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PROCEEDINGS OF A MEETING ON THE BIOLOGY AND MANAGEMENT OF THE INTRODUCED SEASTAR *ASTERIAS AMURENSIS* IN AUSTRALIAN WATERS

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CONTENTS

EXECUTIVE SUMMARY

- *Asterias amurensis* was introduced inadvertently into Australian waters about 20 years ago. There are now estimated to be about 30 million *A. amurensis* in Tasmanian waters, largely restricted to the Derwent estuary. Recently, *A. amurensis* was also found on commercial mussel lines in Port Phillip Bay, Victoria.

- The seastar is a voracious carnivore which seriously affects commercial shellfisheries in its native range. In the Derwent estuary, it significantly impacts native shellfish populations, although it has not yet overlapped with commercial fisheries. However, its range is now expanding and may soon overlap with shellfish farms. In Victoria, its recent appearance on commercial mussel ropes has heightened concern about its ability to spread around much of southern Australia and caused concern about its probable impact on commercial fisheries if it becomes established in Port Phillip Bay.

- Apart from physically removing seastars, there are no methods currently used in Australia to control *A. amurensis*.

- The utility of any control method is determined by its effectiveness, safety, practicality, and social and political acceptability. Potential control methods for the seastar include physical control, chemical control, environmental rehabilitation, biological control, genetic control and education. These methods were screened by participants at this meeting for their likely utility to control *A. amurensis*.

- It is crucial to determine whether *Asterias amurensis* drives or tracks the dynamics of the system in which it occurs i.e. whether the high seastar densities in the Derwent estuary are a partial cause, or a symptom, of a degraded environment. Until this is known, it is unclear whether control efforts should be targeted at the seastar directly or on rehabilitation of the environment.

- Fundamental knowledge of the population dynamics of *A. amurensis* in Australian waters is required to determine which phase(s) of the life cycle of the seastar would be most susceptible to control. Modelling of the seastar population in the Derwent estuary is recommended to address this gap.

- Physical removal remains the most socially and politically acceptable method to control *A. amurensis*. It is not effective against the large population of *A. amurensis* in the Derwent estuary, but could be useful in local areas. It was not recommended to remove large numbers of seastars by dredging in the Derwent estuary because it would resuspend heavy metals trapped in the sediments. It is also unlikely to make any difference to seastar populations.

- Methods are urgently needed to prevent the translocation of juvenile seastars on commercial mussel lines. The effectiveness of quicklime, freshwater and air exposure to kill seastars on mussel lines requires testing. These methods should be introduced in conjunction with management strategies to restrict translocation of seastars by other methods (e.g. by fishing and recreational vessels).

- The use of native Australian predators and parasites to control *A. amurensis* requires continued investigation. Biological control agents from the native range of the seastars were considered less acceptable, but were acknowledged as likely to be effective.

- Genetic methods provide the only identified control technique that could eradicate *A. amurensis* in the long-term, with little or no risk to native seastars. Transgenic techniques for pest control are being developed at CSIRO. An assessment of species-specific compounds was recommended to determine whether such compounds could be integrated into seastar populations, using transgenic techniques, to weaken or eradicate them.

- Commercial exploitation of *Asterias amurensis* in Australia seems remote

- It is possible that the large numbers of *A. amurensis* in the Derwent estuary are a symptom of a degraded environment. If so, then rehabilitation of the Derwent estuary should be a high priority. Improvement of environmental quality would benefit many users and may increase the numbers of native predators and parasites which in turn, could impact seastar densities. Ongoing research will determine the impact of native predators on juvenile *A. amurensis*.

- Supply of food from anthropogenic sources causes seastars to aggregate, thereby improving their fertilisation success. The size of their gonads also decreases when seastars have limited food. Educating the public and commercial operators to limit food input could force seastars to disperse to seek food: it could also cause a decrease in seastar gonad size, which may have a dramatic effect on reproductive success.

1. Introduction

INTRODUCTION

The northern Pacific seastar *Asterias amurensis* is native to the coasts of China, Korea, Russia and Japan, extending across the Bering Sea to the coast of Alaska. It is not native to Australia. It was inadvertently introduced into Australian waters about 20 years ago, probably in the ballast water of ships originating from Japan (Ward & Andrew, 1995). Currently, there are estimated to be about 30 million *A. amurensis* in the Derwent estuary, Tasmania and recently, about 100 juvenile seastars were found in Port Phillip Bay, Victoria. It is unknown whether a breeding population of seastars has been established in Port Phillip Bay, but the discovery of *A. amurensis* in Victorian waters has raised fears that the seastar could be about to rapidly expand its range in Australian waters.

In its native range, *Asterias amurensis* is found from 1-200m depth. It is a voracious predator and affects the viability of commercial shellfish industries. In 1954, an outbreak of *A. amurensis* in Tokyo Bay caused a loss of marketable shellfish worth approximately 400 million yen (Kim, 1968). In Australia, it alters benthic communities by eating molluscs, tunicates, bryozoans, sponges, crustaceans and even other seastars.

In some areas of the Derwent estuary, *A. amurensis* are found at densities of more than seven per square metre (Grannum *et al.*, 1996). This is equivalent to seastar densities overseas during 'outbreaks'. However, seastar 'outbreaks' overseas quickly subside. In contrast, densities of *A. amurensis* in the Derwent estuary have remained at high levels for at least five years and show no signs of declining. There appear to be several factors which allow the seastar to thrive in the Derwent estuary including: low environmental quality; circulation patterns which entrain larvae within the estuary; lack of parasites; and an abundance of food for both larvae and adults. Whether one or more of these factors could be manipulated to reduce seastar numbers is uncertain.

Asterias amurensis becomes sexually mature in about one year, breeding from June to September in Tasmanian waters. A single female can release about 20 million eggs (Kasyanov, 1988), which when fertilised, survive as planktonic larvae for 6-16 weeks before settling to the sea floor. In the Derwent estuary, densities of larval *A. amurensis* are among the highest found anywhere in the world (see Bruce, p. 36). Therefore, to prevent the importation of *A. amurensis*, New Zealand has legislated to stop ballast waters, collected from the Derwent River, being discharged in their territorial waters (Biosecurity Act 1993, Annex 1). This legislation also prohibits the discharge of waters from Port Phillip Bay, Victoria, within New Zealand territorial waters. There is concern that such restrictions could be extended to other ports overseas.

Little is known about the factors which, until recently, have limited the spread of *A. amurensis* in Australian waters. Indeed, the recent discovery of *A. amurensis* in Victoria has highlighted our inability to manage this introduced marine pest. Information about the factors which drive its population dynamics and more importantly, effective control methods, are lacking. Effective long-term management of northern Pacific seastars in Australian waters hinges on accurate information.

A meeting, sponsored by the Centre for Research on Introduced Marine Pests (CRIMP), CSIRO, was held on 19 May 1998 at the CSIRO Marine Laboratories in Hobart. Representatives from CRIMP, The Department of Primary Industry and Fisheries (DPIF),

the Tasmanian Aquaculture and Fisheries Institute (TAFI), Fisheries Victoria, The University of Tasmania, the Department of Environment and Land Management (DELM), the Tasmanian Museum and Art Gallery and marine farmers attended.

The meeting reviewed the current information on populations of the northern Pacific seastar in Australian waters. We also determined and prioritised information required to manage the seastar, particularly to restrict its further spread. Control options were discussed and we identified those which we considered worth pursuing.

I thank the workshop participants for providing results from current research and entering enthusiastically into discussions. I thank Dick Martin for chairing the sessions and directing discussions, Sue Spinks for assistance in organising the workshop and Chad Hewitt for assistance with figures. Thanks also to Nic Bax, Craig Johnson, Dick Martin and Ron Thresher for comments on drafts of this manuscript.

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2. History and biology of *Asterias amurensis* in Australian waters

GROPING IN THE DARK!

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Most of us here today know the basic background of the introduction and subsequent research of the northern Pacific seastar, *Asterias amurensis*, but only those who have been involved from the beginning may know the background story.

Several seastars were first brought into the Tasmanian Museum in 1986 by a woman who had found them at Rosny in the River Derwent. At the time I presumed they were *Uniophora granifera*, a species which varies in colour from orange to brown with mauve highlights. Over the next few years occasionally looking down into the water, I noticed a build-up around the Hobart wharves, but still thought it was *U. granifera* making a come-back in a cleaner River Derwent.

In 1992, thanks to Wolfgang Zeidler and Karen Gowlett-Holmes of the South Australian Museum, we realised that it was *Asterias amurensis*, a native seastar of the northern Pacific Ocean. Generally now accepted to be the result of introduction through ballast water; in 1992 this was a new experience. Although much research had been done by Gustaff Hallegraeff on toxic dinoflagellates and their relationship to ballast water, this was probably the first time in Australia that an exotic marine animal visible to the naked eye had been discovered in such huge quantities due to accidental introduction.

Still not quite realising its significance, I mentioned the discovery a few days later while giving a public talk on introductions of marine invertebrates by the oyster trade from New Zealand about a hundred years ago. An ABC journalist was present at this lecture and reported the story the same evening. Suddenly all hell broke loose. The media and public could not hear enough about it - the authorities went underground. And so started a totally frustrating six months, attempting to make those in responsible positions take any notice at all.

At this stage I use the title of the talk 'Groping in the Dark' because in 1992, only six years ago, there was no precedent for the recognition and treatment of a problem of this obvious magnitude, and almost no guidelines. At the time Australia was considered a leader in the world in ballast water recognition, and the Scientific Working Group on Ballast Water had been active for some time, but nothing had prepared us for a marine animal that could potentially damage a multi-million aquaculture industry.

While the local, interstate and even international media and tabloids were in a frenzy, some with a responsible attitude and others with headings like 'Man-eating seastars!', it seemed impossible to gain any recognition by local and federal governments, although I received calls from banks (was it wise to give loans to potential aquafarmers?), and even the Australian Stock Exchange. Throughout this time, the Tasmanian Museum kept to its policy to present known facts, and not speculate on possible damage to industry.

Finally, after six months of utter frustration, I called a meeting of all those with any interest in, or responsibility for, marine issues in the Hobart area. About 33 people attended and

were presented with facts, photographs and a video of the numbers and voracious habits of the seastar. Out of that meeting grew the *Asterias amurensis Steering Committee*, with Professor Michael Stoddard of the University of Tasmania in the Chair. From then on, state and federal authorities gradually became aware of the extent of the problem. We even managed to educate them to call the animals seastars, and not starfish (a confusing name which Queenslanders still adhere to).

A public talk was attended by about 100 people with interests in marine issues, and educational talks and demonstrations were given to interested organisations. Federal funds were obtained for the employment of Margie Morrice at the Tasmanian Museum, to study the distribution and reproduction of *A. amurensis*. In the winter of 1993, with the help of Department of Primary Industries and Fisheries (DPIF), CSIRO and other agencies, we staged two major dives from the Hobart wharves. The first resulted in the collection of 6000 seastars from a 300 metre stage off Princes Wharf. The second and much larger dive covered strategic locations around the whole of Sullivans Cove, and resulted in 24,000 seastars piled up on the wharf. These went by truck for composting trials by the University of Tasmania.

Local, interstate and Japanese media attended the second dive, and as soon as a SCUBA-clad Jeff McMullen from *Sixty Minutes* emerged from the water holding a seastar and said 'It's scary down there', we knew we had the attention of the remaining relevant federal politicians.

Soon after the DPIF took control and formed the National Seastar Task Force, and the CSIRO became officially involved. By this stage the whole issue had become extremely political, with authorities concerned about the effect of any adverse publicity on the multi-million aquaculture industry. However, these concerns were allayed as *A. amurensis* did not spread as first feared, and care was taken by aquafarmers to avoid infestation.

The Tasmanian Museum acquired further funding for two researchers and later, in conjunction with the University of Tasmania, two more researchers were employed to study the impact of the seastar on the Derwent benthic fauna. The DPIF researched trapping, and the Centre for Research on Introduced Marine Pests (CRIMP) was formed and went forth and multiplied.

The introduction of *Asterias amurensis* was really the catalyst of the change in attitude and awareness of the problems of ballast water and other means of introduction of exotic marine animals in Australia. In only six years we have gone from the wilderness of a problem with no relevant precedent or guidelines, to national guidelines, a well-structured network of contingency awareness plans, and means of public/official reporting and communication. We still have millions of seastars in the Derwent and surrounding waters, but we do know more about their biology, ecology and impact, and hopefully work will still continue on a control method.

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INVASIVENESS AND IMPACT OF THE NORTHERN PACIFIC SEASTAR *ASTERIAS AMURENSIS* ON NATURAL COMMUNITIES IN SE TASMANIA

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Summary

The establishment of the northern Pacific seastar *Asterias amurensis* in southeast Tasmania has the potential to profoundly affect native benthic marine communities. To assess the threat of exotic marine invertebrates, and to develop effective management strategies, it is necessary to assess (1) the impact of adult animals on native communities, (2) the capacity of the exotic species to invade assemblages of native species via recruitment of newly settled juveniles, and (3) their dispersal through hydrodynamic transport of larvae. Despite the strong rhetoric and publicity surrounding the establishment of *A. amurensis* in Tasmania (e.g. McLoughlin & Thresher, 1994), none of these aspects has been examined *directly* or quantitatively. However, observations of seastar foraging behaviour, examination of gut contents, and estimates of feeding electivity (Buttermore *et al.*, 1994; Morrice, 1995; Grannum *et al.*, 1996; S. Lockhart, unpublished data) suggest the potential for considerable impact on assemblages of native species in Tasmania. The work outlined here is part of a project to quantify (1) the impact of adult seastars on native communities in SE Tasmania, and (2) the invasiveness of *A. amurensis* to native assemblages via recruitment of newly settled juveniles. The work provides an important case study of invasion ecology in temperate coastal systems and contributes to both empirical and theoretical knowledge of the phenomenon of invasion. Most importantly, it addresses the immediate pragmatic concerns of conservation of marine communities and management of *A. amurensis* in Tasmania.

Aims and Outcomes

The specific aims of the project are to:

- Assess quantitatively the short-term impact of *A. amurensis* on the structure of several distinct natural communities in south eastern (SE) Tasmania.

- Assess the ability of juvenile seastars to invade several kinds of habitat by examining survivorship of newly settled animals in native habitats, partitioning mortality into components due to predation and non-predatory sources.

The work proposed will define both the impact of *A. amurensis* on, and its threat to, communities of native benthic species in SE Tasmania, and contribute to the establishment of a theoretical framework for invasion ecology.

Methods (and some preliminary results)

Two tasks will be conducted in parallel:

Task #1 *Impact of seastars on natural communities*

We will assess the impact of the seastar on natural communities using two methods: (1) surveys to examine the relationship between infaunal assemblages and seastar abundance at several sites throughout SE Tasmania and, (2) experimental manipulation of seastar abundance. The surveys will examine the correlation between seastar abundance and community parameters e.g. species richness, species diversity, and other multivariate descriptors of community structure. The experimental manipulations will quantify the direct impact of the seastar on natural communities in SE Tasmania. Using the two methods in tandem will provide, for the first time, a quantitative assessment of the impact of the seastar on native benthic assemblages in SE Tasmania.

1. Infaunal and seastar survey

In November 1996, seastar and infaunal abundance were measured at 17 sites in SE Tasmania: nine sites were located inside, and eight outside, the Derwent River. Sites located in the Derwent River were: Kangaroo Bay, Howrah Beach, Sandy Bay Beach, Blinky Billy Point, Ralphs Bay (north), Ralphs Bay (south), Opossum Bay, Kingston Beach and Halfmoon Bay. Outside the Derwent sites were at Gypsy Bay, Breaknock Bay and Murdunna in Norfolk Bay; Barnes Bay, Simpsons Bay and Whaleboat Rock in the D'Entrecasteaux Channel; Cygnet and Port Arthur.

In November 1997, the survey was repeated at three sites: Howrah Beach, Ralphs Bay (south) and Opossum Bay. Sloping Island in Frederick Henry Bay was also surveyed because adult seastars had been discovered. This survey will be repeated in November 1998.

2. Experimental manipulations

Seastars will be manipulated in small (1×1m) completely enclosed cages, and in large (3x3m) netted enclosures with walls (and inward folding skirts top and bottom) but no top. Both designs have been tested extensively to ensure that seastars do not escape. Experiments using the small cages will be repeated at several sites to determine spatial variation in community responses to the seastar. The large enclosures will be used at a single site to test for non-linear and scale-dependent responses to seastar density. The experiments will run at sites in the D'Entrecasteaux Channel, Frederick Henry and Norfolk Bay regions that do not currently support seastar populations. Pilot work conducted in 1997 indicates that experiments of two months duration are sufficient to detect impacts.

1. *Experiments in $1m^2$ enclosed cages.* To examine spatial variation in community responses to the seastar, adult seastars will be added at a density of $1m^{-2}$ to small cages at four different locations. This experiment has been completed at Murdunna in Norfolk Bay, and at Ralphs Bay in the Derwent River. The experiment will be repeated at North West Bay in the D'Entrecasteaux channel in mid 1998 and at Sloping Island in Frederick Henry Bay in early 1999. At each site treatments include (i) cages containing seastars, (ii) cages without seastars, and (iii) uncaged $1m^2$ areas (control). Preliminary data from the survey undertaken in November 1996 (see above) indicate that the distribution of infauna in sediment is patchy at a scale of 10^1-10^2 m, therefore a randomised complete block design is preferred over a completely randomised design.

The scale of the experiment precludes replicating treatments within 'blocks'.

2. *Experiments in 9m² netted enclosures.* Two important questions are whether results from experiments conducted at small scales (e.g. in the 1m² plots outlined above) can be extrapolated linearly to larger areas, and whether the impact of the seastar is linearly related to its density over the range of densities likely to be encountered in nature. To test for non-linear responses to both the scale of the experiment and seastar density, adult seastars will be added to 9m² netted enclosures at low ($0.1m^{-2}$), medium ($0.5m^{-2}$) and high ($1.0m^{-2}$) densities. Additional treatments will be enclosures without seastars, and 9m² areas without either seastars or enclosures (a control for the effect of the enclosures on the community). This experiment will be conducted at Sloping Island in early 1999 where the small cages will be deployed. This will test for non-linear responses to seastar density (impacts at zero, low, medium and high densities), and whether the effects detected in the small cages can be scaled linearly to those detected in the larger enclosures at the same density ($1.0m^{-2}$) of seastars.

Preliminary analysis of data from the experiment conducted at Murdunna indicates effects of the seastar on some species but not on others, and no significant effect of the presence of the cage (Table 1; the complete analysis will include data for 40-50 other infauna species yet to be extracted from the samples). It is necessary to establish the generality of this response to the seastar by repeating the experiment at several sites and in several distinct communities.

Table 1: Mean abundance (no. m^{-2}) of live and empty bivalves (*Fulvia tenuicostata* and *Katelysia scalarina)* and live echinoids (*Echinocardium cordatum)* in three treatments of cage with seastar added, cage without seastars, and uncaged control, in the experiment at Murdunna, April-June 1997. The significance of the treatment effect ascertained by ANOVA based on a randomised complete block design are listed. In all cases in which a significant treatment was detected, there were significantly fewer live prey, and significantly greater remains of prey, in treatments containing seastars relative to cages without seastars. Differences in prey density in cages without seastars and in uncaged control plots were not significant.

Species	**Seastar** (Mean ± SE)	**Cage Control** (Mean ± SE)	**Uncaged Control** (Mean ± SE)	**Results of ANOVA for treatment effect** (df =2,4)
Fulvia tenuicostata				
Alive	3.3 ± 2.33	81.7 ± 20.19	84.3 ± 14.25	$F = 23.10$ $P = 0.0063$
Empty Shell	50.7 ± 9.39	14.0 ± 5.51	5.3 ± 2.60	$F = 12.44$ $P = 0.0192$
Katelysia scalarina				
Alive	0.3 ± 0.33	6.0 ± 1.00	7.3 ± 0.67	$F = 18.20$ $P = 0.0098$
Empty Shell	6.3 ± 1.33	0.6 ± 0.67	0.3 ± 0.33	$F = 19.19$ $P = 0.0089$
Echinocardium cordatum (alive)	34.0 ± 11.93	54.3 ± 29.81	42.0 ± 19.35	$F = 0.80$ $P = 0.5109$

Task#2 *Impact of natural communities on seastars: Survivorship of newly settled seastars*
Mortality of juvenile seastars will be assessed in controlled experiments in the field. This will determine whether: (1) predators have a significant impact on juvenile seastars; (2) predation rates on juveniles differ between habitats (soft sediment *versus* rocky reef) and; (3) predation rates within particular habitats differ between sites with different levels of anthropogenic disturbance and seastars and predator population levels. Over two years, and at several sites, juvenile seastars will be deployed in the field in plastic cages (500 x 250 x 100mm) arranged in a variety of designs. Similar designs have been used successfully by Keesing & Halford (1992) in experiments with the crown-of-thorns seastar *Acanthaster*

planci.

In the first year, predation rates in a rocky reef habitat were compared with soft sediment habitats at Opossum Bay in the Derwent estuary. The experiment partitioned mortality into non-predatory sources and mortality due to the combined effects of infaunal, epibenthic and demersal predators. The four treatments in the experiment included:

1. exposure of juvenile seastars to infaunal, epibenthic and pelagic predators (open cages with low sides and without lids which allow access to all three guilds of predators);

2. protection from all predators (closed cages with lids to exclude all predators; a 1mm mesh in the lids permits water flow);

3. control for emigration of seastars from open cages (open cage within a larger closed cage and predators excluded from both);

4. control for caging effects (closed cage within a larger cage and predators excluded from both).

Results indicate that mortality due to predation is significant when compared to mortality due to other factors, and is approximately 3% per day on soft sediment and 5.5% per day on rocky reef (Fig. 2). The difference in mortality between the two habitats was not significant ($P = 0.057$), however, considering the low power of the analysis, it suggests that the trend of higher predation on rocky reef than on areas of soft sediment is real.

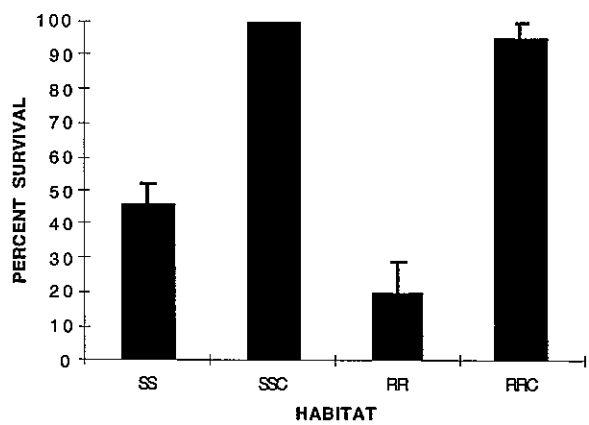

Figure 2. Percent survival of *Asterias amurensis* in treatments allowing access to predators and offering protection from predators in two habitats. SS = soft sediment, SSC = soft sediment control, RR = rocky reef, RRC = rocky reef control. Data are adjusted for emigration of seastars from open cages at a rate of 36.3% on soft sediment and 5% on rocky reef.

In 1999, the experiment will be extended to examine whether predation on juveniles differs among sites. We will test whether mortality of juveniles is lower in areas with high levels of anthropogenic disturbance compared with sites at the periphery of the range which support a relatively diverse assemblage of native species. In conjunction with ongoing research on settlement cues of *A. amurensis* (see Morris & Johnson, p. 32), this work will help define the susceptibility of natural communities to invasion of the seastar via recruitment from the plankton.

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Comments

This presentation stimulated interest in the use of predators to control *A. amurensis*. Several animals eat larval and juvenile seastars. Galtsoff & Loosanoff (1939, *U.S. Fish Wildl Fish. Bull.* **49**:75-132) found high levels of cannabalism among juvenile *Asterias forbesi*. It is possible that predation of juveniles is higher in pristine areas than areas disturbed by humans. This may restrict *A. amurensis* to disturbed areas where there are fewer native predators. If this were the case, seastar numbers may be more effectively reduced by enhancing native communities rather than trying to destroy the seastar. Jeff Ross will address the question of survival of juvenile seastars at disturbed and pristine sites during work for his PhD degree.

Crabs and seastars can injure adult asteroids (see Lawrence, J.M. 1991 **In:** Scalera-Liaci, L., Canicatti, C. (eds) *Echinoderm Research.* Balkema, Rotterdam). In aquaria, pie-crust crabs *Cancer novaezelandiae* will snip the arms of small *A. amurensis*. The strigulating hermit crab *Trizopagurus strigimanus* can also inflict sub-lethal (?lethal) damage to *A. amurensis* in aquaria. In the wild, the seastars *A. amurensis* and *Coscinasterias muricata* eat adult *A. amurensis*. There was great interest in the possibility of the use of *C. muricata* as a biological control agent. However, it is unknown whether *C. muricata* normally eats *A. amurensis* or simply displaces it. Enhancing native populations of *C. muricata* was suggested as a possibility for biological control of *A. amurensis*, however, the impact of *C. muricata* on populations of *A. amurensis* in the Derwent is unclear.

DISTRIBUTION AND IMPACT OF *ASTERIAS AMURENSIS* IN VICTORIA

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- A mature female *Asterias amurensis* was discovered off Pt Cook in Port Phillip Bay in August 1995. A survey of the area found no other specimens. A second adult seastar was found off Portarlington in September 1995 and a third adult at the same site in August 1996. All were found by scallop fishers who were dredging the areas extensively. They found no other specimens (Fig. 1).

- A fourth adult seastar was found under Victoria Dock in April 1997.

- Fisheries Victoria, through Marine and Freshwater Resources Institute (MAFRI), have been undertaking annual dive surveys in Port Phillip Bay (PPB) for ten years which include up to 70 locations across PPB (Fig. 3). Initially these were scallop abundance surveys, but due to the focus on exotic species since 1995, they also included surveys for exotic pests, specifically *Sabella spallanzanii* and *Asterias amurensis*.

- Since 1997, the surveys were specifically focused on detecting the presence of exotic species. The survey now involves dive transects at 30 locations and examination of pier pylons around PPB (Fig. 3).

- The first juvenile seastars were found on "growing" ropes at a mussel farm in Dromana Bay in January 1998. A dive survey of the farm and surrounding seabed found another two small seastars on the mussel ropes. The mussel farmer reported finding further specimens over the following weeks (Fig. 1).

- The 1998 Bay wide survey occurred during February and found only two juveniles off Beaumaris.

- In 1998, a total of 103 juvenile seastars have been found almost exclusively on ropes at the Dromana Bay mussel farm. Seven juveniles were also found at Beaumaris.

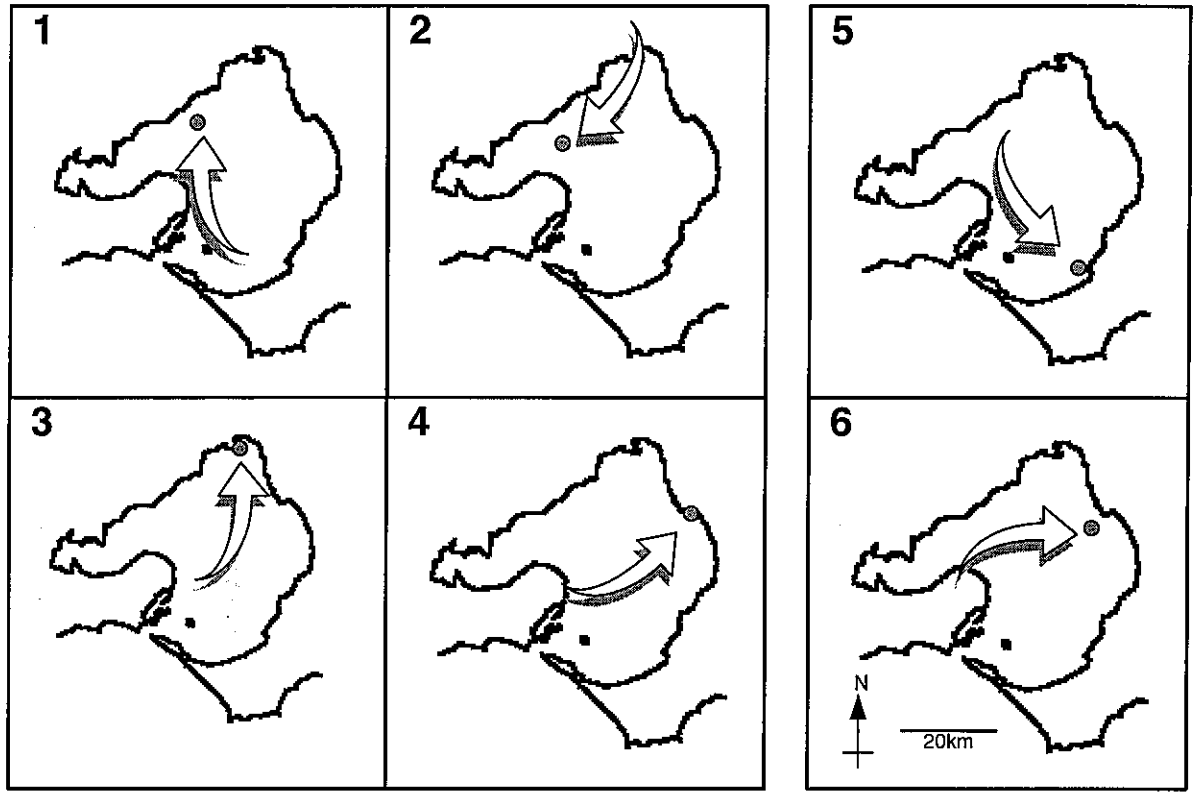
- Fisheries Victoria has introduced a targeted program to look for *A. amurensis* in Port Phillip Bay with funding provided by Environment Australia. The program involves:

1. Deployment of seastar traps around the Bay. Initially these were under piers but after the loss of 29 traps within one week another strategy was required. Commercial fishers offered to monitor the traps and are now deploying approx. 20 around PPB.

2. Targeted surveys using volunteer recreational divers to focus on specific areas.

ADULT SEASTARS

JUVENILE SEASTARS



LEGEND

1. Date: 21/08/95
Position: Off Pt. Cook - no fix
One mature female caught in scallop dredge. Vic Museum F77603

2. Date: 27/09/95
Position: Off Portarlington - no fix
Stored Vic Museum F80981
Also one found 09/08/96 off Portarlington - 23cm diameter animal caught in scallop dredge. Frozen at MAFRI.

3. Date: 17/04/97
Position: 37°49'10S 144°56'30E
One mature animal caught by diving in Vic. Dock, Berth 9. Vic Museum F80982

4. Date: 11/03/98
Position: 37°59'60S 145°02'50E
One 21 cm animal at Keefer's Boat Shed - Beaumaris. Live to MAFRI.

5. Date: 21/01/98
Position: 37°59'80S 145°02'60E
Four 3-4cm diam. on mussel farm lines. (NB Over 100 juveniles found by 30/04/98 on culture ropes, ranging from 7-14cm diam.)

6. Date: 26/02/98
Position: 38°00'81S 145°02'40E
38°02'13S 145°00'30E
One 10cm diam. juvenile found by MAFRI dive survey off Beaumaris at each of two sites in 12m and 17m of water.

Figure 1. Records of the exotic northern Pacific seastar *Asterias amurensis* in Port Phillip Bay from November 1995 to April 1998 (from MAFRI report - in prep).

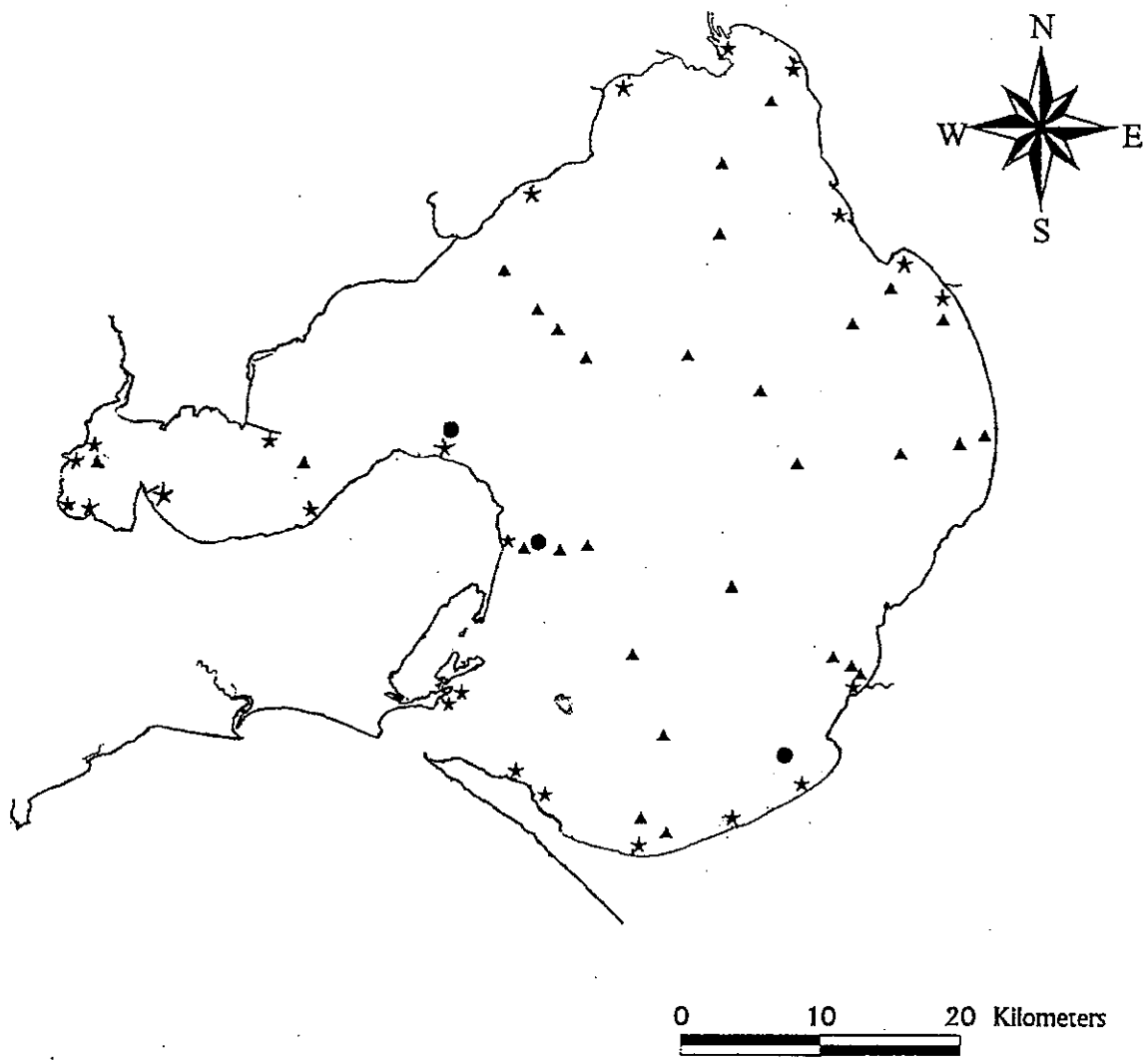


Figure 3. Search program for *Asterias amurensis* in Port Phillip Bay, Victoria. ● Scallop dredge, ★ pier survey, ▲ baywide survey.

Comments

Port Phillip Bay is not being searched for larval *A. amurensis*. Larval echinoderms are difficult to identify accurately because they are morphologically plastic. Therefore, genetic techniques were considered more accurate than morphology to identify *A. amurensis* larvae. Restriction Fragment Length Polymorphism (RFLP) analyses can be used to diagnose *A. amurensis* (see Murphy & Evans, p. 22) although these analyses take three days. Other genetic techniques (e.g. PCR alone, genetic "dipsticks") would be more rapid but have not yet been developed.

Seastars were found on mussel ropes at Dromana which had been in the water since late last year and had not been relocated. Therefore, the seastars were estimated to be about four or five months old (n = 21, mean arm radius = 51mm). (Mussels are also collected at Beaumaris and the ropes are not relocated). Settlement of shellfish at Dromana is also usually heavy. Therefore, it appears circulation in Port Phillip Bay favours larval settlement in this area.

John Garnham commented that adult seastars were easier to locate using divers than traps.

No adult seastars, however, were found on the sea floor or on native mussel beds at Dromana despite extensive dive surveys.

John Garnham noted that the settlement of seastars is patchy: they are usually found away from the influence of fresh water flowing out of the Yarra River and other fresh water flowing into the Bay further south.

The possibility of transferring seastars on commercial mussel ropes was considered a major problem. In Tasmania, ropes of mussels are often translocated (e.g. Huon River to Norfolk Bay) although seastars have not yet been found on these ropes. In Victoria, mussel ropes are moved from Port Phillip Bay to the adjacent Western Port. It is essential to find a way to kill seastars on commercial mussel ropes to avoid their translocation. The use of freshwater was considered preferable to the use of chemicals to sterilise the ropes. Barry Bruce noted freshwater would be effective against seastar larvae but would probably be less effective against settled juveniles. Marsh (1993. The Biology and Distribution of the Introduced Seastar *Asterias amurensis* (Lutken) (Echinodermata: Asteroidea) in Tasmania, Unpublished Honours thesis, University of Tasmania, Hobart) found *A. amurensis* could tolerate 26ppt but tolerance was considerably reduced at 24ppt: all seastars exposed to 24ppt were dead in nine days. Therefore, fresh water is likely to kill juvenile seastars rapidly. Recent work by mussel growers in Port Phillip Bay indicates that 24h air exposure kills small (50-70mm arm length) *Coscinasterias muricata* and many epiphytic biota on mussel ropes. This could also be explored as a control mechanism for juvenile *A. amurensis*.

GENETIC ORIGIN OF AUSTRALIAN POPULATIONS OF *ASTERIAS AMURENSIS*

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Introduction

The northern Pacific seastar *Asterias amurensis* is native to the coasts of eastern Russia, the Korean Peninsula and Japan. It is also found along the coasts of Alaska and Canada although some consider these populations were introduced. In the late 1970s or early 1980s, *A. amurensis* was introduced into the Derwent River estuary, Tasmania. In 1995, the seastars were also found on mainland Australia in Port Phillip Bay, Victoria. Before 1997, less than five adult seastars had been found in Port Phillip Bay, however, in 1998, about 100 juveniles were found on commercial mussel lines.

Restriction Fragment Length Polymorphism (RFLP) analysis was used to determine the origin of seastars in Tasmania and Port Phillip Bay. RFLP analysis produces a restriction profile which differs between species due to nucleotide sequence variation (Matsuoka & Suzuki, 1987). It can therefore be used to diagnose species.

Methods

Between 1993-1996, *A. amurensis* were collected from Japan, Russia and Tasmania (Fig. 4, Table 2). In 1998, seastars were also collected from Port Phillip Bay, Victoria (n=21, mean arm radius 51mm) and Hobart, Tasmania (n=10) (Table 2). Either the gonad or tube feet were preserved in 100% ethanol or the whole seastars were frozen at -80°C. DNA was extracted from the gonad or tube feet using phenol/chloroform and precipitated with ethanol (Sambrook *et al.*, 1989).

Table 2: Collection sites, dates and number (n) of *Asterias amurensis* collected.

COUNTRY	COLLECTION SITE	COLLECTION DATE	n
AUSTRALIA	Derwent River, Tasmania	March, 1996	27
	Derwent River, Tasmania	May, 1998	10
	Triabunna, Tasmania	May, 1994	23
	Port Phillip Bay, Victoria	Feb/May, 1998	21
JAPAN	Nemuro Bay, Hokkaido	August, 1993	21
	Yoichi, Hokkaido	August, 1993	24
	Mutsu Bay, Honshu	October, 1993	25
	Suruga Bay, Honshu	June, 1994	24
	Tokyo Bay, Honshu	August, 1993	23
	Ariake Sea, Kyushu	August, 1994	25
RUSSIA	Vladivostok	October, 1993	15

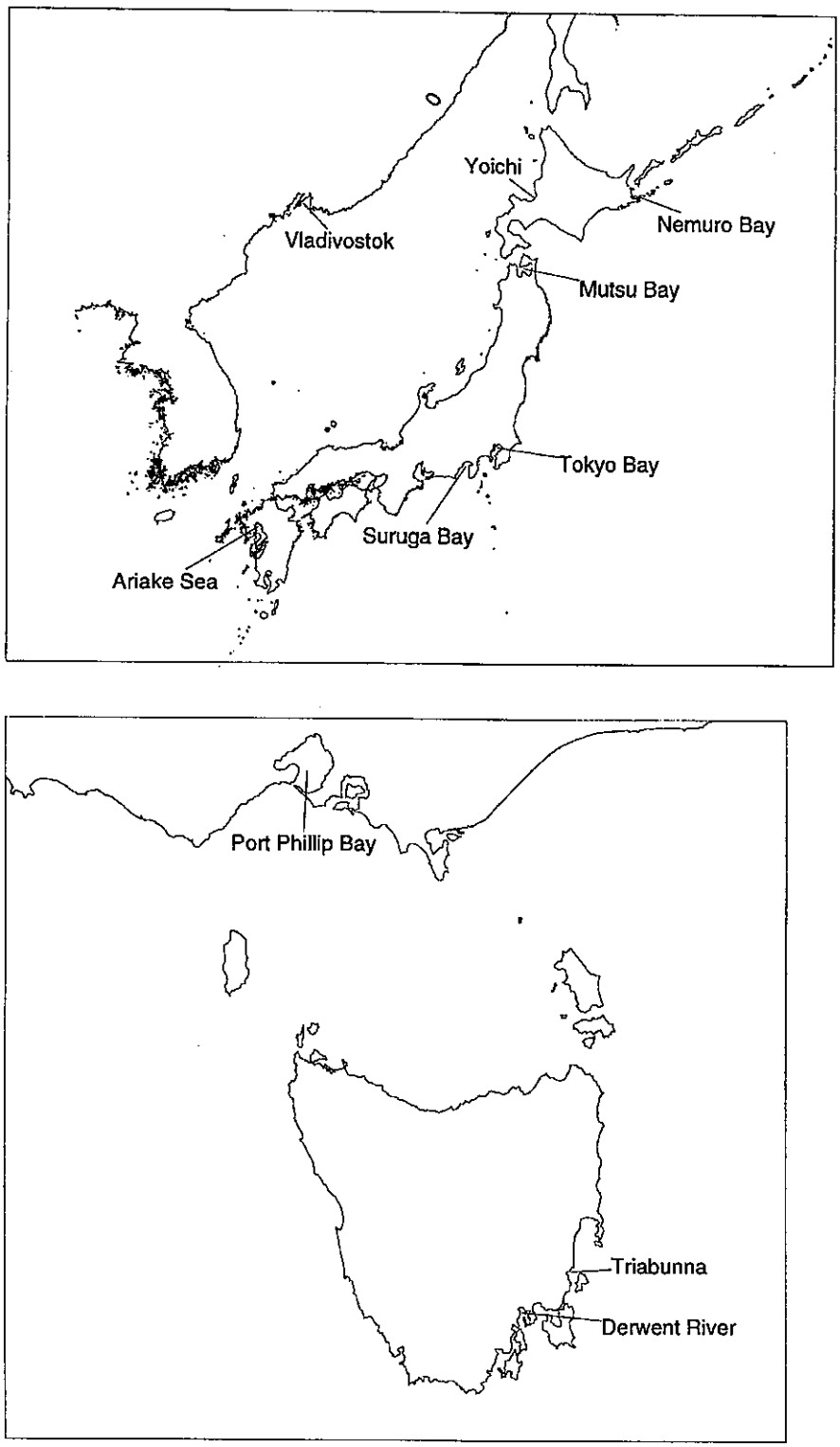

Figure 4. Sites of collection of *Asterias amurensis*

A region of mitochondrial DNA (mtDNA) was amplified by polymerase chain reaction (PCR) from all isolates. This region comprised the 12S ribosomal RNA (rRNA) gene and the 16S rRNA gene containing the putative origin of replication and two transfer RNAs (tRNAs). PCR products were digested using restriction enzymes *Dra I*, *Bfa I* and *Hpa II*, and then electrophoresed through a 2% TBE agarose gel.

Restriction profiles for each enzyme were designated a one letter description. Individual seastars were described by a composite haplotype of three letters representing the restriction profiles produced from the enzymes *DraI*, *BfaI* and *HpaII* respectively.

Results

Nine haplotypes were found in Japanese and Russian populations of *A. amurensis* (Table 3). Tasmanian and Port Phillip Bay populations of *A. amurensis* were distinguished by two of these nine haplotypes: AAA and BAB (Table 3). The BAB haplotype was most common in central and southern Japan, while the AAA haplotype occurred only in central Japan in low numbers. Both haplotypes AAA and BAB were common in seastars from Vladivostok, Russia.

Table 3. Composite haplotypes of *Asterias amurensis* from 11 sites. Sites are: 1, Derwent River, Tasmania 1996; 2, Derwent River, Tasmania 1998; 3, Triabunna, Tasmania; 4, Port Phillip Bay, Victoria; 5, Vladivostok, Russia; 6, Suruga Bay, Japan; 7, Tokyo Bay, Japan; 8, Ariake Sea, Japan; 9, Nemuro Bay, Japan; 10, Yoichi, Japan, 11, Mutsu Bay, Japan.

	TASMANIA			VIC	RUSSIA	JAPAN					
Haplotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
AAA	15	2	18	11	5	1	1				
BAA					2				3		
BAB	12	8	6	10	5	22	19	24			
BAD							1				
BBA									3		
BBB											1
BBC					1				15	24	24
BCB					2	1	2				
BDB								1			
TOTAL	27	10	24	21	15	24	23	25	21	24	25

Discussion

Origin of Tasmanian populations

The haplotypes from Tasmanian seastars were not found in *A. amurensis* from northern Japan, which eliminates this region as a source. *Asterias amurensis* from Tasmania shared haplotypes with central and southern Japan and Vladivostok, Russia. However, previous allozyme analysis (Ward & Andrew, 1995) indicated Vladivostok was an unlikely source of Tasmanian populations. Therefore, the most likely source of *A. amurensis* in the Derwent estuary was central and southern Japan.

Origin of Victorian populations

The haplotype ratio of AAA to BAB in Port Phillip Bay is near 50:50 which is similar to that from seastars from Vladivostok, Russia (Table 3). However, the source of *A. amurensis* in Victoria is unlikely to be Russia because no ships were recorded to have sailed from Vladivostok to Victoria during 1996/97 (Far East Shipping Company, personal communication).

Regular shipping takes place between both Hobart and central Japan into Port Phillip Bay. However, the frequency of visits is higher between Hobart and Port Phillip Bay (56 visits during 1994/95) than between Japan and Port Phillip Bay (13 visits during 1994/1995) (Walters, 1996). Haplotype AAA is rare in central Japan, while AAA and BAB occur in similar frequencies in both Port Phillip Bay and the Derwent estuary, Tasmania (Table 3). Therefore, the most likely source of Port Phillip Bay populations is Tasmania, with its similar haplotype ratio and greater ship movement. This does not, however, eliminate the possibility of a separate introduction from Japan.

Acknowledgements

Asterias amurensis from Port Phillip Bay were kindly supplied by MAFRI, Victoria.

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PORT PHILLIP BAY SEASTARS - RESULTS OF ALLOZYME ANALYSIS

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Twenty northern Pacific seastars *Asterias amurensis* from Port Phillip Bay, Victoria, and ten seastars from the Derwent estuary, Tasmania, were examined for variation in allele frequency at 13 loci. These loci comprised all but one of the variable loci used in a previous study to determine the source of Tasmanian populations of *A. amurensis* (Ward & Andrew, 1995). In the previous study, three Tasmanian populations were compared with one Russian and six Japanese populations.

Methods

Asterias amurensis from Port Phillip Bay were transported to Hobart in dry ice. Local specimens were collected from the Derwent River and all samples stored at -80°C. The local samples were used as controls to ensure that alleles were correctly identified.

Extracts were prepared by grinding pyloric caecae and tube feet in homogenising solution and spinning at 10 000g for three minutes. The supernatant was electrophoresed on cellulose acetate plates in either Tris-glycine or Tris-citrate buffer (Table 4). Further details of electrophoretic methods can be found in Ward & Andrew (1995).

Table 4. Enzymes, loci, tissue and electrophoretic conditions. TG - Tris-glycine buffer; TC - Tris-citrate buffer; pc - pyloric caecae; tl - tube legs.

ENZYME	LOCUS	TISSUE	BUFFER	RUN TIME (min)
Malate dehydrogenase	MDH-1, MDH-2	tl	TC	65
Isocitrate dehydrogenase	IDH	pc	TC	50
Phosphogluconate dehydrogenase	PGDH	pc	TC	55
Xanthine oxidase	XO	pc	TG	25
Aspartate aminotransferase	AAT	tl	TG	25
Hexokinase	HK	tl	TC	60
Arginine phosphokinase	APK	tl	TG	25
Esterase D	EST-D	tl	TG	15
Peptidase (val-leu)	PEP VL-1, PEP VL-2	tl	TC	60
Peptidase (leu-pro)	PEP LP-2	tl	TG	23
Glucosephosphate isomerase	GPI	tl	TC	90
Phosphoglucomutase	PGM	tl	TG	25

Allele frequencies of the Port Phillip Bay population were calculated (Table 5). Allele frequencies for Hobart, Triabunna, Japanese and Russian populations were taken from Ward & Andrew (1995). The sample from Cygnet was not included in these analyses.

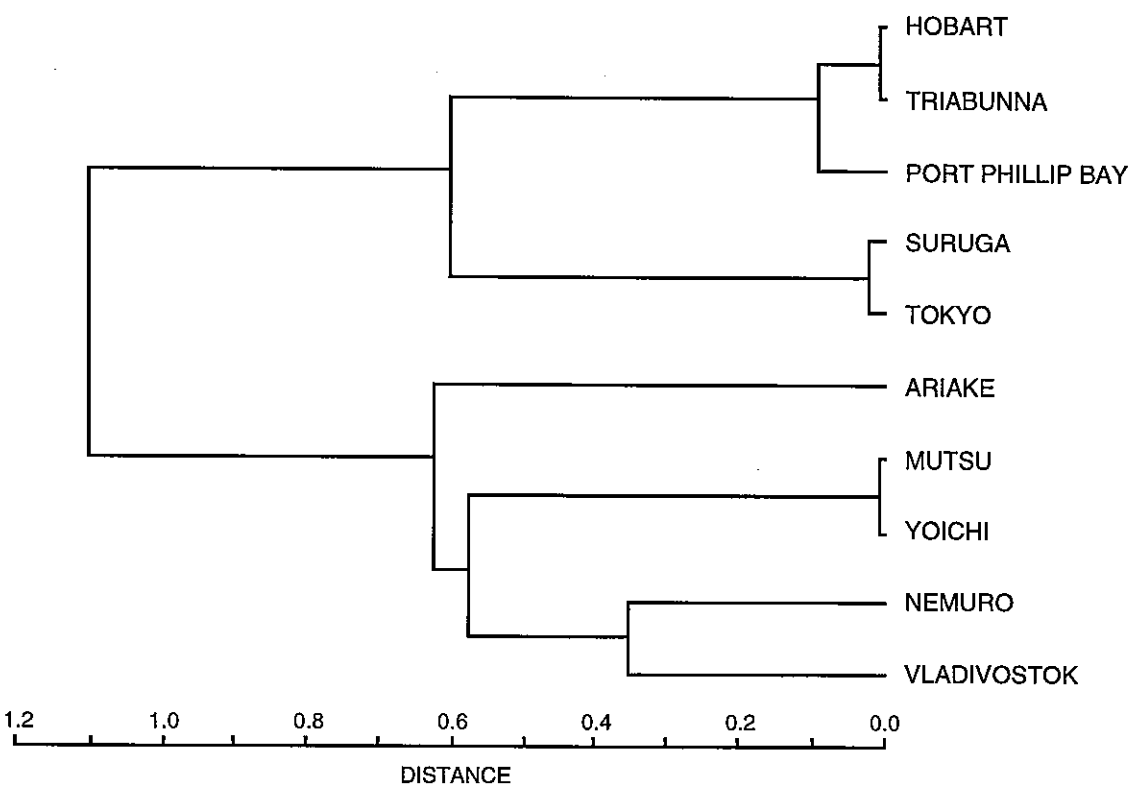
BIOSYS-1 software was used to obtain unbiased genetic distances between the populations and produce a dendrogram of population relationships by cluster analysis (Fig. 5).

Results

All alleles found in the Port Phillip Bay samples were also present in the Hobart and/or Triabunna populations. In general, allele frequencies from Port Phillip Bay seastars were very similar to those of seastars from Hobart and Triabunna, although frequencies at three loci (APK, PEP VL-1 and PGM) varied slightly. The MDH-1 locus is of interest in that all the Australian populations have the slower migrating allele at frequencies ranging from 0.235 to 0.305, whereas this allele is rare in the Japanese and Russian populations, which have frequencies between 0 and 0.01.

Relationships between the populations can be assessed from the dendrogram derived from cluster analysis of genetic distance data (Fig. 5). The tree diagram shows tight clustering of all three Australian populations.

Figure 5. Dendrogram of genetic distances among populations - UPGMA tree of Nei (1978) unbiased genetic distance.



Discussion

The Port Phillip Bay population is more closely related to the Tasmanian populations than to the Japanese and Russian populations used in the previous study (Ward & Andrew, 1995). In addition, the Port Phillip Bay population has MDH-1 allele frequencies which are very similar to those of seastars from Hobart and Triabunna and markedly different from all Russian and Japanese populations thus far analysed. This supports the hypothesis that the Port Phillip Bay population originated from Tasmania. The presence of alleles in seastar populations from Russia and Japan which have not been found in Australian populations also supports this hypothesis. However, it is possible that other populations of *A. amurensis* exist overseas which have similar genotypes to those found here.

The small differences in frequency at three loci that were observed between the Tasmanian and the Port Phillip Bay populations most likely reflect a sampling effect: either the Port Phillip Bay population is descended from a relatively small number of immigrants from Tasmania, or the Port Phillip Bay specimens analysed are the actual immigrants from Tasmania. If the former, the differences could be the result of genetic drift; if the latter, the differences would have resulted from sampling error alone. Discriminating between these hypotheses is not possible at present.

Table 5. Allele frequencies in ten populations at different loci (abbreviations as in Table 1) where populations are: 1, Hobart; 2, Triabunna; 3, Ariake; 4, Suruga; 5, Tokyo; 6, Mutsu Bay; 7, Yoichi; 8, Nemuro; 9, Vladivostok; 10, Port Phillip Bay

	POPULATION									
Locus	1	2	3	4	5	6	7	8	9	10
MDH-1										
(N)	100	100	58	50	50	50	39	54	50	20
A	.000	.000	.026	.030	.020	.030	.013	.019	.000	.000
B	.695	.765	.974	.970	.970	.960	.987	.982	1.000	.750
C	.305	.235	.000	.000	.010	.010	.000	.000	.000	.250
MDH-2										
(N)	100	100	58	50	50	50	39	54	50	20
A	1.000	1.000	1.000	1.000	1.000	.100	1.000	.991	.980	1.000
B	.000	.000	.000	.000	.000	.000	.000	.009	.020	.000
IDH										
(N)	92	99	58	50	50	50	38	54	50	18
A	.000	.000	.000	.000	.010	.000	.000	.000	.000	.000
B	.990	1.000	.897	1.000	.990	.980	1.000	1.000	1.000	.972
C	.010	.000	.103	.000	.000	.020	.000	.000	.000	.028
6PGDH										
(N)	97	100	58	50	50	50	28	53	50	17
A	.000	.000	.026	.000	.030	.010	.000	.009	.050	.000
B	.500	.450	.767	.530	.570	.490	.411	.453	.540	.530
C	.500	.550	.207	.470	.400	.500	.589	.528	.410	.470
D	.000	.000	.000	.000	.000	.000	.000	.009	.000	.000
XO										
(N)	100	100	58	46	44	47	40	50	46	20
A	.000	.000	.000	.000	.000	.000	.000	.010	.000	.000
B	.125	.095	.388	.207	.318	.319	.425	.330	.359	.050
C	.730	.715	.371	.587	.546	.489	.425	.400	.402	.850
D	.130	.165	.164	.185	.125	.149	.138	.230	.228	.100
E	.015	.025	.078	.022	.011	.043	.013	.030	.011	.000
AAT										
(N)	100	100	58	50	50	50	18	43	50	20
A	.000	.000	.017	.010	.010	.000	.000	.000	.010	.000
B	1.000	1.000	.983	.990	.990	1.000	1.000	.988	.990	1.000
C	.000	.000	.000	.000	.000	.000	.000	.012	.000	.000
HK										
(N)	100	100	58	50	50	50	39	54	50	20
A	1.000	1.000	.802	.570	.530	.500	.423	.500	.270	1.000
B	.000	.000	.198	.400	.470	.480	.577	.491	.730	.000
C	.000	.000	.000	.030	.000	.020	.000	.009	.000	.000
APK										
(N)	100	100	58	50	50	50	39	54	50	20
A	.930	.905	.216	.880	.920	.080	.026	.000	.050	.750
B	.070	.095	.690	.110	.070	.210	.128	.037	.570	.250
C	.000	.000	.086	.010	.010	.690	.782	.843	.380	.000
D	.000	.000	.009	.000	.000	.020	.064	.120	.000	.000
EST-D										
(N)	100	100	58	50	50	49	28	54	34	20
A	.000	.000	.035	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	.966	1.000	1.000	1.000	1.000	1.000	1.000	1.000

APvl1										
(N)	100	100	58	50	50	50	39	54	50	20
A	.265	.220	.009	.030	.030	.000	.013	.019	.010	.125
B	.475	.435	.517	.460	.460	.390	.628	.519	.470	.700
C	.260	.345	.474	.480	.440	.580	.321	.417	.480	.175
D	.000	.000	.000	.030	.070	.030	.039	.046	.040	.000
APvl2										
(N)	100	100	58	49	50	49	38	54	50	20
A	.000	.000	.000	.000	.030	.000	.000	.009	.000	.000
B	.000	.000	.164	.316	.380	.449	.276	.130	.240	.000
C	1.000	1.000	.836	.684	.590	.551	.724	.861	.760	1.000
APlp										
(N)	100	100	57	50	50	50	35	53	50	20
A	.000	.000	.000	.030	.000	.060	.000	.000	.000	.000
B	.000	.000	.061	.220	.110	.200	.129	.123	.160	.000
C	1.000	1.000	.912	.740	.870	.620	.800	.717	.560	1.000
D	.000	.000	.026	.010	.020	.090	.057	.142	.260	.000
E	.000	.000	.000	.000	.000	.030	.014	.019	.020	.000
PGI										
(N)	100	100	58	50	50	50	39	54	50	20
A	.000	.000	.000	.010	.000	.030	.013	.009	.000	.000
B	.000	.000	.043	.020	.020	.040	.038	.000	.010	.000
C	.000	.000	.000	.080	.060	.190	.052	.102	.090	.000
D	1.000	1.000	.922	.880	.920	.740	.897	.880	.890	1.000
E	.000	.000	.035	.010	.000	.000	.000	.009	.010	.000
PGM										
(N)	100	100	58	49	50	50	39	54	50	20
A	.000	.000	.009	.000	.000	.000	.000	.000	.030	.000
B	.180	.165	.138	.071	.120	.070	.103	.148	.030	.050
C	.000	.000	.216	.122	.100	.050	.064	.120	.180	.000
D	.355	.375	.586	.633	.520	.720	.731	.602	.470	.275
E	.000	.000	.026	.051	.110	.070	.051	.083	.200	.000
F	.465	.460	.026	.112	.150	.090	.051	.046	.070	.675
G	.000	.000	.000	.010	.000	.000	.000	.000	.020	.000

Acknowledgements

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Comments

The analyses indicate that seastars from Port Phillip Bay are more similar to Tasmanian populations than to Japanese populations. RFLP analyses indicate there were at least two females (or two separate introductions) which gave rise to *A. amurensis* in Port Phillip Bay. However, data from allozymes and RFLP analysis of mitochondrial DNA cannot determine unambiguously whether there is a spawning population of *A. amurensis* in Port Phillip Bay. The seastars in Port Phillip Bay could have originated either from a spawning population in the Bay or from a load of ballast water from the Derwent estuary which contained larvae from more than one female.

FEEDING OF *ASTERIAS AMURENSIS* IN THE DERWENT ESTUARY

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Preliminary investigation into the feeding periodicity and selectivity of the introduced sea star, *Asterias amurensis* (Lütken), in Tasmania, Australia

In the field, the seastar *Asterias amurensis*, recently introduced into Tasmania, fed over the entire 24h period with no obvious peaks and no periods of 100% activity or inactivity. Percentage of seastars feeding ranged from 17.2% at 1600h to 58.2% at 2000h. Feeding periodicity did not correlate with the time of day or the height of the tide. A significantly higher proportion of juvenile than adult seastars were feeding at any given time. A comparison of prey items found in the stomachs, with the availability of prey items in sediments, revealed that, in the field, *A. amurensis* selected some prey species and avoided others. A total of 15 species were consumed; molluscan prey was the most important (always over 60%) in winter and spring and at the two depths studied (2m and 5m). Therefore, *A. amurensis* is an opportunistic generalist predator which shows some specialisation in local populations. The percentage of seastars holding at least one prey item within the stomach folds was almost double the percentage of sea stars found actively feeding in the field. All bivalves at the field site were found to be juveniles, just a few millimetres in length. Whether this is a natural phenomenon or due to the presence of *A. amurensis* cannot be ascertained because no studies of local native communities have been conducted. However, the resilience of the community is likely to be low as a result.

Size selectivity and energy maximisation of the introduced sea star *Asterias amurensis* in Tasmania, Australia

The selectivity of *Asterias amurensis* for different sizes of prey was investigated with the aim of predicting the impact this species will have on the age structure of native prey species and thus, survival of prey populations. The energy maximisation capabilities of *A. amurensis* were assessed. The time *A. amurensis* spent handling the mussel *Mytilus edulis planulatus* generally increased exponentially with increases in mussel size. Mussels of the size class 20-29mm were preferred, although this preference was not significantly greater than for sizes <10mm, 10-19mm and 30-39mm. The energy content of six mussel size classes was divided by the handling time of each to give a prey value. The optimal mussel size class was calculated to be 30-39mm. Thus, *A. amurensis* did not maximise its energy by consuming mussels of a size that gave the greatest energy return for the energy expended. Smaller seastars consumed a greater percentage of their body weights (4.97%) than did larger seastars (2.57%).

Feeding rates of the introduced seastar *Asterias amurensis* (Lütken) in Tasmania

Proceedings 9th International Echinoderm Conference held in San Francisco, USA.

August, 1996 (in press).

Feeding rates of *Asterias amurensis* on four prey varied considerably. The seastars did not feed on the gastropod *Fusinus novaehollandiae* even though this species was a prey item in the field. The hermit anopmuran *Pagurixus handrecki* which resides in the shells of *Nassarius nigellus*, was also not consumed in the laboratory. The gastropod *N. nigellus* was consumed at a rate of 0.23% of wet body weight per seastar per day. The echinoid *Echinocardium cordatum* was consumed at a rate of 11.46% of wet weight per seastar per day. The variation in feeding rates on *N. nigellus* and *E. cordatum* were attributed to variations in the ease of capture. The energy contents of the four prey species were measured and compared to the rates of feeding.

Comments

Anecdotal evidence suggests *Asterias amurensis* prefer *Electroma* sp. to mussels in the field. Some native bivalves escape predation by *A. amurensis* in the field because they have habitat refuges e.g. they settle in areas which are exposed at high tide.

FERTILISATION AND RECRUITMENT DYNAMICS OF *Asterias amurensis*

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Background

Information on the fertilisation and recruitment dynamics of the seastar *Asterias amurensis* is limited to few data from the indigenous Japanese population. There is a strong likelihood that these data do not apply to the Derwent Estuary population, given temporal and spatial variability in fertilisation parameters recorded in other echinoderms and differences observed between Japanese populations of *A. amurensis* (Babcock *et al.*, 1994; see Davenport & McLoughlin, 1993 for review).

An important component of recruitment dynamics of the seastar, critical in assessing invasiveness, is to identify the substrata that induce seastar settlement and metamorphosis and assess the distribution of highly inductive substrata. Knowledge of the substrata that induce high rates of metamorphosis and settlement could be used in management because habitats which provide strong cues for settlement can be identified and considered for control measures.

Knowledge of the fertilisation ecology of *A. amurensis* can identify critical densities, dispersion patterns, and sex ratios and gonad conditions that lead to high fertilisation success. Moreover, non-linear fertilisation responses to changes in any of these parameters can be important information for management to minimise the likelihood of highly successful reproductive events.

Spawning

The Australian population of *A. amurensis* spawn from June to September at temperatures of approximately 10-12°C (Morrice, 1995; pers obs). The spawning season in Japan varies with latitude and tends to be induced by temperature increases at low temperatures (approximately 10°C). Spawning peaks in seastars in Tokyo Bay (35°N) and Sendai Bay (38°N) from February to March at temperatures of 6.2-13.6°C (Takashi *et al.*, 1955). In Mutsu Bay (41°N) and Hokkaido (43°N) spawning occurs at 5-10°C and 6-14°C respectively (Kim 1968). There is some evidence of multiple spawnings of individuals in Peter the Great Bay and Japan (see Davenport & McLoughlin, 1993, for discussion).

Seastars become sexually mature in approximately one year when ray lengths are greater than 46mm (Japan) and 55mm (Australia) (Ino *et al.*, 1955; Hatanaka & Kosaka, 1958; Morrice 1995). Sex ratios are approximately 1:1 in Japanese and Derwent River populations (Hatanaka & Kosaka, 1959; Nemoto & Ishida, 1983; Louise Goggin, personal communication).

Fecundity

One-year-old females produce 0.4-2.8 million eggs and two-year-olds produce 5.3-15.5 million eggs (Hatanaka & Kosaka, 1958). In Japan, adult females can produce up to 19 million eggs (Kasyanov, 1988). Adult female *A. amurensis* in the Derwent River have a

mean fecundity of two million eggs (pers obs).

Induction of metamorphosis by natural substrata

A range of natural substrata were tested for their ability to induce settlement and metamorphosis in competent brachiolaria. Late brachiolaria without morphological abnormalities and with well-developed sensory papillae and seastar primordium, were deemed competent. Ten competent larvae were placed in wells containing 16.5ml filtered seawater and the substrate being tested (mud, bare rock, sand, non-geniculate coralline algae (NCA), mussel *Mytilus edulis* shell, and filtered seawater (control)). There were seven replicates of each trial: each trial ran for seven days and settlement was scored each day. NCA induces high rates of metamorphosis in competent larvae (100% on day 2). Lower rates of settlement were induced by mud and rock (39% and 35% respectively on day 7) but settlement on sand and on mussel did not differ significantly to that in the controls (Fig. 6).

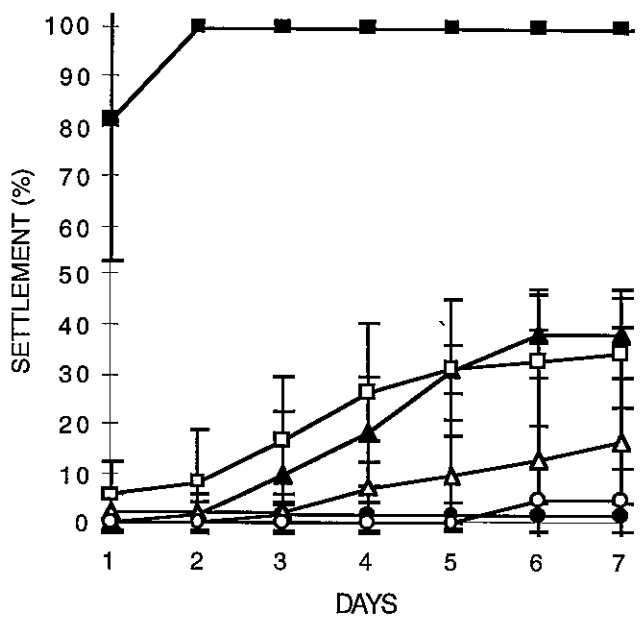

Figure 6. Percentage settlement of competent *Asterias amurensis* larvae over time (mean and 95% confidence interval) in wells with various substrates where ■ = NCA (non-geniculate coralline algae), ▲ = mud, ❑ = rock, Δ = mussel, ● = sand, ○ = control

Settlement

Settlement collectors of 100 bioballs (37 x 29mm) enclosed tightly in a mesh bag were deployed at ten sites in the Derwent River in October 1997 and collected in December 1997. Three replicate collectors were deployed at each site. Samplers were deployed in sand, mud, rock and seagrass habitats, at sites in the upper and lower Derwent. High settlement indices were observed at Macquarie Wharf and Battery Point. There was low settlement at all sites on the eastern shore (0.5-6.7 seastars per collector) (Table 6). Sites with high mean rates of settlement were also characterised by large variances, suggesting that the availability of larvae in the water column may be patchy at scales of $10m^2$.

Table 6. Settlement of *Asterias amurensis* in collectors containing 100 bioballs. Collectors were deployed approximately 0.5m above the substratum (n=3 per site).

SITE	SUBSTRATUM TYPE	MEAN No. JUVS PER COLLECTOR	STANDARD DEVIATION
Kangaroo Bay	Rock	3.3	0.6
Howrah	Sand	1.0	1.0
Howrah	Rock	0.5	0.7
Tranmere	Mud	6.7	1.5
Tranmere	Rock	0.7	1.2
Battery Point	Mud	17.0	6.0
Macquarie Wharf	Mud	18.7	16.0
Halfmoon Bay	Sand	0.5	0.7
Halfmoon Bay	Seagrass	0.5	0.7

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Comments

Bacteria is required for high rates of settlement and metamorphosis of the crown-of-thorns seastar *Acanthaster planci* on crustose coralline algae (Johnson, C.R., Sutton, D.C. 1994. Bacteria on the surface of crustose coralline algae induce metamorphosis of the crown-of-thorns starfish *Acanthaster planci. Mar. Biol.* **120**:305-310). Bacteria on non-geniculate coralline algae (NCA) may also be required to induce settlement of *A. amurensis* at high rates. It was suggested that mussel lines may offer an attractive settlement surface for *A. amurensis* because of their bacterial cover. However, work with the crown-of-thorns seastar suggested that although the bacteria produced the morphogenic cue, the pre-cursor compound was provided by the algae (but not by other substrata). Thus, in this case, the morphogenic signal required both the bacteria and the algae.

Des Whayman commented that in Tasmania, *A. amurensis* appear to settle on the fine algae which grows on the lines rather than on the ropes and settle prior to mussels. Further experiments are required to determine whether seastars settle on the ropes or the algae and whether there are differences between settlement on ropes in Tasmania and Victoria.

Settlement of seastars into collectors was lower on the eastern shore than on the western shore of the Derwent estuary. There is a freshwater lens (~2m deep) which flows out of the estuary, hugging the eastern shore. However, the low settlement of seastars on the eastern shore was unlikely to be due to the freshwater lens because the settlement collectors were at 3-5m depth which is below the freshwater. It was also pointed out that the low settlement of larvae into bioball samplers on the eastern shore does not correspond to low numbers of juveniles and adult seastars in this area.

Alice Morris will continue her work to determine larval behaviour at various salinities and further elucidate the preferred settlement substrates of *A. amurensis*.

A SUMMARY OF CSIRO STUDIES ON THE LARVAL ECOLOGY OF *ASTERIAS AMURENSIS*

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CSIRO conducted a series of studies on the early life history of *Asterias amurensis* between 1993 and 1996. There were two main components:

- Laboratory rearing to provide: reference material for identification (both morphological and genetic); estimates of larval duration and; larvae for temperature and salinity tolerance tests

- Field sampling to examine: larval evidence for spawning activity (complementary to adult sampling by the Tasmanian Museum and the University of Tasmania); larval duration in the field; seasonal availability at major ballasting sites and; broad scale and vertical distribution of larvae in the Derwent estuary.

Methods and results are reported in Bruce *et al.* (1995) and Sutton & Bruce (1996). The following notes summarise the major findings of the work.

Larvae

Asterias amurensis spawn small pelagic eggs approximately 150µm in diameter that hatch and develop through a series of stages (coeloblast, gastrula, bipinnaria, brachiolaria) typical of asteroids with planktotrophic larvae. Larval duration in the laboratory was protracted and variable ranging from 90-150d at 12°C.

Identification of larvae

Two other asteroids with planktotrophic larvae are common in the Derwent: *Coscinasterias muricata* and *Patiriella regularis*. Larvae of *Uniophora granifera*, a species superficially similar to *A. amurensis*, are lecithotrophic and are thus readily separated. Larvae of *C. muricata* and *P. regularis* were reared to provide reference material for morphological and genetic identification. Linear discriminate function analyses based on seven morphological measurements initially proved promising in separating larvae of the three species. However, the morphological plasticity of asteroid larvae (George, 1994) limits the ability of such analyses to provide unequivocal identification.

A more definitive test of identification, based on the PCR amplification of mitochondrial DNA, was developed by Evans *et al.* (in press). The technique successfully typed laboratory-reared larvae of *A. amurensis*, *C. muricata* and *P. regularis* and successfully identified alcohol-preserved larvae from plankton samples.

Seasonal availability of larvae in Derwent estuary

Asteroid larvae were sampled every two weeks at CSIRO, Elizabeth St Pier and the Domain from March 1995 to March 1997 (Fig. 7). A striking feature of the samples was the extremely high abundance of asteroid larvae, with values in excess of 1100 larvae/m^3.

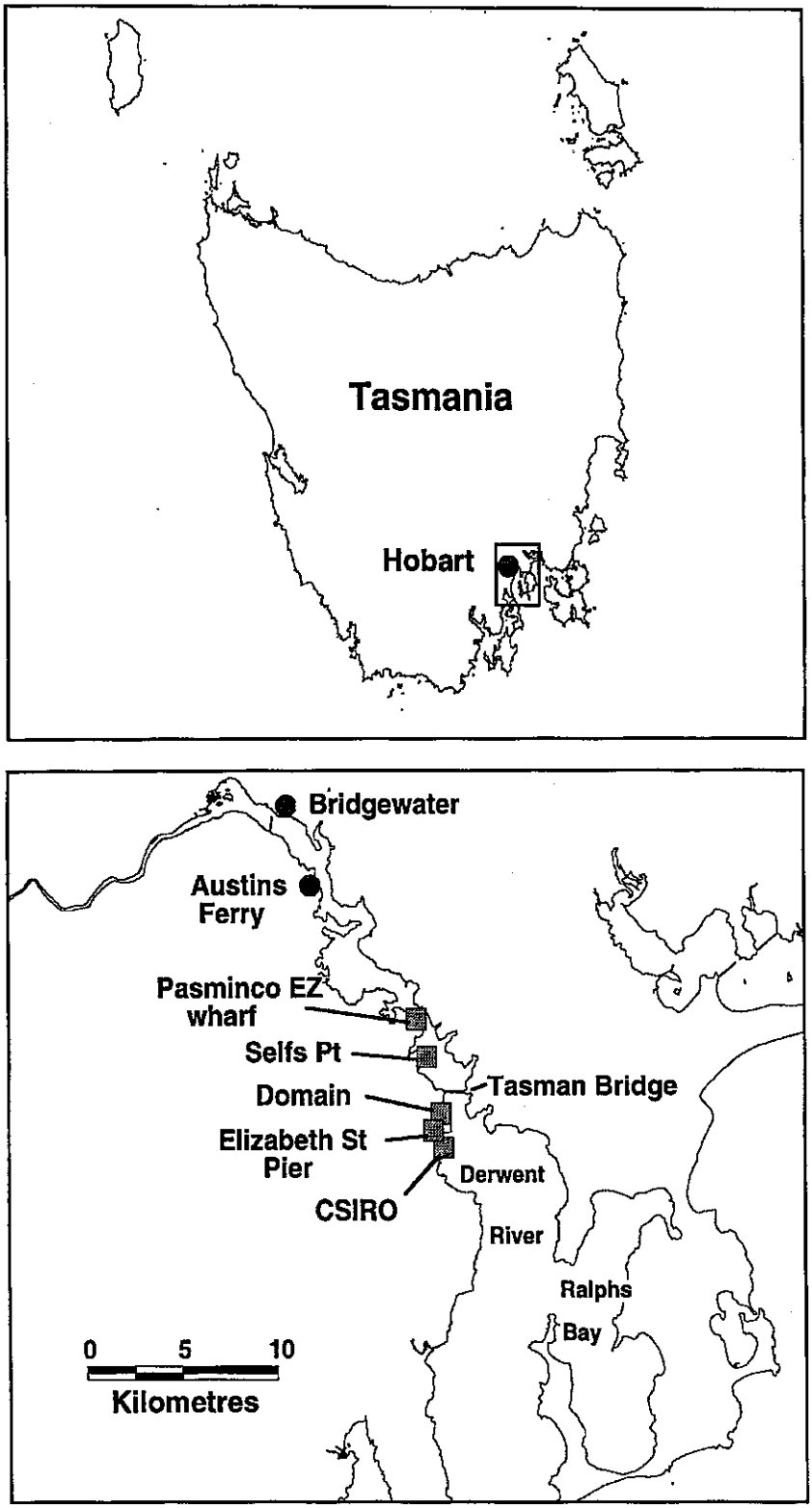

Figure 7. Sites where larval *Asterias amurensis* were collected

There was considerable variability in abundance between sampling dates, with similar patterns observed at each site. Asteroid larvae were most common between June and March with low abundances during April and May. Samples between June and October were dominated by early stage larvae, indicating a period of prolonged spawning activity. Major peaks in abundance (400-1100 larvae/m^3) occurred in early August and late September, shortly after peaks in the gonosomatic index reported for adult *A. amurensis* by Byrne *et al.* (1997). Minor peaks (200-250 larvae/m^3) in larval abundance occurred in November, January and February. Larvae of all stages (coeloblast-brachiolaria) were recorded. Peaks in abundance were dominated by early (coeloblast-early bipinnaria) stage larvae indicating recent spawning activity.

Genetic typing confirmed that 97% of asteroid larvae collected in August were *A. amurensis*. These were mostly gastrula and early bipinnaria with small numbers of late bipinnaria. The remaining larvae were typed as gastrula of *C. muricata*. All larvae tested from September were typed as *A. amurensis*. No larvae were typed during the June-July period, however, we observed ripe adults at that time and in August, captured late-stage *A. amurensis* bipinnaria which suggests these larvae were also *A. amurensis*. By November, the percentage of larvae genetically-typed as *A. amurensis* had fallen to 54% and comprised late bipinnaria and brachiolaria. The remaining larvae included *C. muricata* and *P. regularis*. In January, 11% of larvae were typed as *A. amurensis*, and the remaining larvae typed as either *C. muricata* or *P. regularis*.

The larval data thus confirm the observations of Byrne *et al.* (1997) that *A. amurensis* has a prolonged winter/spring spawning period from July to October in the Derwent estuary. *Asterias amurensis* larvae were present in the Derwent estuary from June to January with peaks in larval abundance in August and September. The presence of larvae of all stages suggests that a considerable number of larvae complete development within the estuary despite the protracted larval duration. Brachiolaria stages were first recorded in mid-October, eight weeks after the August spawning peak, and larvae were still present in January. This suggests the settlement period of the larvae may also be protracted and extend from October until at least January. *Coscinasterias muricata* commenced spawning in late winter although larvae were not abundant prior to November. *Patiriella regularis* commenced spawning in late spring, and continued spawning during the summer months.

Distribution of asteroid larvae in the Derwent estuary during the Asterias amurensis spawning season

Major ballasting sites

In addition to the three seasonal monitoring sites, larvae were sampled at Selfs Point and the Pasminco EZ wharf, Risdon, during September and October 1995 (Fig. 7). These samples covered all major ballasting points in the estuary. Asteroid larvae were abundant at all sites, although there was a decline in abundance with distance up-river. Given the dominance of *A. amurensis* larvae at other sites during this period, it is reasonable to assume that the larvae at Selfs Point and EZ wharf were also *A. amurensis*. The abundance of larvae at Selfs Point and the EZ wharf followed the same fortnightly patterns observed at the three seasonal monitoring sites indicating a broad spatial concordance in larval distribution and abundance throughout this section of the estuary. The high abundance of *A. amurensis* larvae at all sites indicates considerable potential for their uptake by vessels taking on ballast water in the Derwent estuary.

Broad scale distribution of larvae

Two broad scale sampling exercises were carried out during the *A. amurensis* spawning season to determine the distribution of asteroid larvae throughout the Derwent estuary. The first exercise (below Tasman Bridge) sampled 32 stations on eight transects between the Tasman Bridge (Hobart) and the mouth of the estuary; the second (above Tasman Bridge) sampled 12 stations between the Tasman Bridge and Bridgewater. Larvae were sampled at each site using a free-fall vertical drop net.

(i) **Below Tasman Bridge:** Asteroid larvae were widespread and abundant in all sections of the estuary below the Tasman Bridge with the exception of the eastern side of the river below the entrance to Ralphs Bay. Vertical profiles and horizontal contours of salinity were consistent with previously descriptions of Derwent estuary circulation (Guiler, 1955; Thomson & Godfrey, 1985). The Derwent is a stratified salt wedge estuary with net flows of low salinity water to seaward at the surface and higher salinity flows landward at depth. The extent of vertical stratification varies spatially with the Coreolis effect limiting the low salinity outflow to the eastern margin of the lower estuary below Ralphs Bay. Therefore, asteroid larvae were least abundant in water exiting the estuary.

(ii) **Above Tasman Bridge:** Asteroid larvae were present in samples from the Tasman Bridge to Austins Ferry with abundances decreasing upstream. Vertical salinity profiles were dominated by a shallow low salinity surface layer (1-2m in depth) overlying a salt wedge. The salt wedge extended upstream to Austins Ferry, thus matching the distribution of asteroid larvae.

Vertical distribution of larvae

The vertical distribution of larvae was examined at two sites on the highly stratified eastern side of the lower estuary and at five sites on the relatively poorly stratified western side. The vertical distribution of larvae was significantly different on either side of the estuary. Larvae were absent from low salinity, seaward flowing, surface waters on the eastern side but abundant within the underlying salt wedge. Larvae were relatively abundant at all depths on the western side of the estuary.

Despite their extended pelagic life, *A. amurensis* larvae in the estuary were found in all developmental stages (particularly near Hobart) which suggests that large numbers complete their development within the Derwent. The vertical distribution of larvae suggests that they are retained in the estuary because they avoid the low salinity, seaward flowing, surface waters. Larval retention is obviously not complete because low numbers of larvae were found in waters exiting the Derwent near the mouth of the estuary. Thus whilst the Derwent undoubtedly acts as a source of larvae for southern Tasmania, the export is probably limited by the hydrological structure of the estuary. The vertical stratification of the Derwent becomes progressively less distinct south of Ralphs Bay because the low salinity layer mixes with the underlying salt wedge. Because of this breakdown in vertical stratification, larvae produced from populations in this area of the Derwent are less likely to be retained within the system. Thus, the export of larvae from the Derwent estuary may increase as spawning populations of *A. amurensis* increase in abundance on the eastern shore below Ralphs Bay.

Temperature and salinity tolerance of larvae

The salinity and temperature tolerance of *A. amurensis* larvae was investigated to determine the risks of introducing the species to broad areas of mainland Australia. The optimal temperature range for *A. amurensis* larvae from the Derwent estuary population was <8°C - 16.5°C. Growth rates were significantly reduced above 16.5°C and larvae failed to develop normally above 20°C. Larvae did not survive when exposed to temperatures above 26°C.

The optimal salinity for *A. amurensis* larvae was 32‰. The tolerance to temperatures above 16.5°C was significantly reduced at high (35‰) and low (28‰) salinities. Larvae were adversely affected by 10min exposures to salinities < 17.5‰ and did not survive exposures to salinities <8.75‰. Extensive cellular damage occurred within 1-2min exposure to salinities < 8.75‰. These data suggest that *A. amurensis* larvae are most likely to survive in waters of <16.5°C. Survival in waters up to 20°C is possible, but less likely, due to significantly reduced growth rates.

The spawning period of source populations of *A. amurensis* and the temperature tolerance of larvae may help explain the successful introduction of the seastar into the Derwent estuary and its absence (until recently) from mainland ports that receive higher quantities of ballast water. Based on genetic analyses, central eastern Honshu, Japan, is the most likely source of *A. amurensis* in Tasmania (Ward & Andrew, 1995). The peak spawning period and hence the peak larval abundance of *A. amurensis* from this region, is between February and March (Kim, 1968). At this time, water temperatures in Australia are at a maximum. If the 16.5°C isotherm limits larval survival, then southern Tasmania is the only region in Australia where larval *A. amurensis* could survive at that time of year. Therefore, the risk of successfully introducing *A. amurensis* larvae from central Japan into Australian mainland ports is low. However, *A. amurensis* in Tasmania has now aligned its spawning to the austral winter. Based on the winter position of the 16.5 °C isotherm, the risk of ballast water mediated introductions from the Derwent estuary to southern Australian ports between Eden and Albany is high. Therefore, populations of *A. amurensis* in Tasmania pose a more serious threat to mainland Australia than do native populations of seastars in Japan or Russia.

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Comments

A recent report (*Ability of Target Organisms to Survive in Ballast Water*, AQIS, Australian Museum Business Services Consulting, February 1998) suggested *A. amurensis* could not invade Australia in ballast water because the temperature of water in ballast tanks would kill seastar larvae as the ship transits the Equator. Caroline Sutton commented that ships have ballast tanks in different configurations. Temperatures in the outer tanks of a bulk carrier climbed from 24.8°C to 30.3°C in a voyage from Port Kembla to Port Hedland (Rigby, G., Hallegraeff, G., Sutton, C. 1997. *Ballast Water Sampling Trials on the BHP Ship M.V. 'Iron Whyalla' in Port Kembla and en-route to Port Hedland.* AQIS report, October 1997. 40pp). However, the cargo hold of some vessels can also be filled with seawater to increase draft (e.g. during unloading at some ports at high tide and during cyclones). The water in the cargo hold would be more insulated than seawater in the outer ballast tanks and may not reach outside water temperatures. However, there are no data on the fluctuation of temperatures in the cargo hold during a transit of the Equator. It is unknown whether temperatures in the hold would kill *A. amurensis* larvae during such a voyage.

Although most *A. amurensis* larvae are entrained in the Derwent estuary, many larvae exit the system. Populations of seastars have expanded toward the mouth of the Derwent estuary which increases the chance of the larvae exiting the system because there is greater mixing of the freshwater lens and saltwater wedge near the mouth of the estuary.

More asteroid larvae were found along the western than the eastern shore of the Derwent estuary, however, larvae from asteroids other than *A. amurensis* may be found on the western shore.

Abundance of seastar larvae varied considerably between weeks and is likely to vary greatly between years (although only one year of data has been analysed to date). Larval densities found in the Derwent estuary in 1995 are some of the highest recorded for asteroid larvae anywhere in the world. Occasional peaks in larval abundance are often recorded for invertebrates although the stimulus for these peaks is unknown. Indeed, our ignorance of the factors which drive the population dynamics of the seastars was highlighted.

3. Options for control of *Asterias amurensis*

CONTROL OF SEASTARS BY PHYSICAL REMOVAL

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Physical removal is the only method currently used to reduce seastar numbers in near-shore coastal environments. It was routinely practised by the oyster industry on the US Atlantic coast to reduce the impacts of *Asterias forbesi* (Galtsoff & Loosanoff, 1939) and is still an integral part of scallop culture in parts of northern Japan (Ito, 1991). In Australia, community dives to collect large numbers of seastars have been used to raise public awareness of *Asterias amurensis* in Tasmania, and trials have been undertaken to assess the effectiveness of locally designed seastar traps.

Control of Asterias forbesi by the US oyster industry

Galtsoff & Loosanoff (1939) record the methods used by the oyster industry to control the seastar *A. forbesi* in Buzzards Bay, Narragansett Bay, Long Island Sound and lower Chesapeake Bay during the early 1900s. The levels of infestation in these areas varied and was reflected in the numbers of seastars caught during control operations. For example, in Narragansett Bay during the four years 1929–32, over 33 million seastars were removed from a 600ha oyster lease where only seven years earlier in 1922, seastar numbers were too low to be recorded. Seastar control was practised by individual companies and usually confined to the lease areas. On some leases, control measures were continued for ten to 12 months of the year but on most leases, they were only used when seastars appeared in large numbers.

The most commonly used device for catching seastars was a mop consisting of 12 to 16 large rope yarn brushes, about 1.5m long, attached to a 3m long iron bar. A modified version was also developed which could be used over rocky or uneven ground. Mops were usually deployed from either side of the towing vessel and were operated in much the same way as a dredge. Seastars were killed by lowering the brushes, with the entangled seastars, into troughs containing hot water. Catch rates were dependent on seastar density but were less than $2.27 m^3 day^{-1}$ (Galtsoff & Loosanoff, 1939). Seastar moppping is still practised 5-6 days per week around oyster leases in Long Island Sound. Catch rates have declined to around 2-3 seastars per day and mopping will probably be discontinued in the near future (Clyde Mackenzie, personal commmunication).

In the late 1930s suction dredges were introduced to remove seastars from oyster beds. These could achieve catch rates of up to $2 m^3 h^{-1}$ in areas where seastars were abundant (Galtsoff & Loosanoff, 1939).

Control of Asterias amurensis during HOTAC scallop culture

In Nemuro Bay, Hokkaido, northern Japan, physical removal of *A. amurensis* is routinely practised as part of the "Hotate"-aid-conglomerate (HOTAC) method of scallop enhancement and culture (Ito, 1991). The HOTAC scallop culture technique uses plot rotation in which areas of the sea floor (plots) are cleared of seastars prior to seeding with juvenile scallops and again, but with lesser intensity, during the scallop grow-out period.

Seastars reinvade the cleared areas but do not achieve pre-clearance densities, therefore, a significant number of scallops can be harvested by the end of the three year grow-out period.

Seastars are removed prior to scallop reseeding using scallop dredges and traps; 1–3,000 tonnes of seastars are removed annually from rotational scallop grounds in Nemuro Bay. This reduces maximum seastar densities from around 1.4m^{-2} to 0.4m^{-2} (Ito, 1991). Seastar removal is continued after reseeding using rope trawling and trapping (McLoughlin & Bax, 1993).

Diver collections of Asterias amurensis in the port of Hobart

In 1993, a number of community dives were organised by dive clubs and the Tasmanian Museum to collect seastars from the wharves near Hobart. The first dive took place on 10 July 1993 and involved 22 divers. More than 6,000 seastars were collected from an area 300m x 20m adjacent to Princes Wharf and it was estimated that about 60% of seastars in the area were removed (Morrice, 1995).

A second, more extensive, community dive was carried out at the Hobart wharves on 22 August 1993. About three tonnes of seastars (approximately 24,000 individuals) were collected (Morrice, 1995). Seastars collected during both dives were used for composting trials carried out by the Department of Agricultural Science at the University of Tasmania (Line, 1994).

Trapping trials using the Whayman/Holdsworth seastar trap

Between April and May 1994, Tasmanian DPIF carried out trials to assess the effectiveness of the Whayman/Holdsworth seastar trap. They tested the effects of trap size, mesh size and soak time on seastar catch rates, and investigated escape from the traps. The trials showed that:

- most seastars were caught within the first 24–48h;
- catch rates were highly variable;
- catches were dominated by larger individuals and small mesh (26mm) traps caught more seastars than large mesh (65mm) traps;
- the effective fishing area for each trap was estimated to be approximately 30m^2 (Anon, 1994).

More extensive trials were carried out by DPIF in 1996 (Andrews *et al.*, 1996). The aims of these trials were to determine whether the traps provided an effective method of controlling *A. amurensis* infestations around marine farms, and to assess the value of the traps for commercial applications. The study involved fish-down experiments, an assessment of perimeter trapping, an evaluation of different bait types and a comparison between trap and diver control.

Intensive trapping in areas with low/moderate and high seastar densities failed to control seastars within the trapped area. In the low/moderate density site 1160% of the original population was removed over a 51 day period; at the high density site, 53% of the pre-

fishdown population was removed. At both sites, seastars immigrated rapidly and persistently into the trap area. Mark-and-recapture studies indicated that seastars were capable of moving at least 20m in 24h.

Bait trials indicated only minor differences in catch rates using different bait types. Pilchards were slightly more attractive but only for shorter (24–48h) soak times.

The potential for using traps to control the migration of seastars was tested by trapping at the perimeter of an area which was cleared of seastars by divers. Perimeter trapping, even with traps spaced only 2.5m apart, did not prevent seastars entering the cleared area.

Trapping was more cost effective than diving to control chronic infestations, regardless of density or depth. For seastar infestations which are sporadic over time and when densities are below 1.5-2/m, diver control appears to be more appropriate. Diver collection was also preferred in such situations because intensive trapping may attract seastars into an area. Diver control was, however, not cost effective at depths greater than 12m.

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Comments

Physical removal is effective only as a tactical control at local sites and is not suitable for strategic / large scale control. There was concern about the impact of "mopping" on flora and fauna on the sea floor. Dredging of the Derwent estuary was not considered appropriate as heavy metals in the sediments could be resuspended (Coughanowr, C. 1997. *State of the Derwent estuary: a review of environmental quality data to 1997.* Supervising Scientist Report 129, Supervising scientist, Canberra).

THE INDUSTRY PERSPECTIVE

Des Whayman

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Industry now recognises that eradication of the northern Pacific seastar *Asterias amurensis* is impossible unless by some stroke of luck or genius. The best we can hope for is that marine farm management and control of stock movement can restrict the further spread of larvae and juveniles.

However, this would be fruitless unless fishing vessels (both professional and amateur) do not transfer larvae - particularly around boat ramps by amateurs, and harbour-to-harbour by professionals. The proposed methods regarding commercial ballast water are too late for species which have already been introduced but could be useful for controlling future invasions.

I will list the actions which we may be able to take in a program.

- Limit the movement of stock during the larval period.
- Discourage DPIF and prospective marine farm (shellfish) applicants from taking occupancy in potential problem areas.
- Assist in training farmers to participate in larval monitoring.
- Require more stringent records of sitings of seastars and other introduced pests.
- Provide space on statistical returns for such information.
- Encourage farm hands to notify CSIRO of sitings.
- Have a notice board placed at boat ramps and public jetties with relevant information.
- Visit schools and ask pupils to give information to Principals.

I suggest that there has been very little interest taken by those concerned with the environment and thus very little interest by politicians. Unfortunately, industry must take most of the blame for this - I have noticed a very lethargic attitude which has been a common problem during my 50 years in the fishing industry. If information about introduced species was provided as part of an educational program at schools, this would form part of a solution. The work-for-the-dole scheme could also be useful and also provide participants with an interesting occupation. This could be organised by marine farmers and may enable participants to gain employment in the industry.

Although we have not seen any seastars in Great Bay, we find large European crabs *Carcinus maenas*. The crabs appear to be arriving in 50mm oysters because they were found in cages suspended on longlines (one large crab had eaten six 70 to 80mm oysters). Therefore, it is probably only a matter of time before the seastar arrives.

I will conclude by saying that because I have followed this problem more closely than my fellow marine farmers, I feel quite vulnerable. I believe that until farmers suffer substantial losses, they generally do not worry. However, there are some farmers in every growing area who are responsible and I will target those for help with any suggested program or plan.

What Industry Must Do

- Suggest that Port Phillip Bay mussel farmers do not relocate mussel ropes to Western Port.
- Basic training for young marine farmers and farmhands using a microscope to identify larvae. This could be done regularly to determine whether seastars settle on the rope, weed or mussels. Fishing Industry Training Board (FITB) could include this in their courses for trainees.
- Provide this information to CSIRO and the Department of Primary Industries and Fisheries.

CHEMICAL APPROACHES TO MANAGING POPULATIONS OF THE NORTHERN PACIFIC SEASTAR

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"Plagues" of exotic seastars are a relatively new problem. However, native seastars have long been considered pests due to their perceived impacts on valuable shellfish resources. Stories of fishers cutting up seastars and throwing them overboard, in a misguided attempt to reduce their numbers, are literally legend.

One of the first attempts to control seastars on commercial shellfish grounds, dating from the turn of the century, used toxic chemicals. Three approaches to chemical control of seastars has been, or could be, used.

Broadcast application of seastar-icides

Lime, in either slake or hydrated form, is highly alkaline and corrodes the carbonate test of seastars. After long-term (24-48h) contact, lesions form and seastars usually die within two weeks. Because quicklime has industrial uses, obtaining suitable quantities for broadcast application was not considered expensive. In Tasmania, quicklime currently costs about $200 per tonne.

Quicklime has been proposed for use in two ways: as a barrier around production facilities and for broadcast application. As early as 1908, quicklime (CaO_2) was suggested as an effective barrier to the movement of seastars, to protect areas of commercial significance (Wood, 1908). Quicklime was deployed in porous bags, through which particles diffused, and repelled (?) or killed seastars that crawled over them. This approach is still used (but discouraged) in Korea to destroy *A. amurensis* and was used until recently in Canada against *A. vulgaris* and *A. forbesi*. The broadcast application of quicklime has not been used except in experimental trials. The key to success apparently is the dispersal of a relatively uniform layer of the lime over extensive areas. Seastars are exposed to the corrosive particles either as the lime settles to the bottom, or as they crawl over it. Experimental studies in the laboratory indicate that the lime remains effective for several weeks after deposition, but at a much reduced kill rate. In field trials, lime was dispersed by shovelling or hosing it into the water, which resulted in an uncertain dispersal pattern but caused mortalities as high as 70% using circa 200 pounds of quicklime per acre (Loosanoff & Engle, 1942). A device designed to spread the material uniformly is described in Loosanoff & Engle (1942), although apparently never constructed.

Key issues regarding broadcast application of lime are its 1) effectiveness, 2) impact on other marine organisms, and 3) safety. Regarding effectiveness, it appears widely accepted that quicklime does kill seastars, and can do so cheaply and effectively over large areas. As noted, field trials in the US in the 1940s indicate more than 70% of seastars were killed from a single application. The treatment is effective because the seastars live in an essentially two dimensional habitat and are therefore unable to evade the quicklime. However, Birkeland & Lucas (1990) report a considerably lower success rate (28%) when quicklime was used against *Acanthaster planci* in a field trial on the Great Barrier Reef.

They attribute the low kill rate in part to the seastars inhabiting a complex structure with numerous shelters. In a laboratory trial, short-term exposure (about 5h) to quicklime slurry caused no apparent ill effects to a single *A. amurensis* from the Derwent estuary, and the seastar was apparently healthy several months later (Goggin, unpublished data). Therefore, seastars must contact the quicklime for lengthy periods to ensure they are killed.

The two safety issues, regarding effects on other biota and effects on humans, present a more substantial deterrent to use of the material. Loosanoff & Engle (1942) report only slight effects on molluscs (which was critical, given their targeted application), but also note that quicklime has severe effects on crabs (but not barnacles or prawns), larval crustaceans, fish eggs and adult flatfish (bothids?). It would also effect all other echinoderms. Birkeland & Lucas (1990) noted that the quicklime trial against *A. planci* also killed 10-20% of living coral, which is hardly surprising given their carbonate skeletons.

Effects of quicklime on humans could also be significant. Quicklime is a substantial corrosive, and special efforts would be needed to ensure minimum contact with bare skin.

Galtsoff & Loosanoff (1939) report laboratory and field trials with other broadcast chemicals. Apart from quicklime, the most effective was copper sulphate. All were considered unsuitable because of the negative effects on other biota and a low kill rate.

Injected seastar-icides

A prominent and locally effective method to control *Acanthaster planci* has been the injection, by pole-spear, of toxicants into the seastar. Toxicants trialled, and proven to be effective, include formalin (in various concentrations), copper sulphate, hydrochloric acid and ammonia. Of those trialled, copper sulphate was recommended as the safest and easiest to use. Kill rates were usually close to, if not actually 100%, depending upon the toxicant used. Environmental impact of the approach was considered slight. There was no indication that release of the poison following the death of the seastar had any significant impact on local biota.

The principle limitation of the pole-spear approach is the rate at which it can be applied. Birkeland & Lucas (1990) report maximum rates of about 140 *Acanthaster planci* per hour, when seastars were densely packed. More typically, rates are less than 100 per hour. In those cases, total kills were typically from thousands, to tens of thousands, of seastars (Birkeland & Lucas, 1990).

Reproductive inhibitors / stimulants

The reproductive endocrinology of echinoderms has been studied in considerable detail. Japanese biologists, in particular, have published numerous papers on the chemical biology of development of *A. amurensis*. Two classes of compounds could potentially be used against the seastars.

First, pheromones stimulate release of gametes in *Asterias forbesi* and presumably synchronise spawning activity (Miller, 1989). A similar compound is likely in *A. amurensis*. Whether or not such a compound is species-specific has not been determined. In principle, such a compound could be used to disrupt the natural spawning behaviour and synchronicity in *A. amurensis*, reducing its fertilisation success and perhaps leading to a decline in its abundance.

Second, several compounds have been identified that inhibit reproduction by *A. amurensis* (Ikegami, *et al.*, 1972; Nemoto & Ishida, 1983). One group, the asterosaponins, are apparently species-specific (see comments in McLoughlin & Bax, 1993). Again, in principle, dispersal of such compounds into the environment, either in water-soluable form or in the diets of the seastars, could prevent seastars from reproducing, and collapse the population. If the asterosaponins prove species-specific, collateral damage to other species, including native seastars, would be slight.

Application to Asterias amurensis in Australia

From a control perspective, anything that kills a pest is preferable to something that only disables it or reduces its fecundity. Consequently, the apparent ability of lime to kill large numbers of asteroid seastars when broadcast in two dimensional habitats at moderate concentrations is appealing. Potentially, it would be possible to kill a large proportion of the seastars in the Derwent with one or two applications of a relatively cheap compound.

In practice, however, using lime to control seastar numbers in a broadcast sense faces substantial practical problems, not least of which are potential effects on human health and collateral damage to other marine biota. Therefore, it could realistically only be used in areas of exceptional significance to the seastar (critical nursery areas?) and under rigidly controlled conditions to small habitats. Given potential health effects, even this is likely to prove politically unacceptable, unless there were extraordinary benefits attributed to its use.

Pole-spear injection of toxicants into seastars in the Derwent estuary was suggested as a potential control measure by the National Seastar Task Force, but not followed up because of the cost and the impracticality of spearing millions of seastars. These remain formidable obstacles to its implementation except for tactical removal of seastars in high value areas (marine parks?, near mariculture operations). The techniques, which were well developed for use against *A. planci*, might be of value in containing incursions of the seastar into new locales, where removing a small number of seastars could have a substantial effect on population viability.

The most promising use of chemicals to control seastars is likely to involve their reproductive endocrinology. The possibility of species-specific compounds that inhibit reproduction in *A. amurensis* was noted by the National Seastar Task Force, but largely dismissed as the basis for a practical control option because of the difficulties in delivering high enough doses in the Derwent estuary to effectively disrupt seastar reproduction. However, it could be possible to deliver these doses using other than water soluble compounds. For example, lacing prey items or artificial baits with species-specific reproductive inhibitors could disrupt reproduction of the seastars with few environmental side effects. Genetic technology also offers the possibility of breeding prey items (small, non-commercial bivalves) that themselves produce compounds that inhibit seastar reproduction (by splicing the appropriate genetic code from the seastar into the prey species). Such technology would require substantial discussion and widespread support before application, but could constitute a means of reducing the reproductive potential of the seastar over its entire range.

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Comments

There was little support for the broadcast application of quicklime to kill seastars. Most participants felt the adverse effect on the environment did not warrant its use. The injection of seastars with chemicals *in situ,* and leaving many seastar carcasses on the sea floor, was also questioned. Seastars can be composted (Line, M.A. 1994. Composting of seastar (*Asterias amurensis*) wastes. Dept of Agricultural Science, University of Tasmania, Hobart), but this is unlikely to provide a long-term solution.

The possibility of using quicklime slurry to kill seastar larvae and juveniles on mussel ropes was raised. The use of fresh water was preferred because it would be less expensive and toxic to the environment. However, the tolerance of juvenile seastars to freshwater is unknown.

OPTIONS FOR BIOLOGICAL CONTROL OF *ASTERIAS AMURENSIS*

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Introduction

Biological control has been used successfully against agricultural pests for over 100 years. In the terrestrial environment, it is usually combined with physical removal and chemical control to reduce pest numbers. This is termed Integrated Pest Management (IPM). In the oceans, however, the prospects for IPM of marine pests are severely restricted because biological and chemical control methods have not been thoroughly investigated. Indeed, biological control of marine pests is in its infancy. Furthermore, the application of biological control agents in the oceans is likely to differ from their use in agriculture. Biological control agents used against marine pests would require higher levels of safety and lower levels of efficacy than those used against agricultural pests (see Kuris, 1997, for rationale).

I am investigating the options for biological control of the seastar *Asterias amurensis* to enable integrated management of this marine pest. For effective biological control of agricultural or marine pests, a parasite must effect the fecundity, fitness or survival of its host. However, few parasites are reported which deleteriously effect asteroid seastars (Jangoux, 1990). Below, I list parasites which may be effective as biological control agents of *A. amurensis*. I have not included viruses or bacteria because of the problems with containment of these organisms. To date, much of my work has focussed on the ciliate *Orchitophrya stellarum* as a possible biological control agent. I review the results of my research to date.

Ciliate

The scuticociliate *Orchitophrya stellarum* is being investigated as the most likely candidate for biological control of *A. amurensis* because it castrates these seastars in Japan (Byrne *et al.*, 1997) and is associated with mortalities of *Pisaster ochraceus* (Leighton *et al.*, 1991). However, the same species of ciliate has been described from four genera and seven species of asteroid seastars from around the world (although it has not been found in over 2000 *A. amurensis* nor 400 native asteroids from Tasmania, Goggin, unpublished data). This indicated that *O. stellarum* was not host specific and therefore would not be useful for biological control of introduced populations of *A. amurensis* because it could infect Australian native seastars. I used morphological and molecular data as well as experimental infections to determine the host specificity of isolates of *O. stellarum* from four seastar hosts from around the world.

TAXONOMY

The taxonomy of scuticociliates is based primarily on oral structures and the pattern of insertion of body ciliature (somatic kinetids). We compared these structures between isolates of *O. stellarum* collected from *Asterias amurensis* (Hokkaido, Japan), *Asterias rubens* (Europe), *Asterias vulgaris* (Prince Edward Island, Canada) and *Pisaster ochraceus* (Vancouver, Canada). Our results are not yet finalised but preliminary work has found no variation between these isolates. This indicates that these features may not be sufficient to

distinguish between isolates of *O. stellarum* (Goggin & Morado, in prep). The length of these four isolates, taken direct from testes, did not differ significantly. It appears that length may not be taxonomically useful because it decreased significantly with time in seawater culture.

Molecular data (495bp) from the Internal Transcribed Spacers 1 (ITS1) and 2 (ITS2) from the ribosomal RNA (rRNA) gene cluster were collected from ciliates isolated from the four seastar hosts listed above. This region has been used extensively to discriminate protozoans, trematodes, and insects (see Goggin & Newman, 1996). The ITS are subjected to concerted evolution which tends to homogenize sequences among individuals and among populations and enhances their ability to diagnose species. There were no differences in the nucleotide sequence from the ITS regions of these four isolates. It may be possible to discriminate these isolates using molecular data from another region of the genome; we have begun investigating the use of RAPDs of whole genomic DNA for this purpose.

Therefore, neither morphological nor molecular data could adequately distinguish isolates of *O. stellarum* from *A. rubens*, *A. amurensis*, *A. vulgaris* and *P. ochraceus*, suggesting that these isolates belong to a single species.

EXPERIMENTAL INFECTIONS

Preliminary experimental infections were conducted in Japan using seastars imported from Australia (native asteroids *Coscinasterias muricata* and *Uniophora granifera* and the introduced seastar *Asterias amurensis*). In these experiments, *O. stellarum* infected *A. amurensis* from Australia in 12 days (when placed in a ciliate 'bath') and *A. amurensis* and *U. granifera* in ten and 11 days respectively (when injected into the gonad). However, whether *U. granifera* could become infected via the normal route (rather than by injection) and would sustain an infection is unknown.

HOST SPECIFICITY IN JAPAN

Orchitophrya stellarum has not been found in the gonads of 15 other asteroid species in Japan (n=231) (Goggin, unpublished). This apparent host specificity is surprising given that *O. stellarum* is recorded from six asteroid species from around the world and four of these isolates could not be discriminated by morphological and molecular data. Sample sizes may need to be increased to find the ciliate in other asteroid species in Japan. *Orchitophrya stellarum* survived and increased in size when fed sperm from *Distolasterias nipon* and *Uniophora granifera*, therefore the sperm from seastars, which are not naturally infected with *O. stellarum*, is not toxic to the ciliates. The mechanism which prevents infection of these seastars, therefore is unclear. It is possible that ecological (?habitat preference) or morphological (?genital pore position) differences could prevent invasion. Further surveys for *O. stellarum* in other asteroids are needed to confirm this apparent host specificity in Japan.

EFFECT ON THE HOST

The ciliate is not usually found in all ten testes in an individual *A. amurensis* (Goggin & Bouland, 1997). Therefore, natural infections do not completely castrate this host: sperm output from an infected host would be reduced, but it is unknown what level of infection is required to reduce recruitment of seastars to the population.

Infection by *O. stellarum* does not kill every *A. amurensis*. Infected seastars in Japan were found in all size classes (Goggin, unpublished data). Furthermore, a single *A.amurensis*, which was infected, survived and was uninfected when biopsied several months later, after it had spawned. To compare the survival of infected and uninfected seastars, other *A. amurensis* need to be tagged and their survival monitored.

INFECTION PROGRESS

Orchitophrya stellarum (identified from nucleotide sequence data) was collected free-living in Usujiri harbour, Japan (using ciliate traps). The ciliate, isolated from seastar gonads, also survived up to 50 days in seawater culture. Therefore, *O. stellarum* is an opportunistic and not an obligate parasite. It probably survives free-living in the ocean between reproductive cycles of the seastars, invading the gonads via the genital pore when gametogenesis commences and multiplying as gametogenesis progresses and the gonads increase in size. The ciliate is probably flushed from the gonads when the seastars spawn and survives free-living on bacteria until gametogenesis recommences. This hypothesis would explain the prevalence of *O. stellarum* in *A. amurensis* which changes with the reproductive cycle of the seastar, increasing with gametogenesis and declining after the seastar spawns and the testes regress (Fig. 8). It would also explain the incomplete infection of all gonads in a single seastar.

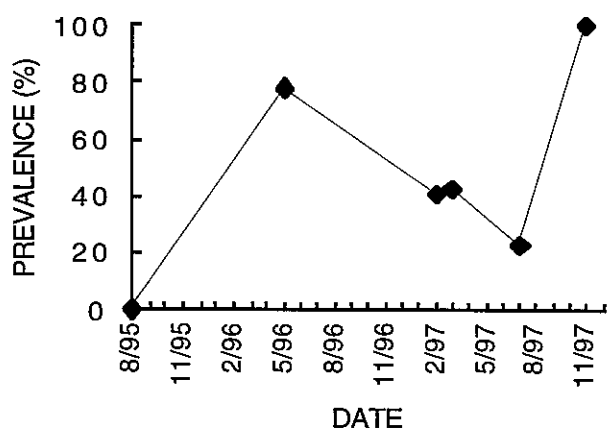

Figure 8. Prevalence of *Orchitophrya stellarum* in male *Asterias amurensis* from a site 30m outside Usujiri harbour, Japan in 5m depth on various dates (data from August 1995 from Kuris *et al.*, 1996, February 1996 from Goggin & Bouland, 1997, other data from Goggin, unpublished)

FUTURE RESEARCH

Morphological and molecular data indicate that the same species of *Orchitophrya* is found in seastars around the globe: *Asterias rubens* (Europe), *Asterias amurensis* (Japan) *Asterias vulgaris* (Prince Edward Island, Canada) and *Pisaster ochraceus* (British Columbia, Canada). Therefore, the host specificity of this ciliate appears to be low. It is possible that molecular data from other regions of the genome (other than the ribosomal RNA gene cluster) could discriminate these isolates, thereby indicating a higher host specificity of these ciliates. However, the final test of its host specificity will lie in experimental trials. In preliminary trials, a single Australian native seastar *Uniophora granifera* became infected when injected with the ciliate. Whether this seastar could become infected by the natural

route (i.e. via the genital pore) and would sustain an infection is unknown. Further trials, for longer and with more seastars, are needed to determine whether this is the case. However, the ciliate has only been found in a single host species in Japan which suggests high host specificity for *O. stellarum*. This may be due to ecological or morphological barriers which could protect Australian seastars from infection in the field. Further experimental trials are required to determine the infectivity of the ciliate to Australian native seastars and the reason for refractivity of some native Japanese seastars to infection

The lack of infection in all testes of *A. amurensis* and the ability of a single seastar to recover from infection suggests that *O. stellarum* in its native envoronment may not effect the reproduction or fitness of the northern Pacific seastar sufficiently to control the population. Disease progress and the survival of infected seastars needs to be monitored.

Therefore, the ability of *O. stellarum* to reduce the numbers of *A. amurensis* in Australian waters is not clear. The ciliate does not appear to be an obligate parasite and may infect seastars in Australia which occupy a similar ecological niche to *A. amurensis*. It is possible that markers can be found which discriminate the ciliates found in different hosts around the world, indicating a higher host specificity than currently suggested. It is surprising that ciliates from separate oceans share genetic sequence in the ITS regions. Perhaps this is because *O. stellarum* has been introduced into the Pacific Ocean. If this were the case, then its effect on populations of *A. amurensis* in Japan should be monitored - this would indicate the likely impact of the ciliate if it were introduced into Australia as a biological control agent.

Sporozoan

In 1985, crown-of-thorns seastars *Acanthaster planci* were found dying on reefs near Fiji. The seastars had eroded stomachs, atrophy of the pyloric caecae and ulceration of the aboral body wall of the central disc. An intracellular protozoan (possibly a sporozoan) was associated with degenerative change in the pyloric caecum of these seastars (Birkeland & Lucas, 1990). However, it was not established whether the protozoan was the primary cause of disease because the outbreak was coincident with starvation of the juvenile seastars. Further mortalities of crown-of-thorns seastars have not been reported. This is one of the few (?the only) mass mortalities recorded for asteroid seastars that may have been caused by a pathogenic agent, therefore, the agent needs to be identified.

Dendrogastrids (Crustacea: Ascothoracida)

Dendrogastrids are highly modifed intracoelomic parasites of seastars. There are about 25 species described from around the world, some of which castrate infected seastars e.g. dendrogastrids in *Zoroaster fulgens* and *Bathyaster vexillifer* (Tyler & Pain, 1982; Tyler *et al.*, 1984). In Tasmania, *Dendrogaster tasmaniensis* is found in the seastar *Allostichaster polyplax*, causing an aboral swelling but no other effect (Hickman, 1959). No dendrogastrids have been found in *A. amurensis* in Japan despite some considerable effort (Kuris *et al.*, 1996). It is possible dendrogastrids may be found in populations of *A. amurensis* from China, Korea or Alaska.

Eulimid Gastropods

Eulimid gastropods are usually ectoparasites of echinoderms and can occasionally cause castration of the host. They can also produce lesions on the test and suppress the automizing ability of the arm which they parasitise. *Parvioris astropectenicola* is an ectoparasite of *A. amurensis* from Tokyo Bay (Waren, 1981), however, its ability to reduce seastar populations is likely to be limited.

Conclusions

The ciliate *O. stellarum* remains the most likely candidate for biological control of *A. amurensis*. However, its host specificity and ability to reduce populations of *A. amurensis* in Australian waters is doubtful.

Thorough searches of *A. amurensis* for parasites have not been undertaken outside Japan. Therefore, it is possible that seastars from Alaska, China or Korea could harbour parasites that have not yet been discovered and have potential for biological control of *A. amurensis* in Australian waters. Searching at the edges of the native range may increase the chances of finding parasites that could be useful for biological control. My intention is to target populations of seastars at these sites to search for possible control agents.

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Comments

The importation of exotic parasites as control agents for *A. amurensis* was considered less attractive than the use of native parasites from native Australian echinoderms. Therefore, it was recommended that native echinoderms be thoroughly checked for possible control agents. Louise Goggin has checked over 400 native seastars for parasites and found only the turbellarians *Acholades asteris* encysted in the feet of *Coscinasterias muricata*, *Marcusiella pallida* in the gut of the sea urchins *Heliocidaris erythrogramma* and *Amblyneustes ovum* and the dendrogastrid *Dendrogaster tasmaniensis* in the coelomic cavity of *Allostichaster polyplax*. These turbellarians are unlikely to be useful as biological control agents for *A. amurensis*. The ability of the dendrogastrid to infect *A. amurensis* and to decrease its population size is unclear.

The ability of a biological control agent to limit seastar numbers was questioned. A biological control agent would not eradicate pest populations but reduce their numbers to manageable levels. It would remain in the pest population and limit further incursions of the pest. Infection by some natural enemies may need to be artificially augmented to effectively control some pests.

The ability of a parasite, found in *A. amurensis* at the edges of their native range, to impact *A. amurensis* in Australian waters, was queried. The edges of the native range of an animal are likely to be set by several factors including environmental tolerance and parasite load. At the edges of its native range, parasites may transfer from their usual hosts to the pest. These parasites could function as effective biological control agents in very dense seastar populations.

POTENTIAL MOLECULAR APPROACHES TO THE ENVIRONMENTALLY BENIGN MANAGEMENT OF THE NORTHERN PACIFIC SEASTAR

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Conventional methods for managing pest populations include physical removal, selective (preferably) application of toxicants and poisons, and use of natural predators or parasites to reduce pest numbers and impacts (classic biological control). For species like the northern Pacific seastar, physical eradication and local application of toxicants are likely to be of value only in localised areas or for specific short-term control (i.e., protecting commercial leases) or, possibly, at the early stages of an invasion into new locales (Port Phillip Bay?). The sheer number of individuals in the Derwent Estuary, however, makes such techniques unsuitable for attempting to control the population as a whole. Rather, a broad-acting, more cost-effective approach is called for. At the moment, this equates with some form of biological control.

To date, biological control of pest populations is carried out using some variant of natural or classical biological control. This involves searching for, testing the safety and effectiveness of, and then releasing (should a 'winner' pass the requisite tests) a natural pathogen or predator to attempt to reduce pest numbers. Despite a few conspicuous failures of this technique, most of which pre-date modern approaches (eg., cane toads and mongoose), classical biological control has a very impressive safety record, with some outstanding success stories (prickly pears and rabbits, as examples).

For the approach to work, however, one needs to find a suitable, ideally species-specific parasite or pathogen, which has a substantial impact on the pest population when released. In many cases, suitable natural control agents are either not available or not obvious. An alternative approach to biological control, which is developing rapidly, by-passes this stage and instead applies modern genetic technology to either modify the pest population itself and render it more manageable or to create highly targeted control approaches using, for example, the prey species taken by the pest. Although still in early stages of development, a recent national conference on the future of pest management in Australia (1998, Australian Academy of Sciences, Canberra) was dominated by talks on the apparent future and state of development of molecular approaches. CSIRO, among other organisations, is investing substantially in developing this technology. Although applications to date are almost entirely directed at terrestrial and agricultural objectives, there is no reason why similar approaches would not also apply to control of marine pests.

In brief, three broadly different approaches have been suggested that could be applied to the seastar.

Inducible Fatality

The inducible fatality approach is based on the development of a transgene that combines a lethal element with an inducible promoter. The latter inhibits the expression of the former

until explicitly triggered by a special compound provided in, for example, artificial baits or delivered by an otherwise benign viral disease. When triggered, the gene is immediately and invariably fatal. A number of suitable lethal genes and inducible promoters are well known in the scientific literature and readily available, but have not been combined or applied to this class of problem. In essence, these techniques can be used to breed into a target population susceptibility to a substance that is otherwise harmless, that is, a species-specific poison that does no collateral damage to other species in the environment. The basic approach is detailed by Grewe (1996), while the dynamics of transgene introgression is modelled by Davis & Fulford (in press).

To control seastar populations, the transgenic construct could be inserted into the embryos, using standard techniques, which would then be reared, bred en masse and stocked into the wild. The stocked animals would breed with the feral ones, with the result that the IFG would introgress into the wild population at a rate dependent on stocking densities and strategies and number of IFGs per genome. Modelling studies indicate introgression rates of 80-90% could be achieved in as little as four or five generations (which in seastars would equate to about 10-15 years). When the introgression level was considered high enough, the lethal gene would be activated by, for example, spreading bait containing the promoter substance, producing a mass die-off. Animals not containing the IFG (other species, for example) would get a free feed, but otherwise be unaffected by the promoter. Alternatively, the promoter process could be triggered by unusual environmental conditions, which reduces the need to distribute a promoter substance, but which also results in less control of the introgression process.

Repressible sterility

'Repressible sterility' is simple in concept, and uses techniques well established in molecular science, though not previously combined for this purpose. Using methods such as subtractive hybridisation and analysis of expressed sequence tags, genes are located that are activated only during embryonic development and/or gametogenesis and are crucial to it. Using one of these crucial genes as a template, DNA constructs are built and inserted into the target genomes. The DNA construct is composed of a blocker (that is, something that blocks the expression of the targeted genetic sequence) and a genetic switch to control its function. In captivity, the blocker can be inactivated using a repressor protein (via diet or in soluble form), so that reproduction and development occur as normal. In the wild, however, where the repressor is not available, the blocker remains active and disrupts the function of the critical gene, leading to sterility or death, depending upon the gene selected. The key is the development of animals which are easy to breed in captivity (where the repressor is present), but which produce non-viable offspring in the field. CSIRO is currently involved in a multi-year, multi-divisional effort to develop this technology, with a applications currently targeted at zebrafish (as a carp equivalent), Pacific oysters and mice.

The principle objective of this technology is to produce animals that can breed in captivity, but are incapable of forming new feral populations if they escape. However, logically it can also be used to control existing exotic pests, in two ways. First, it would be possible to breed and release massive numbers of the equivalents of "sterile male fruit flies", that is, males (and females) which reproduce as normal, but which produce non-viable eggs and larvae. This technique, using irradiated flies, is standard practice in managing fruit fly outbreaks, in which large amounts of the reproductive output of the female flies is wasted because of the male sterility. In theory, and depending upon their fertilisation dynamics, a similar

approach could be used against the seastars and might lead to a substantial reduction in their annual reproductive success.

The second approach relies on targeting the fatal flaw at a later stage in development (e.g. sexual maturation). The logic is to produce individuals that use up resources otherwise available to the wild types, but that themselves do not contribute to adult numbers. We suspect that in seastars (like most high fecundity pests) annual recruitment is likely to be determined principally by survival during the juvenile stage, which in turn depends on the resources available in nursery areas. Because resources are limited, only a set number (N) of juveniles survive, irrespective of how many eggs are spawned. N varies annually depending on environmental conditions, but is always low relative to fecundity.

Using repressible sterility, we could routinely produce large numbers of juveniles that are competitively superior (and hence dominate N each year), but then die after out-competing wild type juveniles. The nett effect is a decrease in recruitment to the adult population, the size of the decrement a function of the number of transgenic juveniles stocked and their competitive ability relative to the wild types. Increased competitiveness could be genetic (growth enhancers), phenotypic (hatchery-reared juveniles released larger than their wild relatives) or temporal (juveniles released before the wild fish hatch, so that nursery habitats are fully occupied when the latter arrive or, even better, the transgenic individuals consume their later arriving wild relatives). The competitively superior, but flawed juveniles could be stocked out directly each year (bred from repressibly sterile adults).

This basic approach is conceptually similar to virally-vectored immunocontraception, a molecular technique being developed by CSIRO Division of Wildlife and Ecology, for control of mice, foxes and other low fecundity mammals. In these low fecundity species, analyses indicate that reducing litter size and breeding rate can significantly reduce population sizes. In a highly fecund species like the seastar, the objective is the same, but the target is reducing survival through the juvenile stage.

We have tested the concept of a repressible sterility gene as a pest management option using a simple, but realistically parameterised, model of a carp population (another high fecundity species). Results indicate that carp populations begin a slow, but irreversible decline once the flawed juveniles constitute more than 75% of N. The rate of decline is determined by the natural mortality rate of the adults, and hence can be accelerated by, for example, a targeted fishery. The models suggest that, given a consistent sterilisation rate in excess of 75%, the targeted populations eventually drop to very low levels.

This application of repressible sterility technology requires good knowledge of the population dynamics of the targeted population and an ability to produce repressibly sterile juveniles in large numbers. It is practical and cost-effective, however, once the repressible sterility technology is available.

Prey or parasite-vectored reproductive inhibition

Recent work in Florida on control of mosquitoes has suggested that a physiological inhibitor can be bred into the algae on which the mosquitoes feed, which effectively causes them to starve to death despite abundant food supplies. This concept, though not the specific approach, could also be applied to seastars. In particular, work in Japan report a number of compounds inhibit spawning of *A. amurensis* (Ikegami *et al.*, 1972; Nemoto &

Ishida, 1983). A group of these compounds, known as asterosaponins, appear to be species-species (McLoughlin & Bax, 1993). The difficulty in using these compounds directly against the seastars is that of producing sufficiently large amounts of it to affect the Derwent population. An alternative approach is to insert the gene sequence for this inhibitor into a prey item favoured by the seastar (small, non-commercial bivalves?) or a parasite that targets the seastar, and then release this altered organism into the Derwent. By consuming these prey, the seastars maintain high levels of reproductive inhibitors, which effectively sterilises them. The risks of permanently introducing the transgene into the native prey populations could be reduced, if not eliminated by 1) using sterile feral technology on the released prey, 2) aiming for episomal gene transmission, which is silenced after a few generations, and 3) mass-transforming late stage embryonic or larval prey, so that the transgene is incorporated into the somatic tissue, rather than the germ line.

In theory, this approach could largely eliminate seastars from the Derwent in a single seastar generation, with no mass die-offs and no collateral damage to other organisms, including other echinoderms, in the system.

Critical issues and research needs

In theory, any one of these three approaches has the potential to lead to the environmentally benign (i.e. little or no impact on other species) decline and possible eradication of the northern Pacific seastar in Australia. Of the three, prey-vectored reproductive inhibition appears to be the most appealing, as it has a direct effect on the target population and relies least on uncertainties of the biology underpinning regulation of seastar numbers. In all three cases, however, ground breaking technology is being applied. The central issues with any of these technologies are 1) the desirability and public and political acceptability of using transgenic technology for solving an environmental problem, and 2) development of the appropriate technology.

Regarding the former, there is an understandable sense of public un-ease about applications of transgenic technology, fanned by sensationalist media reporting of cloning humans, and the like. This will almost certainly translate into political unwillingness to take chances with these approaches. In their favour, however, the regulatory environment regarding genetically modified organisms is changing rapidly in Australia, and the issue is being currently discussed extensively at state and federal government levels. The perceived benefits of transgenic technologies, in terms of environmental remediation and enhancement of production, may make it difficult to resist its selective and well regulated use. In the case of the seastar, if this technology can be shown to lead to solving a major environmental problem, with no long term consequences either to the native communities or to human health or use of the environment, then it may become an acceptable option. In any case, extensive public and political consultation would be essential prior to any release. Hence, the current issue becomes one of evaluating and possibly developing options for decision makers, rather than forcing the decisions themselves.

The second issue is the state of the technology. Two different components are required here: good information on the genetics of *A. amurensis*, which could be used to create effective genetic blockers and/or reproductive inhibitors, and an ability to transform either the seastar itself or, in the case of the prey or parasite-mediated approaches, the prey or parasite. Regarding the former, the developmental genetics and biochemistry of seastars are extraordinarily well studied, though reported in literature almost impenetrable by marine

ecologists. It is highly likely that suitable genetic sequences have already been identified, or could be by using standard techniques (e.g. EST analysis).

With regard to transgenic technology, thus far little work appears to have been done on seastars. A number of the precursor steps have been developed however, such as development of culture techniques for cell lines (e.g. Kaneho *et al.*, 1995). In contrast, considerable work has been done on the related sea urchins (Herrera, 1998), most of which would almost certainly be directly transportable to seastars. Integration rates of foreign DNA in urchins are still low, but as in most groups, techniques are improving rapidly. With specific reference to genetic transformation of potential bivalve prey that could carry reproductive inhibitors, work is currently underway overseas (e.g. Gomez-Chiarri *et al.*, 1998) and at the CSIRO to develop transgenic techniques for oysters. Initial results are promising, and it is highly likely that the same techniques could be applied to native Australian species.

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Comments

Some participants were nervous about the use of transgenic technology. The legality of its use was also queried.

Asterosaponins may only need to be delivered for one month of the year to prevent reproduction in *A. amurensis*. These compounds are normally in high levels in the seastars

and the levels drop just prior to spawning. However, whether asterosaponins would be destroyed if they were ingested by the seastars is unknown and would need to be determined.

It is possible to use a parasite or genetically-engineered prey item to deliver a species-specific "poison" to the seastar. A non-commercial bivalve, which is a preferred food item (e.g. *Electroma* sp.), could be genetically engineered to continually produce and deliver the "poison". These bivalves would not necessarily need to be alive when fed to seastars.

It was recommended that a review of the literature be undertaken to seek species-specific compounds that could be useful for transgenic techniques.

4. Summary of discussion

SUMMARY OF DISCUSSION

Currently, there are few methods used to limit the numbers and spread of *Asterias amurensis* although several novel methods were suggested at the meeting. It was decided that the utility of any control method for the seastar would be determined by its effectiveness, safety, practicality and social and political acceptability: a control method must be both socially and politically acceptable to be useful. Therefore, we used these criteria to screen possible control options and determine which ones warranted further research. We considered physical control, chemical control, environmental management, biological control, genetic control and education (Table 7).

Table 7. Methods considered as control options for *Asterias amurensis* with their perceived effectiveness (EFFECTIVE), safety (SAFE) to both the environment (ENVMT) and humans (HUMAN), practicality and faesability (PRACT), social (SOCIAL) and political (POLIT) acceptability and the likely time frame required prior to their instigation (S, short-term; M, medium-term; L, long-term)

METHOD	EFFECTIVE	SAFE		PRACT	SOCIAL	POLIT	TIME
		ENVMT	HUMAN				
PHYSICAL CONTROL							
Trapping	small scale chronic	✓	✓	✓	✓	✓	S
Hand collection	small scale sporadic	✓	?shallow	✓	✓	✓	S
Dredging	medium/small scale high densities	✖	✓	✓	✖	✖	S
Mopping	medium scale high densities	✖ ?	✓	✓	?	?	S
Barrier	small scale	✓	✓	✓	✓	✓	S
CHEMICAL CONTROL							
Broadcast	medium scale	✖	?	✓	✖	✖	S
Injection	small scale	✓	✓	✓?	✓	✓	S
Barrier	small scale (farm)	✓	✓	?	?	✖	S
Dipping	farm	✓	✓	?	✓	✓	S
Poison	?	?	?	? ✖	✖	? ✖	S
ENVIRONMENTAL CONTROL							
Strategic-rehabilitate	?	✓	✓	?	✓	?✓	LL
Strategic - spread	?	✓	✓	?	✓	✓	LL
Tactical	✖ ?	✓	✓	✓	✓	✓	L
Manipulation of inputs	?	✓	✓	?	✓	?	S
BIOCONTROL							
Native predator	??	✖ ?	✓	?	✓	✓	L
Exotic predator	??	✖	✓	?	✖	✖	L
Native parasite	✓?	?	✓	?	✓	✓	L
Exotic parasite	✓?	✓	✓	✓	✖ ?	✖ ?	LL
GENETIC CONTROL							
Inducible fatality gene	✓	✓	✓	?	?	?	LLL
Repressible sterility	✓	✓	✓	?	?	?	LLL
Reproductive suppression	✓	?	?	✓?	??	??	LL
EDUCATION	selected areas	✓	✓	✓	✓	✓	S

Physical control

At present, physical removal is one of the few methods used to limit *A. amurensis*. Trapping or hand collection can effectively and rapidly remove small numbers of seastars in localised areas, possibly preventing the need to remove massive numbers of seastars at a later date. However, it may not be feasible to remove large numbers of seastars, or seastars spread over large areas, by trap or hand: hand collection is limited to water less than 12m depth. Dredging was not considered practical in the Derwent estuary because it would resuspend heavy metals in the sediments (Coughanowr, C. 1997. *State of the Derwent estuary: a review of environmental quality data to 1997.* Supervising Scientist Report 129, Supervising scientist, Canberra). Furthermore, the impact of "mopping" on sediments was not clear.

Large numbers of seastars could be composted on land. Line (1994, Composting of seastar (*Asterias amurensis*) wastes. Dept of Agricultural Science, University of Tasmania, Hobart) found that seastar compost performed well in plant growth trials although it was unsuitable as a constituent of potting mix. Line (1994) also suggested that "seastar compost would be very suitable for retail as a mulch of good nutrient and water holding status". Tas Crays Pty Ltd market a similar product composed of fish waste composted with bark.

Physical barriers are being designed to prevent adult seastars invading marine farms. Jeff Ross trialled a number of physical barriers and found a lip of copper mesh prevented escape of juvenile *A. amurensis* from cages. A lip of nylon netting on the cages contained adult seastars.

Chemical control

The broadcast application of chemicals and the use of chemical barriers was not considered socially or politically acceptable. Injecting seastars with chemicals was also not considered an effective or efficient method to control seastars because it would only be possible on a small scale and in shallow waters (less than 12m). Leaving large numbers of injected seastars to rot on the sea floor was not considered acceptable: it might also attract other seastars to feed.

There is a significant risk of spreading *A. amurensis* by translocating mussel ropes infected with juvenile seastars. Quicklime slurry could kill seastar larvae on mussel ropes, although the dose required and its effect on juvenile mussels is unknown. Quicklime may be more effective than freshwater although it would be more expensive and more toxic to the environment.

Environmental management

It is critical to determine whether *A. amurensis* drives or tracks the system in which it occurs i.e. whether the high seastar densities in the Derwent estuary are a partial cause, or a symptom, of a degraded system. Until this is known, it is unclear whether rehabilitation of the environment will reduce seastar numbers and where management effort would most effectively be focussed. If the seastar drives community dynamics i.e. is a key threatening process, then control and management need to focus on the seastar directly: rehabilitating other components of the system will not alleviate the problem. However, if *A. amurensis* tracks system dynamics (a much less threatening scenario), then it may be more sensible to focus on managing the processes that the seastar is tracking. Current evidence indicates that

A. amurensis tracks system dynamics.

Processes which prevent the further spread of *A. amurensis* may not be the same as those governing rehabilitation of areas already impacted by the seastar. For example, preliminary evidence suggests that high environmental quality will maintain native predators. These predators feed on juvenile seastars and may prevent their further spread. However, it is unclear whether these predators could decrease existing populations of seastars in the Derwent estuary. It is also unknown whether a decrease in larval and juvenile survival (due to predation) would affect adult seastar densities in the Derwent estuary. Furthermore, it is unknown whether a decrease of anthropogenically-derived nutrients in the Derwent estuary (from sewage treatment plants and land run-off) would lower phytoplankton biomass and hence decrease survival of larval seastars. These questions can be addressed by experiment.

The main conclusion was that we still know too little about the biology of the seastar to identify its critical links to the environment. Nontheless, rehabilitation of the Derwent estuary was considered a valuable objective in its own right, even though it may be of limited value as a control option.

Biological control

There was much interest in the use of native predators to control *Asterias amurensis*. This was considered more socially and politically acceptable than importing exotic organisms. The seastar *Coscinasterias muricata* was considered most likely to affect populations of adult *A. amurensis*. *Coscinasterias muricata* eat adult *A. amurensis* in the field, although it probably competes with *A. amurensis* for food.

Some participants queried whether gill netting in Tasmanian waters kills native predators which would otherwise feed on larval seastars. It was suggested that predation of juvenile *A. amurensis* is higher in disturbed areas than in pristine areas. This will be addressed by Jeff Ross during work for his PhD. If there are differences in predation rates between disturbed and pristine sites then the most effective method to limit *A. amurensis* may be to enhance the ecosystem rather than seek control strategies against the seastar.

The use of exotic parasites for biological control were considered less politically and socially acceptable than the use of native parasites. Few participants agreed with the introduction of a new exotic species to resolve the problem of the introduced pest. Exotic parasites would have to pass rigorous safety tests. However, results from agricultural biological control have found that exotic control agents are often most effective against an introduced pest. Therefore, the continuation of investigations into biological control agents from the native range of *A. amurensis* was considered worthwhile.

Genetic control

Genetic methods could potentially eradicate the seastars and are inherently safe because they are species-specific. More research and assessment is required before the political and social acceptability of these methods can be established. It is unknown whether a species-specific asterosaponin (which, in theory would inhibit meioisis in *A. amurensis*) could be delivered by bait. The asterosaponin may be destroyed if it was ingested by the seastars. Ingestion was considered the most effective method to deliver a poison. Therefore, it was recommended that a literature review is undertaken to seek a species-specific compound that could be ingested.

Genetic methods were recognised as long-term strategies to control *A. amurensis*.

Education

Seastars aggregate on food and disperse when it is unavailable. When seastars disperse, their fertilisation success declines which may have a dramatic impact on population densities. Therefore, point sources of food cause seastars to aggregate, can increase the condition of the gonad, improve their fertilisation success and maintain high seastar densities. Food sources include mussels and oysters scraped from the hulls of boats at marinas, or dropped under marine farms, and fish scraps dumped overboard by commercial and recreational fishers. The public and marine industries need to be educated about the significance of food to seastars.

The public could also provide information on new incursions and range extensions of the northern Pacific seastar if they were educated to recognise it. Rapid response to new incursions could prevent the necessity for massive control efforts at a later date.

Commercial harvesting

We did not discuss the possibility of commercial exploitation of seastars during the meeting. However, a financial reward could provide incentive to collect them. For example, in China, *A. amurensis* are sold for about $1US per seastar for human consumption (I am told they are delicious when steamed). Unfortunately, there is no market for seastars for human consumption in Australia.

Dr Lyndon Llewellyn (Australian Institute of Marine Science, Townsville) found no bioactive compounds in *A. amurensis* from the Derwent estuary. Therefore, there is no incentive to harvest them for pharmaceutical products.

Asterias amurensis could be dried and painted and replace painted seastars which are currently imported from Asia and sold in interior decorator shops and markets. However, this is unlikely to significantly reduce densities of seastars in the Derwent estuary nor raise much revenue. Furthermore, it has been suggested that it would trivialise the problem of introduced pests.

Whether the seastars could be harvested for fish meal is unknown: high levels of asterosaponins may reduce their utility for fish meal products. The seastars can be composted and used as mulch (Line, 1994). However, dredges could not be used to collect large numbers of *A. amurensis* from the Derwent estuary because this would resuspend heavy metals in the sediment.

Therefore, a commercial use for *A. amurensis* in Australia seems remote.

Other avenues for research

Other information was considered necessary to enable effective management of *A. amurensis* populations.

- *A. amurensis* larvae are morphologically plastic. Genetic methods provide more accurate identity of the larvae. Rapid genetic methods are needed to accurately identify *A. amurensis* larvae.

- Could *A. amurensis* larvae survive a voyage across the Equator in the cargo holds of vessels which are filled with water for ballast? The fluctuation of water temperatures in cargo holds during such as voyage needs to be determined.

- Are seastars prevented from settling near freshwater inflow? If so, is it salinity or water movement? What is the tolerance of adult seastars to freshwater?

Conclusion

Modern management of agricultural pests combines several control methods to provide Integrated Pest Management (IPM). Similarly, no single method is likely to effectively control *A. amurensis* nor be appropriate for all applications. There are currently few methods available to control of *A. amurensis*. Research is required to identify further control or management options. Population models of *A. amurensis* are needed to target and estimate efficacy of the identified options.

It is unknown which phase of the life cycle of *A. amurensis* should be targeted (or whether *A. amurensis* needs to be targeted directly at all), and what level of control needs to be achieved, to reduce population numbers. For example, if fecundity of each *A. amurensis* was cut by half, would this reduce adult seastar densities? Most fisheries data suggest that reduced adult fecundity does not affect population densities until fecunity is reduced by 50% or more. A population dynamics model of seastars in the Derwent estuary was considered a high priority if it could determine which phase of the life cycle is most vulnerable to manipulation. It was recognised that asteroid seastars are plastic and can decrease their arm length and resorb their gonads in response to starvation. These responses make estimating the age of asteroids using length frequency data problematic. Therefore, extensive monitoring of seastar populations would be needed to provide data for such a model

The possibility of spreading seastars on commercial mussel ropes was considered a major threat, requiring immediate research and education. Therefore, techniques to kill juvenile seastars on commercial mussel ropes and other aquaculture facilities must be investigated. The use of quicklime slurry or freshwater were both considered worth investigating.

The participants agreed that biological control and in particular, the use of native predators warranted further research. It was suggested that more effort be directed to seek parasites in Australian echinoderms that could impact *A. amurensis*. Continuing research on parasites from the native range of *A. amurensis* was also considered worthwhile because results from terrestrial biological control suggest agricultural pests are most effectively controlled by exotic parasites.

It was also thought that habitat rehabilitation was likely to increase the numbers of native predators (and parasites) which could limit *A. amurensis* densities and spread. Habitat rehabilitation was considered to provide many other benefits.

It was agreed that species-specific compounds which could be used in genetic manipulation of seastars should be investigated. Transgenic technology is currently being investigated for control of other feral animals by the CSIRO Divisions of Marine Research, Entomology and Wildlife and Ecology. This research is likely to provide techniques which could be applied to seastars.

In conclusion, we identified five areas where research effort should be directed:

- ascertain the role of *Asterias amurensis* in the community i.e. whether the species drives or tracks the system dynamic. This is funadamental in deciding whether to target the species directly or not.
- minimise spread of seastars on commercial mussel ropes and other aquaculture facilities
- determine the risk and vessels involved in spreading seastar larvae from the Derwent to other Australian ports and develop methods to reduce this risk.
- population dynamics model of *A. amurensis* in the Derwent to determine the most vulnerable life history stages
- seek biological control agents for seastars (in particular native predators and parasites)
- assess species-specific compounds which could be used in genetic manipulation of seastars

5. Participant list

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