mM MgCl2, 1X AmpliTaq Gold® buffer, and 0.625 units AmpliTaq Gold® (Applied Biosystems). Thermal cycling conditions were: 94°C for 9 minutes then 35 cycles (94°C, 30s / 61°C, 30s / 72°C, 15s) followed by 72°C for 5 minutes. Aerosol-resistant pipette tips were used with all PCR solutions and negative control reactions were performed with each PCR cocktail. Amplified products were electrophoresed on a 1.8% agarose gel and visualised on a UV transilluminator. The new primers were found to be specific to the genus Asterias.

5.2.1.5. Real-time PCR assay conditions and cycle threshold

Preliminary optimisation of reaction conditions was carried out using SYBR green real time quantification assay (a cost saving measure). The SYBR green assay reaction contained SYBR Green PCR master mix (Applied Biosystems), primer (Ast_TaqF and Ast_TaqR) concentrations in the range of 50 to 400 nM and template concentration in the range of 1pg to 100 ng per reaction. Optimal concentrations for SYBR green assay were 200 nM of both primers and <1.4 ng template DNA.

The TaqMan MGB probe PCR conditions were also optimised with regard to concentration of primers (300 to 1200nM), probe (50 to 250nM) and amount of template DNA (1pg to 5 ng). Optimal concentrations for TaqMan PCR assays were 900 nM of both primers, 250 nM of probe and <1.4 ng template DNA. For increased sensitivity a nested PCR approach was adopted to quantify DNA from environmental samples. Additional care was taken to optimise the nested PCR. This involved optimisation of primary amplification cycle numbers (10 to 25) and template concentration. For primary enrichment PCR, 15 amplification cycles and < 1.4 ng of template DNA was found to be suitable.

Standard TaqManMGB probe amplification reactions were completed in a 25 µl volume containing, 1X TaqMan universal PCR master mix (Applied Biosystems), 250nM TaqMan MGB probe, 900nM each of primers (Ast_TaqF and Ast_TaqR), and 1µl primary PCR product. All real time amplification reactions were run on an ABI 7500 Real Time PCR System using the following settings; 2 min at 50ºC, 10 min at 95ºC, 50 cycles of 15 sec at 95ºC, 1 min annealing at 60ºC. All tests were performed in triplicates and 10 fold dilutions of standards were included in each run. Negative controls were also added to each run.

Data analysis was carried out with an ABI 7500 sequence detection software SDS package, version 1.2.1. The cycle threshold (CT) was calculated as the cycle number at which the reaction became exponential. The cycle threshold of each sample was then compared to a standard curve and the results were expressed as a numerical value of the number of target copies (DNA) in the sample.

5.2.1.6. Standard curve

DNA of stage 1 bipinnaria larvae of *Asterias* were used as standards and the concentration was calculated using UV spectrophotometer. *Asterias* DNA was subjected to 10 fold serial dilution (1030pg, 103pg, 10.3pg, 1.03pg & 0.103pg) and nested amplification reactions were carried out. The real time PCR profiles of these concentrations are given in Figure 18. The baseline, threshold and CT values were calculated and the standard curves were generated by ABI 7500 SDS software (Figure 19). The threshold cycle was determined to obtain a slope between –3.12 and -3.29; and regression coefficient values (r^2) were always above 0.97.

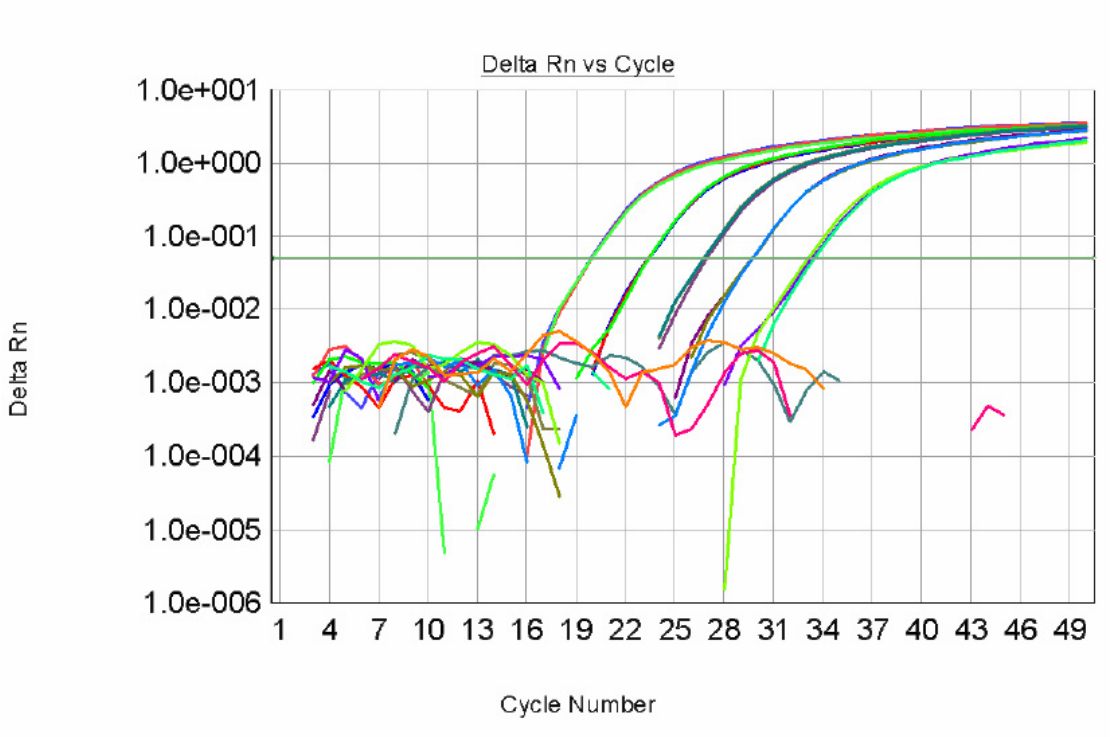

Figure 20. Real-time PCR plots of standards (10-fold serial dilution), showing number of cycles necessary for concentration to reach the threshold (horizontal line).

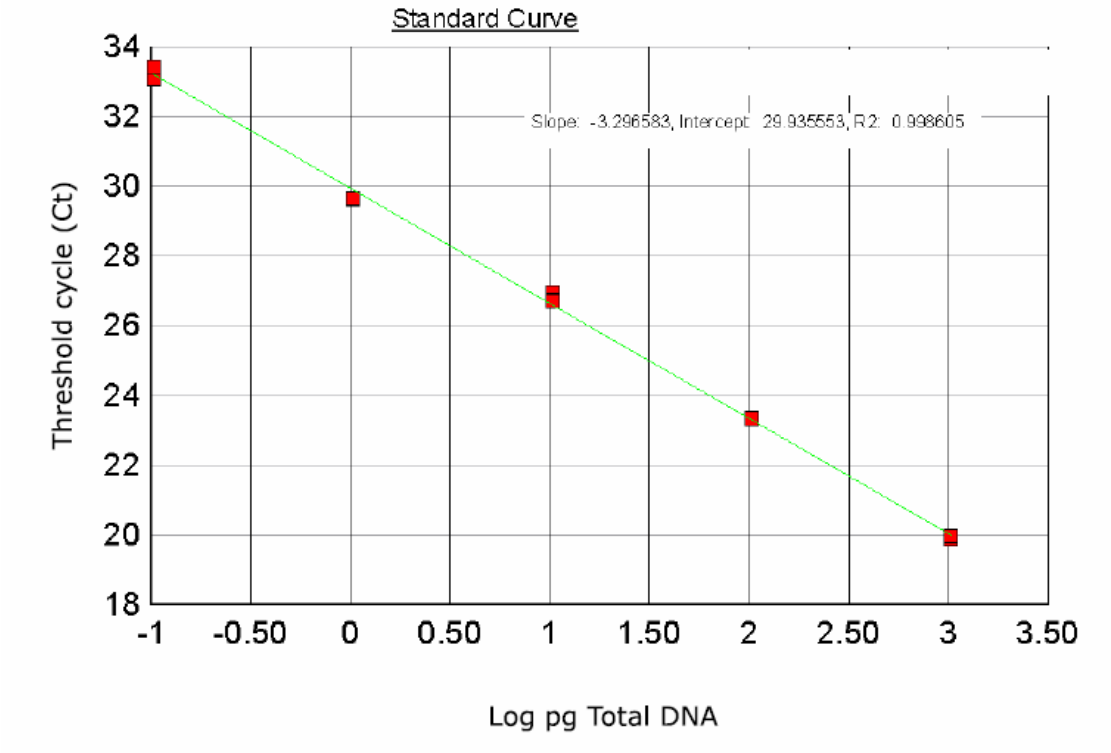

Figure 21. Standard curve generated from Figure 18, showing number of cycles to reach threshold vs DNA concentration of standard.

5.2.2. Quantification of *Asterias* DNA in reared larvae and environmental samples.

A two-step nested PCR was used for plankton samples to enhance the sensitivity of the test. Therefore, 10-fold dilutions of standards and samples of reared eggs and garstrula larvae were also analysed with nested PCR. Primary enrichment PCR was conducted using the universal primer pair ECOLa-F (Knott and Wray 2000) and HCO-R (Folmer et al., 1994). Standard PCR reactions were done in a 25 µl volume containing 0.2 µM of each primer, 0.2 mM dNTPs, 2 mM MgCl2, 1X AmpliTaq Gold® buffer, and 0.625 units AmpliTaq Gold® (Applied Biosystems). Thermal cycling conditions were: 94°C for 9 minutes then 15 cycles (94°C, 30s / 54°C, 30s / 72°C, 1 minute) followed by 72°C for 5 minutes.

Secondary TaqMan Real time PCR amplifications were carried out according to optimised primer/ probe concentrations and thermal cycling conditions given in section "Real Time PCR assay conditions and cycle threshold". Data analysis was carried using ABI 7500 sequence detection software (see details above).

5.2.3. Field program

Larval *A. amurensis* have been recorded in the Derwent estuary between May and January, with major peaks in abundance in early August and late September (Bruce et al. 1995). Larval densities are significantly higher at the surface and 5m compared to 10m. Depth distribution of *Asterias* larvae appears to be independent of ontogeny in the Derwent, although brachiolaria larvae were rare and late stage brachiolaria absent from the samples. It is unknown if larval vertical distribution offshore is the same as in an estuarine environment. The only information we have regarding the vertical distribution of larvae offshore is that large numbers of juvenile *A. amurensis* settle in scallop spat-collectors set between 6-8m in Great Oyster Bay (north of Triabunna).

5.2.3.1. Tasmania

Plankton samples were taken on three cross shelf transects between September and November 2004 (Figure 20). Five stations were sampled along each transect. Transects at St. Helens and Tasman Island transects were sampled twice and the Maria Island transect three times (Table 6).

Single oblique tows from 10m depth to the surface used paired 70 cm diameter, 100µm mesh bongo nets towed from the CSIRO Marine Research shark-cat *Explorer*. A General Oceanics mechanical flowmeter was fitted to the net frame and used to estimate the volume of water filtered for each tow (calibrated at 36.7 counts / meter). Tow length was initially set at 10 minutes but was reduced to 2 minutes due to the large biomass of the samples. Each tow filtered on average $63m^3$ of seawater, with biomass ranging between 25ml to 400ml in wet weight volume per sample. After each tow, the nets were rinsed down using a bilge pump and the sample from each net washed into

separate small 100 µm mesh net-sieves to remove as much seawater as possible. Samples were then rinsed into sample jars with SET buffered reagent grade ethanol, ensuring that the ratio of biomass to SET buffered ethanol was no more than 1:3.

5.2.3.2. Victoria

Larval *A. amurensis* were recorded in Port Phillip Bay sites, between late May and late October 2002 (Dommisse and Hough 2004).

Plankton samples were taken with a vertical 70cm diameter, 100µm mesh plankton net with choke collar (to prevent sampling upon retrieval). Samples were vertically integrated samples from the surface to approximately 1m from the bottom, at a sinking rate of sinking ~ 1m min^{-1}. Three replicates were taken at 5 sites (Table 7). Samples were taken in late October, close to the end of larval presence in the water column. Net contents were washed down and concentrated in the codend, drained in the net sieve to remove as much of the water as possible, washed into the sample jar with SET buffered ethanol, topped up so that organic matter was no more than 1/3 volume.

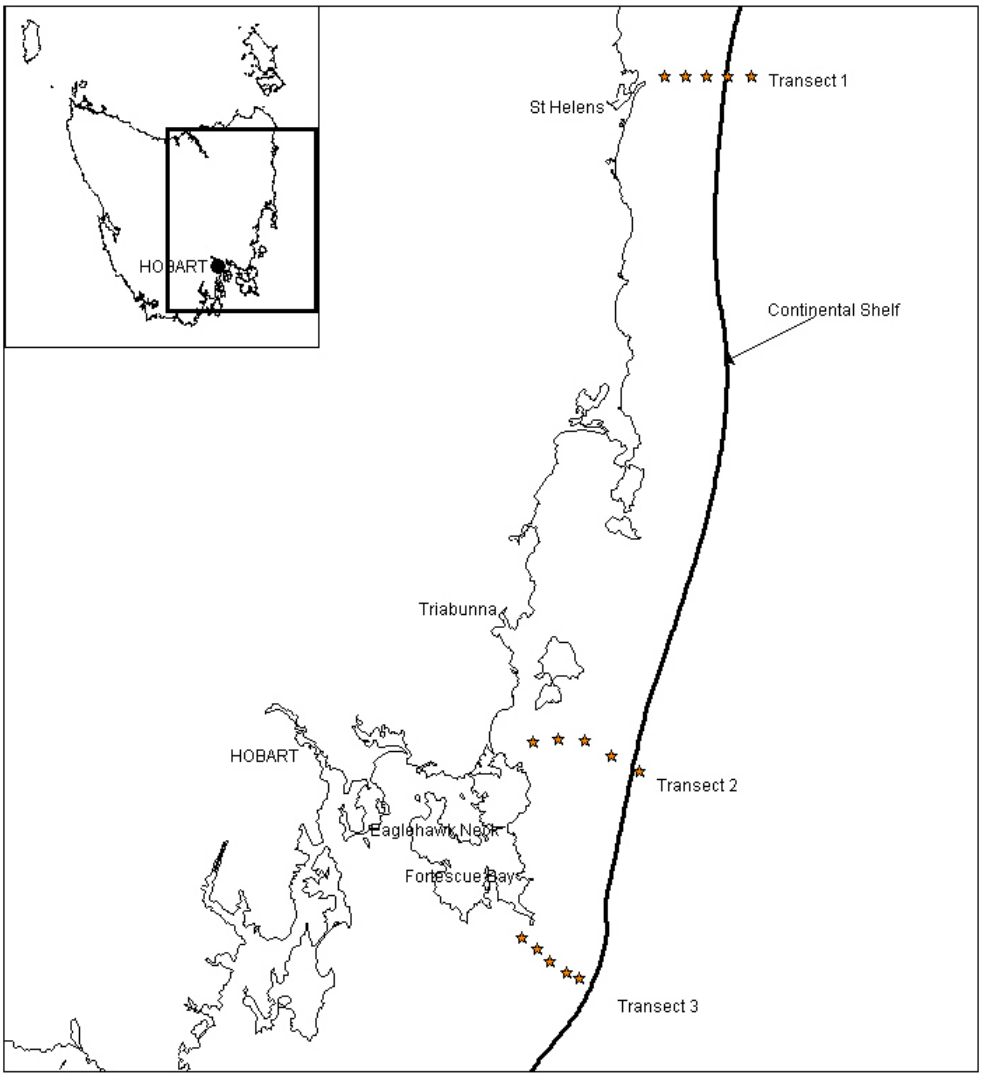

Figure 22 Map of study area showing cross-shelf transect locations.

Table 6 Samples collected in Tasmania

	Maria Island	Tasman Peninsula	St. Helens	Survey Total
Transect Dates **No. of Stations**	22 September 19 October 26 November 5, 5, 5	11 October 9 November 5, 5	29 September 29 October 5, 4	
Total No. Samples	30	20	18	68

Table 7 Samples collected in Victoria between 28/10/04 and 29/20/04

	Port Phillip Bay	Westernport	Andersons Inlet	Survey Total
Site	Point Lonsdale Williamstown, Anne St pier	Flinders Pier St Remo	Inverloch Jetty	
Total No. Samples	3 + 3	3 + 3	3	15

5.2.3.3. South Australia

Dropnet samples were collected in late October from Adelaide Harbour immediately following the arrival of a ship discharging ballast water taken on board in Port Phillip Bay. Unfortunately, the samples were either lost in transit or lost on arrival at the CSIRO Marine Laboratories.

5.2.4. Environmental sample processing

Bongo net samples were too large for DNA extraction using standard approaches, so the samples were split using a Fulsom plankton splitter, until each split was less than 1 gm wet weight, which could be processed in a maxitube (up to 7 times for some samples, leading to 1/128th original volume). Densities of plankton in the samples were low and manual plankton sorting was not attempted on all samples, being restricted to samples where high abundance was indicated by the quantitative PCR. For split samples, total number of larvae per sample was estimated by multiplying the amount found by the split factor.

5.2.4.1. Conversion of DNA to individuals

The real-time PCR measures the absolute amount of target DNA (or RNA) in a sample but provides no direct information on the number of individuals in the samples, because individuals at different stages in their development will have different numbers of cells and different amounts of target DNA. The amount of DNA per individual at various life history stages was determined and used to convert sample DNA to number of individuals. DNA content of eggs and gastrula/stage1 bipinnaria were determined with the real time PCR (Section: “Quantification of *Asterias* DNA in reared larvae and environmental samples”). DNA of bipinnaria larvae was measured with UV spectrophotometry (Section: “Standard curve”).

5.3. Results

Larvae of *A. amurensis* were found in trace to very small amounts off the Tasman Peninsula, the southern most site, in September (the 4 inshore

stations) and October (the one innermost station). No larvae were detected in November (Table 8)

Larvae were more abundant at the Maria Island transect that is further north and further away from the main larval source – the Derwent Estuary. Larvae were detected on the four offshore stations with abundance highest at stations 2 and 3. Three of the five stations sampled in October, and one of the five stations sampled in November had trace or very small amounts of *A. amurensis* DNA.

Trace or small amounts of larval DNA were detected on all 5 stations off St Helens in September and the most offshore station only in October. No samples were taken in November.

Based on the DNA content of an individual stage 1 bipinnaria larvae being 1000 pg and given the caveats associated with the non-uniform sample splitting this suggests that there were between 50 and 1000 larvae per tow off Maria Island in September and 1 larvae or less per tow (ie. a fragment) in all other areas and times.

Of the five areas samples in Victoria, only one – Ann St pier in Williamstown, Port Phillip Bay – was positive for *A. amurensis* (Table 9). All three samples were positive. Given the late stage in the season, it would be expected that the larvae were at least stage 1 bipinnaria, indicating that larval densities were of the order or 1 or 2 per sample.

Table 8. Results for quantitative PCR of *Asterias amurensis* off Eastern Tasmania.

			Total wt	< 250um		Fraction	Sample wet wt(g)			Total DNA (ug)			Asterias DNA (pg)				Total DNA	Asterias DNA (pg)					Asterias DNA (pg)
Transect	Date	Station	(gm)	gm	Fraction	wt (gm)	1	2	3	1	2	3	1	2	3	CV	combined (ug)	1	2	3	mean	cv	original sample
Maria Island	9/21/2004	1	26.3	23	1/32	0.73	0.19	0.19	0.20	11	12	13											
		2	44.0	36	1/64	0.56	0.23	0.11	0.10	79	41	51	1220	24	3800	0.7	167	15720	11310	16890	14427	0.05	923341
		3	32.5	26	1/32	0.82	0.15	0.24	0.29	79	105	64	182	8050	209	0.6	250	15300	12810	12540	13495	0.02	431847
		4	56.8	50	1/64	0.78	0.25	0.19	0.14	95	57	52	972	956	170	0.3	199	861	723	609	724	0.04	46320
		5	53.1	26	1/32	0.81	0.11	0.10	0.16	51	38	69	33	510	1210	0.5	156	2865	2982	2505	2776	0.02	88844
	10/19/2004	1			1/64	0.62	0.09	0.10	0.10	37	31	25											
		2			1/64	0.52	0.06	0.08	0.09	33	10	13	positive				57	positive					
		3			1/64	0.62	0.09	0.12	0.14	4	3	3											
		4			1/64	0.55	0.07	0.07	0.07	4	3	4	positive				14	positive					
		5			1/64	0.56	0.18	0.15	0.17	32	19	28			positive		82	positive					
	11/26/2004	1	39.6	21	1/32	0.65	0.38	0.21	0.16	21	34	26	3	positive	4		91	positive					
		2	66.3	9	1/16	0.59	0.16	0.23	0.17	35	38	25											
		3	33.0	9	1/16	0.57	0.11	0.14	0.21	13	13	16											
		4	48.9	18	1/32	0.56	0.07	0.12	0.11	21	28	26											
		5	38.4	13	1/16	0.84	0.25	0.29	0.34	57	69	83											
Tasman	10/11/2004	1		20	1/32	0.61	0.15	0.11	0.13	11	11	12	positive	positive									
		2	24.3	15	1/16	0.93	0.41	0.47	0.48	58	61	71											
		3	28.7	17	1/16	1.08	0.33	0.28	0.32	70	97	79											
		4	39.6	28	1/32	0.88	0.27	0.27	0.31	24	45	52											
		5	33.7	25	1/32	0.77	0.16	0.19	0.24	35	23	28											
	11/9/2004	1		31	1/32	0.98	0.31	0.37	0.58	84	100	86	positive		4								
		2	61.4	37	1/64	0.57	0.16	0.17	0.25	33	30	37	positive		positive								
		3	50.0	36	1/32	1.11	0.32	0.36	0.35	129	91	91	3	positive	positive								
		4	85.6	38	1/64	0.59	0.17	0.15	0.20	54	28	27		2									
		5	41.8	24	1/32	0.75	0.19	0.21	0.22	124	88	105											
	12/13/2004	1	42.9	27	1/32	0.85	0.17	0.17	0.23	32	31	17											
		2	40.7	27	1/32	0.83	0.10	0.07	0.12	32	27	21											
		3	36.7	16	1/16	0.99	0.19	0.29	0.26	97	71	85											
		4	35.6	21	1/32	0.65	0.11	0.13	0.12	30	25	28											
		5	66.2	32	1/32	0.99	0.11	0.11	0.11	25	29	28											
St Helens	9/29/2004	1	38.7	26	1/32	0.80	0.08	0.06	0.10	3	3	4	1	14	1	0.7	9	2	1	1	1	0.18	42
		2	67.8	50	1/64	0.79	0.09	0.14	0.11	9	16	12	2	2	9	0.4	35	6	5	4	5	0.08	316
		3	62.7	50	1/64	0.78	0.11	0.07	0.08	7	8	5	1	1	0	0.4	18	2	2	1	2	0.16	104
		4	81.5	79	1/128	0.62	0.05	0.04	0.03	8	6	6	positive	positive			20	positive					
		5	67.3	43	1/64	0.67	0.11	0.09	0.14	29	21	18	89		positive		67	positive					
	10/29/2004	2	96.7	21	1/32	0.64	0.10	0.11	0.12	7	17	12											
		3	130.3	26	1/32	0.81	0.12	0.10	0.13	5	5	6											
		4	113.1	15	1/16	0.95	0.19	0.26	0.22	6	6	6											
		5	658.1	45	1/64	0.70	0.14	0.15	0.17	10	8	9	3	2	5	0.2	28	8	8	4	7	0.16	417

Table 9. Results for quantitative PCR of *Asterias amurensis* off Southern Victoria

Location	Date	Sweep lengh (m)	Sample wet wt (gm)	Total DNA (ug/sample)	Asterias DNA (pg/sample)
PPB-Pt Lonsdale	10/28/2004	9	0.64	92.646	
		9	1.1	149.22	
		9	0.38	81.335	
PPB-Williamstown, Ann St Pier	10/28/2004	6.5	0.37	80.155	1790
		6.5	0.32	35.095	3120
		6.5	1.1	62.188	165
Western Port-Flinders Pier	10/29/2004	10	0.66	74.637	
		10	0.68	188.57	
		10	0.95	156.75	
Western Port-San Remo	10/29/2004	10	3.35	53.517	
		10	1.87	75.379	
		10	3.29	188.57	
Inverloch jetty	10/29/2004	NA	2.33	259.8	
		NA	5.17	226.26	
		NA	4.08	215.82	

5.3.1. Conversion of DNA to individuals

An individual's DNA content varies as it size and number of cells increases (Table 10). It is difficult to rear *A. amurensis* larvae beyond the bipinnaria stage without special facilities. In this instance, *A. amurensis* eggs were reared to bipinnaria stage 1, which corresponds to 3 to 14 days, at which time they weighed on average 1000 pg.

If we adjust the increase in individual weight by the fraction of individuals surviving per day (using mortality estimates from the MSE model) we can estimate that the biomass of a cohort of *A. amurensis* increases over time (Figure 19) – at least over the first 15 days of post hatch. If the biomass of a cohort had remained constant over time (individual growth exactly compensates for loss from death and predation) then the level of DNA in a sample would represent the fraction of a cohort surviving, and we could compare between samples without correcting for the age/size of individuals. As this is not the case, we need to know the stages of larvae contained in a sample so that we can convert the DNA concentration to number of individuals. We are currently sorting the samples taken off the East coast of Tasmania that tested positive for *A. amurensis* to determine the larval stages contained in the sample.

Based on the time of year that these samples were taken, and the expectation that they were transported to the east coast from the Derwent estuary, we would expect the larvae in the samples to be bipinnaria stage 1 larvae or later, implying that an individual would weigh 1000 pg or more.

Table 10. DNA content of reared *Asterias* eggs and larvae

Asterias stage	Rearing days	Technique	Number individuals	*Asterias* DNA pg/individual	Mean	SD
Fertilised eggs	0	RT-PCR	100	50	39	10
			100	53		
			100	48		
			100	49		
			100	40		
			100	34		
			100	41		
			100	33		
			100	27		
			100	32		
			100	30		
			100	27		
late gastrula/bipinnaria	2 to 4	RT-PCR	100	89	85	7
			100	84		
			100	92		
			100	98		
			200	79		
			200	76		
			200	79		
			200	85		
stage 1 bipinnaria	3 to 14	UV-Spec	1,000	900	1,000	190
			1,000	800		
			1,000	1,200		
			1,000	1,200		
			1,000	800		
			1,000	1,100		

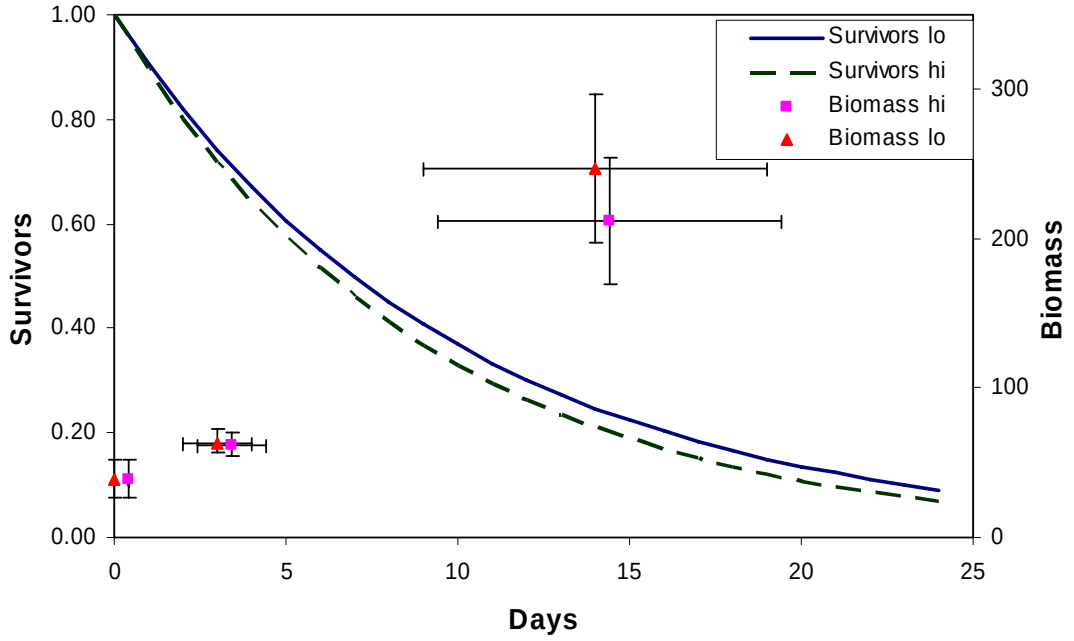

Figure 23. Proportion surviving of an idealised *Asterias* population, and resulting DNA biomass, where x-axis error bars represent the range of possible days to reach a defined larval stage and y-axis error bars represent range in weight of individuals at that stage.

5.3.2. Sampling variability

Where DNA concentrations were high (eg. Maria Island sites in September), it was clear that there was high within sample variability introduced by the splitting process. The amount of DNA detected varied over two orders of magnitude for supposedly equivalent 1/32 or 1/64 splits from the same samples and coefficients of variation on the logged data varied between 0.2 and 0.7. A similar between-spilt variability was observed off St Helens in the same month.

To determine whether this variability was introduced by the splitting process or the first stage PCR amplification, we pooled the remainder of the final splits (more than 50% of the original final split in all instances), mixed the combined sample and processed three samples with the first stage and real time PCR[1].

Between-subsample variability was greatly reduced, with coefficients of variation (logged data) reduced to between 0.02 and 0.18, indicating the first stage PCR amplification did not contribute substantially to the previously observed between-split variability.

[1] The real-time PCR does not introduce this variability as amplification per cycle is monitored against standard concentrations.

Between-split variability was not high for the total DNA recorded, indicating that the splitting process was adequately apportioning biomass between the splits. However, evidently the (rather rare) *A. amurensis* larvae were not distributed evenly in the sample before splitting. Because the final 1/32 or 1/64 splits followed 4 or 5 previous splits with variability introduced at each stage[2], the final DNA concentrations can not be considered reliable at this stage.

DNA analyses typically use small quantities of product. In this study we used Maxikits that extract DNA from 1gm of wet biomass, whereas typically only 0.1 gm of wet biomass is used as the sample. Specialised equipment is available for disrupting and homogenising tissue in larger samples (eg. the Qiagen TissueLyser system that can provide rapid tissue disruption in sample sizes from 2*96 samples in microtubes, 2*24 samples in 2.0 ml microcentrifuge tubes, or 2* larger samples 4-10 ml of sample per jar). This would have enabled effective tissue disruption and homogenisation in the < 250 µm fraction of bongo net samples collected in this study following between 0 and 3 splits, instead of the 4 to 7 splits that were needed before cell disruption and DNA extraction using standard techniques.

5.4. Discussion

The advantages of real-time PCR over standard PCR, which shows only presence/absence, are clear from the problems that it has identified in the subsampling of large plankton samples. It is clear from the variability in results in *A. amurensis* DNA concentration between splits that the splitting process does not distribute *A. amurensis* evenly between the different splits. If we were using standard PCR all samples with any *A. amurensis* DNA would have scored positive, September larval concentrations off Maria Island and St Helens would have been indistinguishable from each other and from other positive samples later in the year, and we would not have discovered the high sub-sampling variability that will have influenced the final result. This has led us to recommend that larger plankton samples (especially where the target organism is relatively rare) need to be well mixed before splitting. Given the matrix of organic material that may prevent adequate mixing of rare particles stuck to the matrix, we anticipate that mechanical disruption of the tissues will be needed first.

At the same time the real-time PCR technique does have some disadvantages. First, it required that the *Asterias* probe be redesigned, although this might have some advantages as it appears that the new probe is specific for *A. amurensis* and not just *Asterias.* Second at the moment, it appears that there can be cross-contamination of readings in the real-time PCR if probes for more than one species are used at one time. This may be surmountable by using blank cells between samples, but this would reduce throughput.

[2] This is not strictly true as the 4 final splits comprise the 2 final splits from one penultimate split and one final split from the other penultimate split

Alternative approaches for estimating the abundance of target organisms in a sample need to be examined. These options need to be compared against the results from the real-time PCR and compared with the level of information required to inform specific management decisions.

In the case of the Port of Hastings study (Patil et al 2004), added information on the amount of DNA in samples would have been informative. While in that study, we were able to show where ballast water considered low-risk by the risk assessment DSS contained the target species. We were not able to determine the target species concentration, however, and in some comparisons this restricted our ability to distinguish a failure in the ballast water management system from an acceptable reduction in risk. Reanalysis of these samples with the real-time PCR could be a goal of future work.

6. MOLECULAR CHARACTERISATION OF GENE(S) INVOLVED IN SEX DETERMINATION AND DIFFERENTIATION PATHWAYS OF THE SEASTAR, *A. AMURENSIS*.

Integrated pest management approaches with a genetic mechanism at its core appear to be a most promising pest control option (Bax and Thresher in review). In particular self-perpetuating genetic mechanisms that exert negative selection pressure on reproductive output, survival and hence the recruitment to adult population have been the subject of interest/scrutiny to control a variety of pests. As a prelude to the potential evaluation of one or more of genetic options for the control of *A amurensis* (see modelling section for details) we have begun a systematic characterisation of the molecular pathways involved in sex differentiation and determination in the species. An understanding of these molecular pathways will facilitate developing genetic control strategies.

The recent identification of a conserved DNA binding domain (the DM domain) in a family of transcription factors important for sexual determination or differentiation has indicated a degree of molecular conservation in these events across phyla. The initial finding was that the *Drosophila* Doublesex (DSX) DM domain is shared by the MAB-3 protein of *Caenorhabditis elegans* (Raymond et al. 1998). The DSX protein, which lies at the terminus of the fly sex determination pathway, is present in male and female specific forms (Burtis and Baker 1989), whereas MAB3 appears to be primarily important in male development (Shen and Hodgkin 1988; Yi et al. 2000). Vertebrates contain a family of genes encoding DM domain transcription factors, one of which, DMRT1, has been studied in greatest detail. DMRT1 expression in the genital ridge of a variety of animals, including mammals, reptiles, and fish, correlates with testis differentiation (reviewed by Zarkower 2001). It therefore seems that DM proteins are involved in sex determination or sexual differentiation in many if not all higher metazoans. We developed a small project to determine if DM-containing proteins are present in *Asterias amurensis*.

First, we obtained the sequences of all the DM domain genes in the public database, aligned these and designed several degenerate primers pairs spanning the most conserved regions. Adopting standard molecular tools we successfully amplified target fragments from *A. amurensis* genomic DNA. These amplified products were cloned and sequenced to determine their identity. Subsequently more specific primers were designed and the entire cDNA for two genes sharing the DM domain were successfully amplified using the standard 5' and 3' RACE cDNA synthesis approach. In a preliminary study one of the genes appears to be sex specific. However this needs to be further evaluated for its expression pattern during early larval ontogeny and in other adult tissues.

7. EXTENSION OF MSE FRAMEWORK TO OTHER HIGH PROFILE MARINE PEST SPECIES

7.1. MSE Framework

One advantage of the Management Strategy Evaluation (MSE) framework is that it is based on a rigorously defined computer model, that once defined is readily transferable to other situations – in this case, other pest species. Australia's marine pests will be exposed to the same oceanographic currents at the same time of the year; they have the potential to be carried by the same vectors between the same ports and estuaries with identical environmental properties; and control methods – physical removal, ballast water management, genetic control, etc. – will be effected through the same biological and physical models. What will differ is the way in which each pest species interacts with physical current, vectors, and control methods. This is defining the pest species' characteristics, something that is required before the value of imposing vector controls or other management options can be assessed.

The cost of applying the MSE framework to other pest species, is that being based on a quantitative model, it requires the characteristics of each pest species and the way they interact with vectors and control options to be defined explicitly. The same quantitative requirement applies to management objectives, strategies, performance actions and indicators. While this may seem an onerous task, it is realistically the logical extension of the risk-based approach that has been used to identify those pest species which need managing in the first instance (Hayes et al 2004). And in a way, the information required by the MSE framework is exactly the information needed to make management decisions. The MSE framework requires that the information needed for informed management decisions be collected and interpreted in a transparent and rigorous manner.

At the moment the MSE framework is limited to southern Australia. This spatial restriction was based on the availability of oceanographic information at the time that this project started in 2003. Since that time, CSIRO Marine (and Atmospheric) Research have invested considerable effort in developing the BlueLink model for the Australian Navy. This model provides a full oceanographic model for all Australian waters, nested within a global coupled oceanographic climatological model. The model has the capacity to provide finer resolution of particular areas and has been used to recreate ocean currents around Australia since 1992. Future extensions of the MSE framework should include ocean currents from this model, providing the ability to simulate the movement of marine pests all around Australia.

Existing components of the MSE framework could be easily updated to match this wider geographical distribution – estuary retention times are available for all Australian estuaries from the SERM II database, commercial shipping

traffic is available for all of Australia, Commonwealth fishing vessel traffic is available. The main database that is lacking is the movement of recreational vessels. Several projects have attempted to get this information but products from those projects have either focussed on general attributes of the traffic, or have provided actual vessel movements but only for a restricted area. The lack of recreational vessel traffic was not a major issue for the North Pacific seastar, as we did not have evidence that this vector could spread the seastar. This will not be the case for other species, and recreational vessel traffic will need to be defined. This would be most realistically achieved through a statistical model that extends the characteristics of the limited data available to other areas.

Once the MSE framework has been extended to cover the entire Australian coast, it will be possible to evaluate management strategies for performance over the range of marine pest species deemed high risk to Australia. This will provide a means to link the management strategies, actions and indicators for all National Control Plans so that marginal benefits for each species and overall benefits for all species can be determined.

7.2. Consultation

Management strategy evaluation has been applied in several situations where the consequences of a range of management strategies or options cannot be determined by qualitative examination, either because the underlying system is too complicated, or because the social and management values are too contentious to be resolved in a qualitative, value-laden framework. Thus the MSE approach has been used in the International Whaling Commission and is mandated in the US for fisheries in recovery mode and in Canada for all fisheries. This quantitative approach is at its most powerful where the views and values of different stakeholders differ markedly.

Early in the project it became clear that the MSE framework provided more than a mechanism to assist the development of clear management objectives, strategies and performance measures for managing marine pests. The process involved in developing these attributes also identified the values and beliefs of diverse stakeholders. And perhaps as importantly allowed those stakeholders to be included (and to be felt to be included) in the management process.

There has been steady progress in defining suitable management objectives, strategies and actions. Early discussions with managers (as part of an earlier NHT-funded project) started with “maintaining biodiversity” as the management objective. Since biodiversity is neither clearly defined nor readily measurable it was clear that it could not provide the necessary quantitative rigour. The next iteration of “reducing the spread of invasive species” was an improvement as it provided a measurable objective, but it did not differentiate between highly-valued and less-valued habitats, nor between areas where a pest species could be expected to spread anyway (in prevailing currents) and areas where it could only reach on(in) an anthropogenic vector. In the first

workshop held as part of this project, management objectives were further honed to “stopping human-mediated dispersal” and “protecting high-value habitats”. Progress was also made on defining potential management strategies.

In a public forum held in April 2005 a more structured approach was taken to developing objectives, strategies, actions and indicators. After some interpretation, this resulted in 4 core objectives, 8 strategies, and 23 actions with associated indicators.

Ideally there will be several similar workshops in other affected States to ensure that we have a broad input to defining objectives, etc, but also as a process to include a diverse group of stakeholders from different areas. Ensuring that stakeholders feel included in the process is an important component of developing the National System as many of the vector management measures will be voluntary depending on stakeholder goodwill. Experience from other areas suggests that involving these stakeholders in the process to determine why and which management measures are needed, will be an effective approach to achieving high uptake.

7.3. Presentation of MSE results

An important component of the future development of the MSE framework is the effective communication of results. As the various stakeholder groups become better informed, and as the management strategies and performance measures become better defined, it will be necessary to evaluate tradeoffs in the context of the held values of the different groups. The complexity of the problem, and the many options available, have the potential to deflect the necessary debate on how to achieve effective compromise between differing opinions. One way to reduce the perceived complexity of the problem is to communicate the results of the MSE in a simple and unambiguous format that makes the tradeoffs between different options clear. We have made a first attempt at this using the results of alternative ballast water management arrangements.

Different ballast water management options were rated according to their effectiveness at reducing the median probability of invasion to a density of 10^{-4} per m^2. This is about the density at which the seastars would be first detectable. The results clearly show that managing ballast water by the DSS is as effective as requiring all vessels to reballast. The results show no effect of increased monitoring on reducing the probability of invasion. This results from a management rule that considers a port invaded once a seastar is found, even if a self-sustaining population has yet to establish. The value of more frequent monitoring (up to the level of diminishing returns) would presumably be found if a port were declared invaded for outgoing ballast water, but clear for incoming ballast water, until it was clear that a self-sustaining population had established.

The results also show the value of reballasting the equivalent of 5 or 7 tanks compared to the 'standard' three tanks that provides an expected 95% reduction in home port organisms. Paradoxically, reballasting the equivalent of 1 tank appears equivalent to reballasting 3 tanks. These results will be specific to *A. amurensis* that has a very long planktonic life history (~ 3 months).

Table 11. Report card for alternative ballast water management options in reducing the median probability of invasion for *A. amurensis.*

Depth or distance offshore (1 cell = 20km)	***Number of tanks exchanged***	***Number of monitoring samples taken if risk tables used***	***Reduction in median invasion probability***
0 cells (coastal)	3	NA	★★
1 cell	3	NA	★★
2 cells	3	NA	★★
200m depth	3	NA	★★
1 cell	1	NA	★★
1 cell	5	NA	★★★
1 cell	7	NA	★★★★
1 cell	3	2	★★
1 cell	3	5	★★
1 cell	3	10	★★
1 cell	3	20	★★
☆ <= 10% reduction ★ 11 – 20% reduction ★★ 21 -30% reduction ★★★ 31-50% reduction ★★★★ 51-95% reduction ★★★★★ >= 95% reduction			

The results for genetic control are presented in Table 11. Care must be taken in comparing tables 11 and 12 as the simulations differed. However, by comparing the relative efficiencies of "standard" reballasting of 3 tanks with no route deviation with genetic control it is apparent that at an early stage in the invasion, controlling the source populations is the most efficient method of control. Ballast exchange and adding transgene seastars to selected estuaries to reduce the probability of further spread achieve similar results.

As the species becomes more established throughout southern Australia, the benefits of controlling the increasing number of source populations. Controlling the spread will be more important and genetic control in selected estuaries (or ballast water exchange) becomes preferred options.

Table 12. Report card for comparing ballast water management and genetic options in reducing the median probability of invasion for *A. amurensis.*

Depth or distance offshore (1 cell = 20km)	***Number of tanks exchanged***	***Genetic control technique***	***Number of transgene animals added***	***Reduction in median invasion probability***
1	3	NA	0	★
NA	0	Sonless	10% of established populations	★★★
NA	0	Sonless	1000 into high retention estuaries	★
NA	0	Daughterless	10% of established populations	★★
NA	0	Daughterless	1000 into high retention estuaries	★

☆ <= 10% reduction ★ 11 – 20% reduction ★★ 21 -30% reduction ★★★ 31-50% reduction ★★★★ 51-95% reduction ★★★★★ >= 95% reduction

7.4. Costing alternative management options

Section 4.4.2 dealt with the direct costs associated with ballast control compared with the costs of no control (Table 3 repeated below). Using the risk tables to determine the reballasting requirements achieved a similar reduction in invasion probability to the best outcomes for mandatory exchange with costs at least 10 times lower than mandatory exchange. The cost of monitoring did not significantly increase the cost of management when the risk tables were used, despite the assumption that monitoring occurred in all 104

ports present in Southern Australia. Using risk tables to manage ballast water limits the number of times ballast water must be exchanged and controls the spread of an invasive species as efficiently as mandatory exchange. However, the effectiveness of risk tables is very dependent on accurate monitoring of ports and prompt management responses when an invasion is detected.

Table 3. The median journey duration and cost of ballast water control compared with no control.

	Normal Shipping	***Exchange coastal***	***Exchange 1 cell***	***Exchange 2 cells***	***Exchange 200m***	***Risk Tables (2 samples)***
Median Time (h)	14	36.8	38.5	40.2	55.6	14
Cost ($AU ,000,000)	95	139	154	154	231	99
Exchange cost ($AU ,000,000)	-	44	99	99	136	4

A similar reduction in median invasion probability could be achieved by daughterless or, preferably sonless, genetic control of the existing population. The costs of achieving this are hard to estimate, but we could guess that the initial research would be a one-off cost of $10 million, initial construction of aquaculture facilities to produce 10% of background settlement in the Derwent Estuary and Port Phillip Bay might cost $10 million and the ongoing production might cost an annual $1 million.

At first glance development of genetic techniques would appear to be the better investment, however, this analysis does not account for the role of ballast water exchange in reducing the invasion probability of other invasive species, nor does it take into account the risks associated with the genetic control, in particular that hatchery-reared seastars would not be able to compete with naturally produced seastars.

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Appendix 1. List of attendees at forum

Organisation	Individual
State Governments	
Fisheries Victoria	Dr James Andrews
Parks Victoria	Dr Anthony Boxshall
	Sonia Lloyd
DEH Victoria	Dr Michaela Dommisse
	Ingrid Holliday
	Dr Martina Doblin
	Don Hough
NSW Fisheries	Rebecca Chapman
Commonwealth Government	
DEH Australia	Warren Geeves
DAFF Australia	Dr Naomi Parker
Government Advisory Bodies	
Central Coastal Board	Sarah Canham
Gippsland Lakes Coastal Board	Brett Millington
Commercial fishing peak bodies	
Seanet	Jim Newman -SeaNet Extension
Commercial shipping peak bodies	
ASA	Angela Gillham
Dive Industry peak bodies	
Dive Industry of Victoria Assocn.	Geoffrey Sparkes
Key Academics and Professional bodies	
Australian Marine Sciences Assoc.	Robby Mort
CSIRO	Dr Nic Bax
	Dr Piers Dunstan
Key Consultants	
PriceWaterhouseCoopers	Vartguess Markarian
Conservation Peak bodies	
Reefwatch Victoria	Wendy Roberts
Earthcare St Kilda	Andrew McCutcheon

Appendix 2 Population Biology of Asterias amurensis

Growth and Mortality

The current distribution of adult A. amurensis is limited to two estuaries in southern Australia, the Derwent estuary in southern Tasmania and Port Phillip Bay, in southern Victoria. It is estimated that there are 4 x 10^6 seastars with a ray length of 5.5 cm in the Derwent estuary (Ling *et al.* unpublished manuscript) and 9 x 10^7 seastars in Port Phillip Bay (Bruce et al 1995). To predict the spread of A. amurensis across southern Australia it is necessary to understand the dynamics of these populations and their reproductive output.

In 2000, CSIRO, with help from community groups, held a seastar cleanup around the docks in the Derwent Estuary. Seastars were collected by divers, and the ray length of each starfish (to the nearest 5mm) was measured for a proportion of those collected. This data set provided the best data to assess the growth and mortality of *A. amurensis* in the Derwent. Data collected on starfish from Bellerive yacht club, CSIRO docks and Kings Wharf were used in this analysis.

The size frequency distribution of seastars collected from Bellerive yacht club was bimodal, with a mode at approximately 5 cm and a second mode at 11 cm. It was assumed that the first model represented one year old seastars. Similar estimates have been found in other studies (REF). It was further assumed that the spread of starfish sizes around the first mode represented variation growth rates. The von Bertalanffy growth coefficient, K,

$$K = \log\left(\frac{L_\infty - l_t}{L_\infty - l_{t+1}}\right) \qquad \text{eq 1}$$

was calculated for the data in the first mode, with limits from 3 cm to 8.5cm (L_∞ = maximum ray length (21cm), l_t = length a time t, l_{t+1}=length at time t+1). The distribution was resampled with replacement 1000 times to generate a distribution of sizes in the first mode, and subsequently a distribution of values for K . A pert distribution (modified beta distribution, parameters a = minimum, b= most likely, c = maximum and w = weight, REF), was fitted to the boot strapped K values using maximum likelihood estimates for a beta distribution. The minimum and maximum values were specified from the data (a and c) and the values of b and weight (most-likely value and the spread of the distribution) were estimated. This distribution was then used to define the range of growth rates for *A. amurensis*.

The dynamics of established populations were modelled as a age structured, constant mortality model,

$$N_t^a = N_{t-1}^{a-1} e^{-Z} \quad \text{eq(2)}$$

where N_t^a is the population of size class *a*, at time *t*, N_{t-1}^{a-1} is the population in the previous time and age class , and *Z* is the mortality rate from time *t – 1* to *t*. The seastars will live for eight years and have monthly age classes, yielding a total of 96 age classes. Solving the von Bertalanffy growth equation for age and inserting into the population model yields

$$N(l) = N_c \left(\frac{L_\infty - l}{L_\infty - l_c} \right)^{\frac{Z}{K}} \quad \text{eq 3}$$

This equation was fitted against the total size frequency distribution (Belrieve, CSIRO, and Kings combined) with 10000 K values drawn from the pert distribution to yield a distribution of Z values. The distribution of Z values were fitted using maximum likelihood estimation to estimate values of b and weight for a pert distribution, fixing values for a and c. The pert distribution can be used to define the dynamics of established and new populations using equation (2). This approach has many assumptions, but in the absence of any other developed population model and without any additional field data, offers the best estimate of population dynamics.

For an established population N_c must also be calculated. The abundance of Asterias with a ray length > 5.5cm in the Derwent is estimated at 4,000,000 (Ling *et al.* unpublished manuscript). For this population the N_c is the abundance at age 0. Equation (2) can be integrated for starfish between a ray length of 5.5cm (using the mode of the K pert distribution to convert length to age) and an age of 8 years and the result can be solved for N_c by holding all other parameters constant so that the sum of the starfish greater than 5.5cm 4,000,000. This process is repeated for Port Phillip Bay, assuming that the number of starfish greater than 5.5 cm is 90,000,000.The abundances for age classes 0 to 8 year, within a specified number of bins in each year can then be calculated over the inter-bin ranges for all age classes, using the parameters calculated (N_c and Z).

Density and Fertilisation

As *A. amurensis* is an externally fertilised broadcast spawning species, the density of reproductively mature adults is critical in determining the proportion of eggs fertilised. The fundamental reproductive characteristics of *A. amurensis* have been estimated from seastar populations in the Derwent estuary. Morris (2002) estimated that an adult female produced an average of 10600 eggs per gram of dry gamete. Grannum et al. (1996) estimated the

length for weight relationship and Morris (2002) the weight/dry gamete released relationship. Combining these two relationships with the estimate of egg numbers yields with the von Bertalanffy growth curve gives

$$E_a = 3059 * R^{2.6} - 274540$$
$$R = L_\infty - L_\infty e^{-Ka} \qquad \text{eq 4}$$

where R is the ray length, K is the von Bertalanffy growth coefficient, and E_a is the number of eggs for age a. By rearranging this equation, we can determine that females do not produce eggs until they have a ray length of greater than 5.52 cm, similar to the estimate given in Byrne *et al.* (1997). Length can be calculated for each age class using the pert distribution of K values. From the population model specified in equation (2), using the pert distributions for K, the total reproductive output of the female portion of the population (separated into appropriate age classes) can be calculated.

The density of individuals is critical to determining fertilisation success. The fertilisation success of populations of starfish across different densities has been calculated using a 3-dimensional fertilisation model (Morris 2002 pp. 52). For large numbers of seastars:

$$F_D = 0.165 \times \ln(D) + 0.609 \qquad \text{eq 5}$$

where F_D is the fertilisation rate of density D. The most accurate, recent survey of densities of *A. amurensis* was reported in Ling *et al.* (unpublished manuscript). In this study, seastar densities were calculated for transects across a range of depths in the Derwent estuary. From this data, we fitted a mixture of two negative binomials distributions. The parameters of the negative binomials are mean (mu) and size, the measure of dispersion. The larger the value of size the tighter the distribution is around mu. In this parameterisation, $\sigma^2 = mu + mu^2/size$, thus as the variance decreases, the value of size increases for a given value of mu. The parameters will be $p_1 = 0.903$, $mu_1 = 12.79$, $size_1 = 0.6798$ for the 1st (left hand) distribution and $p_2 = 0.097$, $mu_2 = 114.55$, $size_2 = 93.97$ for the 2nd (right hand) curve (fitted with an EN algorithm) for the current distribution of densities in the Derwent estuary ($D_{Derrwent}$) .

To simulate the reduced levels of aggregation in less dense populations the relative contribution of the right-hand curve was reduced. For densities less than $D_{Derwent}$, the contribution of the 2nd distribution to the mixed distribution is reduced linearly to 0 with average density in the estuary ($D_{estuary}$) such that:

$$p_2 = D_{estuary} \times 0.9329$$
$$p_1 = 1 - p_2$$

The proportion of the population at each density between D_{min} and D_{max} can then be calculated. D_{min} and D_{max} are the maximum and minimum densities found in the Derwent estuary respectively. As the density of a population increases beyond the density of the Derwent, mu1 and mu2 are scaled in proportion to the increase in density. This is done to preserve the relationship between high density populations and the distribution of densities within those populations.

The capacity of an area to support a population is not unlimited and abundances cannot increase forever. The upper limits of the population was not set by the adult population but by the fecundity of the adult populations, implicitly limiting the self-replacement rates for high densities. The average density of seastars in the Derwent estuary is 0.103 m^{-2} and this density is used to scale the fecundity of all populations so that as density increases beyond 0.103m^{-2} the fecundity decreases. The fecundity of the total population (F) is defined as,

$$F = 2 - \frac{(D_t + D_{t-1})}{2 * 0.103} \qquad \text{eq 6}$$

where D_t and D_{t-1} are the densities at times t and t-1. Values of F greater than 1 and less than 0 are rounded to 1 and 0 respectively. Defined this way, fecundity will be 1 at densities up to 0.103m^{-2}. At densities greater than the density of the Derwent, fecundity will be reduced as the population increases to 0 when the density is twice the density of the Derwent (0.207m^{-2}).

Combining our estimates of density with fertilisation, fecundity, egg number, population age structure and a value of K drawn from the pert distribution will give the reproductive output of that estuary for that particular year. The number of larvae produced in a location in a given year will be

$$L = \sum_{a=0}^{a=96} \sum_{D=D_{min}}^{D=D_{max}} A_f^a \times E_a \times F \times F_D \qquad \text{eq 7}$$

where A_f is the female proportion of the population (0.5 * total population) and L is the total number of larvae produced by that location for the year.

Larval release in the model occurs between 1st July and 15th October each year, with the maximum release occurring on the 15th August. Given that the maximum observed larval period is 120 days, larvae will be present in the model from July to mid February the following year corresponding with predicted larval presence in the Derwent estuary (Bruce *et al.* 1993 and unpublished data).

Larval Duration

The larval duration of A. amurensis varies with temperature (Bruce et al. 1995). At low temperatures (11 ^{o}C) larval duration is approximately 119 days, but as the water temperature increases larval duration decreases.

$$LarvalDuration = e^{-0.11*tempature+5.58} \quad \text{eq 8}$$

Larval Mortality and Settlement Probability

Estimates of larval survival and settlement success are difficult to obtain. Laboratory studies of *A. amurensis* have suggested mortality rates between 12-17% per week (Sutton and Bruce 1996). Likewise, settlement success appears to vary between 0 and 100% depending on the substrate type (Morris 2002) in laboratory studies. The link between these studies and oceanic systems is uncertain.

Larval dynamics were simulated using an age-structured, constant hazard model, as for the adult population (eq 2). Once the age of the larvae exceeded larval duration (as defined in eq 8) , larvae were competent to settle and remained so for one week (the period over which larval morality and age were updated). A larval mass could settle if it were adjacent to the coastline or in an estuary. The larvae settled with a fixed probability, tested against a uniform distribution.

In the absence of estimates of either larval mortality (Z_L) or settlement probability (S), a simplified model of the Derwent estuary was constructed. In this sub-model the Derwent estuary (a salt wedge estuary with 5 cells) was linked to a single oceanic cell. This model had a complete biological model (as described above). The values for larval mortality and settlement success were varied across a range of possible values (0.1<=settlement success<=0.9; 0.001<=larval mortality<=0.2) to determine which combinations of S and Z_L generated a final population closest to the existing abundances in the Derwent estuary. Larvae were exchanged with the adjacent oceanic cell at the rate used in the full MSE model (retention = 15.43 days). The model runs of 100 years were repeated 50 times and the final populations averaged, for each combination of S and Z_L . The final abundances were compared with the existing population size in the Derwent estuary and appropriate values of S and Z_L were selected. When S was below 0.5, with an appropriate Z_L value,

populations had extreme and unrealistic oscillations. Above this level the population dynamics were similar, but settlement success of 80%-90% seems excessive. Consequently, vales of 0.5 for S and 0.1 for Z_L were chosen.

The effect of larval mortality (Z_L) on the number of larvae in the model was simulated using the same functional relationship as for adult *Asterias* (eq 5), and was applied on a weekly basis. Once the larvae were competent to settle, settlement occurred in suitable habitat with a probability of S (uniformly distributed R=0,1).