Molecular surveillance to support domestic ballast water management in Australia

Kathryn Wiltshire, Di Barton,
Danièle Giblot-Ducray, Kelly Hill,
Marty Deveney

February 2025

Australian Government
Department of Agriculture, Fisheries and Forestry

Government of South Australia
Department of Primary Industries and Regions

SARDI
South Australian Research and Development Institute

Why survey invasive aquatic species (IAS)?

IAS can cause:

- Environmental harm
- Economic impacts
- Human health impacts & loss of amenity

Established IAS usually ineradicable

- Continued management/control costs
- Continued impacts/ risk of spread

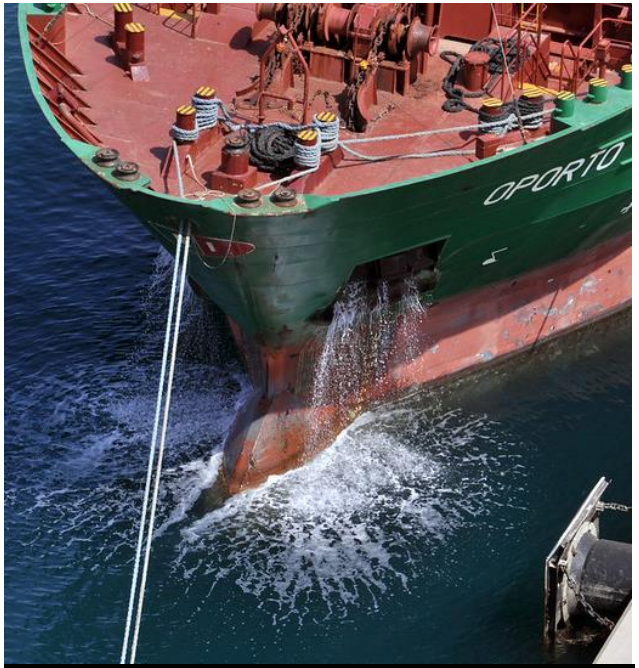
Surveillance important

- Detect new incursions and range extensions
- Understand distributions to inform management

Mytella strigata Photo: Andy Shinn, Inve Benchmark Thailand

Didemnum vexillum Photo: Ian Davidson, Smithsonian Environmental Research Centre

Ballast water and IAS



Ship discharging ballast water
CC BY-NC-ND 2.0 Chris Bentley
https://www.flickr.com/photos/cementley/19109972043

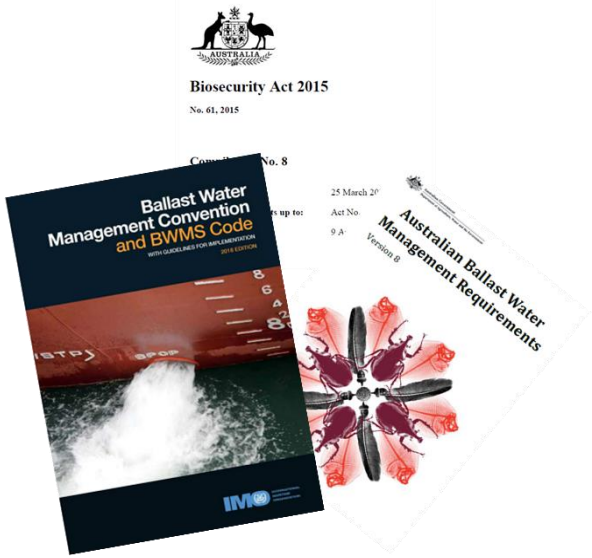
Ballast water a known key risk vector for new IAS introductions

IMO *Ballast Water Management Convention* (2004)

- Ballast water exchange (BWE)
  - Mid-ocean
- Ballast Water Management System (BWMS)
  - Reg D-2 performance discharge standard
  - Biological counts across 3 size classes

Ballast water management in Australia

- Management, movement and discharge of ballast water in Australia is legislated under the *Australian Biosecurity Act 2015 (Cwlth)*
  - International and domestic ballast water
- It is an **offence to discharge** ballast water (s270) unless it has been **managed** [BWE/BWMS] or a **low-risk exemption** has been provided



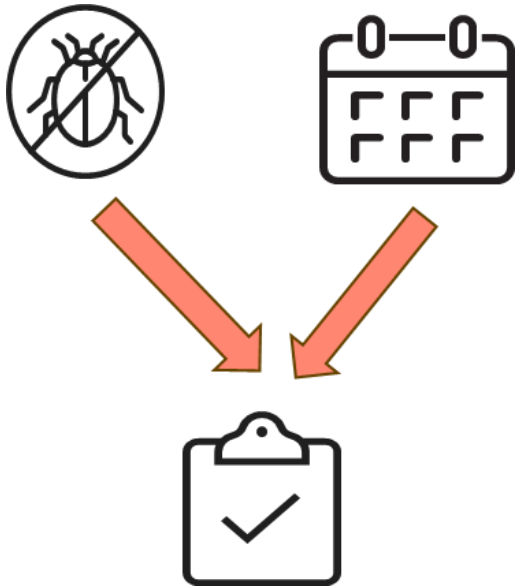
Domestic Risk Assessment Outcome (DRAO)

- Provides indication of risk for movement of ballast water between specific ports

Domestic Risk Assessment Tool

Based on

- **Status of marine pests** in uptake *and* discharge ports
- **Date** of uptake *and* discharge
- **Biology/ecology** of marine pests
  - Reproductive biology
  - Capacity to establish if introduced
  - Species Range Mapping/Sea Surface Temperature



Ballast water management

- Ballast water exchange (BWE)
  - Phased out by IMO
  - Still available for some/Contingency measure
- Ballast Water Management System (BWMS)
  - >90% vessels in Australian waters

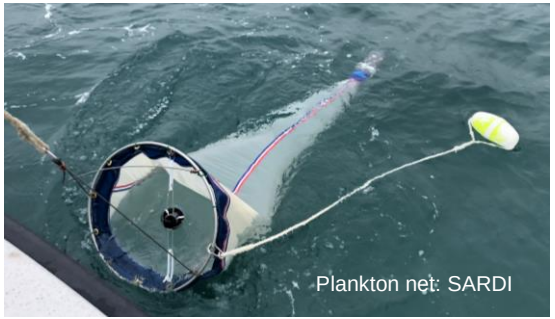
DRAO developed for BWE

Issues with D-2 compliance

➡ **Risk assessment of ballast water movement still required**

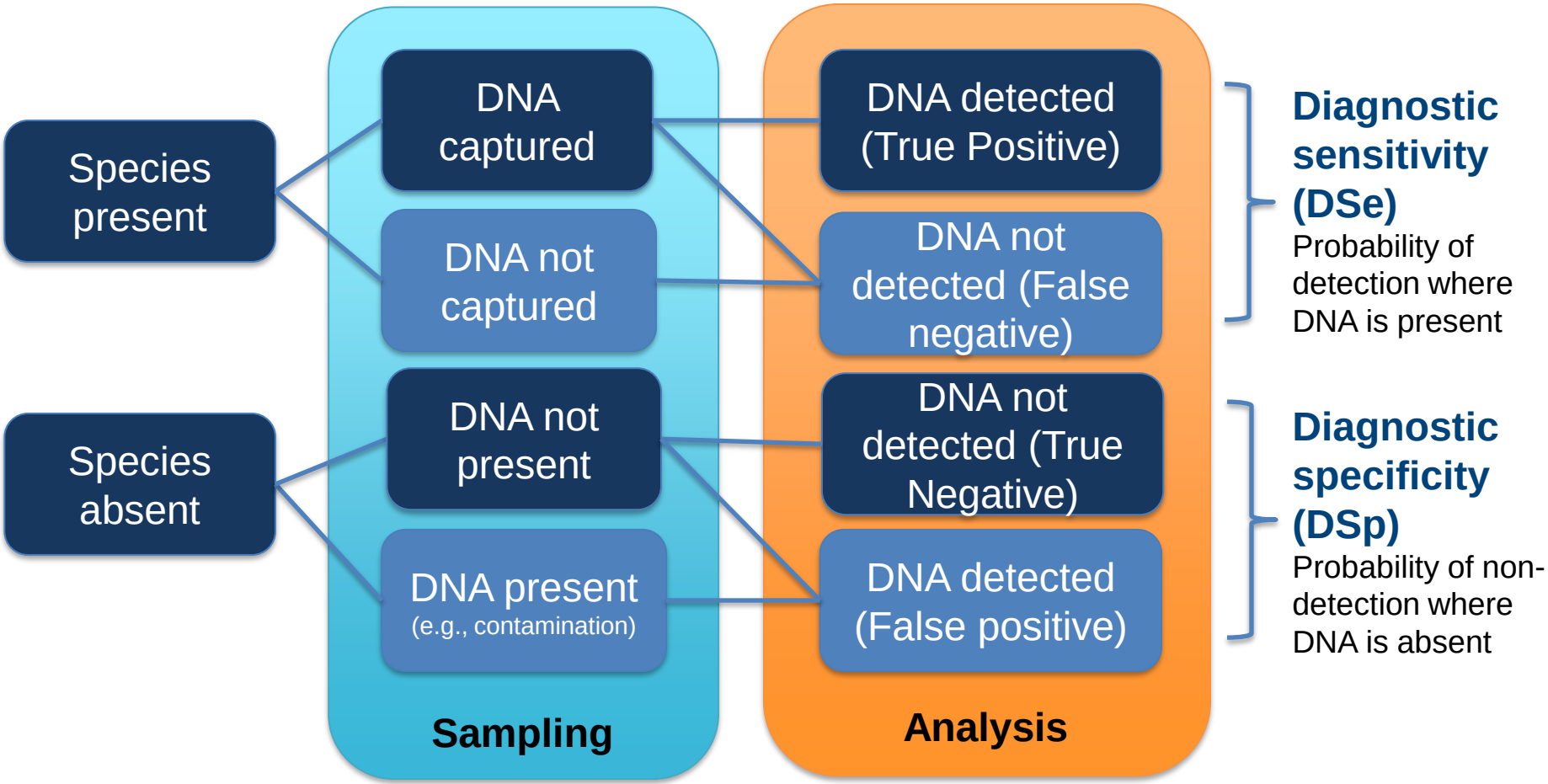
Molecular survey method - Plankton

- 100 m plankton tow, 50 µm mesh net
- Filtered and freeze-dried
- DNA extracted, tested using qPCR
- Assays available for 19 IAS, including 9 considered in ballast water risk management:
  - Northern Pacific sea star *Asterias amurensis* (Vic, Tas)
  - European green crab *Carcinus maenas* (SA, Vic, Tas, NSW)
  - Asian shore crab *Hemigrapsus sanguineus* (Vic)
  - European clam *Varicorbula gibba* (Vic, Tas)
  - Asian date mussel *Arcuatula senhousia* (Vic, Tas, WA)
  - Pacific oyster *Magallana gigas* (SA, Vic, Tas, NSW)
  - New Zealand screwshell *Maoricolpus roseus* (Tas, Vic)
  - European fan worm *Sabella spallanzanii* (WA, SA, Vic, Tas, NSW)
  - Japanese seaweed *Undaria pinnatifida* (Vic, Tas)


Plankton net: SARDI


DNA structure: Pixabay licence CC0 1.0

Understanding survey performance



Operational assay performance: method 1

Multiple tests in samples with unknown pest status

- Minimum 3 tests, or 2 tests with samples from 2 ‘populations’
- Need samples with and without pest DNA present
- With 2 tests, ‘populations’ must have different prevalence (proportion of samples containing target DNA)

Sabella spallanzanii Photo: CSIRO
http://www.scienceimage.csiro.au/image/880
licence: CC-BY-3.0

Operational assay performance: method 2

One or more tests in samples with known pest status

- ‘constructed samples’
- Can test a range of doses (need to be relevant!)

Statistical analysis (both methods)

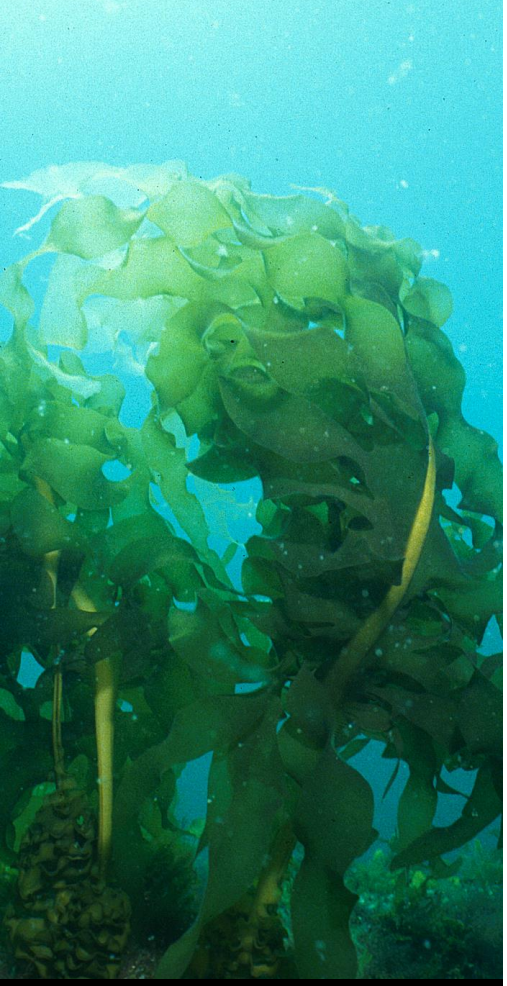
Bayesian Latent Class Modelling (BLCM)

Carcinus maenas Drawing: Rene Campbell, Flinders University/DAFF

Constructed plankton samples

Maoricolpus roseus
John Barkla CC BY 4.0

- NZ screwshell, Asian shore crab
- Plankton samples without IAS presence
- Freeze-dried pest tissue homogenised into sand
  - 6 Sand stocks with varying doses of IAS tissue
- Spiked sand dosed into 240 plankton samples
  - 40 samples per target without added pest tissue



Undaria pinnatifida Photo: CSIRO
http://www.scienceimage.csiro.au/image/867
licence: CC-BY-3.0

Multiple tests: qPCR + HTS

- 5 established IAS:
  - Northern Pacific sea star, European green crab, Pacific oyster, European fan worm, Japanese seaweed
- Plankton samples (205) from 2 ports with IAS present
- Samples from each season
- DNA extracts tested by:
  - qPCR
  - High-throughput sequencing (18S & COI for invertebrates, rbcL for *Undaria*)

Multiple tests: alternative qPCR assays

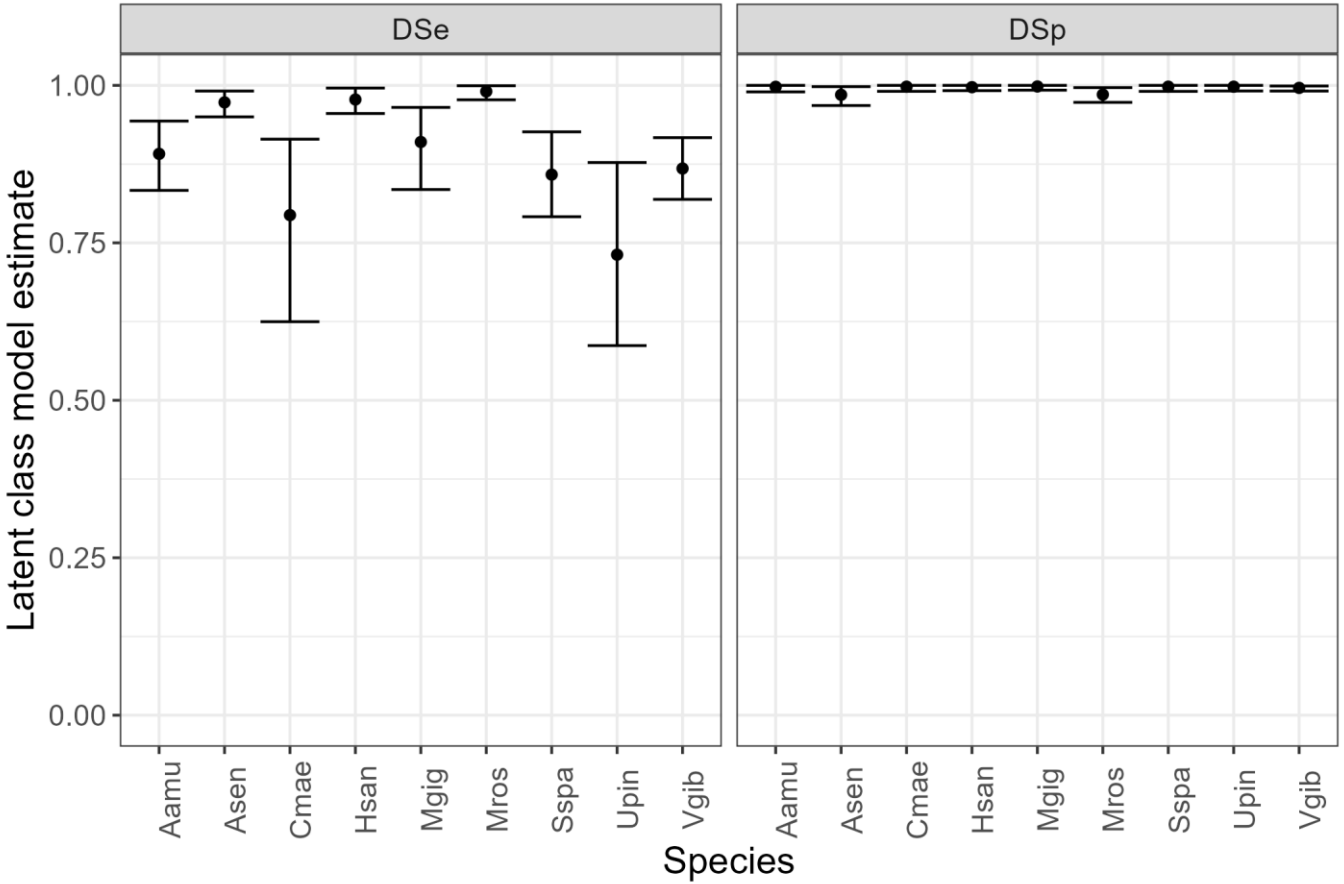
- Asian date mussel and European clam
- Plankton samples (368) from 18 ports with and without IAS present
- DNA extracts tested by two qPCR assays per species (28S and COI for each)

- BLCM
  - Models allowing for covariance between tests
  - Prevalence by season or location

Arcuatula senhousia
Auckland Museum MA92031 CC-BY 2.0

Varicorbula gibba
Y. Mollaoğlu CC-BY-NC-SA 4.0

Diagnostic performance in field samples



DSe:

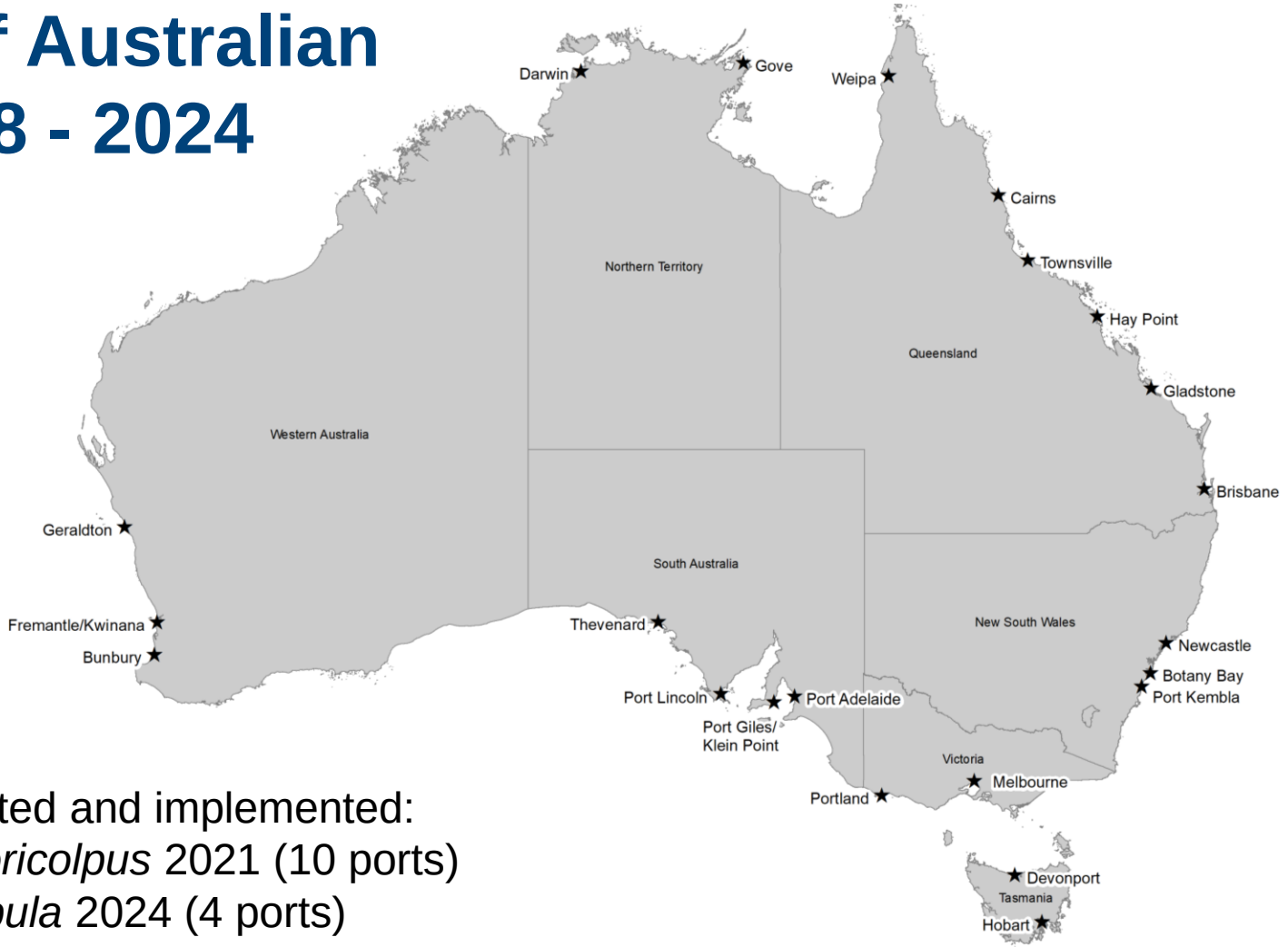
- Above 0.73 for all assays

DSp:

- > 0.95, close to 1 for most assays

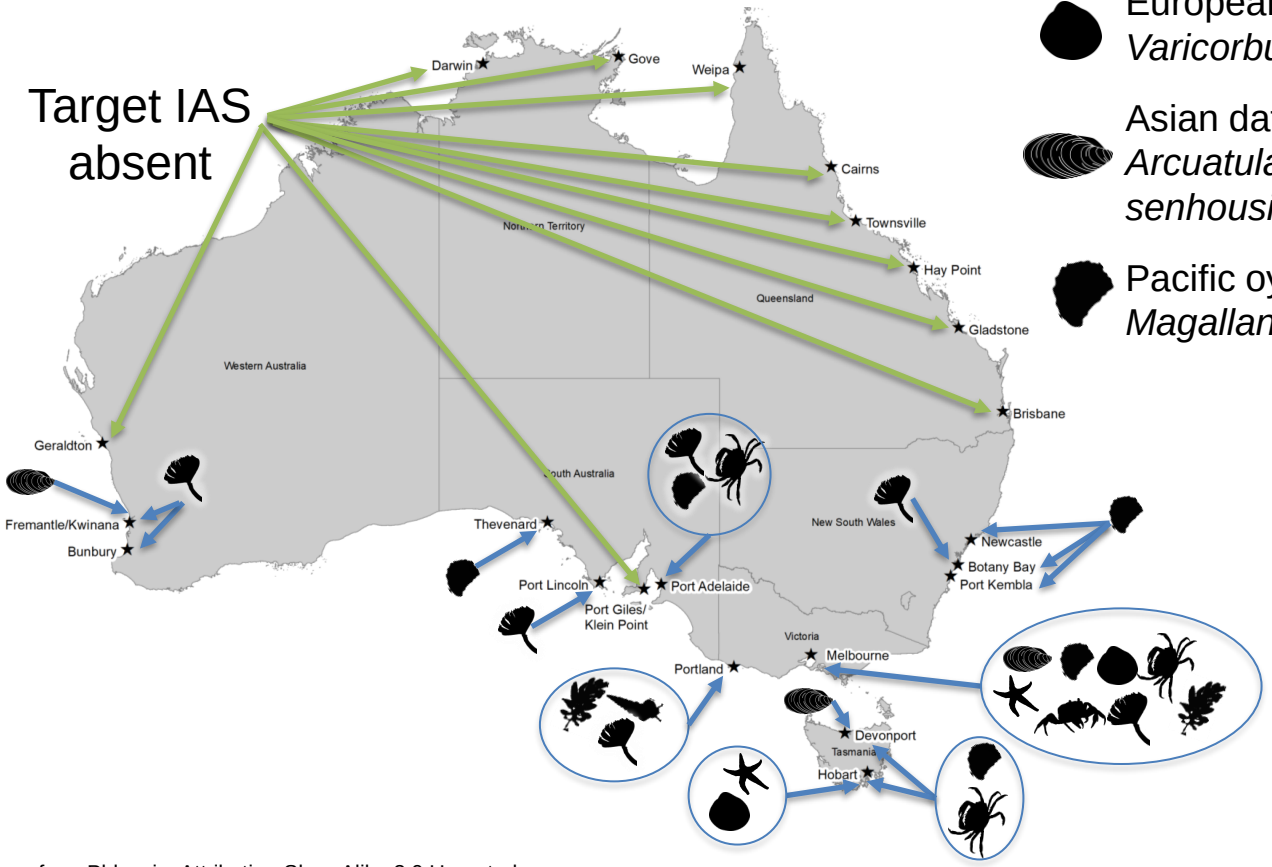
Surveys of Australian Ports: 2018 - 2024

24 ports surveyed for ballast water IAS of concern



New assays validated and implemented:
Hemigrapsus, Maoricolpus 2021 (10 ports)
Arcuatula, Varicorbula 2024 (4 ports)

IAS status at surveyed ports



European clam
Varicorbula gibba

Asian date mussel
Arcuatula senhousia

Pacific oyster
Magallana gigas

Northern Pacific sea star
Asterias amurensis

New Zealand screwshell
Maoricolpus roseus

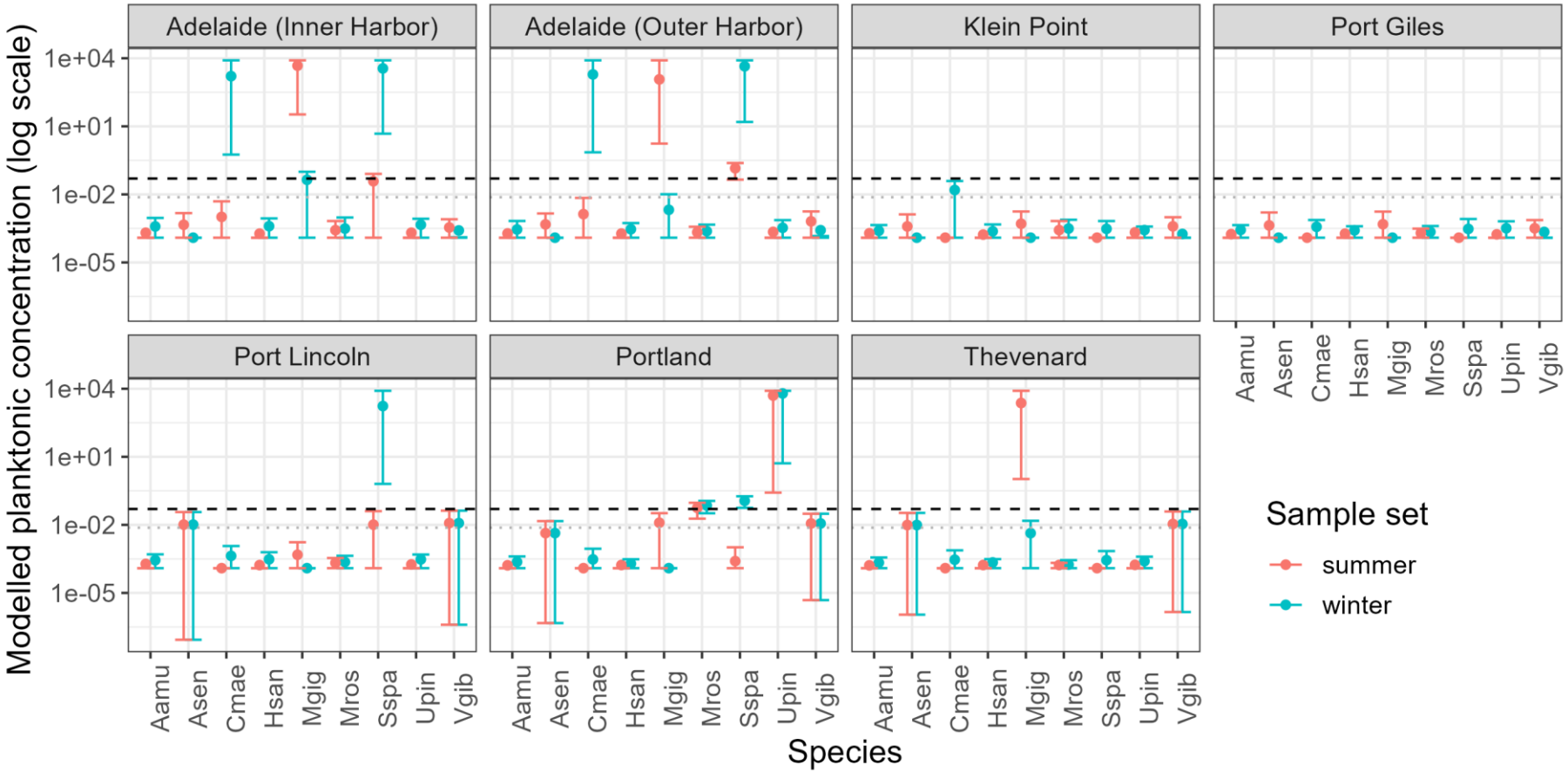
European green crab
Carcinus maenas

Asian shore crab
Hemigrapsus sanguineus

European fan worm
Sabella spallanzanii

Japanese seaweed
Undaria pinnatifida

Interpretation of survey results



Acknowledgements:

Work presented was funded by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF)

Thanks to:

- DAFF staff who supported this work, including Peter Stoutjesdijk, Sonia Gorgula, Cian Foster-Thorpe, Nick Fahy, Jason Bayly-Stark, Tim Carew, James Forwood, Melissa Kinsela *et al*.
- Individuals and institutions who provided tissue or pest specimens for assay validation work:
    - DPIRD WA, Agriculture Vic, MAGNT, Andy Shinn (Benchmark Inve, Thailand), NRE Tasmania, Woodbridge School Marine Discovery Centre Tasmania, Biosecurity SA, SA Museum, Museums Victoria, Deakin University
- SARDI Crop Sciences Molecular Diagnostics staff for DNA extractions, qPCR and project support: Herdina, Russel Burns, Danuta Pounsett, Tracy How, Ina Dumitrescu, Nigel Percy, Alan McKay, and Kathy Ophel-Keller
- SARDI Aquatic Sciences staff for sample collection, processing and project support: Mandee Theil, Sharon Drabsch, Ian Moody, Alex Dobrovolskis, Sunira Reardon, Jonathon Western, Jason Nichols, Leonardo Mantilla, Simon Goldsworthy, Jason Tanner, Mike Steer
- Traditional custodians of the lands where this work was performed, particularly the Kaurna people where the SARDI Adelaide sites are located
- Everyone who has assisted with this and related projects

Australian Government
Department of Agriculture, Fisheries and Forestry

Government of South Australia
Department of Primary Industries and Regions

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE