Australian Government
Department of Industry, Innovation and Science

Business Cooperative Research Centres Program

SOUTH AUSTRALIAN RESEARCH & DEVELOPMENT INSTITUTE
PIRSA

Bonamiasis in farmed Native Oysters (*Ostrea angasi*)

FRDC Project number: 2015/001

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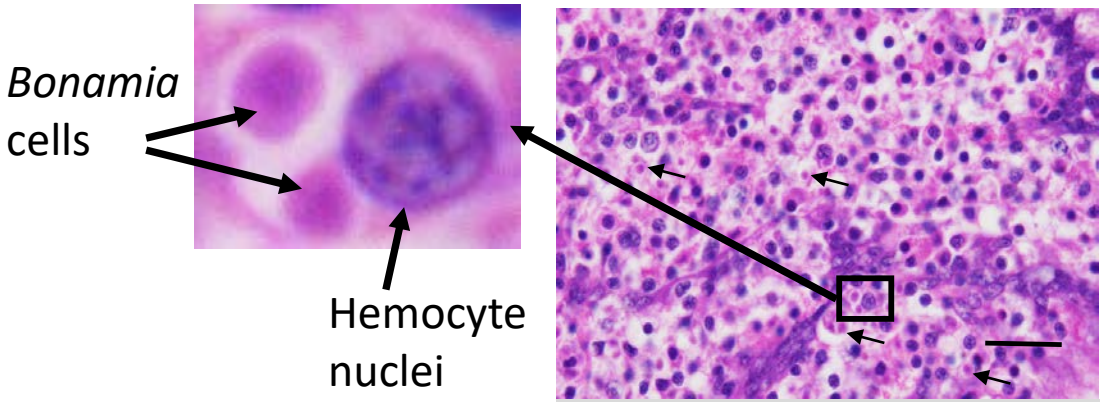
Background and aims

Bonamia

- Protozoan, 2–5 µm, infects oyster hemocytes
- Causes disease in Flat Oyster, Australia: *Ostrea angasi*
- Large body of work in Europe, NZ and North America, but not in Australia

Ostrea angasi (Native Oyster) from South Australian farm



Histology section with H and E staining. *Bonamia exitiosa*. (black arrows) in *Ostrea angasi*, Australia. Scale bar = 20µm.

Diagnostic tests - background

- Dec 2015 – Jan 2016 initial SA farm screening:
  - Coffin Bay/ Streaky Bay positive for *Bonamia exitiosa* (no *B. ostreae*)

<u>Aims</u>

1) **Assess prevalence and intensity on farm**
2) **Compare diagnostic tests (qPCR/histology/heart smear) to define:**

- DSe (Diagnostic Sensitivity) Proportion of true (+)
- DSp (Diagnostic Specificity) Proportion of true (-)

3) **Identify Australian *Bonamia* isolates (AAHL)**

Diagnostic tests - results/implications

- AAHL: *Bonamia* isolate in southern Australia (NSW/VIC/SA): *Bonamia exitiosa*

Prevalence and intensity at the three farm sites

	Coffin Bay (1)	Coffin Bay (2)	Streaky Bay
Prevalence (%) (credible intervals)	0.90 (0.78–0.99)[a]	0.90 (0.78–0.99)[a]	0.59 (0.46–0.72)[b]

- >50% prevalence in SA
- Best single test: histology; best combined tests: histology/qPCR
- DSe/DSp data form basis for survey designs
- Larger animals, >2 years: highest intensity and clinical disease
- High intensity correlated with low meat to shell ratio

Transmission - background

- Infection dynamics of *B. exitiosa* in *O. angasi* unknown
- Laboratory studies designed to assess infection

<u>Aims</u>

<u>South Australia</u>

1) **Assess *Bonamia* infection dynamics in *O. angasi***
   - **Time to first infection**
   - **Changes in intensity over time**
2) **Create infection model for *Bonamia* studies**

<u>Victoria</u>

1) **Assess which husbandry stressors ↑ mortality and *B. exitiosa* infection in *O. angasi***

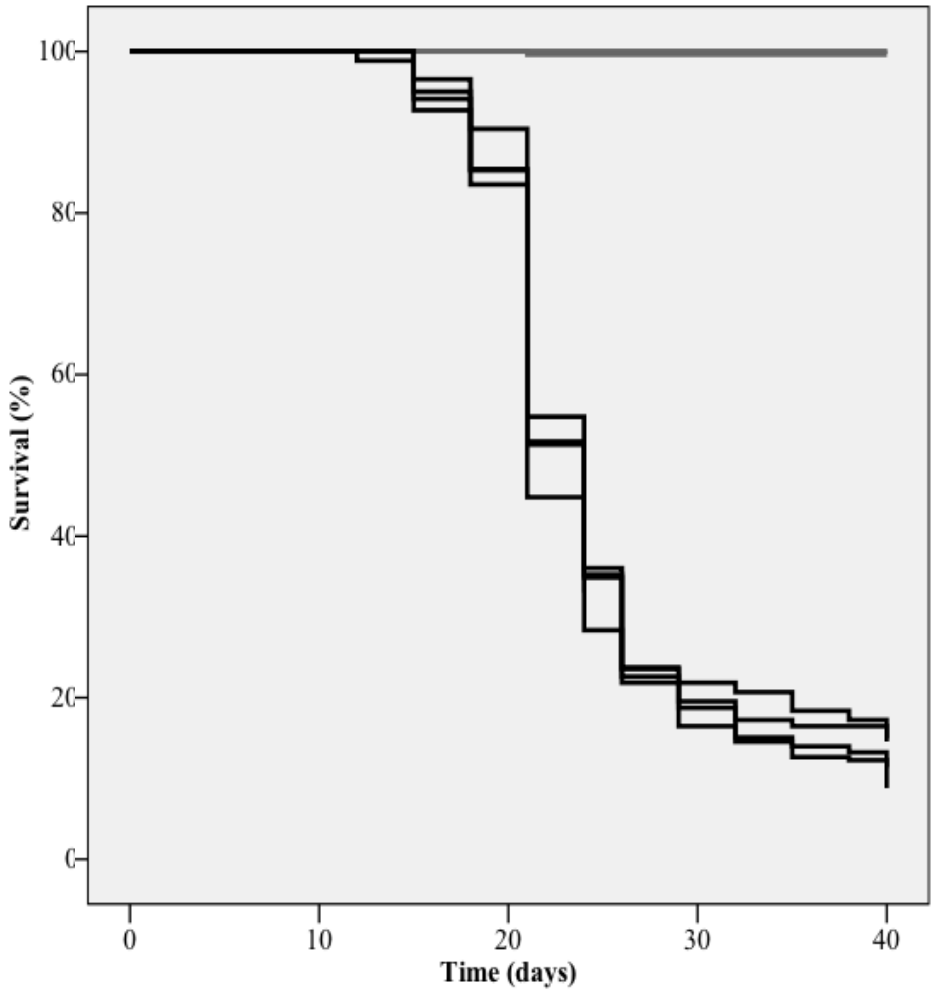
Transmission - results

Victoria

- Stressors ↑ mortality and ↑ *B. exitiosa* infection:
  - Heat (22°C), starvation (56 d no food) and tumbling

South Australia

- Control
  - Negative for *B. exitiosa*
- Exposed
  - **Day 12:** First mortalities
  - **Day 15:** Mortalities in all tanks
  - **Day 21:** 44.2–55.2% mortality
  - **Day 40:** 85.1–91.2% mortality
  - **Day 10:** 0.51 prevalence
  - **Day 11+:** 0.9 prevalence



Kaplan-Meier survival curve for *O. angasi* spat over 40 days. Black lines represent exposed treatments and grey lines represent control un-exposed treatments. n=300. Log-rank and Breslow tests.

Transmission - implications

Victoria

- Regular exposure to physical stress, starvation & exposure to increased temperature should be avoided

South Australia

- *Ostrea angasi*-*Bonamia exitiosa* infection dynamics:
  - Rapid and lethal infection in juvenile *O. angasi*: faster than other oyster-*Bonamia* spp. systems
  - Direct infection between live oysters
  - *Bonamia exitiosa* infects hosts of different ages
- Provides infection model for work on *B. exitiosa*
  - Breeding program for resistance

Farm trials - background

- Farm seasonality and other *B. exitiosa* infection dynamics in *O. angasi* unknown
  - Important for farm management
- Field trials

<u>Aims</u>

<u>Victoria</u>

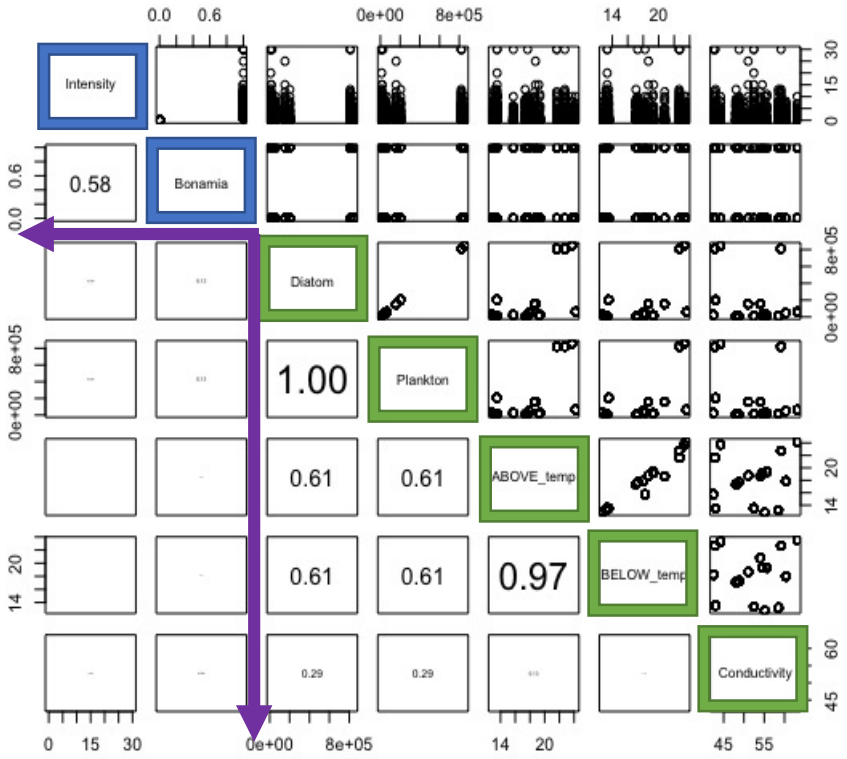
1) Effect of basket depth and fouling on *O. angasi* mortality (Port Phillip Bay, VIC)

<u>South Australia</u>

2) Assess seasonality of *B. exitiosa* infection in *O. angasi* in SA

- Monitor *O. angasi* cohort seasonally at 4 SA farm sites (2 x Coffin Bay, Cowell and Streaky Bay)
- Measure *B. exitiosa* prevalence and intensity

Farm trials - environmental influences



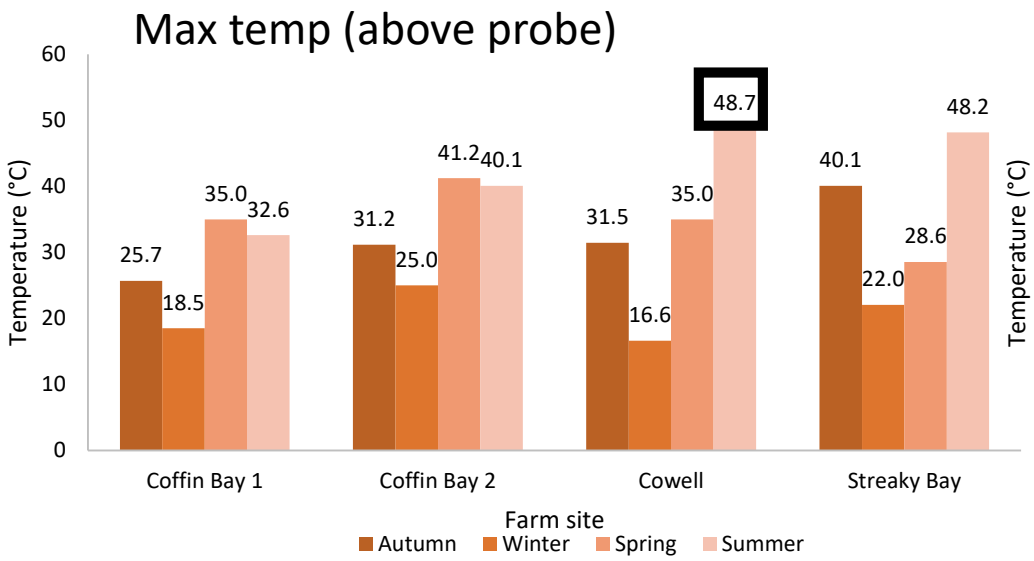
South Australia

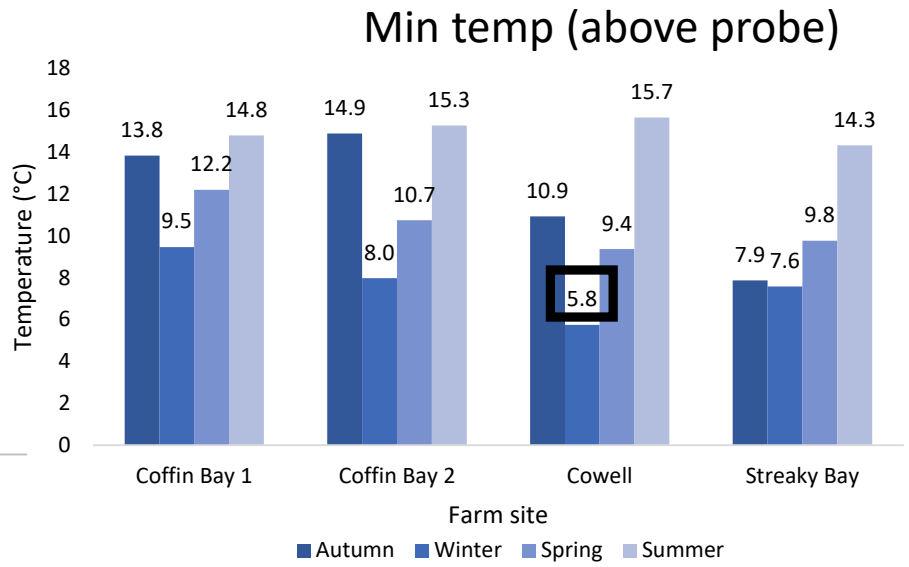
- Intertidal environmental parameters did not influence infection

Victoria

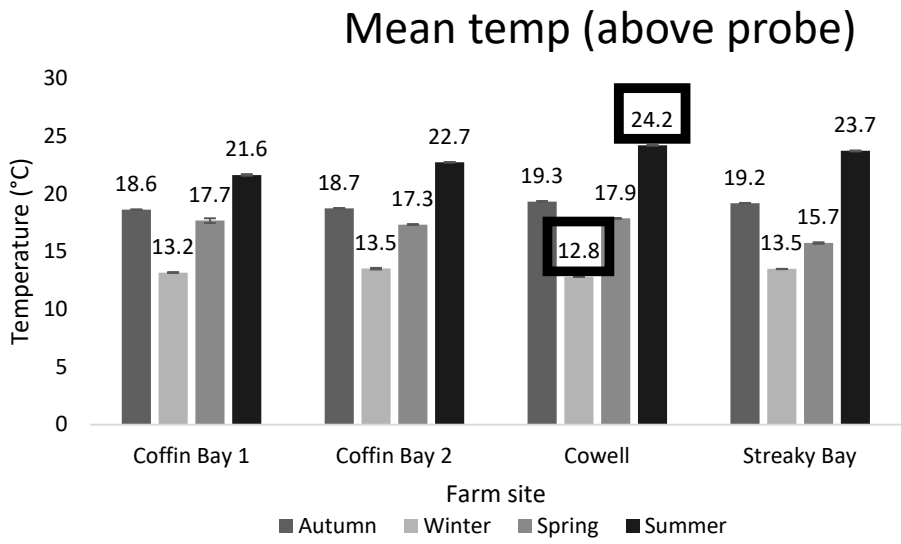
- Subtidal sites: Deeper and cleaner baskets ↓ *O. angasi* mortalities

Farm trials - SA temperature





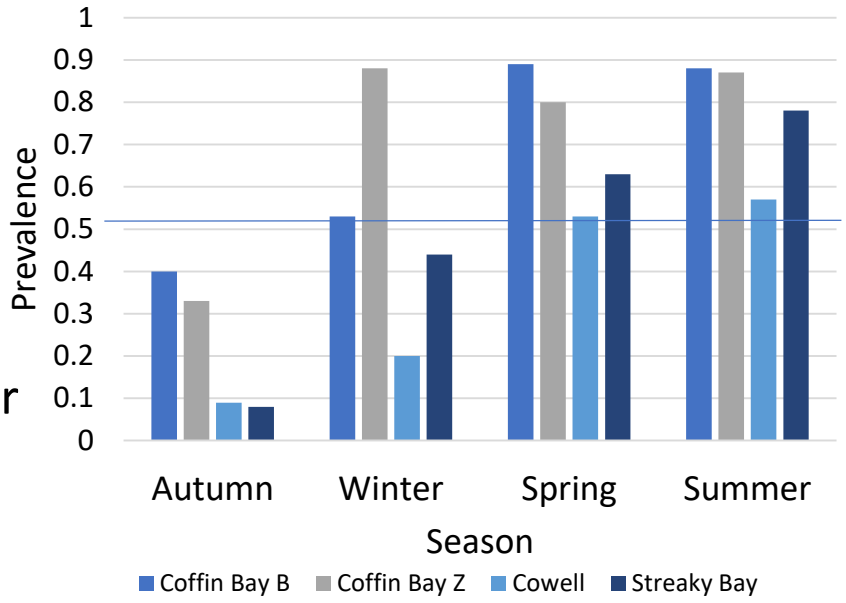
- Cowell had highest max summer T°C
  - On this day: >42°C for 2 h
- Cowell lowest min winter T°C
- SA intertidal systems are harsh environments
- Suitability for *O. angasi*



Farm trials - prevalence & intensity

South Australia

- Prevalence
  - Increases over time, no seasonality
- Rapid prevalence increase in Coffin Bay
  - Coffin Bay sites: >0.50 after 6 mo.
  - Streaky Bay/Cowell: >0.50 after 9 mo.
  - Cowell: Lowest prevalence after 1 year
  - Helps select farming regions



- Intensity
  - Low (3–7 cells/slide), no clear increase over 12 months
  - In general:
    - Spring/summer – earlier/lighter stages of infection
    - Autumn/winter – heavier stages of infection
    - No uniform pattern across all sites

Farm trials - implications

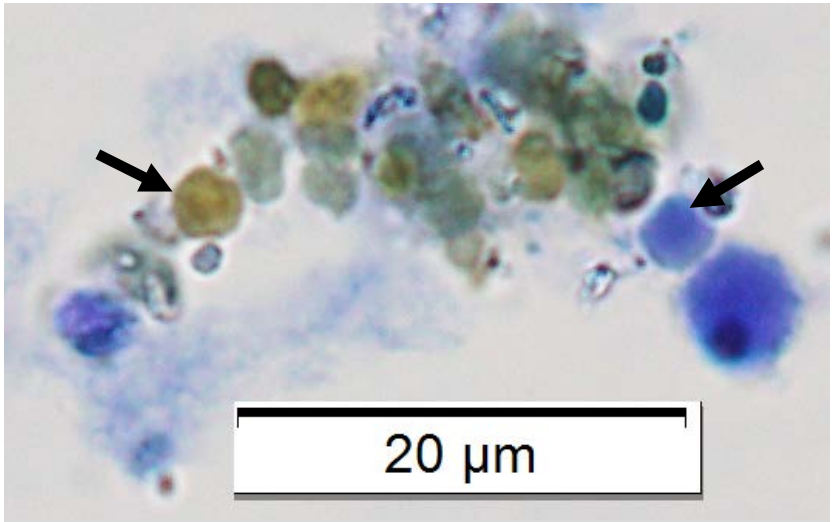
- Need to consider *B. exitiosa* in industry development / reef restoration
- *Bonamia exitiosa* prevalence increases over time (0.57–0.88)
- Seasonal intensity patterns
- High temperature variability in intertidal systems (max: 48.7°C / min 5.8°C)
  - Sub-tidal sites worth exploring
- Prevalence increased faster at some sites (Coffin Bay)
  - Differences oyster population densities
  - New sites should be assessed for *B. exitiosa* prior to development
  - Suitable culture sites: slower prevalence increase
  - Industry expansion: consider development of a breeding program

Decontamination - background

- No decontamination criteria for *Bonamia exitiosa*
- Important for farm management and translocation

<u>Aims</u>

1) **To assess concentration and exposure criteria for deactivation of *B.* exitiosa in sea water using sodium hypochlorite (NaOCl), Detsan, or Agri dyne**



Live *Bonamia exitiosa* cells (golden/brown) and dead *Bonamia* cells (blue) stained with Trypan Blue.

Decontamination - results

Two treatments achieved 100% efficacy:

- **1) Sodium hypochlorite (NaOCl): 40,000 ppm (4%) free chlorine for 10 min**
  - 40,000 ppm free chlorine = **320 mL NaOCl (12.5% free chlorine) / 1 L water**
  - $18 for 5 L (12.5% NaOCl) excl. delivery & GST ($3.6 / L) -> Chem-Supply
  - Corrosive, sensitive to organic matter
- **2) Iodine based disinfectant (Agri dyne): >2000 ppm (0.2%) iodine for 1 min**
  - 2000 ppm free iodine = **125 mL Agri dyne (1.6% free iodine) / 1 L water**
  - $163 for 25 L, excl. delivery, & GST ($6.52 / L) -> Tasman Chemicals
  - Less corrosive, more stability with organic matter

Detsan (quaternary ammonium compound)

- Maximum efficacy: 27% -> not recommended for decontamination, useful cleaning product (prior to disinfection)
- $110 for 25 L, excl. delivery & GST ($4.4/L) -> Chemetall

Decontamination - implications

- Data for *B. exitiosa* decontamination for hatcheries and farms
- Effective for *B. exitiosa* decontamination:
  - 40,000 ppm (4%) free chlorine for 10 min (NaOCl)
  - >2000 ppm (0.2%) free iodine for 1 min (Agri dyne)
- Agri dyne is more cost effective and less corrosive
- Detsan was not effective but useful for cleaning
- Minor use permits (PER 14029 and PER 82160) for oyster pathogens require amendment for use against *B. exitiosa*
  - Permit 14029: Authorises 1% free chlorine (NaOCl)
  - Permit 82160: Authorises 0.1% iodine (Agri dyne) (for POMS only)

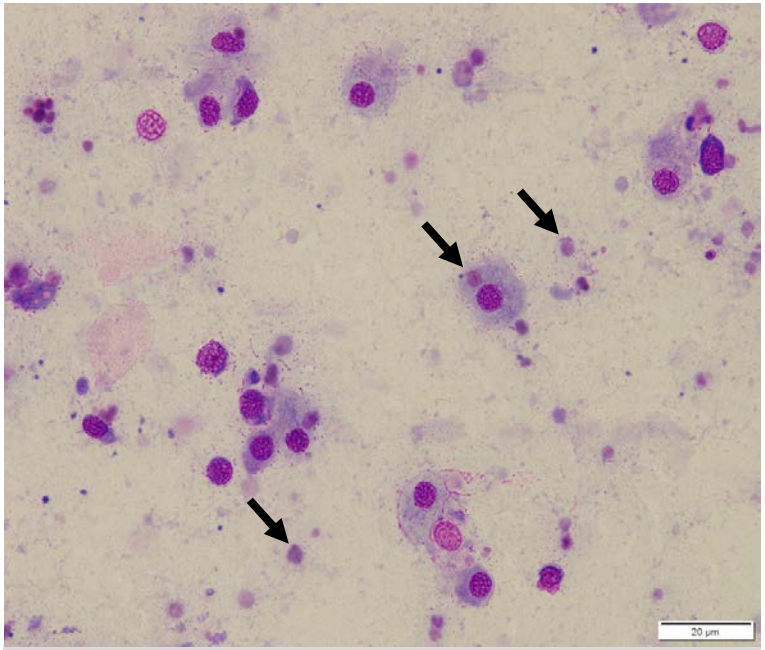
Species susceptibility - background

- *Bonamia* at low prevalence in *Crassostrea gigas* (Europe) (Lynch et al. 2010) and *Saccostrea glomerata* (Georges River) (Spiers et al., 2014)
- No clinical disease
- Susceptibility of *C. gigas and Saccostrea* sp. to *B. exitiosa* infected *O. angasi* unknown

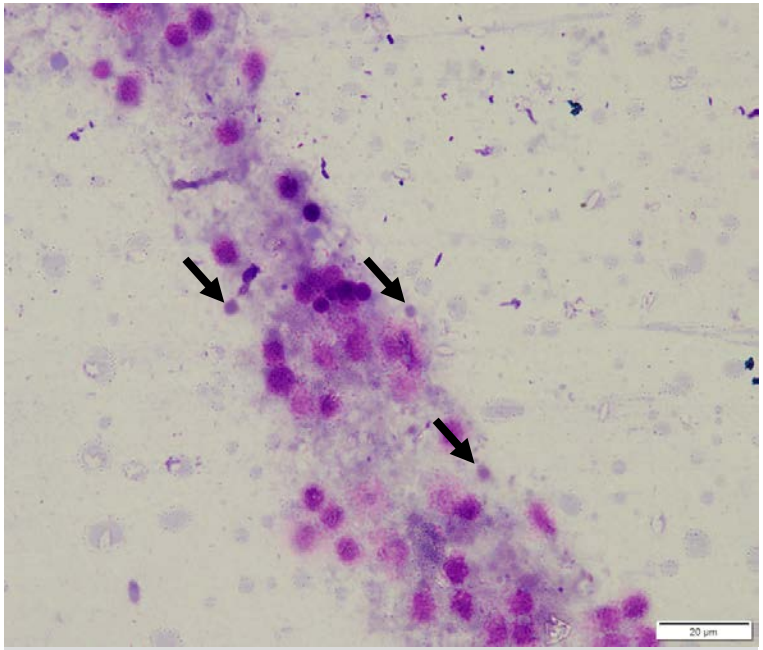
Aims

1) **Expose *C. gigas* and *Saccostrea* sp. to infected *O. angasi***
2) **Assess *Bonamia* susceptibility of *C. gigas* and *Saccostrea* sp.**

Species susceptibility - results



Heart smear staining with Hemacolor blood kit. *Bonamia* (black arrows) in *Saccostrea* sp. Scale bar = 20µm.



Heart smear staining with Hemacolor blood kit. *Bonamia* (black arrows) in *Crassostrea gigas* Scale bar = 20µm.

- 20 days: *Bonamia* in both species
- Higher intensity and prevalence in *Saccostrea* sp. than *C. gigas*
- Prevalence did not increase over time
- Low mortality:
  - *Crassostrea gigas*: 0.2% / *Saccostrea* sp.: 0.27%
  - Oysters were not dying due to disease by *Bonamia* after 60 days

Species susceptibility - implications

- *Crassostrea gigas and Saccostrea* sp. are susceptible to infection with *Bonamia*
- Negligible mortality indicates infection does not cause disease over 60d, but *C. gigas and Saccostrea* sp. are likely carriers of *Bonamia*
- Assess if *C. gigas* and *Saccostrea* sp. can transmit *Bonamia*
- Physical arrangement of *O. angasi* farms important:
  - Total oyster biomass (not just *O. angasi*) contributes to *Bonamia* infection

Crassostrea gigas.

Saccostrea sp.

Ostrea angasi.

Outcomes for farmers

- Diagnostic information to inform surveillance programs
- *Bonamia exitiosa* infects rapidly and causes disease and mortality
- Death is not required for transmission
- Assess sites for *Bonamia* before commencing *O. angasi* culture
- ↓ mortality ↑ depth: investigate suitable culture systems
- Infection model - created controllable exposures and infection
- Other means of management:
  - Choose sites with low infection
  - Subtidal site selection with uni-directional flow
  - Decontaminate using NaOCl/Agri dyne
  - Consider total biomass when choosing sites (*Saccostrea*/*Crassostrea*/*Ostrea*)
    - *C. gigas/Saccostrea* sp. influence *Bonamia* and *O. angasi* health

Future work

- Understand role of cupped oysters in *Bonamia* transmission
- Breed for resistance

Acknowledgements

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Thank you for listening