Marine Ecosystems

Molecular surveys for priority marine pests in 2021-22 for Thevenard, Port Lincoln, Port Giles, Klein Point, Port Adelaide, South Australia, and Portland, Victoria

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

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Report to the Department of Agriculture, Fisheries and Forestry

Government of South Australia

Department of Primary Industries and Regions

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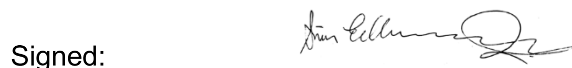
Signed: 

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Cover photo: Plankton sampling at Port Giles © SARDI

EXECUTIVE SUMMARY

Shipping is a major vector for marine pest introductions, and ports are therefore at relatively high risk of pest incursion. Knowledge of pest occurrence around ports is also required for management, including to ensure compliance with ballast water regulations. Molecular techniques for marine pest surveillance offer cost and time savings over traditional techniques (e.g. dives, trawls/dredges, trapping). SARDI has developed a molecular surveillance method using plankton samples analysed with qPCR assays for species-specific detection of key marine pests, including seven priority pests for ballast water management (Japanese sea star *Asterias amurensis*, European shore crab *Carcinus maenas,* Pacific Oyster *Magallana gigas*, Asian bag mussel *Arcuatula senhousia,* basket shell clam *Varicorbula gibba,* European fanworm *Sabella spallanzanii* and Japanese kelp *Undaria pinnatifida*). This system has been field tested and demonstrated to provide higher confidence of pest detection with less than half the person-hours and field cost of traditional surveillance. Applying this molecular survey method, collecting and analysing 35 samples per location in each of two seasons (winter – early spring and summer – early autumn) would provide a survey confidence of 80% for detection of planktonic pests at a concentration representative of an emerging incursion and close to 100% confidence in detection of established pests.

Ports at Thevenard, Port Lincoln, Port Giles, Klein Point, and Adelaide, South Australia, and Portland, Victoria were surveyed for marine pests using molecular methods. Surveys targeted the seven species of concern for domestic ballast water; three species that are established in parts of Australia: New Zealand screwshell, *Maoricolpus roseus,* which occurs in Tasmania and south-eastern mainland Australia; Japanese soft-shell clam *Mya japonica*, which occurs in eastern Tasmania; and Asian shore crab *Hemigrapsus sanguineus*, which occurs in Port Philip Bay, Victoria; and nine species currently exotic to Australia. Surveys were designed for detection of a planktonic concentration of target pests in the range expected for emerging incursions, providing 80% confidence of detection of this concentration, and close to 100% confidence in detection of established pests. Samples were collected in September – October 2021 (winter-spring) and February – March 2022 (summer-autumn), with 35 samples per location collected in each sample set. Each port comprised a single location except Adelaide, where 35 samples per set were collected in each of Outer and Inner Harbor.

Carcinus maenas, Magallana gigas and *Sabella spallanzanii* were detected in Adelaide, *S. spallanzanii* in Port Lincoln, *M. gigas* in Thevenard, and *U. pinnatifida* and *S. spallanzanii* in

Portland. These pests are established at these locations. The detections further suggest that *Maoricolpus roseus* occurs in Portland and that *Arcuatula senhousia* occurs in Thevenard. These species are not previously recorded at these locations. There were no target pests known to occur at the survey locations that were not detected. Following patterns observed in previous surveillance, *M. gigas* and *A. senhousia* were detected primarily in summer, and *C. maenas, U. pinnatifida* and *S. spallanzanii* in winter.

A Bayesian modelling approach was used to assess survey results, taking into account assay performance, sample volumes and potential effects of PCR inhibition. The model provided estimates of planktonic concentration of each pest for comparison with the survey design threshold. Model results showed that the established pests detected by the survey all occurred at concentrations confidently above the threshold, as did the newly detected occurrences of *Maoricolpus roseus* in Portland and *Arcuatula senhousia* in Thevenard. Estimated concentrations of undetected species were all confidently below the survey threshold, demonstrating that there were no measured issues with surveillance that would have prevented their detection.

Keywords: Marine pests, qPCR, plankton, surveillance, South Australia, Victoria, ballast water.

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). Surveillance is a key component of managing incursion risk. Early and reliable detection maximises the likelihood of successful responses to incursions, minimises risk of spread for established pests, and supports targeted sustainable management of marine systems (DAWE 2018). A surveillance strategy for Australian ports was established in the 2000s, based on traditional methods such as dredge sampling, trapping and visual surveys (National System for the Prevention and Management of Marine Pest Incursions 2010a, b), but few surveys were completed. A review of that monitoring strategy identified that the lack of implementation of the system was largely due to the expense of traditional surveillance methods (Arthur *et al.* 2015).

Molecular techniques for marine pest surveillance offer cost and time savings over traditional techniques. 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; DAWE 2018). New international and domestic regulations for ballast water management came into force in September 2017. In Australia, surveillance is required to support port status for the assessment of Australian Sourced Ballast Applications (ASBA) in the Maritime Arrivals Reporting System (MARS), which focuses on seven species (Japanese sea star *Asterias amurensis*, European shore crab *Carcinus maenas,* Pacific Oyster *Magallana gigas*, Asian bag mussel *Arcuatula senhousia,* basket shell clam *Varicorbula gibba,* European fanworm *Sabella spallanzanii* and Japanese kelp *Undaria pinnatifida*) that have established populations in Australia (Arthur *et al.* 2015; DAWE 2018). A molecular surveillance system was therefore established to assess the occurrence of priority pests at ports where ballast water transfer occurs.

The South Australian Research and Development Institute (SARDI) has developed qPCR assays for detection of key marine pests, and established plankton sampling, preservation, and extraction methods for molecular marine pest surveillance (Wiltshire and Deveney 2011; Giblot-Ducray and Bott 2013; Deveney *et al.* 2017). Field performance of this molecular surveillance system was initially assessed by the Australian Testing Centre for Marine Pests (ATCMP) project (Deveney *et al.* 2017), which involved application of assays for the seven ballast water pests of concern to plankton samples collected from six ports around Australia in two seasons each. All established

target pests were reliably detected in Adelaide, Melbourne, Hobart and Sydney. The *Varicorbula gibba* assay, however, displayed problems with specificity when applied to samples from Cairns and Darwin due to cross-reaction, probably with a native tropical corbulid (Deveney *et al.* 2017). Further field validation of the molecular methods was carried out by conducting parallel molecular and traditional surveys targeting six of the priority pests (all except *Varicorbula gibba*) in four ports (Gladstone, Brisbane, Melbourne and Hobart) over 2017 – 2018 (Wiltshire *et al.* 2019a). The parallel surveys demonstrated that the molecular approach is fit-for-purpose for marine pest surveillance. Molecular methods provide higher survey sensitivity than traditional methods, while requiring less than half the person-hours and 22 – 45% of the field cost of traditional surveillance. Detection likelihood of most of the target pests varied between seasonal sampling sets, with the highest likelihood of detection for the species with pronounced seasonal patterns of detectability corresponding to the known spawning season (Wiltshire *et al.* 2019a).

In addition to the assays for the seven priority pests for domestic ballast water management (Ophel-Keller *et al.* 2007; Bott *et al.* 2010a; Bott and Giblot-Ducray 2011a, b), SARDI has developed assays for three pests currently exotic to Australia but of concern for introduction: New Zealand (NZ) greenlip mussel (*Perna canaliculus*), black-striped mussel (*Mytilopsis sallei*) (see Bott and Giblot-Ducray 2011b; Bott *et al.* 2012), and charru mussel (*Mytella strigata*) (see Wiltshire *et al.* 2021b); and three pests that have established in parts of Australia and pose a risk of further spread: *Hemigrapsus sanguineus* (Asian shore crab) (Wiltshire *et al.* 2021a), *Maoricolpus roseus* (NZ screw shell) and *Mya japonica* (Japanese soft-shell clam) (Giblot-Ducray *et al.* 2022). Assays developed elsewhere for six additional exotic pests have also been implemented: the Andersen *et al.* (2018) Chinese mitten crab (*Eriocheir sinensis*) assay, and the Simpson *et al.* (2018) assays for Asian paddle crab (*Charybdis japonica*), Harris mud crab (*Rhithropanopeus harrisi*), brown mussel (*Perna perna*), Asian green mussel (*Perna viridis*) and carpet sea squirt (*Didemnum vexillum*).

For application to management, it is important to understand the performance of molecular surveys and any potential sources of error. Field validation (Deveney *et al.* 2017; Wiltshire *et al.* 2019a) quantified field efficiency of the molecular method and demonstrated that the overall performance of the system was high, but full validation of the system also requires an understanding of diagnostic performance for each molecular assay. This knowledge permits appropriate survey design, and, combined with appropriate quality assurance/quality control also provides confidence in results. Diagnostic performance has been assessed for all 19 priority pest

assays in SARDI's system (Wiltshire *et al.* 2019b; Wiltshire *et al.* 2021b; Wiltshire *et al.* 2022), allowing surveys to be designed to achieve a known confidence of detection for target pests.

A tool for designing molecular surveillance was developed by Wiltshire (2021), who analysed a compiled set of data from surveys using the method validated by Wiltshire *et al.* (2019a). The design tool calculates the required number of samples per location to achieve a target survey confidence (= likelihood of detection of target species in at least one sample from a survey) for a given planktonic pest concentration. The analysis also provided information on the planktonic pest concentration for areas with established or emerging pest populations and on seasonality for the species of ballast water concern in the locations where these occur. The seasonal analysis showed that sampling in a combination of winter (defined as July – September) and summer (January – March) would maximise detection likelihood across this suite of pests (Wiltshire 2021). Data are not available on the best season to sample for all other priority pests, but sampling in two, opposite, seasons is a good strategy to allow detection across species that reproduce in different seasons or where reproductive seasonality is unknown or variable (Rey *et al.* 2019; Wiltshire *et al.* 2019a).

Compounds that inhibit PCR, and may consequently impair detection by molecular methods, occur inconsistently in environmental samples (Schrader *et al.* 2012; Goldberg *et al.* 2016; Sidstedt *et al.* 2020). PCR inhibition can reduce detection likelihood and impair the performance of the marine pest assays in some cases, although the effects observed in SARDI testing have been generally minor, and detections have been recorded in plankton samples even with high levels of inhibition (Deveney *et al.* 2017; Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019b; Wiltshire *et al.* 2021b; Wiltshire *et al.* 2022). It is important, however, to assess PCR inhibition in environmental samples to identify cases where detection may be compromised.

Six ports were surveyed using molecular methods in 2021-22: Thevenard, Port Lincoln, Port Giles, Klein Point and Adelaide, South Australia (SA), and Portland, Victoria (Vic), with design informed by Wiltshire (2021) and following the methods applied in Wiltshire *et al.* (2019a). Two locations: Outer Harbor and Inner Harbor, were surveyed in the port of Adelaide, while other ports each comprised a single location. Plankton samples were collected in winter and summer at each location. Samples were tested for the seven pests for domestic ballast water management and for the 12 other priority pests for which assays are available. Testing included assessment of PCR inhibition and sampling quality assurance controls.

Results were analysed using Bayesian models that included effects of sampling volume, PCR inhibition and assay performance. This analysis provides an estimate of true prevalence and planktonic concentration for each target species, while accounting for known issues that affect detection. Outputs of the analysis were compared to the target planktonic pest concentration used for survey design to determine the confidence of occurrence for each species, including those that were not detected.

1.2. Objectives

- Apply molecular surveillance for priority marine pests to Thevenard, Port Lincoln, Port Giles, Klein Point, Adelaide, SA, and Portland, Vic;
- Map detections and compare occurrences to previous records of detected species;
- Assess results with consideration of test diagnostic performance, sampling volume, and PCR inhibition.
- Obtain further data on the seasonality of pest detections.

2. METHODS

2.1. Survey design

Survey design followed the guidelines developed by Wiltshire (2021) and considered the seven target species used for guiding ballast water management:

- Northern Pacific Seastar (*Asterias amurensis*)
- Asian Date Mussel (*Arcuatula senhousia*, formerly *Musculista senhousia*)
- European Green Crab (*Carcinus maenas*)
- Japanese kelp (*Undaria pinnatifida*)
- European Fan Worm (*Sabella spallanzanii*)
- Pacific Oyster (*Magallana gigas*, also known as *Crassostrea gigas*)
- Basket Shell Clam (*Varicorbula gibba*, formerly *Corbula gibba*)

The sample number calculator[1] developed by Wiltshire (2021) was used to determine the required number of samples per survey location. The calculator requires selection of a target planktonic pest concentration and survey confidence and specification of a minimum assay diagnostic sensitivity. Diagnostic sensitivity (DSe), the likelihood of an assay detecting target DNA when present in a sample, has been characterised for each of the 19 priority marine pest assays implemented in the SARDI system (Wiltshire *et al.* 2019b; Wiltshire *et al.* 2021b; Wiltshire *et al.* 2022) and ranges from 73 to 99%. The planktonic pest concentration is the number of discrete detectable units of target species DNA m^{-3}, with these detectable units ostensibly being larvae or other propagules (Wiltshire 2021). It should be noted, however, that other DNA sources (e.g. shed adult cells) may also be detected. The planktonic pest concentration is also different to the DNA concentration (in copies or mass of DNA per sample) because larvae, propagules and other detectable units will contain highly variable amounts of DNA. It is the concentration of planktonic pests, however, rather than the total DNA present, that determines the likelihood of capture in a sample and therefore detection (Wiltshire 2021).

[1] https://sardi-mar-biosec.shinyapps.io/surveydesign/

A target concentration of 0.0075 planktonic pests (pp) m^{-3} was used, with target survey confidence (= likelihood of detection of a target in at least one sample) of 80%, and minimum DSe of 73%, based on that of the *Undaria pinnatifida* assay (Wiltshire et al. 2019b), which has the lowest DSe of an assay applied in the survey. The target concentration chosen is in in the range relevant for detection of emerging incursions (Wiltshire 2021), and a confidence of 80% was selected because this is the default used in the survey design tool (Monitoring Design Excel Tool) that was used to design previous surveys (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c, 2020b). The selected target confidence and planktonic pest concentration were regarded as acceptable by the Department of Agriculture, Fisheries and Forestry, who commissioned the surveys. Using these parameters, 35 samples are required per location (sample number calculator). The 80% confidence is of detecting the target concentration, and the likelihood of detecting pests that occur at higher concentration (e.g. established species) is greater. This number of samples provides 99.5% confidence of detecting a concentration of 0.025 pp m^{-3}, which is at the lower end of the range observed for established pests (Wiltshire 2021)

2.2. Ports surveyed and sample locations

The ports of Thevenard, Port Lincoln, Port Giles, Klein point and Adelaide, SA, and Portland, Vic were surveyed in 2021-22. At each port, wharves where ballast exchange is likely to occur were targeted for surveillance. The analysis of Wiltshire (2021) suggested that samples should be collected within ~ 1 km around areas of interest, and hence areas of interest separated by more than 2 km should be considered as separate locations, with a separate set of samples collected in each. Two locations: Outer Harbor and Inner Harbor, were therefore surveyed in the port of Adelaide, while other ports comprised a single location each.

Electronic Navigational Chart (ENC) cells obtained from the Australian Hydrographic Office for each port were processed in ArcGIS 10.7.1 (Esri Inc.) to create shapefiles delimiting the survey area of interest for each location. For enclosed or partially enclosed ports (Portland, Inner and Outer Harbors Adelaide), survey areas were defined as being from the low tide line to a maximum distance 500 m from wharves of interest or to the harbour entrance, while for the remaining ports, the area was defined using the low tide line with a 500 m buffer around wharf areas used to define the outer (seaward) extent.

To assign proposed sample locations for plankton tows, we used the *samplePts* function from the *R* (R Core Team 2021) package *geospt* (Melo et al. 2012) to generate the required number of sample points using a hexagonal grid over the area of interest for each location. The area within

which points were placed was derived from a shapefile of the survey areas, but with a 50 m buffer around wharves and the shoreline (low tide mark) to reduce the likelihood of sample locations falling within areas that would be infeasible or inaccessible to sample. The proposed sample points for Adelaide are shown in Figure 1 and for the other ports in Figure 2.

2.3. Plankton sample collection

Plankton samples for molecular analysis were collected based on the methods developed by Giblot-Ducray and Bott (2013) and refined by Deveney *et al.* (2017). Sampling in Portland, Vic, was carried out by CTS Environmental, while SA samples were collected by SARDI Aquatic Sciences. A conical mesh plankton net with mouth diameter 0.5 m, length 1.5 m and 50 µm mesh (Sea-Gear 90-50x3-50 in SA and custom net made to the same specifications in Portland) fitted with a flowmeter (Sea-Gear MF315 in SA, General Oceanics 230R in Portland) was towed behind a vessel at a speed of ~1 – 1.5 m s^{-1} and depth of 0.5 – 1 m for a target distance of 100 m. After collection, plankton samples were concentrated 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 gel ice packs or refrigerator after collection and for delivery to the South Australian Aquatic Sciences Centre (SAASC) where they were stored in a cool room at ≤ 4 °C until processing (see section 2.5).

Proposed sampling points (see section 2.2) were provided as a guide only and could be altered if necessary due to field conditions, access or logistical constraints. In all cases, samples were distributed as evenly as possible through the sampling area, and plankton tow start and end waypoints were recorded with a handheld or vessel GPS. Sampling waypoints, plankton tow flowmeter readings, and notes pertaining to field conditions and individual samples were recorded for each sample set. In Portland, field data were recorded by CTS and provided to SARDI for compilation.

Five sampling quality controls (SQCs), used to check for sample degradation, were collected at the start of most sample sets. SQCs consisted of a 50 µL aliquot of *Artemia salina* (Ocean Nutrition™ Instant Baby Brine Shrimp; hereafter *Artemia*) in 80 mL preservation buffer to which 40 mL of seawater from the sampling location was added at the time sampling commenced. SQCs were then kept with subsequently collected plankton samples under the same storage conditions until processing. SQCs were not collected for the first sample sets in Portland or Thevenard due to unavailability of *Artemia* at the time of preparation of the sample jars for those sets.

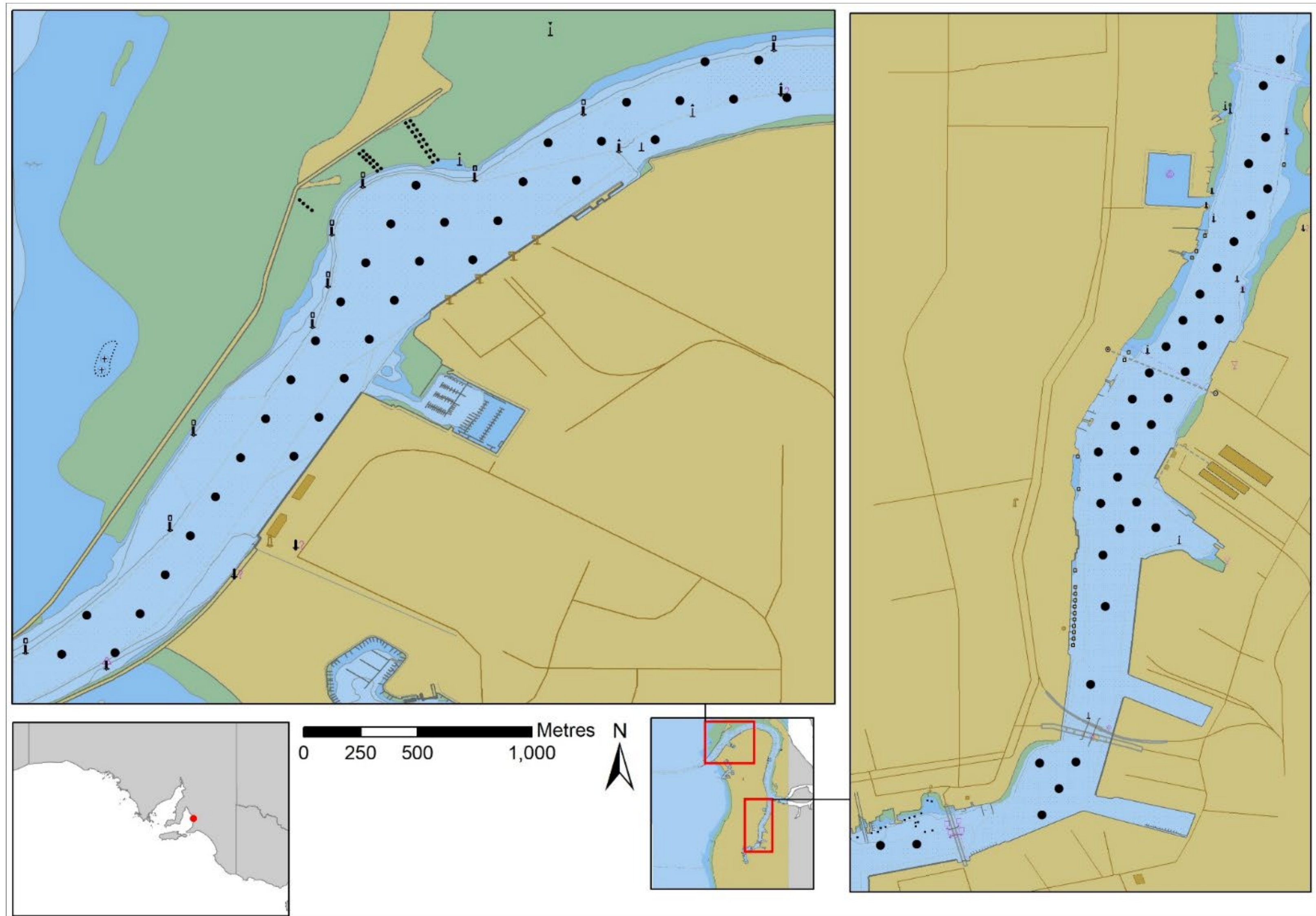

Figure 1. Proposed plankton sampling points in Adelaide: Outer Harbor (left) and Inner Harbor (right).

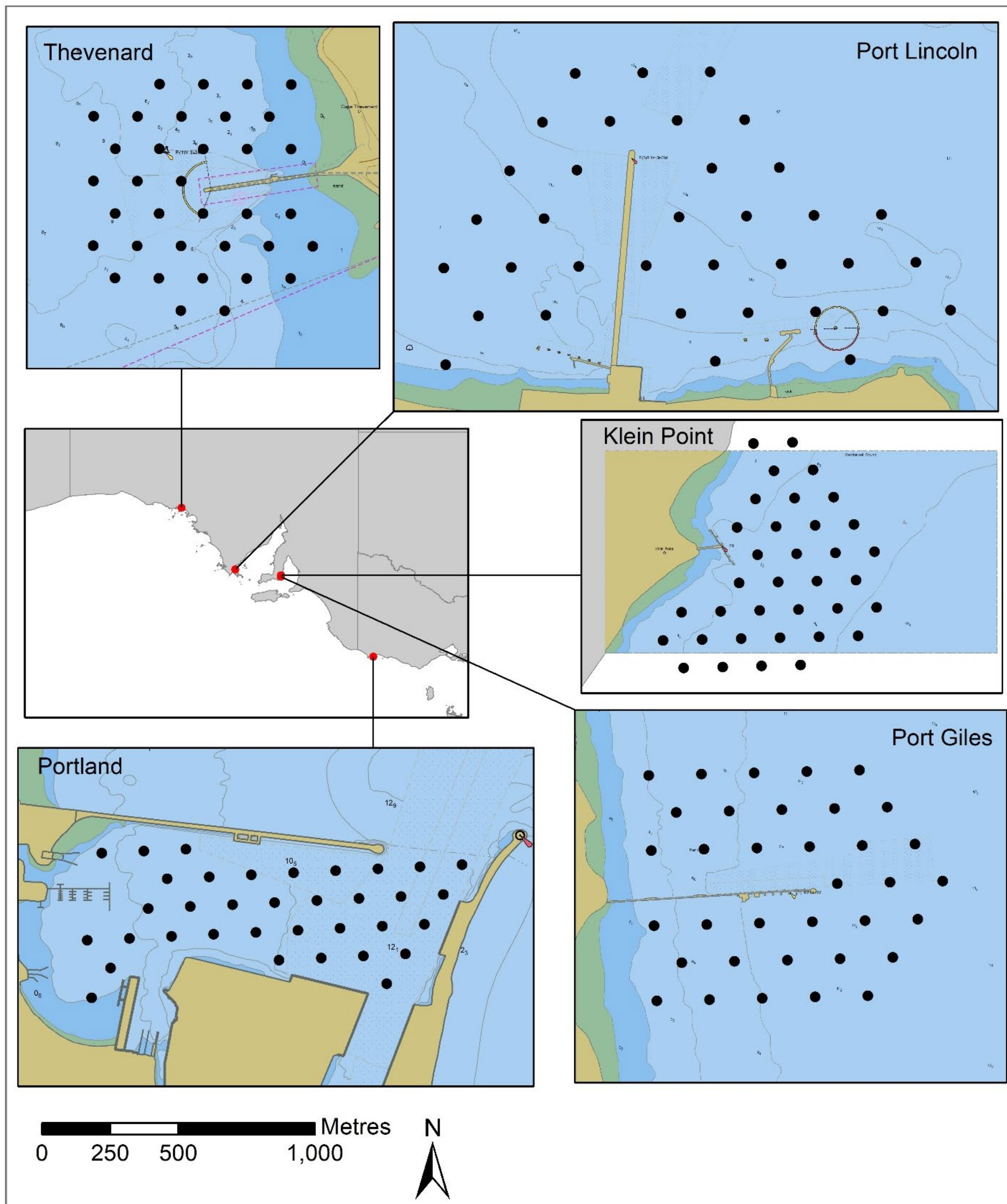

Figure 2. Proposed plankton sampling points for Thevenard, Port Lincoln, Klein Point and Port Giles, SA, and Portland, Vic.

2.4. Processing and analysis of molecular samples

Plankton samples were filtered in the laboratory at SAASC using a manifold and sterile single-use filter cups with 0.45 µm filters (Pall Microcheck® or Thermo Scientific™ Nalgene™). Filter papers were transferred to 50 mL centrifuge tubes, frozen at −20 °C and freeze-dried until completely dehydrated. DNA extraction and qPCR analysis were carried out by the SARDI Molecular Diagnostics laboratory. DNA was extracted using 20 mL of DNA extraction buffer containing an inhibition control (standardised quantity of an exogenous organism) per sample and physical disruption (Ophel-Keller *et al.* 2008). This extraction method has been demonstrated to be efficient and consistent in application to environmental samples (Haling *et al.* 2011). Final elution volume of the DNA was 160 µL in elution buffer. Each DNA extract was tested in singleplex quantitative polymerase chain reaction (qPCR) performed on ViiA 7 or QuantStudio7 real-time PCR systems (Applied Biosystems, Foster City, CA, USA) using species-specific qPCR assays for the target marine pests (Table 1) plus the inhibition control. Testing also included negative controls and the appropriate calibration standard for each target pest.

For each PCR analysis batch, reference samples known not to 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 in the reference samples to that detected in the sample. The scale factor for a sample is used as a multiplier to correct the apparent DNA yield of a sample as calculated from the C_T value for the effects of inhibition (Ophel-Keller *et al.* 2008).

An *Artemia* qPCR assay (Mackie and Geller 2010) was applied to DNA from SQC samples. *Artemia* yield from the SQCs was compared to that of two sets of laboratory control samples, which were prepared at the same time as preparation of SQCs for each sample set. The laboratory controls consisted of a 50 µL aliquot of *Artemia* in 80 mL preservation buffer with 40 mL filtered natural seawater added. One of the laboratory sets was stored at ≤ 4 °C while the other was stored at ambient indoor temperature at SAASC until processing. SQCs and laboratory control samples were processed immediately after the completion of processing of plankton samples from each set.

To avoid cross-contamination between samples from different locations, samples from different locations were processed on different days, and all benchtops and apparatus, including freeze-drier shelving, decontaminated using LookOut® DNA Erase between sample sets.

2.5. Mapping and analysis of results

Field data collected during plankton sampling and qPCR analysis results were compiled to link qPCR results to recorded field sampling locations. For results presented in this report, tow midpoint (average of start and end latitude and longitude) was used to map sample locations. Maps were generated using ArcGIS 10.7.1. (Esri Inc).

2.5.1. Spatio-temporal analysis

For each species with ≥ 5 total detections in at least one port, we applied spatial zero-added Gamma (ZAG) models to investigate patterns in detection likelihood and DNA yield. ZAG models consist of two components, a binary component (detected/non-detected) and a continuous component (DNA yield in samples with detection), with the latter modelled using the Gamma distribution, which is suitable for continuous, strictly positive, data (Zuur *et al.* 2017; Zuur and Ieno 2018). Spatial ZAG models account for spatial correlation by including spatial random fields in both model components.

ZAG models were fitted using a Bayesian hierarchical modelling approach with integrated nested Laplace approximations (Rue *et al.* 2009) for model inference and Matérn correlation structure using the stochastic partial differential equation (SPDE) approach (Lindgren *et al.* 2011) for spatial effects. Models were run with the *R-INLA* package (Martins *et al.* 2013; Lindgren and Rue 2015; Rue *et al.* 2017) using *R* statistical software v4.1.0 (R Core Team 2021) and following Zuur and Ieno (2018). We applied barrier models (Bakka *et al.* 2018; Bakka *et al.* 2019) to account for coastal features and prevent smoothing of spatial effects over land. A complementary log-log (cloglog) link was used for the binary component with the response being detection/non-detection, and the default log link was used for the Gamma distribution of the continuous component, with the response variable being DNA yield calculated from the assay standard curve before correction for inhibition.

Table 1. Assays for priority marine pests available in the SARDI testing system and reference for each assay's development. ‡Species considered in risk tables for domestic ballast water management (Zhao *et al.* 2012). †Species on the Australian priority marine pest list[2]. *Species on the National Priority List of Exotic Environmental Pests, Weeds and Diseases[3].

Species	Common name	Assay name	Assay development reference
Arcuatula (= *Musculista*) *senhousia*‡	Asian Bag Mussel	Asen	Bott and Giblot-Ducray (2011b)
Perna canaliculus†*	NZ greenlip mussel	Pcan	Bott and Giblot-Ducray (2011b)
Perna perna†*	Brown mussel	Pper	(Simpson *et al.* 2018)
Perna viridis†*	Asian green mussel	Pvir	(Simpson *et al.* 2018)
Mytella strigata (= *M. charruana*)†	Charru mussel	Mstr	(Wiltshire *et al.* 2021b)
Mytilopsis sallei†*	Black-striped false mussel	Msal	(Bott *et al.* 2012)
Varicorbula (=*Corbula*) *gibba*‡	European basket shell	Vgib	Bott and Giblot-Ducray (2011b)
Magallana (= Crassostrea) *gigas*‡	Pacific Oyster	Mgig	(Bott and Giblot-Ducray 2012)
Mya japonica	Japanese soft-shell clam	Mjap	(Giblot-Ducray *et al.* 2022)
Maoricolpus roseus	New Zealand screw shell	Mros	(Giblot-Ducray *et al.* 2022)
Asterias amurensis†‡	Northern Pacific Sea star	Aamu	(Bott *et al.* 2010a)
Carcinus maenas†‡	European shore crab	Cmae	(Bott and Giblot-Ducray 2011a)
*Charybdis japonica**	Asian paddle crab	Cjap	(Simpson *et al.* 2018)
Rhithropanopeus harrisii†*	Harris mud crab	Rhar	(Simpson *et al.* 2018)
*Hemigrapsus sanguineus**	Asian shore crab	Hsan	(Wiltshire *et al.* 2021a)
Eriocheir sinensis†*	Mitten crab	Esin	(Andersen *et al.* 2018)
Sabella spallanzanii‡	European fanworm	Sspa	(Ophel-Keller *et al.* 2007)
*Didemnum vexillum**	Carpet sea squirt	Dvex	(Simpson *et al.* 2018)
Undaria pinnatifida†‡	Japanese kelp	Upin	(Bott and Giblot-Ducray 2011a)

[2] https://www.marinepests.gov.au/what-we-do/apmpl
[3] https://www.agriculture.gov.au/biosecurity-trade/policy/environmental/priority-list#marine-pests

A separate model was run for each species. For this analysis, we considered the two Adelaide locations as being a single port, with differences between these captured in the spatial field. Data was included only for ports where ≥ 5 detections occurred across sample sets. Where a species had five or more detections at more than one port, port was included as a fixed factor in both model components. Where models included data from > 2 ports, differences between ports were assessed using post-hoc tests implemented with the *inla.make.lincombs* function following Gómez-Rubio (2020). Factor levels were considered significantly different where 95% credible intervals of the difference between posterior estimates of those factors did not contain zero. For species with at least one detection in each set (season), sample set was applied as a fixed factor in both model components, otherwise, data was only included for the sample set with detections. PCR inhibition was included in models using the natural logarithm of scale factor (lnSF) as a fixed effect in both components. The natural logarithm of scale factor was used, as per Wiltshire *et al.* (2019a), to account for the multiplicative effect of scale factor on DNA yield. In the absence of inhibition, scale factor = 1, and hence lnSF = 0 for these samples.

For the spatial field of each model, we used penalised complexity priors (Fuglstad *et al.* 2019) with probability of range < 1 km and of standard deviation > 1 set to 0.05. The predicted spatial range of each model is the distance at which the estimated Matérn correlation between predictions is ~ 0.1 (Bakka *et al.* 2019), i.e. the distance beyond which predictions are effectively independent. The resulting spatial field shows the spatial random effect (standard deviation around mean predictions). Spatial fields for the two components of each model were mapped by using the *inla.mesh.project* and *inla.mesh.projector* functions to generate a grid of model predictions across the survey area, the *raster* package (Hijmans 2021) to create rasters from prediction grids, and ArcGIS 10.7.1 (Esri Inc.) to compile maps.

Gaussian priors with mean zero and precision of 0.025 were used for fixed effects in the binary component. This precision was chosen because it provides 95% confidence that changes to the linear predictor are < |12|, with resulting probability being between ~$5E^{-6}$ (effectively zero) and ~1, preventing numerical overflow. Default Gaussian priors were used for fixed effects in the continuous component of the model, except for lnSF, where we used a mean of -1 and precision of 4, This reflects the fact that reduction in pest DNA yield in response to inhibition is expected to be the same as for the inhibition control, but allows for some variation, specifically, this prior indicates 95% probability that the log scale factor effect on DNA yield is between −0.02 and −1.98. Fixed effects were considered to be statistically significant where the 95% credible interval of the effect did not include zero.

2.5.2. Estimated planktonic pest concentration

In environmental surveillance, prevalence is the likelihood that a sample will contain a target, i.e., the frequency of occurrence of target DNA in plankton samples. Apparent prevalence is the proportion of samples returning a positive detection of a target, but where assay diagnostic performance has been assessed, an estimate of true prevalence, which adjusts apparent prevalence for possible false positives and false negatives, can be made (Speybroeck *et al.* 2013; Devleesschauwer *et al.* 2014). Diagnostic performance has been characterised for each of the assays implemented in the SARDI system (Wiltshire *et al.* 2019b; Wiltshire *et al.* 2021b; Wiltshire *et al.* 2022), and these data were used to estimate true prevalence in previous molecular surveillance (Wiltshire *et al.* 2020b).

Site occupancy detection models (SODM) expand on the true prevalence modelling approach to infer the likelihood of species occurrence at both the site and individual sample level (Chambert *et al.* 2015; Guillera-Arroita *et al.* 2017). Bayesian analysis provides estimates with credible bounds (based on 95% of the posterior probability), given the number of samples and assay performance (Speybroeck *et al.* 2013). The upper credible limit shows the maximum plausible prevalence or likelihood of site occurrence, which provides the basis for Bayesian proof of freedom approaches for species that are not detected (Low-Choy 2013; Stanaway 2015). SODM approaches proposed for analysis of molecular survey data (Guillera-Arroita *et al.* 2017; Tingley *et al.* 2020; Diana *et al.* 2021), however, do not incorporate some factors that may affect detection, such as sample volume, which influences the likelihood of capture for planktonic pests, and PCR inhibition, which may decrease effective assay sensitivity in individual samples. Incorporating information on individual sample volume into models also allows planktonic pest concentration to be estimated (Royle and Dorazio 2009; Wiltshire 2021), permitting direct comparison with the target concentration used for survey design.

A Bayesian SODM approach (detailed in section 2.5.3) was therefore developed to estimate planktonic pest concentrations from patterns of detection while also correcting likelihood of detection for assay performance, including inhibition. The outputs of the model provide an estimated planktonic concentration (pp m^{-3}) for each pest at each location, and a multiplicative difference in concentration between sample sets for each pest. The estimated planktonic pest concentration should be interpreted as per the survey design, i.e. as the concentration of discrete detectable units (or particles) of DNA, with these discrete units being larvae, other propagules, shed adult cells or DNA adsorbed to particulate matter, rather than the total concentration (copies

or mass) of DNA. The mean and 95% highest density interval (HDI) of the estimated planktonic pest concentration in each case show the most likely value and plausible range (i.e. there is 95% confidence that true concentration lies in this range). Mean and 95% HDI limits of predicted concentration for each species and location in each sample set were compared to the target concentration used for survey design (see section 2.1), i.e., 0.0075 pp m^{-3}, to determine where detected pests were likely to occur at greater than this threshold concentration. Results were categorised as shown in Table 2.

Table 2. Categories of pest occurrence based on SODM results. Mean, lower HDI and upper HDI are of the predicted planktonic pest concentration, with each compared to the threshold concentration of 0.0075 pp m-^{3}.

Mean	Lower HDI	Upper HDI	Classification of pest concentration
< 0.0075	< 0.0075	< 0.0075	Confidently below threshold
< 0.0075	< 0.0075	≥ 0.0075	Probably below threshold
≥ 0.0075	< 0.0075	≥ 0.0075	Probably above threshold
≥ 0.0075	≥ 0.0075	≥ 0.0075	Confidently above threshold

2.5.3. SODM approach

SODM code was built on published true prevalence and SODM code that accounts for both potential false negatives and false positives (Devleesschauwer *et al.* 2014; Chambert *et al.* 2015). The SODM code was modified to estimate concentration from sample volume and prevalence as per Wiltshire (2021), but using adjusted rather than apparent prevalence. The code was also modified to allow simultaneous estimation of prevalence and concentration for multiple species and locations, and to include the effect of inhibition on DSe. Beta priors were used for the DSe intercept of each assay (i.e. DSe in the absence of inhibition), and for diagnostic specificity (DSp), with beta parameters calculated using the *prevalence* package *betaExpert* function (Devleesschauwer *et al.* 2014). For the assays assessed by Wiltshire *et al.* (2019b), i.e., Aamu, Came, Mgig, Sspa and Upin, priors were assigned based on results from from using the lowest (most conservative) estimate in each case. Priors for DSe and DSp of the Mstr assay were based on results of Wiltshire *et al.* (2021b), and for remaining assays on the results of Wiltshire *et al.* (2022). The effect of inhibition, as measured by the scale factor, on effective DSe in each sample was modelled using a cloglog link and the natural logarithm of scale factor (lnSF) as per Wiltshire *et al.* (2022). The coefficient of the lnSF effect was given a normal prior with mean of -0.3 and precision of 100, based on the mean lnSF effect across assays and variation in the effect between assays found by Wiltshire *et al.* (2022). Prevalence (= likelihood of a sample containing target

DNA) was related to modelled log concentration with a cloglog link as per Wiltshire (2021), including a coefficient to estimate concentration separately for each sample set. For each species and location, the intercept of log concentration was given a vague normal prior with mean zero and precision of 0.001, indicating 95% confidence that this parameter is < |62|. The sample set effect for each species was given a normal prior with mean zero and precision of 0.01, indicating 95% confidence that this parameter is < |20|. The higher precision for the seasonal effect reflects the fact that species detected commonly in only one of the two sample sets are believed to have been present, but at a lower than detectable planktonic concentration, in the other sample set. Parameters on the cloglog scale were bounded by < |12|, to prevent numerical overflow. This limits probability of species DNA occurrence in a sample (prevalence) to between $6.1E^{-6}$ and 1.

The SODM uses the volume sampled by each plankton tow to infer concentration from prevalence. Sample volume was calculated from the net dimensions and tow length as recorded by the flowmeter. Flowmeter data was however missing for some samples from each sample set and from the winter sample set from Port Lincoln (see results section 3.4). A regression analysis was therefore used to impute missing flow meter distances, using location, sample set, tow length as measured by GPS, and filtered sample wet weight as predictors. Linear regression was run with *R-INLA* (Martins *et al.* 2013; Lindgren and Rue 2015; Rue *et al.* 2017), and fitted values from this model were used to calculate tow volume for any sample with missing flow meter data. The natural logarithm of tow volume was used in the model to estimate concentration as an offset following Wiltshire (2021). Estimated true prevalence of each species at each location and sample time was determined using the model estimate of concentration and the average sample volume the relevant sample set at that location.

The SODM analysis was run in JAGS (Plummer 2017) via the *R2jags* package (Su and Yajima 2015) with 10 000 MCMC iterations thinned at a rate of 10, following 40 000 for burn-in. Convergence was assessed using the Gelman-Rubin convergence statistic, and confirmed by visual inspection of trace, density and autocorrelation plots generated using the *MCMCplots* package (McKay Curtis 2015). HDIs demonstrating 95% of the probability mass for posterior estimates were calculated using the *HDIinterval* package (Meredith and Kruschke 2018).

3. RESULTS

3.1. Samples collected and analysed

The sampling dates for each location and sample set are shown in Table 3. In contrast to some recent surveys where a subset of collected samples were processed and analysed (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c), all collected samples were analysed. Winter – spring sampling (hereafter ''winter') took place in September – October 2021, when water temperatures in southern Australia are typically at their lowest, while summer – autumn sampling ('summer') took place in February – March 2022. During collection of samples in Portland on 2nd March 2022, the net was torn after ten samples had been collected, preventing collection of the remaining samples until the net could be repaired. The other 25 Portland summer samples were collected on 27th March 2022.

Table 3. Sample dates for each sampling event.

Port (Sublocation)	Winter - Spring	Summer - Autumn
Adelaide (Inner Harbor)	27 Sep 2021	17 Feb 2022
Adelaide (Outer Harbor)	24 Sep 2021	10 Mar 2022
Klein Point	29 Sep 2021	3 Mar 2022
Port Giles	8 Oct 2021	2 Mar 2022
Port Lincoln	10 Sep 2021	15 Feb 2022
Thevenard	22 Sep 2021	23 Feb 2022
Portland	9 – 10 Sep 2021	2 & 27 Mar 2022

3.2. PCR inhibition, plankton tow sampling volume and sample dry weight

PCR inhibition, as measured by scale factor, occurred in some samples from most sampling events, but was typically negligible (scale factor < 2; Figure 3). Minor inhibition, defined as scale factors 2 – 5, occurred in a small number of samples from Inner Harbor (one per sample set) and Klein point (two in winter), and in winter samples from Outer Harbor (11) and from Port Giles (14). Moderate to high inhibition (scale factor > 5) occurred in four summer samples from Inner Harbor, where the highest inhibition (scale factor 3810) across the surveys was recorded, and 15 winter samples from Port Giles, where the maximum scale factor was 87.4 (Table 4). The majority of winter samples from Port Giles, however, had scale factor < 10 (Figure 3).

No detections were recorded in the two samples from Inner Harbor that had scale factor > 100. The level of inhibition in other samples is unlikely to have prevented detection, with previous

studies demonstrating that most of the assays are not severely affected by inhibition at scale factors < 10 or even to ~ 100 (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019b; Wiltshire *et al.* 2022).

Table 4. Number of samples with minor inhibition (scale factor 2 – 5) and moderate to high inhibition (scale factor >5) and maximum scale factor for each sample set.

Sample location and time	Samples with minor inhibition	Samples with moderate – high inhibition	Maximum scale factor
Inner Harbor Summer	1	4	3810
Inner Harbor Winter	1	0	2.5
Outer Harbor Summer	0	0	1.9
Outer Harbor Winter	11	0	3.3
Klein Point Summer	0	0	1.5
Klein Point Winter	2	0	2.6
Port Giles Summer	0	0	1.9
Port Giles Winter	14	15	87.4
Port Lincoln Summer	0	0	1.4
Port Lincoln Winter	0	0	1.6
Thevenard Summer	0	0	1.6
Thevenard Winter	0	0	1.5
Portland Summer	0	0	1.3
Portland Winter	0	0	1.0

Tow distances calculated from start and end GPS coordinates were generally close to or just above the target of 100 m (Figure 4). Effective tow volumes calculated from flow meter distances were similar across locations and sample sets (Figure 4). A tow of 100 m length would sample a volume of 19.6 m^3 if 100% filtration efficiency is assumed, but tow volumes were mainly < 10 m^3, indicating that net clogging occurred. A few samples had volume of > 20 m^3, which can occur if samples are collected against currents. Effective tow volumes were slightly lower than the average volume (8.6 m^3) but within the range of previous (2015 – 2020) sampling, as calculated by Wiltshire (2021). Flow meter data was not obtained for the Port Lincoln winter samples due to failure of the flow meter, but no excessive net clogging was observed. Tow volume for this sample set for use in calculating planktonic pest concentration was estimated by regression (see section 2.5.2), and this estimate is shown in Figure 4 for comparison.

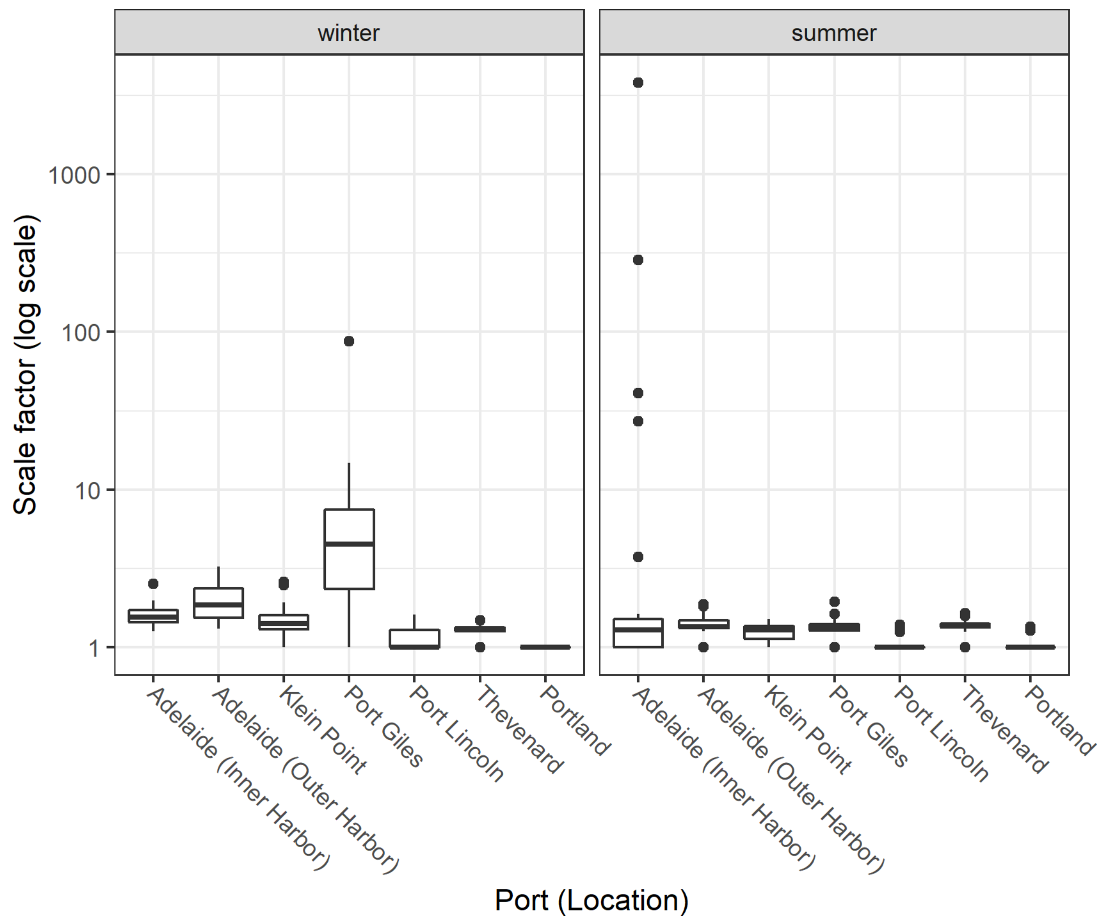

Figure 3. Boxplot of scale factor by port and collecting event.

Sample wet and dry weights were generally lower in summer than winter but similar between locations within each sample set. In winter, however, sample wet and dry weights were somewhat higher in Outer Harbor and Portland than other locations (Figure 5). Sample weights were within typical ranges of previous sampling, although the mass of summer samples was at the lower end of the previously observed range (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c, 2020b).

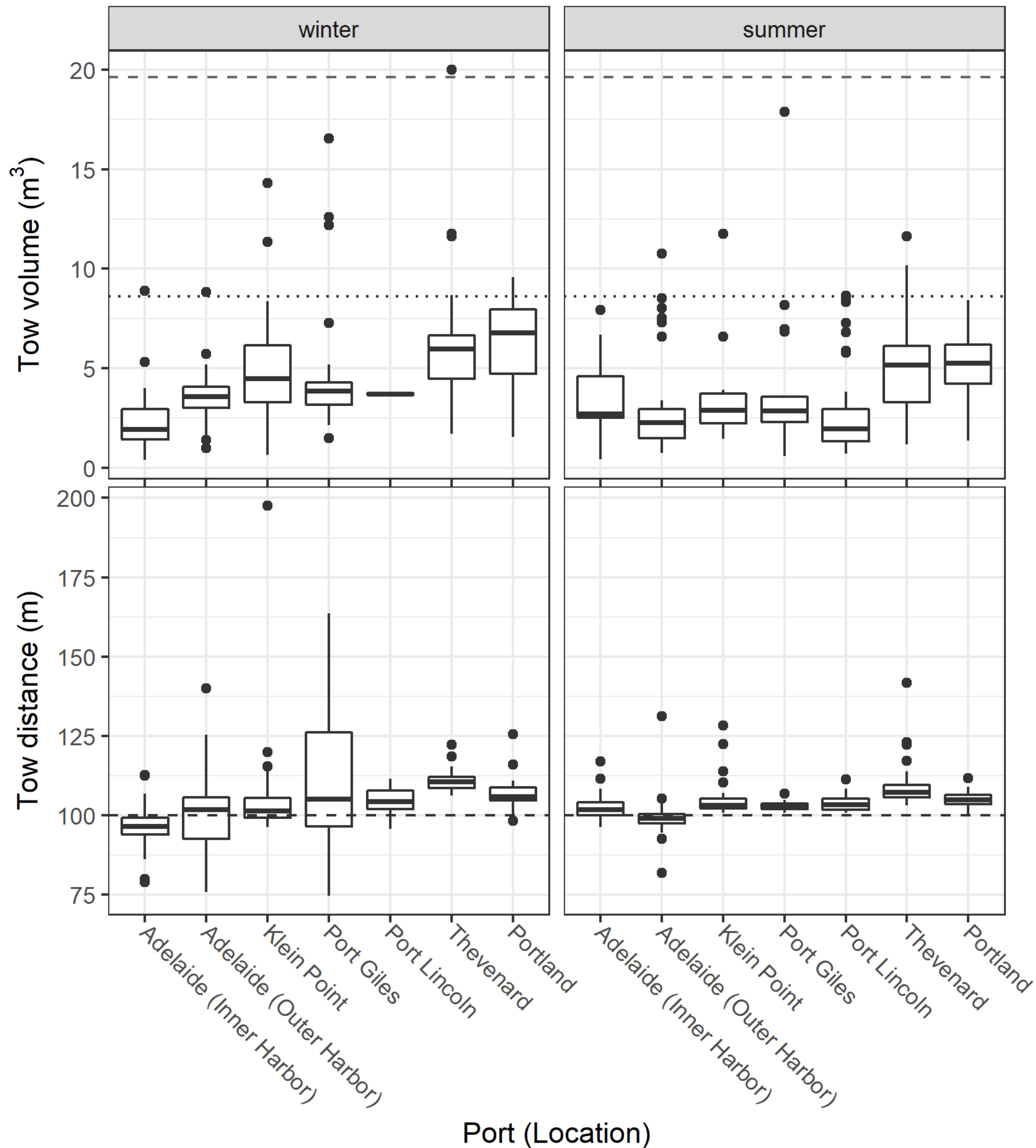

Figure 4. Boxplot of sample water volume based on flow meter readings (top panel) and of tow distance based on GPS coordinates (bottom panel), by location and collecting event. Dashed lines show the volume that would be collected by a 100 m tow with no net clogging (top panel) and a tow distance of 100 m (bottom panel). Dotted line in top panel shows average tow volume from previous surveillance as determined by Wiltshire (2021). Winter tow volume in Port Lincoln was estimated as described in section 2.5.2.

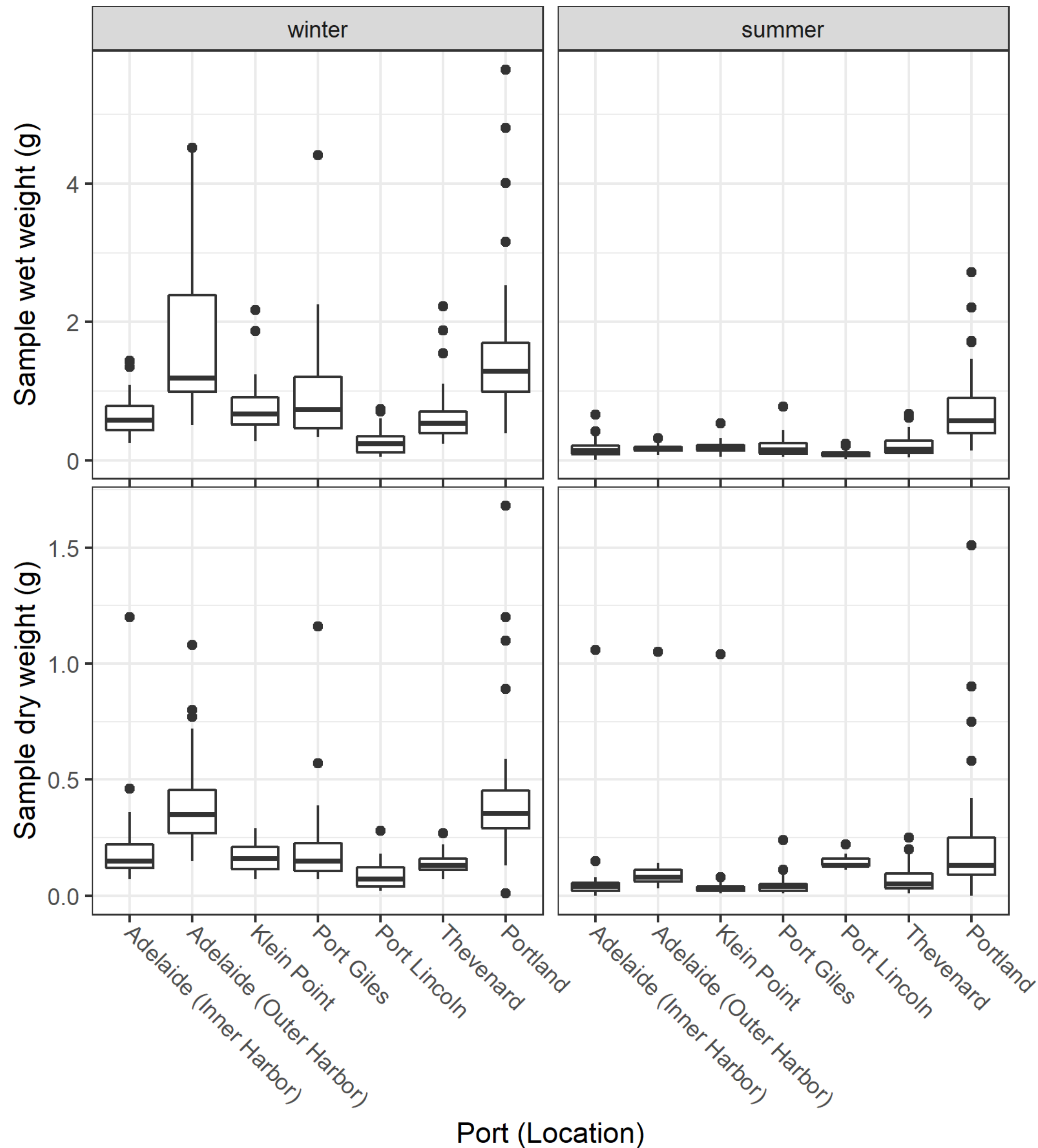

Figure 5. Boxplot of filtered sample wet weight (top panel) and dry weight (bottom panel) by port and collecting event.

Artemia yield from field SQC samples was similar to that of laboratory controls in all cases indicating that there was no evidence of sample degradation in the relevant sample sets. Normal *Artemia* yield and low PCR inhibition indicate that sample preservation and storage effects would not compromise detections.

3.3. Species detections

3.3.1. Maps of sample locations and detection results

Maps of sample points and detections by location and sample set are shown in Figures 6 – 14. In each map, the size of each sample point is scaled to the total number of species detected. Detections in each sample are coloured by assay, with codes as in Table 1; multiple species detections are shown as a pie-chart with a different coloured segment per assay, while samples with no detection appear in black.

3.3.1. Detections by location and sample set

Carcinus maenas, Magallana gigas and *Sabella spallanzanii* were all widely detected in Adelaide in both Inner Harbor and Outer Harbor (Figures 3 - 6). Detections of *Carcinus maenas* and *Sabella spallanzanii* occurred predominantly in the winter samples, while detections of *Magallana gigas* were more common in summer. There were four detections by the Vgib assay in Outer Harbor samples from summer, and one detection by the Mstr assay in a winter sample from Inner Harbor.

At Klein Point, *Carcinus maenas* and *Sabella spallanzanii* were detected in two samples each, with the Cmae detections in winter (Figure 10) and Sspa detections in summer (Figure 11). The Vgib assay returned three detections from Klein Point, one in winter and two in summer, and there were also two detections by the Mjap assay and one by the Mros assay in summer samples. In Port Giles, there was one detection each by the Mstr and Mjap assays in winter samples, and no detections in summer samples.

Arcuatula senhousia and *Magallana gigas* were widely detected in summer samples from Thevenard (Figure 13), with one Mgig detection in winter (Figure 12). *Sabella spallanzanii* was widely detected in winter samples from Port Lincoln, with two summer detections. There were several Vgib detections in both Thevenard and Port Lincoln, particularly in summer samples. One Mjap detection was recorded in a winter sample from Port Lincoln.

In Portland, *Undaria pinnatifida* was widely detected in both sample sets (Figures 10, 11), and *Sabella spallanzanii* was widely detected in the winter samples, with two detections in summer. There were 12 Mros detections in winter and nine in summer samples. A total of five Vgib detections were recorded in Portland samples, comprising two from winter and three from summer. Two Mgig detections were recorded in summer.

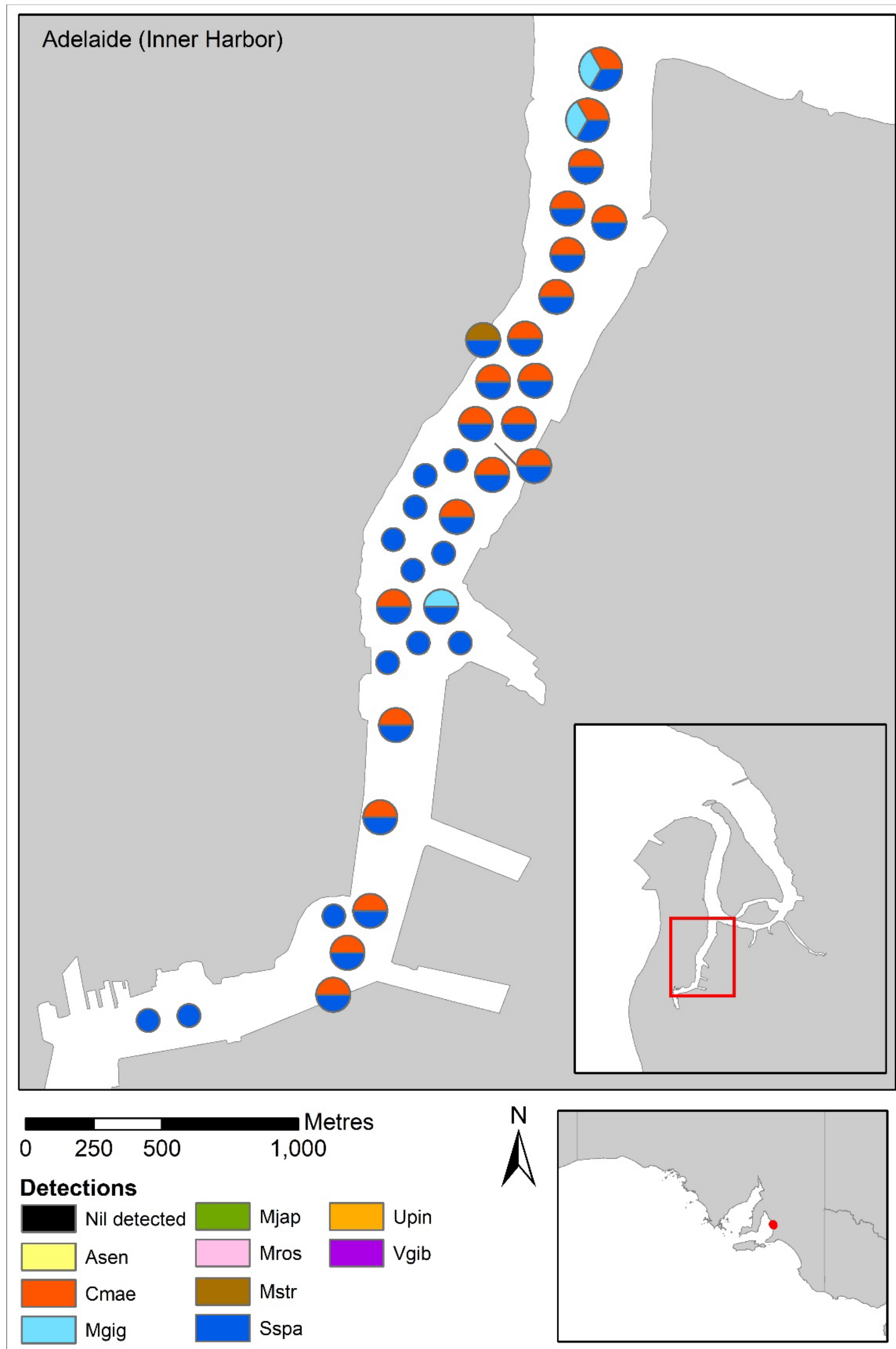

Figure 6. Map of sample locations and detections for Adelaide Inner Harbor in winter. See Table 1 for species (assay name) codes.

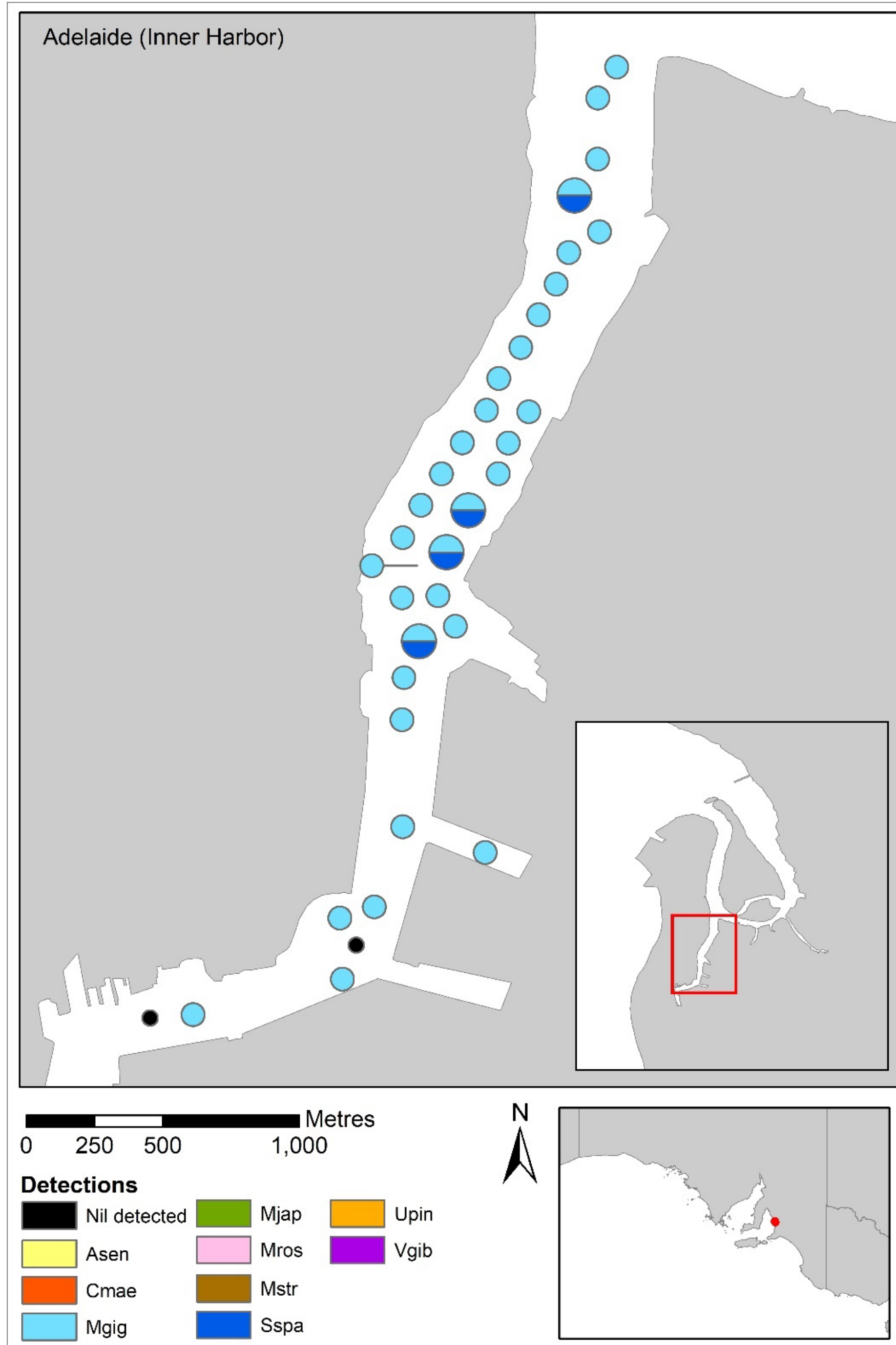

Figure 7. Map of sample locations and detections for Adelaide Inner Harbor in summer. See Table 1 for species (assay name) codes.

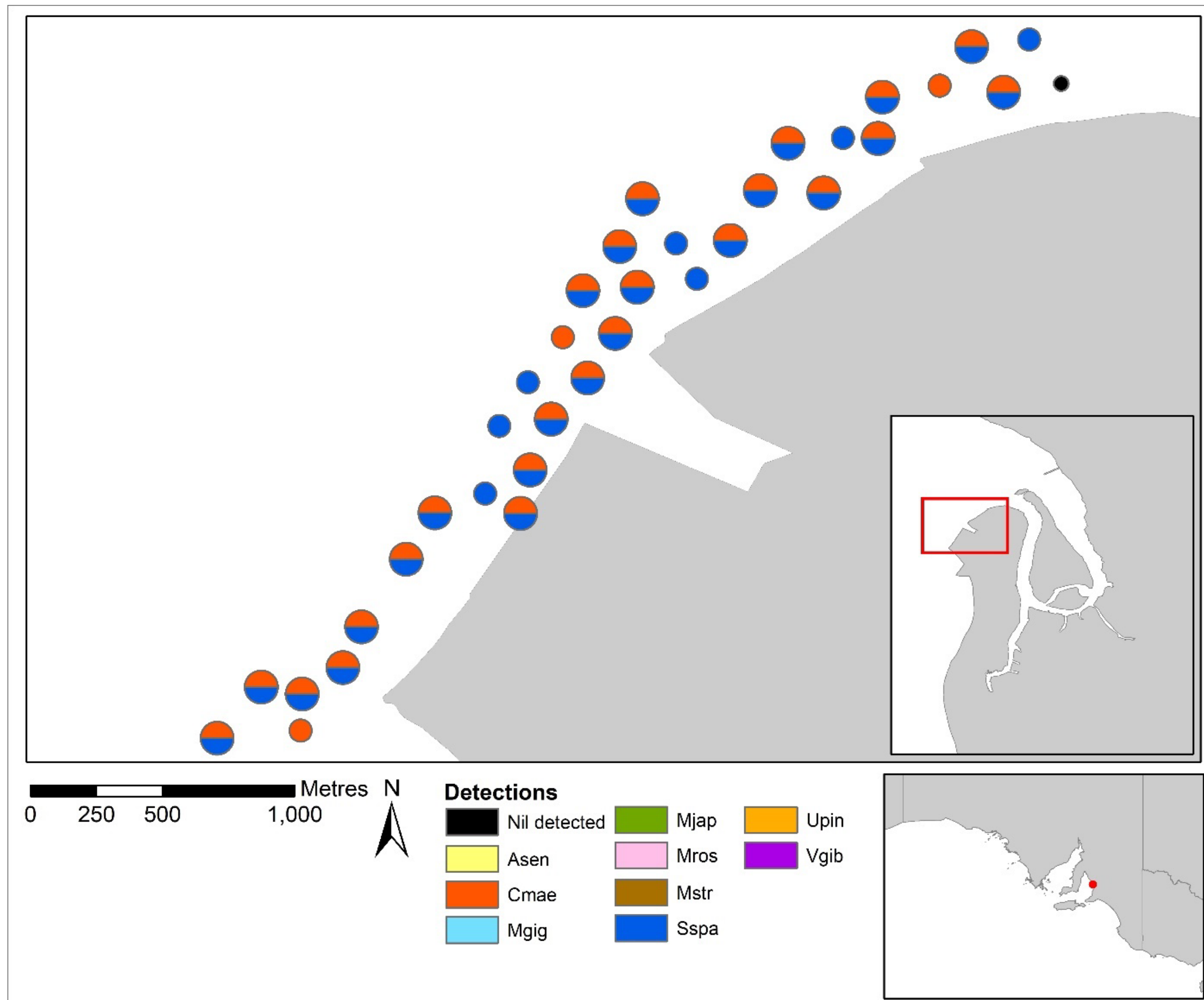

Figure 8. Map of sample locations and detections for Adelaide Outer Harbor in winter. See Table 1 for species (assay name) codes.

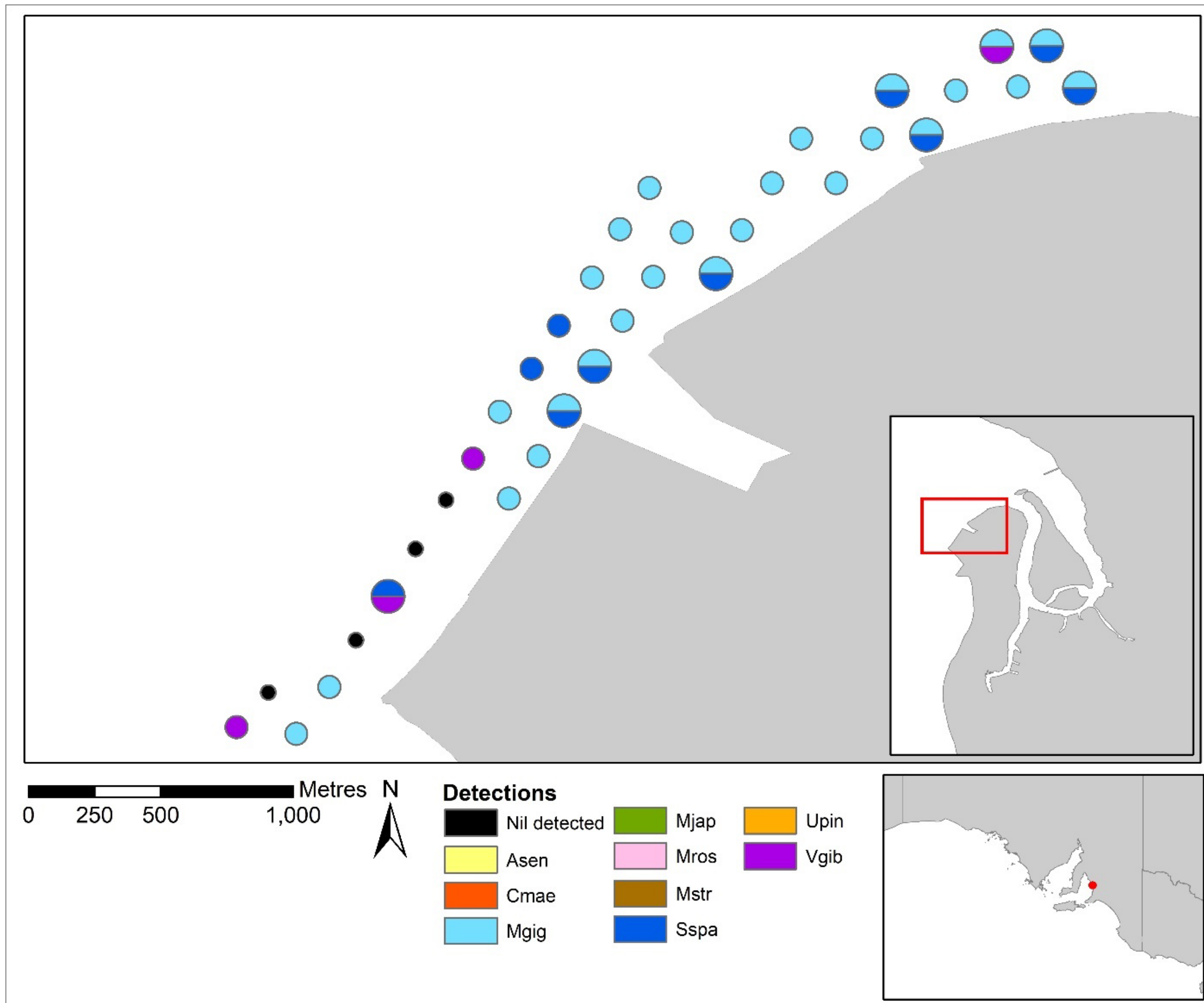

Figure 9. Map of sample locations and detections for Adelaide Outer Harbor in summer. See Table 1 for species (assay name) codes.

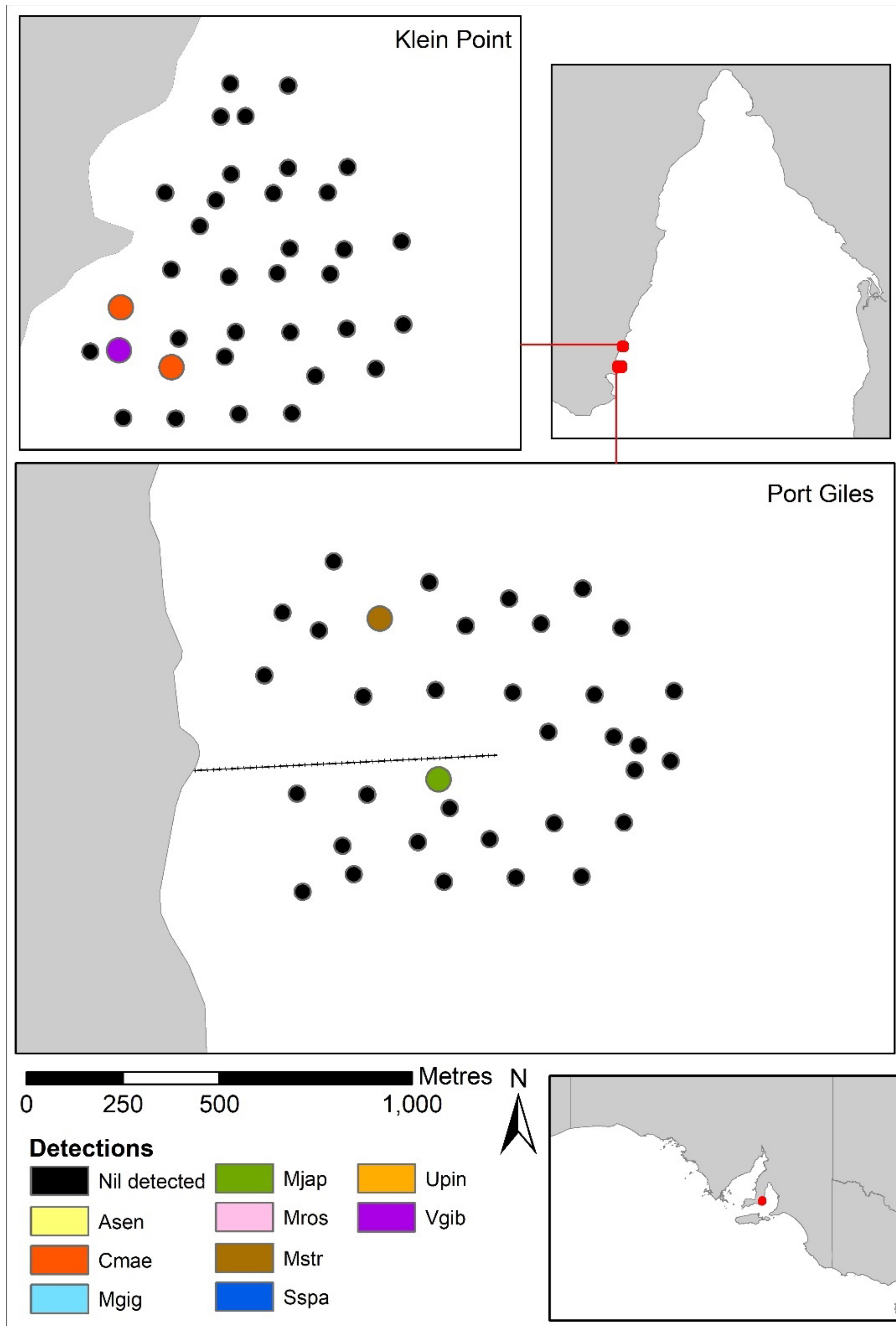

Figure 10. Map of sample locations and detections for Klein Point (top) and Port Giles (bottom) in winter. See Table 1 for species (assay name) codes.

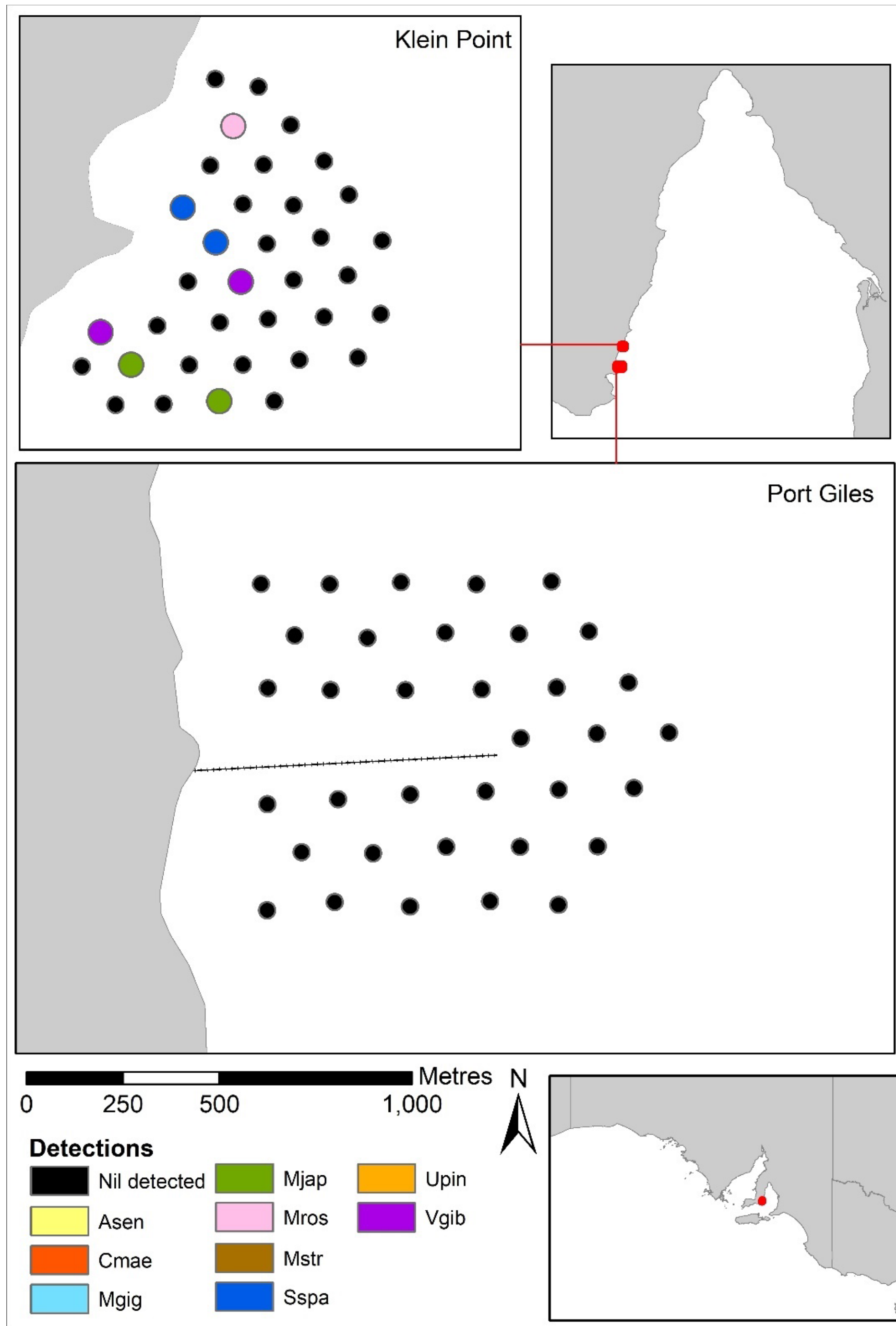

Figure 11. Map of sample locations and detections for Klein Point (top) and Port Giles (bottom) in summer. See Table 1 for species (assay name) codes.

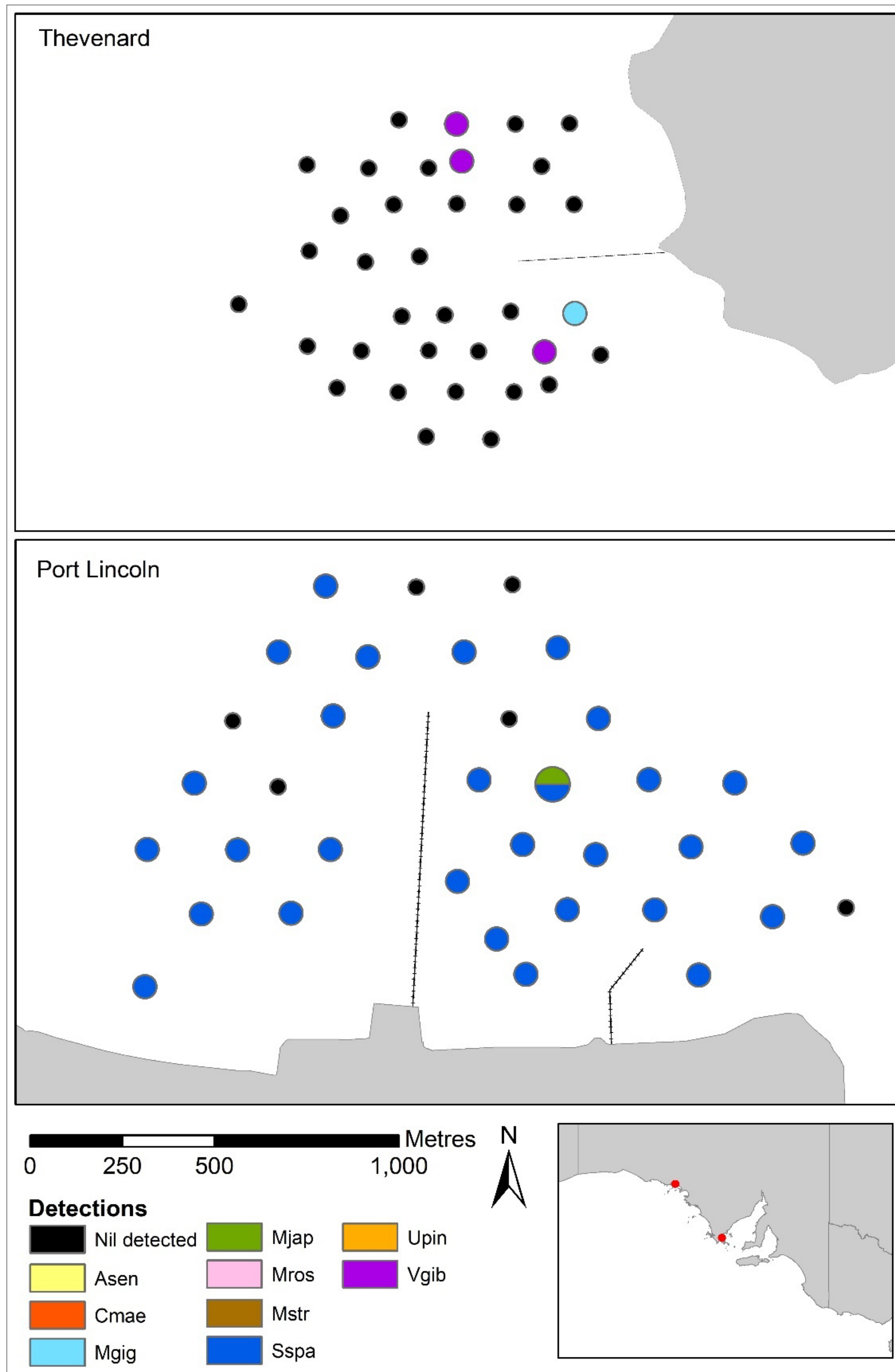

Figure 12. Map of sample locations and detections for Thevenard (top) and Port Lincoln (bottom) in winter. See Table 1 for species (assay name) codes.

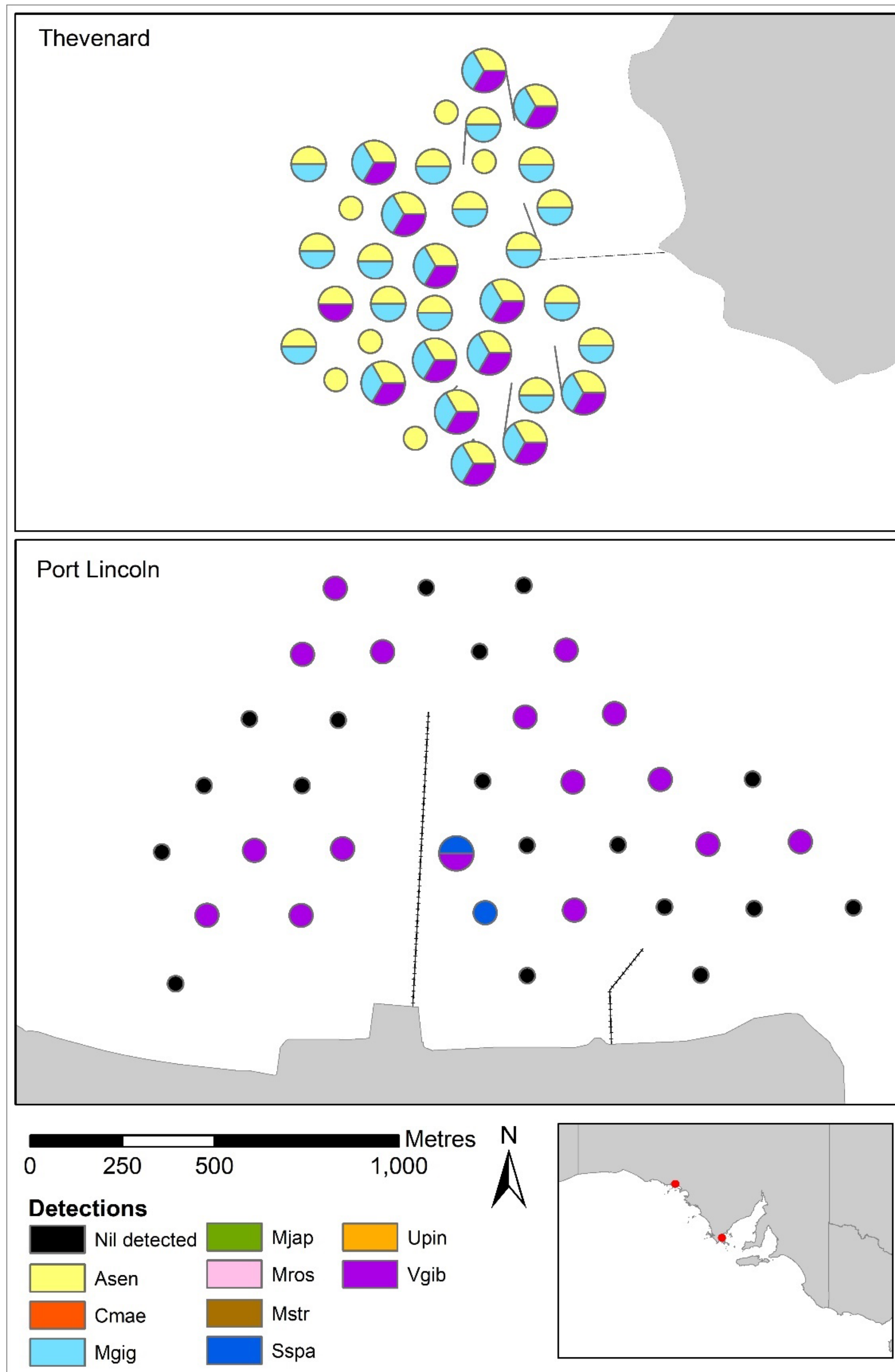

Figure 13. Map of sample locations and detections for Thevenard (top) and Port Lincoln (bottom) in summer. See Table 1 for species (assay name) codes.

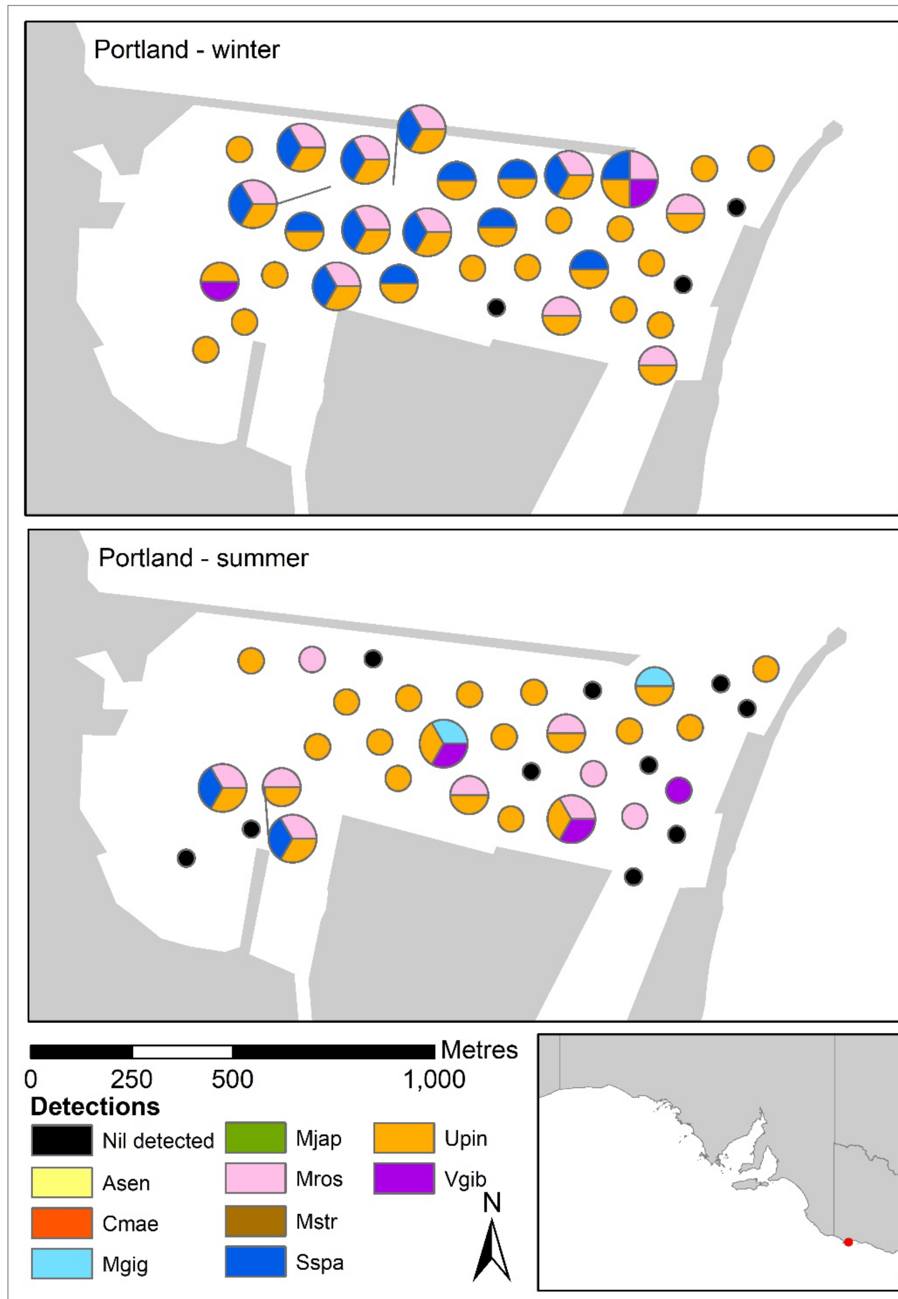

Figure 14. Map of sample locations and detections for Portland in winter (top) and summer (bottom). See Table 1 for species (assay name) codes.

3.4. Spatio-temporal patterns in detection and DNA yield

ZAG models were run for six species, being those with more than five detections across sample sets in at least one port. For these models, data was included for each port with > 5 detections and from each sample set with at least one detection. The species, and the ports and sample sets included in each model, were: *Arcuatula senhousia* in Thevenard for summer only;

Carcinus maenas in Adelaide for winter only; *Magallana gigas* in Adelaide and Thevenard for both sample sets; *Sabella spallanzanii* in Adelaide, Port Lincoln and Portland for both sample sets; and *Maoricolpus roseus* and *Undaria pinnatifida* in Portland for both sample sets. Species detected in Adelaide all had detections in both Inner and Outer Harbors. The models did not include separate Adelaide locations as factors, but the spatial effect illustrates differences between these locations within Adelaide. In each case the spatial field shows variation around the model average, i.e., after accounting for the fixed effects of port and sample set where included.

Arcuatula senhousia was detected in all summer samples from Thevenard, therefore, no effects on detection likelihood could be estimated, and the binary component was excluded from the model, making this a Gamma regression rather than ZAG model. Scale factor, which was the only fixed effect in this model given detections were from a single port and sample set, did not have a significant effect on DNA yield (Table 5). The spatial field showed lower DNA yield near the jetty and along the shipping channel, with high DNA yield in samples to the south of the survey area (Figure 15).

Table 5. Parameter estimates of Gamma regression investigating scale factor effect on DNA yield for *Arcuatula senhousia.*

Model component: effect	mean estimate (95% HDI)
DNA yield: Intercept	6.35 (5.53 – 7.18)
DNA yield: log(scale factor)	1.18 (−0.80 – 3.12)

There was no effect of scale factor on either binary or continuous model components in the ZAG model for *Carcinus maenas* (Table 6). This species had > 5 detections only in Adelaide in winter. The spatial field of detection likelihood was high in Outer Harbor and to the northern end of Inner Harbor, and low in central Inner Harbor and at the western end of the upper Port River (Figure 16). The spatial field for DNA yield (Figure 17) was similarly high at the northern end of Inner Harbor and low in central Inner Harbor. At Outer Harbor, the spatial field of DNA yield was more homogenous, but with generally higher values closer to the wharves and marina on the southern side of the channel (Figure 17).

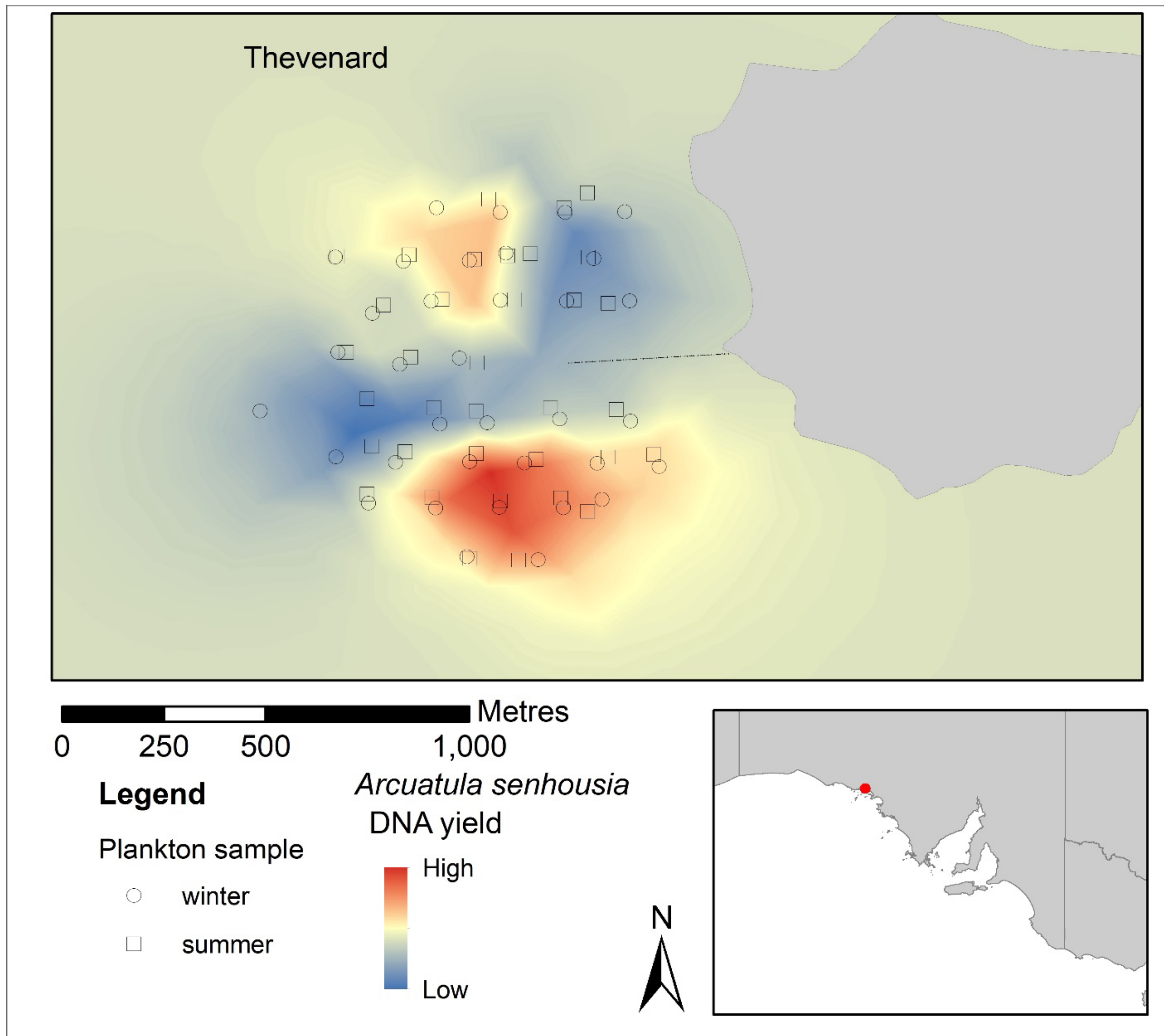

Figure 15. Spatial field of DNA yield for *Arcuatula senhousia* in Thevenard.

Table 6. Parameter estimates of ZAG model investigating scale factor effect on detection likelihood and DNA yield for *Carcinus maenas.*

Model component: effect	mean estimate (95% HDI)
Detection likelihood: Intercept	0.51 (-0.58 – 1.67)
Detection likelihood: log(scale factor)	−0.39 (−2.02 – 1.15)
DNA yield: Intercept	5.42 (3.62 – 7.15)
DNA yield: log(scale factor)	−0.38 (−2.54 – 1.71)

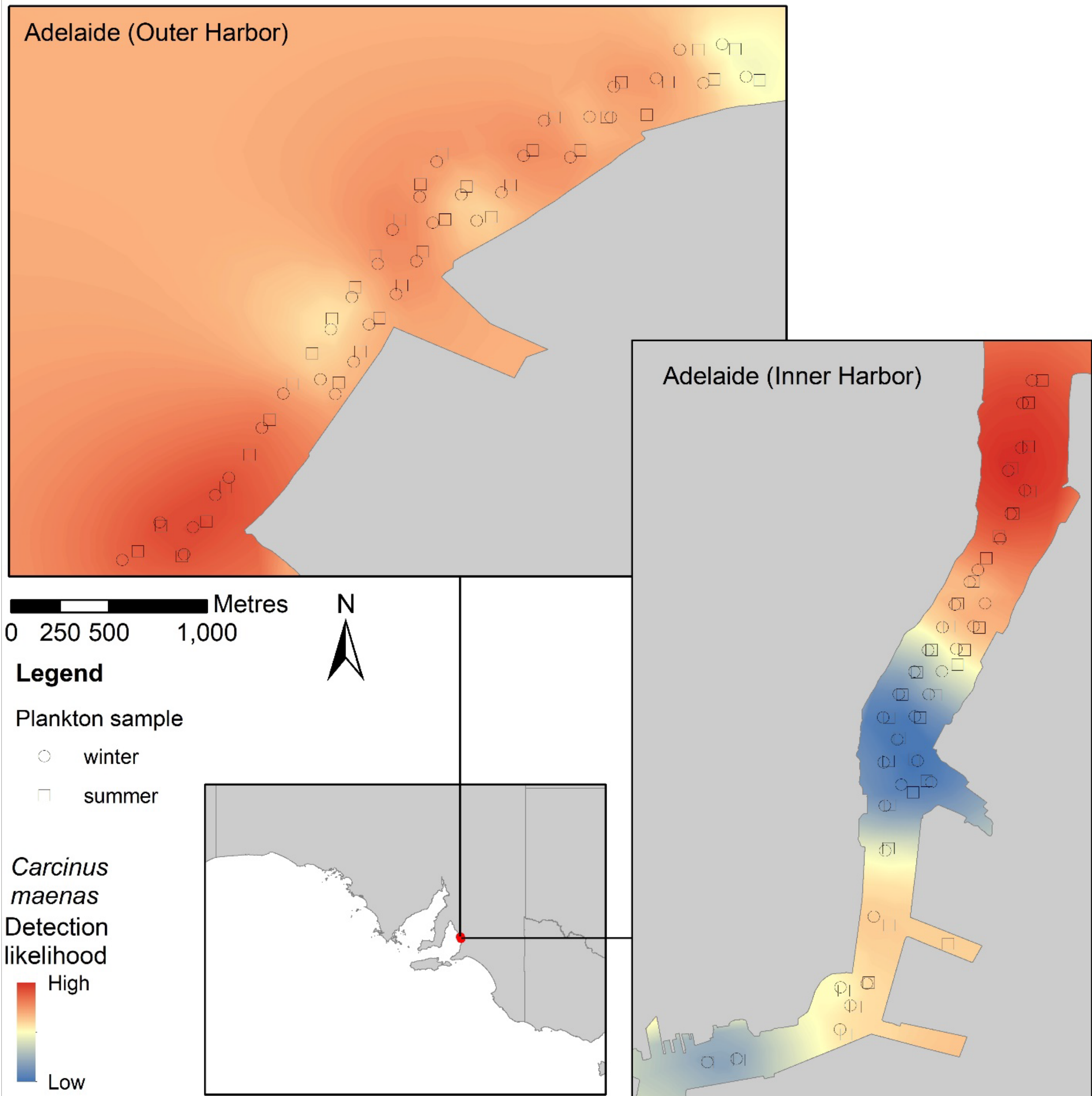

Figure 16. Spatial field of detection likelihood for *Carcinus maenas* in Adelaide.

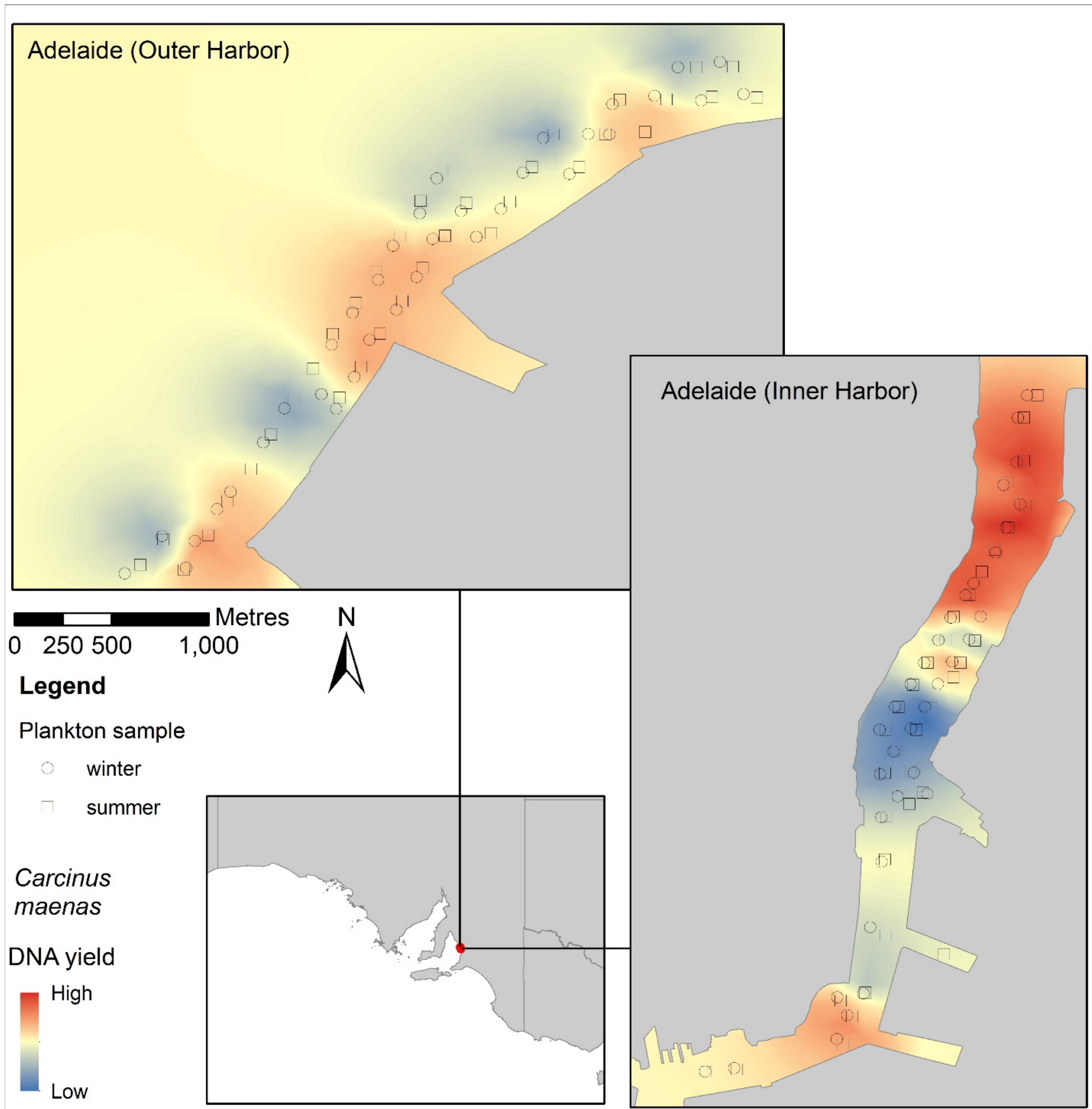

Figure 17. Spatial field of DNA yield for *Carcinus maenas* in Adelaide.

The ZAG model for *Magallana gigas* showed a negative scale factor effect on detection likelihood, i.e. a lower probability of detection at higher scale factors, but there was no scale factor effect on DNA yield (Table 7). There was no significant difference in either detection likelihood or DNA yield between the ports of Thevenard and Adelaide, but both were higher in the summer than winter sample set (Table 7). The spatial field showed higher than model average detection likelihood from central to northern Inner Harbor, with lower detection likelihood to the south of Inner Harbor

and in Outer Harbor (Figure 18). There was little variation, however, across the Thevenard survey area. The spatial field for DNA yield showed a similar, although less pronounced, pattern (Figure 19).

Table 7. Parameter estimates of ZAG model investigating scale factor, sample set and port effect on detection likelihood and DNA yield for *Magallana gigas.* *Parameter statistically different from zero.

Model component: effect	**mean estimate (95% HDI)**
Detection likelihood: Intercept	−3.38 (−6.21 – −0.55)
Detection likelihood: log(scale factor)	−0.58 (−1.22 – −0.11)*
Detection likelihood: Thevenard - Adelaide	−1.60 (−8.12 – 4.03)
Detection likelihood: summer - winter	5.43 (3.89 – 7.49)*
DNA yield: Intercept	2.26 (0.58 – 3.98)
DNA yield: log(scale factor)	−0.42 (−1.04 – 0.24)
DNA yield: Thevenard - Adelaide	−0.51 (−3.29 – 2.25)
DNA yield: summer - winter	3.35 (2.29 – 4.26)*

For *Sabella spallanzanii,* detection likelihood and DNA yield were both lower in summer than winter, but there was no significant effect of scale factor in either model component (Table 8). Detection likelihood was lower in Portland than in both Adelaide and Port Lincoln, while other differences in either parameter between ports were not significant (Table 8). The spatial field for detection likelihood showed a peak in western Portland Harbor with higher than model average values also inshore at Port Lincoln, central Inner Harbor, and near the marina entrance at Outer Harbor (Figure 20). Highest values for the DNA yield spatial field were in Inner Harbor, with lower values at Outer Harbor and offshore at Port Lincoln (Figure 21).

The ZAG model for *Maoricolpus roseus*, which had > 5 detections only in Portland, showed higher DNA yield in summer than winter, but no significant difference in detection likelihood between sample sets (Table 9). There was no significant effect of scale factor in either model component (Table 9). The detection likelihood spatial field showed highest values to the north-west of the harbour, while highest values for the DNA yield spatial field were to the south-east (Figure 22).

Detection likelihood and DNA yield of *Undaria pinnatifida* in Portland were higher in winter than summer, with no significant effect of scale factor in either component (Table 10). The spatial fields for both model components showed higher values to the west of the harbour, particularly for detection likelihood (Figure 23).

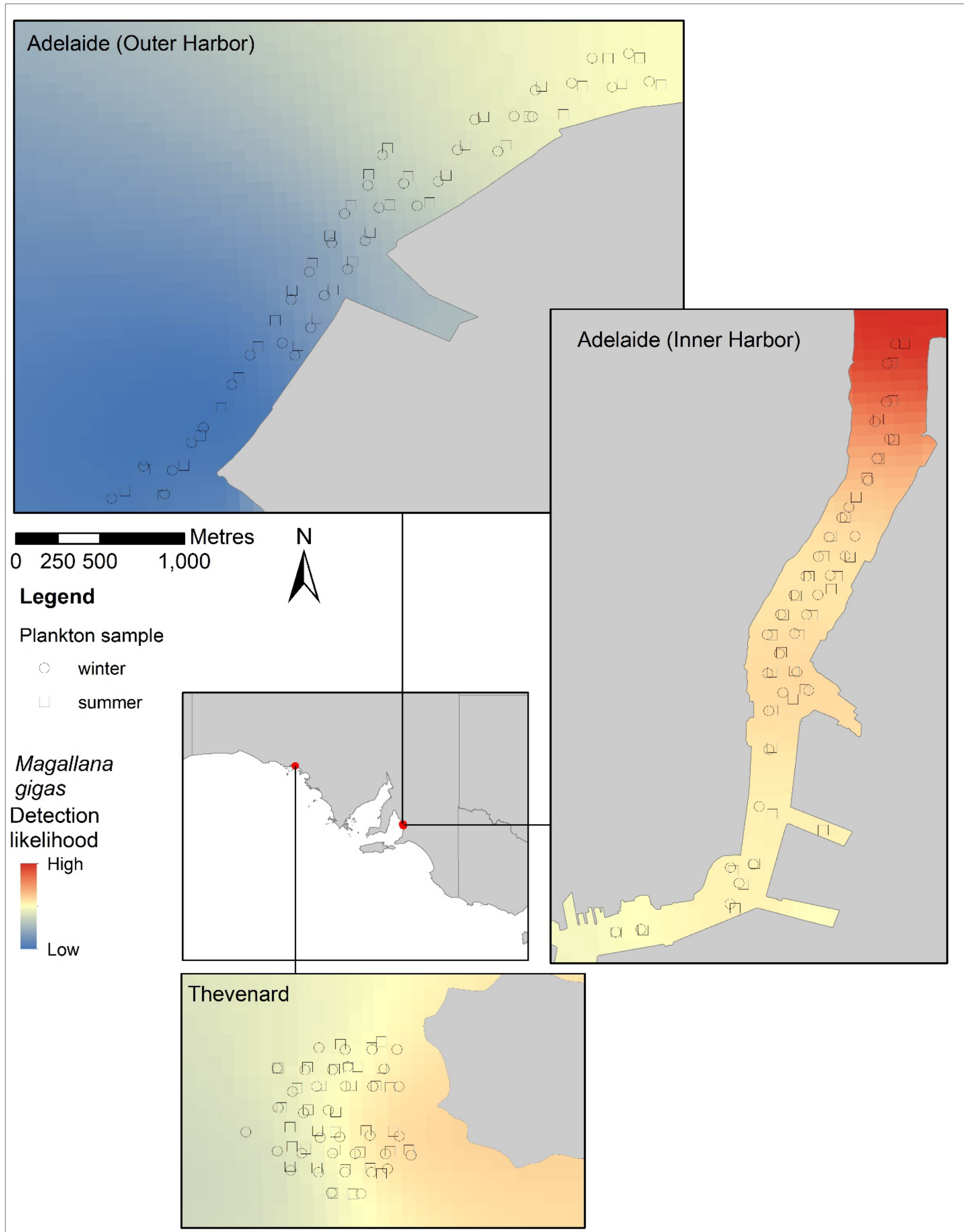

Figure 18. Spatial field of detection likelihood for *Magallana gigas* in Adelaide and Thevenard.

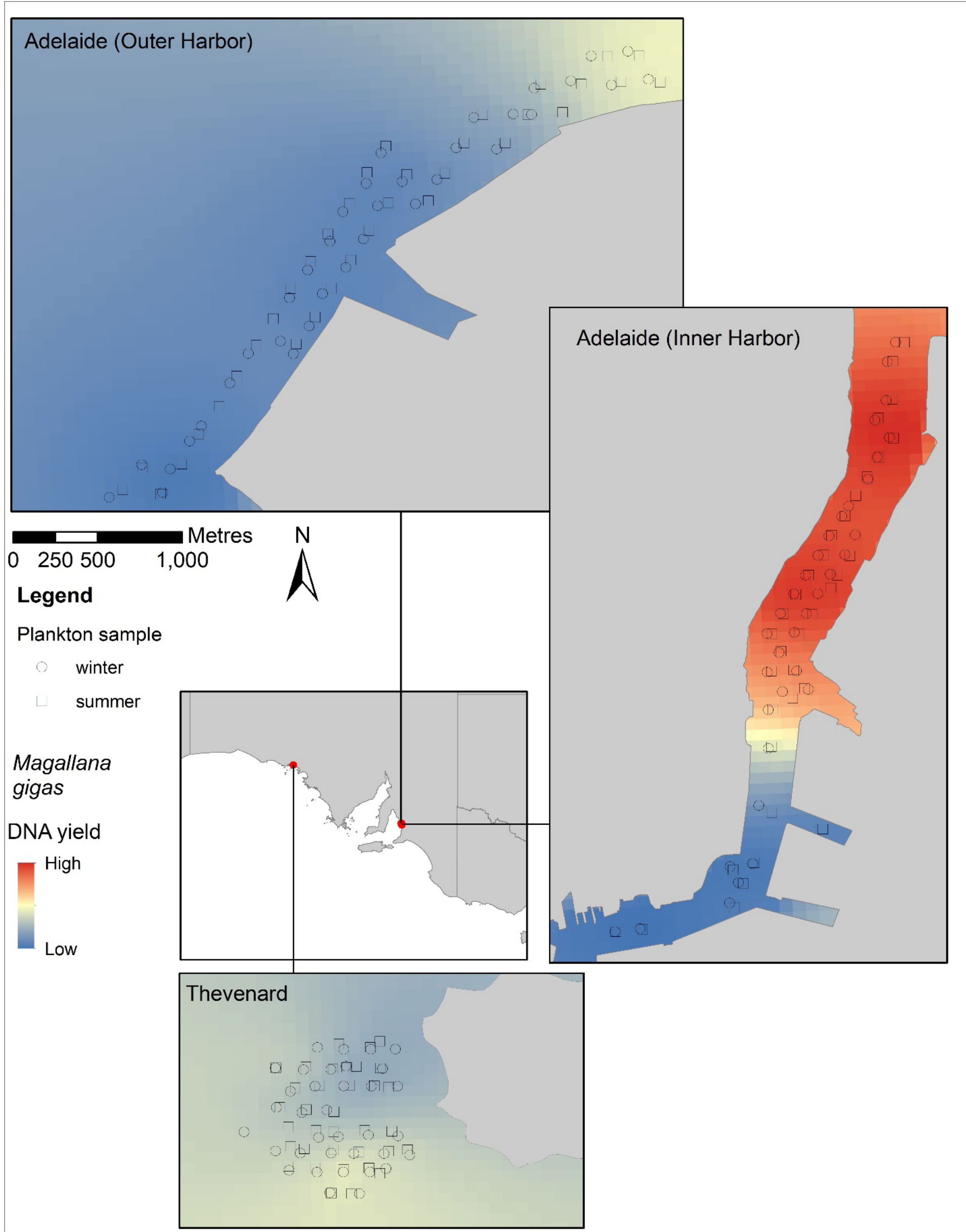

Figure 19. Spatial field of DNA yield for *Magallana gigas* in Adelaide and Thevenard.

Table 8. Parameter estimates of ZAG model investigating scale factor, sample set and port effect on detection likelihood and DNA yield for *Sabella spallanzanii.* *Parameter statistically different from zero.

Model component: effect	**mean estimate (95% HDI)**
Detection likelihood: Intercept	1.32 (0.79 – 2.00)
Detection likelihood: log(scale factor)	-0.29 (-1.11 – 0.30)
Detection likelihood: Port Lincoln - Adelaide	-0.67 (-1.37 – 0.04)
Detection likelihood: Portland - Adelaide	-1.98 (-3.06 – -1.14)*
Detection likelihood: Port Lincoln - Portland	1.31 (0.49 – 2.37)*
Detection likelihood: summer - winter	-2.74 (-3.36 – -2.21)*
DNA yield: Intercept	2.41 (1.23 – 3.73)
DNA yield: log(scale factor)	-0.32 (-1.39 – 0.77)
DNA yield: Port Lincoln - Adelaide	0.70 (-0.79 – 2.35)
DNA yield: Portland - Adelaide	-0.06 (-2.64 – 2.43)
DNA yield: Port Lincoln - Portland	0.76 (-1.62 – 3.41)
DNA yield: summer - winter	-1.33 (-1.87 – -0.77)*

Table 9. Parameter estimates of ZAG model investigating scale factor and sample set effect on detection likelihood and DNA yield for *Maoricolpus roseus.* *Parameter statistically different from zero.

Model component: effect	**mean estimate (95% HDI)**
Detection likelihood: Intercept	-0.95 (-1.99 – -0.17)
Detection likelihood: log(scale factor)	4.46 (-2.11 – 10.15)
Detection likelihood: summer - winter	-0.53 (-1.50 – 0.38)
DNA yield: Intercept	1.25 (-0.37 – 2.58)
DNA yield: log(scale factor)	2.21 (-2.73 – 7.70)
DNA yield: summer - winter	1.41 (0.55 – 2.32)*

Table 10. Parameter estimates of ZAG model investigating scale factor and sample set effect on detection likelihood and DNA yield for *Undaria pinnatifida.* *Parameter statistically different from zero.

Model component: effect	**mean estimate (95% HDI)**
Detection likelihood: Intercept	0.95 (-0.32 – 2.11)
Detection likelihood: log(scale factor)	3.85 (-2.05 – 10.16)
Detection likelihood: summer - winter	-1.32 (-2.23 – -0.57)*
DNA yield: Intercept	5.54 (4.41 – 6.48)
DNA yield: log(scale factor)	2.11 (-1.78 – 6.48)
DNA yield: summer - winter	-1.13 (-1.65 – -0.58)*

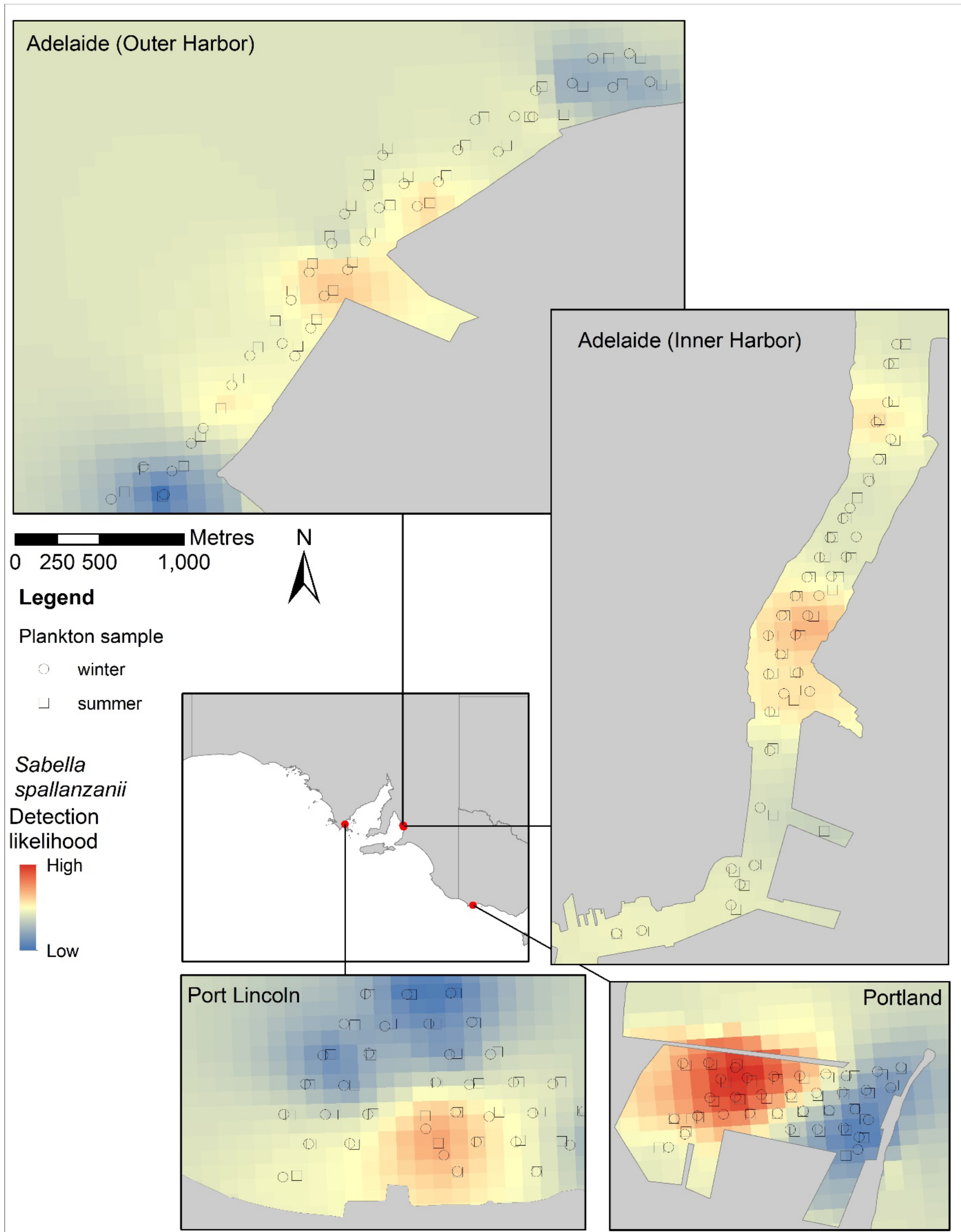

Figure 20. Spatial field of detection likelihood for *Sabella spallanzanii* in Adelaide, Port Lincoln and Portland.

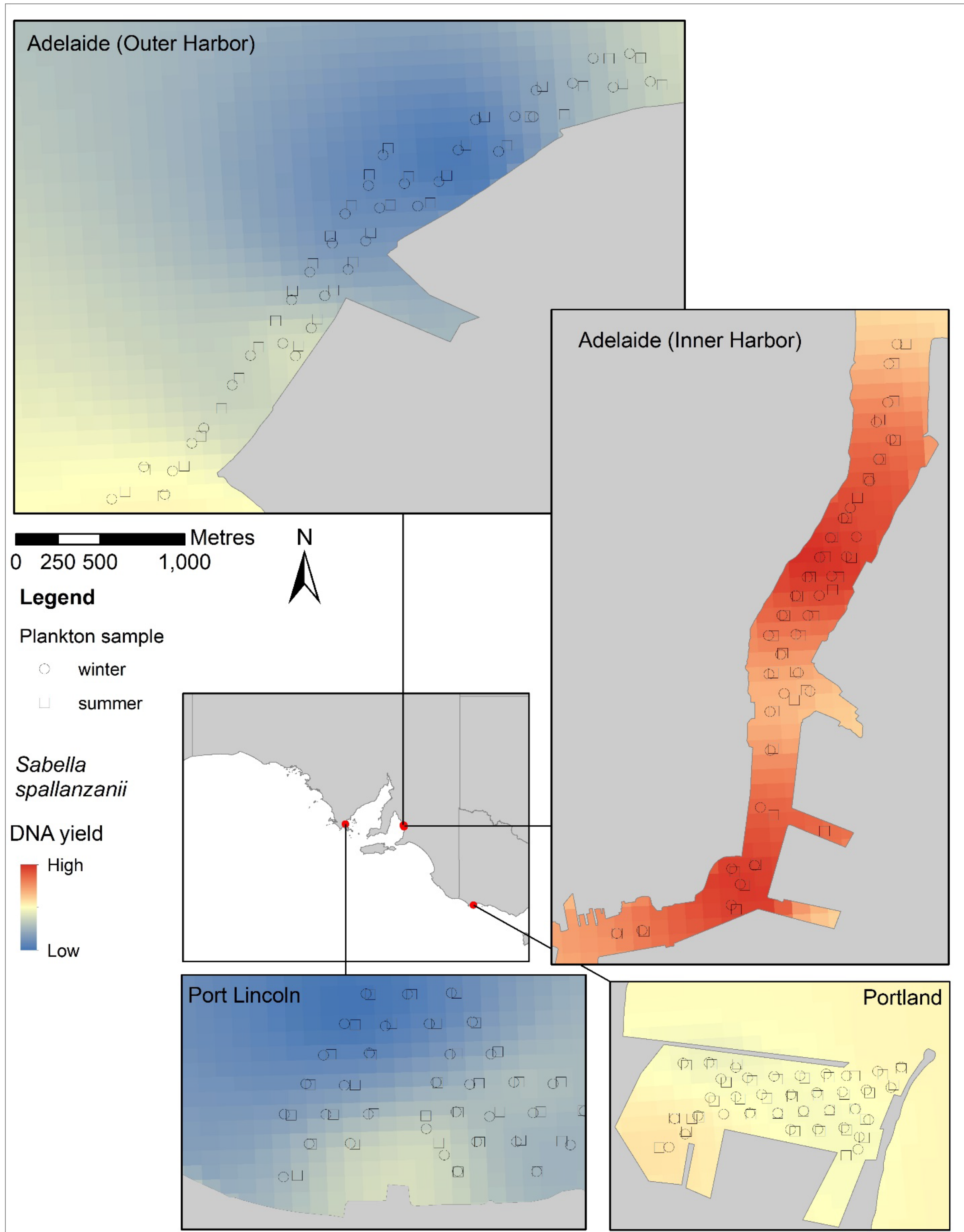

Figure 21. Spatial field of DNA yield for *Sabella spallanzanii* in Adelaide, Port Lincoln and Portland.

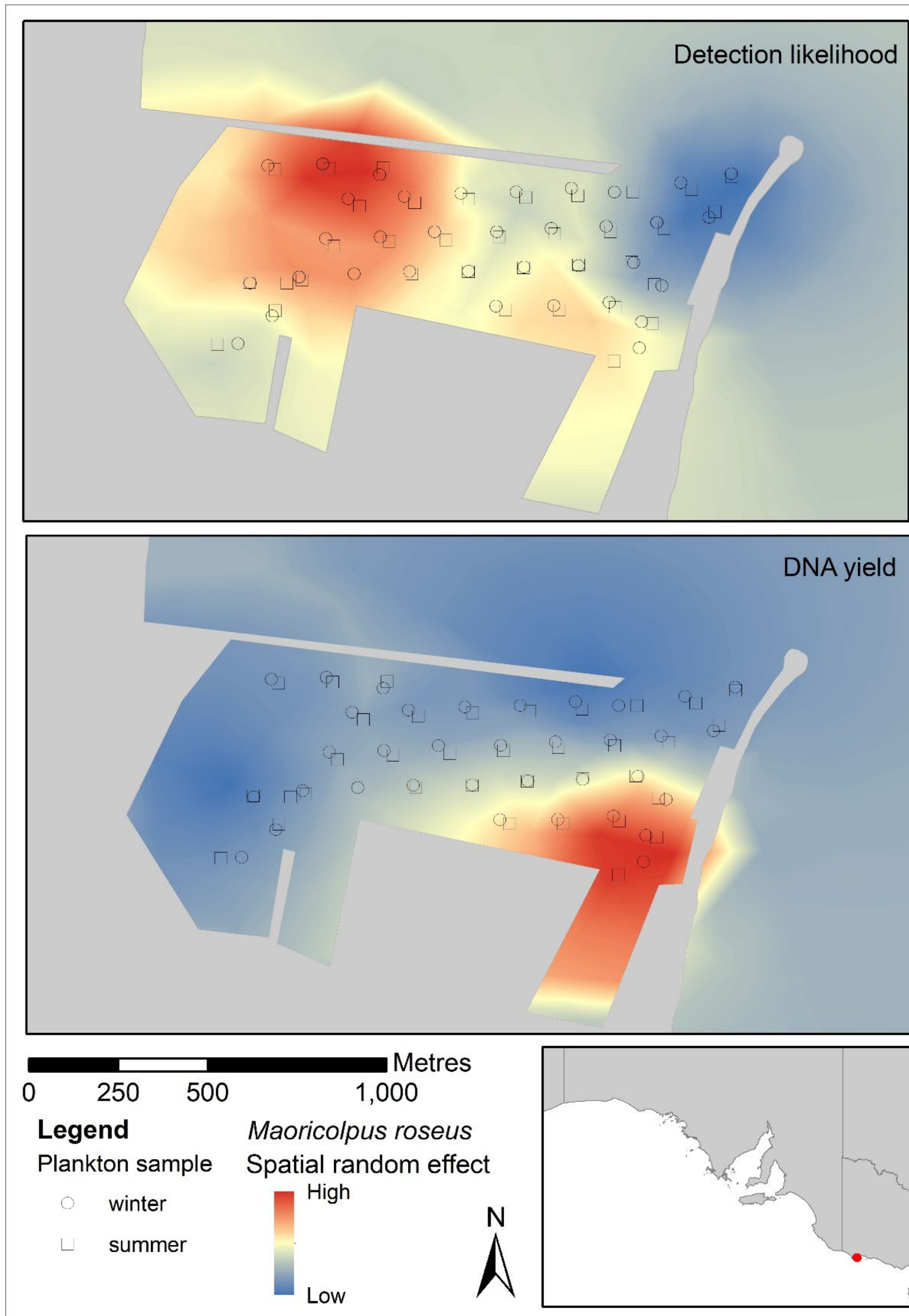

Figure 22. Spatial fields of detection likelihood (top) and DNA yield (bottom) for *Maoricolpus roseus* in Portland.

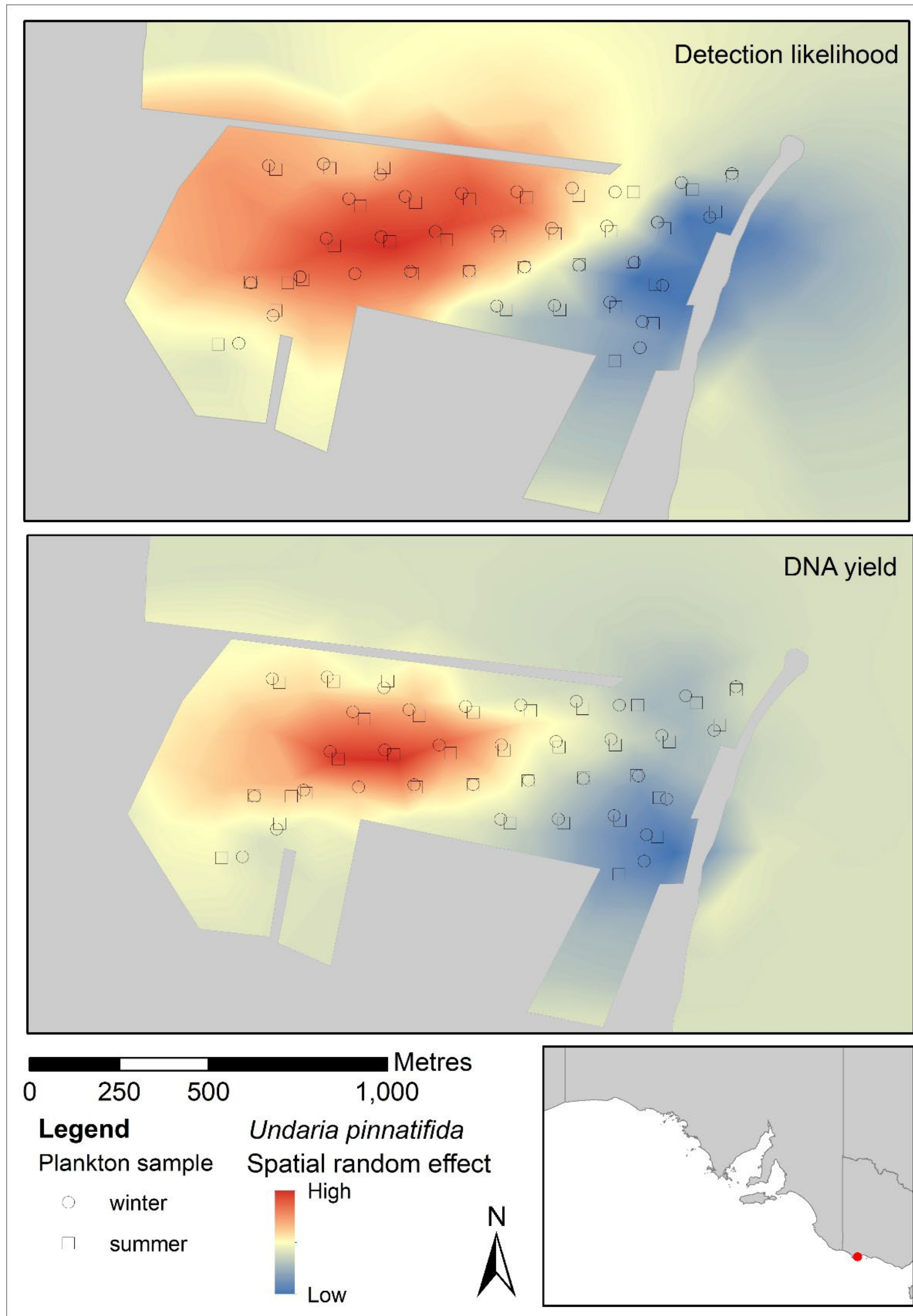

Figure 23. Spatial fields of detection likelihood (top) and DNA yield (bottom) for *Undaria pinnatifida* in Portland.

3.5. Estimated true prevalence and planktonic pest concentrations

SODM results showed that the species detected in multiple samples and known to be present at the survey locations all occurred at concentrations confidently above the threshold in at least one sample set (Appendix section 7.1). Planktonic pest concentrations results were generally > 1000 pp m^{-3} in at least one of the two sample sets for the relevant locations (Figure 24; Appendix section 7.1) and were higher in each case for the sample set with more detections, especially for the species that showed strong seasonal patterns in detection. In Adelaide, the mean predicted concentrations for *Magallana gigas* and *Sabella spallanzanii* were higher than those for *Carcinus maenas* in the optimum season for each (Figure 24). Predicted winter concentration was similar between Inner and Outer Harbor for *Carcinus maenas* (1600 *c.f.* 1900 pp m^{-3}; Table 14) and *Sabella spallanzanii* (3600 *c.f.* 4400 pp m^{-3}; Table 27). *Magallana gigas*, however, had higher mean predicted summer concentration in Inner than Outer Harbor (4700 *c.f.* 1200 pp m^{-3}; Figure 24; Table 18). For Thevenard, the predicted summer concentration of *Magallana gigas* was 2300 pp m^{-3} (Figure 24; Table 18), and, in Port Lincoln, *Sabella spallanzanii* had a predicted winter concentration of 1700 pp m^{-3} (Figure 24; Table 27). In Portland, the predicted mean concentration of *Sabella spallanzanii* in winter was 0.11 pp m^{-3} (Figure 24; Table 27), the lowest for any species with confirmed occurrence in this survey, but within the range observed for established species elsewhere (Wiltshire 2021). Mean predicted winter concentration was 6200 pp m^{-3} for *Undaria pinnatifida* in Portland (Figure 24; Table 28).

Estimated true prevalence, which can be considered as the likelihood of any given sample containing at least one planktonic pest, was close to 100% in the sample set with more detections for these confirmed species at relevant locations (Figure 25; Appendix section 7.1), except for *Sabella spallanzanii* in Portland, where estimated true prevalence in winter was 51.2% (Figure 25; Table 27)

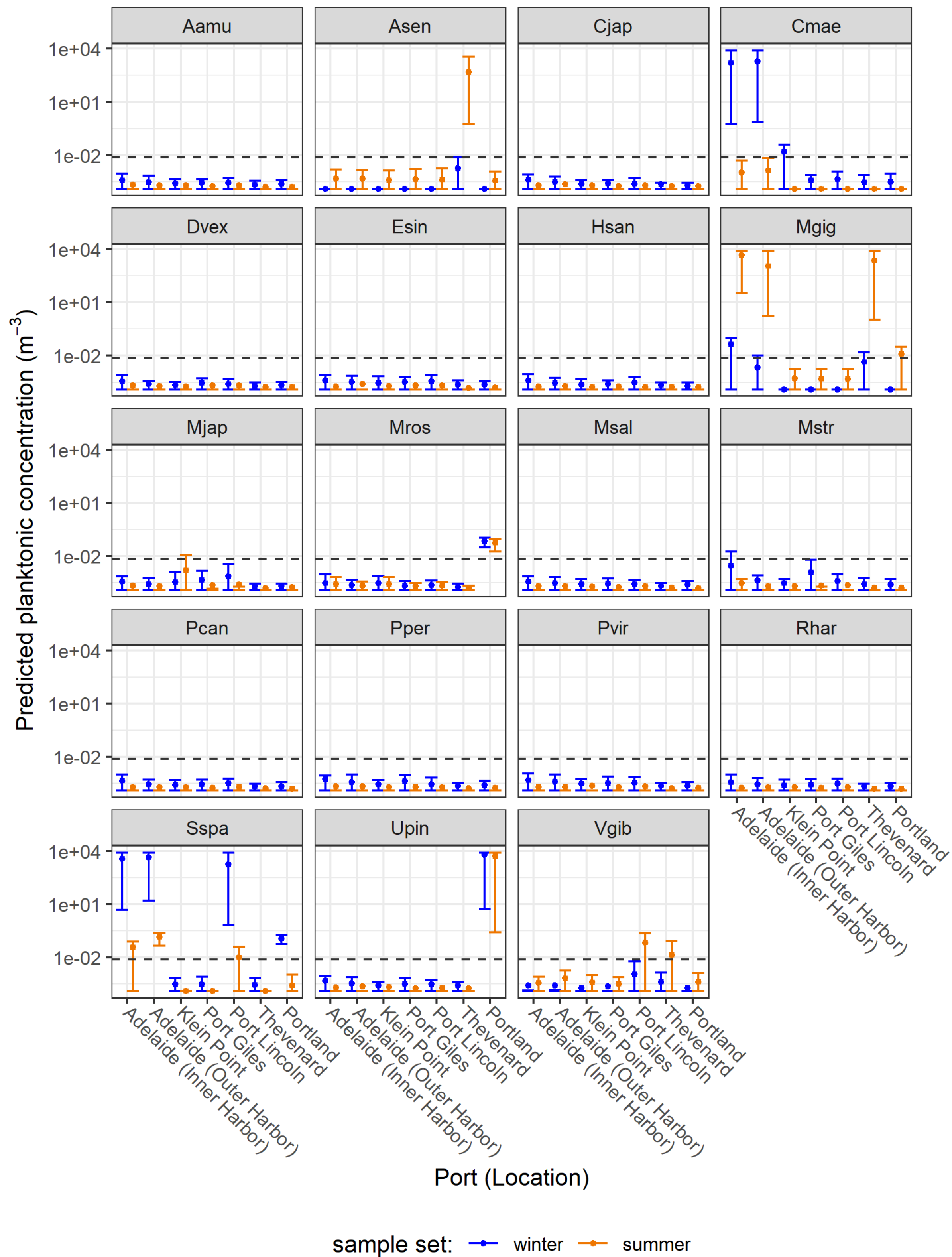

Figure 24. Modelled planktonic pest concentration (mean and 95% HDI) for each species at each location and sample time from SODM analysis. Dashed line shows the threshold concentration of 0.0075 pp m^{-3}.

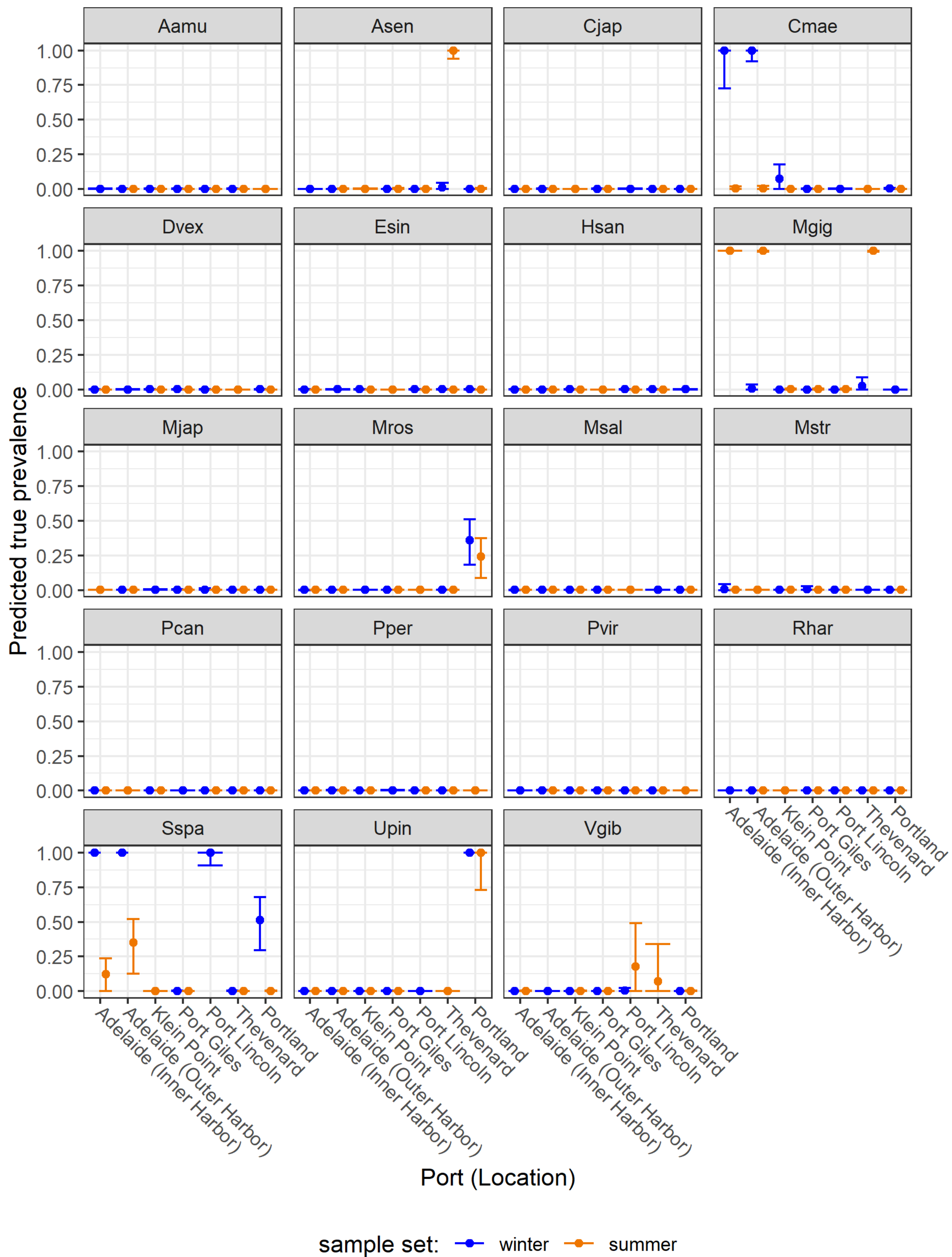

Figure 25. Modelled true prevalence (mean and 95% HDI) for each species at each location and sample time.

Species that were not detected all had predicted planktonic concentrations confidently below the threshold. These species were: *Asterias amurensis* (11), *Charybdis japonica* (Table 13), *Didemnum vexillum* (Table 15), *Eriocheir sinensis* (Table 16), *Hemigrapsus sanguineus* (Table 17), *Mytilopsis sallei* (Table 21), *Perna canaliculus* (Table 23), *P. perna* (Table 24), *P. viridis* (Table 25) and *Rhithropanopeus harrisii* (Table 26). The upper limit of the 95% HDI can be considered as the maximum plausible concentration. Across locations and sample sets for the species that were not detected, this maximum plausible concentration was 0.0011 pp m^{-3}. The mean estimated true prevalence for this set of species was ≤ 0.18% with the upper limit of the 95% HDI being ≤ 0.41% (Figure 25; Appendix section 7.1).

For the species that were detected at locations where they were not previously recorded, the model estimates of concentration and prevalence provide a guide to the true likelihood of species presence and maximum plausible level of occurrence. For *Arcuatula senhousia* in Thevenard, predicted concentration in summer, when all detections occurred, was confidently above the threshold, being (mean and 95% HDI): 480 (0.55 – 3400) pp m^{-3} (Figure 24; Table 12), with estimated true prevalence of 100 (93.9 – 100) % (Figure 25; Table 12). In Portland, estimated concentration of *Maoricolpus roseus* was confidently above the threshold in both sample sets: 0.071 (0.035 – 0.12) pp m^{-3} in winter and 0.056 (0.023 – 0.10) pp m^{-3} in summer (Figure 24; Table 20), with estimated true prevalence of 35.9 (18.4 – 50.9) % and 24.2 (8.87 – 37.3) % respectively (Figure 25; Table 20).

At Klein Point, where *Carcinus maenas* was detected in two winter samples, predicted concentration was probably above the threshold: 0.016 (0.00012 – 0.039) pp m^{-3} (Figure 24; Table 14), with estimated true prevalence of 7.5 (0.06 – 17.7) % (Figure 25; Table 14). The predicted summer concentration for *Varicorbula gibba* in both Port Lincoln and Thevenard was also probably above the threshold concentration (Figure 24; 29). The low diagnostic specificity of the Vgib assay resulted in estimated true prevalence for this species being lower than apparent prevalence in sample sets with detections. In the summer sample sets for Port Lincoln and Thevenard, estimated true prevalence was 17.9 (0.04 – 49.0) % and 6.91 (0.06 – 34.1) %, compared with apparent prevalence of 45.7 and 40.0 % respectively (Figure 25; 29). For all other locations with detection of this species, predicted concentration was confidently below the threshold, with predicted true prevalence being < 1% in each case (29).

For the other species with 1 – 2 detections at a location: *Mya japonica* at Klein Point and Port Giles, *Maoricolpus roseus* and *Sabella spallanzanii* at Klein Point, and *Mytella strigata* at

Adelaide Inner Harbor and Port Giles, mean predicted concentration was between 0.00044 and 0.0016 pp m^{-3} (Figure 24, Appendix section 7.1). Predicted concentration was confidently below the threshold in these cases, except for *Mya japonica* at Klein Point (Table 19) and for *Mytella strigata* at both Adelaide Inner Harbor and Port Giles (Table 22) where predicted concentrations were probably below the threshold. Predicted true prevalence in these instances was 0.51% for *Mya japonica* at Klein Point (Table 19) and 0.55 – 0.67% for *Mytella strigata* at Adelaide Inner Harbor and Port Giles respectively (Table 22). Predicted true prevalence was 0.20% for *Mya japonica* at Port Giles (Table 19) 0.09% for *Maoricolpus roseus* at Klein Point (Table 20) and 0.04% for *Sabella spallanzanii* at Klein Point (Table 27).

4. DISCUSSION

These molecular surveys provide updated information on the status of seven pests of concern for domestic ballast water management in the ports of Thevenard, Port Lincoln, Port Giles, Klein Point and Adelaide, SA, and Portland, Vic, and also assessed occurrence of twelve other priority pests. Molecular surveillance detected pests known to occur at each location: *Carcinus maenas, Magallana gigas* and *Sabella spallanzanii* in Adelaide, *Sabella spallanzanii* in Port Lincoln, *Magallana gigas* in Thevenard, and *Undaria pinnatifida* and *Sabella spallanzanii* in Portland. The detections further suggest that *Maoricolpus roseus* occurs in Portland and that *Arcuatula senhousia* occurs in Thevenard. There were no target pests known to occur at the survey locations that were not detected.

Carcinus maenas, Magallana gigas and *Sabella spallanzanii* are established in Adelaide, where they have been detected by previous traditional and molecular surveillance (Wiltshire *et al.* 2010; Wiltshire and Deveney 2011; Dittmann *et al.* 2016; Deveney *et al.* 2017; Dittmann *et al.* 2017; Deveney *et al.* 2020; Wiltshire 2021). In Thevenard, it is unclear whether *Magallana gigas* detections were driven by the naturalized population on the wharf infrastructure, or advected material from farmed populations located at Denial Bay, ~ 7 km to the north-west. *Sabella spallanzanii* is established in Port Lincoln and recorded in molecular and diver visual surveys (Wiltshire *et al.* 2017; Baker *et al.* 2019). *Undaria pinnatifida* and *Sabella spallanzanii* both are recorded in Portland harbour, with *Sabella spallanzanii* occurring since 2011 (Hirst *et al.* 2012), and both species detected during recent surveys for marina infrastructure works (Glenelg Shire 2021). SODM results showed that the planktonic concentration of these pests in these locations is confidently above the threshold in at least one of the survey seasons.

Maoricolpus roseus is established in south-eastern Australia, with extensive populations in Tasmania and eastern Victoria (Bax *et al.* 2003; Reid 2010; Barton *et al.* 2012). It is not, however, known to occur west of Wilsons Promontory, and was not detected in testing of recent samples from Melbourne with the Mros assay (Wiltshire *et al.* 2022). The Mros assay is newly developed (Giblot-Ducray *et al.* 2022), but field and laboratory specificity testing did not identify any problems with specificity for this assay when used on Australian samples (Giblot-Ducray *et al.* 2022; Wiltshire *et al.* 2022). The considerable number of *Maoricolpus roseus* detections across both sample sets in Portland suggest a population may be present, with modelled concentration being confidently above the threshold.

Arcuatula senhousia is not recorded from Thevenard, but we could not identify any data from surveys of this location that used methods appropriate for detection of this pest. The Asen assay has demonstrated likely problems with specificity in tropical locations (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c), but in temperate regions, this assay has returned detections only where this pest is established (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2020b; Wiltshire *et al.* 2022). There were no other detections by the Asen assay in this survey. It appears likely, therefore, that detections in Thevenard are of *Arcuatula senhousia.* Sequencing approaches will, however, be explored to confirm this result. Modelled planktonic concentration for this species, accounting for the probability of false positives, was confidently above the threshold.

Patterns of detection across sample sets generally followed established trends (Wiltshire 2021); *M. gigas* and *A. senhousia* were detected primarily in summer, and *C. maenas, U. pinnatifida* and *S spallanzanii* in winter. ZAG models showed higher detection likelihood and DNA yield for *M. gigas* in summer and for *S. spallanzanii* and *U. pinnatifida* in winter. Testing of Tasmanian samples for initial validation of the Mros assay did not detect *Maoricolpus roseus* in samples from July – August, and found that detection likelihood and DNA yield were generally higher in December – March than in October – November (Giblot-Ducray *et al.* 2022). That project did not, however, test any samples from September, when winter samples were collected from Portland in this project. Detection likelihood was similar between the winter and summer sample sets, but DNA yield was higher in summer. It is possible *Maoricolpus roseus* spawning starts or peaks earlier in Portland than in Tasmania, but data from the current project support that summer is likely to be the best season for detection, given higher DNA yields during the summer sampling. Where pests are particularly abundant, as was observed for *M. gigas* in Thevenard and Port Adelaide, *S. spallanzanii* in Port Adelaide and Port Lincoln and *U. pinnatifida* in Portland, pests can be detected out of their 'typical' season. The assays detect DNA whether present from gametes, larvae, or tissue shed from adult organisms, and it is not possible to determine the specific source of a detection. It is likely that different taxa shed DNA via different mechanisms, and that all taxa are likely to produce detectable DNA by more than one means.

The two Sspa assay detections in Klein Point both had very high C_T values (> 42), suggesting these detections could be of transient DNA. The Accolade II, which transits daily between Adelaide Inner Harbor and Klein Point, was in port at Klein Point during each survey of this location, and may have been the source of transient *Sabella spallanzanii* DNA from either hull fouling or ballast release. It is, however, possible that *S. spallanzanii* occurs at Klein Point in low abundance. This species has been recorded on the Yorke Peninsula since 2010 and is

established at Port Vincent (Baker *et al.* 2019), ~ 20 km north of Klein Point. Modelled planktonic concentration for *S. spallanzanii* at Klein Point was confidently below the threshold in both sample sets. The Cmae detections in Klein point each had a moderately high C_T (34.7 – 36.8), and could also be either of transient DNA or due to presence of a small population of crabs. *Carcinus maenas* was recorded in Port Vincent and in Edithburgh (~ 14 km south of Klein Point) in 2008, although it was not detected in 2009-10 (Wiltshire *et al.* 2010). Where this species occurs at low abundance it is difficult to detect with traditional methods (i.e., traps), and the molecular method provides reliable detection of much smaller populations than traditional surveys (Wiltshire *et al.* 2019a). Modelled concentration of *C. maenas* at Klein Point was probably above the threshold in winter at this location. The detections of *S. spallanzanii* in summer, with no detection in winter, when this species was more commonly detected elsewhere, supports that the detections are more likely to be transient. In contrast, detections of *C. maenas* only in winter, consistent with the pattern of detection in Adelaide, suggest that a small population may be present that is only detectable during the reproductive season. Although *C. maenas* and *S. spallanzanii* may occur in Klein Point, the small number of detections with high C_T value for each, supported by SODM results, shows they are not abundant. *Magallana gigas* appears to be absent from Klein Point despite regular vessel movements from Adelaide Inner Harbor where it is common and the farm populations at Stansbury, < 10 km to the north. It is possible that vessel traffic does not effectively transport these pests from Adelaide to Klein Point, and/or that the environment at Klein Point is not conducive to their establishment and proliferation.

The Asian paddle crab, *Charybdis japonica*, which is regarded as exotic to Australia, has been occasionally detected in Adelaide (Wiltshire *et al.* 2020a) and Perth (Hewitt *et al.* 2018) but does not appear to have established in either location. A lack of Cjap detections in these surveys provides further support that this species has not established a population in Adelaide, or at the other surveyed locations. *Arcuatula senhousia* was common in Adelaide Outer Harbor *circa* 2000 (Cohen *et al.* 2002) but subsequently became locally extinct (Wiltshire *et al.* 2010). A lack of Asen detections in 2015-16 (Deveney *et al.* 2017) and this survey supports that this species is below the detection threshold and is probably absent from the Adelaide region. Modelled concentrations were confidently below the threshold for *Charybdis japonica* at all locations, and for *Arcuatula senhousia* in all cases aside from Thevenard in summer.

Further assessment is required for some detections. The detections of *Mytella strigata* in Port Adelaide and Port Giles, of *Mya japonica* in Port Lincoln, Klein Point and Port Giles, and *Maoricolpus roseus* at Klein Point could reflect transient DNA, contamination, or problems with

the specificity of the relatively new Mstr, Mjap and Mros assays. Each of these assays has been tested for specificity using genomic DNA from a range of related native species (Wiltshire *et al.* 2021b; Giblot-Ducray *et al.* 2022) and environmental DNA from plankton (Wiltshire *et al.* 2021b; Wiltshire *et al.* 2022). No problems with specificity were identified, but the testing did not include plankton samples from the areas where detections occurred in this project. Sequencing approaches will be explored to investigate these detections. Although the source of these detections is unclear, SODM results show that these species are either probably or confidently below the threshold concentration at any of these locations.

The presence of *Varicorbula gibba* at the surveyed locations is also unclear. The Vgib assay has displayed problematic specificity in tropical locations, and has also returned detections in multiple temperate areas where this species is not known to occur (Deveney *et al.* 2017; Wiltshire *et al.* 2022). It is possible that this assay cross-reacts with DNA of more than one other species, but further investigation is needed to determine what taxa may be responsible for cross-reactions and to obtain data to enable either redesign of this assay or development of a confirmatory test (Wiltshire *et al.* 2022). The greatest number of Vgib detections occurred at Port Lincoln and Thevenard, and SODM results, after accounting for poor assay specificity, suggest the species may occur at these two locations, with concentrations probably above the threshold. Interpretation of these results is complicated, however, because it is possible that detections and associated model predictions are driven by a different, native species that cross-reacts with this assay. Sequencing approaches are currently being explored for investigation of Vgib detections.

Policy frameworks to guide management responses to molecular surveillance should be informed by risk and relative cost, including the risk and potential cost of inaction if a pest is regarded as absent (undetected or detection is considered transient) where it is present, and the risk and cost of a response or further surveys where a pest is absent or is present but at sufficiently low abundance that impacts are unlikely (Sepulveda *et al.* 2020). The relative likelihood of detections indicating occurrence of a pest population, as opposed to transient DNA or the result of a cross-reaction or contamination, increases with increasing number and prevalence of detections, especially where supported by validated methods, appropriate quality assurance controls and statistical analyses (Goldberg *et al.* 2016; Sepulveda *et al.* 2020; Jerde 2021). Wiltshire *et al.* (2019a) provide some guidelines for the interpretation of new molecular detections and suggest potential responses, which escalate with increasing likelihood of the detections indicating an incursion based on the number of detections and their C_T values. Those recommendations, however, we based on preliminary data and were made prior to the availability of data and

modelling approaches that allow more sophisticated analyses of results such as applied here. Wiltshire (2021) analysed a compiled data set of molecular detections, including from locations where detections were likely transient and locations where detections were of emerging incursions, providing further information to guide policy decisions, including relevant ranges of planktonic pest concentrations that occur where pests are established or potentially emerging. Bayesian modelling, as applied to detection results in this study, allows estimation of planktonic concentration from patterns of detection while accounting for influences on surveillance results, and provides a level of confidence around these estimates. Model estimates can be compared to the target concentration used for survey design, as done here, but other thresholds could be used to inform the level of risk, e.g. model estimates could also be compared to the concentration range expected for established pests. We have demonstrated here a potential modelling approach that can assist in interpreting survey results and informing management, but the approach should be tested on a wider range of surveillance data, and potentially refined, prior to being applied for this purpose. Additional information should also be considered in interpretation of survey results, such as potential sources of transient detection, and further detail on assay performance, such as the variation in performance of the Asen assay in temperate and tropical locations.

In considering whether a result is likely to be transient material, possible sources (vectors) for the detection should be identified, including an assessment of the likelihood of a cross-reaction or contamination. If a potential vector, e.g., a vessel with possible hull-fouling or discharging ballast water, was present in the vicinity at the time of sampling, it supports that detections may be transient. With ballast water controls in place, the risk of pest transport in ballast water is reduced, but DNA may still be detected. Detectable DNA may come from non-viable organisms, including those killed by ballast treatment systems, as well as from organisms that remain despite ballast water management or from hull fouling. The possibility of cross-reactivity or contamination as sources for detections can be explored using sequencing or other confirmatory tests if available, although these approaches are yet to be developed for most target pests. Results should be interpreted with respect to assay diagnostic performance, which has now been assessed for all assays in the SARDI testing system (Wiltshire *et al.* 2019b; Wiltshire *et al.* 2021b; Wiltshire *et al.* 2022). The modelled predictions of true prevalence and planktonic pest concentration use these data on diagnostic performance to aid interpretation of results from the current survey.

Where analysis of results based on assay performance indicates that pest prevalence or concentration at the new site is within the range recorded at sites with populations of the target pest, an incursion should be considered more likely, especially in the absence of potential vectors

or other sources for detection. Repeating molecular surveillance, ideally within the optimum sampling season for the newly detected pest (where this is known), can also assist in elucidating if detections are transient, with repeated detections across sample sets providing further evidence of pest occurrence. Traditional surveillance should be used to confirm occurrence and a specimen of the organism deposited in an appropriately curated collection before regarding these taxa as established at the new site. Specimen collection by traditional methods remains the 'gold standard' to demonstrate presence of living organisms at a site but molecular methods are typically able to detect populations at a much lower abundance than traditional methods (Jerde *et al.* 2011; Díaz-Ferguson and Moyer 2014; Smart *et al.* 2015; de Souza *et al.* 2016; Wilcox *et al.* 2016; Hinlo *et al.* 2017). There is a risk, therefore, that traditional methods may fail to confirm pest occurrence in emerging incursions detected by molecular methods, and delaying management in these situations may result in establishment and further spread of pests (Darling and Mahon 2011). Precautionary management following molecular detections could, however, include regarding a pest as present, pending confirmation, where molecular evidence suggests a population is likely to occur (Wiltshire *et al.* 2019a).

The spatial field of the ZAG models provide an indication of the distribution of target species DNA in plankton within the survey areas, but should be interpreted in combination with knowledge of the sampling methods used and of hydrodynamics. For species detected in both sample sets, the fields show the spatial variation in detection likelihood and DNA yield after accounting for the effect of sample set (season). The fields therefore provide an overview of spatial structure across sample sets, but results for each species are likely to be strongly influenced by data from the sample set with more detections. The relationship between areas of higher DNA yield and detection likelihood and source locations for DNA (e.g. adult populations) will depend on patterns of water movement, particularly at the sampling time when most detections occurred. To inform ballast water risk, however, the occurrence of larvae or other propagules around wharves (i.e. within the areas surveyed) is more important than the location of adults. Although DNA detections may be of material other than propagules, both detection likelihood and DNA yield are likely to correlate highly with the presence of target-species propagules. Knowledge of hydrodynamics would, however, help to identify likely areas of adult occurrence to guide traditional surveys for species delimitation or confirmation.

PCR inhibition, which can occur in environmental samples, was detected in some plankton samples collected during the surveys but was not at a level likely to have prevented detection in more than a few samples. This is supported by the ZAG modelling results (section 3.4) which

showed no effect of scale factor on detection likelihood except for a small negative effect on *Magallana gigas* detection. This effect was driven by two summer samples from Inner Harbor with high (scale factor > 100) inhibition and no detection, while *Magallana gigas* was detected in all other samples from that set. Inhibition should, however, continue be assessed in environmental sampling to determine cases where detection likelihood may be compromised. In addition to ZAG modelling, potential effects of inhibition on detection were considered in the SODM. This approach therefore provides estimated pest concentrations and quantifiable confidence in absence of undetected species, accounting for any effects of PCR inhibition and assay performance.

5. CONCLUSIONS

The surveys provide information on the status of 19 pests in the ports of Thevenard, Port Lincoln, Port Giles, Klein Point and Adelaide, SA, and Portland, Vic. *Carcinus maenas, Magallana gigas* and *Sabella spallanzanii* were detected in Adelaide, *Sabella spallanzanii* in Port Lincoln, *Magallana gigas* in Thevenard, and *Undaria pinnatifida* and *Sabella spallanzanii* in Portland. These pests are all recorded at these locations and the extent and frequency of molecular detections confirms their continued presence. Detections by this survey further suggest that *Maoricolpus roseus* occurs in Portland and that *Arcuatula senhousia* occurs in Thevenard, with model results suggesting these pests are present at a prevalence and planktonic concentration comparable to known established pests. *Carcinus maenas* and *Sabella spallanzanii* may be present at low abundance in Klein Point, but detections of these species at this location could also be of transient DNA from vessel fouling or ballast water, or advected from populations elsewhere on Yorke Peninsula. Following expected patterns, *M. gigas* and *A. senhousia* were detected primarily in summer, and *C. maenas, U. pinnatifida* and *S spallanzanii* were detected primarily in winter.

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7. APPENDIX

7.1. Predicted true prevalence and concentration

Tables 11 – 29 show model predictions (mean and 95% HDI) of true prevalence and planktonic concentration for each species at each location and for each sample set. Adelaide locations are Inner Harbor (IH) and Outer Harbor (OH), other ports comprise a single location. Tables show: AP = Apparent prevalence (proportion of samples with a detection), TP = True prevalence (probability of a sample containing ≥ 1 planktonic pest), and predicted concentration (in pp m^{-3}). Concentrations are shown using scientific notation, where E±00 indicates x $10^{\pm 00}$. Predicted concentrations above threshold (= survey target: 0.0075 pp m^{-3}) are indicated with: *upper highest density interval (HDI) concentration > target, i.e., concentration probably below threshold; ** mean > target, i.e. concentration is probably but not confidently above the threshold; ***lower HDI > target, i.e. concentration is confidently above the threshold. Where the upper HDI is < 0.0075 pp m^{-3}, the species can be considered as being confidently below the threshold concentration.

Table 11. Prevalence and planktonic pest concentration model results for *Asterias amurensis*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.09% (0.03% – 0.21%)	3.9E-04 (1.2E-04 – 9.2E-04)
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.10% (0.04% – 0.24%)	2.9E-04 (1.2E-04 – 6.7E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.12% (0.06% – 0.22%)	2.5E-04 (1.2E-04 – 4.4E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.12% (0.06% – 0.20%)	2.7E-04 (1.2E-04 – 4.4E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.10% (0.05% – 0.19%)	2.8E-04 (1.2E-04 – 5.0E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.15% (0.08% – 0.25%)	2.3E-04 (1.2E-04 – 4.1E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.13% (0.08% – 0.23%)	2.2E-04 (1.2E-04 – 3.6E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)

Table 12. Prevalence and planktonic concentration model results for *Arcuatula senhousia.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.03% (0.03% – 0.03%)	1.2E-04 (1.2E-04 – 1.2E-04)
Adelaide (IH)	summer	0.0%	0.16% (0.04% – 0.51%)	4.6E-04 (1.2E-04 – 1.5E-03)
Adelaide (OH)	winter	0.0%	0.04% (0.04% – 0.04%)	1.2E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	summer	0.0%	0.14% (0.04% – 0.44%)	4.8E-04 (1.2E-04 – 1.4E-03)
Klein Point	winter	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)
Klein Point	summer	0.0%	0.12% (0.04% – 0.42%)	3.9E-04 (1.2E-04 – 1.3E-03)
Port Giles	winter	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Giles	summer	0.0%	0.15% (0.04% – 0.56%)	4.3E-04 (1.2E-04 – 1.6E-03)
Port Lincoln	winter	0.0%	0.05% (0.05% – 0.05%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	summer	0.0%	0.12% (0.04% – 0.52%)	4.1E-04 (1.2E-04 – 1.8E-03)
Portland	winter	0.0%	0.08% (0.08% – 0.08%)	1.2E-04 (1.2E-04 – 1.2E-04)
Portland	summer	0.0%	0.17% (0.06% – 0.59%)	3.5E-04 (1.2E-04 – 1.2E-03)
Thevenard	winter	0.0%	1.06% (0.08% – 4.43%)	1.7E-03 (1.2E-04 – 7.3E-03)*
Thevenard	summer	100.0%	100.0% (93.9% – 100.0%)	4.8E+02 (5.5E-01 – 3.4E+03)***

Table 13. Prevalence and planktonic concentration model results for *Charybdis japonica.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.09% (0.03% – 0.17%)	4.2E-04 (1.2E-04 – 7.6E-04)
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.11% (0.04% – 0.22%)	3.1E-04 (1.2E-04 – 6.2E-04)
Adelaide (OH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.2E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.12% (0.06% – 0.18%)	2.4E-04 (1.2E-04 – 3.7E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.11% (0.06% – 0.19%)	2.5E-04 (1.2E-04 – 4.1E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.7E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.09% (0.05% – 0.18%)	2.4E-04 (1.2E-04 – 5.0E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.13% (0.08% – 0.17%)	2.0E-04 (1.2E-04 – 2.7E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.13% (0.08% – 0.17%)	2.2E-04 (1.2E-04 – 2.7E-04)
Thevenard	summer	0.0%	0.09% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 14. Prevalence and planktonic concentration model results for *Carcinus maenas.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3}))
Adelaide (IH)	winter	60.0%	100.0% (72.5% – 100.0%)	1.6E+03 (5.7E-01 – 8.1E+03)***
Adelaide (IH)	summer	0.0%	0.34% (0.04% – 1.66%)	1.0E-03 (1.2E-04 – 4.9E-03)*
Adelaide (OH)	winter	77.1%	100.0% (92.3% – 100.0%)	1.9E+03 (7.2E-01 – 8.1E+03)***
Adelaide (OH)	summer	0.0%	0.41% (0.04% – 2.11%)	1.3E-03 (1.2E-04 – 7.0E-03)*
Klein Point	winter	5.7%	7.53% (0.06% – 17.68%)	1.6E-02 (1.2E-04 – 3.9E-02)**
Klein Point	summer	0.0%	0.04% (0.04% – 0.04%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.17% (0.06% – 0.34%)	3.7E-04 (1.2E-04 – 7.4E-04)
Port Giles	summer	0.0%	0.04% (0.04% – 0.04%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.16% (0.05% – 0.43%)	4.3E-04 (1.2E-04 – 1.2E-03)
Port Lincoln	summer	0.0%	0.04% (0.04% – 0.04%)	1.2E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.19% (0.08% – 0.55%)	3.0E-04 (1.2E-04 – 8.9E-04)
Portland	summer	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.18% (0.08% – 0.47%)	2.9E-04 (1.2E-04 – 7.5E-04)
Thevenard	summer	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)
Adelaide (IH)	winter	0.0%	0.08% (0.03% – 0.18%)	3.6E-04 (1.2E-04 – 7.8E-04)

Table 15. Prevalence and planktonic concentration model results for *Didemnum vexillum.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.09% (0.04% – 0.13%)	2.6E-04 (1.2E-04 – 3.6E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.11% (0.06% – 0.16%)	2.2E-04 (1.2E-04 – 3.2E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.13% (0.06% – 0.24%)	2.9E-04 (1.2E-04 – 5.3E-04)
Port Giles	summer	0.0%	0.07% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.09% (0.05% – 0.18%)	2.5E-04 (1.2E-04 – 4.9E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.14% (0.08% – 0.21%)	2.2E-04 (1.2E-04 – 3.3E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.12% (0.08% – 0.19%)	1.9E-04 (1.2E-04 – 3.0E-04)
Thevenard	summer	0.0%	0.09% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 16. Prevalence and planktonic concentration model results for *Eriocheir sinensis.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.09% (0.03% – 0.18%)	4.0E-04 (1.2E-04 – 8.1E-04)
Adelaide (IH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.12% (0.04% – 0.27%)	3.2E-04 (1.2E-04 – 7.5E-04)
Adelaide (OH)	summer	0.0%	0.08% (0.04% – 0.04%)	2.5E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.14% (0.06% – 0.35%)	2.8E-04 (1.2E-04 – 7.0E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.14% (0.06% – 0.28%)	3.2E-04 (1.2E-04 – 6.3E-04)
Port Giles	summer	0.0%	0.07% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.13% (0.05% – 0.31%)	3.5E-04 (1.2E-04 – 8.5E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.14% (0.08% – 0.22%)	2.3E-04 (1.2E-04 – 3.5E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.5E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.14% (0.08% – 0.25%)	2.3E-04 (1.2E-04 – 4.0E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.5E-04 (1.2E-04 – 1.2E-04)

Table 17. Prevalence and planktonic concentration model results for *Hemigrapsus sanguineus.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.09% (0.03% – 0.20%)	4.0E-04 (1.2E-04 – 8.7E-04)
Adelaide (IH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.10% (0.04% – 0.19%)	2.9E-04 (1.2E-04 – 5.4E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.12% (0.06% – 0.24%)	2.4E-04 (1.2E-04 – 4.7E-04)
Klein Point	summer	0.0%	0.05% (0.04% – 0.04%)	1.7E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.12% (0.06% – 0.18%)	2.6E-04 (1.2E-04 – 3.9E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.11% (0.05% – 0.23%)	3.0E-04 (1.2E-04 – 6.2E-04)
Port Lincoln	summer	0.0%	0.05% (0.04% – 0.04%)	1.7E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.12% (0.08% – 0.19%)	2.0E-04 (1.2E-04 – 3.0E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.14% (0.08% – 0.19%)	2.2E-04 (1.2E-04 – 3.1E-04)
Thevenard	summer	0.0%	0.09% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 18. Prevalence and planktonic concentration model results for *Magallana gigas.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	8.6%	9.55% (0.03% – 20.29%)	4.4E-02 (1.2E-04 – 9.9E-02)**
Adelaide (IH)	summer	94.3%	100.0% (100.0% – 100.0%)	4.7E+03 (3.3E+01 – 8.1E+03)***
Adelaide (OH)	winter	0.0%	0.74% (0.04% – 3.56%)	2.1E-03 (1.2E-04 – 1.0E-02)*
Adelaide (OH)	summer	74.3%	100.00% (99.5% – 100.0%)	1.2E+03 (1.7E+00 – 8.1E+03)***
Klein Point	winter	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)
Klein Point	summer	0.0%	0.16% (0.04% – 0.57%)	5.1E-04 (1.2E-04 – 1.8E-03)
Port Giles	winter	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Giles	summer	0.0%	0.17% (0.04% – 0.61%)	4.8E-04 (1.2E-04 – 1.7E-03)
Port Lincoln	winter	0.0%	0.05% (0.05% – 0.05%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	summer	0.0%	0.14% (0.04% – 0.50%)	4.8E-04 (1.2E-04 – 1.7E-03)
Portland	winter	0.0%	0.08% (0.08% – 0.08%)	1.2E-04 (1.2E-04 – 1.2E-04)
Portland	summer	5.7%	5.97% (0.06% – 14.96%)	1.2E-02 (1.2E-04 – 3.3E-02)**
Thevenard	winter	2.9%	2.64% (0.08% – 8.90%)	4.3E-03 (1.2E-04 – 1.5E-02)*
Thevenard	summer	80.0%	100.0% (99.5% – 100.0%)	2.3E+03 (1.0E+00 – 8.1E+03)***

Table 19. Prevalence and planktonic concentration model results for *Mya japonica.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.09% (0.03% – 0.16%)	3.8E-04 (1.2E-04 – 7.0E-04)
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.2E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.09% (0.04% – 0.21%)	2.6E-04 (1.2E-04 – 5.8E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.17% (0.06% – 0.66%)	3.4E-04 (1.2E-04 – 1.3E-03)
Klein Point	summer	5.7%	0.51% (0.04% – 3.64%)	1.6E-03 (1.2E-04 – 1.1E-02)*
Port Giles	winter	2.9%	0.20% (0.06% – 0.65%)	4.4E-04 (1.2E-04 – 1.4E-03)
Port Giles	summer	0.0%	0.08% (0.04% – 0.05%)	2.4E-04 (1.2E-04 – 1.4E-04)
Port Lincoln	winter	2.9%	0.27% (0.05% – 1.25%)	7.3E-04 (1.2E-04 – 3.4E-03)
Port Lincoln	summer	0.0%	0.07% (0.04% – 0.05%)	2.4E-04 (1.2E-04 – 1.9E-04)
Portland	winter	0.0%	0.13% (0.08% – 0.18%)	2.0E-04 (1.2E-04 – 2.8E-04)
Portland	summer	0.0%	0.09% (0.06% – 0.06%)	1.8E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.13% (0.08% – 0.17%)	2.1E-04 (1.2E-04 – 2.8E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.5E-04 (1.2E-04 – 1.2E-04)

Table 20. Prevalence and planktonic concentration model results for *Maoricolpus roseus.*

Port (location)	**sample set**	**AP (%)**	**predicted TP (%)**	**predicted concentration (pp m^{-3})**
Adelaide (IH)	winter	0.0%	0.07% (0.03% – 0.22%)	3.1E-04 (1.2E-04 – 9.6E-04)
Adelaide (IH)	summer	0.0%	0.09% (0.04% – 0.23%)	2.7E-04 (1.2E-04 – 6.7E-04)
Adelaide (OH)	winter	0.0%	0.09% (0.04% – 0.17%)	2.4E-04 (1.2E-04 – 4.6E-04)
Adelaide (OH)	summer	0.0%	0.07% (0.04% – 0.11%)	2.1E-04 (1.2E-04 – 3.8E-04)
Klein Point	winter	0.0%	0.16% (0.06% – 0.38%)	3.1E-04 (1.2E-04 – 7.6E-04)
Klein Point	summer	2.9%	0.09% (0.04% – 0.22%)	2.7E-04 (1.2E-04 – 6.7E-04)
Port Giles	winter	0.0%	0.10% (0.06% – 0.18%)	2.2E-04 (1.2E-04 – 4.0E-04)
Port Giles	summer	0.0%	0.07% (0.04% – 0.11%)	2.0E-04 (1.2E-04 – 3.1E-04)
Port Lincoln	winter	0.0%	0.09% (0.05% – 0.16%)	2.3E-04 (1.2E-04 – 4.3E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.10%)	2.1E-04 (1.2E-04 – 3.5E-04)
Portland	winter	34.3%	35.93% (18.39% – 50.94%)	7.1E-02 (3.2E-02 – 1.1E-01)***
Portland	summer	25.7%	24.21% (8.87% – 37.28%)	5.6E-02 (1.9E-02 – 9.4E-02)***
Thevenard	winter	0.0%	0.11% (0.08% – 0.17%)	1.8E-04 (1.2E-04 – 2.8E-04)
Thevenard	summer	0.0%	0.09% (0.06% – 0.11%)	1.7E-04 (1.2E-04 – 2.1E-04)

Table 21. Prevalence and planktonic concentration model results for *Mytilopsis sallei.*

Port (location)	**sample set**	**AP (%)**	**predicted TP (%)**	**predicted concentration (pp m^{-3})**
Adelaide (IH)	winter	0.0%	0.08% (0.03% – 0.16%)	3.7E-04 (1.2E-04 – 7.0E-04)
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.11% (0.04% – 0.23%)	2.9E-04 (1.2E-04 – 6.5E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.13% (0.06% – 0.25%)	2.6E-04 (1.2E-04 – 5.0E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.13% (0.06% – 0.25%)	2.8E-04 (1.2E-04 – 5.5E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.10% (0.05% – 0.17%)	2.7E-04 (1.2E-04 – 4.6E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.15% (0.08% – 0.25%)	2.4E-04 (1.2E-04 – 3.9E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.13% (0.08% – 0.19%)	2.2E-04 (1.2E-04 – 3.1E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)

Table 22. Prevalence and planktonic concentration model results for *Mytella strigata.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	2.9%	0.67% (0.03% – 4.23%)	3.0E-03 (1.2E-04 – 1.9E-02)*
Adelaide (IH)	summer	0.0%	0.11% (0.04% – 0.18%)	3.1E-04 (1.2E-04 – 5.3E-04)
Adelaide (OH)	winter	0.0%	0.15% (0.04% – 0.30%)	4.1E-04 (1.2E-04 – 8.3E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.15% (0.06% – 0.27%)	3.0E-04 (1.2E-04 – 5.3E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	2.9%	0.55% (0.06% – 2.93%)	1.2E-03 (1.2E-04 – 6.6E-03)*
Port Giles	summer	0.0%	0.08% (0.04% – 0.07%)	2.2E-04 (1.2E-04 – 1.9E-04)
Port Lincoln	winter	0.0%	0.14% (0.05% – 0.35%)	3.9E-04 (1.2E-04 – 9.4E-04)
Port Lincoln	summer	0.0%	0.07% (0.04% – 0.04%)	2.3E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.15% (0.08% – 0.32%)	2.5E-04 (1.2E-04 – 5.1E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.17% (0.08% – 0.37%)	2.7E-04 (1.2E-04 – 6.0E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 23. Prevalence and planktonic concentration model results for *Perna canaliculus.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.10% (0.03% – 0.23%)	4.5E-04 (1.2E-04 – 1.0E-03)
Adelaide (IH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.10% (0.04% – 0.18%)	2.7E-04 (1.2E-04 – 5.1E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.13% (0.06% – 0.23%)	2.6E-04 (1.2E-04 – 4.7E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.13% (0.06% – 0.24%)	2.9E-04 (1.2E-04 – 5.2E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.12% (0.05% – 0.22%)	3.3E-04 (1.2E-04 – 5.9E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.13% (0.08% – 0.23%)	2.1E-04 (1.2E-04 – 3.7E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.13% (0.08% – 0.19%)	2.2E-04 (1.2E-04 – 3.1E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.6E-04 (1.2E-04 – 1.2E-04)

Table 24. Prevalence and planktonic concentration model results for *Perna perna.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.12% (0.03% – 0.20%)	5.3E-04 (1.2E-04 – 8.9E-04)
Adelaide (IH)	summer	0.0%	0.08% (0.04% – 0.04%)	2.2E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.13% (0.04% – 0.35%)	3.7E-04 (1.2E-04 – 9.7E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.14% (0.06% – 0.24%)	2.8E-04 (1.2E-04 – 4.7E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.18% (0.06% – 0.41%)	4.1E-04 (1.2E-04 – 9.0E-04)
Port Giles	summer	0.0%	0.07% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.10% (0.05% – 0.24%)	2.8E-04 (1.2E-04 – 6.4E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.15% (0.08% – 0.27%)	2.4E-04 (1.2E-04 – 4.4E-04)
Portland	summer	0.0%	0.09% (0.06% – 0.06%)	1.8E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.14% (0.08% – 0.21%)	2.3E-04 (1.2E-04 – 3.3E-04)
Thevenard	summer	0.0%	0.09% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 25. Prevalence and planktonic concentration model results for *Perna viridis.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.11% (0.03% – 0.26%)	4.8E-04 (1.2E-04 – 1.1E-03)
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.14% (0.04% – 0.35%)	3.8E-04 (1.2E-04 – 9.8E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.15% (0.06% – 0.26%)	3.1E-04 (1.2E-04 – 5.3E-04)
Klein Point	summer	0.0%	0.07% (0.04% – 0.04%)	2.2E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.15% (0.06% – 0.33%)	3.3E-04 (1.2E-04 – 7.4E-04)
Port Giles	summer	0.0%	0.07% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.12% (0.05% – 0.26%)	3.3E-04 (1.2E-04 – 7.0E-04)
Port Lincoln	summer	0.0%	0.06% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.14% (0.08% – 0.22%)	2.3E-04 (1.2E-04 – 3.6E-04)
Portland	summer	0.0%	0.09% (0.06% – 0.06%)	1.8E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.14% (0.08% – 0.20%)	2.3E-04 (1.2E-04 – 3.3E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 26. Prevalence and planktonic concentration model results for *Rhithropanopeus harrisii.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.09% (0.03% – 0.22%)	3.8E-04 (1.2E-04 – 9.8E-04)
Adelaide (IH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.7E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.10% (0.04% – 0.23%)	2.8E-04 (1.2E-04 – 6.4E-04)
Adelaide (OH)	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.12% (0.06% – 0.26%)	2.4E-04 (1.2E-04 – 5.1E-04)
Klein Point	summer	0.0%	0.06% (0.04% – 0.04%)	1.9E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.12% (0.06% – 0.25%)	2.6E-04 (1.2E-04 – 5.5E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.7E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.11% (0.05% – 0.21%)	2.9E-04 (1.2E-04 – 5.6E-04)
Port Lincoln	summer	0.0%	0.05% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Portland	winter	0.0%	0.13% (0.08% – 0.20%)	2.1E-04 (1.2E-04 – 3.1E-04)
Portland	summer	0.0%	0.08% (0.06% – 0.06%)	1.5E-04 (1.2E-04 – 1.2E-04)
Thevenard	winter	0.0%	0.14% (0.08% – 0.18%)	2.2E-04 (1.2E-04 – 3.0E-04)
Thevenard	summer	0.0%	0.08% (0.06% – 0.06%)	1.5E-04 (1.2E-04 – 1.2E-04)

Table 27. Prevalence and planktonic concentration model results for *Sabella spallanzanii.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	100.0%	100.0% (100.0% – 100.0%)	3.6E+03 (4.7E+00 – 8.1E+03)***
Adelaide (IH)	summer	11.4%	12.08% (0.04% – 23.79%)	3.8E-02 (1.2E-04 – 8.0E-02)**
Adelaide (OH)	winter	88.6%	100.0% (100.0% – 100.0%)	4.4E+03 (1.6E+01 – 8.1E+03)***
Adelaide (OH)	summer	28.6%	35.04% (12.70% – 51.97%)	1.4E-01 (4.5E-02 – 2.4E-01)***
Klein Point	winter	0.0%	0.15% (0.06% – 0.33%)	3.0E-04 (1.2E-04 – 6.7E-04)
Klein Point	summer	5.7%	0.04% (0.04% – 0.04%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.14% (0.06% – 0.37%)	3.0E-04 (1.2E-04 – 8.2E-04)
Port Giles	summer	0.0%	0.04% (0.04% – 0.04%)	1.2E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	82.9%	100.0% (90.6% – 100.0%)	1.7E+03 (6.4E-01 – 8.1E+03)***
Port Lincoln	summer	5.7%	2.93% (0.04% – 11.08%)	1.0E-02 (1.2E-04 – 4.0E-02)**
Portland	winter	42.9%	51.15% (29.70% – 67.96%)	1.1E-01 (5.6E-02 – 1.8E-01)***
Portland	summer	5.7%	0.13% (0.06% – 0.51%)	2.5E-04 (1.2E-04 – 1.0E-03)
Thevenard	winter	0.0%	0.17% (0.08% – 0.43%)	2.8E-04 (1.2E-04 – 7.0E-04)
Thevenard	summer	0.0%	0.06% (0.06% – 0.06%)	1.2E-04 (1.2E-04 – 1.2E-04)

Table 28. Prevalence and planktonic concentration model results for *Undaria pinnatifida.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.10% (0.03% – 0.19%)	4.6E-04 (1.2E-04 – 8.4E-04)
Adelaide (IH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.0E-04 (1.2E-04 – 1.2E-04)
Adelaide (OH)	winter	0.0%	0.12% (0.04% – 0.26%)	3.4E-04 (1.2E-04 – 7.3E-04)
Adelaide (OH)	summer	0.0%	0.07% (0.04% – 0.04%)	2.2E-04 (1.2E-04 – 1.2E-04)
Klein Point	winter	0.0%	0.13% (0.06% – 0.19%)	2.7E-04 (1.2E-04 – 3.8E-04)
Klein Point	summer	0.0%	0.07% (0.04% – 0.04%)	2.1E-04 (1.2E-04 – 1.2E-04)
Port Giles	winter	0.0%	0.15% (0.06% – 0.29%)	3.2E-04 (1.2E-04 – 6.5E-04)
Port Giles	summer	0.0%	0.06% (0.04% – 0.04%)	1.7E-04 (1.2E-04 – 1.2E-04)
Port Lincoln	winter	0.0%	0.11% (0.05% – 0.18%)	3.0E-04 (1.2E-04 – 4.9E-04)
Port Lincoln	summer	0.0%	0.05% (0.04% – 0.04%)	1.8E-04 (1.2E-04 – 1.2E-04)
Portland	winter	91.4%	100.0% (100.0% – 100.0%)	6.2E+03 (5.2E+00 – 8.1E+03)***
Portland	summer	60.0%	100.0% (72.9% – 100.0%)	5.0E+03 (2.6E-01 – 8.1E+03)***
Thevenard	winter	0.0%	0.15% (0.08% – 0.24%)	2.5E-04 (1.2E-04 – 4.0E-04)
Thevenard	summer	0.0%	0.09% (0.06% – 0.06%)	1.7E-04 (1.2E-04 – 1.2E-04)

Table 29. Prevalence and planktonic concentration model results for *Varicorbula gibba.*

Port (location)	sample set	AP (%)	predicted TP (%)	predicted concentration (pp m^{-3})
Adelaide (IH)	winter	0.0%	0.06% (0.03% – 0.03%)	2.6E-04 (1.2E-04 – 1.3E-04)
Adelaide (IH)	summer	0.0%	0.12% (0.04% – 0.27%)	3.5E-04 (1.2E-04 – 8.0E-04)
Adelaide (OH)	winter	0.0%	0.09% (0.04% – 0.05%)	2.6E-04 (1.2E-04 – 1.5E-04)
Adelaide (OH)	summer	11.4%	0.20% (0.04% – 0.54%)	6.5E-04 (1.2E-04 – 1.8E-03)
Klein Point	winter	2.9%	0.09% (0.06% – 0.06%)	1.8E-04 (1.2E-04 – 1.2E-04)
Klein Point	summer	5.7%	0.13% (0.04% – 0.32%)	3.9E-04 (1.2E-04 – 9.8E-04)
Port Giles	winter	0.0%	0.10% (0.06% – 0.06%)	2.2E-04 (1.2E-04 – 1.2E-04)
Port Giles	summer	0.0%	0.11% (0.04% – 0.26%)	3.2E-04 (1.2E-04 – 7.4E-04)
Port Lincoln	winter	0.0%	0.40% (0.05% – 2.12%)	1.1E-03 (1.2E-04 – 5.8E-03)*
Port Lincoln	summer	45.7%	17.91% (0.04% – 48.98%)	6.8E-02 (1.2E-04 – 2.3E-01)**
Portland	winter	5.7%	0.12% (0.08% – 0.08%)	1.9E-04 (1.2E-04 – 1.2E-04)
Portland	summer	8.6%	0.20% (0.06% – 0.62%)	4.1E-04 (1.2E-04 – 1.2E-03)
Thevenard	winter	8.6%	0.25% (0.08% – 0.83%)	4.0E-04 (1.2E-04 – 1.4E-03)
Thevenard	summer	40.0%	6.91% (0.06% – 34.09%)	1.4E-02 (1.2E-04 – 8.2E-02)**

7.2. JAGS code for prevalence and concentration model

```
model{
	for(i in 1:N){
		# N = number of data rows
		# n_sp = number of species/assay results
		for(j in 1:n_sp){
			# Y = detect/non-detect
```

```
            # AP = Apparent prevalence
            # TP = True prevalence
            # lambda = expected number of planktonic pests
            # lc.int = log concentration in season 1
            # Bseas = seasonal covariate for species j
            # loc = location for row i
            # season = sample season for row i
            # logV = log sample volume
            # a = se intercept on cloglog scale
            # bSF = scale factor coefficient
            # lnSF = log Scalefactor for row i
            Y[i,j] ~ dbern(AP[i,j])
            AP[i,j] <- se[i,j] * TP[i,j] + (1 - SP[j])*(1 - TP[i,j])
            cloglog(TP[i,j]) <- max(min(12, lc.int[loc[i],j] + Bseas[j] * season[i] + logV[i]),-12)
            cloglog(se[i,j]) <- max(min(12, a[j] + bSF[j] * lnSF[i]),-12)
        }
    }
    # Priors
    # with scale factor effect on SE
    for(j in 1:n_sp){
        SE[j] ~ dbeta(Sa[j],Sb[j])T(1 - SP[j],)
        a[j] <- max(min(12, cloglog(SE[j])), -12)
        bSF[j] ~ dnorm(bSF.mean,10)
        SP[j] ~ dbeta(Ca[j],Cb[j])T(0.6,)
        Bseas[j] ~ dnorm(0,0.01)
        for(i in 1:n_loc){
            #n_loc = number of locations
            lc.int[i,j] ~ dnorm(0,0.001)
        }
    }
    bSF.mean ~ dnorm(-0.3,100)
    # Posterior calculation of abundance
    for(j in 1:n_sp){
        for(i in 1:n_loc){
            # winter
            logconc[i,j,1] <- min(max(lc.int[i,j],-9),9)
            #summer
            logconc[i,j,2] <- min(max((lc.int[i,j] + Bseas[j]),-9),9)
            conc_1[i,j] <- exp(logconc[i,j,1])
            conc_2[i,j] <- exp(logconc[i,j,2])
        }
    }
}
```