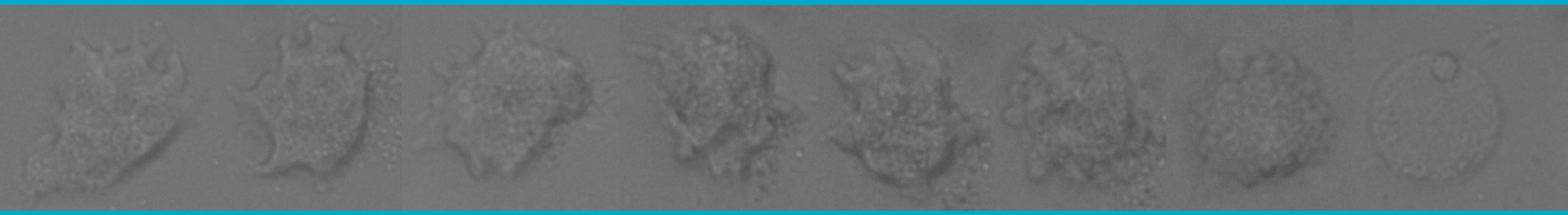


From contractile vacuoles to pseudocyst formation: adaptation mechanisms for survival in the marine parasitic amoeba *Paramoeba perurans*.

Paula Lima, Richard Taylor, Mathew Cook

Gill Health Initiative - Galway
16th April 2015

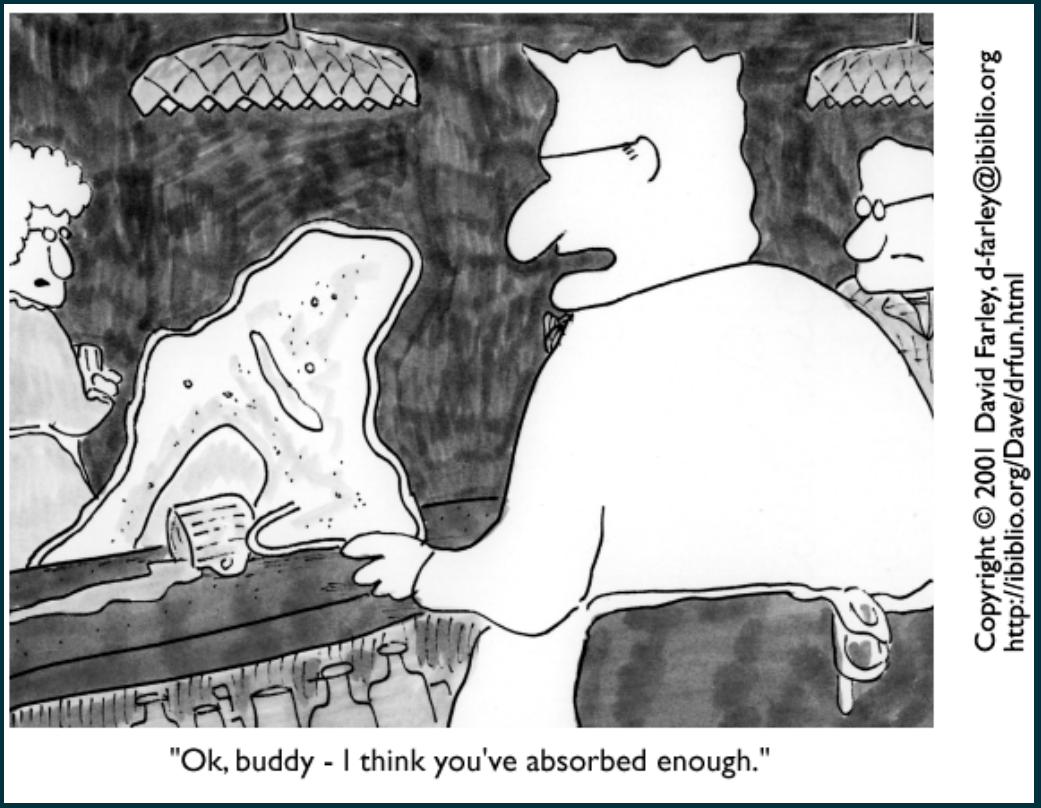
CSIRO/TASSAL AGD CRA: Amoeba biology

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

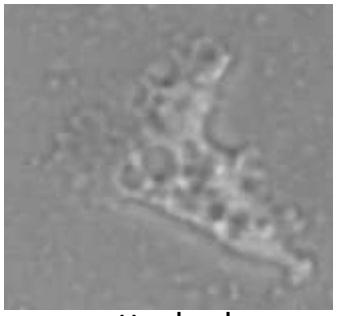


"Ok, buddy - I think you've absorbed enough."

Osmoregulatory mechanism/s in *Paramoeba perurans?*

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

Paramoeba perurans:



attached

floating

constant exposure to salinity variations

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

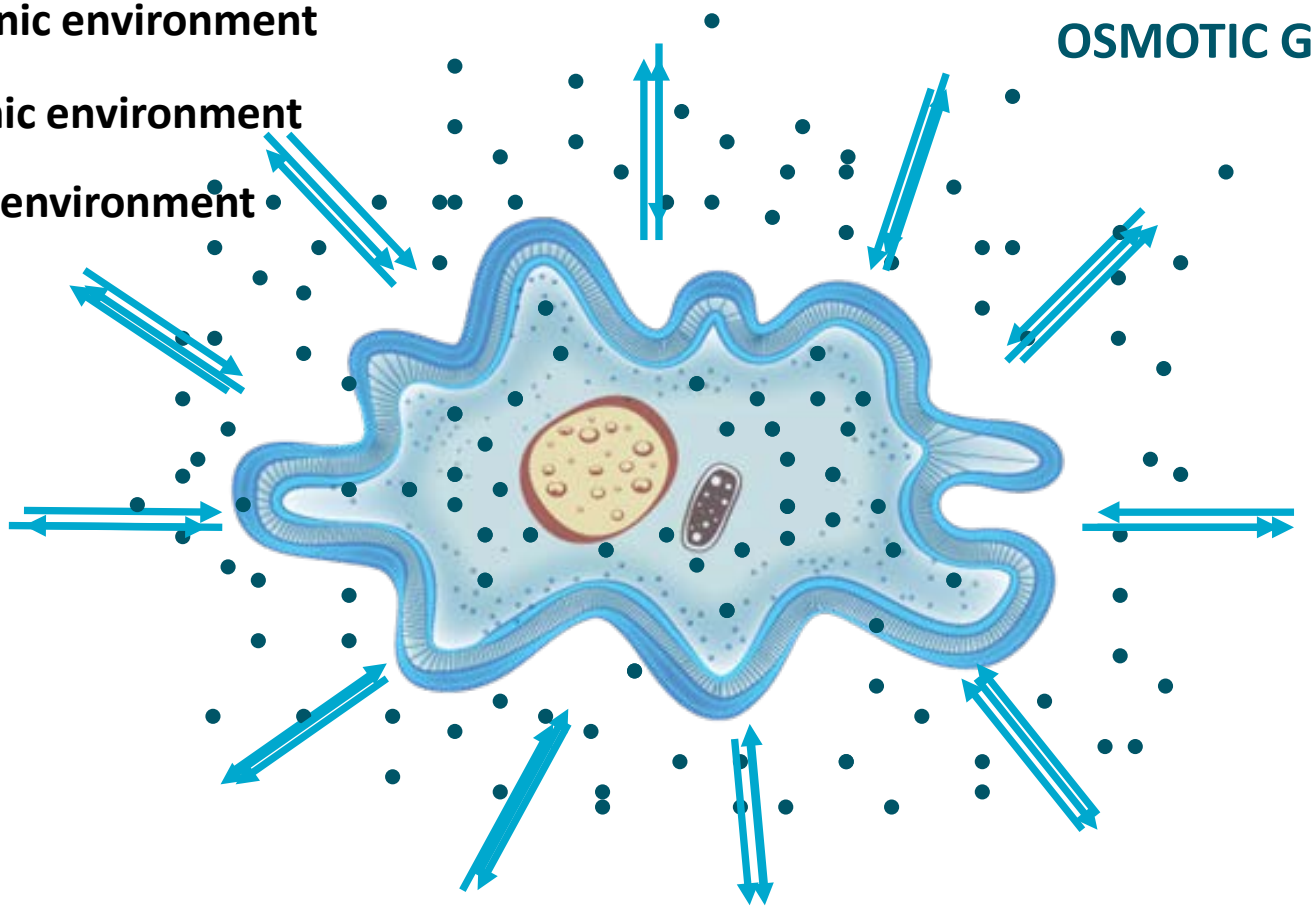
Membrane permeability in unicellular Protists

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

Hypertonic environment

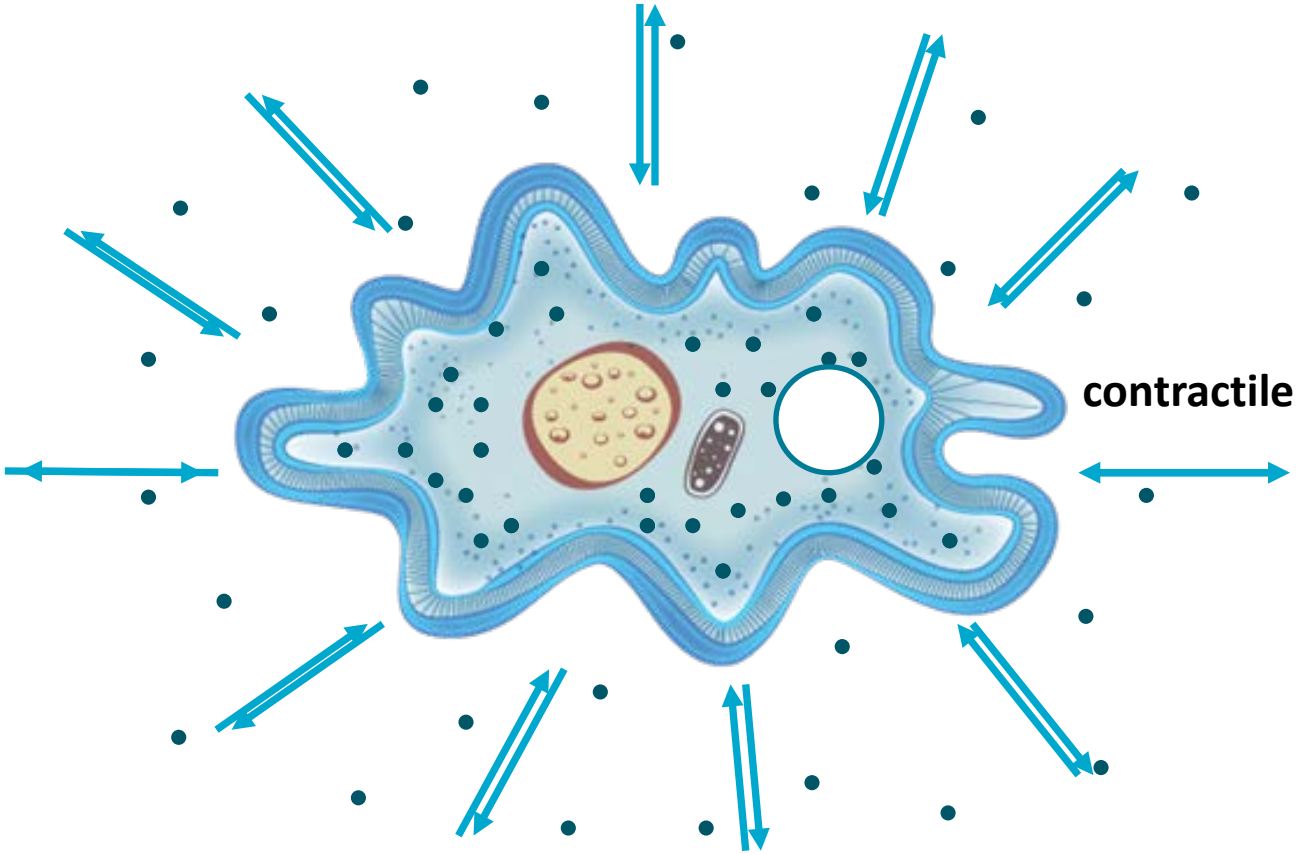
Hypotonic environment

Isotonic environment



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

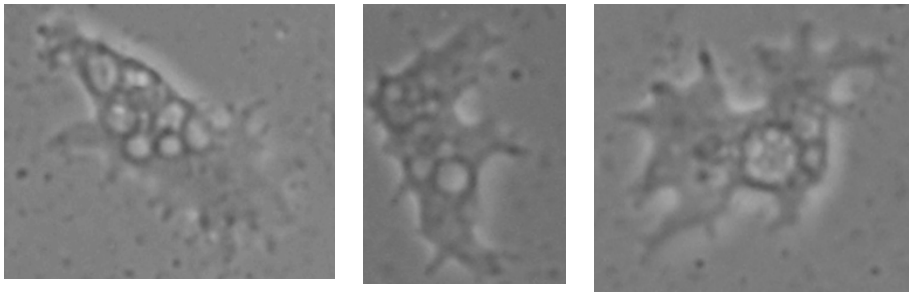
Contractile vacuole in *Paramoeba perurans*

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*)

no further research

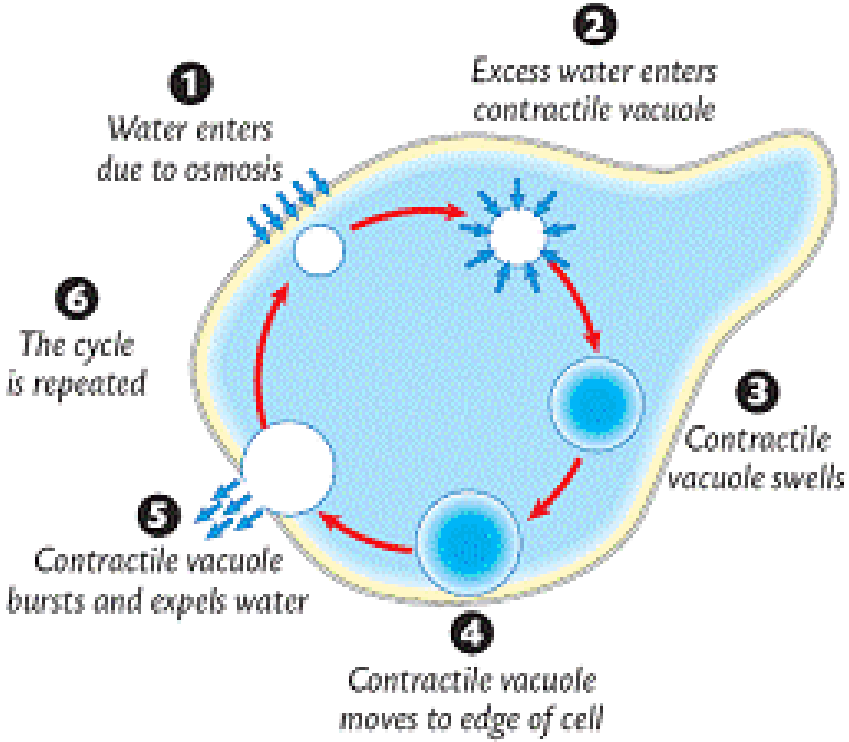
***In vitro* culture in our lab** → vacuolar formation often observed, particularly following water changes



Hypothesis → CVs are involved in the osmoregulation process of *P. perurans*

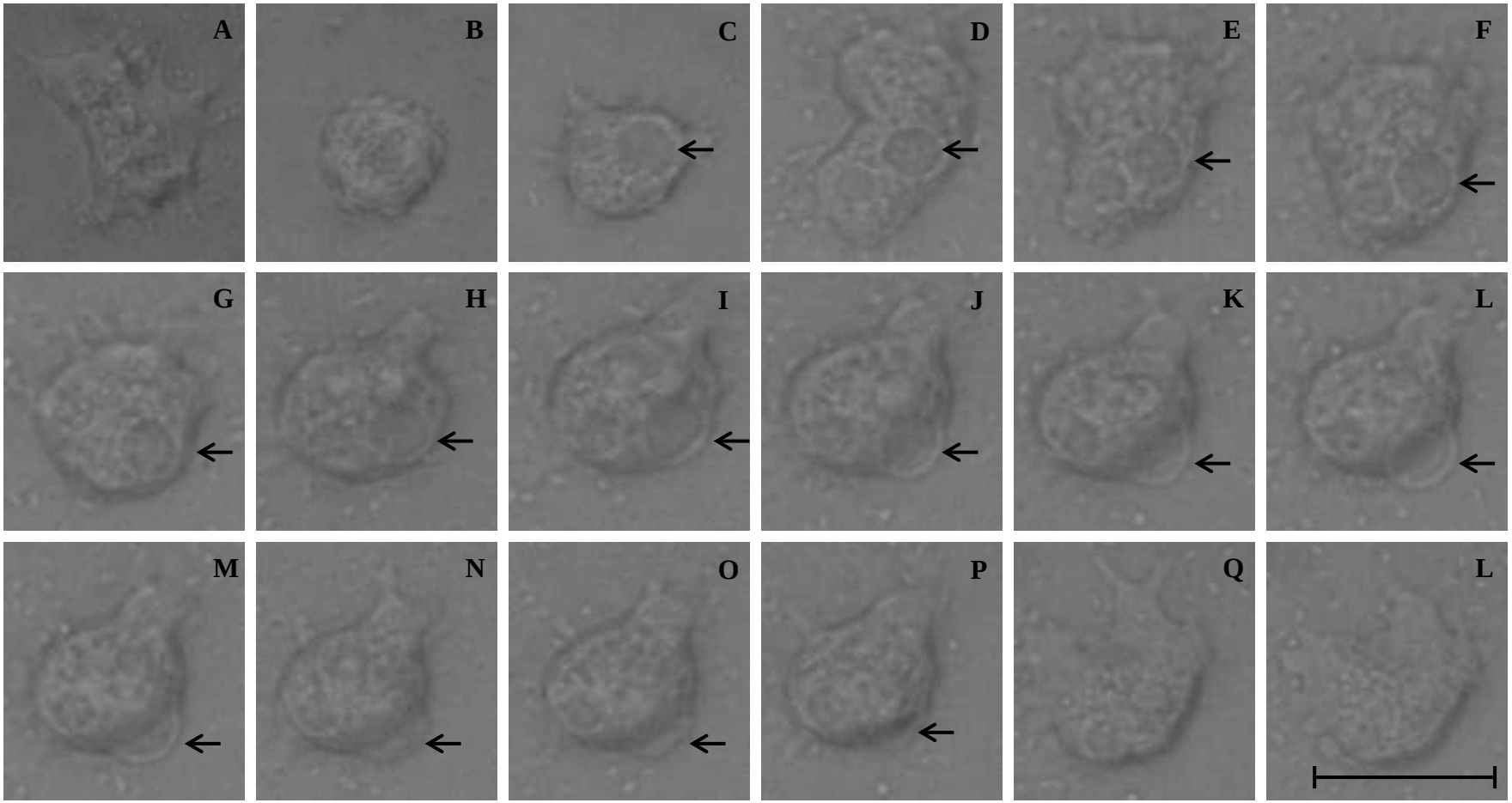
Contractile vacuole in *Paramoeba perurans*

20% salinity reduction → CV formation and activity similar to freshwater amoeba species



Contractile vacuole in *Paramoeba perurans*

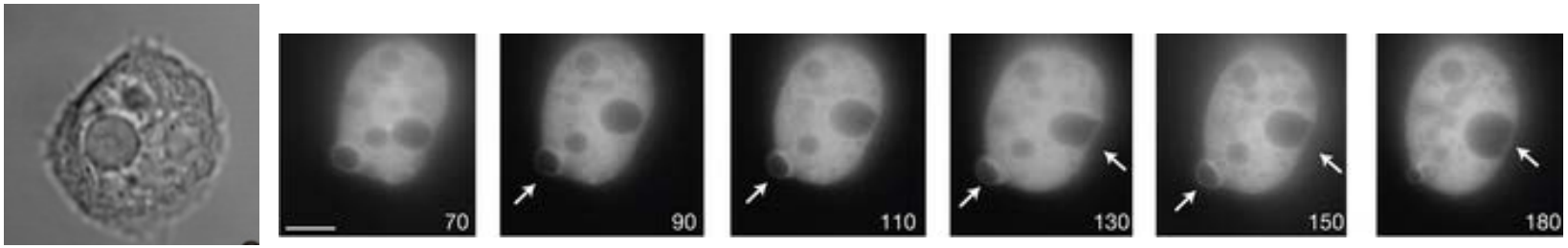
20% salinity reduction → CV formation and activity similar to freshwater amoeba species



Contractile vacuole in *Paramoeba perurans*

20% salinity reduction → CV formation and activity similar to freshwater amoeba species

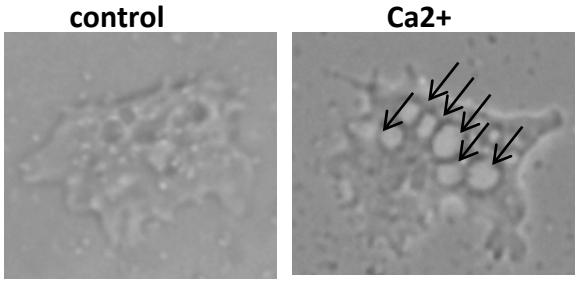
Dictyosteliurn discoideum → Ca2+ involved in osmoregulation by regulating the CVs contractions (Moniakis et al 1999, Malchow et al 2006)

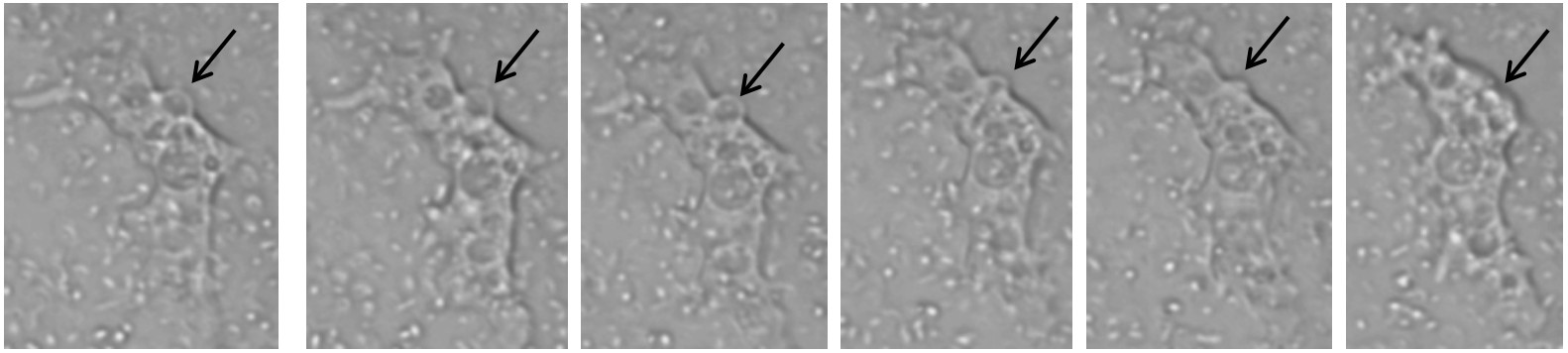


Involvement of Ca2+ in *Paramoeba perurans* osmoregulation?

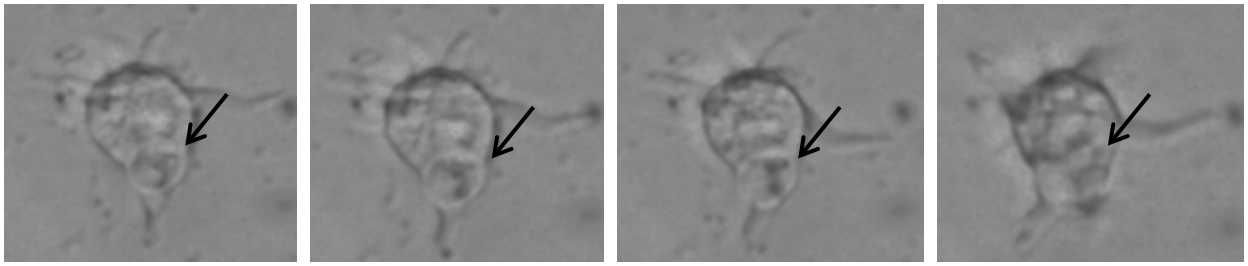
***P. perurans* incubated in CaCl2 (200ppm)**

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





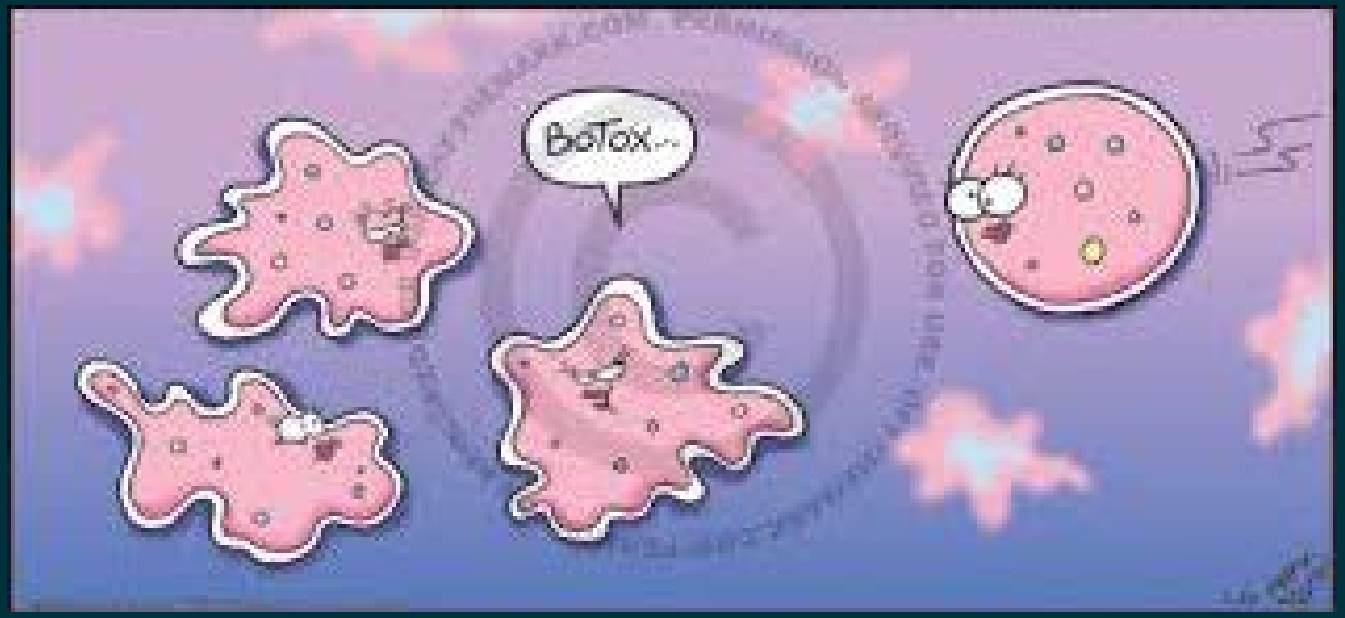
- Vacuolar activity also observed in floating cell (very unusual)



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

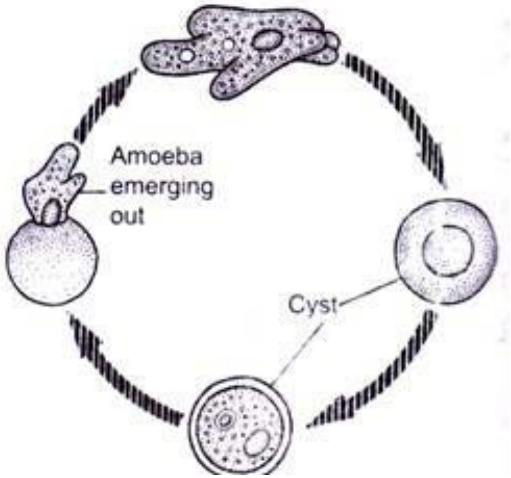
Pseudocysts



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

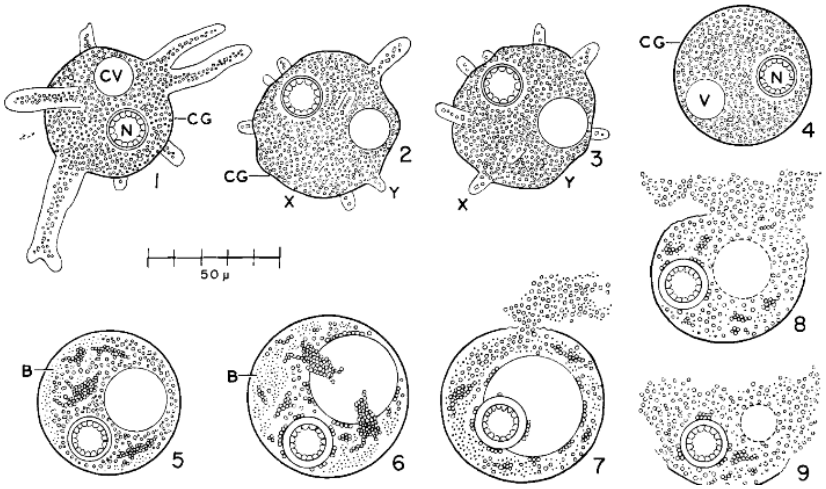
Pseudocysts:

- absence of cyst wall → reduction in survival capacity
- survives for shorter periods of time without any evidence of activity

Pseudocysts in different amoeba species:

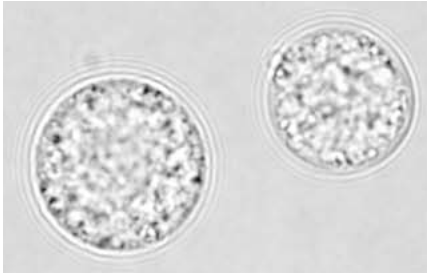
Hydramoeba hydroxena → starvation (Beers 1963)

- 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)

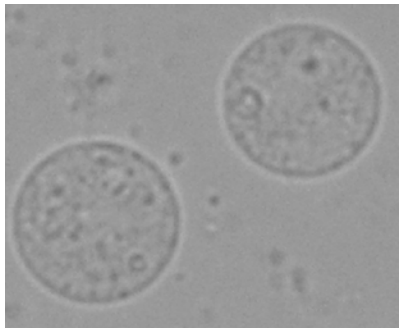
- single-layered amorphous mannose/glucose coat
- dissolved in acidic pH
- reverse to viable trophozoites after 24h exposure



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)

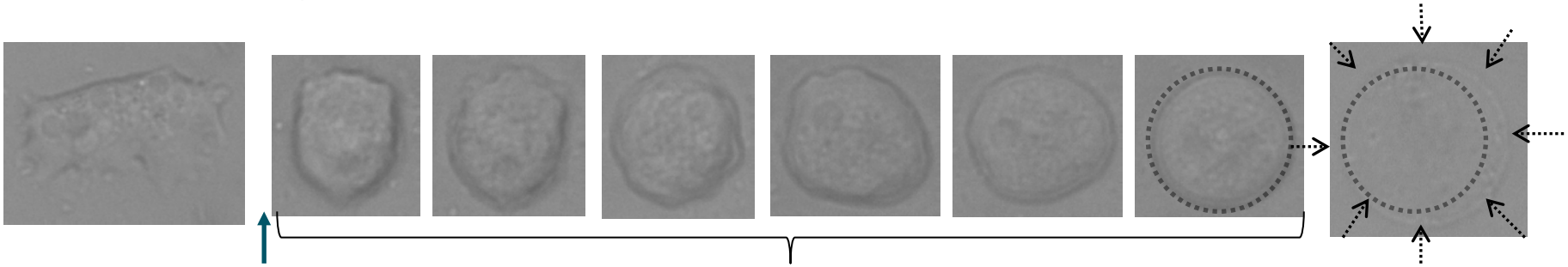


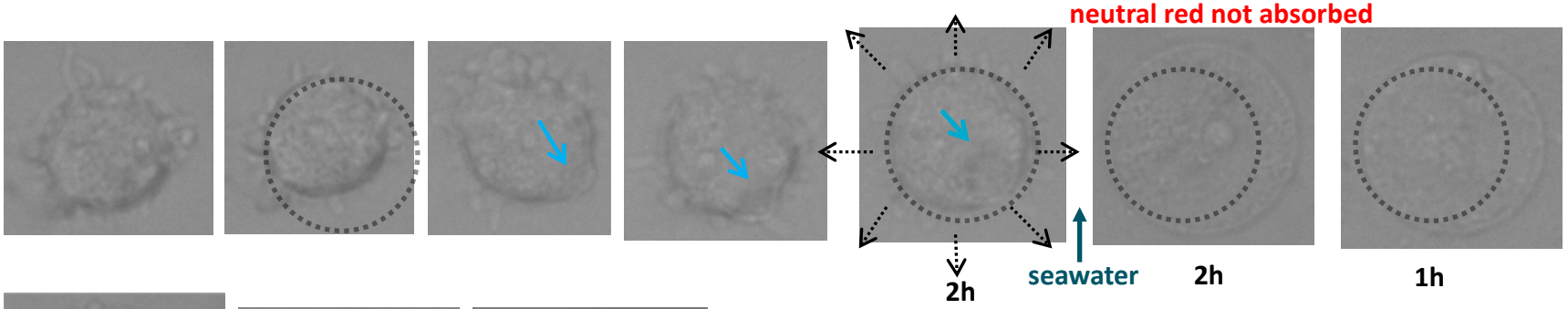
Are the spherical cells actually dead?

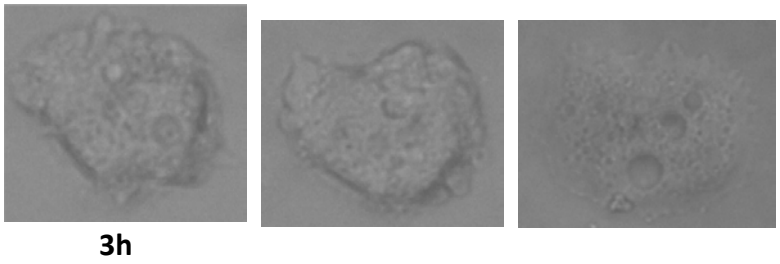
Could they revert to viable trophozoites if returned to optimum culture conditions?

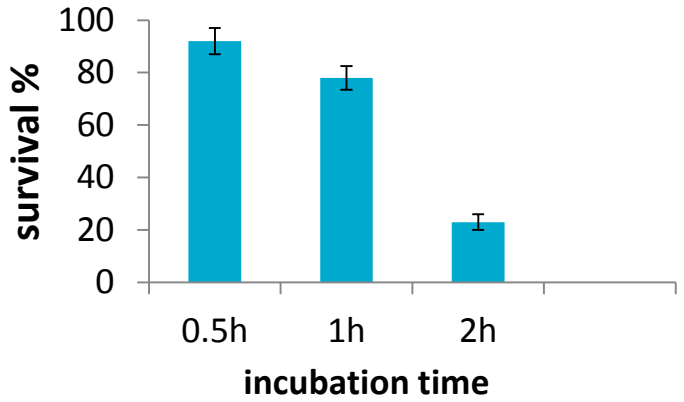
Hypothesis → non-motile cyst-like forms are an immediate cell response to acute threat of plasma membrane damage or cell death

Paramoeba perurans vs. freshwater









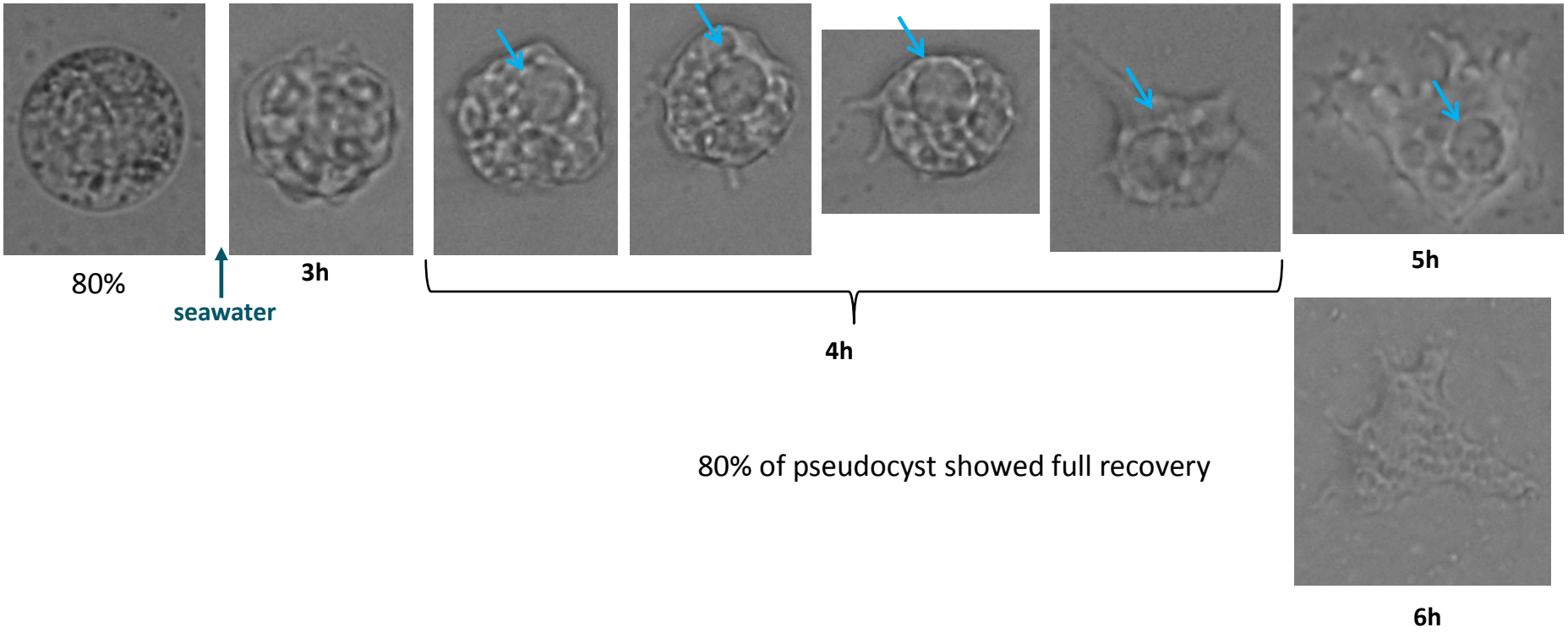
spherical cell submitted to 40% SW for 7 days → fully recovered once returned to standard culture conditions

Paramoeba perurans vs. DMSO

10% DMSO → 20 min

not resistant to KOH and detergent

neutral red not absorbed

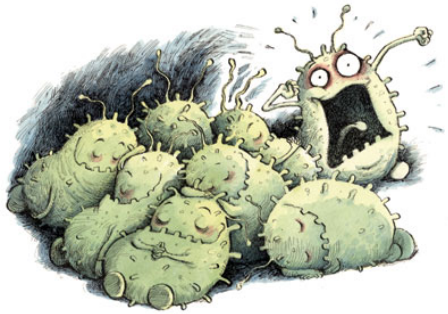


80% of pseudocyst showed full recovery

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?

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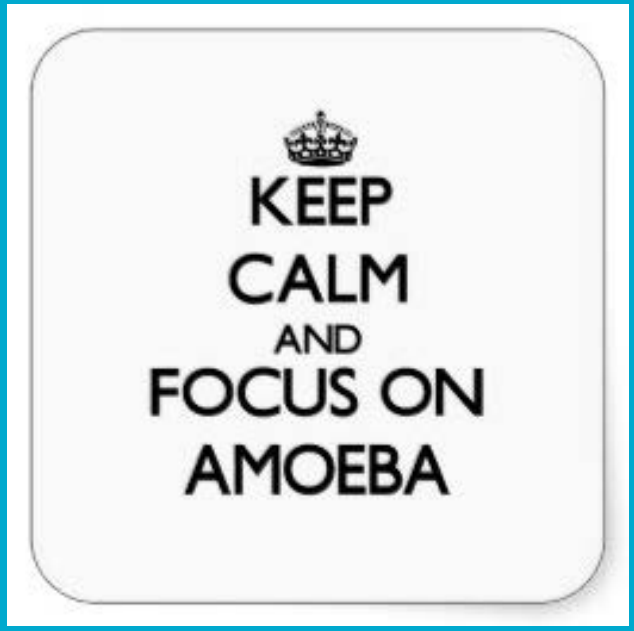
KEEP
CALM
AND
FOCUS ON
AMOEBA