Thermal stress alters the allergen and proteome profile of the redclaw crayfish (*Cherax quadicarinatus*)

Emily Jerry [1-5], Shaymaviswanathan Karnaneedi [1-5], Sahel Heidari [1-5], Kelly Condon [3, 4], **Andreas L. Lopata [1-6]**

[1] Molecular Allergy Research Laboratory, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia, [2] Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia, [3] ARC Research Hub for Supercharging Tropical Aquaculture through Genetic Solutions, James Cook University, Townsville, Australia, [4] Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering, James Cook University, Townsville, Australia, [5] Centre for Food and Allergy Research, Murdoch Children's Research Institute, Melbourne, Australia, [6] Tropical Futures Institute, James Cook University Singapore, Singapore

Introduction

- **Rising temperatures** and **demand** for sustainable **protein** are ↑ **farming** and **translocation** of species like the Australian Redclaw Crayfish.
- **Temperature** strongly **affects** the **physiology**, **metabolism**, and **stress responses** of ectothermic organisms such as **shellfish**.
- Despite this, the **impact** of **temperature** on **food protein allergenicity**, particularly in shellfish, remains **poorly understood**.

Aim:

To investigate the effect of **rearing temperature** on the **allergenicity** and **protein abundance** of the Australian **Redclaw** Crayfish

Methods

- Redclaw groups were reared at either **17°C**, **28 °C** or **32°C** for four weeks.
- Extracts were generated and used to identify rearing temperature-based:
- **1.** Differences in shellfish-allergic patient IgE-binding using:
  - SDS-PAGE & Western blot
- **2**. Gene Ontology (GO) group differential expression, **AND**
- **3**. Protein differential abundance using:
  - In-solution digestion
  - Quantitative mass spectrometry
  - Differential GO expression/protein abundance bioinformatic analysis

Results

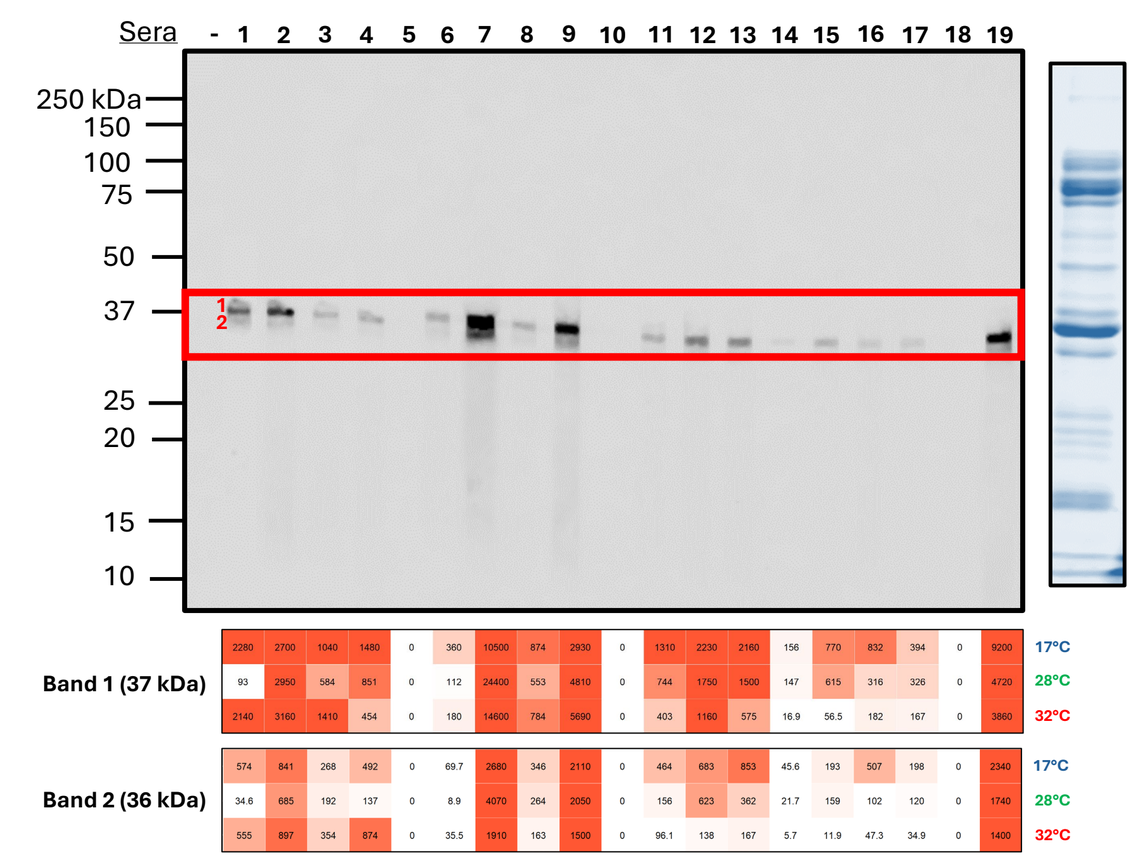


Figure 1. IgE-binding and binding intensity to redclaw extract across temperatures. Bands at 36 - 37 kDa elicited IgE-binding at all temperatures, with stronger binding at lower temperatures.

Table 1. Significantly (adj.p > 0.05) upregulated GO groups (adj.p > 0.05) and associated allergens. HSP70, AK, PM, and others were upregulated within their respective GO groups.

17°C vs 28°C Comparison	
17°C	**28°C**
Protein Folding, Maturation & Binding • **Heat Shock Protein 70 (HSP70)**	Extracellular Region • **Arginine Kinase (AK)**
Nucleoside Metabolism/Catabolism • **Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Fructose-Bisphosphate Aldolase (FBA), Enolase**	Ribosomal Components
	Cytoskeletal and Structural Molecules

28°C vs 32°C Comparison *This comparison was not deemed statistically significant (adj.p-value > 0.05)	
28°C	**32°C**
Protein Folding Chaperone • **HSP70**	Supramolecular Fiber • **Paramyosin**
System Development	Transmembrane Transport
Oxidoreductase Activity	Proteasome Complex

17°C vs 32°C Comparison	
17°C	**32°C**
Protein Folding & Maturation • **HSP70**	Ribonucleoprotein Complex
Nucleoside Metabolism/Catabolism • **GAPDH, FBA, Enolase**	Membraneless Organelle
	Extracellular Region

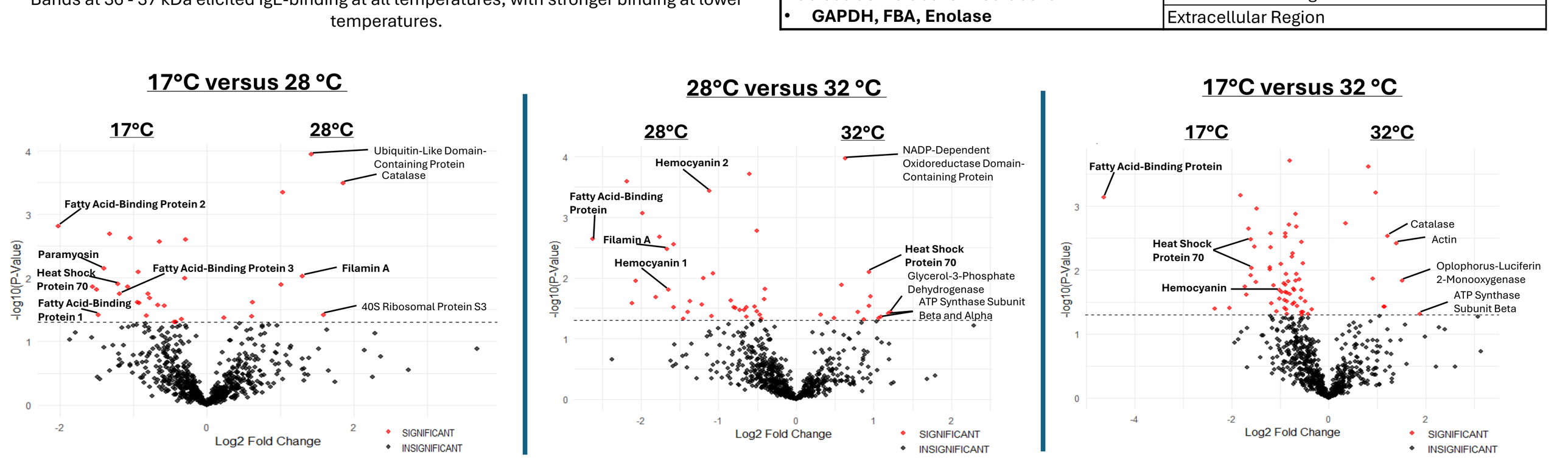


Figure 2. The top four individual proteins considered as most significantly (p-value > 0.05) upregulated in each temperature comparison, allergens are bolded. Allergens including HSP70, fatty acid-binding protein (FABP), PM and hemocyanin (HC) were especially upregulated at colder temperatures, apart from HSP70 at 28°C versus 32°C.

Summary & Conclusion

- Rearing temperature influenced **IgE-reactivity** and **allergen abundance**.
- Shellfish-allergic patient **IgE-binding ↑ at 17°C** and **↓ at 32°C**.
- **Protein folding** (HSP70) and **metabolic** (GAPDH, enolase and FBA) GO groups ↑ at lower temperatures.
- **FABP** and **HSP70** notably ↑ at lower temperatures. Major shellfish allergen **tropomyosin** was **NOT affected.**
- This study provides the **first evidence** of **rearing temperature influence** on **shellfish allergenicity**.

Acknowledgements

Prof. Andreas Lopata:
andreas.lopata@jcu.edu.au

Emily Jerry:
emily.jerry@my.jcu.edu.au