# ADAPTATIONS TO THERMAL VARIATION IN TWO MEDITERRANEAN LIMPETS

## cardiac response and haemocyte lysosomal stability

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# Introduction

The limpets *Patella rustica* and *Patella caerulea* are found at different tidal heights in the Mediterranean. This study investigated whether their zonation on the shore is a result of their differential physiological adaptations to thermal stress. The lower zoned *P. caerulea* should have reduced upper thermal limits when compared with higher zoned *P. rustica*, which is predicted to be more tolerant. Arrhenius break temperatures (ABTs) of cardiac performance were used as a sensitive parameter to estimate species critical thermal limits, together with analysis of haemocyte lysosomal stability.

# Material and methods

**Heart rates** were measured using the infrared sensor fixed onto the limpet shell directly over the heart (**Fig 1**). The sensor consisted of an infrared-light-emitting diode from which the signal were filtered, amplified and recorded (**Fig 2**). Each animal was placed into a beaker in a water bath where the temperature was continuously increased 3°C per 15 min. Real time heart rates (**Fig 3**) of five individuals of each species were recorded every 5 min until no regular beats could be detected. Their ABTs (the temperature at which heart rate decreases dramatically) were determined using regression analyses.

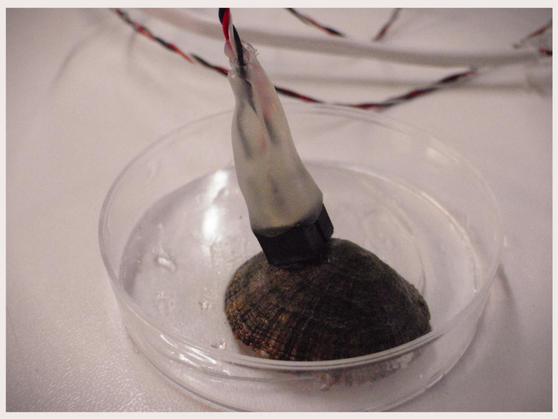

**Fig 1.** Infrared sensor fixed onto the limpet shell

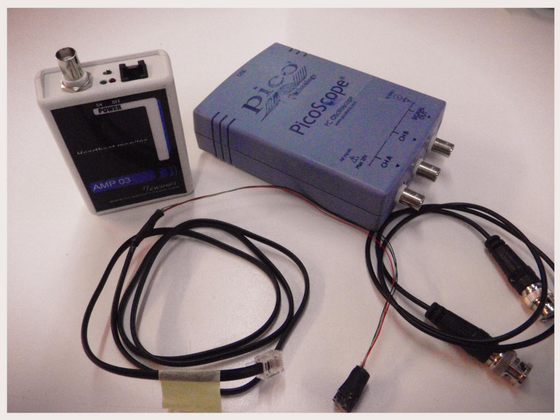


**Fig 2.** Equipment for measuring heart beats

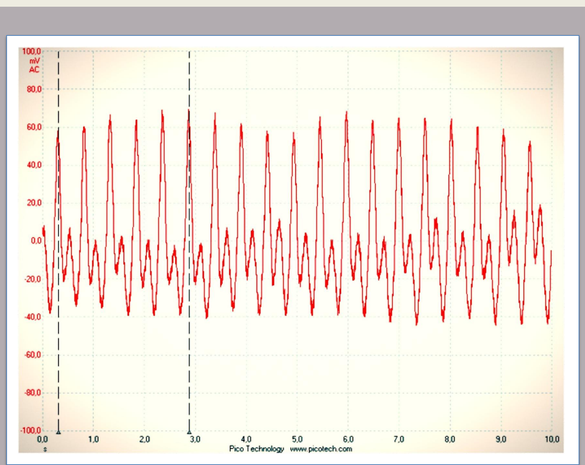


**Fig 3.** Recorded signal in 10 sec of non stressed limpet (between two dashed lines are 5 beats)

**Haemocytes lysosomal stability** was assessed using the neutral red uptake (NRU). Only live and undamaged cells are able to absorb NR. Limpets were sampled at 20 (control), 32, 38 and again at 20°C to test possible recovery. Haemolymph samples were taken by direct puncture of the foot muscle (**Fig 4**) and the NRU procedure was done according to the Test Method Protocol NHK, 2003 (**Fig 5**). The release of NR pinocytosed within the cells was measured using microplate reader (**Fig 6**) and expressed as optical density values (OD).

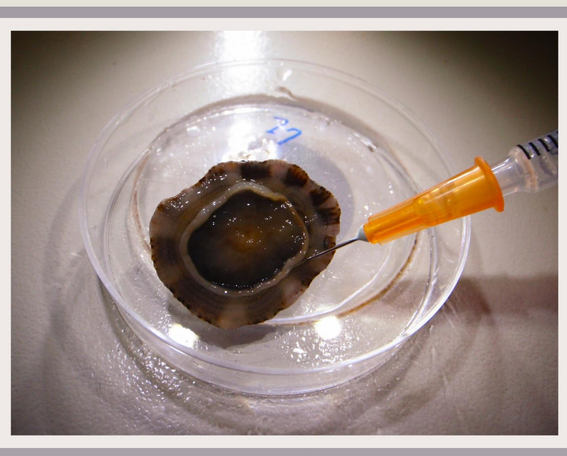

**Fig 4.** Extracting haemolymph from limpet

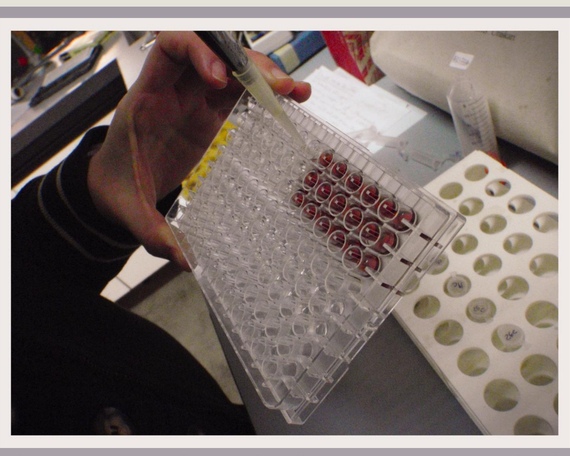

**Fig 5.** Microtiter plate with cell suspension in neutral red

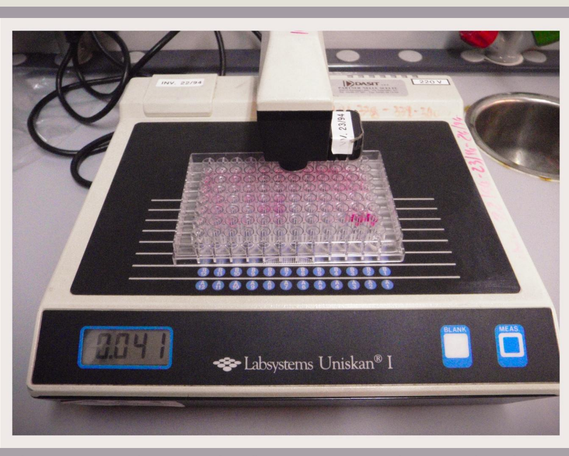


**Fig 6.** Microplate reader for pinocytosed neutral red

# Results and Conclusion

**We can conclude that under present conditions the different strategies of *P. rustica* and *P. caerulea* are successful, but with rising temperatures *P. caerulea* appears to be more sensitive as it is already living very close to its physiological limits.**

The cardiac performance (**Fig 7**) clearly showed that *P. rustica* (ABT= 37.86 ± 2.07 °C) is able to tolerate higher temperatures than *P. caerulea* (ABT= 35.92 ± 2.60 °C). Relatively high ABT of *P. rustica* indicates that individuals could maintain normal oxygen supply and cardiac function at the temperatures higher than 35°C, explaining the vertical zonation these two species have on the shore.

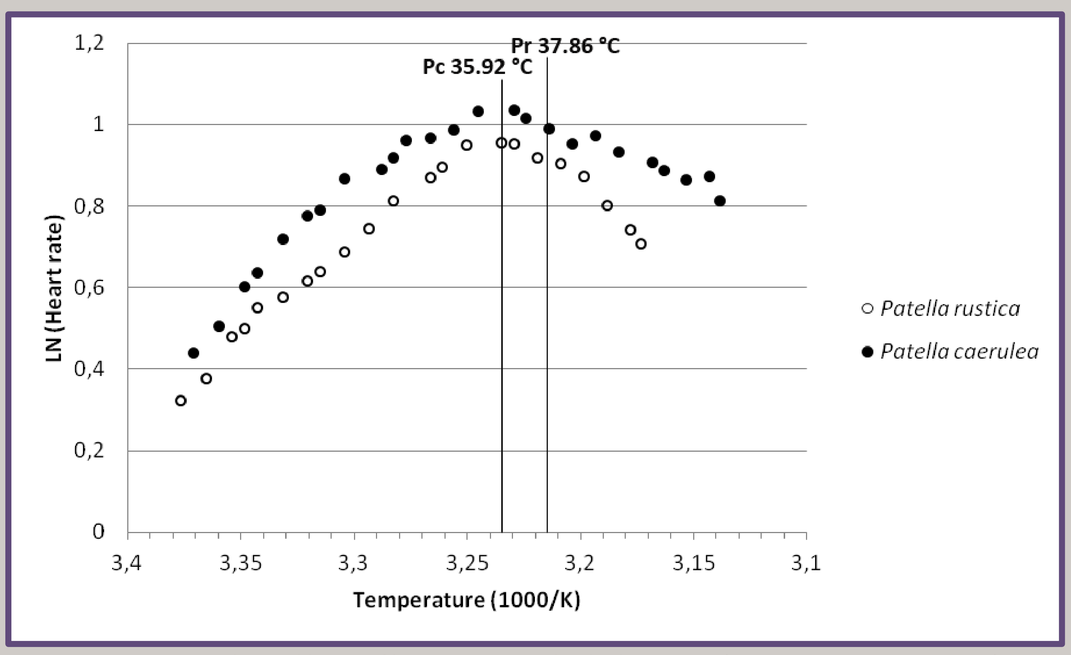


**Fig 7.** Representative thermal limits of heart rates in individual *P. rustica* (37.86 ± 2.07 °C, mean ± SD) and *P. caerulea* (35.92 ± 2.60 °C, mean ± SD)

Temperature significantly influenced dye uptake by haemoctes in *P caerulea* showing the highest OD values at 38 C (0.072), while both species had reduced values when returned again to spray seawater, indicating that recovery is not possible or takes more time (**Fig 8**).

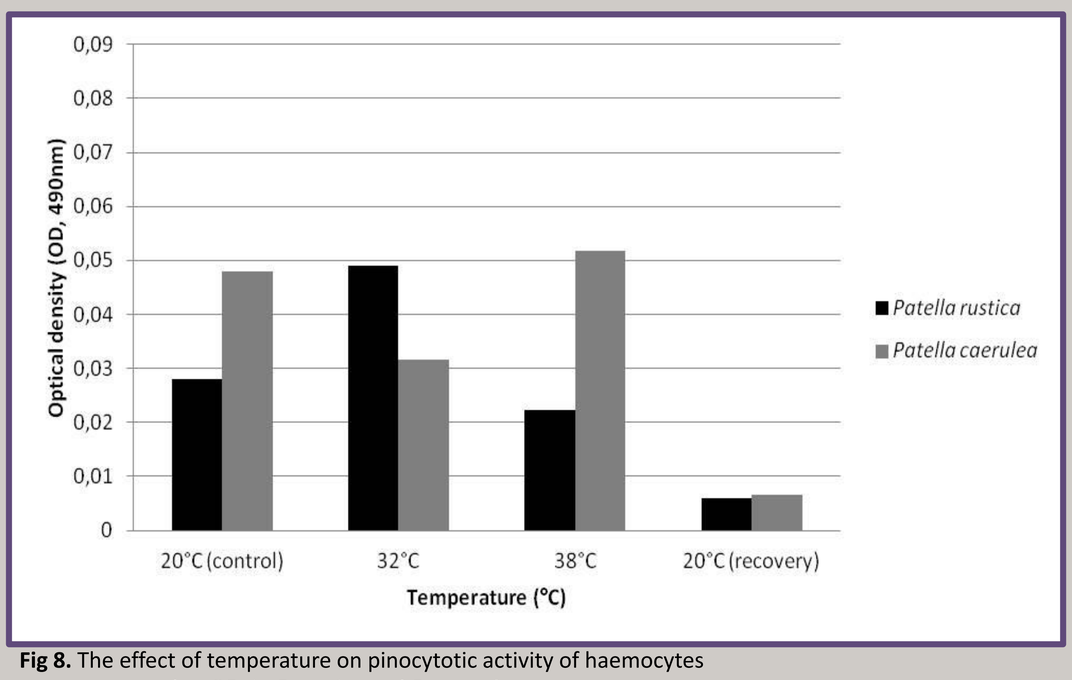


**Fig 8.** The effect of temperature on pinocytotic activity of haemocytes expressed as OD, in *P. rustica* and *P. caerulea*