Energetics of the lecithotrophic larvae of *Laternula elliptica* under pH and temperature stress

Christine Bylenga*[1], Vonda Cummings[2], and Ken Ryan[1]

1: School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand
2: National Institute of Water and Atmospheric Research, Private Bag 14901, Wellington, New Zealand
*: *Christine.Bylenga@vuw.ac.nz*

Introduction

Laternula elliptica is an infaunal bivalve found at a range of depths throughout the Southern Ocean. Experiments have shown elevated temperatures (+2°C) largely accelerate development in the larvae, potentially reducing time spent at vulnerable stages[(1)]. Reduced pH (pH 7.80 and 7.65) significantly impairs larval shell development and integrity (Figure 1)[(2)]. Responses in adult *L. elliptica* indicate there is an energetic and metabolic cost at low pH and elevated temperature [(3,4,5)], however, such effects on the larvae are unstudied.

Larval Energetics

Larvae under reduced pH conditions may experience higher energetic costs as they maintain calcification and cellular gradients [(6,7)]. Additionally, altered patterns of development may place additional strain on these resources.

L. elliptica, with non-feeding, lecithotrophic larvae, are fully reliant on maternally provided energetic reserves until metamorphosis. This study investigated the use of lipid and protein energy reserves and metabolic responses, during development and under stress.

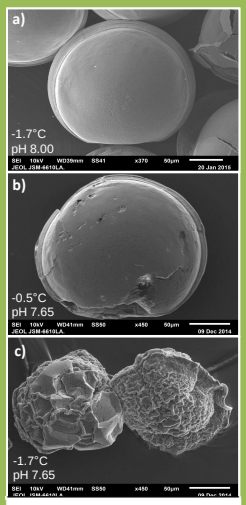

Figure 1. SEM imagery of *L. elliptica* D-larvae under (a) ambient and (b, c) reduced pH conditions[(2)]

Methods

- *L. elliptica* were fertilised and raised to D-larval (shell forming) stage at exp. temperature and pH (Table 1)
- Quantified:
  - Lipid classes and concentrations (thin layer chromatography/flame ionisation detection system)
  - Protein content (Bradford assay)
  - Depletion of protein and lipid between 24h post-fertilisation and D-larvae stage
  - Respiration rates in D-larvae

Table 1. Seawater conditions for each experimental treatment, average temperature and pH (total hydrogen scale; n = 22,765), Ω_{Ar} calculated from A_T (2290.1 ± 2.4 µmol kg^{-1}, n = 3) and pH; salinity 34.7 ppt.

Temp (°C)	pH	Ω_{Ar}
-1.7 ± 0.01	8.00 ± 0.001	1.08 ± 0.04
	7.64 ± 0.001	0.53 ± 0.00
-0.5 ± 0.01	7.99 ± 0.001	1.18 ± 0.02
	7.65 ± 0.001	0.59 ± 0.01
0.5 ± 0.01	7.99 ± 0.001	1.20 ± 0.05
	7.66 ± 0.001	0.62 ± 0.01
1.5 ± 0.01	7.99 ± 0.001	1.24 ± 0.04
	7.65 ± 0.001	0.59 ± 0.02

Results

Lipids

- ~200 ng total lipid in newly fertilised larvae, eight classes
- Dominated by triacylglycerol (TAG) and phospholipids (PL): 64 and 30% of total lipids, respectively
- Significant reserves remained at D-larvae stage (151 ng; >75% of initial pool).
- Significant depletion in both TAG (25%) and PL (36%)
- At ambient temperature, less lipid use was observed under reduced pH (Figure 2a, p = 0.016); no other pH or temperature effect

Protein

- Protein content was variable at 24 h post-fertilisation (8.8 - 38.5 ng $larvae^{-1}$), generally increased in D-larvae (27.1 - 67.1 ng $larvae^{-1}$; Figure 2b)
- Significant increases in protein content at -0.5°C ($p < 0.001$; small initial reserve)

Respiration

- Increase in oxygen consumption with initial temperature elevation (to -0.5°C; Figure 3)
- No additional increase in with further temperature elevation
- No pH effect

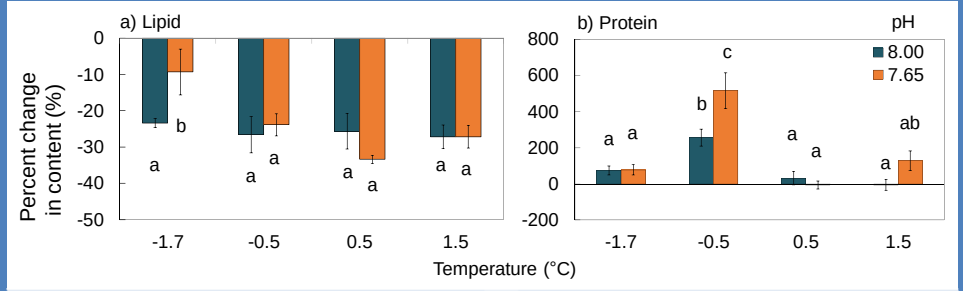

Figure 2. Percent change in (a) lipid and (b) protein per larva in development from fertilisation to the D-larval stage; error bars are standard error, different letters indicate significant differences at $p < 0.05$

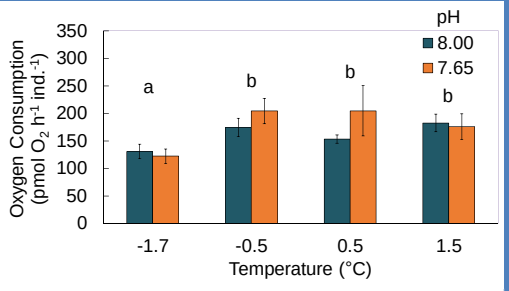

Figure 3. Respiration in D-larvae; error bars are standard error, different letters indicate significant differences at $p < 0.05$

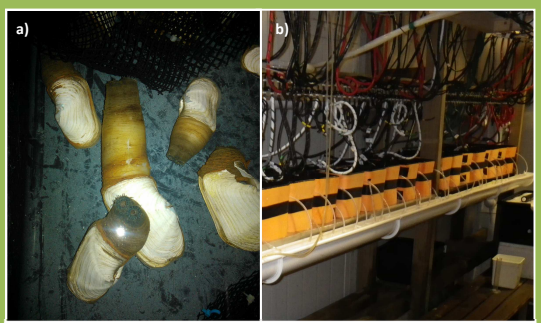

Figure 4. a) Adult *L. elliptica* and b) holding tanks for developing larvae

Conclusions

- *L. elliptica* larvae are well provisioned, with lipids the primary energetic source (specifically TAG and PL)
- Significant lipid reserves remain at the D-larvae stage, even under stress
- Protein is not a major energetic reserve for larval development in this species
- Lack of change in oxygen consumption rates with elevations in temperature above -0.5°C indicates larvae may be approaching temperature thresholds and thermal limitation
- No correlation between oxygen consumption and lipid use
- Rather than increasing the use of finite energy stores, larvae may be prioritising their energy use:
  - Diverting from less immediate functions, e.g. shell development, somatic growth, with potentially negative implications for larval condition and survival at settlement
  - May account for previous observations of lower shell integrity at reduced pH[(2)]

Future directions

- Are responses similar in other species?
- Planktotrophy vs. lecithotrophy – does feeding strategy influence response?
- Longer term exposure – will these metabolic responses deplete resources?

Acknowledgements
Thanks to Prof. Mary Sewell and Josefina Peters-Didier (University of Auckland) for use of equipment and training for lipid analysis; Yasmin Gabay (VUW) for assistance with the protein analysis; to Neill Barr, Graeme Moss (NIWA) and Sonja Hempel (VUW) for all their help in and around the lab. This research was funded by VUW Grant 100241, the Victoria Doctoral Scholarship Fund, The ARC Endowed Development Fund, the Royal Society of NZ Marsden Fund to VC (NIW1101).

References
1 Bylenga *et al.* (2015). *Mar. Ecol. Prog. Ser.* **536**: 187-201
2 Bylenga, et al. (in prep)
3 Cummings *et al.*, 2011. *PLoS ONE* **6**(1): e16069.
4 Peck *et al.*, 2004. *Funct Ecol* **18**(5):625-630
5 Hempel 2015. Master's Thesis, VUW
6 Stumpp *et al.* 2011 *Comp. Biochem. Phys. A.* **160**:331-340
7 Matson *et al.* 2012 *Biol. Bull.* **223**:312-327