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Molecular surveillance for Asian Paddle Crab in the Port River – Barker Inlet

Wiltshire, K.H., Theil, M.J., Giblot-Ducray, D. and Deveney, M.R.

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SARDI Aquatics Sciences
PO Box 120 Henley Beach SA 5022

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EXECUTIVE SUMMARY

Two adult specimens of the Asian paddle crab (APC), *Charybdis japonica*, were captured in Gulf St Vincent (GSV) in 2019 by commercial and recreational fishers, including one specimen captured near Port Adelaide. Crabs of the genus *Charybdis* have been introduced and formed invasive populations in several countries, with negative impacts of these introductions resulting from competition with native crabs and spread of crustacean diseases. APC has established invasive populations in New Zealand, but is not established in Australia. A commercial fishing industry for the Blue Swimmer Crab *Portunus armatus* is based in GSV. In combination with recreational effort, it is likely fishing would detect further APC if a population was established in GSV. Port Adelaide, however, is not commercially fished and is a likely location for first entry, establishment and breeding of APC. While APC specimens have been captured by recreational fishers, the fishing effort in Port Adelaide and extent of communication to recreational fishers regarding APC is unquantified, and it is unclear if recreational fishing effort is sufficient to detect an APC population.

Establishment of APC in Port Adelaide would pose a risk of spread to GSV by natural or human-mediated dispersal, and to other ports via ballast water or biofouling. To assess the APC status of Port Adelaide we surveyed the Port River – Barker Inlet system in March 2020 using molecular methods to detect APC. Molecular methods provide greater sensitivity for detection of many marine pests, including the European shore crab, *Carcinus maenas.* Crab traps were also deployed opportunistically coincident with the molecular survey. The molecular survey employed plankton tows, with samples tested by qPCR for APC and other pests: Northern Pacific sea star (*Asterias amurensis*)*,* European shore crab (*Carcinus maenas*)*,* Pacific Oyster (*Crassostrea* gigas), New Zealand greenlip mussel (*Perna canaliculus*)*,* European fanworm (*Sabella spallanzanii*), and the Japanese kelp (*Undaria pinnatifida*)*.* These additional pests are of interest for ballast water management and tests were applied to obtain updated data on the status of these pests in Port Adelaide, as well as to obtain further data on performance of the molecular surveillance system.

DNA extracted from plankton samples collected in Port Adelaide between February 2017 and April 2019 was also retrospectively tested using the same set of pest assays. APC was not detected in the March 2020 surveillance. Traps captured several Blue Swimmer Crabs but no non-native crabs. Three pests that are established in Adelaide were detected by qPCR in the March 2020 samples: European shore crab, Pacific Oyster and European fanworm. These pests

were widely detected in the plankton samples from 2017 – 2019. The detection of these pests supports performance of the molecular surveillance method for detection of marine pests. Retrospective testing in May 2020 of two samples from November 2017 also returned positive qPCR detections for APC. The 2017 detections may have been of transient DNA from ballast water or hull-fouling, but demonstrate that APC may have been in the Port River in 2017. The capture of a third adult specimen by a recreational fisher in April 2020 demonstrates that the species may continue to be present in the Port River. The scarcity of molecular detections, and lack of detection in the March 2020 survey, however, suggest that if an APC population exists in Port Adelaide it is small, and that widespread spawning, which would be indicative of a self-sustaining population, is not occurring.

Keywords: Marine Pests, qPCR, Plankton, Asian paddle crab, Port Adelaide

1. INTRODUCTION

1.1. Background

Marine pests affect fishing and aquaculture, amenity and infrastructure, undermining recreational, community and indigenous values of marine systems, and placing communities that depend on those systems at risk (Hayes and Sliwa 2003; Schaffelke and Hewitt 2007; Molnar *et al.* 2008; Hewitt *et al.* 2011). Over 70 crab species have been transported and become established outside their native ranges, with many having considerable negative impacts in their introduced ranges (Brockerhoff and McLay 2011). Crabs of the genus *Charybdis* are among the worst invaders, (Brockerhoff and McLay 2011); these include the Indo-Pacific swimming crab *C. helleri*, which is invasive in the Mediterranean (Galil 2008) and western Atlantic (Dineen *et al.* 2001), and the Asian paddle crab (APC), *C. japonica*, which is invasive in New Zealand (NZ) (Gust and Inglis 2006; Fowler *et al.* 2013). In addition to establishing multiple invasive populations, *Charybdis* species have negative impacts in invaded areas through competitive interactions with native crabs and by contributing to spread of crustacean diseases, particularly white-spot syndrome virus (Gust and Inglis 2006; Brockerhoff and McLay 2011; Hewitt *et al.* 2011).

APC has been identified as having a high risk of establishment in Australia, with potentially severe impacts if introduced (Hayes and Sliwa 2003; Hewitt *et al.* 2011). Although not established in Australia, APC has periodically been detected. In December 2000, one adult male APC was captured by a recreational fisher in the Port River, South Australia (SA), although an intensive trapping survey carried out subsequent to this detection did not capture any further APC (Hooper 2001). Since 2010, a small number of adult APC have been captured in the Swan and Peel River estuaries of Western Australia (Hourston *et al.* 2015). In July 2019, an adult male APC was captured by a commercial fisher in Gulf St Vincent (GSV) near Port Gawler, approximately 20 km from the Barker Inlet estuary which is connected to the Port River. Following communication regarding this detection, Primary Industries and Regions SA (PIRSA) Biosecurity received photographic confirmation of an additional adult male APC captured in western GSV in early 2019. Commercial fishing effort for Blue Swimmer Crab is likely to be effective for detecting APC if a population exists in GSV, but the Port River, which receives international and domestic shipping and provides suitable habitat for APC, is not subject to commercial fishing. APC have been captured by recreational fishing in both SA (Hooper 2001; PIRSA data) and WA (Hourston *et al.* 2015), but the level of recreational fishing within the Port River is undefined, and may be insufficient for effective detection of an APC population. The Port River has been surveyed for

European shore crab (Dittmann *et al.* 2016; Dittmann *et al.* 2017), but this surveillance is not ongoing, and the suitability of the shore crab survey method for APC detection is not established. An established population of APC in Port Adelaide would provide a source population that would spread to GSV by natural and human-mediated dispersal, and to other ports via ballast water and biofouling. It was therefore a priority to assess if a population of APC had established in the Port River – Barker Inlet system.

The APC detection in Port Adelaide in 2000 was followed by a large-scale trapping survey to assess if it was established (Hooper 2001). Trapping is an effective means of surveillance for crab species, including APC (e.g. Archdale *et al.* 2006; Gust and Inglis 2006; Archdale 2008), but molecular surveillance methods developed since 2010 have demonstrably higher sensitivity for detection of invasive European shore crab *Carcinus maenas* than trapping (Wiltshire *et al.* 2019a). Molecular techniques for marine pest surveillance offer cost and time savings over traditional techniques, and technical advances have provided a platform for the development of practical, specific, sensitive and rapid molecular surveillance tools for marine pests (Bott *et al.* 2010b; Deveney *et al.* 2017; Department of Agriculture and Water Resources 2018). The South Australian Research and Development Institute (SARDI) has developed and validated quantitative polymerase chain reaction (qPCR) assays for detection of 10 marine pest species (Ophel-Keller *et al.* 2007; Bott *et al.* 2010a; Bott and Giblot-Ducray 2011a, b, 2012), and developed and refined plankton sampling and preservation methods and quality controls for molecular marine pest surveillance (Wiltshire and Deveney 2011; Giblot-Ducray and Bott 2013; Deveney *et al.* 2017). A qPCR assay developed for detection of APC (Simpson *et al.* 2018) was recently implemented into the SARDI testing system, enabling molecular surveillance for this species.

Detection likelihood using molecular surveillance varies seasonally for most marine pests and species with pronounced seasonal patterns of detectability have highest likelihood of detection corresponding to their reproductive seasons (Wiltshire et al. 2019a). To maximise the likelihood of detection, molecular surveillance should therefore be carried out during the period where propagules of the target pest (spores, gametes or larvae) are most likely to be present. APC spawn primarily in summer – early autumn, although spawning can occur from mid-spring (Kolpakov and Kolpakov 2012; Wong and Sewell 2015; Kobayashi and Vazquez-Archdale 2018). Larvae develop in the plankton for approximately four weeks (Han et al. 2015), therefore propagules of this species, either gametes or larvae, are most likely to be present in summer – autumn.

Three pests of concern for ballast water transport: Pacific oyster (*Crassostrea gigas*), European fanworm (*Sabella spallanzanii*) and European shore crab (*Carcinus maenas*) are established in Port Adelaide, but these species, with the exception of Pacific oyster, have not been surveyed since the 2010 – 2011 Port Adelaide pest surveys (Wiltshire and Deveney 2011). Plankton samples were opportunistically tested for these pests, and for the other temperate pests of ballast water concern: Northern Pacific sea star (*Asterias amurensis*), New Zealand greenlip mussel (*Perna canaliculus*), and the Japanese kelp (*Undaria pinnatifida*) which are not established in Port Adelaide. This testing provides updated information on the status of these pests and additional data to assess performance of the molecular survey.

1.2. Objectives

- Survey the Port River – Barker Inlet for APC;
- Assess results in comparison to other molecular surveillance, considering effects of sampling volume, sample mass and PCR inhibition;
- Obtain updated information on additional pests of concern in Port Adelaide.

2. METHODS

2.1. Plankton tow collection

Crab larvae are active swimmers and many species display negative phototaxis (Dittel and Epifanio 1982; dos Santos *et al.* 2008). Samples for the APC molecular survey were therefore collected near the benthos, using a plankton net mounted on a sled (Figure 1). Plankton samples for molecular analysis were otherwise collected following the methods developed by Giblot-Ducray and Bott (2013) and refined by Deveney *et al.* (2017). A conical mesh plankton net with mouth diameter 0.5 m, length 1.5 m and 50 µm mesh (Sea-Gear 90-50x3-50) fitted with a flowmeter (Sea-Gear MF315) was towed behind a vessel at a speed of ~1–1.5 m s^{-1} for a target distance of 100 m. After collection, plankton samples were concentrated down to a volume of ~40 mL by filtering through the mesh windows of the plankton net cod end and transferred to 120 mL tubes containing 80 mL sulfate based preservation buffer (similar to Stanford University 2015). Samples were kept cool in an insulated container with ice after collection and for transport to the South Australian Aquatic Sciences Centre (SAASC) where they were stored in a cool room at ≤ 4 °C until processing (see section 2.2). 60 plankton tow samples were collected through the Barker Inlet between St Kilda and North Arm, and the Port River between Inner Harbour and Outer Harbor (Figure 2) on March 17 – 18th, 2020. Start and end waypoints for each tow were recorded by GPS (Garmin 78sc).

2.1. Processing and analysis of molecular samples

The plankton samples were filtered in the laboratory at SAASC using a manifold and sterile single-use filter cups with 0.45 µm filters (Thermo Scientific™ Nalgene™). Filter papers were transferred to 50 mL centrifuge tubes, frozen at −20 °C and freeze dried until completely dehydrated prior to DNA extraction. Sample mass was determined by weighing each sample tube prior to and after addition of the filtered plankton, and again after drying. DNA extraction and qPCR analysis were carried out by the SARDI Root Disease Testing System (RDTS) laboratory. DNA was extracted from samples using the method developed by SARDI Molecular Diagnostics, with 20 mL of DNA extraction buffer containing an internal control (a standardised amount of exogenous organism added to each sample) added to each sample before physical disruption (Ophel-Keller *et al.* 2008). The efficiency and consistency of SARDI's method to extract DNA from environmental samples has been confirmed in comparison to commercial methods (Haling *et al.* 2011). Final elution volume of the DNA was 160 µL in elution buffer. Each DNA extract was then tested in singleplex quantitative polymerase chain reaction (qPCR) performed on QuantStudio7 real-time

PCR system (Applied Biosystems, Foster City, CA, USA) using SARDI developed assays for six marine pests (Table 1) plus the Asian Paddle crab assay (Simpson *et al.* 2018) and the exogenous organism that was added to samples as a control to test for PCR inhibition.

Figure 1. Plankton net mounted on benthic sled for collection of near-bottom plankton samples.

Table 1. SARDI developed qPCR assays applied to extracted DNA, assay gene target, and reference for assay.

Species	Gene target	Reference
Northern Pacific sea star *Asterias amurensis*	Cox1	Bott et al. (2010a)
European shore crab *Carcinus maenas*	Cox1	Bott et al. (2010a)
Pacific Oyster *Crassostrea gigas*	Cox1	Bott and Giblot-Ducray (2012)
NZ greenlip mussel *Perna canaliculus*	IGS	Bott and Giblot-Ducray (2011b)
Eurpean fanworm *Sabella spallanzanii*	28S rDNA	Ophel-Keller et al. (2007)
Asian kelp *Undaria pinnatifida*	Cox1	Bott et al. (2010a)

Testing included negative controls and the appropriate calibration standard for each target pest. Reference samples that are known to not cause inhibition were also extracted after addition of the inhibition control and tested by qPCR. A scaling factor was calculated for each plankton sample by comparing the yield of inhibition control DNA detected in that sample to that in the reference samples. For those assays with a standard curve the scale factor for a sample is used as a multiplier to correct the apparent DNA concentration as calculated from the cycle threshold (C_T)value for the effects of inhibition (Ophel-Keller *et al.* 2008).

To avoid cross-contamination, all benchtops and apparatus, including freeze-drier shelving, were decontaminated using LookOut® DNA Erase prior to and after each use.

2.2. Retrospective testing of Port River plankton samples

Plankton samples were collected from the Port River, Adelaide, between January 2017 and April 2019 for a project investigating the potential use of molecular testing of plankton for OsHV-1 surveillance (Deveney *et al.* 2020). Sampling locations for that project targeted known areas of occurrence for *Crassotrea gigas*, specifically: the upper Port River, Inner Harbour in the vicinity of Dock 2, North Arm near the Port River junction, and Lipson reach in the area between the Quarantine Station jetty and Osbourne wharves (Table 2; Figure 3). Samples were also collected in the vicinity of Outer Harbor in some sampling sets. For that project, samples were tested for Pacific Oyster DNA and for OsHV-1, but were not tested for other pests. Additional tests were applied in May 2020 to the DNA extracted from these Port Adelaide plankton samples as part of a project to assess prevalence and seasonality of pests established in Australian ports (Wiltshire *et al.* in prep). The retrospective tests were for the pests outlined in Table 1 (excluding *Cr. gigas*, which was not re-tested), plus APC. Tests were applied to 15 sets of samples comprising 247

plankton samples in total (Table 2). Results from the tests applied to these samples were used to calculate prevalence of each pest (see section 2.5).

Table 2. Collection dates and areas for previously collected plankton samples.

Collection date(s) / Location	Upper Port	Inner Harbour	North Arm	Lipson Reach	Outer Harbor	TOTAL
28/02/2017	5	5	5	5		20
8/05/2017	5	5	5	5		20
14/11/2017	5	5	5	5		20
21/02/2018	5	5	5	5		20
26/03/2018	4	4	4	4	4	20
24/04/2018	4	4	4	4	4	20
30/05/2018	4	4	4	4	4	20
1/08/2018	4	4	4	4	4	20
22/01/2019	3	3	3	3		12
8/02/2019		5	5			10
25/02/2019	3	3	3	3	3	15
12/03/2019		5	5			10
26/03/2019	3	3	3	3	3	15
9/04/2019		5	5			10
29/04/2019	3	3	3	3	3	15

2.3. Trapping

Sixteen box-style crab traps, baited with sardines (*Sardinops sagax*) and weighted with cement blocks, were deployed for a soak time of ~ 24 hours between March 17 and 18, 2020. Trap locations are shown in Figure 4.

2.4. Mapping and statistical methods

Diagnostic performance of five of the qPCR assays applied in the current project (each species in Table 1 except *P. canaliculus*) was assessed by Wiltshire *et al.* (2019b). Specifically, estimates were obtained for the diagnostic sensitivity (DSe) and specificity (DSp). DSe is the likelihood that a test will detect a target if present in the sample, while DSp is the likelihood that a detection is correct. Knowledge of test diagnostic performance aids the interpretation of survey results, allowing estimation of true prevalence (proportion of samples containing target DNA) from the

number of positive and negative test results in each case (= apparent prevalence). The relationship between target DNA prevalence in plankton and adult pest population size is unknown, but estimation of true prevalence allows standardised comparison of results across species where test performance is not identical, and, where a target is not detected, estimation of the maximum prevalence that may be present. This maximum plausible prevalence, given the sampling effort and diagnostic performance, provides the basis for Bayesian proof of freedom approaches (Low-Choy 2013; Stanaway 2015). Estimated true prevalence for each tested species was determined in a Bayesian framework using code modified from the *truPrev* function of the *prevalence R* package (Devleesschauwer *et al.* 2014) to allow simultaneous estimation of prevalence for multiple species. An uninformative beta (1,1) prior was used for true prevalence of each species; this prior allows equal probability for any prevalence between 0 and 1. True prevalence was calculated separately for the March 2020 APC survey and using the combined results of retrospective testing, thus providing a prevalence estimate for each species for the APC survey (2020) and average for the period February 2017 – April 2019. Beta priors were used for diagnostic sensitivity (DSe) and specificity (DSp) of each species assay based on results from Wiltshire *et al.* (2019b), with beta parameters calculated using the *prevalence* package *betaExpert* function (Devleesschauwer *et al.* 2014). Diagnostic performance has not been assessed for the assays for *P. canaliculus* or APC*,* but testing spiked samples for APC by Simpson *et al.* (2018) provided an initial estimate of performance for that assay, while for *P. canaliculus* the lowest estimates of DSe and DSp of the other assays was used to provide the most conservative (highest) estimate of true prevalence. Priors are described in Table 3.

The prevalence analysis was run in JAGS (Plummer 2017) using *R* (R Core Team 2019) and package *R2jags* (Su and Yajima 2015) with 10,000 MCMC iterations thinned at a rate of 10, following 50,000 for burn-in. Convergence was assessed by Gelman-Rubin convergence statistic, and confirmed by visual inspection of trace, density and autocorrelation plots generated using the *MCMCplots* package (McKay Curtis 2015). Highest density intervals (HDIs) demonstrating 95% of the probability mass for posterior estimates were calculated using the *HDIinterval* package (Meredith and Kruschke 2018).

Posterior estimates from the Bayesian models were used to calculate the number of samples that would be required to detect DNA of APC at the predicted prevalence (π) with 60% and 80% confidence (= survey sensitivity, S_{SE}). The number of samples, n, is given by: $n = \log(1 - SSE)/\log(1 - DSe \times \pi)$. Prevalence, which is a proportion, will depend on the abundance (i.e. average number of molecules) of DNA in plankton samples. If DNA is randomly

distributed, prevalence is related to DNA abundance (λ) as: $\pi = 1 - \exp(-\lambda)$. This relationship can be used to infer the relative abundance of DNA between two species having different prevalences.

Maps of results were produced using ArcMap 10.6 (Esri Inc). Plankton sample locations are mapped as the midpoint of each tow.

Table 3. Description of priors used for DSe and DSp of each assay for estimation of true prevalence. Prior estimate shows most likely value and 95% credible range. Parameters were assigned a beta prior: beta(a,b), with a and b values calculated using *betaExpert* (Devleesschauwer *et al.* 2014).

Species	Parameter	Prior estimate	a	b
Asterias	DSe	0.89 (0.83 – 0.94)	110	13.4
	DSp	1.00 (0.99 – 1.00)	273	0.625
Carcinus	DSe	0.79 (0.62 – 0.91)	21.9	5.68
	DSp	1.00 (0.99 – 1.00)	273	0.625
Charybdis	DSe	0.83 (0.70 – 0.95)	30.4	7.03
	DSp	0.96 (0.90 – 1.00)	71.1	3.92
Crassostrea	DSe	0.91 (0.83 – 0.97)	65.0	6.42
	DSp	1.00 (0.99 – 1.00)	273	0.625
Perna	DSe	0.73 (0.59 – 0.88)	25.8	9.50
	DSp	1.00 (0.99 – 1.00)	273	0.625
Sabella	DSe	0.86 (0.79 – 0.93)	90.2	14.9
	DSp	1.00 (0.99 – 1.00)	273	0.625
Undaria	DSe	0.73 (0.59 – 0.88)	25.8	9.50
	DSp	1.00 (0.99 – 1.00)	273	0.625

Sample mass and volume were assessed to determine whether they fell within typical ranges based on previous sampling (Deveney *et al.* 2017; Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c) using the same protocols. Likelihood of detection generally increases with increasing sample mass and volume, although the effect of these factors is minor within the typical ranges (Wiltshire *et al.* 2019a). Very low sample volumes or mass, could, however, indicate issues with sampling, especially if pest detections do not result.

3. RESULTS

3.1. Species detections

Plankton samples collected for the March 2020 survey returned detections for the three pests known to be established in Port Adelaide: *Ca. maenas* (detected in 4 samples), *Cr. gigas* (57) and *S. spallanzanii* (54) (Figure 2; Table 4). All *Ca. maenas* detections were in samples collected in the vicinity of Outer Harbor, while the only samples without *S. spallanzanii* detection were in Barker Inlet. These three species were all commonly detected in the plankton samples from 2017 – 2019 (Table 4). There were no detections of *A. amurensis* or *P. canaliculus* in any of the sample sets. APC was detected by the retrospective testing in two samples from November 2017, and *U. pinnatifida* in a single sample from February 2017. The APC and *U. pinnatifida* detections were all from Lipson reach (Figure 3). These pests were not detected in the 2020 survey.

The crab traps captured 19 native Blue Swimmer Crabs (*Portunus armatus*), with the highest numbers recorded in traps near the junction of Angas Inlet and Barker Inlet (Figure 4). No other species were captured.

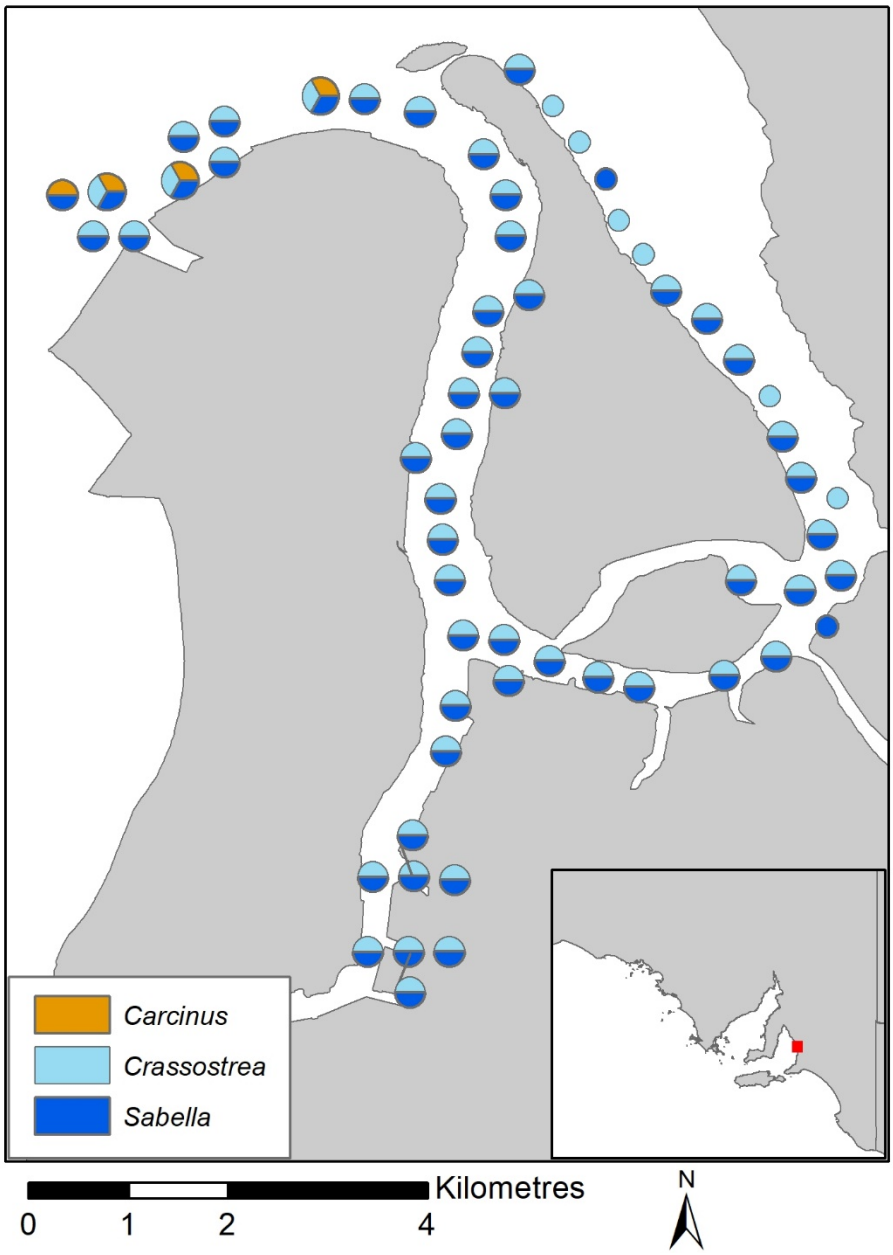

Figure 2. Location of plankton tows for the March 2020 paddle crab survey showing pest detections

Table 4. Summary of number of samples with pest DNA detections by sample set.

Collection date(s)	**Number of detections**					
Species	***Ca. maenas***	***Ch. japonica* (APC)**	***Cr. gigas***	***S. spallanzanii***	***U. pinnatifida***	**Samples**
Paddle crab survey						
17 – 18/03/2020	4		57	54		60
Retrospective testing						
28/02/2017			16	4	1	20
8/05/2017	5			19		20
14/11/2017	13	2	19	2		20
21/02/2018	1		20	4		20
26/03/2018			16	18		20
24/04/2018	3		2	3		20
30/05/2018	16		11	15		20
1/08/2018	4		7	17		20
22/01/2019	1		12	9		12
8/02/2019			10	4		10
25/02/2019			15	2		15
12/03/2019			8	1		10
26/03/2019			13	5		15
9/04/2019			7	10		10
29/04/2019	2		2	14		15

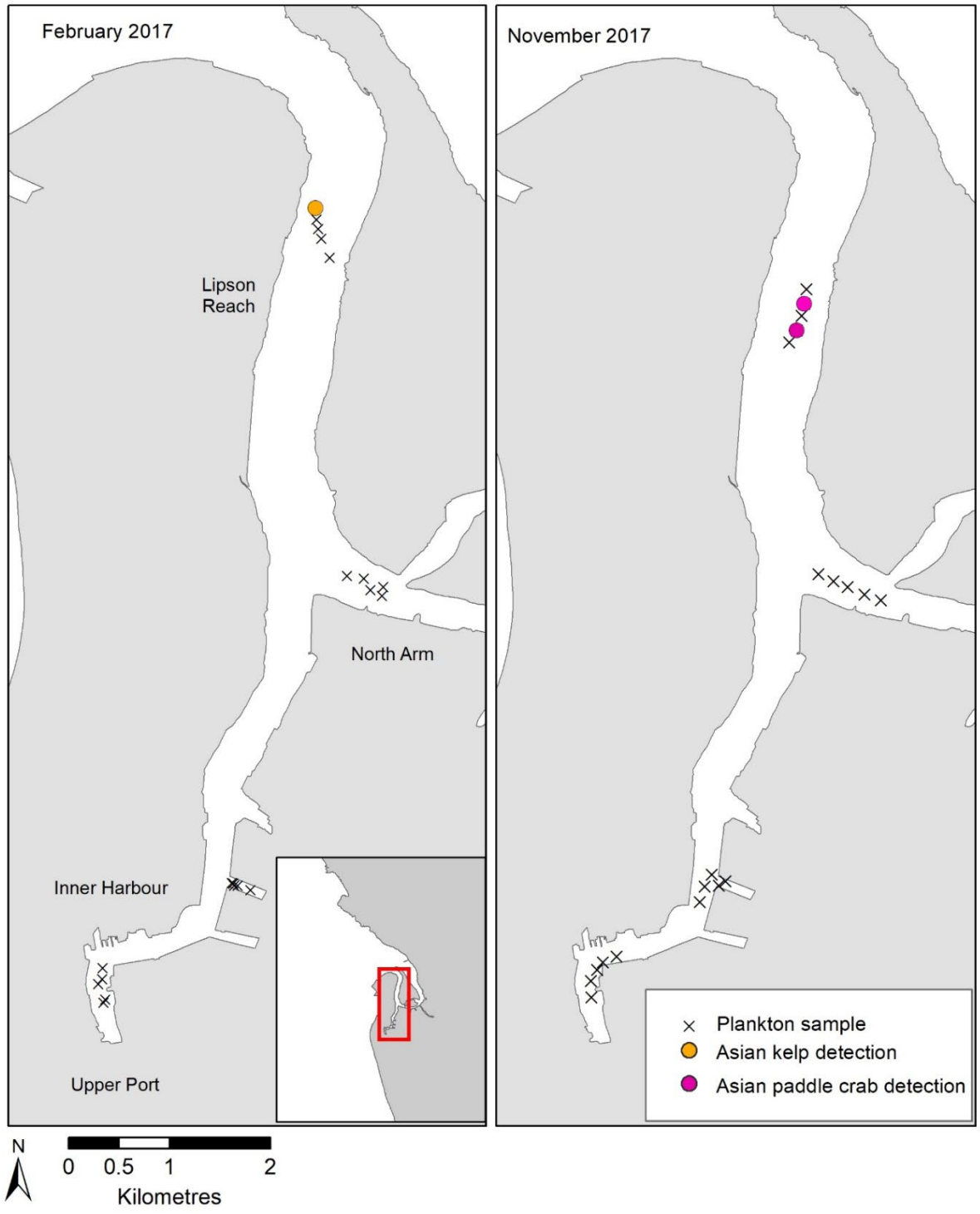

Figure 3. Location of detections for Asian kelp (left) and Asian paddle crab (right) in retrospectively tested samples, plus location of plankton samples collected on the same sampling dates.

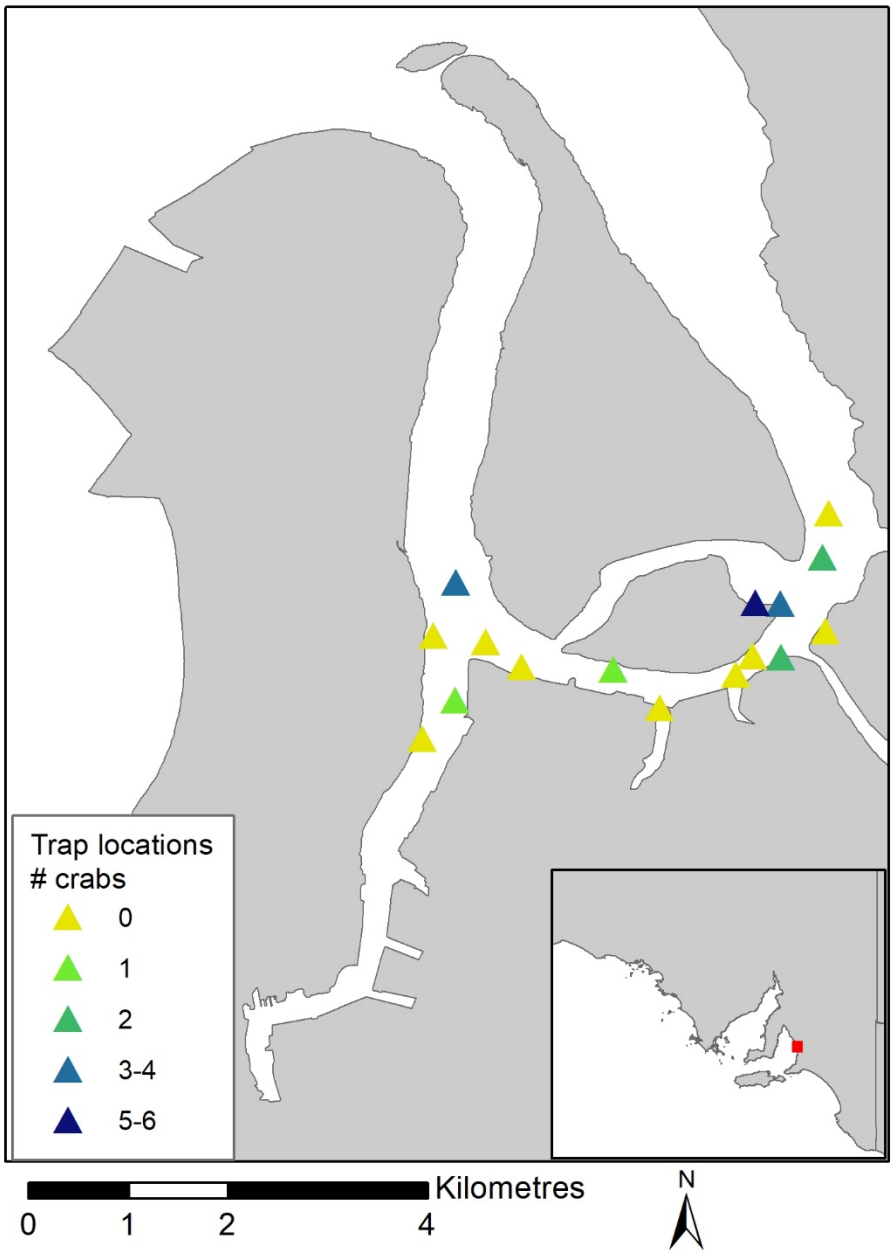

Figure 4. Location of traps showing number of Blue swimmer crabs captured. No introduced crabs were captured by trapping.

3.2. PCR inhibition, sample mass and volume

PCR inhibition, as measured by scale factor (section 2.2), occurred in several samples from the paddle crab survey, but at a low level, such that detection of pest DNA was unlikely to be impaired. 26 samples had scale factors > 1.5, with four of these having scale factor > 2, and a maximum

scale factor of 2.9. Scale factors > 5 may reduce likelihood of detection by some of the pest assays (Wiltshire *et al.* 2019b). At scale factors > 2 but < 5, calculation of DNA concentration from C_T values may be less accurate than in samples with lower scaling, but detection is unlikely to be compromised.

All tows targeted a distance of 100 m, measured by GPS. The average distance of tows based on actual start and end way points was (mean ± s.d.) 99.5 ± 11.1 m. Clogging of the plankton net during a tow is typical, leading to flow meter distances less than the distance travelled (Deveney *et al.* 2017; Wiltshire *et al.* 2019a). During the paddle crab survey, the average flow meter distance was 46.1 ± 25.1 m, resulting in net efficiency (ratio of flow meter to actual distance) of 46.9 ± 24.0 %. An average of 1.19 ± 0.92 g filtered dry weight was collected in each sample. Flow meter distance, net efficiency and sample mass for the paddle crab survey are within typical ranges for plankton sampling in Port Adelaide and other Australian temperate ports (Deveney *et al.* 2017; Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c; SARDI data).

3.3. Estimation of true prevalence

For APC, which may not be established in Adelaide but captures show adult specimens are periodically present, the true prevalence estimates provide an indication of the maximum DNA prevalence that could credibly be present but not detected given the survey sample numbers and assay performance (Low-Choy 2013; Stanaway 2015). The upper limit of the HDI indicates 95% confidence that prevalence of APC DNA was < 0.06 at the time of the APC survey, and < 0.02 across the retrospectively tested samples. Mean predicted prevalence was 0.02 and 0.008 respectively. The lower HDI limit in each case was zero, indicating credible support for absence of APC DNA.

For *Cr. gigas* and *S. spallanzanii*, which are established in Port Adelaide and were widely detected by qPCR in the 2020 survey, true prevalence estimates were > 0.96 (Table 5), indicating probable occurrence of target DNA in all samples. Prevalence of pest DNA in plankton varies seasonally for most species. This survey was undertaken in early autumn, which is the best time of year for detection of *Cr. gigas*, and equal best for *S. spallanzanii,* which also has high prevalence in late autumn – early winter (Wiltshire *et al.* 2019a). True prevalence from the combined results of retrospective testing for these species shows average prevalence across the seasons in which these samples were collected; the lower estimates obtained (0.60 for *S. spallanzanii*, 0.70 for *Cr. gigas*) reflect this averaging rather than indicating an increase in prevalence between 2017 – 2019 and the current survey. *Carcinus maenas*, the other species established in

Port Adelaide, occurred at lower prevalence than *Cr. gigas* and *S. spallanzanii*, with a prevalence estimate of 0.10 in the current survey and 0.23 in retrospectively tested samples. Seasonality of *Ca. maenas* detections is less pronounced than for some pests, but peaks in detection occur in late autumn and in spring (Wiltshire *et al.* 2019a). Data from the retrospective testing also supports this seasonal pattern (Table 4). The APC survey therefore occurred outside the peak time for detection of *Ca. maenas.* The lower limit of the HDI, which shows the minimum plausible DNA prevalence for each of these established pests was ≥ 0.02, and was lowest for *Ca. maenas* in this survey.

To provide 60% confidence of detecting the predicted prevalence of APC DNA in the 2020 survey, 892 samples would be needed, while 1567 samples would be needed to provide 80% confidence of detection. This can be compared to the number needed to detect the predicted prevalence of *Ca. maenas*: 15 samples to provide 60% confidence, and 26 samples to provide 80% confidence. The prevalence estimates for *Ca. maenas* and APC suggest that *Ca. maenas* DNA was 71 times more abundant than that of APC at the time of the 2020 survey, and 326 times more abundant in the retrospective testing.

Table 5. Predicted true prevalence from Bayesian models using data from the paddle crab survey, and combined results of retrospective testing of samples collected 2017 – 2019.

	Predicted true prevalence (95% HDI)	
Species	**Paddle crab survey**	**Retrospective testing**
Asterias	0.018 (0.000 – 0.054)	0.005 (0.000 – 0.014)
Carcinus	0.098 (0.021 – 0.188)	0.233 (0.161 – 0.309)
Charybdis	0.020 (0.000 – 0.060)	0.008 (0.000 – 0.022)
Crassostrea	0.969 (0.916 – 1.000)	0.703 (0.614 – 0.793)
Perna	0.022 (0.000 – 0.067)	0.005 (0.000 – 0.016)
Sabella	0.961 (0.895 – 1.000)	0.601 (0.511 – 0.683)
Undaria	0.023 (0.000 – 0.069)	0.010 (0.000 – 0.024)

For *A. amurensis* and *P. canaliculus*, which were not detected and which are regarded as absent from the survey area, the estimates also show the DNA prevalence that could plausibly be present without being detected. In the case of *U. pinnatifida*, which returned a single detection in retrospective testing but is also regarded as absent, the estimate shows the maximum plausible DNA prevalence. The upper HDI limit for each of these species was < 0.07 in the 2020 survey,

and < 0.02 in the retrospective testing. The lower HDI limit in each case was zero, indicating credible support for absence of target DNA.

4. DISCUSSION

To assess if APC are established in Port Adelaide, molecular surveillance and limited trapping was undertaken in the Port River – Barker Inlet. Assays for other marine pests were also applied, providing an opportunity to assess results in comparison to molecular surveillance for other pest species. The 2020 survey did not detect APC specimens or DNA in the Port River – Barker Inlet, but did detect DNA of another pest crab, *Ca. maenas*, along with detections of *Cr. gigas* and *S. spallanzanii.* Retrospective testing of plankton samples collected in 2017 – 2019 returned two qPCR detections of APC in samples from November 2017 and a single detection of *U. pinnatifida* in February 2017. These detections demonstrate the suitability of the method for detection of marine pests generally, and that detection of crab DNA, including of APC, is feasible. Data on PCR inhibition, sample mass and volume from the 2020 molecular survey also support that the method was effective. Of the other pests known to occur in Port Adelaide, only *Ca. maenas* may feasibly have been caught by trapping, because *Cr. gigas* and *S. spallanzanii* are sessile. Parallel molecular surveillance and trapping in Melbourne and Hobart, where *Ca. maenas* also occurs, demonstrated that the molecular method was more effective than trapping for detecting this species (Wiltshire *et al.* 2019a). For the APC survey, we used a trap type and chose locations more suitable for APC than for *Ca. maenas,* which is smaller and is most abundant intertidally. The lack of *Ca. maenas* detections by trapping is therefore unsurprising despite its continued presence in Port Adelaide.

Despite the lack of APC detections during the 2020 survey, an adult male APC was caught by a recreational fisher in the North Arm of the Port River in April 2020. It is unclear, however, whether this, or other APC, were present at the time of the survey, because crabs are highly mobile, and adult APC might be introduced regularly by shipping. The lack of APC specimens or DNA detections in the 2020 survey suggest that if present, APC numbers in the Port River – Barker Inlet are low, and, if spawning is occurring, it is limited, leading to low DNA prevalence.

Exponentially greater sampling effort is required to achieve equivalent confidence in detection with decreasing prevalence (Hayes *et al.* 2005; Furlan *et al.* 2016). Detecting a very low prevalence is therefore not cost-effective, and, where a target is truly absent, it cannot be detected, regardless of the number of samples collected. It is therefore not possible to completely prove absence of a pest species, rather, absence of detection provides a level of confidence that a population, if present, is below a given population size/prevalence. While a larger pest population is likely to provide a greater DNA prevalence, the exact nature of this relationship is

unknown, and it is likely to vary widely between species due to differences in fecundity, larval size and period, and quantity of DNA shed by adult pests. It is therefore difficult to infer population size from prevalence, or to make direct comparisons between species.

Some qualitative comparison can, however, be made between similar species such as *Ca. maenas* and APC, although differences in fecundity and larval period make direct comparison difficult. *Carcinus maenas* is established in the Port River – Barker Inlet, and, while it undergoes population fluctuations, it has been commonly found in the area since 2015 (Dittmann *et al.* 2016; Dittmann *et al.* 2017). Likelihood of detection and DNA concentration in plankton varies seasonally for most pests (Wiltshire *et al.* 2019a). We targeted late summer/early autumn to maximize likelihood of detecting APC, but late autumn is likely to be a better time for *Ca. maenas* detection. Given that the 2020 survey was outside the peak season for detection of *Ca. maenas*, the estimate that *Ca. maenas* DNA is ~70 times more abundant than that of APC is conservative. A more accurate representation may be given by the retrospective testing, where samples covered multiple times of year providing prevalence estimates indicating that *Ca. maenas* DNA is > 300 times more abundant than that of APC. These estimates are of DNA abundance in plankton, and may not reflect relative adult population sizes.

The detection of APC DNA in November 2017 suggests that APC may have been present in the Port River at that time, but the detections could have been of transient DNA, i.e. from a ballast water release, dead material or DNA shed from crabs in hull or niche fouling that did not leave the vessel. DNA may have been produced by crabs spawning either in vessel fouling or in the Port River; while late summer is typically the peak spawning time, APC can spawn from mid-spring (Kolpakov and Kolpakov 2012; Wong and Sewell 2015; Kobayashi and Vazquez-Archdale 2018). It is not possible, however, to determine if qPCR detections are of gametes, larvae, or shed adult DNA.

Detections of APC in SA and WA have all been adult crabs (Hooper 2001; Hourston *et al.* 2015; PIRSA data), suggesting that introductions comprise transport of adults, potentially in niche fouling (e.g. sea chests) of vessels, rather than larval transport or from spawning of adults in fouling. Fouling of vessel niche areas is a known vector for crab introductions, and adult *Charybdis* spp. have been found in sea chests, including in NZ (Dodgshun and Coutts 2002; Brockerhoff and McLay 2011). The 2019-2020 captures of APC in SA are the first detections of this species in SA since 2000. In combination with the sporadic WA detections since 2010 (Hourston *et al.* 2015) the SA detections suggest an increase in the frequency of introduction of

APC to southern Australia. This may be due to a change in vector type, frequency or source of shipping from areas where APC are present, either in NZ or their native range in Asia. Increased APC abundance in one or more donor ports could also contribute to an increased rate of introduction. Alternatively, a larval recruitment event in ~2017 – 2018 could be responsible for the appearance of adult crabs in 2019 – 2020 in SA, given that 18 – 24 months is required for APC to reach adult size; and adult crabs are more likely to be trapped than juveniles (Hewitt *et al.* 2018). Increased captures could also result from an increase in fishing effort, but effort in the commercial Blue Swimmer Crab fishery in GSV declined from 2000 – 2010 and has been stable since 2010 (Beckmann and Hooper 2019). A large recent increase in recreational fishing effort appears unlikely, and, while communication regarding APC detections may have prompted additional reports from recreational fishers (Hourston *et al.* 2015), initial detections occurred prior to publicity.

The APC assay is new, was not developed by SARDI and, unlike the assays developed by SARDI, has not been field validated or had its diagnostic performance formally assessed (Deveney et al. 2017; Wiltshire et al. 2019a; b). The initial investigations of Simpson *et al.* (2018), suggest that the APC assay has DSp close to 1 and high DSe, similar to the SARDI assays, but further assessment is required to validate its performance.

The single detection of *U. pinnatifida* from 2017 is likely to be of transient DNA from erosion or spawning of hull-fouling material, or from a ballast-water release. The detection occurred in summer, which is the primary reproductive season for *U. pinnatifida*, but surveys in Melbourne, where this species is established, show that its DNA is detected year-round (Wiltshire *et al.* 2019a). Erosion of adult algae probably contributes environmental DNA in addition to spore release. *Undaria pinnatifida* was not detected in Adelaide samples collected after February 2017. Sporadic summer detections of *U. pinnatifida* have been recorded in molecular surveillance of other ports where this species is absent but which receive shipping from areas with native or invasive populations (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c).

5. CONCLUSIONS

Surveillance in March 2020 using qPCR applied to plankton, and opportunistic trapping did not detect APC in the Port River – Barker Inlet. Retrospective testing of plankton samples collected between February 2017 and April 2019 returned two APC detections from November 2017. Following capture of 2 APC in GSV in 2019, a single adult male APC was captured by a recreational fisher in the Port River in April 2020, with no other APC captured in SA since 2000. Together, these results indicate that APC are transiently present in the Port River, and were possibly present in 2017, but that numbers are low, providing limited DNA prevalence. The lack of DNA detections suggest that it is unlikely that there is widespread spawning of APC, or a self-sustaining population in the survey area. The risk of establishment of an APC population cannot, however, be excluded. Detections of this species in both WA and SA demonstrate that vectors are introducing this species to southern Australia, and environmental conditions in SA are conducive to its establishment if sufficient crabs are present (Hayes and Sliwa 2003; Hewitt *et al.* 2011). Impacts of invasive APC are likely to correlate with range and abundance of this species (Gust and Inglis 2006), hence, obtaining additional knowledge of the distribution of this species where it occurs would assist management. The molecular method is viable for detection of APC, although further validation of assay performance would provide additional confidence in results of molecular surveillance using this assay. Seasonal sampling of a temperate region where APC is established would assist in validating seasonal patterns. Future molecular surveillance could be applied for APC, and would provide high survey sensitivity for detection of increased abundance or a spawning population in the Port River – Barker Inlet, but is unlikely to be cost-effective for large-scale or long term surveillance, especially where APC occur at low density. Detections of adult APC by commercial and recreational fishers have been facilitated by education and community engagement, in both SA (PIRSA data) and WA (Hourston *et al.* 2015). Continued publicity, education, and engagement with the public and with commercial crab fishers would assist in leveraging existing fishing effort for detection of APC with minimal expense. Ongoing promotion of the pest reporting module in the SA Fishing App (formerly the SA Recreational Fishing Guide App) would improve reporting quality by providing photographs for identification, and reducing staff time for each reported detection of a potentially invasive crab.

6. REFERENCES

Archdale MV (2008) Invasive swimming crabs: Development of eradicating trapping gear and methods. In 'Invasive Species: Detection, Impact and Control'. (Eds CP Wilcox and RB Turpin). (Nova Science Publishers Inc: New York)

Archdale MV, Añasco CP, Hiromori S (2006) Comparative fishing trials for invasive swimming crabs *Charybdis japonica* and *Portunus pelagicus* using collapsible pots. *Fisheries Research* 82, 50-55.

Bott NJ, Giblot-Ducray D (2011a) Molecular tools for detection of marine pests: Development of diagnostic PCR assays for the detection of significant marine pests: *Carcinus maenas, Ciona intestinalis* and *Undaria pinnatifida*. Report prepared for Adelaide & Mt Lofty Ranges Natural Resources Management Board. South Australian Research and Development Institute (Aquatic Sciences), SARDI Publication No. F2011/000503-1. SARDI Research Report Series No. 587, Adelaide.

Bott NJ, Giblot-Ducray D (2011b) Molecular tools for detection of marine pests: *Musculista senhousia, Corbula gibba* and *Perna canaliculus* quantitative PCR assays. Report prepared for Biosecurity SA. . South Australian Research and Development Institute (Aquatic Sciences), SARDI Publication No. F2010/000991-1. SARDI Research Report Series No. 522, Adelaide.

Bott NJ, Giblot-Ducray D (2012) Development of a qPCR assay for the Pacific Oyster, *Crassostrea gigas.* Report prepared for Biosecurity SA. . South Australian Research and Development Institute (Aquatic Sciences), SARDI Publication No. F2012/000210-1. SARDI Research Report Series No. 641, Adelaide.

Bott NJ, Giblot-Ducray D, Deveney MR (2010a) Molecular tools for detection of marine pests: Development of putative diagnostic PCR assays for the detection of significant marine pests: *Asterias amurensis, Carcinus maenas, Undaria pinnatifida* and *Ciona intestinalis*. South Australian Research and Development Institute (Aquatic Sciences), SARDI Publication No. F2010/000669-1. SARDI Research Report Series No. 509, Adelaide.

Bott NJ, Ophel-Keller KM, Sierp MT, Herdina, Rowling KP, McKay AC, Loo MGK, Tanner JE, Deveney MR (2010b) Toward routine, DNA-based detection methods for marine pests. *Biotechnology Advances* 28, 706-714.

Brockerhoff A, McLay C (2011) Human-Mediated Spread of Alien Crabs. In 'In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts'. (Eds BS Galil, PF Clark and JT Carlton) pp. 27-106. (Springer Netherlands: Dordrecht)

Department of Agriculture and Water Resources (2018) Marine Pest Plan 2018–2023: the National Strategic Plan for Marine Pest Biosecurity. Department of Agriculture and Water Resources, Canberra.

Deveney MR, Giblot-Ducray D, Wiltshire KH (2017) Marine pests testing methods: contributing to establishment of an Australian Testing Centre for Marine Pests. SARDI Aquatic Sciences, SARDI Publication No. F2017/000273-1. SARDI Research Report Series No. 963, Adelaide.

Deveney MR, Wiltshire KH, Lieu YN, Buss JJ, Moody NJG, Mohr PA, Ellard KA (2020) Advanced surveillance for OsHV-1 microvariant. In 'Future Oysters CRC-P: Advanced aquatic disease surveillance for known and undefined oyster diseases'. (Eds MR Deveney and KH Wiltshire) pp. 1 - 43. (Fisheries Research and Development Corporation Deakin, ACT)

Dineen JF, Clark PF, Hines AH, Reed SA, Walton HP (2001) Life History, Larval Description, and Natural History of *Charybdis hellerii* (Decapoda, Brachyura, Portunidae), an Invasive Crab in the Western Atlantic. *Journal of Crustacean Biology* 21, 774-805.

Dittel AI, Epifanio CE (1982) Seasonal Abundance and Vertical Distribution of Crab Larvae in Delaware Bay. *Estuaries* 5, 197-202.

Dittmann S, Baring R, Campbell R, Jessup-Case H, Evans-Sanchez K (2016) Assessment of presence and distribution of the European shore crab *Carcinus maenas* in near-shore habitats of Gulf St. Vincent 2015/16. Report for the Adelaide & Mount Lofty Ranges NRM Board. Flinders University, Adelaide.

Dittmann S, Baring R, Jessup-Case H, Campbell R (2017) Monitoring and morphometric studies of the European shore crab *Carcinus maenas* in Gulf St. Vincent 2016/17. Report for the Adelaide & Mount Lofty Ranges NRM Board. Flinders University, Adelaide.

Dodgshun T, Coutts A (2002) Ships' sea chests: a "side door" for marine pests? Cawthron Institute, Nelson, New Zealand.

dos Santos A, Santos AMP, Conway DVP, Bartilotti C, Lourenço P, Queiroga H (2008) Diel vertical migration of decapod larvae in the Portuguese coastal upwelling ecosystem: implications for offshore transport. *Marine Ecology Progress Series* 359, 171-183.

Fowler AE, Taylor RB, Muirhead JR (2013) Early stages of a New Zealand invasion by *Charybdis japonica* (A. Milne-Edwards, 1861) (Brachyura: Portunidae) from Asia: behavioral interactions with a native crab species. *Journal of Crustacean Biology* 33, 672-680.

Galil B (2008) Alien species in the Mediterranean Sea—which, when, where, why? *Hydrobiologia* 606, 105-116.

Giblot-Ducray D, Bott NJ (2013) Development of a plankton sampling protocol for molecular testing of marine pests. Report prepared for Biosecurity SA. . South Australian Research and Development Institute (Aquatic Sciences), SARDI Publication No. F2013/000281-1. SARDI Research Report Series No. 734, Adelaide.

Gust N, Inglis GJ (2006) Adaptive Multi-scale Sampling to Determine an Invasive Crab's Habitat Usage and Range in New Zealand. *Biological Invasions* 8, 339-353.

Haling RE, Simpson RJ, McKay AC, Hartley D, Lambers H, Ophel-Keller K, Wiebkin S, Herdina, Riley IT, Richardson AE (2011) Direct measurement of roots in soil for single and mixed species using a quantitative DNA-based method. *Plant and Soil* 348, 123-137.

Han Z, Zheng W, Zhu W, Yu C, Shui B, Gao T (2015) A barrier to gene flow in the Asian paddle crab, *Charybdis japonica*, in the Yellow Sea. *ICES Journal of Marine Science* 72, 1440-1448.

Hayes KR, Sliwa C (2003) Identifying potential marine pests - a deductive approach applied to Australia. *Marine Pollution Bulletin* 46, 91-98.

Hewitt CL, Campbell ML, Coutts ADM, Dahlstrom A, Sheilds D, Valentine J (2011) Species biofouling risk assessment. . Department of Agriculture Fisheries and Forestry, Canberra.

Hewitt MJ, Hourston M, McDonald JI (2018) A long way from home: Biosecurity lessons learnt from the impact of La Nina on the transportation and establishment of tropical portunid species. *PLoS One* 13, e0202766.

Hooper G (2001) Assessment of the potential translocation of an exotic crab, *Charybdis japonica*, in the Port River, South Australia. Report to PIRSA Marine Habitat Program. SARDI Aquatic Sciences, Adelaide.

Hourston M, McDonald J, Hewitt M (2015) Public engagement for the detection of the introduced marine species *Charybdis japonica* in Western Australia. *Management of Biological Invasions* 6, 243-252.

Low-Choy S (2013) Bayesian modelling of surveillance and proof of freedom: the mathematical, logical & psychological challenges.

McKay Curtis S (2015) mcmcplots: Create Plots from MCMC Output. R package version 0.4.2. https://CRAN.R-project.org/package=mcmcplots.

Meredith M, Kruschke J (2018) HDInterval: Highest (Posterior) Density Intervals. R package version 0.2.0. https://CRAN.R-project.org/package=HDInterval.

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the Global Threat of Invasive Species to Marine Biodiversity. *Frontiers in Ecology and the Environment* 6, 485-492.

Ophel-Keller K, Loo M, Bott N, McKay A (2007) Development of gene probes for introduced marine pest species (*Musculista senhousia, Corbula gibba, Sabella spallanzanii*). A report for Natural Heritage Trust. South Australian Research and Development Institute, Adelaide.

Ophel-Keller K, McKay A, Hartley D, Herdina, Curran J (2008) Development of a routine DNA-based testing service for soilborne diseases in Australia. *Australasian Plant Pathology* 37, 243-253.

Plummer M (2017) JAGS Version 4.3.0 user manual.

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schaffelke B, Hewitt CL (2007) Impacts of introduced seaweeds. *Botanica Marina* 50, 397-417.

Simpson T, Bunce M, Stat M, Huerlimann R, Veileux H, Jerry D, Rojahn J, Gleeson D, Furlan E, Deveney M, McDonald J (2018) Partial validation of molecular diagnostic assays for priority exotic marine pests significant to Australia. Final report to the Department of Agriculture and Water Resources. Department of Agriculture and Water Resources, Canberra.

Stanaway M (2015) Evidence of absence for invasive species: roles for hierarchical Bayesian approaches in regulation. In 'Biosecurity Surveillance: Quantitative Approaches'. (Eds F Jarrad, S Low Choy and K Mengersen) pp. 265. (CAB International: Oxfordshire, England)

Stanford University (2015) Recipe for an RNA-later like buffer solution Accessed: 27 November 2015. URL: http://sfg.stanford.edu/RNAbuffer.pdf

Su Y-S, Yajima M (2015) R2jags: Using R to Run 'JAGS'. R package version 0.5-7. https://CRAN.R-project.org/package=R2jags.

Wiltshire KH, Deveney MR (2011) Final monitoring report for the 2010-2011 marine pest survey of the Port of Adelaide, South Australia. South Australian Research and Development Institute (Aquatic Sciences), SARDI Publication No. F2011/000386-1. SARDI Research Report Seres No. 575, Adelaide.

Wiltshire KH, Giblot-Ducray D, Prowse T, Deveney MR (2019a) Validation of molecular surveillance: parallel traditional and molecular surveys for priority marine pests in the ports of Gladstone, Brisbane, Melbourne and Hobart. Report to Department of Agriculture and Water Resources. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, SARDI Publication No. F2019/000040-1. SARDI Research Report Series No. 1019. .

Wiltshire KH, Prowse T, Buss JJ, Giblot-Ducray D, Lieu YN, Deveney MR (2019b) Diagnostic performance of molecular tests used to detect priority marine pests in plankton samples. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, SARDI Publication No. F2019/000337-1. SARDI Research Report Series No. 1036. .

Wiltshire KH, THeil MJ, Giblot-Ducray D, Deveney MR (2019c) Molecular surveys for priority marine pests in 2018-2019 for the ports of Gove, Weipa, Hay Point, Newcastle, Botany Bay, Port Kembla and Devonport. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, SARDI Publication No. F2019/000406-1. SARDI Research Report Series No. 1044.