From contractile vacuoles to pseudocyst formation: adaptation mechanisms for survival in the marine parasitic amoeba *Paramoeba perurans*.

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General objectives:

- Deliver fundamental knowledge on *Paramoeba perurans* biology
- Determine amoebae biological tolerances / environmental factors associated with AGD
- Identify key virulence factors using genomic sequence data
- Investigate the *in vitro / in vivo* effect of different alternative treatments

“| I DON'T KNOW WHAT THIS IS, BUT YOU SHOULD SEE HOW FAST IT'S GROWING!”
Contractile Vacuoles

"Ok, buddy - I think you've absorbed enough."
Osmoregulatory mechanism/s in *Paramoeba perurans*?

- AGD outbreaks → increasing temperature and salinity (>32 ppt)

*Paramoeba perurans*:

- Estuarine sheltered areas → significant spatial-temporal salinity fluctuations
  - freshwater and tidal inflow
  - wind stress
  - seasonal rainfalls
  - evaporation

The development of an osmoregulatory mechanism/s to survive in a wide range of environmental salinities should be expected for the species
Membrane permeability in unicellular Protists

Selectively semi-permeable membrane → allows certain molecules or ions to pass through it by means of active or passive transport (osmosis)

Hypertonic environment
Hypotonic environment
Isotonic environment

OSMOTIC GRADIENT
Osmoregulation in freshwater amoeba

Constantly have to cope with permeation of water across their plasma membrane

Marine species assumed to exist in isotonic equilibrium with their environment
Contractile vacuole in *Paramoeba perurans*

- **Kent, Sawyer & Hendrick (1998)** → prominent cytoplasmic vacuoles at 8 to 9 ppt salinity (*Neoparamoeba pemaquidensis*)

- **Powell and Clark (2003)** → rounding of amoeba and formation of a vacuole within the cell in low ionic solutions (*Neoparamoeba pemaquidensis*)

- **In vitro culture in our lab** → vacuolar formation often observed, particularly following water changes

- **Hypothesis** → CVs are involved in the osmoregulation process of *P. perurans*
Contractile vacuole in *Paramoeba perurans*

- 20% salinity reduction → CV formation and activity similar to freshwater amoeba species
Contractile vacuole in *Paramoeba perurans*

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Contractile vacuole in *Paramoeba perurans*

- 20% salinity reduction $\rightarrow$ CV formation and activity similar to freshwater amoeba species

- *Dictyostelium discoideum* $\rightarrow$ Ca2+ involved in osmoregulation by regulating the CVs contractions (Moniakis et al 1999, Malchow et al 2006)
Involvement of Ca2+ in *Paramoeba perurans* osmoregulation?

*P. perurans* incubated in CaCl2 (200ppm)

- Increased vacuolar activity
- Reduction of the CV cycle duration
- Smaller vacuoles also active (unusual)

Vacuolar activity also observed in floating cell (very unusual)
Conclusions:

CVs are involved in the osmoregulatory mechanism of *P. perurans*

• possible explanation for the amoeba occurrence at salinity levels as low as 7.2ppm in TAS (Clark & Nowak 1999)

• high salinities the main environmental risk factor associated with AGD ???

Ca2+ appears to have a role on CV regulation

• might explain why the reduction of total hardness enhances the efficacy of freshwater bathing (Powell & Clark 2003, Roberts & Powell 2003)

• ↑Ca2+, ↑ CV activity, ↑ osmoregulation efficiency, ↑ survival under osmotic stress condition
Pseudocysts
Cyst vs. Pseudocyst:

- **Free-living protists** → constantly exposed to environmental factors that challenge their survival → evolved distinct adaptation mechanisms

- **Encystation** → major survival strategy which allows some species of amoeba to survive under long-lasting, unfavourable environmental conditions

**Cysts:**
- highly resistant → double-layered wall
- multiple nuclei
- remain metabolically inert until conditions are favourable for hatching

**Pseudocysts:**
- absence of cyst wall → reduction in survival capacity
- survives for shorter periods of time without any evidence of activity
Pseudocysts in different amoeba species:

**Hydramoeba hydroxena** → starvation (Beers 1963)
- dissolved in KOH
- survival capacity → 72h
- weakened, starved, moribund amoebae

**Acanthamoeba keratitis** → contact-lens solutions, methanol, acetone and DMSO (Kliescikova et al 2011a and 2011b)
- single-layered amorphous mannose/glucose coat
- dissolved in acidic pH
- reverse to viable trophozoites after 24h exposure
Does *Paramoeba perurans* develop into pseudocysts?

Round shape amoeba often observed in our *in vitro* cultures

Spherical cells previously reported → freshwater and DMSO → **not stained by neutral red** → counted as dead cells (Powell and Clark 2003, Crosbie et al 2014)

Are the spherical cells actually dead?

Could they revert to viable trophozoites if returned to optimum culture conditions?

**Hypothesis** → non-motile cyst-like forms are an immediate cell response to acute threat of plasma membrane damage or cell death
Paramoeba perurans vs. freshwater

Freshwater cell submitted to 40% SW for 7 days → fully recovered once returned to standard culture conditions.

Survival over time:
- Freshwater: 5 min, 30 min
- Seawater: 2h, 1h

Neutral red not absorbed after treatment with KOH / detergent.
Paramoeba perurans vs. DMSO

10% DMSO → 20 min

not resistant to KOH and detergent
neutral red not absorbed

80% of pseudocyst showed full recovery
**Conclusions**

**Pseudocyst** → reversible stress-induced dormant stage → triggered by certain external stress factors

- reduce the surface area exposed to the stress source
- least resistance to flow → more efficiently transported by currents/tides
- attenuate the ability of stressors to enter the cell

Mechanism of protection to rapidly escape undesirable environmental conditions and, therefore, improve the chances of being passively transported to a more favourable substratum

↑ exposure time ↓ viability

↑ stress source intensity ↓ pseudocyst durability and ability to recover
General conclusions:

- The marine parasitic amoeba *P. perurans* has evolved cell response mechanisms to adapt to osmotic pressure (*contractile vacuoles*), as well as to survive under acute stress conditions (*pseudocyst*).

- Could this increase amoebae survival and allow rapid reinfection levels following FW bathing?

- **Pseudocyst** → should be taken into account when assessing the effect of treatment strategies.

- **Neutral red assay** → CAUTION! might be subject to misinterpretation of results → return cells to optimum conditions and perform assay after 24h.

Are current treatments possibly selecting for resistant amoeba?
References


