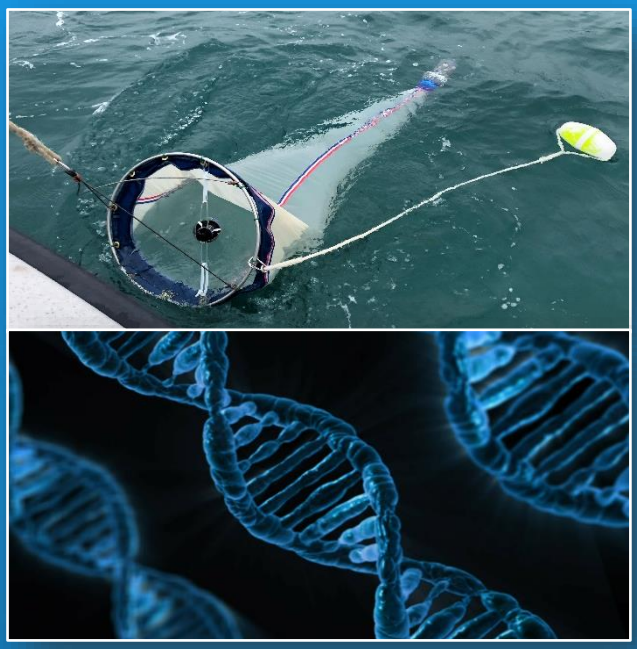
Marine Ecosystems

Statistical modelling approaches for the interpretation of molecular surveillance data



Wiltshire, K.H.

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SARDI Aquatics Sciences
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June 2023

Report to the Department of Agriculture, Fisheries and Forestry

Government of South Australia
Department of Primary Industries and Regions

Australian Government
Department of Agriculture, Fisheries and Forestry

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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EXECUTIVE SUMMARY

Molecular techniques for marine pest surveillance offer cost and time savings over traditional techniques, but for molecular surveillance to be routinely applied and used in management frameworks, data from molecular surveys need to be appropriately interpreted. Molecular surveys out-perform traditional methods but false negatives (FN, failure to detect a pest when present) and false positives (FP, incorrectly detecting a pest that is not present) may still occur. Where FN and FP rates are known, occupancy models (OMs) can be applied to provide unbiased estimates of the likelihood of species presence while accounting for FN and FP errors. OM methods have therefore been suggested as a useful tool for interpretation of molecular surveillance data.

OM is commonly applied to predict species occurrence likelihood across sites, but low occurrence likelihood estimates will result where a species is present at low abundance, and interpretation of such outputs for use in management may be difficult. Occupancy-abundance models (OAM) use the relationship between detectability and abundance to estimate abundance from patterns in detection. Estimates of abundance from OAMs are likely better suited to incorporation in management frameworks than likelihood estimates from OMs, but OAMs require additional sampling data and can be more difficult to fit.

To determine the most suitable modelling approach to interpret data from molecular surveys carried out using the plankton tow and qPCR method, a range of OMs and OAMs were run using data from molecular surveys of Australian ports for priority pest species. Models were compared based on precision and accuracy of parameter estimates, applicability of outputs to use in management frameworks, and feasibility to run.

All assessed models were feasible to run although more complex models, particularly those estimating concentration rather than occurrence likelihood, took considerably longer (hours *c.f.* minutes) to fit. Most assessed models were able to delineate present from absent species, but multi-scale models did not provide clear delineation or else resulted in implausible estimates. An OAM approach including FPs, seasonal and PCR inhibition effects is likely to be most useful for interpreting molecular surveillance. Planktonic concentration estimates from this model account for factors that may impact survey performance, are clearly related to the risk of propagule uptake in ballast water and can be directly compared with the target concentration used for survey design.

Keywords: Marine pests, molecular surveillance, plankton sampling, qPCR, occupancy modelling.

GLOSSARY

Table 1. Definition of abbreviations, symbols and technical terms used in the text.

Term	Definition
BW/ BWM	Ballast water / Ballast water management
C_T	Cycle threshold. In qPCR, the PCR cycle at which fluorescence exceeds a threshold and a detection is recorded. C_T is inversely related to the quantity of target DNA present (i.e., greater DNA content results in faster amplification and lower C_T)
DSe	Diagnostic sensitivity = likelihood of detection by a test when target is present
DSp	Diagnostic specificity = likelihood of non-detection by a test when target is absent
FN	False negative, failure to detect a target that is present
FP	False positive, apparent detection of a target not actually present
Prevalence	Proportion of samples containing a target (equivalently, likelihood of a given sample containing the target)
HDI	Highest density interval, the smallest interval containing a given probability mass (e.g., 95%) of a distribution
IMS	Introduced marine species
JAGS	Software used for model fitting using MCMC
LCM	Latent class model
MCMC	Markov chain Monte Carlo, a method for obtaining model estimates
MDeT	Monitoring design Excel template, a tool developed to design Australian port surveys
OAM	Occupancy-abundance model
OM	Occupancy model
PCR/ qPCR	Polymerase chain reaction/ quantitative PCR. A method for amplifying and detecting target DNA. qPCR measures target DNA amplification at each PCR cycle using fluorescence to quantify target DNA
z	Indicator of species presence (0 = absent, 1 = present)
ψ	Occurrence likelihood = probability of species occurrence in a sample (also known as prevalence) or at a site
θ	Availability = likelihood that a species occurs in (or is captured by) a sample, given presence at the site level

1. INTRODUCTION

1.1. Background

Introduced marine species (IMS) have wide ranging impacts on ecosystems, marine industries, infrastructure and amenity (Hayes *et al.* 2005; Schaffelke and Hewitt 2007; Molnar *et al.* 2008; Katsanevakis *et al.* 2014). Shipping is a major vector for IMS introductions via propagules in ballast water or hull-fouling (Hewitt *et al.* 2007; Molnar *et al.* 2008; Minchin *et al.* 2009; Hewitt and Campbell 2010) and ports are at high risk of shipping-mediated introductions and act as nodes for further spread of pest species (Glasby *et al.* 2007; Ojaveer *et al.* 2014; Lehtiniemi *et al.* 2015; Couton *et al.* 2019). The International Convention for the Control and Management of Ships' Ballast Water and Sediments 2004 (the BWM convention) includes ballast water management (BWM) measures that aim to prevent the spread of marine pests and human pathogens by this vector (IMO 2019).

Australia is a signatory to the BWM Convention and vessels operating domestically within Australia are required to manage ballast water risk by using low-risk ballast water, an approved ballast water treatment system, or exchanging ballast water (DAWE 2020). Vessels are eligible for BWM exemptions on domestic voyages where the ballast transfer is low risk (DAWE 2020). The risk assessment framework for domestic ballast water considers seven target IMS (Zhao *et al.* 2012; Arthur *et al.* 2015b): the seastar *Asterias amurensis* Lütken, 1871, the crab *Carcinus maenas* (Linnaeus, 1758), the fanworm *Sabella spallanzanii* (Gmelin, 1791), the brown seaweed *Undaria pinnatifida* (Harvey) Suringar, 1873, and the bivalves *Arcuatula senhousia* (Benson, 1842)*, Magallana gigas* (Thunberg, 1793) and *Varicorbula gibba* (Olivi, 1792). These IMS are established in some jurisdictions of Australia and pose a risk of further spread and impacts (Hayes *et al.* 2005). Where target IMS status at a port is unknown, a precautionary approach is applied to BWM exemptions (Zhao *et al.* 2012). Knowledge of target IMS status at ports is therefore required to assess risk and avoid unnecessary imposition of management and costs.

Molecular methods for pest detection are of interest for IMS surveillance to manage risk because they provide results rapidly and are cheaper than traditional methods (Bott *et al.* 2010b; Lehtiniemi *et al.* 2015). The South Australia Research and Development Institute (SARDI) has developed a molecular surveillance system using plankton tows tested with quantitative polymerase chain reaction (qPCR) assays for priority IMS (Giblot-Ducray and Bott 2013; Deveney *et al.* 2017). Performance of this molecular survey system and of qPCR assays for priority IMS has been

quantified, providing confidence that the method is suitable for surveillance to support risk-based BWM in Australia.

To assist implementation of molecular surveys for BWM, Wiltshire (2021) analysed available molecular survey data to estimate the planktonic concentration of target IMS at infested sites and developed a web-based sample number calculator[1] tool. The estimates of planktonic IMS concentration help inform a suitable minimum concentration for use in survey design, while the calculator tool can be used, given a target planktonic IMS concentration, to determine the number of samples required to achieve a desired survey confidence (Wiltshire 2021). For integration of molecular survey results into management systems, decision support tools to interpret survey results are also required. IMS managers need to understand the level of risk posed by detected species, and the potential likelihood of species presence despite non-detection, to determine what management actions are appropriate (Sepulveda *et al.* 2020b).

Molecular surveys can detect IMS at much lower abundance than traditional surveys but false negatives (FN, failure to detect a pest when present) and false positives (FP, incorrectly detecting a pest that is not present) may still occur. DNA detections do not always indicate species presence because DNA may be present from transient sources or contamination, or assays with inadequate specificity may cross-react with non-target DNA (Díaz-Ferguson and Moyer 2014; Goldberg *et al.* 2016; Hinlo *et al.* 2017; Stoeckle *et al.* 2017; Baillie *et al.* 2019). Issues with assay specificity should be identified and rectified when assays are assessed prior to implementation. The risk of contamination can be minimised through implementation of best-practice protocols for sampling and laboratory processing (Sepulveda *et al.* 2020a; Burian *et al.* 2021; Hutchins *et al.* 2022), but the potential for detection of transient DNA remains (Burian *et al.* 2021).

Uncertainty about viability of detected species complicates management decisions. Confirmation of species presence by obtaining specimens is the gold standard for detection, but may be impractical because traditional methods can fail to capture species that occur with low abundance, and delaying management based on inability to capture organisms can permit IMS to establish, spread, or cause preventable impacts (Darling and Mahon 2011; Sepulveda *et al.* 2020b; Jerde 2021). Management measures also can have economic, political and social costs, and managers therefore need robust data to support and justify decisions (Sepulveda *et al.* 2020b; Jerde 2021). Statistical methods that analyse surveillance results while accounting for inherent uncertainty can provide robust estimates of the likelihood of viable species presence and guide management

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

decisions. The applicable statistical methods belong to a large family of finite mixture models, which also includes latent class models (LCM) that are commonly used to assess diagnostic test performance (Branscum *et al.* 2005; Bermingham *et al.* 2015; Wang *et al.* 2020), or to estimate disease prevalence (Lewis and Torgerson 2012; Speybroeck *et al.* 2013; Buss *et al.* 2019). Where used to predict species occurrence from surveillance data, the approach is commonly referred to as site occupancy detection modelling or, more generally, occupancy modelling (OM).

OM comprises a broad range of models, with formulation dependent on the type of survey data, survey design, whether false positive errors are considered, and whether covariates are included for occurrence likelihood and/or detectability. Survey data may be binary (i.e., detected/not detected) or quantitative (e.g., counts), and OM approaches can be applied in either case (Royle and Nichols 2003). Survey designs may include nesting of survey sites within broader locations and varying levels of spatial or temporal replication, each of which influence OM formulation. Multi-scale models can be formulated to estimate occurrence while accounting for uncertainty on multiple levels, e.g., probability of occurrence at location, site and/or sub-site, probability of capturing the target, probability of accurate detection where captured (Nichols *et al.* 2008; Stanaway 2011; Guillera-Arroita *et al.* 2017). Such multi-scale models, however, require sufficient replication at each level of interest (Mackenzie and Royle 2005; Buxton *et al.* 2021). Models often consider only a single species and survey method, but the OM approach can be extended to jointly model multiple species and/or methods (Nichols *et al.* 2008; Schmelzle and Kinziger 2016; Chambert *et al.* 2018; Keller *et al.* 2022). OM approaches can also be extended to include spatial effects (Burian *et al.* 2021; Doser *et al.* 2022).

The most common application of OM is to predict likelihood of species occurrence across sites (MacKenzie *et al.* 2003; Miller *et al.* 2011; Schmidt *et al.* 2013), which is a useful measure in many studies, e.g., where the aim is to determine the percentage of sites in an area used by a species, or to explore how covariates influence occupancy likelihood. For some applications, including molecular surveillance, however, predictions of occurrence likelihood may be difficult to interpret. Detectability in a survey depends on the abundance of the target and the performance of the survey method (Royle and Dorazio 2009; McCarthy *et al.* 2013). Low occurrence likelihood estimates result where a species is present but at low abundance (Keller *et al.* 2022). Particularly where an aim of surveillance is early detection of invasive species, the detection of a previously absent species is of concern, even if with low occurrence likelihood in OM estimates. The relationship between detectability and abundance allows the OM approach to be extended to infer abundance where suitable sampling data (typically, sample volume data) are available (Royle

and Nichols 2003; Royle and Dorazio 2009; Furlan *et al.* 2016; Keller *et al.* 2022). These models are referred to as occupancy-abundance models (OAM). OAM approaches may provide estimates that are better suited to incorporation in management frameworks than OM, but in addition to requiring additional data about samples, these models can be more difficult to fit (Royle and Nichols 2003; Royle and Dorazio 2009). OM or OAM using data from multiple survey methods can also be used to assess the performance of each survey method and estimate FN and FP error rates where these are unknown (Schmelzle and Kinziger 2016; Tingley *et al.* 2020; Dimond *et al.* 2022; Keller *et al.* 2022).

Both OM and OAM typically use Bayesian methods for model fitting, specifically Markov chain Monte Carlo (MCMC) simulation, although frequentist approaches can be applied in some cases (Lahoz-Monfort *et al.* 2016; Guillera-Arroita *et al.* 2017; Tingley *et al.* 2020). Bayesian approaches can incorporate prior information (Stanaway 2011; Griffin *et al.* 2020; Burian *et al.* 2021), which can include test and/or survey method performance. Including prior information also allows estimates to be obtained where models would otherwise not be identifiable (Burian *et al.* 2021; Buxton *et al.* 2021). In particular, prior information is required for model identifiability where both FN and FP errors are considered, and data are from a single survey method (Lahoz-Monfort *et al.* 2016; Guillera-Arroita *et al.* 2017).

Freely available software such as JAGS (Plummer 2017), which provides cross-platform support, or the Windows-only WinBUGS (Lunn *et al.* 2000) can be run through the popular, free R statistical software (R Core Team 2023) to fit models using MCMC. Model fitting in JAGS or WinBUGS requires the model to be specified using the BUGS coding language, which is similar, but not identical, to the R language (Lunn *et al.* 2000; Plummer 2017). Some R packages for OM (Dorazio and Erickson 2018; Stratton *et al.* 2020; Doser *et al.* 2022) include MCMC samplers that run within R, avoiding use of additional software and the necessity of coding models in BUGS. These packages, however, offer limited flexibility in OM formulation, while coding models for JAGS or WinBUGS facilitates customised model specification. The MCMC sampling methods used by JAGS, WinBUGS, and some R packages are, however, computationally intensive, and complex models are computationally demanding and may take hours or days to run (Stratton *et al.* 2020; Doser *et al.* 2022). Alternative methods for Bayesian estimation, including numerical estimation and gradient sampling, are typically faster than MCMC, but these methods are not able to fit most finite mixture models, and are therefore rarely suitable for OM (Stratton *et al.* 2020). Complex models provide more informative outputs than simpler models, but require sufficient data, including suitable replication, to provide estimates and are time-consuming, or potentially

infeasible to run. The value of the additional information provided by more complex models therefore needs to be assessed in relation to available data and computational resources to determine if running more complex models is feasible and warranted.

To determine the most suitable modelling approach to interpret data from molecular surveys carried out using the plankton tow and qPCR method, OM and OAM approaches proposed in the literature were reviewed. A range of OM and OAM models were run using data from molecular surveys of Australian ports for priority pest species. Models were compared based on time taken to run, precision and accuracy of parameter estimates, and applicability of outputs to use in management frameworks.

1.2. Objectives

This project aimed to investigate occupancy modelling approaches for interpretation of molecular survey results, particularly to provide information relevant for IMS management. Specific aims were to:

- Assess the feasibility of applying different OM approaches to typical molecular survey data
- Compare model outputs and performance to determine the most suitable OM approach to inform ballast water risk based on molecular surveillance

2. METHODS

2.1. Data sets used in modelling

Three data sets were used to assess models. Initial assessment of models was carried out using data selected from a compiled set of molecular survey results (Table 3). Data used were from surveys of Australian ports from 2017 to 2022 using the validated SARDI plankton tow and qPCR testing method (Wiltshire *et al.* 2019a). Selected models were further assessed using the data set from the most recent 2021-22 survey of South Australian ports and Portland, Victoria (Wiltshire *et al.* 2022) and a simulated data set.

2.1.1. Port surveillance data

Several Australian ports were surveyed using standardised methods for collection and analysis from 2017 – 2022. Surveys in 2017 – 2019 tested for the seven IMS of ballast water concerns and for the exotic species *Perna canaliculus* and *Mytilopsis sallei*, with subsequent surveillance testing for additional species as new assays were developed and implemented. The ballast water target species and currently available assays are shown in Table 2. Surveys in 2020 applied 13 assays, while those in 2021-22 used 19 assays (Table 3). The data selected from the compiled set for analysis comprised the results for the nine assays that have been applied in all surveys. The Asen and Vgib assays have been demonstrated to lack specificity when applied to samples from at least some Australian locations (Deveney *et al.* 2017; Wiltshire *et al.* 2019a; Wiltshire *et al.* in prep). Results from these two assays were included to explore how the FP detections by these assays influenced model estimates.

Surveys in 2017 – 2020 (Wiltshire and Deveney 2017; Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c, 2020) were designed using the *Monitoring Design excel Template* (MDeT), a tool that was developed to support Australia's previous national marine pest surveillance strategy (DAFF 2010a, b). MDeT was, however, found to underestimate molecular survey performance (Wiltshire *et al.* 2019a), which, in combination with findings of a review of the previous surveillance system (Arthur *et al.* 2015a), prompted development of a survey design tool specifically for the molecular surveillance method (Wiltshire 2021). This molecular survey calculator tool was used to design the 2021-22 surveys (Wiltshire *et al.* 2022).

Table 2. 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. *Species on the National Priority List of Exotic Environmental Pests, Weeds and Diseases.

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)

At each surveyed port, wharves where ballast exchange is likely to occur were targeted for surveillance. Where relevant wharf areas within a port were separated by more than ~ 500 m, these were considered separate sublocations (sites). For surveys designed using the MDeT, which considers a default pest population size and uses site water volume and flushing rates in calculations for plankton sample numbers, sample numbers were calculated separately for each site. The molecular sample number calculator (Wiltshire 2021) uses a target IMS planktonic concentration in calculations rather than population size, because planktonic concentration is a better indicator of ballast water risk (Arthur *et al.* 2015a). This approach means that the same number of samples are collected per site (Wiltshire 2021). Future surveys are likely to use the approach of the 2021-22 survey including a standardised sample size across sites, probably 35 samples as per Wiltshire *et al.* (2022). To assess models, data were therefore selected from ten sites having sample size of between 30 and 45 plankton tows per sampling set (Table 4). The selected sites included some where each of the species established in Australia occur and some from which target species are absent.

Table 3. Molecular surveys of Australian ports 2017 – 2022. Data sets included in the assessment of models were selected from ports marked with an asterisk.

Survey reference	Ports surveyed	Sample sets	Assays applied
Wiltshire *et al.* (2019a)	Hobart, Melbourne*, Brisbane, Gladstone*	4: approximately seasonally July 2017 – June 2018	Aamu, Asen, Cmae, Mgig, Msal, Pcan, Sspa, Upin, Vgib
Wiltshire *et al.* (2019c)	Devonport, Pt Kembla, Botany Bay*, Newcastle, Hay Point, Weipa, Gove	2 per location (summer, autumn): May 2018 – May 2019	Aamu, Asen, Cmae, Mgig, Msal, Pcan, Sspa, Upin, Vgib
Wiltshire *et al.* (2020)	Bunbury, Kwinana*, Fremantle*, Geraldton	2 per location (summer, autumn): February – May 2020	Aamu, Asen, Cjap, Cmae, Mgig, Msal, Pcan, Pper, Pvir, Rhar, Sspa, Upin, Vgib
Wiltshire *et al.* (2022)	Portland*, Adelaide*, Port Giles, Klein Point*, Port Lincoln, Thevenard	2 per location (summer, winter): September 2021 – March 2022	Aamu, Asen, Cjap, Cmae, Dvex, Esin, Hsan, Mgig, Mjap, Mros, Msal, Mstr, Pcan, Pper, Pvir, Rhar, Sspa, Upin, Vgib

Table 4. Data sets included in the assessment of models showing number of samples collected and detections of selected species. See Table 2 for assay name definitions. Detections marked with an asterisk are regarded as uncertain (species not expected to be present).

Site	Sample set	Samples	Aamu	Asen	Cmae	Mgig	Msal	Pcan	Sspa	Upin	Vgib
Port Botany	Feb 2019	33	0	0	0	33	0	0	6	0	11*
Port Botany	May 2019	34	0	0	0	2	0	0	7	0	0
Fremantle	Mar 2020	34	0	33	0	0	0	0	0	0	20*
Fremantle	May 2020	33	0	33	0	0	0	0	0	0	31*
Gladstone coal terminal	July 2017	34	0	2*	0	0	0	0	0	0	2*
Gladstone coal terminal	Nov 2017	33	0	32*	0	0	0	0	0	0	27*
Gladstone coal terminal	Feb 2018	34	0	27*	0	0	0	0	0	0	5*
Gladstone coal terminal	Jun 2018	33	0	4*	0	0	0	0	0	0	5*
Adelaide Inner Harbor	Sep 2021	39	0	0	22	5	0	0	39	0	0
Adelaide Inner Harbor	Feb 2022	36	0	0	0	34	0	0	5	0	0
Klein Point	Sep 2021	36	0	0	2*	0	0	0	0	0	1*
Klein Point	Mar 2022	36	0	0	0	0	0	0	2*	0	2*
Kwinana bulk jetty	Mar 2020	32	0	30	0	0	0	0	0	0	15*
Kwinana bulk jetty	May 2020	32	0	32	0	0	0	0	10	0	22*
Melbourne Yarra River	July 2017	41	41	0	5	5	0	0	24	1	37
Melbourne Yarra River	Nov 2017	44	6	24	0	28	0	0	37	5	41
Melbourne Yarra River	Mar 2018	39	7	36	0	39	0	0	39	0	39
Melbourne Yarra River	Jun 2018	41	41	1	2	3	0	0	41	1	41
Adelaide Outer Harbor	Sep 2021	36	0	0	28	0	0	0	32	0	0
Adelaide Outer Harbor	Mar 2022	36	0	0	0	26	0	0	10	0	4*
Port Lincoln	Sep 2021	35	0	0	0	0	0	0	31	0	0
Port Lincoln	Feb 2022	35	0	0	0	0	0	0	2	0	16*
Portland	Sep 2021	36	0	0	0	0	0	0	16	33	2
Portland	Mar 2022	37	0	0	0	2*	0	0	2	22	3

The 2017-18 surveys that were used to validate the molecular survey method included plankton samples taken in four approximately seasonal sets to obtain preliminary data on seasonality in molecular detections (Wiltshire *et al.* 2019a). Results from that project suggested that sampling in summer and autumn provided high detection likelihood across the target IMS, and this strategy was applied to surveys in 2018 – 2020 (Wiltshire *et al.* 2019c, 2020). Analysis of a larger set of molecular survey data, including from samples collected in Port Adelaide and south-eastern Tasmania by Deveney *et al.* (2020) that were tested for priority IMS, and the molecular port surveys (Table 3), however, showed that a combination of sampling in summer and winter maximised detection likelihood (Wiltshire 2021). The 2021-22 survey therefore used summer and winter sampling (Wiltshire *et al.* 2022).

Analysis of the selected data set provided outputs for sites where target species were absent, rare or well established, allowing comparison of model performance relative to target species status and abundance. This data set, however, included more sites but fewer tested species that are likely to be included in future surveillance. The selected data set also included data from multiple seasons and different seasons per site, whereas future surveys are likely to sample in two opposing seasons (summer and winter in temperate areas), and to sample all sites within the same seasons. Selected models were therefore applied to the full data set from the most recent survey (Wiltshire *et al.* 2022) to assess performance on a typical data set. This analysis also included results from all 19 species tested (Table 3).

2.1.2. Available covariate data

The data available for each sample included relevant covariate data in addition to qPCR results for each tested species. Sampling location (site and GPS co-ordinates) and date of collection were recorded for each sample. Actual tow length (measured from GPS start and end points) and effective tow length (determined from flow meter readings) were available for most samples but missing in some cases. PCR inhibition was assessed during PCR analysis by comparing the DNA yield of an exogenous organism added at extraction in reference samples to that in each plankton sample. The resulting measure, referred to as the scale factor, is 1 where no inhibition is present, with higher scale factors indicating greater potential inhibition.

2.1.3. Simulated data set

A simulated data set was used to assess performance of selected models against known target species presence and planktonic concentration. The simulated data set used detection data

generated for seven species, nominally *Asterias amurensis, Carcinus maenas, Hemigrapsus sanguineus, Magallana gigas, Maoricolpus roseus, Sabella spallanzanii* and *Undaria pinnatifida*, at five locations assuming two sets of 35 samples per location. Species presence at each site was randomly generated using a location-level occurrence probability of 0.8 to ensure that each simulated location had several species present, and that species were each simulated to occur at multiple locations with a range of concentrations. The planktonic concentration of each species per sample set at each location with that species present was generated using ranges of planktonic concentrations for established pests in Australia and of seasonal covariates estimated by Wiltshire (2021). The seasonal covariates used did not necessarily correspond to expected seasonal patterns for the nominal species. Log-concentration was generated and then exponentiated to ensure positive values. Log-concentration for absent species was set to −10 for both sample sets, while for present species log-concentration for the second sample set was adjusted by addition of the seasonal covariate prior to being exponentiated. Likelihood of target presence in each sample was estimated using the simulated concentrations and randomly generated sampling volumes in the range recorded across the compiled data set, and detection likelihood calculated using assay diagnostic sensitivity (DSe) and specificity (DSp) of the assays Aamu, Cmae, Hsan, Mgig, Mros, Sspa and Upin. Detections were generated by random Bernoulli draws.

The effect of PCR inhibition on model performance was assessed using two modified simulated data sets, each using randomly generated scale factors for the simulated samples. For both inhibition-affected data sets, random log scale factors were generated using a gamma distribution to ensure positive values and to allow for a skewed distribution with most values close to 1 (i.e., no to minimal inhibition) while allowing for some large (>10) values, reflecting the pattern observed in most surveys. In the first inhibition-affected data set, inhibition was generated randomly across all simulated samples using the same simulated mean and standard deviation for the gamma distribution, resulting in similar levels of inhibition within each simulated sample set. In the second inhibition-affected data set, the mean of the gamma distribution was varied between sample sets, hence, some sample sets had higher levels of inhibition than others.

A scale factor coefficient for each nominal species was randomly generated using estimates for the relevant assays from the model including scale factor effect estimation applied to data from 2021-22 surveillance (see section 2.2). Adjusted likelihood of detection was calculated including the scale factor effect on DSe and detections generated by random Bernoulli draws using the revised detection likelihoods. Models were applied to simulated data using detection data with

and without inhibition effects on detection for comparison. Code used for simulation and a summary of the simulated data sets are provided in the Appendix (section 6.2).

2.2. Occupancy modelling

2.2.1. Selection of modelling approaches

Relevant literature on potential OM approaches was obtained through Google Scholar searches, using combinations of the terms: “occupancy” or “prevalence”; “model” or “modelling”; “survey” or “surveillance”; and “DNA”, “qPCR”, “PCR” or “molecular”. The most relevant papers returned by the search results were downloaded and reviewed. Relevant studies cited by these papers that were not returned in the search results were also obtained and reviewed. Supplementary material containing modelling code or model specifications was also obtained and reviewed where available.

The review of potential OM approaches considered the applicability of models to the available data. No models were found to be applicable to the molecular survey data without at least some modification. Most OM approaches in the literature considered only a single species and code would need to be extended to simultaneously model multiple species. Other models could not be applied to the molecular survey data without modification because they were formulated for survey designs with different hierarchical structure or required data from repeated testing of DNA from each sample for the same species (technical PCR replicates). The R packages developed for analysis of molecular survey data (Dorazio and Erickson 2018; Griffin *et al.* 2020; Stratton *et al.* 2020; Doser *et al.* 2022) were found to be unsuitable due to also having been designed for single species or requiring technical replicates. The models run by these packages also lacked flexibility to include several components of interest (see Table 5). Custom models were therefore developed using published OM approaches for guidance but without applying specific published models. The models tested aimed to test the feasibility of inclusion and effect on resulting estimates of the options shown in Table 5.

2.2.2. Model formulation

Models were formulated to include combinations of the OM options (Table 5) using code or formulae adapted from MacKenzie *et al.* (2003), Nichols *et al.* (2008), Royle and Dorazio (2009), Speybroeck *et al.* (2013), Furlan *et al.* (2016) and Guillera-Arroita *et al.* (2017). The options considered in each model are summarised in Table 6. Models to estimate abundance or including

inhibition effects need to use individual sample data because the volume of each sample is required to infer concentration and the scale factor of each sample is needed for estimation of inhibition effects. For other models, grouped data was used, except for one model (OM_8) used to assess any difference in model running time or estimates using individual as compared to grouped data.

Beta priors were used for likelihood of FN errors, and for FP where included, based on assessed assay performance (Wiltshire *et al.* 2019b; Wiltshire *et al.* in prep). Beta parameters for DSe (= 1 – FN) and DSp (= 1 – FP) priors were calculated using the *betaExpert* function in the *R* package *prevalence* (Devleesschauwer et al. 2014) from the mean and 95% highest density interval (HDI) of previous estimates of DSe and DSp for each assay (Wiltshire *et al.* 2019b; Wiltshire *et al.* in prep). Calculated Beta parameters are shown in the Appendix.

For models of occurrence likelihood, vague Beta(1,1) priors were used for likelihood of occurrence of each species at the site level. Occurrence likelihood was estimated independently for each sample set in models without seasonal covariates. Where seasonal covariates were included, the complimentary log-log (cloglog) link was used, with the covariate for occurrence of each species in each included season given a normal prior with mean 0 and precision of 0.4, which is a vague prior on the cloglog scale. The seasonal covariate was not estimated separately for each location because the available data did not include sufficient temporal replication to allow this to be estimated. In models to estimate abundance, the intercept of log-concentration for each species at each site was given a normal prior with mean 0 and precision 0.1. The cloglog link was used to estimate likelihood of occurrence at the sample level in these models, using the log-concentration intercept, log sample volume and seasonal covariate. Two variations of multi-scale modelling were applied. For the first, species presence at the site level (z) was modelled as a Bernoulli indicator variable, with probability given by the site-level occurrence likelihood (ψ). Occurrence likelihood at the sample level was given by the product of z and availability, θ. For the second variation, sample-level occurrence likelihood was calculated as ψ x θ. In each case, θ was given a Beta(1,1) prior. A single value of θ was estimated across species/locations.

2.2.3. Model outputs

Models of occurrence likelihood provided estimates of occurrence likelihood of each species per sample set per location, or, of each species per season per location where seasonal covariates were included. Models of abundance provided estimates of planktonic concentration per season

per location. Multi-scale models provided estimates of theta, a parameter also referred to as species availability likelihood. Models including seasonal covariates also provided estimates of the relative occurrence likelihood or concentration as relevant for each species and season. All model outputs include updated estimates of DSe, and of DSp where FN errors are also included.

Table 5. OM options considered.

Model option	**Description**
Multi-scale	Occurrence likelihood at both site and sample level (2 variations)
Grouped data	Model uses total detections of each species per sample set
Individual data	Model uses detect/non-detect data from each individual sample
False negatives (FN)	Include potential false negatives (imperfect detection)
False positives (FP)	Include potential false positive detections
Seasonal covariates	Include seasonal covariates for occurrence likelihood or abundance
Estimate abundance	Infer planktonic concentration using sample volume
Inhibition effect	Include potential effect of PCR inhibition on detection likelihood

Table 6. OM options included in assessed models. Two variations on multi-scale modelling were tested as denoted by superscripts (see text for details).

Model	**Options included**
OM_1	FN, grouped data
OM_2	FN, grouped data, multi-scale[1]
OM_3	FN, grouped data, multi-scale[1], seasonal covariates
OM_4	FN & FP, grouped data, multi-scale[1], seasonal covariates
OM_5	FN & FP, grouped data
OM_6	FN & FP, grouped data, seasonal covariates
OM_7	FN & FP, grouped data, seasonal covariates, multi-scale[2]
OM_8	FN & FP, individual data, seasonal covariates
OAM_1	FN & FP, individual data, estimate abundance
OAM_2	FN & FP, individual data, seasonal covariates, estimate abundance
OAM_3	FN & FP, individual data, seasonal covariates, estimate abundance, multi-scale[2]
OAM_4	FN & FP, individual data, seasonal covariates, estimate abundance, inhibition effect

2.2.4. Data preparation

Each survey aimed to collect samples in specified seasons, but due to logistical and port access issues, samples were not always collected in the target season, and within seasons, samples were collected in different months across sites and projects. For models using seasonal covariates, season was assigned to each sample using the month of collection rather than the nominal collection season where different. Given the typical lag between ocean and air temperature cycles, January – March was considered "summer", April – June as "autumn", July – September as "winter" and October – December as "spring", following Wiltshire (2021). For models requiring sample volume, a regression analysis was used to impute missing flow meter distances, using location, sample set, and tow length as measured by GPS as predictors. Linear regression was run with *R-INLA* (Martins *et al.* 2013; Lindgren and Rue 2015; Rue *et al.* 2017) in *R* statistical software v4.2 (R Core Team 2023), and fitted values from this model were used to calculate tow volume for any sample with missing flow meter data.

2.2.5. Model fitting

All models were fit using Markov chain Monte Carlo (MCMC) simulation in JAGS v. 4.3.0 (Plummer 2017) for parameter estimation. JAGS code for each model applied is provided in the Appendix. MCMC simulations were obtained from three chains using 10,000 iterations thinned at a rate of 10, following 40,000 iterations for burn-in. JAGS was run using the *R2jags* package (Su and Yajima 2015) in *R*. Convergence was assessed by Gelman-Rubin convergence statistic and by visual inspection of trace, density and autocorrelation plots generated using the *MCMCplots* package (McKay Curtis 2015). Highest density intervals (HDIs) were calculated using the *HDInterval* package (Meredith and Kruschke 2018). The time taken for each model to run was assessed using the inbuilt *system.time* function in *R*.

2.2.6. Model performance assessment

Model predictions of occurrence likelihood or planktonic concentration of each species at each location were extracted from outputs. Except for models run on simulated data, the highest estimate across sample sets or seasons for each species/location was considered and assessed relative to the expected status (present/absent) of the species at that location. Model outputs were examined to determine if predictions clearly delimited occurrence likelihood or concentration for species expected to be absent that were not detected (considered as confirmed absences) and those expected to be present that were detected (considered confirmed presences). For models

run using the simulated data, predictions for each sample set or season were assessed relative to the relevant simulated values. The precision of model estimates was assessed using the 95% HDI of MCMC estimates. Posterior DSe and DSp estimates were examined to determine whether these varied from prior estimates used in modelling. A shift in these estimates by one model relative to others would indicate that a model did not effectively estimate occurrence likelihood and instead assumed detections/non-detections were due to assay performance rather than likelihood of species presence. If estimates of DSe or DSp were shifted across multiple different model formulations, this would indicate that the data was not consistent with the priors used.

For models run using the simulated data set, occurrence likelihood or planktonic concentration estimates were compared to the simulated values. Model estimates for seasonal covariates, scale factor (inhibition) effect and posterior DSe and DSp values examined.

The time taken to run was used to assess the feasibility of applying each model. The time taken is only indicative because this will vary with computing power, but is useful to compare models run using the same machine. Time taken to run is also dependent on the size of the data set used in analysis. The analyses of data from the most recent molecular survey provides information on the relative time taken for selected models to run on a typical data set. The time taken to run models can be reduced by employing parallel processing, but individual MCMC chains cannot be split across processing cores, limiting the potential time saving to a factor equal to the number of chains (typically 3). Parallel processing was not used in this study, but, given all models used the three MCMC chains for estimation, relative time taken to run would be unaffected by the application of parallel processing.

3. RESULTS

3.1. Modelling of compiled data set

3.1.1. Summary of outputs and running time

For models run using the compiled data set of ten sites and nine species, running time increased with the number of parameters estimated (Table 7). Models estimated a minimum of two parameter types: an occupancy measure, either as occurrence likelihood or planktonic concentration, and posterior assay sensitivity, DSe. Additional parameters included in a subset of models were posterior assay specificity, DSp, seasonal covariates, availability likelihood (θ), and inhibition effect. The total number of parameters estimated for each model depends on the parameter types specified but also on the nature of the data set, specifically, the number of unique sites, species, and seasons or sample sets. This dependence is due to occupancy being estimated for each species in each location per sample set or season, and DSe (plus DSp and seasonal effects where included) being estimated separately per species.

Table 7**.** Outputs and running time for models using the compiled data set of ten sites and nine species, showing number and types of parameters returned. Occupancy was estimated for each species/site combination by either sample set or season as indicated. Occupancy was estimated as occurrence likelihood or concentration (Conc). Other estimated parameters were: DSe/DSp = posterior estimates of DSe, or both DSe & DSe, θ = availability likelihood, Inhib = inhibition effects estimated.

model	time	Parameter types (total parameters)	Occupancy by:	Conc	DSe/ DSp	θ	Inhib	Data
OM_1	0:00:40	2 (226)	sample set	x	DSe	x	x	grouped
OM_2	0:00:49	3 (227)	sample set	x	DSe	✓	x	grouped
OM_3	0:01:48	4 (407)	season	x	DSe	✓	x	grouped
OM_4	0:02:25	5 (416)	season	x	DSe, DSp	✓	x	grouped
OM_5	0:01:07	3 (235)	sample set	x	DSe, DSp	x	x	grouped
OM_6	0:02:04	4 (415)	season	x	DSe, DSp	x	x	grouped
OM_7	0:01:54	5 (416)	season	x	DSe, DSp	✓	x	grouped
OM_8	0:19:30	4 (415)	season	x	DSe, DSp	x	x	individual
OAM_1	0:46:29	4 (451)	sample set	✓	DSe, DSp	x	x	individual
OAM_2	1:46:58	5 (505)	season	✓	DSe, DSp	x	x	individual
OAM_3	3:37:27	6 (506)	season	✓	DSe, DSp	✓	x	individual
OAM_4	2:53:35	6 (514)	season	✓	DSe, DSp	x	✓	individual

Occupancy-abundance models (OAM) used to estimate planktonic concentration took considerably longer to run (46 min to several hours) than models to estimate occurrence likelihood

(most < 3 minutes). Model OM_8, which was used to test the effect of using individual rather than grouped data, took ~10 times as long to run as the equivalent grouped data model, OM_6 (Table 7). OAMs used individual data by necessity, but this explains only part of the increased running time, with OAM_2 taking ~5 times as long to run as the otherwise equivalent OM_8 (Table 7).

3.1.2. Posterior estimates of assay performance, availability, and seasonality

Modelled estimates of DSe, DSp, θ and seasonal covariates were examined before assessing occupancy estimates because occupancy predictions should be considered with knowledge of these parameters. A model predicting low DSe or θ, for example, would predict higher occurrence likelihood (or concentration) for the same pattern of detection because the model is assuming a lower likelihood of successful detection due to either assay performance parameters (low DSe) or a lack of available targets (low θ). A model estimating low DSp, conversely, would predict lower occurrence likelihood because it assumes a greater proportion of detections are false positives. A consistent shift in DSe and DSp across multiple models would suggest that the priors used were not appropriate.

Predicted seasonal effects provide context for comparison of models that include seasonal effects with those predicting by sample set. A comparison of seasonal predictions across different model formulations also provides insight into how the inclusion of other parameters (potential FPs, availability) influences predicted seasonal effects.

Availability likelihood (θ) estimates varied widely between the different multi-scale models, ranging from ~0.5 to ~1 in models of occupancy likelihood and being close to 0 in OAM_3 (Figure 1). Predicted seasonal effects were generally similar across models where these were included, but with a tendency for higher predictions by OAM_3 than the OAMs that didn't consider θ (Figure 2). The multi-scale models OM_3 and OM_4 provided estimates with wider 95% HDIs than the OMs without estimation of θ, and estimates that differed from the other OMs for some species and season combinations. Seasonal covariates for some species and seasons also varied between model OM_3, which did not consider FPs, and OM_4, which did (Figure 2).

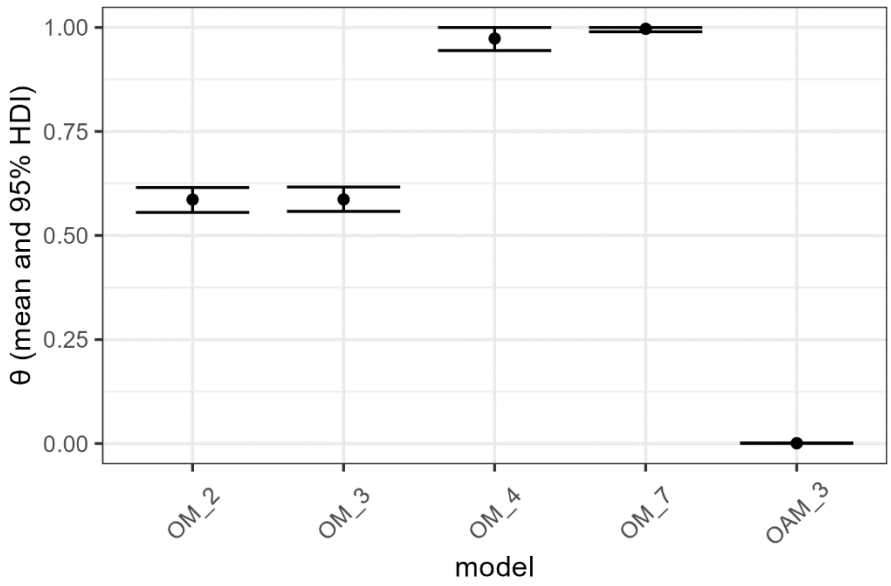

Figure 1. Estimated availability likelihood (θ) in multi-scale models

Posterior estimates of DSe for each assay were lower than prior estimates for some assays in OM_2 and OM_3, the multi-scale models that did not include potential FP errors (Figure 3). DSe estimates were also slightly lower than prior estimates for some assays in models that predicted occurrence by sample set (OM_5, OAM_1) rather than using seasonal covariates. Posterior DSp estimates were marginally higher than the prior estimate for the two assays with lower prior specificity (Asen, Vgib) across all models including FP errors. This suggests that FP rates in the data set used for modelling were slightly lower than expected, however, the posterior DSp estimates for these two assays were still lower than for other assays. Posterior DSp estimates for other assays were > 0.99 and did not vary from the priors, except for in OM_4, which predicted slightly lower DSp for Sspa than the prior estimate (Figure 3).

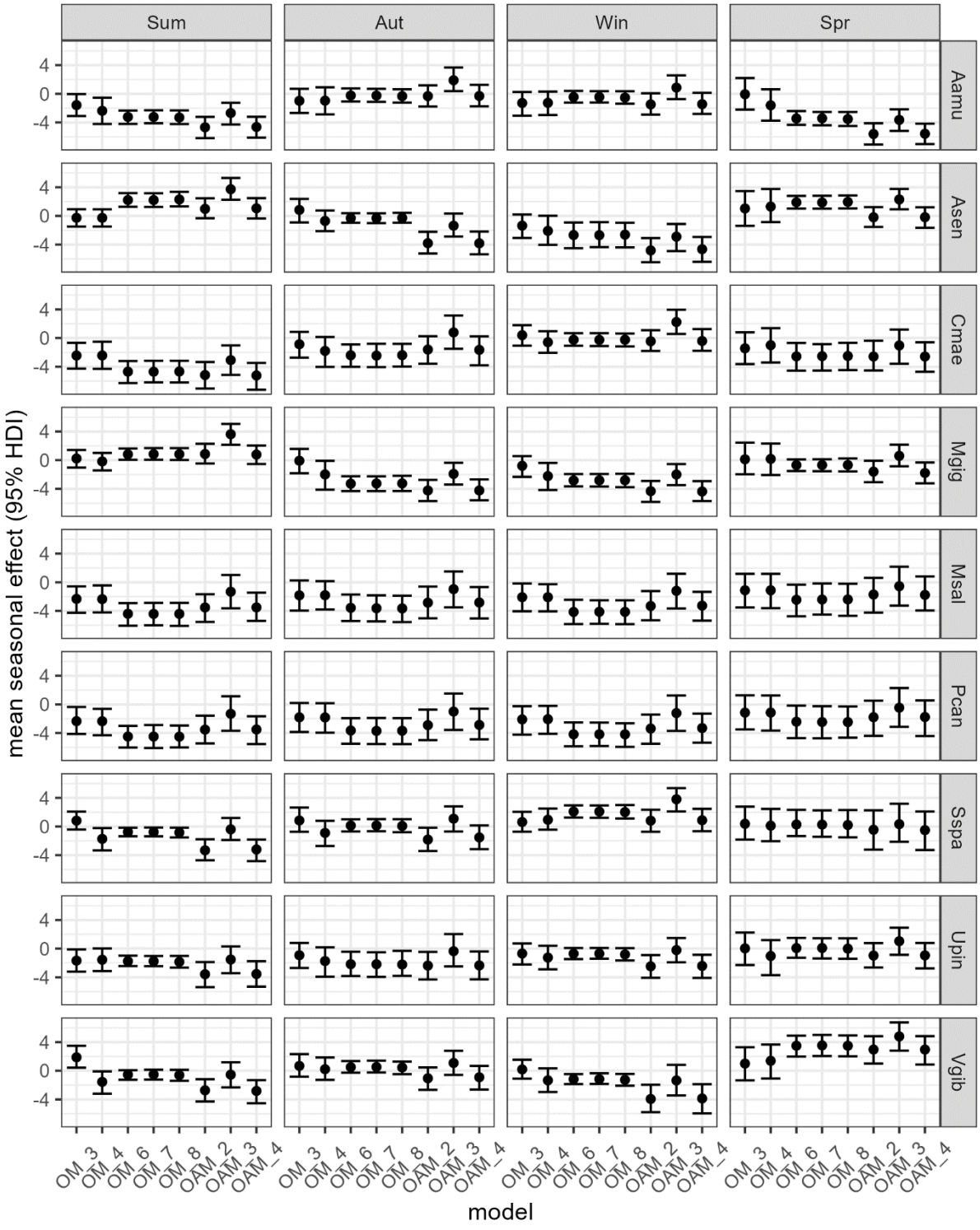

Figure 2. Estimated seasonal covariates for each species and season across models that included estimation of seasonal effects.

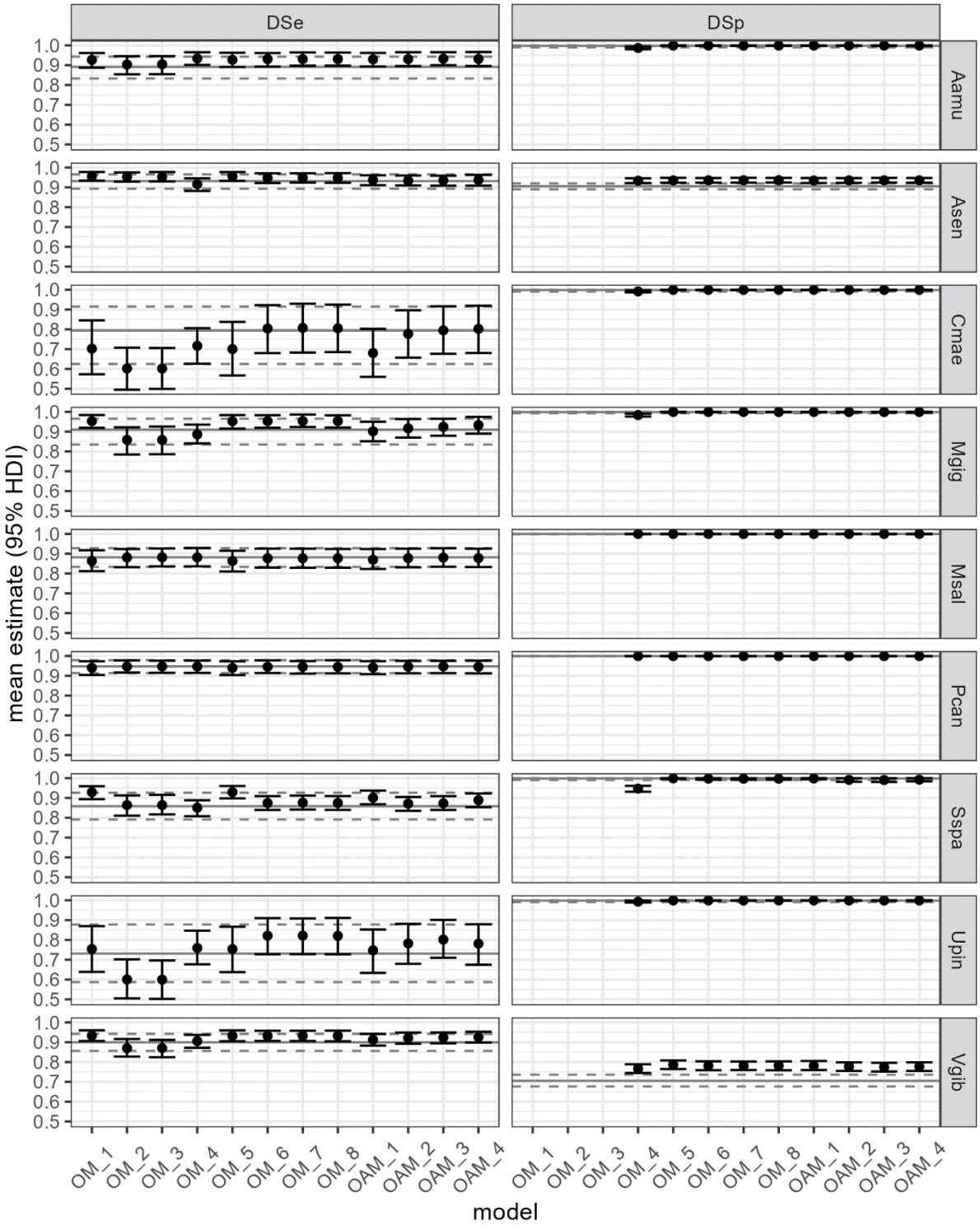

Figure 3. Posterior estimates of DSe and DSp. The prior mean estimate is shown as a solid grey line with dashed lines indicating the 95% probability bounds of the prior.

3.1.3. Occupancy estimates

Multiscale models that used an indicator variable of species presence (OM_2 to 4) did not clearly differentiate the different classes of species occurrence (Figure 4). Mean occurrence predictions were higher for confirmed presences than confirmed absences, but with large overlap of 95% HDIs. Predicted occurrence likelihood estimates for uncertain detections were generally similar to those for confirmed presences in these models. The remaining models showed clear differences in the ranges of predictions for confirmed absences and presences (Figure 4). Occurrence likelihood or concentration estimates were low in these models for some detections regarded as confirmed presences, but these were for species detected in only a small proportion of samples in each case. Estimates for uncertain detections were generally lower than for confirmed presences in these models (Figure 4). Many of the uncertain detections were of Vgib (Table 4). Models including FP errors (except OM_4, as noted above) provided lower occurrence estimates for Vgib due to assuming a relatively high proportion of FP detections, but, for sites with many detections, estimates fell within the range of confirmed positives. Multi-scale OAMs (OAM_3) predicted planktonic concentrations 1 – 2 orders of magnitude higher than other OAMs due to the low value of θ estimated. Inclusion of θ in model OM_7 did not result in any change in occupancy estimates in comparison to the otherwise equivalent OM_6, due to the estimate for θ being close to 1 in this case. The use of individual data in OM_8 did not result in any difference in estimates from the equivalent model using grouped data (OM_6). OMs estimating occurrence by season (OM_6 to 8) showed wider 95% HDIs for some predictions than OM_5, which provided estimates per sample set. There was little difference, however, in 95% HDI width between OAMs using sample set (OAM_1) and season (OAM_2 to 4).

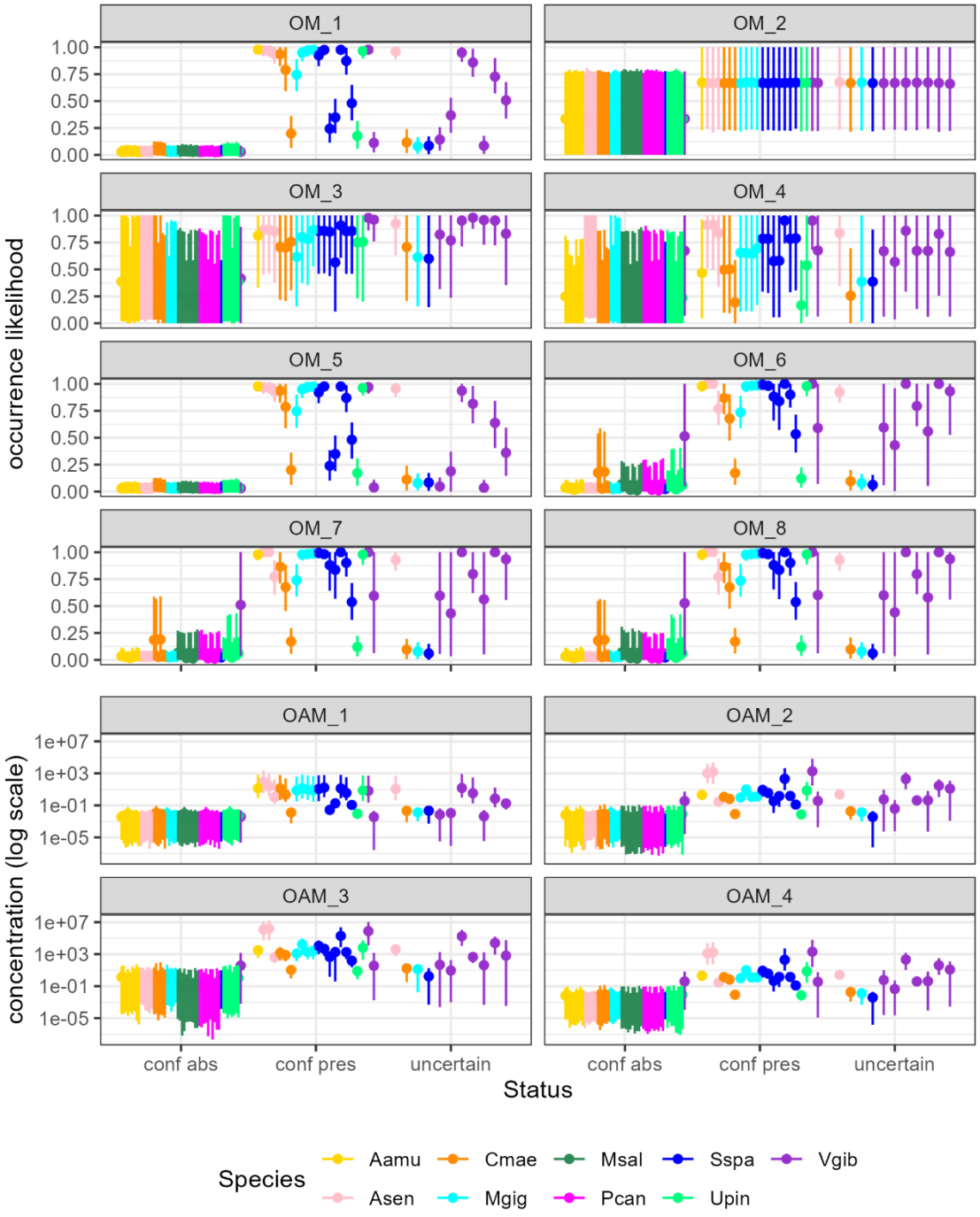

Figure 4. Summary of posterior occupancy estimates. Estimates are shown for: confirmed absences (conf abs) = species not detected and expected to be absent; confirmed presences (conf pres) = detected species expected to be present; and uncertain = detected species not expected to be present.

3.1.4. Models selected for application to additional data

Models OM_2 to OM_4 were not considered further due to their inability to clearly discriminate presence and absence. Model OM_1 was also not considered further because it did not consider FP errors, and including FP errors imposed only a very minor computational burden while likely improving estimates. Predictions from models OM_6 to OM_8 were near identical (within the variation expected by MCMC sampling) and model OM_6 was applied to further data because this was the most efficient (fastest) of these models to run. Models OM_5 and OAM_1 were included to further compare predictions made by sample set rather than by season. Model OAM_3 was not considered because this model assumed very low availability (θ) and hence much higher concentrations than equivalent models without θ while also requiring additional computation time. Models OAM_2 and OAM_4 were included to further compare the influence of including the inhibition effect.

3.2. Modelling of data from 2021-22 surveillance

The total number of parameters estimated for the data set from 2021-22 surveillance, which considered seven sites with sampling in two seasons (= 14 sample sets) and 19 species, was similar to that for the compiled data set despite differences in the number of sites, sample sets and species, and running time was therefore similar for equivalent models (Table 8). As found for the compiled data set, running time was considerably longer for OAMs than OMs. Models that included estimation by season (OM_6, OAM_2 and 4) took approximately twice as long to run as equivalent models estimating by sample set (OM_5 and OAM_1), while inclusion of inhibition effect estimation (OAM_4 *c.f.* OAM_2) resulted in ~50% longer run time (Table 8).

Table 8. Outputs and running time models using the data set from 2021-22 surveillance, showing number and types of parameters returned. Occupancy was estimated for each species/site combination by either sample set or season as indicated. Occupancy was estimated as occurrence likelihood or concentration (Conc). All models included estimation of both DSe and DSp.

model	time	Parameter types (total parameters)	Occupancy by:	Conc	Inhib
OM_5	0:01:05	3 (305)	sample set	x	x
OM_6	0:02:05	4 (343)	season	x	x
OAM_1	0:55:10	4 (571)	sample set	✓	x
OAM_2	1:58:18	5 (476)	season	✓	x
OAM_4	2:55:46	6 (495)	season	✓	✓

Posterior estimates of DSe were consistent with priors, although, as found for the compiled data set analysis, posterior DSe was marginally lower for some assays in OM_5 and OAM_1 compared with models using seasonal covariates, although within the 95% probability bounds of the priors used (Figure 5). Model OAM_2 also estimated slightly reduced DSe for Mgig in comparison with OAM_4, which considered inhibition effects and provides an estimate of DSe in the absence of inhibition. Posterior DSp estimates were consistent with priors for assays with prior DSp > 0.99 (Figure 6). For other assays, posterior DSp was slightly higher than the prior estimate across all models, indicating fewer FPs occurred than expected. As per the analyses of the compiled data set, posterior DSp estimates for these assays were still below those of the highly specific assays.

Model OAM_4 estimates for the effect of inhibition on DSe were generally negative, but with 95% HDIs spanning zero, indicating that the inhibition effect was not significant, except for Mgig (Table 9). Relatively few samples in this data set displayed inhibition (Wiltshire *et al.* 2022), so estimates of inhibition effect are difficult to estimate across assays from these data. The negative predicted scale factor effect for Mgig in OAM_4, however, in combination with reduced DSe predicted for this assay in OAM_2, suggests detections of this species were compromised in some samples by the presence of inhibition.

Models including seasonal covariates estimated similar seasonal patterns (Figure 7). Seasonal patterns could not be estimated for species without detection but were evident for most detected species. Each model predicted higher seasonal occurrence (likelihood or concentration) in summer for Asen and Mgig, and higher occurrence in winter for Cmae and Sspa.

Occupancy likelihoods for detected species varied slightly between models predicting by sample set (OM_5, OAM_1) and season (OM_6, OAM_2 and 4), although with considerable overlap of 95% HDIs for the OMs (Figure 8). Planktonic concentrations estimated by OAM_1 were, however, considerably greater for most detected species than those of OAM_2 or 4. Concentration predictions were near identical for the two OAMs that included seasonal effects (Figure 9) demonstrating that including consideration of inhibition effects did not meaningfully change estimates for this data set.

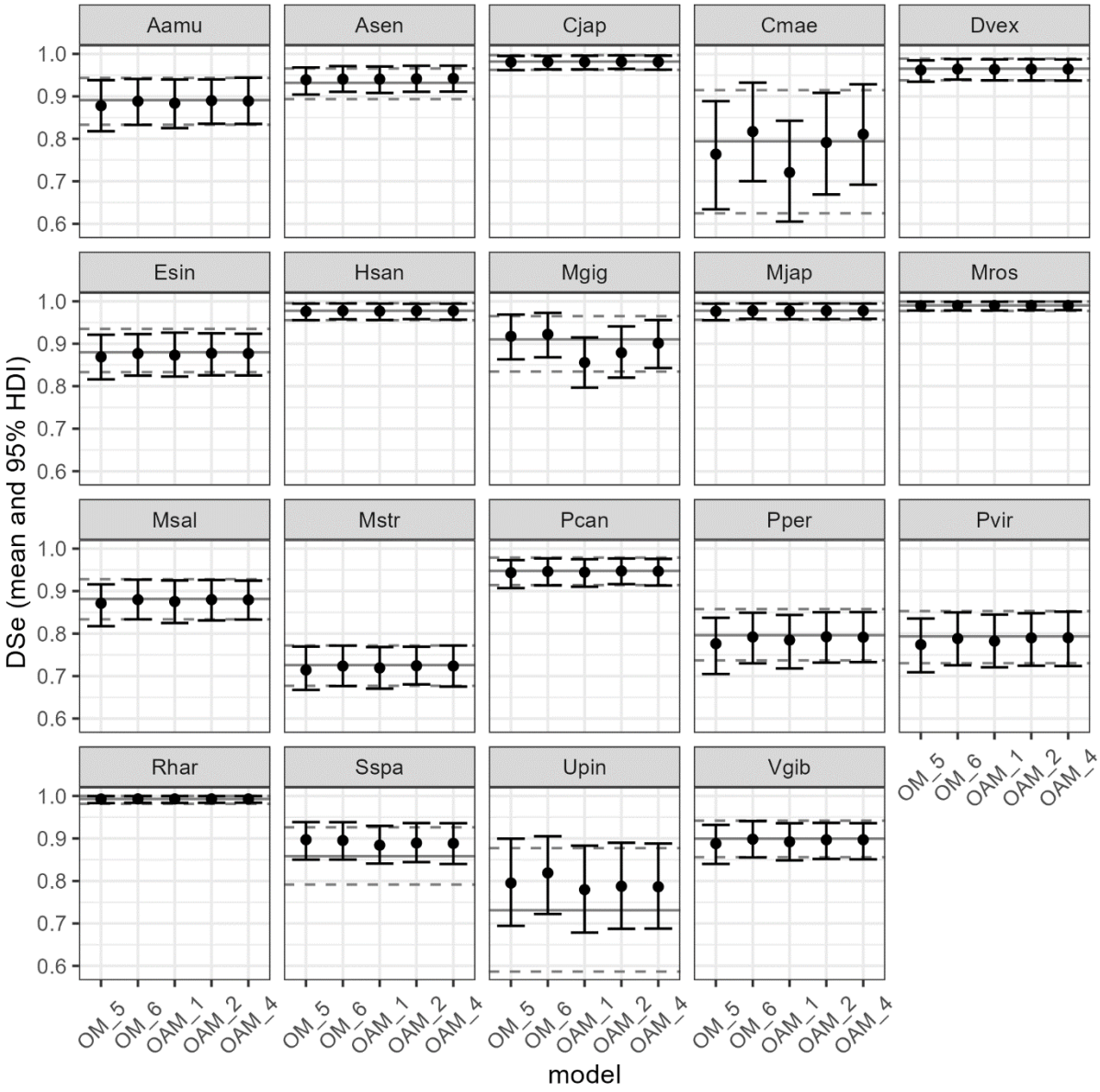

Figure 5. Posterior estimates of DSe for models applied to 2021-22 surveillance data. The prior mean estimate is shown as a solid grey line with dashed lines indicating the 95% probability bounds of the prior.

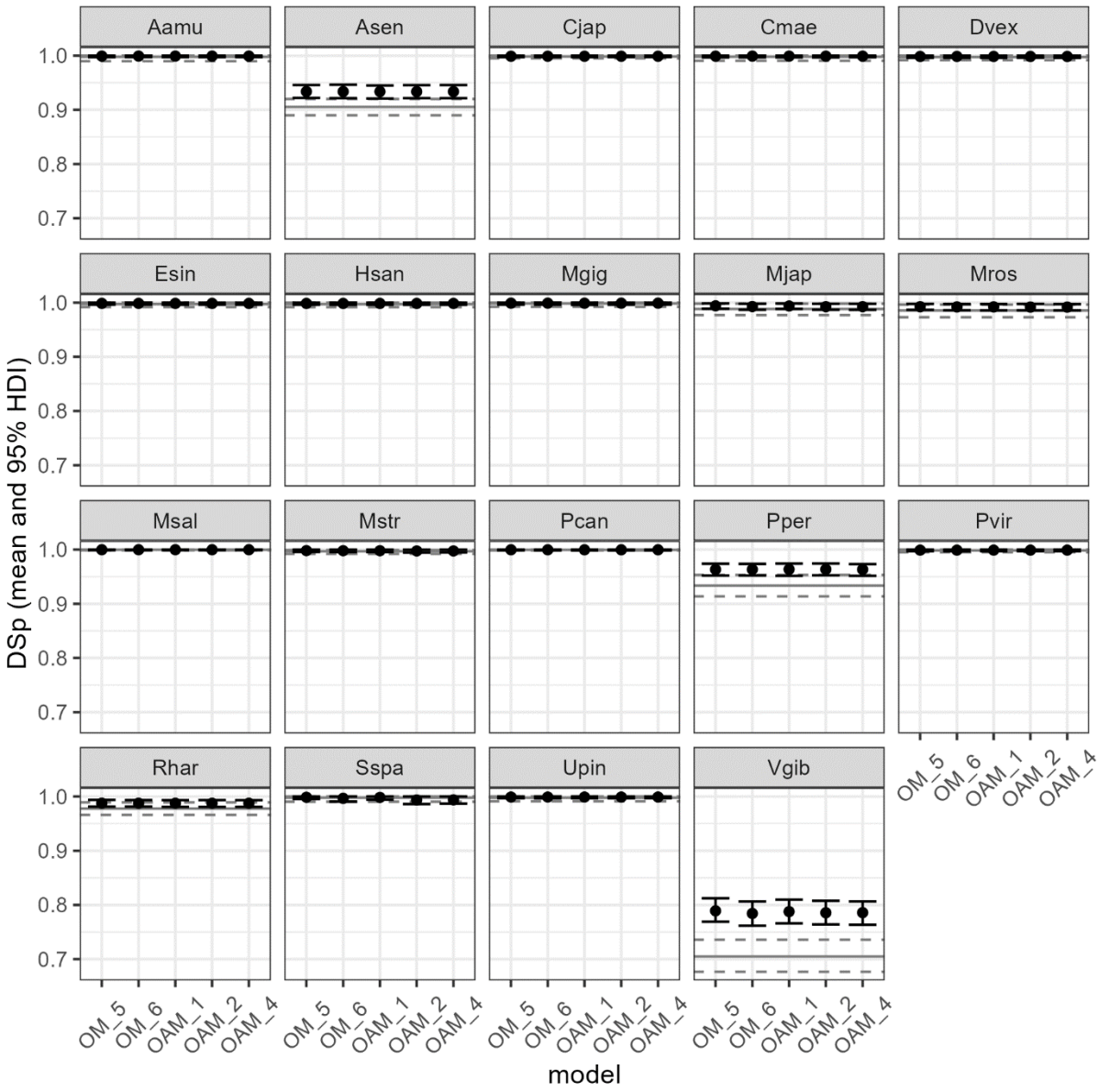

Figure 6. Posterior estimates of DSp for models applied to 2021-22 surveillance data. The prior mean estimate is shown as a solid grey line with dashed lines indicating the 95% probability bounds of the prior.

Table 9. Estimated scale factor effect from model OAM_4 using data from 2021-22 surveillance

Species	Scale Factor effect (mean and 95% HDI)
Aamu	−0.33 (−0.97 – 0.31)
Asen	−0.10 (−0.67 – 0.55)
Cjap	−0.30 (−0.94 – 0.35)
Cmae	−0.24 (−0.79 – 0.29)
Dvex	−0.31 (−1.03 – 0.27)
Esin	−0.34 (−0.96 – 0.30)
Hsan	−0.30 (−0.96 – 0.35)
Mgig	−0.34 (−0.65 – −0.07)
Mjap	−0.30 (−0.93 – 0.33)
Mros	−0.32 (−0.99 – 0.30)
Msal	−0.33 (−0.96 – 0.28)
Mstr	−0.32 (−0.92 – 0.33)
Pcan	−0.32 (−0.95 – 0.33)
Pper	−0.34 (−0.99 – 0.31)
Pvir	−0.34 (−1.00 – 0.33)
Rhar	−0.31 (−1.00 – 0.30)
Sspa	0.01 (−0.44 – 0.54)
Upin	−0.34 (−0.97 – 0.33)
Vgib	−0.34 (−0.97 – 0.29)

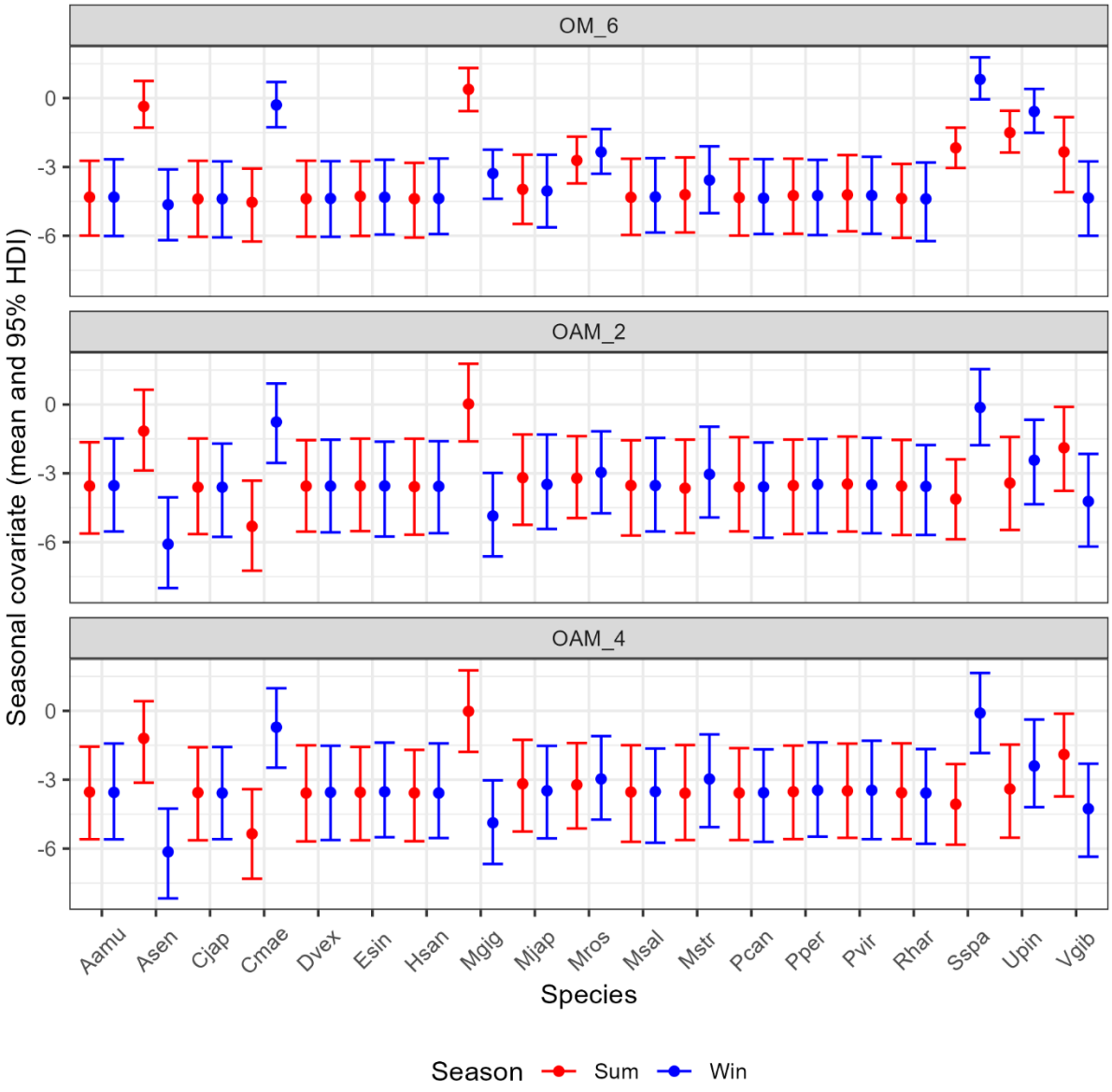

Figure 7. Seasonal covariates estimated by models applied to 2021-22 surveillance data

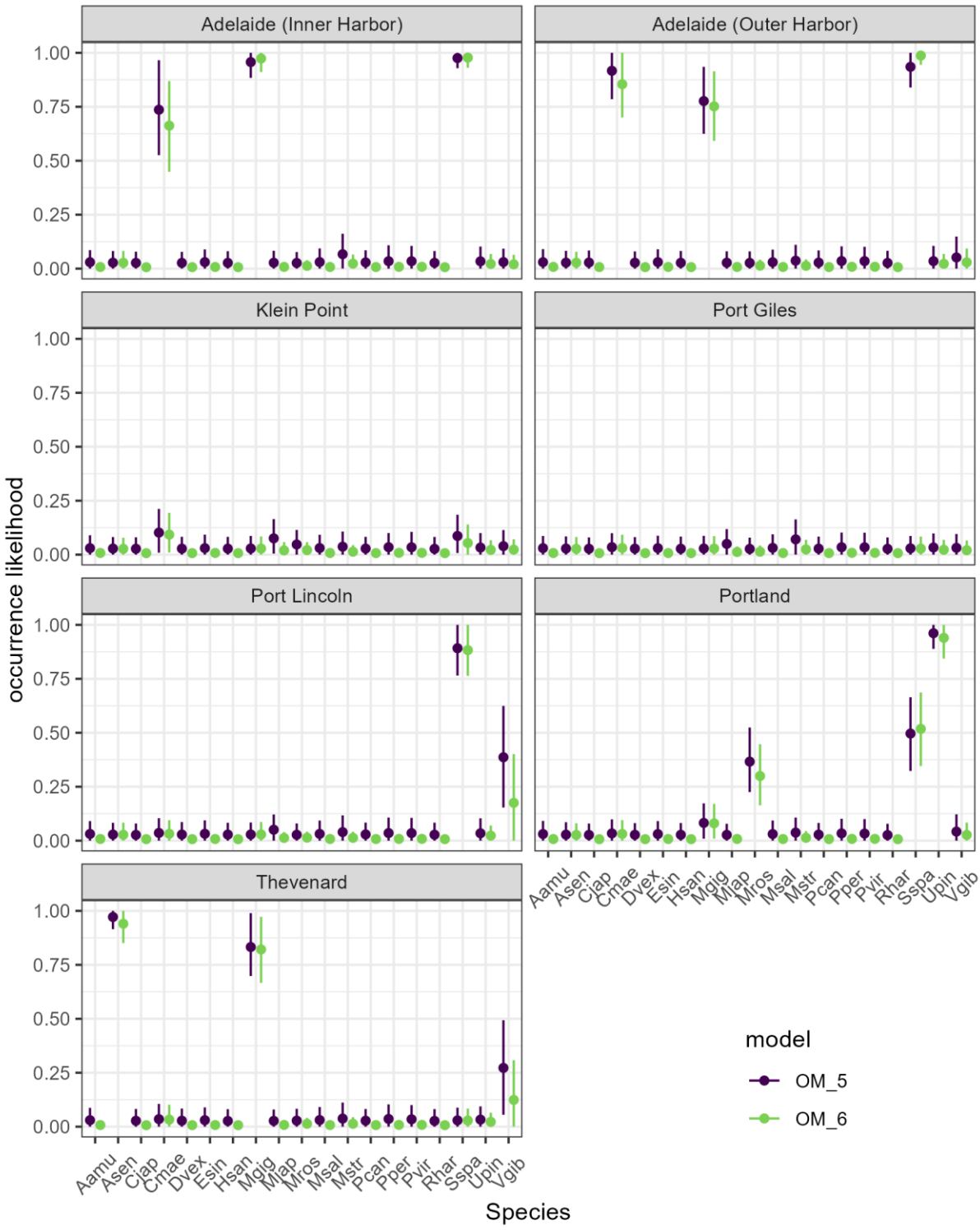

Figure 8. Occupancy likelihood predicted using 2021-22 surveillance data and selected models.

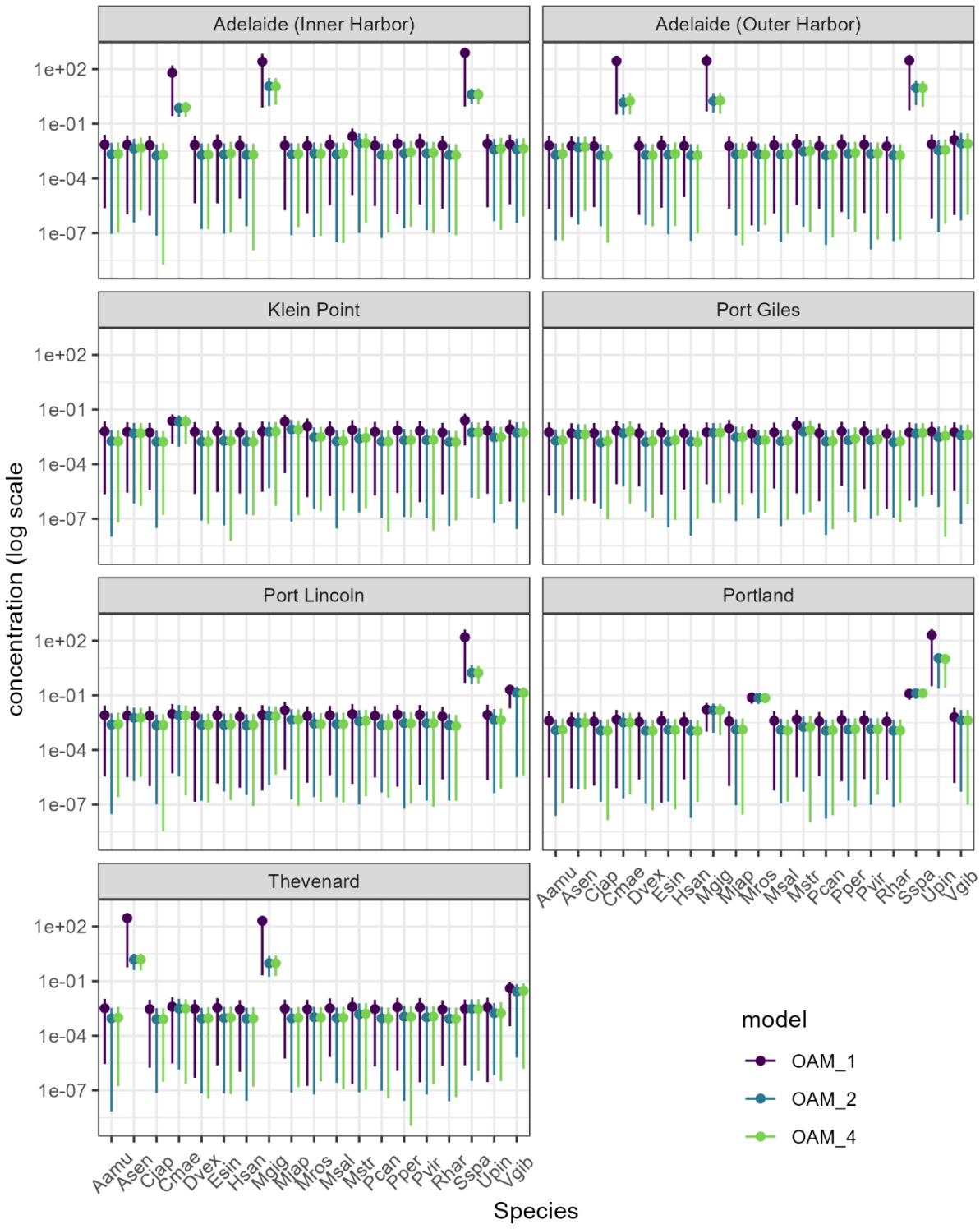

Figure 9. Planktonic concentration predicted using 2021-22 surveillance data and selected models.

3.3. Modelling of simulated data

Analyses of simulated data compared predictions across data sets with and without simulated effects of inhibition on detections. The scale factor (SF) effect across assays was randomly generated and does not reflect the actual effect of scale factor on DSe of these assays. The potential effect of inhibition on detections was considered in model OAM_4, and predictions of SF effect from this model using the inhibition affected data sets reflected the values used for generating data (Figure 10). Model SF effect predictions showed a correlation of 0.87 with values used for simulation using the first inhibition-affected data set (inhib v1), where scale factors were similar across sample sets. Correlation was 0.82 in the second inhibition-affected data set (inhib v2) where scale factors were higher for some sample sets than others.

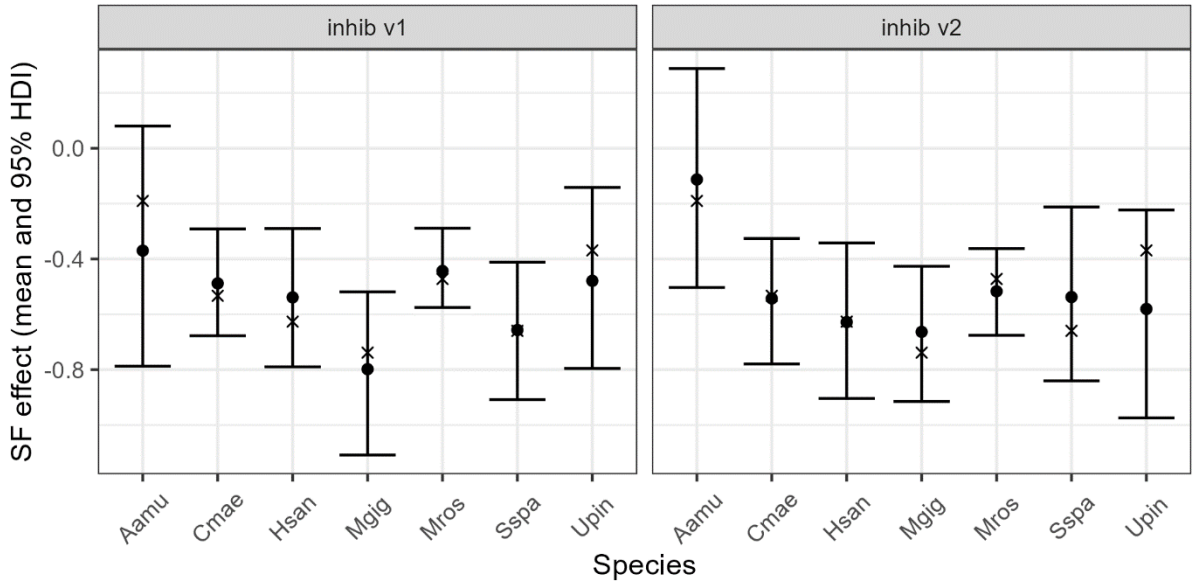

Figure 10. Estimated scale factor (SF) effects from model OAM_4 applied to two simulated data sets (inhib v1 and v2), showing mean and 95% HDI of predictions. Crosses show the value used in simulation.

Posterior estimates of assay DSe were close to the values used for simulation for most models, but slightly lower for some estimates using inhibition-affected data sets, even in the model accounting for inhibition (OAM_4) (Figure 11). The decrease in DSe was, however, less for OAM_4 than other models. For all models, the largest shifts in estimated DSe were for the assays with less informative priors (Cmae and Upin). Posterior estimates of DSp were high in all cases and reflected the values used to generate data (Figure 11), noting that assays with low DSp were not included in the simulation and DSp is not affected by inhibition.

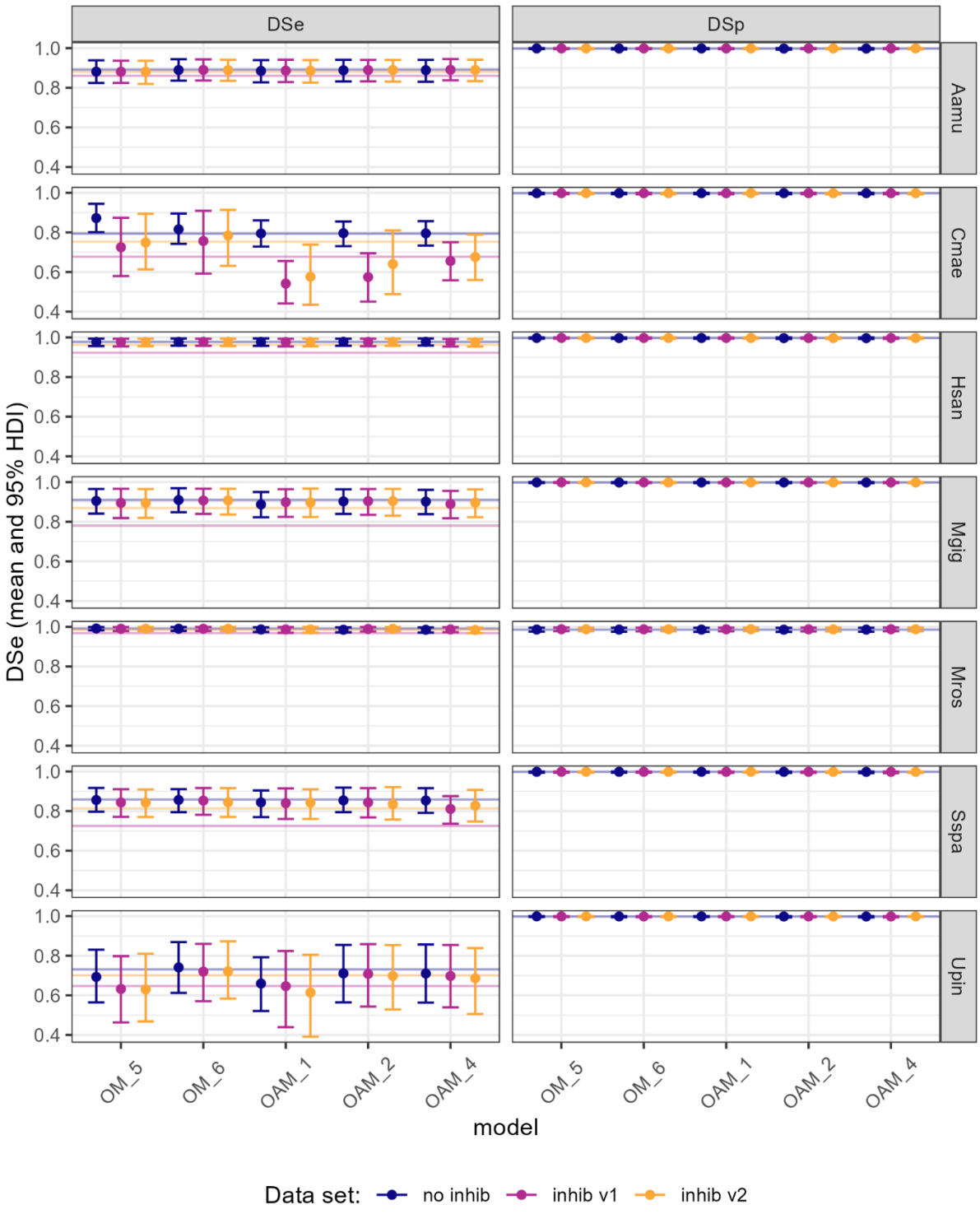

Figure 11. Posterior estimates of DSe and DSp for models applied to simulated data. The value used for simulating detections is shown by horizontal lines coloured by data set. For inhibition-affected data sets, the horizontal line shows effective DSe for each assay at the median simulated scale factor for that data set.

Predicted occurrence likelihood in models OM_5 and OM_6 was affected by the inclusion of inhibition on simulated detections, with a similar effect for both models (Figure 12). Predicted occurrence likelihood was reduced, in some cases being less than half that of models using the data without inhibition effect. Predictions of OAM_1 and 2 were also reduced, but with little change in predictions by OAM_4, which accounted for inhibition effects (Figure 13). Log concentration predictions were examined for OAMs because these are the raw model predictions from which concentration is calculated and because data generation used random values of log concentration to obtain strictly positive values. Where inhibition effects on detection were not included in the data set, both OAM_2 and OAM_4 produced the same log concentration predictions as expected. Some predictions by OAM_1, however, varied from those of the OAMs that included seasonal effects (Figure 13).

Prediction errors were greater for models predicting by sample set than season within each model type (Table 10; Figure 14). Prediction errors increased in the OMs when applied to the inhibition-affected data set but were similar across data sets for the OAMs (Table 10). Although the magnitude of errors was similar between OAM_2 and 4, there was a greater tendency for OAM_2 to under-predict log concentration in the inhibition-affected data sets, while OAM_4 over-predicted in some cases, particularly at higher simulated values (Figure 14).

Table 10. Sum of absolute errors between simulated and predicted values for models applied to data sets with no simulated inhibition or with detections affected by inhibition. Note that absolute errors are only comparable between models with the same type of prediction.

Model	Prediction	No Inhibition	Inhib v1	Inhib v2
OM_5	occurrence likelihood	3.83	6.28	6.13
OM_6	occurrence likelihood	2.45	6.53	6.38
OAM_1	log concentration	206	191	189
OAM_2	log concentration	61.5	70.2	70.3
OAM_4	log concentration	61.5	58.5	70.8

Models including seasonal covariates generally demonstrated the same patterns in estimates for these covariates, capturing the simulated values used. The magnitude of the seasonal effect for Mgig was underestimated by most models (Figure 15) due to the simulated effect being relatively large, while the prior used pulled estimates towards zero. Seasonal covariates were shifted for some species where the data sets with inhibition were used, particularly for the v2 set, where inhibition varied between sample sets (Figure 15).

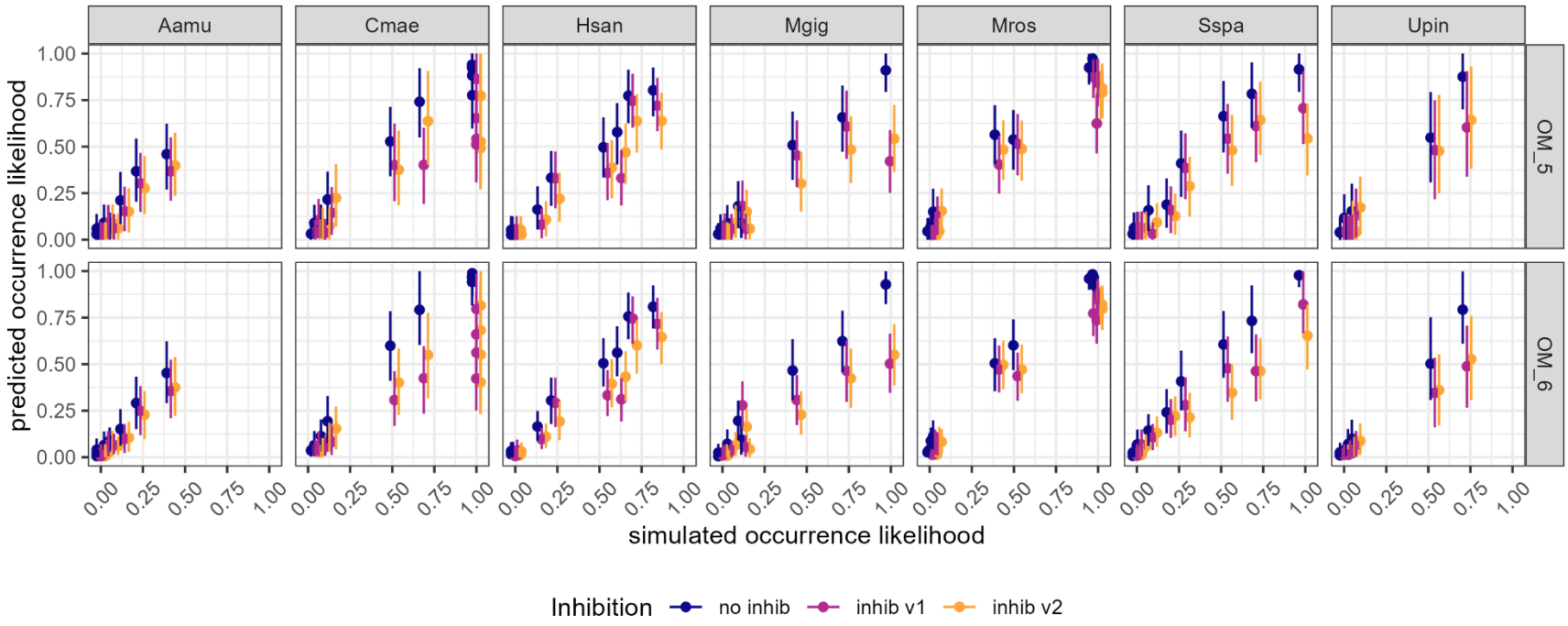

Figure 12. Predictions of occurrence likelihood from models applied to simulated data with or without effects of inhibition on simulated detections.

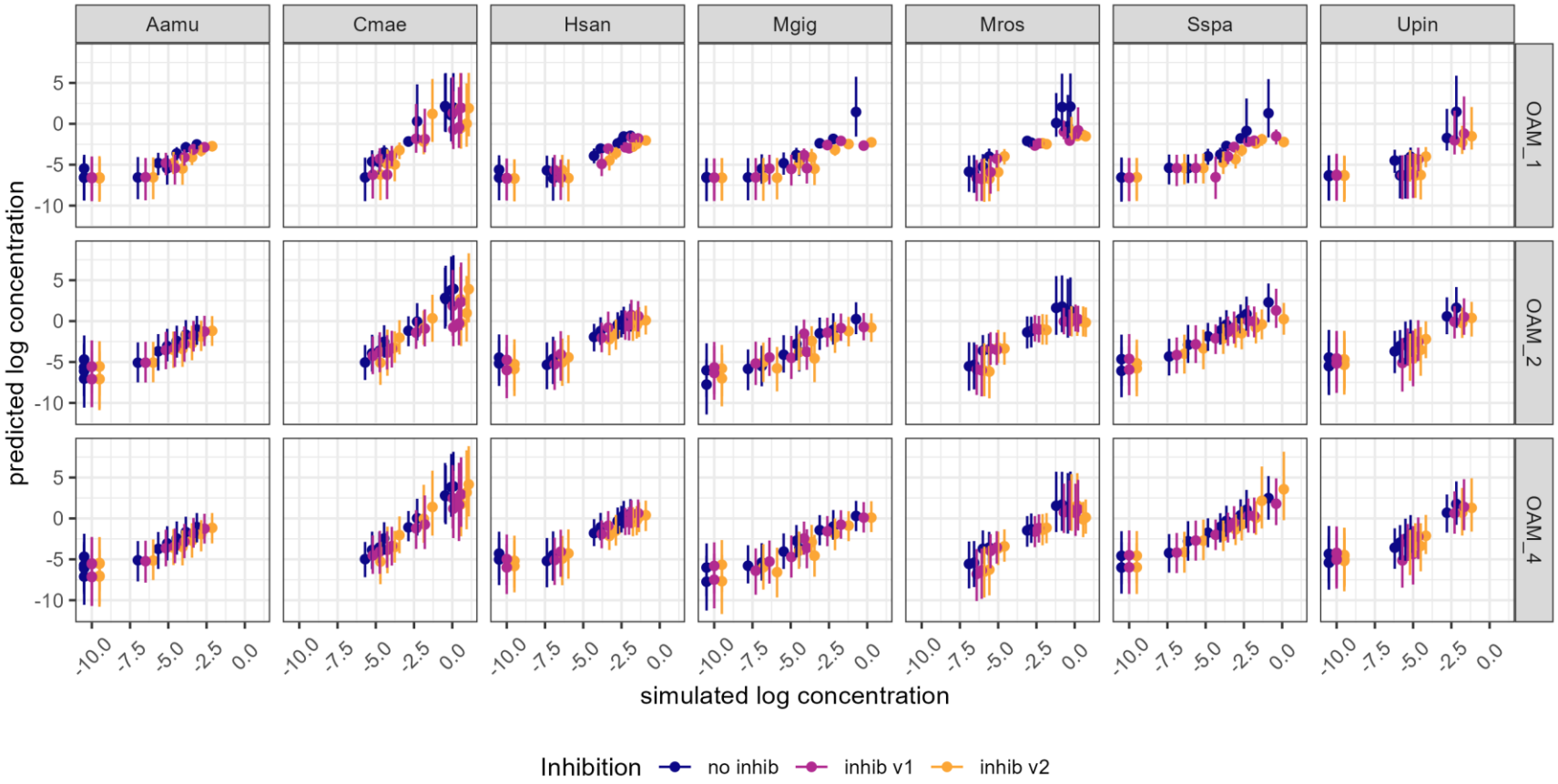

Figure 13. Predictions of log planktonic concentration from models applied to simulated data with or without effects of inhibition on simulated detections.

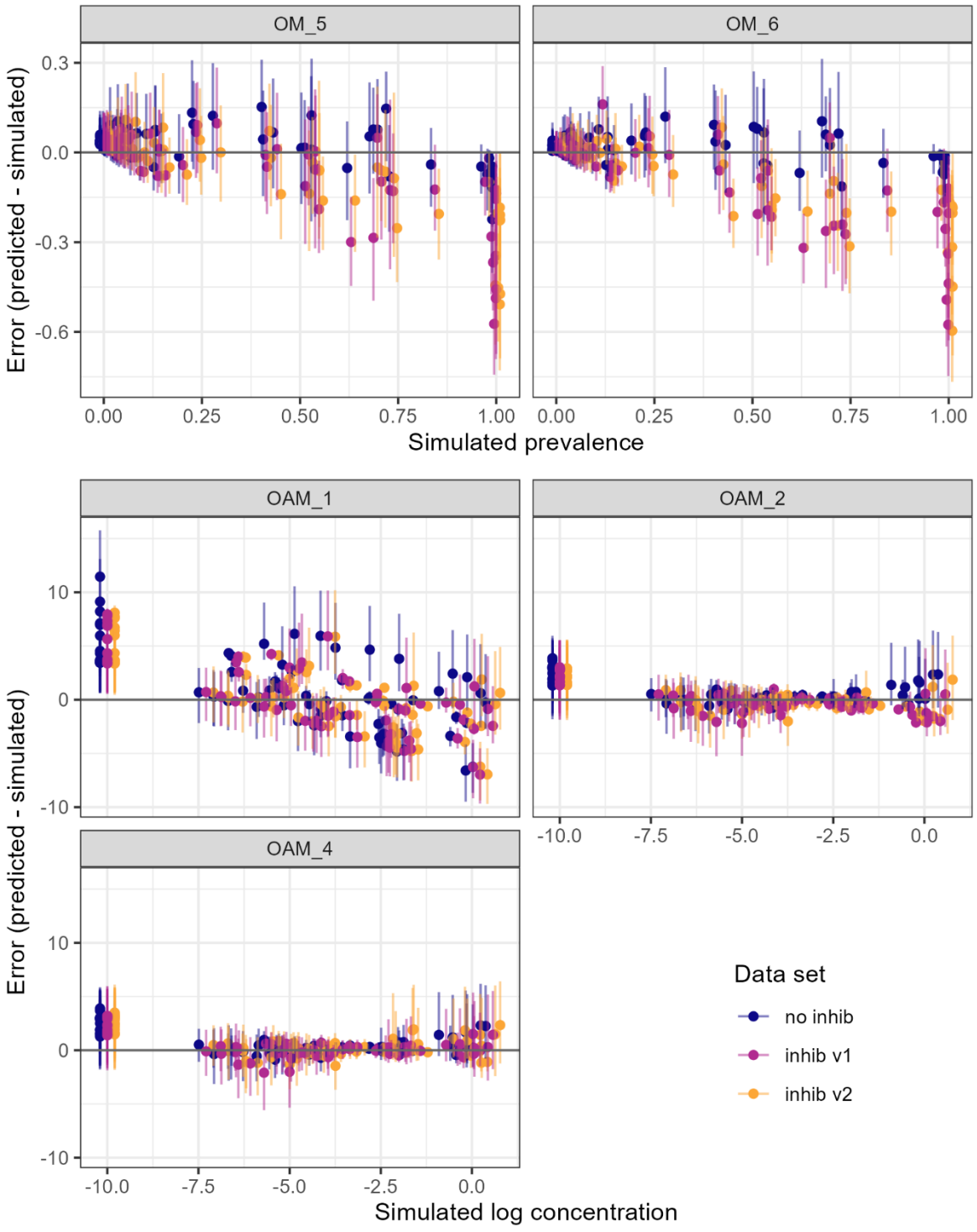

Figure 14. Prediction error for models applied to simulated data with or without effects of inhibition on simulated detections.

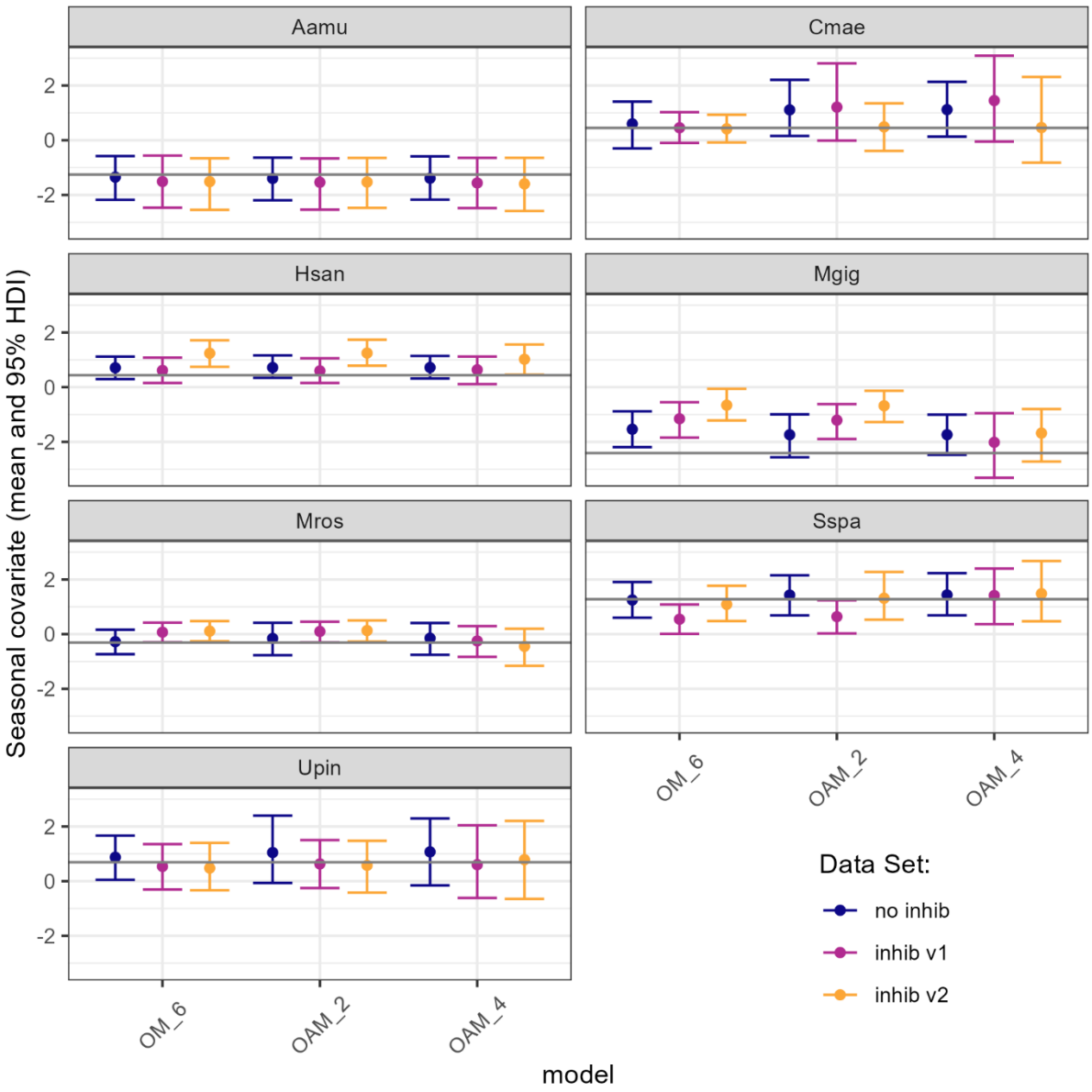

Figure 15. Predictions of seasonal covariates from models applied to simulated data with or without effects of inhibition on simulated detections. The horizontal line shows the value used for simulation for each species. Simulated seasonal effects were randomly generated and do not reflect expected seasonality for the nominal species used.

4. DISCUSSION

The project assessed a range of occupancy modelling approaches of varying complexity. More complex models can provide information on additional parameters of interest, but as expected, more complex models were more computationally intensive to fit. The time taken to fit models, however, while only indicative, did not make any assessed model infeasible to run. OAMs, which estimate planktonic concentration, took ~ 50 times longer to run than equivalent models to estimate occurrence likelihood, but OAMs have several advantages that make them an attractive option despite being relatively computationally intensive to fit.

OAMs and most models of occurrence likelihood (OMs) produced estimates with clear delineation between undetected species that were expected to be absent (confirmed absences) and detected species that were expected to be present (confirmed presences). Predictions of occurrence likelihood, however, could be more difficult to incorporate into management frameworks than the planktonic concentration estimates of OAMs. Posterior OM estimates are of the likelihood of an average plankton tow containing the target species DNA for a specific sample set or location/season, but the likelihood of a sample containing a target depends on sample volume (Royle and Dorazio 2009), and plankton tow volumes are not consistent. Plankton tows collected during the molecular surveys use a standardised tow length of 100 m, but the actual volume sampled by each tow is variable across (range ~ 1 – 20 m^3) and within (average coefficient of variation 0.85) surveys (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019c, 2020; Wiltshire *et al.* 2022). It is important to note that, when operationalising these models, that OM estimates for surveys with different plankton tow volumes are not directly comparable, which makes setting a defined occurrence likelihood threshold for triggering management action difficult. OAMs, however, explicitly leverage the relationship between sample volume and target concentration to provide estimates while accounting for effective tow volumes, with resulting predictions being comparable between surveys and therefore more useful for analysing data for decision making.

For surveys to inform risk-based ballast water management, the estimations made by OAM may also be more relevant to incorporate into management, because in contrast to occurrence likelihood estimates, planktonic concentration is directly related to the risk of propagule uptake in ballast water. For surveys designed using the molecular sample number calculator (Wiltshire 2021), the posterior estimate of planktonic concentration can also be directly compared with the target concentration used for survey design. Regardless of survey design, concentration predictions can also be compared to the ranges estimated for several key species in locations with emerging or established incursions (Wiltshire 2021). In addition to accounting for assay

performance and plankton tow volume, OAMs also allow sample-level covariates such as PCR inhibition to be included, providing estimates that account for factors that may influence detection likelihood in addition to target DNA abundance.

Previous guidelines on the interpretation of molecular survey results were provided by Wiltshire *et al.* (2019a). These guidelines used the number of detections and qPCR cycle threshold (C_T, i.e., the PCR cycle at which detection occurs) values to inform likelihood of species occurrence but were based on the limited data set available at that time. The OM or OAM approach provides for more appropriate interpretation than the raw number of detections in a data set due to incorporation of assay and sampling performance in the resulting estimates. The C_T values of detections provide a guide to the relative DNA yield per sample but comparing C_T values across assays or setting a consistent value for a 'high C_T' detection is difficult due to differences in assay performance (Wiltshire *et al.* 2020; Wiltshire *et al.* in prep). Using C_T values to inform the level of risk is therefore not straightforward. Revised guidelines for the interpretation of molecular survey results based on OAM concentration estimates are provided in section 4.1.

The OAM and, to a lesser extent, OM approaches can assist in the interpretation of survey data, but it is important to recognise the limitations of the model outputs. One limitation of the OAM approach is that, because planktonic concentration is inferred from detection patterns, the models can only estimate concentrations in a limited range. Where a species is abundant enough that detection in every sample is likely, or rare enough that detection is unlikely, further increase or decrease in abundance will not change model estimates. Analysis of the simulated data set showed that log concentration estimated by OAMs was ~ −7 (= 0.001 planktonic pests m^{-3}) for absent species, even though simulated log concentration was set to −10 for these.

Concentration estimates for very rare species could be improved by the collection of more samples, which would also facilitate detection of species at lower abundance, but with increased sampling and processing costs. The lowest concentration possible to estimate using the OAM approach, however, will always be of the same order of magnitude as the lowest concentration detectable by the number of samples used. As planktonic concentration increases, the likelihood of a single sample containing target DNA approaches 100%. At a planktonic concentration > 0.8 m^{-3}, the likelihood of an average plankton sample containing target DNA exceeds 99.9%, and models cannot accurately predict higher concentrations, even with collection of more samples.

Both OM and OAM approaches can provide estimates that account for differences in assay performance and for the potential for both false negative (FN) and false positive (FP) results. The

inclusion of potential FP errors in models is useful to account for the possibility of non-systematic FPs, such as those that may result from the occurrence of transient DNA or sporadic sample contamination but cannot correct results to compensate for poor assay specificity.

The port survey data sets used to assess models featured several detections of pests that were assumed to be absent, most from the Vgib and Asen assays that have low specificity. OMs including potential FPs typically provided lower estimates of occurrence likelihood for these species, particularly *Varicorbula gibba*, than models without FP estimation. Occurrence likelihood was, however, predicted to be high in sample sets where either species was detected in a relatively high proportion of samples. OAMs similarly penalised estimates of concentration for this species due to assuming that some detections were FPs, but several estimates were in the same range as estimates for species regarded as confirmed presences. The Asen assay appears to have poor specificity when applied in tropical ports but to be specific when applied to most temperate locations. Models can only incorporate a single DSp estimate for each assay, hence, DSp is modelled as constant across surveys. For Asen and Vgib, estimates for locations where these species are established were also penalised by the assumption of low DSp, despite detections in these locations likely being correct.

The data sets included a small number of uncertain detections by assays with high specificity, which may have been due to detections of transient DNA or possible sample contamination. Posterior DSp estimates would decrease if the rate of FPs was higher than expected, but posterior DSp estimates remained high, showing that the likelihood of FPs, except for Asen or Vgib, was very low (< 0.1%). Uncertain detections by assays other than Asen or Vgib comprised 1 – 2 detections per sample set. Model estimates for these cases, whether of occurrence likelihood or concentration, were typically in the range of confirmed absences, suggesting that, if these species are present at those survey sites, they occur at very low abundance.

The models that allow for FPs therefore provide estimates while accounting for the likelihood of either transient DNA or sporadic (non-systematic) contamination for assays with typically high specificity, but, where assays lack specificity, models cannot distinguish between locations with target species present and those where non-target detections occur. To date there has been no evidence of systematic contamination in the molecular surveillance carried out, but models would similarly be unable to clearly identify this occurrence and, as a result, would provide estimates suggesting species presence if enough samples were contaminated. Models can also not distinguish between potential causes of FPs. Assessing assay performance, including thorough

investigation of specificity in environmental samples, is critical to ensure that only suitably specific assays are used in surveys to inform management. Even with suitably specific assays, however, additional data may be required in some cases to resolve uncertain detections and provide confidence in model results.

There is some risk of non-specific detection whenever assays are applied to a new geographic area, although this risk is minimised by robust assessment of assays prior to operational use. Sequencing of qPCR positive samples is a potential method to confirm detections or identify FPs due to non-target detection (Díaz-Ferguson and Moyer 2014) but may not be successful in samples with low yield of detected DNA and will not identify FPs due to contamination or presence of transient target species DNA. Sequencing PCR product amplified by the assay would also fail to detect a cross-reaction with a closely related species that shares the target assay region. Amplicons produced by qPCR are short and any sequence producing a cross-reaction is necessarily similar to the target sequence, making non-target sequences difficult to distinguish from target sequences in many cases. A high-throughput sequencing (HTS) approach for confirming qPCR results was trialed by Wiltshire *et al.* (in prep). This approach provides longer amplicons and sequence data from a region spanning that of the relevant assay target, and hence more information to determine species identity for confirmation, or to identify taxa responsible for non-target detections. The additional sequence data provided by HTS can still fail to resolve the identity of detected taxa in groups where limited sequence data exist or which have low genetic divergence in the relevant gene region (Wiltshire *et al.* in prep). The HTS method was shown to be useful in assessing specificity of new assays (Wiltshire *et al.* in prep) but is not practical for confirming detections from routine surveillance where few qPCR positives occur, due to relatively long turn-around times and high per-sample costs, especially for analysis of small (< 50) batches of samples. The method could, however, be useful to apply where sampling returns a relatively large number of detections from an area without known occurrence of a species.

The use of controls in molecular surveillance is important to detect potential sample quality and contamination issues (Goldberg *et al.* 2016; Sepulveda *et al.* 2020a). Controls have been used in the molecular port surveys to check for sample degradation and PCR inhibition, and positive and negative controls are included in all qPCR analyses. These measures assess key factors that may influence FN errors, but do not provide for the detection of systematic FP errors should these occur. The implementation of field and laboratory best practice ensures that the risk of contamination is low (< 0.1%), and an impractically large number (> 500) of negative controls would be needed to detect sporadic contamination at this level (Hutchins *et al.* 2022). The

implementation of a relatively small number (~5) of processing blank controls, would, however allow identification of systematic contamination in a sample set (Hutchins *et al.* 2022), which would provide increased confidence in results or identify if a sample set had been affected. An additional method to detect whether contamination has occurred at the PCR analysis stage is the use of suitably designed 'Q-control' synthetic standards, which can be distinguished from target species DNA if inadvertently present in samples, e.g., from micro-droplets generated by pipetting.

The C_T values or DNA yield of detections are not utilised in the OM or OAM approaches, which use only detection/non-detection data. Differences in performance, and in the type of standards used to calculate DNA yield from C_T value, across assays complicate the interpretation of these data. Examination or analysis of C_T values or DNA yields, however, can provide additional information in some cases to complement OM or OAM output. For example, spatial analyses of DNA yields, such as carried out by Wiltshire *et al.* (2022) can reveal areas with relatively higher or lower yields and may assist in determining the location of an adult population or potential sources for transient DNA. Where detections are due to contamination, DNA yield is likely to be low, and C_T value relatively high, and detections due to non-target amplification may also result in relatively high C_T. Determining a C_T value above which a detection is more likely to be a false than true positive is possible (Caraguel *et al.* 2011) but would need to be done for each assay. Implementing such C_T cut-offs comes with a risk of classing true detections as FPs, with resulting reduction in effective survey confidence. The C_T value or DNA yield of detections, relative to those for the same assay in areas of confirmed species occurrence, may however provide supporting evidence for the status of detections. Implementing the same type of standard, ideally Q-control standards, across assays, would also assist in comparing results across assays.

Definitively determining if transient DNA has been detected, and separating this possibility from that of contamination, is likely impossible, although the implementation of processing blanks and Q-control standards would aid ruling out some types of contamination. The potential for transient DNA occurrence can be informed by investigation of potential sources of transient DNA, such as shipping from relevant regions being present in the port area at the time of surveillance. Such data are not always available, however, and are unlikely to definitively identify the source of target DNA. Where data are available, however, a clear absence of potential transient DNA sources would suggest that contamination may have occurred. It is important to note, however, that an emerging incursion where the target species is present at low abundance could cause a similar pattern of detection(s) to transient DNA or sporadic contamination. Model outputs cannot distinguish between these possibilities but assist by providing an estimate of the likely and

maximum plausible level of occurrence for detected species that can inform the level of risk and therefore the most suitable management action. Where surveillance is carried out in at least two different seasons, models can also use the seasonality of detections to inform the likelihood of detections indicating species presence, because detections in the case of an emerging incursion would be more likely to occur in the season corresponding to the species reproductive period.

OAM estimates can be compared to concentration thresholds, including the planktonic concentration used in survey design, to inform the level of risk, noting limitations on the lowest concentration able to be estimated. The 2021-22 surveys were designed using a target planktonic concentration of 0.0075 m^{-3} (Wiltshire *et al.* 2022), which is in the range expected for emerging incursions (Wiltshire 2021). OAM raw predictions are of log concentration, which is exponentiated to provide concentration estimates. The positive-skewed nature of the resulting concentration predictions means that the median prediction is a better estimate of central tendency than the mean.

Median OAM estimates for the 2021-22 surveys of undetected species that were not expected to occur were all below the survey target concentration (maximum 0.0053 m^{-3}), but median predictions for species where there were 1 – 2 detections across sample sets were > 0.0075 m^{-3} in some cases. Estimates varied across models, as illustrated by estimates for three species, *Carcinus maenas, Sabella spallanzanii* and *Mya japonica*, which each had two detections in one sample set from Klein point, where these species are not known to occur (Wiltshire *et al.* 2022). For Cmae, which was detected in winter, the median estimate was ~ 0.02 planktonic pests m^{-3} in each OAM applied to this data set (OAM_1, 2 and 4); for Sspa, which was detected in summer, OAM_1 provided an estimate of 0.025 while OAM_2 and 4 each estimated < 0.001 planktonic pests m^{-3}; and for Mjap, which was detected in summer, OAM_1 provided an estimate of 0.022 while OAM_2 and 4 each estimated 0.005 planktonic pests m^{-3}.

The discrepancy in predictions for Sspa and Mjap across models, in contrast to Cmae, is due to OAM_1 predicting by sample set rather than season, and to the detections of Cmae occurring in the season consistent with detection of this species at other survey locations, whereas detections of Sspa occurred predominantly in winter across other locations, and besides Klein Point, Mjap was detected in one winter sample each from Port Giles and Port Lincoln (Wiltshire *et al.* 2022). The OM estimates for these occurrences, while not directly comparable to the survey design concentration, show a similar pattern of both models (OM_5 and 6) having consistent estimates (~0.1) for Cmae, but the model predicting by sample set (OM_5) having higher estimates for the

other species (0.08 *c.f.* 0.003 for Ssap; 0.08 *c.f.* 0.02 for Mjap) than OM_6, which included seasonal effects.

The models including seasonal effects accounted for patterns in detection and effectively down-weighted the likely abundance of Sspa given a lack of detection in winter. Transient DNA occurrence is possible for both Cmae and Sspa because the *Accolade II*, which travels from Port Adelaide where both species occur, was in port at the time of each survey. The results suggest, however, that Cmae is more likely to occur than Sspa because Cmae may have been present during both survey periods but only detectable in winter, whereas, had Sspa been present in both seasons, detection in winter would have been likely but did not occur. The models were unable to estimate seasonality for Mjap given the scarcity of detections of this species in the survey, but models accounting for seasonality consider occurrence likelihood across both sample sets for a location, and therefore down-weight occurrence where detections occur only one season in the absence of clear seasonal patterns. Models OM_5 and OAM_1, in contrast, consider each sample set independently, regardless of sample location.

The inclusion of seasonal effects in models can therefore assist in identifying which detections are more likely to be transient, but it should be noted that the models can only estimate seasonal effects for species with variable numbers of detections across seasons, and that seasonal effects are assumed to be the same within each species across the surveyed locations. Seasonality appears to vary across locations for most species, but to be generally similar between locations within the same area or latitude (Wiltshire 2021). Assuming consistent seasonal effects is therefore reasonable where survey data are from ports with similar environmental conditions, as per the 2021-22 surveys, but this approach may not be valid where surveyed ports are from a broad geographic range. For species and location combinations included in both modelling of the compiled data set and of the 2021-22 surveys, model estimates varied in some cases between data sets for models including seasonal effects. This was due to different seasonal effects being estimated for some species in the compiled data set, which included data from a wider geographic range and additional seasons in comparison to the 2021-22 survey data.

The data available from the surveys as carried out does not allow estimation of different seasonal effects by location, because this would require data from multiple surveys per season in each location. Where there are multiple surveyed ports within distinct geographic regions, however, models could be formulated to estimate seasonality separately by region, or separate models run for data from each region. Alternatively, as more data are obtained on the seasonality of species

detections across different regions, informative priors could be used in models to reflect expected seasonality in different survey areas. Such informative priors would need to be carefully selected and supported by robust data. Where suitable data are lacking, the use of vague priors for seasonal effects is recommended, but model outputs should be considered with regard to available knowledge on seasonality.

All models used vague priors for occurrence likelihood (in OMs) or planktonic concentration (in OAMs). Somewhat informative priors could, however, be applied to account for existing knowledge of species occurrence, as per the approach of Keller *et al.* (2022). If surveillance is repeated at the same site, predictions from models of surveillance results could be used to set priors for repeat surveillance. This approach was not assessed here because there are no sites where molecular surveillance has been repeated using the adopted method. The approach of using informed priors for occurrence (or concentration) could, however, assist in determining status for uncertain detections, because detection in repeated surveys would increase the plausibility of species presence, which would be reflected in increasing estimates. Repeated non-detection of recorded species would similarly lead to decreasing estimates, while non-detection or a low level of detection of an established species in a single survey would not necessarily indicate species absence. Further investigation would be required to determine the most suitable priors to use for this approach.

Including potential effects of PCR inhibition also resulted in longer model run times but added valuable information. Modelling of the simulated data set demonstrated that the occurrence of PCR inhibition could bias some model estimates, but model OAM_4, which included estimation of scale factor effects, was less impacted than other assessed models. There was little impact on estimates of planktonic concentration in OAM_2, despite this model not accounting for inhibition, at the levels of inhibition used in the simulated data set, however, accounting for inhibition imposed only a small computational burden and provides a safeguard against biased estimates should higher inhibition occur in a data set.

Priors used for scale factor effects were slightly informative but could be made more informative as further data on impacts of inhibition on assay performance is obtained, to further improve model estimations. PCR inhibition has occurred only in relatively few samples across all surveys but can occasionally be severe where it occurs. Including inhibition effects in models allows identification of cases where inhibition may have impaired detection of targets. Estimation of inhibition effects is therefore worth including in models.

The inclusion of availability likelihood in models, in contrast to inclusion of seasonal and inhibition effects, did not provide useful information. The molecular surveys of Australian ports did not use a hierarchical sampling design, and technical replicates of PCR testing have not been applied. Resulting data were therefore not well suited to multi-scale OM approaches, and a different survey design approach would be needed to apply multi-scale OM and accurately estimate availability. Multi-scale models applied to the port surveillance data either provided imprecise or implausible estimates, or, in some cases, identical estimates to otherwise equivalent single-level models, providing no further information on occurrence. In all models we explicitly modelled differences in occurrence likelihood or abundance by either sample set or season, and this approach provided information more relevant to the interpretation of molecular surveys than estimates of availability. Multi-scale models can be useful to apply to some data sets, and, with suitable study design, can provide information on the relative contribution of sampling and analysis stages to survey errors, but are not the only way to separate these errors. Data on sampling and analysis errors for the molecular surveys are available from previous studies (Wiltshire *et al.* 2019a; Wiltshire *et al.* 2019b; Wiltshire *et al.* in prep), and therefore assessing availability in OMs or OAMs is unnecessary.

The most suitable model of those assessed for application to analysis of molecular survey data is therefore OAM_4. This model provides estimates of planktonic concentration while accounting for assay performance, sample volume, seasonal effects and PCR inhibition. Estimation of these effects means that this model takes ~2 – 3 hours to run, depending on the size and structure (number of sites, species and seasons) of the data set, in comparison to < 5 minutes for simple models. This greater computational burden, however, is insufficient to make the model infeasible to run. Future refinements to this model could include using more informative priors for seasonality and PCR inhibition as more data on these effects are obtained. Assessing this model using informative priors and results from repeated surveillance would also assist in refining the approach.

4.1. Considerations for management application

Risk-based BWM considers the occurrence of target species at ports (Zhao *et al.* 2012; Arthur *et al.* 2015b). A key consideration for management is therefore identification of target species status changes, i.e., a target species establishes in a port from which it was previously absent, or an established species becomes locally extinct. BWM aims to prevent new BW related introductions, but marine pests may be introduced by several vectors, and the rate of introduction is accelerating globally (Williams *et al.* 2013; Cope *et al.* 2015), so the risk of incursions is on-going. Not all

incursions, however, result in successful pest establishment (Geburzi and McCarthy 2018). Marine pests that establish are typically infeasible to eradicate, but pest population sizes can fluctuate, and invasive populations sometimes become locally extinct (Simberloff and Gibbons 2004). Populations may sometimes decrease to the point of being impractical to detect before re-emerging, as has occurred for *Arcuatula senhousia* in Perth (McDonald and Wells 2009; Wiltshire *et al.* 2020) and *Carcinus maenas* in Adelaide (Dittmann *et al.* 2010; Wiltshire *et al.* 2010; Dittmann *et al.* 2017).

A precautionary approach is applied to domestic BWM in Australia, with target species considered present by default. A single survey (consisting of sampling in two seasonal sets) carried out with suitable sensitivity (e.g., designed following Wiltshire 2021) would provide confidence that undetected species not known to occur in the area are currently absent for the purpose of BWM (noting that proving complete absence is infeasible). OAM estimates in these cases would provide confidence that the survey as carried out achieved the target sensitivity or, alternatively, assist in identifying cases where detection may have been compromised by sampling issues or PCR inhibition. Where a species is previously known to occur, however, non-detection by a single survey may not necessarily mean that the species has become locally extinct, because it may be present at a temporarily low (below detectable) abundance. The lack of detection would be reflected in OAM estimates showing that the species does not currently pose a risk for ballast water transport, however, species present at low abundance could potentially re-populate rapidly, whereas a truly absent species would need to both be introduced and multiply sufficiently before becoming a risk.

Any survey provides information on the status of target species that is current at the time of surveillance, but status is subject to change due to new incursions and population changes. Repeated surveillance is therefore required to maintain the currency of pest status determinations. For species that are considered absent, the frequency of surveillance could be informed by the cumulative risk of new incursion over time and the likely lag time (sensu Crooks 2016) between introduction and population expansion to a detectable level. For undetected species potentially present at low abundance based on previous known occurrence, it would be prudent to either consider the species as being present despite the non-detection or else to re-survey sooner than for those considered absent. Repeated non-detection would provide confidence that the previously present species had become functionally extinct, while detection would confirm on-going species presence and provide updated information on current level of occurrence.

For species considered absent that are detected by a survey, the most conservative approach would be to consider the species present even if detected in only 1 – 2 samples. Survey results, however, show that, while rare, sporadic FPs may occur due to transient DNA detection or sample contamination. The OAM approach can provide estimates in these cases that indicate the likely level of species occurrence while accounting for potential FP errors and seasonality. These estimates can be compared with the target concentration used for survey design, or to other concentration ranges, to inform the level of risk.

The target concentration used for survey design is not necessarily the concentration above which a target species would be regarded as at risk of ballast water transport but allows for detection of an emerging incursion or for detection of an established species where surveillance is carried out in a sub-optimal season. All models applied provide a predicted concentration for each sample set or season included in each survey, and therefore, multiple predictions per species for each location. Only the highest estimate across sample sets/seasons was examined in each case, because it is abundance (or occurrence likelihood) in the season where target abundance is greatest, which will typically be the reproductive season, that is relevant for informing BWM risk.

The survey target concentration will be close to the lowest concentration able to be estimated, but as demonstrated by the analysis of the 2021-22 surveillance data, median estimates for undetected species will be below the survey target concentration where surveillance is not unduly affected by inhibition or other issues. In analysis of the compiled data set, estimates for undetected species were > 0.0075 m^{-3} (the target concentration used for 2021-22 surveys) in some cases, but only where fewer than 35 samples had been collected. Where surveillance does not achieve target confidence, e.g., due to inadequate sample volume or issues with inhibition, the collection of additional samples may be required to achieve the desired confidence. In these cases, OAM estimates can assist in showing the level of risk for each species and therefore informing whether additional sampling is needed. If surveillance has been impacted by high levels of PCR inhibition, reanalysis using sample dilution, DNA cleaning, or other methods that may reduce inhibitory effects could be attempted prior to carrying out additional sampling. Reanalysis would cost less than additional sampling but may not be successful dependent on the severity of inhibition.

OAM estimates could alternatively, or additionally, be compared to concentration ranges estimated by Wiltshire (2021) to assess whether species are likely to be established. That analysis showed a concentration of > 0.02 planktonic pests m^{-3} was typically estimated for established

species in appropriately timed sample sets. OAM results for the analyses of the compiled data set and of 2021-22 survey data similarly showed that estimated concentrations were > 0.02 planktonic pests m^{-3} at locations where species were known to occur. The only exceptions were *Carcinus maenas* and *Undaria pinnatifida* in Melbourne, where estimates from OAM_4 were 0.0086 and 0.0075 m^{-3} respectively. The Melbourne location used in the analysis was, however, the Yarra River, where few detections of these species were recorded, despite their common detection at other sites surveyed in the Port of Melbourne. Detections of these species may therefore have been of transient DNA advected from nearby sites with established populations, or the species may occur at low abundance in this area relative to other Melbourne sites. The estimated ranges for established species from OAMs were therefore consistent with those previously estimated but should continue to be revised as more survey data is collected and analysed. Additional information, such as potential sources of transient DNA, relative C_T values or DNA yields, and, if implemented, data from control samples, Q-control standards and sequencing, can further assist with interpretation of modelling results where available.

Even with additional information, however, distinguishing between transient DNA detections and emerging incursions where the species is currently rare is unlikely to be possible, because both scenarios result in low levels of target DNA presence and concomitant low, but non-zero, detection likelihood. For determining BW risk, follow-up molecular surveillance targeting the same sampling seasons in the subsequent 12-month period would assist in determining if species that are detected at a very low level (possibly transient) are present and increasing in abundance. For other purposes, such as delimitation of suspected new incursions or surveys to support eradication, it is likely to be desirable to repeat surveillance sooner than would be required for BWM. In these cases, more extensive surveillance (i.e., covering a wider area and/or using higher sampling effort) and the incorporation of multiple survey methods (molecular and traditional) would be desirable, although it should be noted that traditional methods may fail to detect new incursions where the species is at low abundance (Darling and Mahon 2011; Jerde *et al.* 2011; Wiltshire *et al.* 2019a). Spatial analyses of relative DNA yields may assist in these cases to determine areas within which to focus surveillance.

A summary of the considerations for interpreting survey results based on OAM estimates, species detection and previous species status is provided in Table 11. This table uses ranges of OAM estimates, which should be the highest median estimate for a species at a location across seasons, to define the likely level of species occurrence. Where survey results suggest a possible status change (detection of previously unrecorded species or no detection of previously recorded

species), additional considerations, summarising those discussed above, are shown that may supplement OAM outputs in determining whether additional surveillance is warranted to confirm a change in species status. Considerations for resolving uncertainty or alleviating issues are also shown. A flow chart for interpretation of survey and OAM results for determining species status change and whether to continue routine surveillance or carry out additional surveillance is shown in Figure 16. The decision of whether to carry out additional surveillance, and of how soon this is needed, can be informed by the level of risk suggested by the OAM, species and location involved, and survey costs.

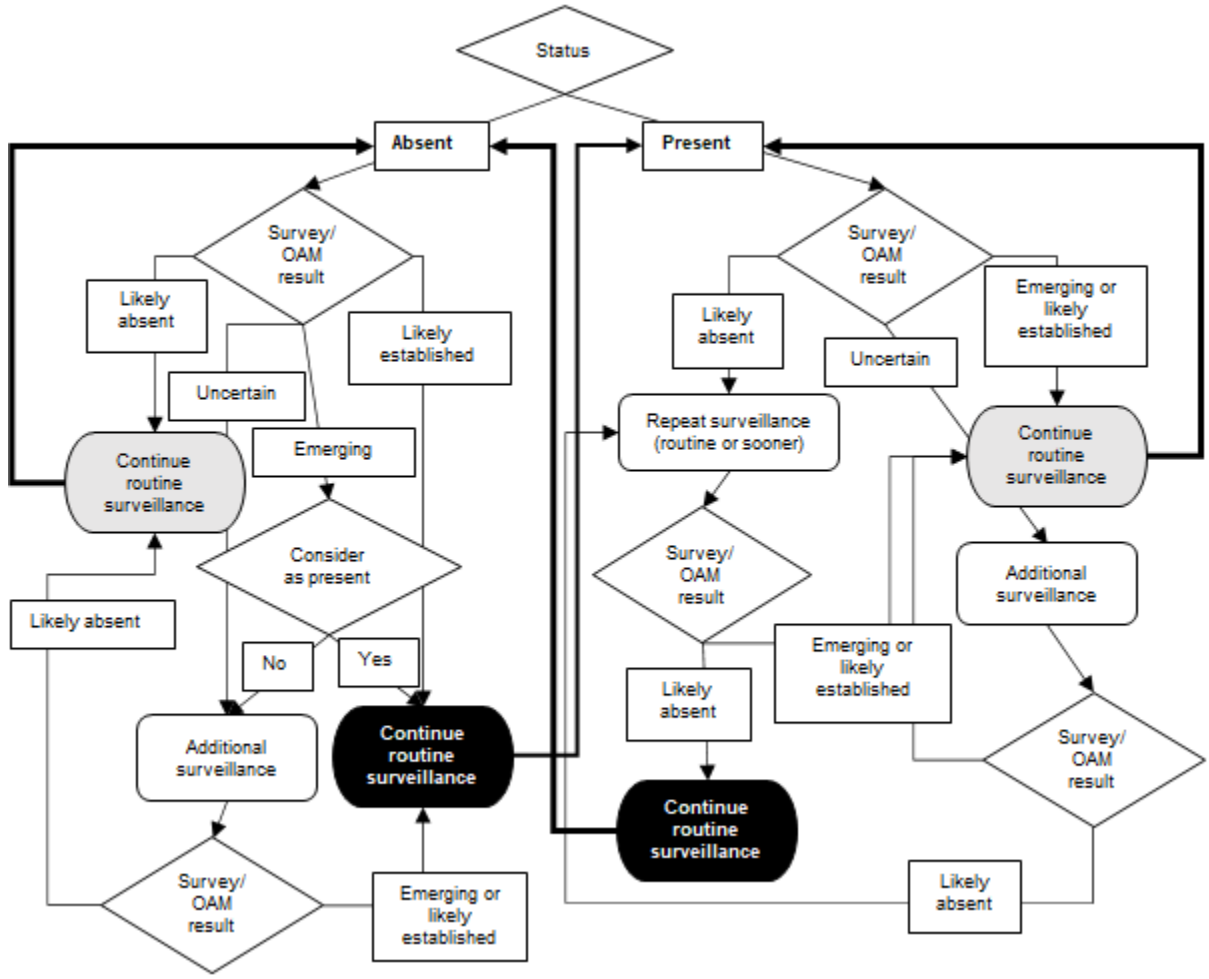

Figure 16. Flow chart for determining changes in species status from surveillance and OAM results and informing when to continue routine surveillance or carry out additional surveillance.

Table 11. Considerations for interpretation of survey results based on estimated concentration and species status.

OAM estimate (median concentration m^{-3})	Species detected	Previous detection/ known occurrence	Likely species status and survey performance	Other considerations	Repeat surveillance
< 0.0075	No	No	Surveillance effective. Species unlikely to be present at above target concentration	nil	Routine/ risk-based
0.0075 – 0.015	No	No	Surveillance did not achieve target confidence. Species may be present at up to estimated concentration but unlikely to be established	Reanalysis may be possible where inhibition is an issue	Consider additional sampling to increase survey confidence
> 0.015	No	No	Surveillance severely compromised. Species may be present despite non-detection	Reanalysis may be possible where inhibition is an issue	As soon as practical to increase survey confidence
< 0.0075	Yes	No	Likely transient detection or false positive. Species unlikely to be present at above target concentration	Results from control samples or Q-control standards where available. Relative C_T value(s)/DNA yield. Potential sources of transient DNA.	Consider repeat sampling to distinguish emerging incursion
0.0075 – 0.015	Yes	No	Species likely to be present but may not be established.	Results from control samples or Q-control standards where available.	Routine/ risk-based if species is considered present or repeat sooner to confirm status change
> 0.015	Yes	No	Species likely to be established.	Consider confirmation by sequencing if assay is applied in a new area	Routine/ risk-based with species considered present

OAM estimate (median concentration m^{-3})	Species detected	Previous detection/ known occurrence	Likely species status and survey performance	Other considerations	Repeat surveillance
< 0.0075	No	Yes	Surveillance effective. Species unlikely to be present at above target concentration but may occur at low abundance given previous presence	Species biology and tendency to boom-bust cycles	Routine/ risk-based if species is considered present or repeat sooner to confirm status change
0.0075 – 0.015	No	Yes	Surveillance did not achieve target confidence. Species may be present at up to estimated concentration	Reanalysis may be possible where inhibition is an issue	Routine/ risk-based if species is considered present or repeat sooner to increase survey confidence
> 0.015	No	Yes	Surveillance severely compromised. Species may be present despite non-detection	Reanalysis may be possible where inhibition is an issue	As soon as practical to increase survey confidence
< 0.0075	Yes	Yes	Species unlikely to be present at above target concentration but may occur at low abundance given previous presence	nil	Routine/ risk- based if species is considered present or repeat sooner to confirm status change
0.0075 – 0.015	Yes	Yes	Species likely present at low abundance	nil	Routine/ risk-based
> 0.015	Yes	Yes	Species likely established	nil	Routine/ risk-based

5. CONCLUSIONS

The project assessed a range of occupancy modelling and determined that the most suitable model for application to analysis of molecular survey is an OAM, which provides estimates of planktonic concentration, while accounting for assay performance (including potential false negatives and false positives), sample volume, seasonal effects, and PCR inhibition. The OAM approach provides estimates that are comparable across species and surveys, and likely to be suitable for incorporation into management frameworks. Models cannot, however, provide accurate estimates where systematic false positives occur such as those due to non-specific detection. Assessment of assay performance is required in any case to provide data for use in OAM approaches, and only suitably specific assays should be implemented for routine surveillance.

OAM outputs can be used to assess the performance of surveillance and ensure that target survey confidence has been achieved, with undetected species therefore unlikely to be present at above the target concentration used for survey design. For detected species, model estimates can assist in determining the level of occurrence, including whether detections are likely to be false positives (transient DNA or contamination), emerging incursions or established species. Implementation of additional control samples and suitable synthetic standards would provide additional ability to detect contamination should this occur to provide additional confidence in results. Distinguishing transient detections from emerging incursions where the species is at very low abundance is, however, difficult, and repeated or additional surveillance may be needed to clarify species status in some cases.

The OAM approach developed makes the most use of currently available data but can be refined as more data are obtained. The modelling framework allows for incorporation of prior information, but assessment would be needed to ensure that any informative priors used are appropriate. Outputs from OAM analyses can also be used to refine knowledge about the planktonic concentration of target species where they occur. These estimates would assist in determining whether survey target concentrations and ranges for emerging or established species are appropriate, and in revising these concentrations if needed.

6. APPENDIX

6.1. JAGS model parameters and code

6.1.1. Model parameter names and data format

Consistent parameter names (Table 12) were used in code across models as far as possible, noting that the exact meaning of some parameters varied dependent on the model type. For models using grouped data, data were provided as the number of detections and number of samples per sample set with one row per sample set per species. Where seasonal effects were included in modelling, location and season for each sample set were provided as numeric covariates. For models using individual sample data, data were provided as a matrix with one row per sample and columns of detect/non-detect (coded as 1/0) per species.

Table 12. Parameter names used in models and their definitions

Parameter	Definition
N	Number of rows in the data set. For grouped data N = number of sets x number of species. For individual data N = number of samples
AP	Apparent prevalence = probability of detection in a sample
Y	Result. For grouped data Y = number of detections in the data set. For individual data Y = detect (1) or non-detect (0)
n	Number of samples in the set for grouped data
sp	Species (as number) for result
n_sp	Total number of species
loc	Location (as number) for result
n_loc	Total number of locations
seas	Season (as number) for the result
n_seas	Total number of seasons
set	Sample set (as number)
n_set	Total number of sets
SE	Assay diagnostic sensitivity, intercept in absence of inhibition for OAM_4
SE.int	SE intercept on complimentary log-log scale
SE.adj	SE adjusted for inhibition
SP	Assay diagnostic specificity
Sa	shape 1 (α) for sensitivity beta prior
Sb	shape 2 (β) for sensitivity beta prior
Ca	shape 1 (α) for specificity beta prior

Parameter	Definition
Cb	shape 2 (β) for specificity beta prior
prev	Prevalence = likelihood of occurrence of target DNA in a sample
TP	True prevalence in the sample set (or by location + season) = expected proportion of samples containing target DNA
TP.int	TP intercept where seasonal effects are included
a	TP.int on complimentary log-log scale
Bseas	Seasonal effect
z	Species occurrence indicator in multi-scale models
theta	Availability in multi-scale models
lp	Linear predictor of log concentration in OAMs
lc.int	Log-concentration intercept where seasonal effects are included
conc	Predicted concentration
logV	Logarithm of sample volume
bSF	Scale factor effect
lnSF	Logarithm of scale factor

6.1.2. Beta parameters used for priors

Table 13. Beta parameters (shape 1 = α, shape 2 = β) used for assay diagnostic sensitivity (DSe) and specificity (DSp) priors in modelling.

Assay	Parameter	Prior estimate (95% HDI)	α	β
Aamu	DSe	0.89 (0.83 – 0.94)	110	13.4
Asen	DSe	0.93 (0.89 – 0.97)	175	12.8
Cjap	DSe	0.98 (0.96 – 1.00)	236	4.36
Cmae	DSe	0.79 (0.62 – 0.91)	21.9	5.68
Dvex	DSe	0.97 (0.94 – 0.99)	200	7.22
Esin	DSe	0.88 (0.83 – 0.93)	145	19.8
Hsan	DSe	0.98 (0.96 – 1.00)	213	4.89
Mgig	DSe	0.91 (0.83 – 0.97)	65	6.42
Mjap	DSe	0.98 (0.96 – 1.00)	226	5.16
Mros	DSe	0.99 (0.98 – 1.00)	302	2.94
Msal	DSe	0.88 (0.83 – 0.93)	160	21.4
Mstr	DSe	0.73 (0.68 – 0.77)	245	92.3
Pcan	DSe	0.95 (0.91 – 0.98)	180	9.95
Pper	DSe	0.80 (0.74 – 0.86)	138	35.3
Pvir	DSe	0.79 (0.73 – 0.85)	132	34.4

Assay	Parameter	Prior estimate (95% HDI)	α	β
Rhar	DSe	0.99 (0.98 – 1.00)	313	2.14
Sspa	DSe	0.86 (0.79 – 0.93)	90.2	14.9
Upin	DSe	0.73 (0.59 – 0.88)	25.8	9.5
Vgib	DSe	0.90 (0.86 – 0.94)	172	19.2
Aamu	DSp	1.00 (0.99 – 1.00)	274	0.625
Asen	DSp	0.91 (0.89 – 0.92)	1000	104
Cjap	DSp	1.00 (1.00 – 1.00)	1000	1.67
Cmae	DSp	1.00 (0.99 – 1.00)	300	0.628
Dvex	DSp	1.00 (0.99 – 1.00)	605	1.64
Esin	DSp	1.00 (0.99 – 1.00)	598	1.65
Hsan	DSp	1.00 (0.99 – 1.00)	606	1.75
Mgig	DSp	1.00 (0.99 – 1.00)	396	0.699
Mjap	DSp	0.99 (0.98 – 1.00)	425	5.04
Mros	DSp	0.99 (0.97 – 1.00)	423	6.26
Msal	DSp	1.00 (1.00 – 1.00)	1000	0.463
Mstr	DSp	1.00 (0.99 – 1.00)	775	2.33
Pcan	DSp	1.00 (1.00 – 1.00)	1000	0.654
Pper	DSp	0.93 (0.91 – 0.95)	574	40.9
Pvir	DSp	1.00 (0.99 – 1.00)	925	1.54
Rhar	DSp	0.98 (0.97 – 0.99)	632	14.3
Sspa	DSp	1.00 (0.99 – 1.00)	293	0.606
Upin	DSp	1.00 (0.99 – 1.00)	346	0.728
Vgib	DSp	0.71 (0.68 – 0.74)	649	272

6.1.3. OM_1

```
model{
  for (i in 1:N){
    AP[i] <- SE[sp[i]]*TP[sp[i],set[i]]
    Y[i] ~ dbin(AP[i],n[i])
  }
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])
    for (j in 1:n_set){
      TP[i,j] ~ dbeta(1,1)
    }
```

```
  }
}
```

6.1.4. OM_2

```
model{
  for (i in 1:N){
    z[i] ~ dbern(TP[sp[i],set[i]])
    prev[i] <- z[i]*theta
    AP[i] <- SE[sp[i]]*prev[i]
    Y[i] ~ dbin(AP[i],n[i])
  }
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])
    for (j in 1:n_set){
      TP[i,j] ~ dbeta(1,1)
    }
  }
  theta ~ dbeta(1,1)
}
```

6.1.5. OM_3

```
model{
  for (i in 1:N){
    z[i] ~ dbern(TP[sp[i],loc[i],seas[i]])
    prev[i] <- z[i]*theta
    AP[i] <- SE[sp[i]]*prev[i]
    Y[i] ~ dbin(AP[i],n[i])
  }
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])
    for (j in 1:n_loc){
      TP.int[i,j] ~ dbeta(1,1)
      a[i,j] <- cloglog(TP.int[i,j])
      for(k in 1:n_seas){
        cloglog(TP[i,j,k]) <- a[i,j] + Bseas[i,k]
      }
    }
    for(k in 1:n_seas){
```

```
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  }
  theta ~ dbeta(1,1)
}
```

6.1.6. OM_4

```
model{
  for (i in 1:N){
    z[i] ~ dbern(TP[sp[i],loc[i],seas[i]])
    prev[i] <- z[i]*theta
    AP[i] <- SE[sp[i]]*prev[i] + (1 - SP[sp[i]])*(1 - prev[i])
    Y[i] ~ dbin(AP[i],n[i])
  }
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for (j in 1:n_loc){
      TP.int[i,j] ~ dbeta(1,1)
      a[i,j] <- cloglog(TP.int[i,j])
      for(k in 1:n_seas){
        cloglog(TP[i,j,k]) <- a[i,j] + Bseas[i,k]
      }
    }
    for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  }
  theta ~ dbeta(1,1)
}
```

6.1.7. OM_5

```
model{
  for (i in 1:N){
    prev[i] ~ dbeta(1,1)
    AP[i] <- SE[sp[i]]*prev[i] + (1 - SP[sp[i]])*(1 - prev[i])
    Y[i] ~ dbin(AP[i],n[i])
  }
```

```
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    }
}
```

6.1.8. OM_6

```
model{
  for (i in 1:N){
    prev[i] <- TP[sp[i],loc[i],seas[i]]
    AP[i] <- SE[sp[i]]*prev[i] + (1 - SP[sp[i]])*(1 - prev[i])
    Y[i] ~ dbin(AP[i],n[i])
  }
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for (j in 1:n_loc){
      TP.int[i,j] ~ dbeta(1,1)
      a[i,j] <- cloglog(TP.int[i,j])
      for(k in 1:n_seas){
        cloglog(TP[i,j,k]) <- a[i,j] + Bseas[i,k]
      }
    }
    for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  }
}
```

6.1.9. OM_7

```
model{
  for (i in 1:N){
    prev[i] <- TP[sp[i],loc[i],seas[i]] * theta
    AP[i] <- SE[sp[i]]*prev[i] + (1 - SP[sp[i]])*(1 - prev[i])
    Y[i] ~ dbin(AP[i],n[i])
  }
  for (i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
```

```
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for (j in 1:n_loc){
      TP.int[i,j] ~ dbeta(1,1)
      a[i,j] <- cloglog(TP.int[i,j])
      for(k in 1:n_seas){
        cloglog(TP[i,j,k]) <- a[i,j] + Bseas[i,k]
      }
    }
    for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  }
  theta ~ dbeta(1,1)
}
```

6.1.10. OM_8

```
model{
  for(i in 1:N){
    for(j in 1:n_sp){
      prev[i,j] <- TP[j,loc[i],seas[i]]
      AP[i,j] <- SE[j] * prev[i,j] + (1 - SP[j])*(1 - prev[i,j])
      Y[i,j] ~ dbern(AP[i,j])
    }
  }
  for(i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for(j in 1:n_loc){
      TP.int[i,j] ~ dbeta(1,1)
      a[i,j] <- cloglog(TP.int[i,j])
      for(k in 1:n_seas){
        cloglog(TP[i,j,k]) <- a[i,j] + Bseas[i,k]
      }
    }
    for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  }
```

```
}
```

6.1.11. OAM_1

```
model{
  for(i in 1:N){
    for(j in 1:n_sp){
      lp[i,j] <- lc.int[j,set[i]] + logV[i]
      cloglog(prev[i,j]) <- max(min(12, lp[i,j]),-12)
      AP[i,j] <- SE[j] * prev[i,j] + (1 - SP[j])*(1 - prev[i,j])
      Y[i,j] ~ dbern(AP[i,j])
    }
  }
  for(i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for(j in 1:n_set){
     lc.int[i,j] ~ dnorm(0,0.1)
     conc[i,j] <- exp(lc.int[i,j])
    }
}
```

6.1.12. OAM_2

```
model{
  for(i in 1:N){
    for(j in 1:n_sp){
      lp[i,j] <- lc.int[j,loc[i]] + Bseas[j, seas[i]] + logV[i]
      cloglog(prev[i,j]) <- max(min(12, lp[i,j]),-12)
      AP[i,j] <- SE[j] * prev[i,j] + (1 - SP[j])*(1 - prev[i,j])
      Y[i,j] ~ dbern(AP[i,j])
    }
  }
 for(i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for(j in 1:n_loc){
     lc.int[i,j] ~ dnorm(0,0.1)
     for(k in 1:n_seas){
      conc[i,j,k] <- exp(lc.int[i,j] + Bseas[i,k])
```

```
     }
  }
  for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
}
```

6.1.13. OAM_3

```
model{
  for(i in 1:N){
    for(j in 1:n_sp){
      lp[i,j] <- lc.int[j,loc[i]] + Bseas[j, seas[i]] + logV[i] +
log(theta)
      cloglog(prev[i,j]) <- max(min(12, lp[i,j]),-12)
      AP[i,j] <- SE[j] * prev[i,j] + (1 - SP[j])*(1 - prev[i,j])
      Y[i,j] ~ dbern(AP[i,j])
    }
  }
  for(i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for(j in 1:n_loc){
     lc.int[i,j] ~ dnorm(0,0.1)
     for(k in 1:n_seas){
      conc[i,j,k] <- exp(lc.int[i,j] + Bseas[i,k])
     }
  }
  for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  theta ~ dbeta(1,1)
}
```

6.1.14. OAM_4

```
model{
  for(i in 1:N){
    for(j in 1:n_sp){
      lp[i,j] <- lc.int[j,loc[i]] + Bseas[j, seas[i]] + logV[i]
```

```
      cloglog(prev[i,j]) <- max(min(12, lp[i,j]),-12)
      cloglog(SE.adj[i,j]) <- max(min(12, SE.int[j] + bSF[j] *
lnSF[i]),-12)
      AP[i,j] <- SE.adj[i,j] * prev[i,j] + (1 - SP[j])*(1 - prev[i,j])
      Y[i,j] ~ dbern(AP[i,j])
    }
  }
  for(i in 1:n_sp){
    SE[i] ~ dbeta(Sa[i],Sb[i])T(1-SP[i],)
    SE.int[j] <- max(min(12, cloglog(SE[j])), -12)
    bSF[j] ~ dnorm(bSF.mean,10)
    SP[i] ~ dbeta(Ca[i],Cb[i])
    for(j in 1:n_loc){
     lc.int[i,j] ~ dnorm(0,0.1)
     for(k in 1:n_seas){
      conc[i,j,k] <- exp(lc.int[i,j] + Bseas[i,k])
     }
  }
  for(k in 1:n_seas){
      Bseas[i,k] ~ dnorm(0,0.4)
    }
  bSF.mean ~ dnorm(-0.3,100)
}
```

6.2. Simulated data

6.2.1. Code used for simulation

```
set.seed(123456) # for replicability
# Five locations
nLOC <- 5
# Seven species
sim.spp <- c("Aamu","Cmae","Hsan","Mgig","Mros","Sspa","Upin")
nSPP <- length(sim.spp)
# Random seasonal difference per species
Bseas.av <- runif(nSPP,-2.5,2.5)
# Simulate some variation between locations
Bseas.sim <- matrix(rnorm(nSPP*nLOC,Bseas.av,0.1),ncol = nLOC)
#se and sp
```

```
Dse <- Bpars$Best[Bpars$par == "DSe" & Bpars$Species %in% sim.spp]
Dsp <- Bpars$Best[Bpars$par == "DSp" & Bpars$Species %in% sim.spp]
# Concentration range where present
conc.sim <- matrix(runif(nSPP*nLOC,-7,0.7),nrow = nSPP) #season 1
conc.sim2 <- conc.sim + Bseas.sim #season 2
# Likelihood of presence to use for simulation
Ppres <- rep(0.8,7)
spp.occ <- array(rbinom(nSPP*nLOC,1,Ppres),dim = c(nSPP,nLOC))
#Set log conc to -10 for absent species
sim.conc[,,1] <- conc.sim * spp.occ
sim.conc[,,2] <- conc.sim2 * spp.occ
sim.conc[sim.conc == 0] <- -10
# sample volume to use for simulation
# Random average volume per sample set
vol.av <- matrix(rnorm(nLOC*2,8.59,2),nrow = nLOC)
#35 samples per set
nsamp <- 35
sim.vol <- array(rnorm(nLOC*2*35,vol.av,1), dim = c(nsamp,nLOC,2))
# Data frame to hold simulated values
sim.dat <- expand.grid(Species = sim.spp,
                       loc = 1:5,
                       seas = 1:2,
                       repl = 1:nsamp,
                       svol = NA,
                       Detect = NA,
                       lnSF = 0,
                       conc = NA,
                       TP = NA,
                       pres = NA)
#Simulate results
for(i in 1:nSPP){
  for(j in 1:nLOC){
    for(k in 1:2){
      selrows <- sim.dat$Species == sim.spp[i] &
        sim.dat$loc == j &
        sim.dat$seas == k
      sim.dat$conc[selrows] <- exp(sim.conc[i,j,k])
      sim.dat$svol[selrows] <- sim.vol[,j,k]
      TP <- 1 - exp(-1 * exp(sim.conc[i,j,k]) * sim.dat$svol[selrows])
```

```
      pres <- rbinom(nsamp,1,TP)
      sim.dat$TP[selrows] <- TP
      sim.dat$pres[selrows] <- pres
      pdet <- Dse[i]*pres + (1 - Dsp[i])*(1-pres)
      sim.dat$Detect[selrows] <- rbinom(nsamp,1,pdet)
    }
  }
}
# Simulated scale factor effect
# Get estimates from OAM_4 applied to data from SA and Portland survey
m.name <- "OAM_4"
out.dir <- paste0("JAGSout/",m.name,"_SAPS")
load(paste0(out.dir,"/jags.Rdata"))
bSF.out <- out$summary[grepl("^b",rownames(out$summary)),c(1:3,7:9)]
all.spp <- unique(Bpars$Species)
bSF.df <- cbind.data.frame(Species = all.spp,bSF.out)
# Estimates for simulated species
bSF.sel <- bSF.df[bSF.df$Species %in% sim.spp,]
# Generate coefficients using mean and standard deviation of estimates
bSF.sim <- rnorm(nSPP, bSF.sel$mean, bSF.sel$sd)
# Patterns of inhibition in previous surveillance
load("Data/PCR_all.Rdata")
PCR.all$lnSF <- log(PCR.all$SF)
samp.sum <- PCR.all[,names(PCR.all) != "geometry"] %>%
group_by(jarLabel) %>%
  summarise(lnSF.s = mean(lnSF))
# Proportion of samples with lnSF > 0
sum(samp.sum$lnSF.s > 0)/nrow(samp.sum) #0.56
# Mode of lnSF (where > 0) ~ 0.9
hist(samp.sum$lnSF.s[samp.sum$lnSF.s > 0])
SF.av <- mean(samp.sum$lnSF.s[samp.sum$lnSF.s > 0])
SF.sd <- sd(samp.sum$lnSF.s[samp.sum$lnSF.s > 0])
# Inhibition v 1: similar across all sample sets
# Generate log scale factor for all samples but then set some to zero
sf.mode <- 0.9
ra <- (sf.mode + sqrt(sf.mode^2 + 4*SF.sd^2))/(2*SF.sd^2)
sh <- 1 + sf.mode*ra
sim.SF <- array(rgamma(nsamp*nLOC*2,sh,ra), dim = c(nsamp,nLOC,2))
# Samples with/without lnSF > 0
```

```
SFpos <- array(rbinom(nsamp*nLOC*2,1,0.6), dim = c(nsamp,nLOC,2))
sim.SF <- sim.SF * SFpos
# Simulate results using lnSF v 1
sim.dat.sf <- sim.dat
for(i in 1:nSPP){
  for(j in 1:nLOC){
    for(k in 1:2){
      selrows <- sim.dat.sf$Species == sim.spp[i] &
        sim.dat.sf$loc == j &
        sim.dat.sf$seas == k
      sim.dat.sf$lnSF[selrows] <- sim.SF[,j,k]
      se.int <- clogloglink(Dse[i])
      se.lp <- se.int + bSF.sim[i] * sim.SF[,j,k]
      SE <- clogloglink(se.lp, inverse = T)
      pres <- sim.dat$pres[selrows]
      pdet <- SE*pres + (1 - Dsp[i])*(1-pres)
      sim.dat.sf$Detect[selrows] <- rbinom(nsamp,1,pdet)
    }
  }
}
sim.dat.sf$Detect[sim.dat.sf$Detect > sim.dat$Detect] <- 0
# Inhibition v2: scale factor affecting selected sets more than others
# Location 1 season 1 and location 4 season 2
sf.set <- c(2,rep(0.9,7),3,0.9)
sh <- sf.set^2/SF.sd^2
ra <- sf.set/SF.sd^2
sim.SF2 <- array(dim = c(nsamp,nLOC,2))
i = 1
for(j in 1:nLOC){
  for(k in 1:2){
    sim.SF2[,j,k] <- rgamma(nsamp,sh[i],ra[i])
    i <- i + 1
  }
}
SFpos2 <- SFpos
SFpos2[1:nsamp,1,1] <- SFpos2[1:nsamp,4,2] <- rep(1,nsamp)
sim.SF2 <- sim.SF2 * SFpos2
sim.dat.sf2 <- sim.dat
# Simulate results using lnSF v 2
```

```
for(i in 1:nSPP){
  for(j in 1:nLOC){
    for(k in 1:2){
      selrows <- sim.dat.sf2$Species == sim.spp[i] &
        sim.dat.sf2$loc == j &
        sim.dat.sf2$seas == k
      sim.dat.sf2$lnSF[selrows] <- sim.SF2[,j,k]
      se.int <- clogloglink(Dse[i])
      se.lp <- se.int + bSF.sim[i] * sim.SF2[,j,k]
      SE <- clogloglink(se.lp, inverse = T)
      pres <- sim.dat$pres[selrows]
      pdet <- SE*pres + (1 - Dsp[i])*(1-pres)
      sim.dat.sf2$Detect[selrows] <- rbinom(nsamp,1,pdet)
    }
  }
}
sim.dat.sf2$Detect[sim.dat.sf2$Detect > sim.dat$Detect] <- 0
```

6.2.2. Summary of simulated data sets

Table 14. Simulated sample volume (mean ± standard deviation) and log concentration by species and sample set. Simulated absent species were assigned a log concentration of -10.

Location	Season	Volume	Aamu	Cmae	Hsan	Mgig	Mros	Sspa	Upin
1	1	7.63 ± 1.95	-2.636	-4.261	-3.793	-3.943	-5.092	-4.349	-10
1	2	7.95 ± 1.95	-3.950	-3.953	-3.367	-6.393	-5.501	-3.143	-10
2	1	7.87 ± 1.72	-5.159	-5.199	-10	-4.960	-6.077	-1.828	-5.703
2	2	7.38 ± 1.78	-6.488	-4.740	-10	-7.290	-6.406	-0.393	-5.003
3	1	7.72 ± 1.78	-3.354	0.029	-2.252	-1.717	0.222	-10	-5.355
3	2	7.84 ± 1.64	-4.572	0.574	-1.855	-4.129	0.055	-10	-4.666
4	1	7.84 ± 1.97	-10	-2.364	-6.873	-10	-2.339	-3.543	-2.291
4	2	7.29 ± 1.92	-10	-1.797	-6.471	-10	-2.602	-2.215	-1.674
5	1	7.59 ± 1.75	-10	0.068	-2.006	-0.230	-0.328	-6.889	-10
5	2	7.99 ± 1.72	-10	0.437	-1.413	-2.605	-0.708	-5.648	-10

Table 15. Simulated number of samples per sample set with occurrence of each species.

Location	Season	Aamu	Cmae	Hsan	Mgig	Mros	Sspa	Upin
1	1	16	4	5	3	5	5	0
1	2	7	7	11	1	1	13	0
2	1	2	2	0	2	0	26	2
2	2	0	2	0	0	0	35	0
3	1	11	35	17	23	35	0	2
3	2	1	35	27	5	33	0	3
4	1	0	19	1	0	20	9	19
4	2	0	26	0	0	18	23	26
5	1	0	35	35	35	35	1	0
5	2	1	35	18	34	33	1	0

Table 16. Simulated number of samples with detection for each species in the no inhibition data set. Log scale factor (lnSF) was set to zero for this set

Location	Season	lnSF	Aamu	Cmae	Hsan	Mgig	Mros	Sspa	Upin
1	1	0.00 ± 0.00	14	2	5	2	5	4	0
1	2	0.00 ± 0.00	6	6	11	1	2	12	0
2	1	0.00 ± 0.00	2	0	1	2	1	24	2
2	2	0.00 ± 0.00	0	2	0	0	1	29	0
3	1	0.00 ± 0.00	11	31	17	21	34	0	0
3	2	0.00 ± 0.00	1	28	27	5	32	0	3
4	1	0.00 ± 0.00	0	16	1	0	19	5	13
4	2	0.00 ± 0.00	0	23	0	0	20	20	23
5	1	0.00 ± 0.00	0	30	20	30	35	1	0
5	2	0.00 ± 0.00	1	24	28	16	33	1	0

Table 17. Simulated log scale factor (lnSF, mean ± standard deviation) and number of samples with detection for each species in the inhibition data set v1.

Location	Season	lnSF	Aamu	Cmae	Hsan	Mgig	Mros	Sspa	Upin
1	1	1.07 ± 1.29	12	1	3	1	5	2	0
1	2	1.13 ± 1.58	4	5	7	0	1	8	0
2	1	0.89 ± 1.32	2	0	0	2	0	19	1
2	2	1.02 ± 1.12	0	1	0	0	0	16	0
3	1	0.99 ± 1.10	8	20	13	15	28	0	0
3	2	0.65 ± 0.90	1	20	22	4	29	0	3
4	1	1.56 ± 1.57	0	9	1	0	17	3	10
4	2	1.15 ± 1.37	0	16	0	0	17	14	14
5	1	1.82 ± 1.93	0	12	16	17	28	1	0
5	2	1.16 ± 1.56	0	13	22	9	31	1	0

Table 18. Simulated log scale factor (lnSF, mean ± standard deviation) and number of samples with detection for each species in the inhibition data set v2.

Location	Season	lnSF	Aamu	Cmae	Hsan	Mgig	Mros	Sspa	Upin
1	1	2.33 ± 1.59	11	0	2	1	4	0	0
1	2	0.45 ± 0.89	4	3	11	1	1	11	0
2	1	0.30 ± 0.66	2	0	0	1	0	18	0
2	2	0.36 ± 0.80	0	2	0	0	0	21	0
3	1	0.89 ± 1.46	9	17	12	19	31	0	0
3	2	0.56 ± 1.62	1	24	26	5	30	0	2
4	1	0.78 ± 1.01	0	10	1	0	18	4	10
4	2	1.42 ± 2.74	0	10	0	0	14	16	13
5	1	2.44 ± 1.79	0	13	11	13	22	1	0
5	2	0.44 ± 0.62	0	14	25	14	31	1	0

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