Australian Government
Australian Institute of Marine Science

NORTH WEST SHOALS TO SHORE RESEARCH PROGRAM

Monitoring of pearl oysters exposed to marine seismic survey source

Cecile Dang, Mark Meekan

Acknowledgements

Funding provided by Santos, helping to understand Western Australia's marine environment

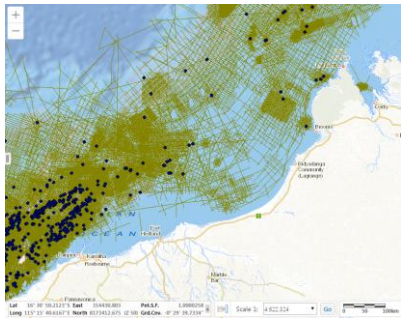
Collaborator Agencies include:

- Paspaley Pearling Company
- U of Tasmania
- Pearl Producers Association

Experimental design

- 5 treatments: 0, 1, 2, 3, or 4 exposures to the seismic source
- 7 locations (-1000, 0, 300, 500, 1000, 2000 and 6000 m)
- 35 groups of samples

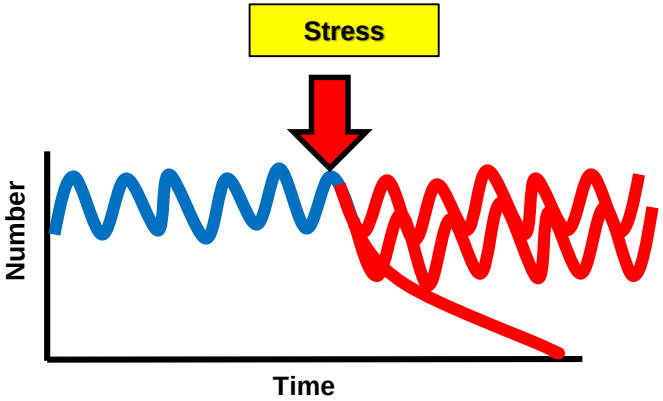
- 10 replicates per treatment for each location
- **~ 360 oysters** sampled at each sampling time
- Sampling frequency: pre-exposure, 0 (after exposure), 4 weeks, 3 months, 6 months



Preparation and logistics

- Logistic of sampling quite complex and challenging – coordination between staff
- Between 10 to 15 staff present in the laboratory in Broome to analyse and preserve samples – AIMS, UTAS, Industry, DPIRD
- DPIRD provided training to AIMS staff in July 2018 on pearl oyster sampling techniques (collection of haemolymph, dissection, etc.)

- Pearl oyster to cope with environmental stressors in their habitat
- Those stressors regulate populations
- Additional stress may induce imbalance and lead to reduction of oyster ability to resist disease, to grow, to heal, to produce a market quality pearl, etc.
- Energy budget of oysters mainly dedicated to growth, reproduction, and maintenance

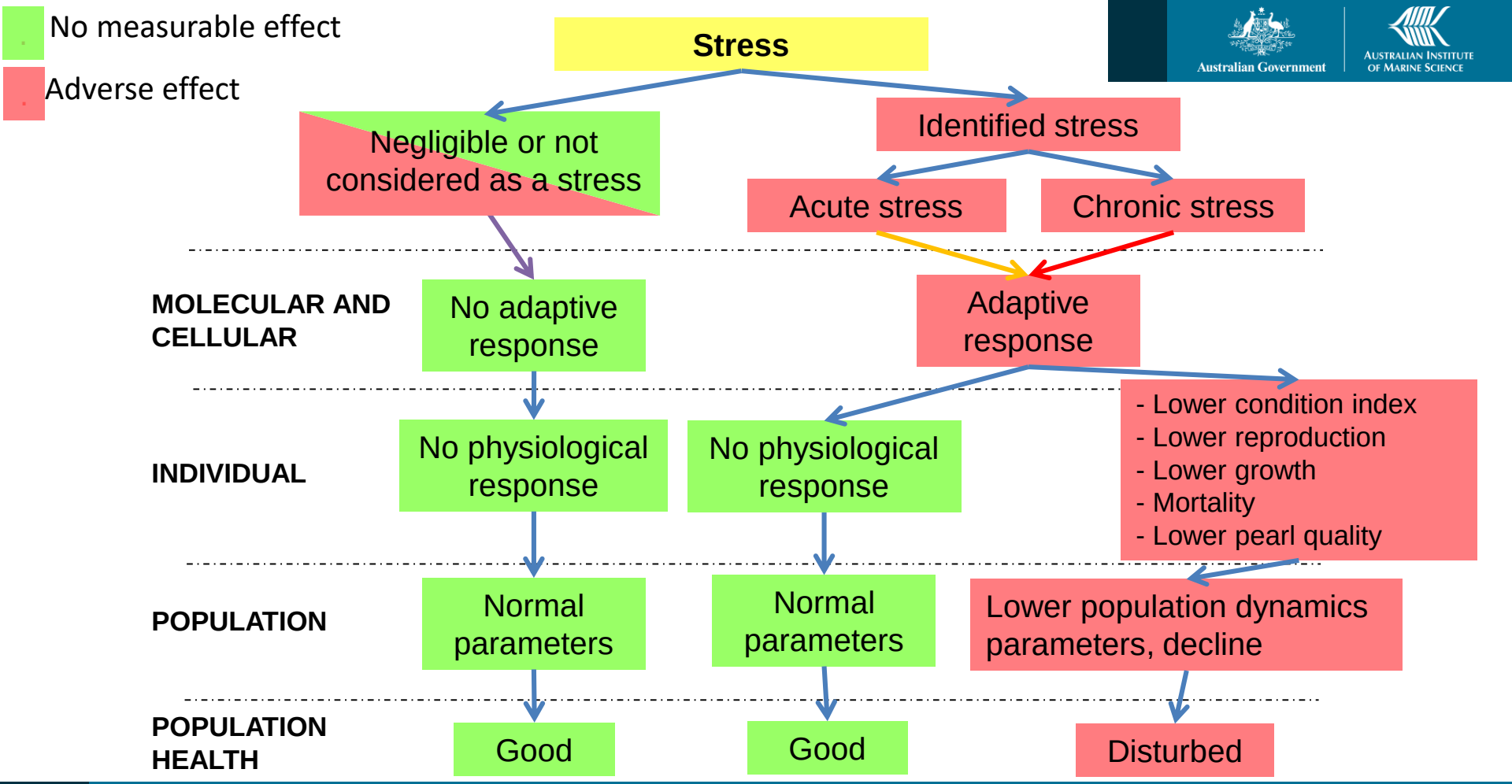


Molecular level
(gene expression, transcriptome, molecular immunity)

Cellular level
(enzyme activity, cellular immunity)

Individual level
(condition index, growth, reproduction, pearl quality, etc.)

Population level


No measurable effect
Adverse effect
Stress
Negligible or not considered as a stress
Identified stress
Acute stress
Chronic stress
MOLECULAR AND CELLULAR
No adaptive response
Adaptive response
INDIVIDUAL
No physiological response
No physiological response
- Lower condition index
- Lower reproduction
- Lower growth
- Mortality
- Lower pearl quality
POPULATION
Normal parameters
Normal parameters
Lower population dynamics parameters, decline
POPULATION HEALTH
Good
Good
Disturbed

Analyses

1. **Cellular functions (immunity, enzyme activity)**
2. **Molecular functions (transcriptomics**)
3. **Histology** (general health status and reproduction)
4. **Physiology** (mortality, growth, condition index, proximal analyses, etc.)
5. **Ability of oysters to produce quality pearls**

1. Cellular functions (immunity, enzymes)

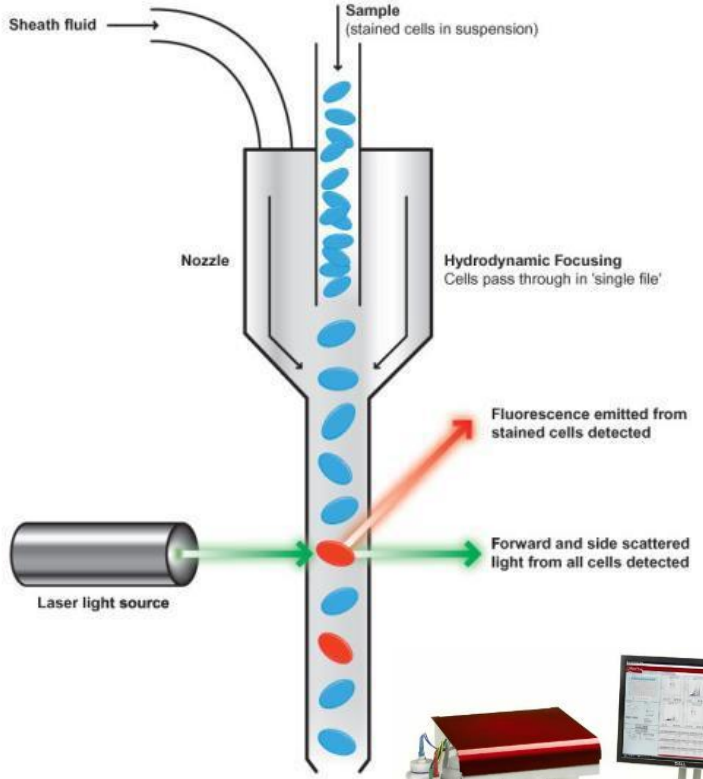
1.1. Flow-cytometry – What is it?

Measures every single particle (cell) in a fluid stream:

– Relative size (Forward Scatter – FSC) related to cell surface area

– Relative granularity or internal complexity (Side Scatter – SSC) - related to cell granularity and complexity

– Relative fluorescence intensity

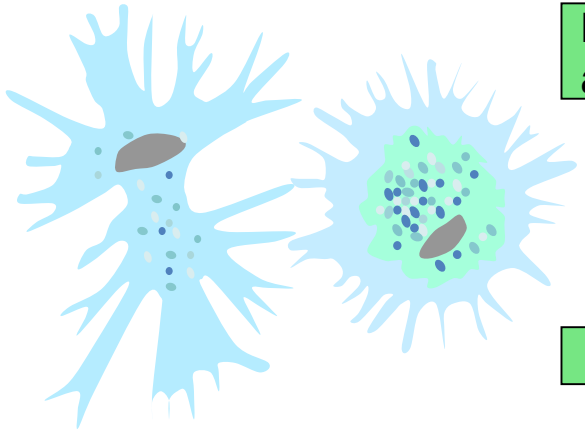


Role of hemocytes: not only involved in defense

Internal defense

Shell repair

Nutrient digestion and transport



Wound repair

Excretion

Haemocytes

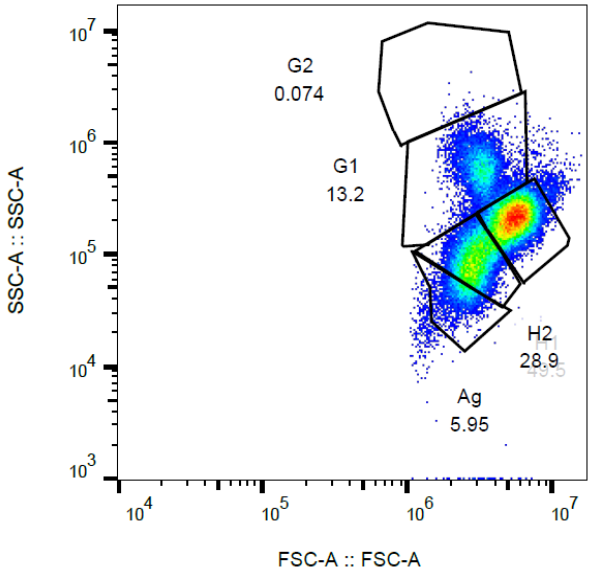
Several studies demonstrated effects of stressors on haemocyte functions

Oyster Haemocytes are the equivalent of our white blood cells
Haemocytes are in oyster blood or haemolymph

Parameters measured during the study using flow-cytometry

- Total and differential haemocyte count
- Proportion of dead cells
- Apoptosis
- Intracellular oxidative activity
- Lysosomal presence and activity
- Phagocytosis
- Mitochondrial activity

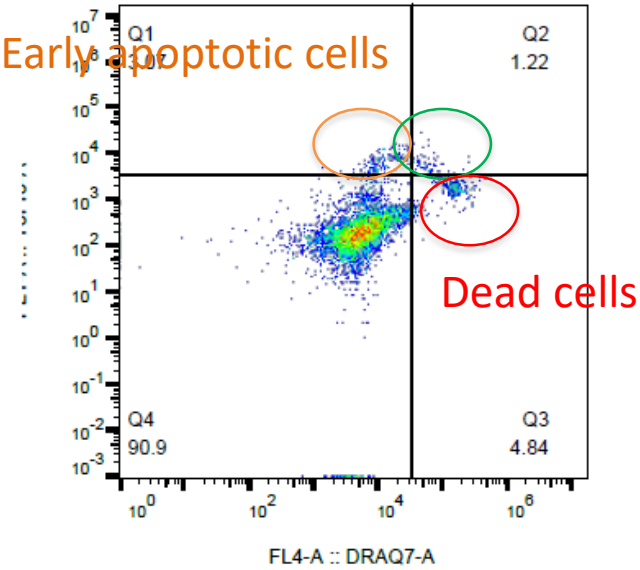
Those parameters will indicate if haemocytes are responding as they should and whether critical functions are impacted



Total haemocyte count

Late apoptotic cells

Early apoptotic cells



Dead cells

Apoptosis

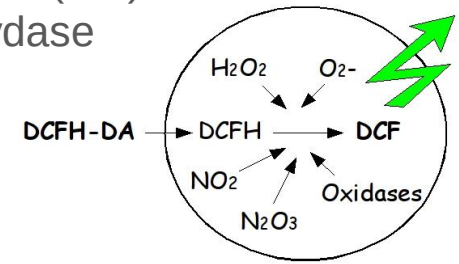
If oyster is stressed:

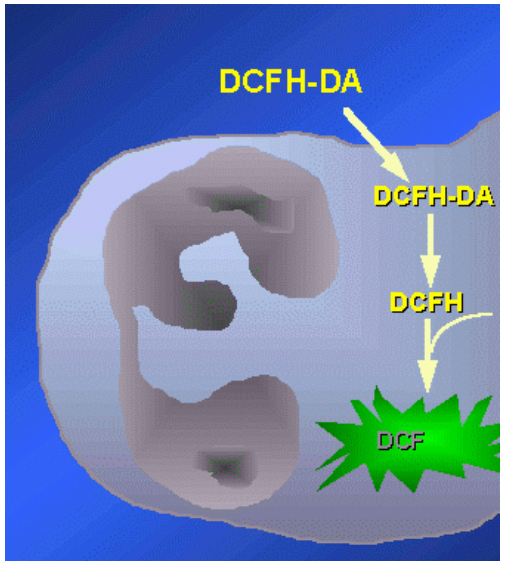
- cell count will vary – indicator of capacity of oyster to defend it self and to carry out biological functions
- Proportion of apoptotic cells is sensitive to stress

Intracellular oxidative activity

- Known indicator of external stress in mollusc
- Detection of free radicals – free radicals degrade microorganisms
- Oysters need to produce sufficient level of free radicals but not too much – otherwise oxidative stress occurs (exceed ability of antioxidant defences)

- ROS: Superoxide anion (O_2^-) Hydrogen peroxide (H_2O_2)
- RON: Peroxynitrite (NO_3^-), Nitric Oxide (NO)
- Enzymes : peroxydase, xanthine oxydase lipoxygenase
- Cytochrome C



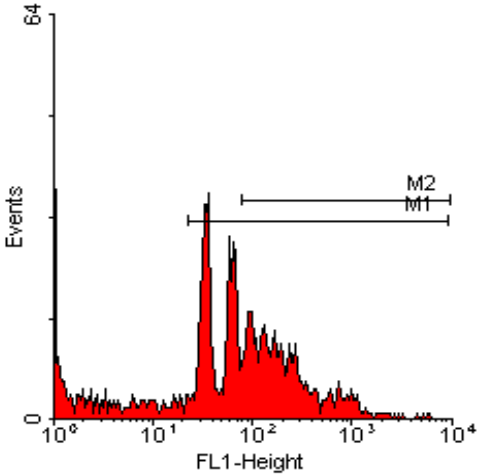


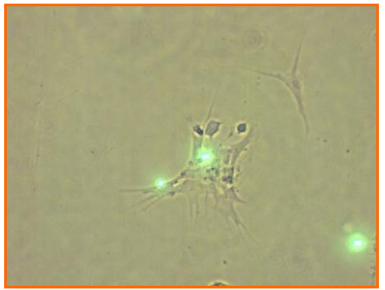
Measure using DCFH-DA dye, which becomes fluorescent (**DCF**) upon oxidation

Phagocytosis

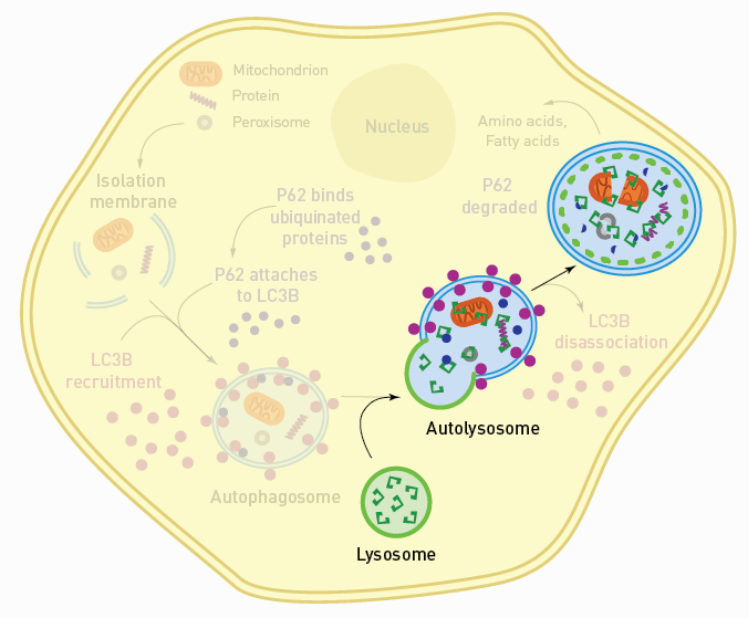
- Ingestion of large particles (bacteria, cell debris, etc.) in order to degrade it
- Most common mechanism to fight microorganisms
- If phagocytosis impacted by stress, it will compromise ability of oyster to fight an infection or clear cellular debris
- Use of fluorescent beads

- Phagocytosis capacity: number of beads per cell (for cells having ingested more than 3 beads)
- Phagocytosis rate: Proportion of cells that have ingested more than 3 beads





Lysosomes



Lysosomes: organelle responsible for intracellular digestion. They contain hydrolytic enzymes to breakdown macromolecules and pathogens. Disruption of this small recycling center can have devastating results for the cell.

Lysotracker & Lysosensor: dyes used to measure the biogenesis and activity of lysosomes

2.2. Biochemical analyses

- Lysozyme: antimicrobial enzyme that damages bacterial walls by attacking peptidoglycans - non specific defense mechanism - *plasma*
- Lactate deshydrogenase (LDH): cytoplasmic enzyme released into plasma by damaged cells – marker of injury - *plasma*
- Cortisol-like steroid: hormones indicator of stress – *gill and DG*
- Phenoloxidase: anti-microbial enzyme, which plays a role in immune defense, wound healing and marker of stress - *haemolymph*
- Oxidative stress (lipid peroxidation): measurement of MDA (malondyaldeide) – *DG and mantle*

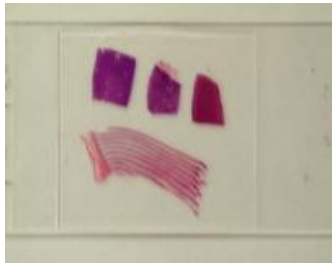
Oxidative stress: imbalance between antioxidant defenses and the production of free radicals leading to DNA damages and lipid peroxidation

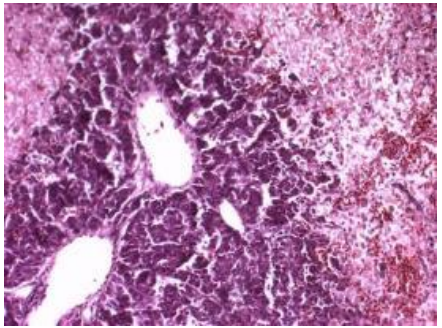
2. Molecular functions (transcriptomic)

- Sequencing transcriptome: image of all the transcripts encoded by the genome.
- Assessment of all down- and up-regulated genes (including immune, stress and nacre-associated genes)
- Collaboration with Pr Jacqui Batley at UWA

3. Histology

- NT government lab – observation of tissues using microscopy
- General health status, reproductive status, sex





4. Physiology

- Mortality rate, post-seeding mortality
- Assessment of apparent health at seeding (mantle retraction, ease of opening, gaping, etc.)
- Growth (length, height, weight)
- Condition index (medium term energetic status of the oyster)
- Gonad index
- Byssal attachment
- Lipids, proteins and carbohydrates
- Electrolytes & minerals in haemolymph (Na, K, Ca, Mg, P, etc.)
- Haemolymph pH
- Haemolymph refractive index (nutritional condition)

5. Pearlability

- Ability of a seismic-treated oyster to produce a market quality pearl
- Oysters were seeded and pearl production data and quality assessed at 1 and 2 year post-exposure: luster, size, shape, surface defect, color, nacre deposition
- % retention from seeding – determined using X-ray at 6 month

Sampling trips in Broome

- 360 oysters processed per trip at a rate of 80 per day – 5 sampling days
- Peter – coordinating role with boat and oyster delivery, time management
- Industry team: opening, weighing, photo
- Haemolymph sampling team: 4 people (AIMS, industry)
- Dissecting team: 4 people (AIMS, industry)
- Flow-cytometry team: 4 people (DPIRD)
- Haemolymph spinning team: 1 person (UTAS)
- Freezing cryotubes: 1 person (AIMS)
- Fixing samples in formalin: 1 person (AIMS)

Thank you

cecile.dang@dpird.wa.gov.au