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Plankton survey of *Asterias amurensis* larvae in coastal waters of Victoria

Final Report

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Victoria
The Place To Be

Plankton survey of *Asterias amurensis* larvae in coastal waters of Victoria (August - September 2012)

Final Report

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Executive Summary

This report was commissioned to investigate the incidence of northern Pacific seastar (*Asterias amurensis)* larvae in Victorian coastal waters, following the discovery of adult seastars at San Remo (Western Port) in September 2011 and in the Tidal River estuary (Wilsons Promontory National Park) in May 2012.

This study used a combination of plankton surveys and hydrodynamic modelling to examine the distribution of *Asterias* larvae in coastal waters between Port Phillip Bay (PPB) and Port Welshpool in eastern Victoria.

- Zooplankton assemblages were sampled during August - September 2012 using a 90 μm mesh plankton net. The quantity of *Asterias* DNA (larvae) in plankton samples was measured using a genetic probe
- The behaviour of *Asterias* larvae in coastal waters between PPB and Cape Liptrap was modelled using hydrodynamic and dispersion models.

Asterias larvae were detected in coastal waters between PPB and Wilsons Promontory during August - September 2012. This finding is supported by hydrodynamic modelling of buoyant particles simulating the behaviour of *Asterias* larvae exported from PPB. The hydrodynamic and dispersion model estimated larvae could reach the coast off Tidal River in approximately 44 days, well within the larval duration of 79–112 days for *Asterias.*

Asterias larvae were not detected in coastal waters east of Wilsons Prom (i.e. Corner Inlet and Port Welshpool) during August - September 2012. As no major currents connect the west and east coasts of Wilsons Promontory, the absence of larvae along this coastline is consistent with the influence of the prevailing currents in Bass Strait and their role in transporting *Asterias* larvae.

Currents in Bass Strait are primarily influenced by the prevailing wind direction (SW) and tides. Prevailing currents in Bass Strait flow principally eastwards, diverging south at Wilsons Promontory flowing across Bass Strait to the north coast of Tasmania and returning to the Victorian coast off Point Hicks as part of a gyre that dominates central Bass Strait.

Asterias larvae were also detected in Western Port, Andersons Inlet and Tidal River during this survey. The most likely source of larvae in these bays and inlets is the intrusion of larvae from the open coast. Whilst there is good evidence that adult populations of seastars have been eradicated from Andersons Inlet and Tidal River estuary, the presence of adult seastars in Western Port can neither be confirmed nor dismissed based on the results of this survey. This is because the *Asterias* larvae detected in Western Port may have either arrived from PPB or originated from a source population within Western Port. *Asterias* larvae may also be present in Shallow Inlet, but this location was not surveyed during this study.

Overall the pattern of *Asterias* incursions at Andersons Inlet, Western Port and Tidal River appears consistent with natural range expansion via larval dispersal; with PPB as the main source of larvae in the region.

Managers will need to be vigilant to prevent the spread of *Asterias* along the coastline between PPB and Wilsons Promontory. The presence of larvae in coastal waters between PPB and Wilsons Promontory indicates further incursions are likely in Western Port, Andersons Inlet, around Wilsons Promontory, but also possibly in systems where *Asterias* has not previously been recorded (e.g. Shallow Inlet). Protected embayments such as Waratah Bay may also be susceptible, given the presence of larvae at relatively high levels in this region.

We recommend further sampling along the west coast of Victoria between PPB and Cape Otway, and further east along the East Gippsland coast to confirm the outputs of the modelling and the pattern of larval dispersal described in this report. Sampling should also be undertaken in Shallow Inlet, to confirm the presence/absence of larvae in this system.

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Introduction

The northern Pacific seastar (*Asterias amurensis)* is an introduced marine pest currently listed on the CIMPE (Consultative Committee on Introduced Marine Pest Emergencies) trigger list (Murphy and Paini 2010). The seastar is native to the north-west Pacific and is likely to have been first introduced to south-east Tasmania from central Japan via ballast water in the 1980s (Buttermore et al. 1994, Ward and Andrew 1995). Genetic evidence indicates *Asterias* was subsequently introduced into Port Phillip Bay (PPB) from the south-east Tasmania in the mid-1990s, most likely also by ballast water (Murphy and Evans 1998).

Asterias populations in Australia are primarily restricted to the south-east coast of Tasmania, particularly the Derwent River estuary, and PPB. In Victoria adult seastar have also been recorded at Andersons Inlet in 2003, and more recently at San Remo (Western Port) in September 2011 and in the Tidal River estuary, (Wilsons Promontory National Park), in May 2012. Follow up removal efforts and monitoring at all three locations indicates that no viable spawning population survived beyond the year of first detection. It is unclear whether these outbreaks represent a natural range extension of the population in Victoria facilitated by planktonic larval dispersal or are the product of physical translocation of non-larvae (juveniles and adults).

The initial introduction of *Asterias* into Australian coastal waters has been linked to the capacity of its planktonic larvae to remain viable in ballast water for sustained periods (Ward and Andrew 1995). *Asterias* possess long-lived planktotrophic (feeding) larvae that are capable of remaining in the water column for up to 120 days prior to settlement (Bruce et al. 1995).

Asterias spawn small eggs, approximately 150 μm long, that hatch and develop through a series of stages (gastrula, bipinnaria, brachiolaria) before settling out of the plankton and metamorphosing into juvenile seastar. Adult *Asterias* spawn during the winter months and fertilization of eggs and sperm occurs externally in seawater (Byrne et al. 1997). Larval development is strongly temperature dependent and at 12°C (typical of Bass Strait in winter) larval duration is estimated to range from 79–112 days (Bruce et al. 1995). Hydrodynamic modelling indicates *Asterias* larvae may be dispersed over relatively large distances by oceanographic processes in south-eastern Australia (Dunstan and Bax 2007).

Although larval dispersal has been implicated in the establishment of *Asterias* populations along both the Victorian and Tasmanian coasts (Bax and Dunstan 2004, Holliday 2005); no previous surveys of *Asterias* larvae have been undertaken outside of Port Phillip Bay or the Derwent River estuary. This is despite predictions from hydrodynamic modelling that *Asterias* larvae are likely to occur in Bass Strait (Dunstan and Bax 2007).

Information on the distribution on *Asterias* larvae in coastal waters is likely to be important in identifying the link between larval dispersal and establishment of new *Asterias* populations along the Victorian coastline; and the role of other potential vectors (e.g. ballast water or the physical translocation of non-larvae).

Aim of this study

Following the discovery of *Asterias* individuals at San Remo and in Tidal River estuary, the Department of Sustainability and Environment (DSE) commissioned the Department of Primary Industries (DPI) to investigate the incidence of *Asterias* larvae in selected coastal waters in August - September 2012. Parks Victoria (PV) also commissioned DPI to survey the Tidal River estuary during August - September 2012.

The aim of this study was to survey the following regions/locations for the presence and abundance of *Asterias* larvae:

- Port Phillip Bay (PPB)
- Western Port (WP)
- coastal waters between PPB and WP
- coastal waters offshore of Walkerville (South Gippsland) and Tidal River, Wilsons Promontory National Park
- Corner Inlet
- coastal waters adjacent to Barry Beach Marine Terminal and Port Welshpool
- Andersons Inlet, and
- Tidal River estuary

Port Phillip Bay, Western Port and the South Gippsland coast are connected by prevailing

currents (Greer et al. 2008), whereas the ports of Port Welshpool and Barry Beach Marine Terminal in Corner Inlet experience high vessel traffic.

A genetic probe developed by SARDI aquatic sciences (Bott et al. 2010) was used to measure the quantity of *Asterias* DNA in zooplankton samples. This approach is the only reliable method of identifying *Asterias* larvae collected in plankton samples (Bruce et al. 1995, Deagle et al. 2003).

This report

- Presents the results of *Asterias* larval surveys undertaken in August - September 2012
- Presents the results of hydrodynamic modelling simulating the behaviour of *Asterias* larvae in Bass Strait
- Discusses the role of larval dispersal in the establishment of new populations of *Asterias* along the Victorian coastline.

Methods

Plankton sampling

Zooplankton assemblages were sampled during August and September 2012. This corresponds with the period where *Asterias* larvae are most abundant in the water column (Bruce et al. 1995, Bax and Dunstan 2004) and follows the peak in spawning during July - August (Byrne et al. 1997). *Asterias* larvae have previously been recorded from June to the end of October in Port Phillip Bay (Bax and Dunstan 2004). A survey conducted by DPI in PPB and WP on 7–8 December 2011, following the outbreak at San Remo, detected no *Asterias* DNA in the water column at this time.

Zooplankton was sampled at 31 locations (Figure 1) from 1 August to 19 September (Table 1). Samples from PPB were collected on two different occasions, at the start of the survey (1 August) and at the end of the survey (19 September) to confirm the presence of *Asterias* larvae in the water column during this period. *Asterias* DNA was recorded at the beginning and end of the survey (see also results).

Zooplankton assemblages were sampled at two locations in PPB at the beginning of the survey, four locations in WP, four locations between PPB and WP, 10 locations off the coast of Walkerville and Tidal River, seven locations in Corner Inlet and off Port Welshpool, a single location in Tidal River estuary, two locations in Andersons Inlet and a single location in PPB at the end of the survey (Table 1). The location of each site was recorded using GPS in the field.

Zooplankton was sampled using a 90 μm-mesh plankton net (mouth diameter 0.48 m; length 3.25 m, with cod-end jar containing 90 μm-mesh windows). *Asterias* eggs are approximately 105 μm in diameter; larvae vary from 150 μm at the gastrula stage to 4.5 mm at the brachiolaria stage prior to settlement (Kashenko 2005). Zooplankton was sampled at each site using a 5 minute surface-tow from a vessel moving at 2–3 knots: a distance of approximately 400 m depending on tides and currents. This method samples surface waters to a depth of 0.5 m. In WP, Corner Inlet and Tidal River estuary plankton samples were collected at high tide or on outgoing tides with the net towed into the outgoing tide.

Plankton samples were washed into a specimen container and fixed with *RNAlater* (a commercial molecular fixative). Three freeze-dried brine shrimp were added to the samples at the time of collection to assess sample preservation and storage. Samples were stored at <4°C until they were transported, via courier, to SARDI's diagnostic laboratory for analysis.

Cross-contamination between samples was minimised by towing the plankton net without the cod-end for one minute at each site, prior to sampling, to wash contents from net. Potential cross-contamination between field-trips was minimised by soaking the plankton net and cod-end in freshwater for two hours, rinsing the net and allowing to dry prior to use. There was no evidence of cross-contamination between samples or field-trips (see results section).

Fixed zooplankton samples were filtered in the laboratory onto qualitative filter paper (Filtech filter paper grade 1803 (47 mm diameter, Cat No 1803-047)) and stored in 5 ml of *RNAlater*. DNA was extracted from filtered plankton samples using a modified variant of SARDI's Root Disease Testing Service (RDTS) commercial DNA extraction method. DNA from the plankton samples was then analysed using three separate quantitative polymerase chain reaction (qPCR) assays for a) evidence of PCR inhibition, b) brine shrimp quality control for sample quality and c) *Asterias* identification (see Bott et al. 2010). Detection limits for the *Asterias* qPCR assay are approximately 2 femtograms (fg)/ul of target DNA (i.e. 1×10^{-15} g), substantially less than a single larva allowing for variation in DNA extraction rates (Bax et al. 2006).

Results were plotted along the Victorian coastline using ArcView GIS software.

Hydrodynamic modelling

The dispersal of *Asterias* larvae in coastal waters between PPB and Cape Liptrap was examined using hydrodynamic and dispersion models – with PPB as a larval source. The dispersal of buoyant particles, simulating the behaviour of *Asterias* larva in coastal waters, was modelled using an 800 m grid, 8-layer, 3D hydrodynamic and dispersion model (Black and Parry 1999, Jenkins et al. 1999, Lee et al. 2012) that covered the region from Cape Patton to Cape Liptrap

and included the Port Phillip and Western Port embayments. The model was run using data for a typical year (2004) for the period July–October using observed forcing conditions (spring/neap tides, SW wind events, localised wave behaviour, rainfall) for this period.

Hydrodynamic modelling generated information on the circulation patterns at hourly intervals and the dispersion model introduced particles into this flow field at a rate determined by expected larval release (pulse with outgoing tide from Port Phillip Bay) and survival (approximately 100 days) . The simulations examined the:

- dispersal and distribution footprint of particles from PPB to Cape Liptrap, and
- time it takes for particles to travel from Port Phillip Heads to Cape Liptrap.

The latter was used to investigate if the time taken by particles to reach Cape Liptrap via passive dispersal was consistent with the larval duration of *Asterias*.

Table 1. Locations sampled in this survey including map codes used in figure 1, date and geographical position.

Sample	Location	Map code	Date	Latitude	Longitude
1	Port Phillip Bay (spill ground)	PPB 1	1/08/2012	-37.9836	144.8858
2	Port Phillip Bay (Fawkner Beacon)	PPB 2	1/08/2012	-37.9486	144.9276
3	Western Port - north	Wport N	13/08/2012	-38.2730	145.3360
4	Western Port - east	Wport E	13/08/2012	-38.3550	145.5090
5	Western Port - south	Wport S	13/08/2012	-38.4500	145.3180
6	Western Port - west	Wport W	13/08/2012	-38.3280	145.2330
7	PPB Heads	BS 1	4/09/2012	-38.3161	145.6296
8	Cape Schank	BS 2	4/09/2012	-38.5151	144.8902
9	Flinders	BS 3	4/09/2012	-38.5027	145.0671
10	Ventnor Channel (WP)	BS 4	4/09/2012	-38.4359	145.1956
11	Cape Liptrap	W1	11/09/2012	-38.9323	145.9218
12	Walkerville South	W2	11/09/2012	-38.8852	146.0098
13	Waratah Bay	W3	11/09/2012	-38.8396	146.0621
14	Off Shallow Inlet	W4	11/09/2012	-38.8724	146.1561
15	2 km south Shallow Inlet	W5	11/09/2012	-38.9251	146.2170
16	Adjacent Norman Is.	NB1	11/09/2012	-39.0016	146.2621
17	Picnic Bay	NB2	11/09/2012	-39.0294	146.2850
18	Norman Bay	NB3	11/09/2012	-39.0414	146.3069
19	Oberon Bay	NB4	11/09/2012	-39.0674	146.3216
20	2 km south Oberon Bay	NB5	11/09/2012	-39.0894	146.3183
21	Corner Inlet	C1	12/09/2012	-38.7680	146.2901
22	Corner Inlet	C2	12/09/2012	-38.8098	146.3680
23	Corner Inlet	C3	12/09/2012	-38.8200	146.3961
24	Barry Beach Terminal	BB1	12/09/2012	-38.7172	146.3502
25	Barry Beach Terminal	BB2	12/09/2012	-38.7142	146.3815
26	Port Welshpool	PW1	12/09/2012	-38.7053	146.4615
27	Port Welshpool	PW2	12/09/2012	-38.7165	146.5438
28	Tidal River estuary	TR	13/09/2012	-39.0285	146.3187
29	Andersons Inlet	AR1	18/09/2012	-38.6418	145.7233
30	Andersons Inlet	AR2	18/09/2012	-38.6396	145.7548
31	Port Phillip Bay (Fawkner Beacon)	PPB 3	19/09/2012	-37.9485	144.9273

Geog co-ords: GDA94

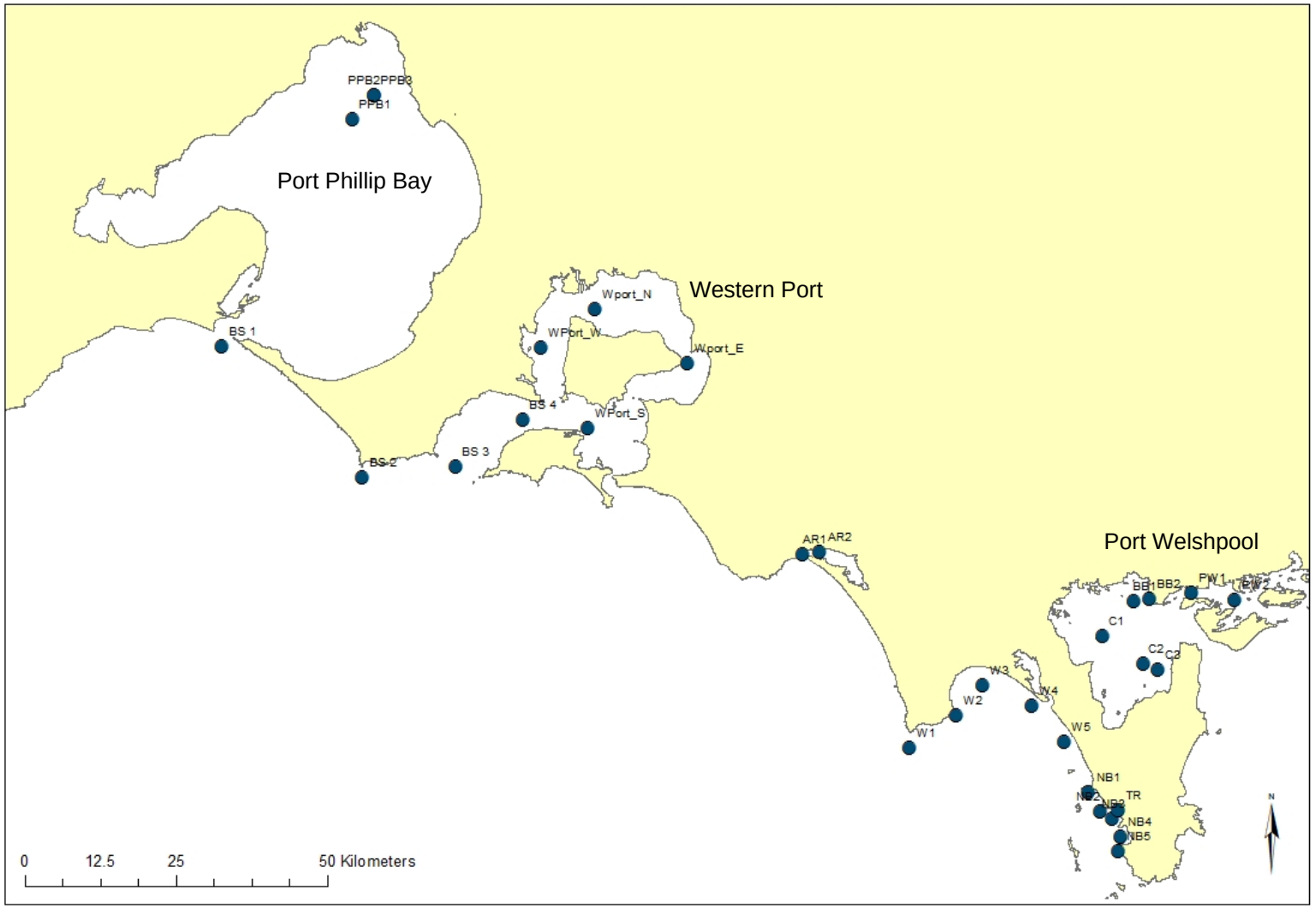

Figure 1. Map of sample locations (for location codes see Table 1).

Results

Plankton sampling

The results of the genetic assays measuring the amount of *Asterias* DNA in each sample are shown in Table 2. PCR results with the internal control show that some of the samples contained inhibitors that negatively affected PCR amplification. This is not unusual for marine plankton samples (N. Bott, pers. comm.). Accordingly, estimates of *Asterias* DNA were scaled to the level of inhibition (i.e. using scaling factors not shown) and this has been incorporated into the final estimate of *Asterias* DNA shown in Table 2. Brine shrimp sample control results were within expected variation for plankton samples (N. Bott, pers. comm.).

Asterias DNA was detected in 20 of the 31 samples collected in this survey (Table 2, Figure 2). DNA was detected in all three samples collected in PPB, in three of four samples collected in WP and in all four samples collected between PPB and WP. *Asterias* DNA was detected in all samples collected between Cape Liptrap and Shallow Inlet (South Gippsland); and in three of the five samples collected between Norman Island and Oberon Bay offshore of Tidal River. In addition, *Asterias* DNA was recorded in samples collected from Andersons Inlet and in Tidal River estuary. No *Asteria* DNA was detected in samples collected in Corner Inlet, off Barry Beach Marine Terminal or in waters adjacent to Port Welshpool (Figure 2).

The quantity of *Asterias* DNA detected in the survey varied by several orders of magnitude (Table 2) indicating high variation in the density of larvae collected in the samples. *Asterias* DNA is measured in picograms (pg or 10^{-12} g) of DNA per sample. The highest amount of *Asterias* DNA was recorded at Cape Schanck (Bass Strait) (25,418 pg) and the lowest amount of DNA (2 pg) was recorded at the eastern site in WP.

Samples collected offshore of Port Phillip Heads contained > 10,000 pg of Asterias DNA (Table 2, Figure 2), whereas samples collected from Cape Liptrap to Oberon Bay (with the exception of Waratah Bay where 1002 pg was recorded) contained <100 pg (Figure 2). *Asterias* DNA was detected in only two out of the five samples collected off Norman Bay (Table 2). The plankton sample collected from the Tidal River estuary contained only 94 pg of DNA suggesting that only low densities of *Asterias* larvae were present.

Major cross-contamination between samples and field-trips appears unlikely. For example, no *Asterias* DNA was detected in sample 3 taken in WP following the detection of 24,950 pg of DNA for the previous sample collected in PPB (see Table 2). Similarly, no *Asterias* DNA was detected in samples collected in Corner Inlet following the detection of *Asterias* DNA in coastal waters off Wilsons Prom and Walkerville on the previous day (Table 2). It is impossible to completely eliminate cross-contamination, particularly between samples. However, there is no evidence results were influenced by cross-contamination either between samples or field-trips in this survey.

Hydrodynamic modelling

The hydrodynamic and dispersal models indicate buoyant particles released from PPB are predominantly transported east and south-east along the coastline to Cape Liptrap (Figure 3). The greatest concentration of particles occurs offshore of Port Phillip Heads, the Mornington Peninsula and Phillip Island. The model simulation displays limited exchange between PPB and WP and lower particle concentrations for the coastline between Cape Patterson and Cape Liptrap.

Hydrodynamic modelling of buoyant particles predicted *Asterias* larvae are transported from Port Phillip Heads to Cape Liptrap via prevailing easterly currents in approximately 800 hours (33 days). This translates to a particle velocity of approximately 170 m/hour (i.e. 136 km/800 hours). At this rate the section of coast between Cape Liptrap and Tidal River (approx. 45 km) can be traversed by buoyant particles in approximately 265 hours — a further 11 days.

Table 2. Genetic assay results showing the presence and quantity of *Asterias* DNA (pg/5 min tow) in each plankton sample.

Sample	Location	Map code	Date	Asterias (pg DNA)
1	Port Phillip Bay (spill ground)	PPB 1	1/08/2012	**738**
2	Port Phillip Bay (Fawkner Beacon)	PPB 2	1/08/2012	**24,950**
3	Western Port - north	Wport N	13/08/2012	0
4	Western Port - east	Wport E	13/08/2012	**2**
5	Western Port - south	Wport S	13/08/2012	**283**
6	Western Port - west	Wport W	13/08/2012	**425**
7	PPB Heads	BS 1	4/09/2012	**10,320**
8	Cape Schank	BS 2	4/09/2012	**25,418**
9	Flinders	BS 3	4/09/2012	**2,138**
10	Ventnor Channel (WP)	BS 4	4/09/2012	**2,023**
11	Cape Liptrap	W1	11/09/2012	**46**
12	Walkerville South	W2	11/09/2012	**95**
13	Waratah Bay	W3	11/09/2012	**1,002**
14	Off Shallow Inlet	W4	11/09/2012	**23**
15	2 km south Shallow Inlet	W5	11/09/2012	**39**
16	Adjacent Norman Is.	NB1	11/09/2012	0
17	Picnic Bay	NB2	11/09/2012	**17**
18	Norman Bay	NB3	11/09/2012	0
19	Oberon Bay	NB4	11/09/2012	**9**
20	2 km south Oberon Bay	NB5	11/09/2012	0
21	Corner Inlet	C1	12/09/2012	0
22	Corner Inlet	C2	12/09/2012	0
23	Corner Inlet	C3	12/09/2012	0
24	Barry Beach Terminal	BB1	12/09/2012	0
25	Barry Beach Terminal	BB2	12/09/2012	0
26	Port Welshpool	PW1	12/09/2012	0
27	Port Welshpool	PW2	12/09/2012	0
28	Tidal River estuary	TR	13/09/2012	**94**
29	Andersons Inlet	AR1	18/09/2012	**74**
30	Andersons Inlet	AR2	18/09/2012	**351**
31	Port Phillip Bay (Fawkner Beacon)	PPB 3	19/09/2012	**472**

Positive detection in bold, UD: undetected

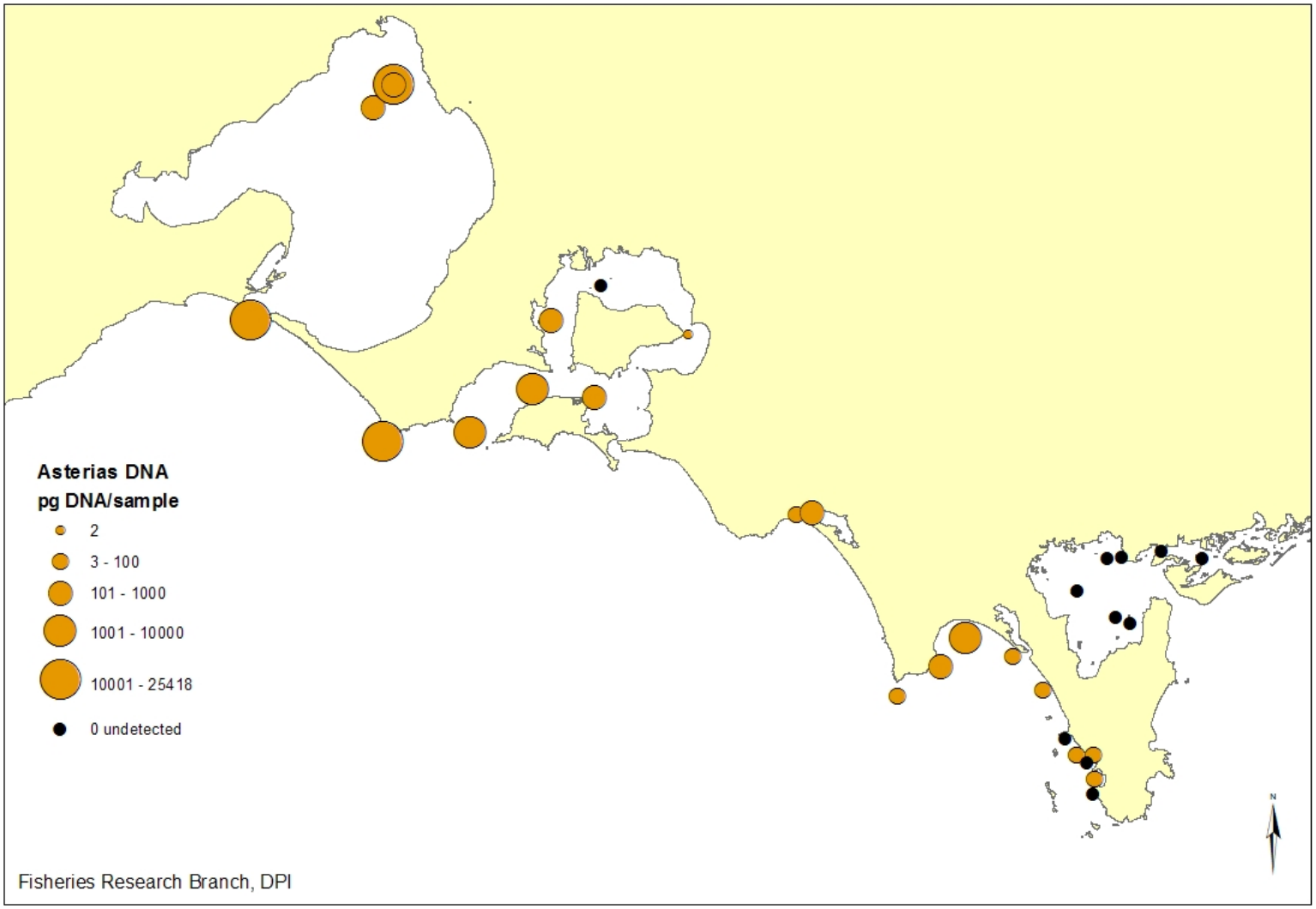

Figure 2. Bubble plot showing the quantity of *Asterias* DNA (pg/ 5 minute plankton tow) recorded at each location between PPB and Port Welshpool.

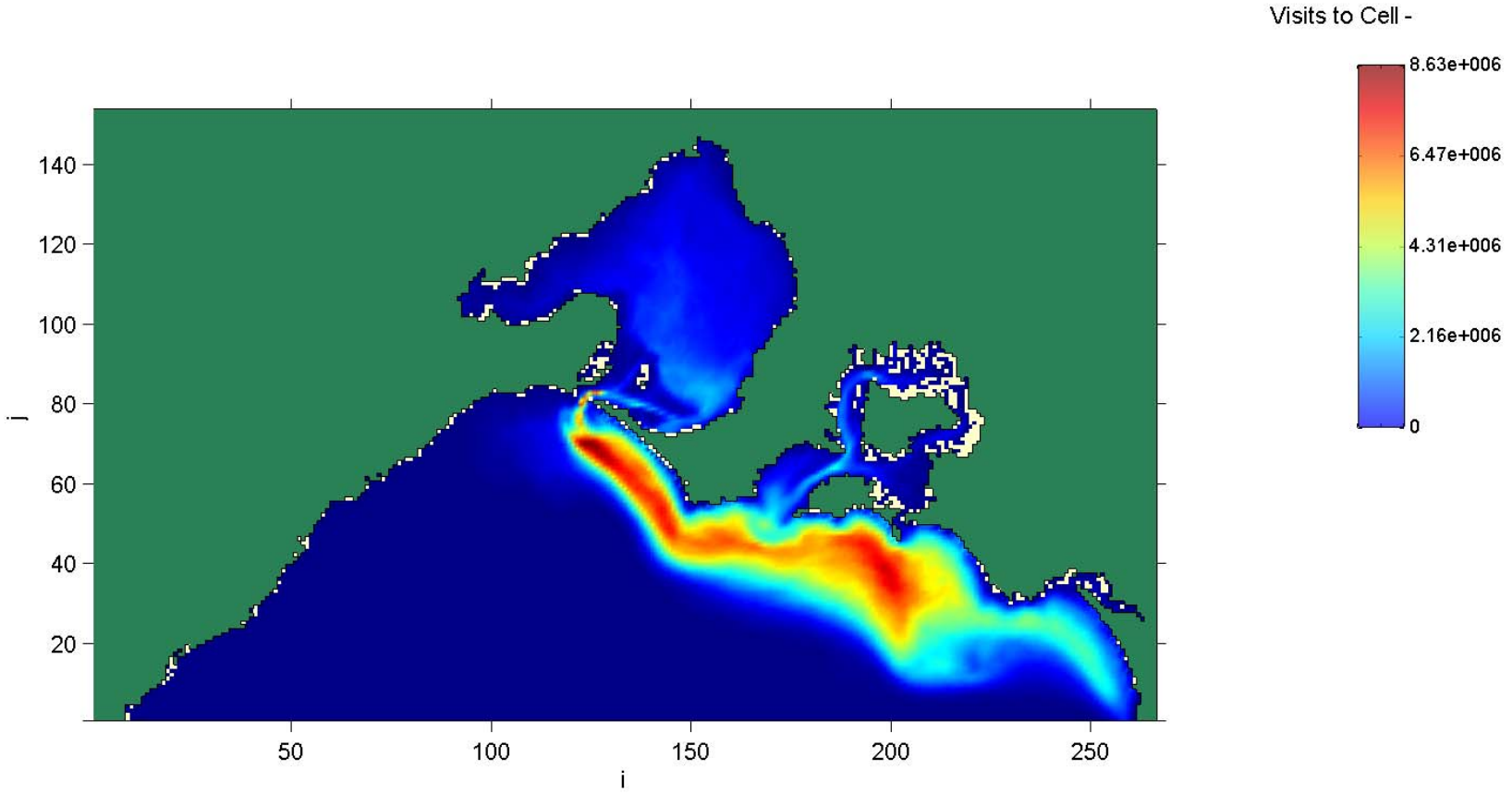

Figure 3. 200 day simulation of continuous larval release (total of 96000 particles) from Port Phillip Bay between July and November shown as cumulative counts of visits per cell. The dispersion model provides an indication of the likelihood of *Asterias* larvae presence and key pathways.

Discussion

Distribution of *Asterias* larvae

This study used a combination of plankton surveys and hydrodynamic modelling to examine the distribution of *Asterias* larvae in coastal waters between Port Phillip Bay (PPB) and Port Welshpool in eastern Victoria. The two approaches suggest *Asterias* larvae occur in coastal waters between PPB and Wilsons Promontory. First, *Asterias* larvae were detected in coastal waters between Cape Liptrap and Oberon Bay. Second, hydrodynamic modelling indicates larvae are likely to occur between Phillip Island and Cape Liptrap, despite the gap in sampling for this stretch of coastline (Figure 2). Moreover, hydrodynamic modelling found that buoyant particles exported from PPB could reach Cape Liptrap (the geographical extent of the model) in approximately 33 days. Assuming a similar rate of transport, it was estimated that larvae would reach the coast off Tidal River in a further 11 days (i.e. 44 days in total). This is well within the estimated larval duration of 79–112 days for *Asterias* (Bruce et al. 1995).

Larval densities declined with increasing distance from PPB, which is consistent with PPB being the primary source of larvae in this region and the outputs of the hydrodynamic/dispersion modelling (see Figure 3). The highest densities of *Asterias* DNA were recorded in PPB, at Port Philip Heads and off Cape Schanck. The high quantity of *Asterias* DNA off Port Phillip Heads and Cape Schanck suggest significant export of larvae into Bass Strait.

Hydrodynamic and dispersion modelling predicts larvae are transported predominately eastwards along the coast by the prevailing winter currents in this region (Figure 3). The currents in this region are influenced primarily by the prevailing wind direction (SW) and the tides, and flow mainly from the west forming a gyre rotating anticlockwise in Bass Strait and following the coastline towards Point Howe (Figure 4).

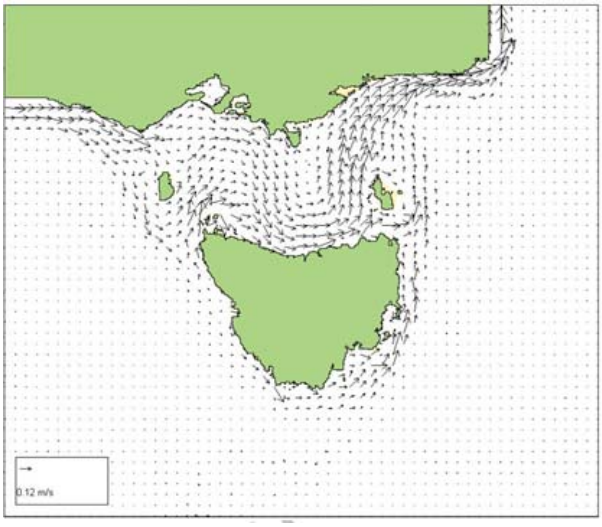

Figure 4. Directionally average currents for Bass Strait displaying residual velocity vectors (m/s). Source: SEA (south-east Australia) hydrodynamic model (Greer et al. 2008)

The amount of *Asterias* DNA recorded between Cape Liptrap and Oberon Bay was several orders of magnitude lower than that recorded off Port Phillip Heads. This is consistent with a dilution effect as larvae are transported further from their original source (see also Figure 3). High values of *Asterias* DNA recorded at Waratah Bay may be due localised accumulation of larvae caused by a local gyre (circulation) effect (R. Lee, EPA pers. comm.). The absence of *Asterias* larvae for Corner Inlet, Barry Beach marine terminal and Port Welshpool is also consistent with the influence of currents in determining the distribution of larvae. This is because currents flow primarily south across Bass Strait to the north coast of Tasmania, rather than around the tip of the Promontory and up into Corner Inlet (Figure 4).

Asterias larvae were also recorded in bays and inlets such as WP, Andersons Inlet and Tidal River estuary. Hydrodynamic modelling does not support strong connectivity between PPB and WP (Figure 3); however, high DNA levels were recorded at locations linking these two systems. Approximately 20 adult seastars were collected at San Remo (WP) between September and November 2011. This discovery raised concerns about the presence of adult *Asterias* populations in WP.

Unfortunately, the results of this survey are equivocal regarding the presence of adult

A*sterias* populations in WP. *Asterias* larvae were present in WP during this survey, particularly in the south and west of the bay and between Shoreham and Phillip Island, but this pattern is largely consistent with larvae arriving from PPB. Consequently, it is impossible to distinguish larvae arriving from PPB from those that may have originated from populations within WP. The survey provides no definitive evidence of adult populations in WP .

The presence of *Asterias* DNA in Andersons Inlet and Tidal River estuary during September 2012 is also most likely due to the intrusion of larvae into these systems from the open coast, rather than a product of existing adult populations. In the case of Andersons Inlet, *Asterias* have not been detected in this system since the eradication of the original outbreak in 2004–2005, and in Tidal River a large flood event is believed to have removed/killed all remaining adults present (S. Howe pers. comm.). Moreover, the low DNA levels recorded in the Tidal River estuary are similar to levels recorded offshore between Norman Island and Oberon Bay in this survey.

The presence of *Asterias* larvae in Bass Strait conforms with the predictions of Dunstan and Bax (2007). Dunstan and Bax (2007) predicted *Asterias* larvae exported from PPB would diffuse across central Bass Strait to the Kent and King Island groups and toward the northern coastline of Tasmania. In contrast, the modelling in this study indicates that larvae are transported predominantly to the east of PPB, past Phillip Island and along the South Gippsland coast. The differences between this and earlier modelling are likely to be due to recent improvements in the oceanographic model for this coastline (e.g. Greer et al. 2008; R. Lee, EPA pers. comm.). The results of the hydrodynamic modelling in this study indicate the risk of larval dispersal by currents along the west coast to Cape Otway is low; however, no sampling of this coastline was undertaken in this study.

It is difficult to know how typical this overall pattern is because this is the only survey of its kind. It is likely *Asterias* larvae have been present in coastal waters between PPB and Wilsons Prom since *Asterias* populations reached their initial peak in PPB in 1999/2000 (Parry et al. 2004); and PPB began to export high densities of larvae into Bass Strait. If this is a normal phenomenon this raises the question of why more outbreaks of *Asterias* have not been recorded along the coastline between PPB and Wilsons Promontory during this time.

Role of larval dispersal in causing new outbreaks of *Asterias*?

Only three adult populations have been recorded outside of PPB in the 14 years since *Asterias* populations peaked in PPB: Andersons Inlet, San Remo and Tidal River. Each appears to be a single, isolated recruitment event. However, the establishment of new populations of *Asterias* along the Victorian coastline is likely to be dependent on factors other than simply the presence of larvae in the water column.

Successful recruitment requires the convergence of a number of factors that influence the survival of both larvae and juvenile seastars. *Asterias* larvae need to be transported to suitable locations and habitat (e.g. inlets and bays), find suitable substratum for settlement, and evade predation and other forms of mortality during their early life. Conditions will also need to be favourable for the growth, reproduction and expansion of adult populations.

Little is known about the early life-history of larval and juvenile *Asterias*. Larvae need to evade predation and find sufficient food to develop and grow. Both factors are likely to be influenced by oceanographic and climatic processes that vary annually. These processes in turn may influence the abundance of larvae present in offshore waters. Mortality during this phase is likely to be both exceptionally high and highly variable (Bax and Dunstan 2004). The vast majority of larvae are unlikely to find suitable habitats for settlement, particularly along the open coast.

Successful recruitment from larvae may be a relatively rare event, requiring the convergence of a range of factors that influence larval transport, survival and settlement. The rate of outbreaks suggests that such conditions may only be infrequently suitable in Victorian waters (i.e. on the basis of three known outbreaks in 14 years). However, future incursions along this coastline seem inevitable: given adult populations appear to have successfully recruited in the past and that larvae will continue to be exported from PPB over the winter months.

Could the geographical pattern of *Asterias* outbreaks in Victoria be explained by other vectors?

Ballast water is the principal vector implicated in the introduction of *Asterias* to Australia, and its subsequent translocation from south-east Tasmania to PPB (Ward et al. 1995, Bax and Dunstan 2004). Ballast water provides a mechanism by which larvae can be transported beyond the range of natural larval dispersal (in terms of distance or prevailing currents). Although, legislation now minimizes the risk of spread via ballast water for large commercial shipping vessels, this still remains the main vector by which *Asterias* could be introduced to other ports within Australia (Bax and Dunstan 2004). Ballast water also remains a significant vector by which *Asterias* could be spread between Victorian ports.

If ballast water is a major vector involved in the spread of *Asterias* along the Victoria coastline then we would expect new outbreaks to occur at locations unlikely to be reached by natural dispersal alone (i.e. the west coast of Victoria or further east along the Gippsland coast beyond Wilsons Promontory). Although, it is not possible to dismiss the role of ballast water in the spread of *Asterias*, the location of outbreaks appears entirely consistent with passive larval transport as the primary mode of dispersal, based on the plankton surveys and hydrodynamic modelling undertaken in this study.

Bax and Dunstan (2004) list a range of vectors that could spread *Asterias* non-larvae (juveniles and adults) around the coast of Australia. These include entanglement in nets/fishing gear, biofouling on vessel hulls and surfaces, and intentional introductions. In general Bax and Dunstan (2004) considered the importance of these vectors low in comparison to ballast water. It is entirely conceivable that *Asterias* could be transported from PPB to WP by such activities, particularly as there is high vessel traffic between the two water bodies. However, as seastar are dioecious (sexes are separate) and fertilization occurs externally, the establishment of a founder population would require the successful translocation of at least one individual of each sex (i.e. one individual would be insufficient to found a new population).

Conclusions

- *Asterias* larvae were detected in coastal waters between PPB and Wilsons Promontory during August and September 2012. This finding is supported by hydrodynamic modelling of buoyant particles simulating the behaviour of *Asterias* larvae exported from PPB
- No *Asterias* larvae were detected in coastal waters east of Wilsons Prom (i.e. Corner Inlet and Port Welshpool) and this is consistent with the influence of the prevailing currents in Bass Strait and their role in transporting *Asterias* larvae along the Victorian coast
- *Asterias* larvae were also detected in bays and inlets such as WP, Andersons Inlet and Tidal River estuary. This is likely to be due to the intrusion of larvae into these systems from the open coast. *Asterias* larvae may also be present in Shallow Inlet, but this location was not surveyed during this study
- The pattern of *Asterias* outbreaks at Andersons Inlet, Western Port and Tidal River appears to be most consistent with natural range expansion by larval dispersal (rather than other vectors); with PPB as the source population
- Managers will need to be vigilant to prevent the spread of *Asterias* along the coastline between PPB and Wilsons Promontory. The presence of larvae along this coastline indicates further outbreaks are likely in Western Port, Andersons Inlet, around Wilsons Promontory and also possibly Shallow Inlet. Protected embayments such as Waratah Bay may also be susceptible, given the presence of larvae at relatively high levels
- **Recommendations regarding further sampling:** We recommend further sampling along the west coast of Victoria between PPB and Cape Otway, and further east along the East Gippsland coast to confirm the outputs of the modelling and the pattern of larval dispersal described in this report. The hydrodynamic model indicates that larvae are predominantly restricted to coastal waters (see Figure 3), however, additional sampling in offshore shipping lanes or in locations where ballast water exchanges occur may be also be informative in quantifying the distribution of *Asterias* larvae in Victorian waters. Sampling should

also be undertaken in Shallow Inlet, to confirm the presence/absence of larvae in this system.

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