The impacts of dietary metal/loid intake and increasing water temperature on Nile tilapia *Oreochromis niloticus*

Cheuk Ming Lam*, Wing Yin Mo, Chun Au Yeung, Ka Wai Chan, Man Hay Choi, Yuk Ki Ling, Kit Ling Lam, Steven Jing liang Xu, Fred Wang Fat Lee
s1297341@live.hkmu.edu.hk*
Hong Kong Metropolitan University, School of Science and Technology

Introduction

- Nile tilapia: important aquaculture species (high economic and nutritional importance)
- Aquaculture system challenge: climate-related temperature changes and environmental metal/loid contamination
- Global warming impacts: increasing water temperature, major lakes warming at 0.31°C/decade (O'Reilly et al., 2015)
- Dietary exposure to metal/loid (As, Cd, Cr, Cu, Hg, Pb, and Zn): common, causes accumulation in food fish
- Knowledge gap: Limited understanding of combined effects of: Thermal stress + dietary metal/loid exposure
- Study focus: effects of combined stressors on 1. immune system performance, 2. proteome responses, 3. growth performance

Aim

- To evaluate about the combined impacts:

1. dietary metal/loid exposure
2. increasing water temperature on Nile tilapia health and performance

Methodology

- Species: Nile tilapia
- Important species to freshwater aquaculture
- Top three farmed finfish species globally (Debnath et al., 2023), high adaptivity, relatively high heat-tolerant



- Number of fish: 10 fish/tank, total: 360 fish
- Experimental duration: 6 months
- Sample dissection: every 2 months
- Dissection: 3 Nile tilapia in each tank
- Replication: triplicate tanks per treatment
- Total: 36 fish tanks
- Sample collection and parameters:

Sample/ Data collected	Parameters
Body Weight	Relative Weight Gain
Blood	NBT assay, RBC count
Plasma	Plasma bactericidal activity, proteomic response

- Diet and temperature treatment:

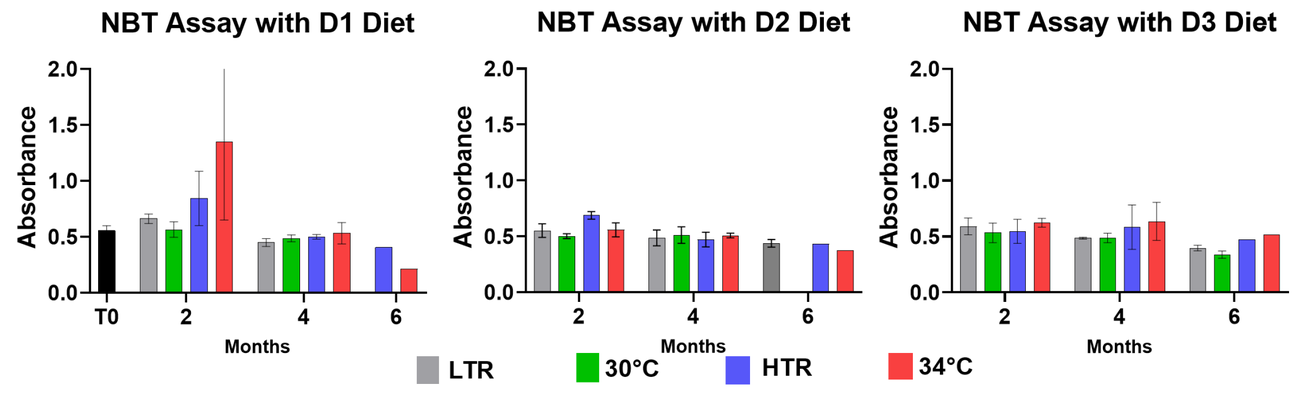
Diet treatment	Temperature treatment#
D1: Control Diet	LTR: 24 °C to 30 °C (increase 1 °C per month) Night: 4°C below daytime
D2: 0.1 × Max Limits* Diet	30°C (Constant for 6 months)
D3: Max Limit* Diet	HTR: 28 °C to 34 °C (Increase 1°C per month) Night: 4°C below daytime
	34°C (Constant for 6 months)

*Max Limit:
(the Hygienic Standard of Feed and the EU guidelines)

HM	Concentration (mg/kg)
As	10
Cd	1
Cr	5
Hg	0.5
Pb	5
Cu	25
Zn	150

Temperature treatment#
- LTR, HTR: simulated gradual seasonal temperature increases
- 30°C, 34°C: chronic warming conditions

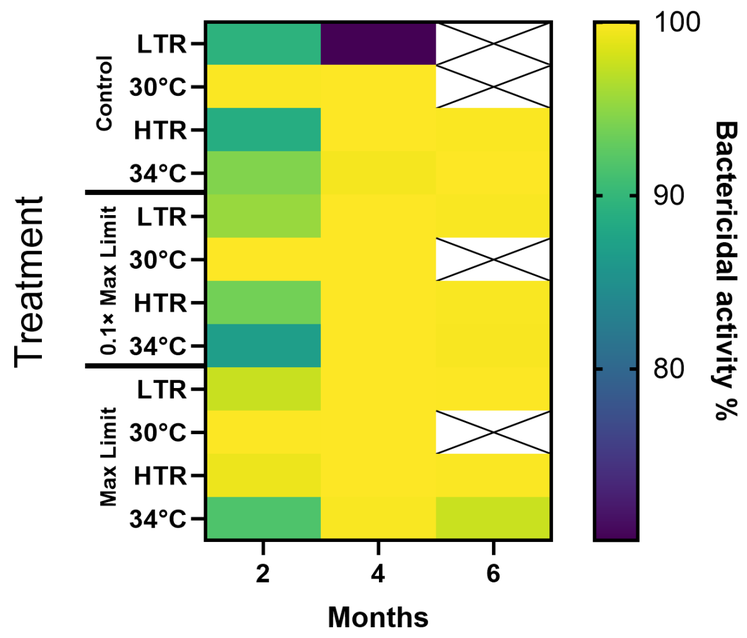
NBT Assay (Anderson & Siwicki, 1995)



- Reflects the respiratory burst activity of immune cells
- D1: higher temperature treatments appeared to stimulate NBT activity at 2 months, but this response was not maintained at later sampling points
- D2/D3: moderate responses, chronic exposure may not consistently enhance respiratory burst activity

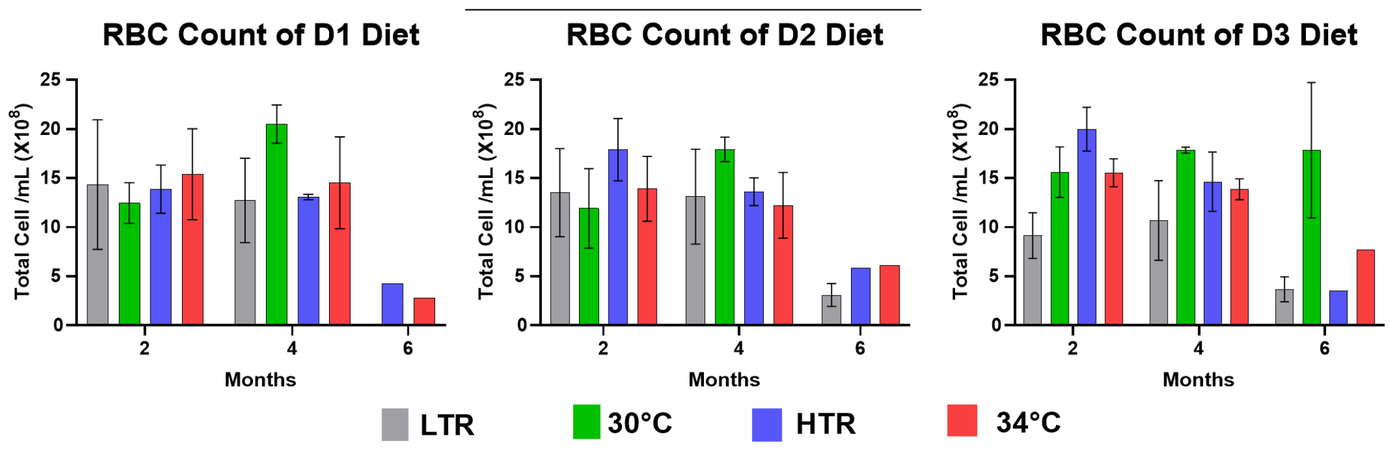
Plasma Bactericidal Activity Against *Aeromonas hydrophila*

(Iida et al., 1989)



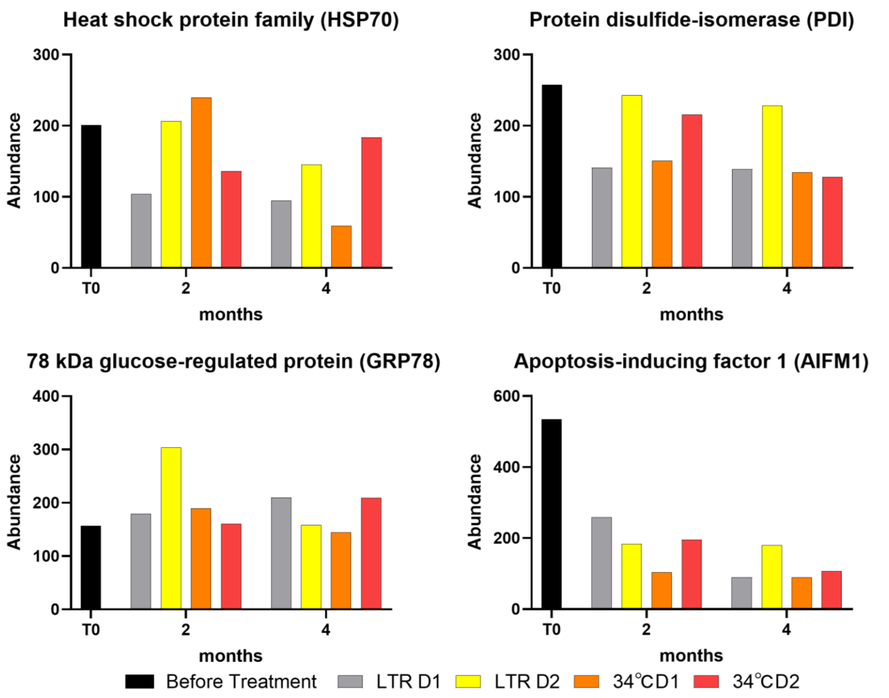
- Measures plasma killing ability against *A. hydrophila*
- High bactericidal activity across most treatment
- No clear treatment-dependent reduction observed
- Possible limitation: low sensitivity for detecting subtle immune changes

Red Blood Cell Count



- Indicator of hematological and physiological stress
- Variation of D3 Diet > Variation of D1/D2 Diet
- Higher metal/loid exposure may indicate blood/physiological stress

Proteomic Response



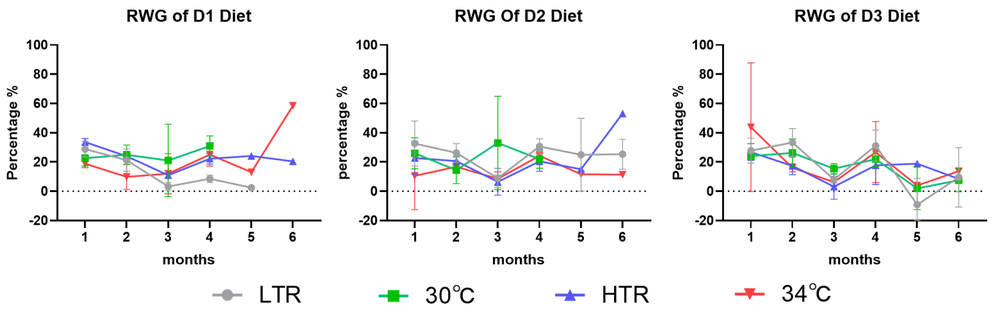
Four differentially expressed stress-related proteins detected in plasma:

Stress-related protein	Introduction of protein
Hsp70	Heat stress protein
PDI	Indicate protein-folding/ER stress
GRP78	Indicate protein-folding/ER stress
AIFM1	Possible cell damage response

- Identify cellular- stress related protein
- Selected treatments compared baseline, control, and extreme chronic exposure
- HSP70: high abundance at 2 months, especially in 34°C D1 and LTR D3
- PDI and GRP78: higher in LTR D3, suggesting ER-stress/protein-folding response
- AIFM1: decreased after exposure compared with before treatment
- HM-spiked feed under temperature stress altered stress-related protein abundance

Results

Growth Performance
Relative Weight Gain of Nile tilapia



- RWG fluctuated across treatments
- No consistent diet- or temperature-dependent pattern
- No clear growth suppression across treatments

Conclusion

- Immune response, RBC count, and proteomic profiles showed treatment-dependent changes.
- Even growth is not affected, higher metal/loid exposure, especially under temperature stress, may increase physiological stress to Nile Tilapia.
- Proteomic changes suggest activation of stress response, protein folding, and cell damage pathways.
- These findings highlight possible risks of climate warming and dietary contamination for Nile tilapia aquaculture and food safety.

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Reference