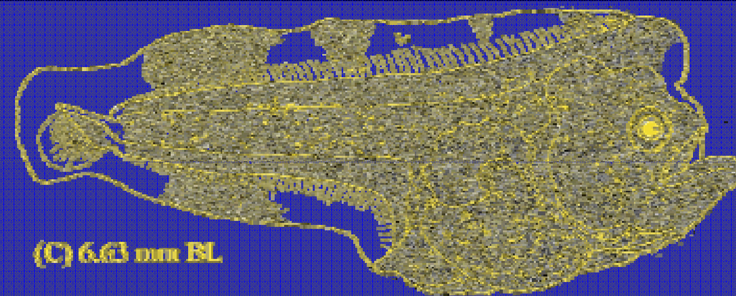
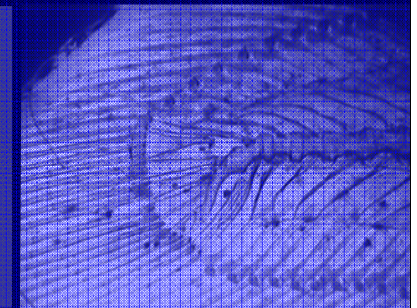
MODELLING THE DIGESTIBILITY OF *Artemia franciscanis* 'IN VITRO' DURING THE EARLY LARVAL STAGES OF MARINE FINFISH: A NOVEL APPROACH.





RHODES UNIVERSITY
Where leaders learn

Ernst Thompson & Tom Hecht

Introduction

- Establishing feeding strategies to match nutritional needs of larvae is NB
  - optimal growth / survival
  - normal development
- Co – feeding strategies; live vs. artificial
- The absorption factor is essential in evaluating fish diets

Understanding digestibility of *Artemia* is NB for successful larviculture, and could contribute towards designing improved replacements diets

Introduction

Digestibility can be determined ‘In vivo’ or ‘In vitro’

Why ‘In Vitro’?

- Error
- Wide range of biochemical techniques
- Relatively inexpensive / quick / simple

Various studies have shown that determining the digestibility of diets in vitro can complement, and in some instances precede or substitute, in vivo digestibility techniques

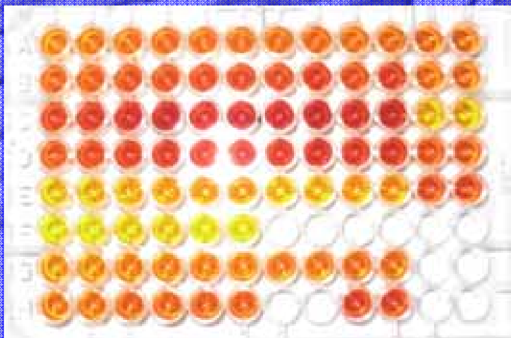
Aims

1. Design a 'In vitro' protocol – holistic view of digestion (predictive power of models)
2. Model digestion of *Artemia* by larvae of three warm temperate finfish species
3. Test the model's predictive power

Enzymes ⟶ Substrate ⟶ Product

Protocol

- Enzyme characterisation – pH & temp
- Specific enzyme activity (U/mg protein) – optimal conditions
- In vitro testing – activity and evacuation (incubation)



Methods & Materials

Source	Enzyme	Characterisation Vary pH and temp	Specific enzyme activity	In Vitro pH = 7.7 *Artemia* substrate Time: 15 – 270 min
Proteins	Alkaline proteases	Haemoglobin (l-tyrosine)	pH = 7.67	Ninhydrin (AA)
Lipids	Lipase	P – nitrophenyl meristate	pH = 8.03	Korn method (glycerol)
Carbs	Amylase	Somogyi – Nelson Starch - Reducing sugars	pH = 7.69	
n		3 x 9	8	64

Methods - Characterisation

- Altering pH and temp during substrate – enzyme incubation
- Activity at diff pH's was modelled using best supported model (AIC) – compared for species using log likelihood ratio test
- Establish optimal pH for specific enzyme activity determination

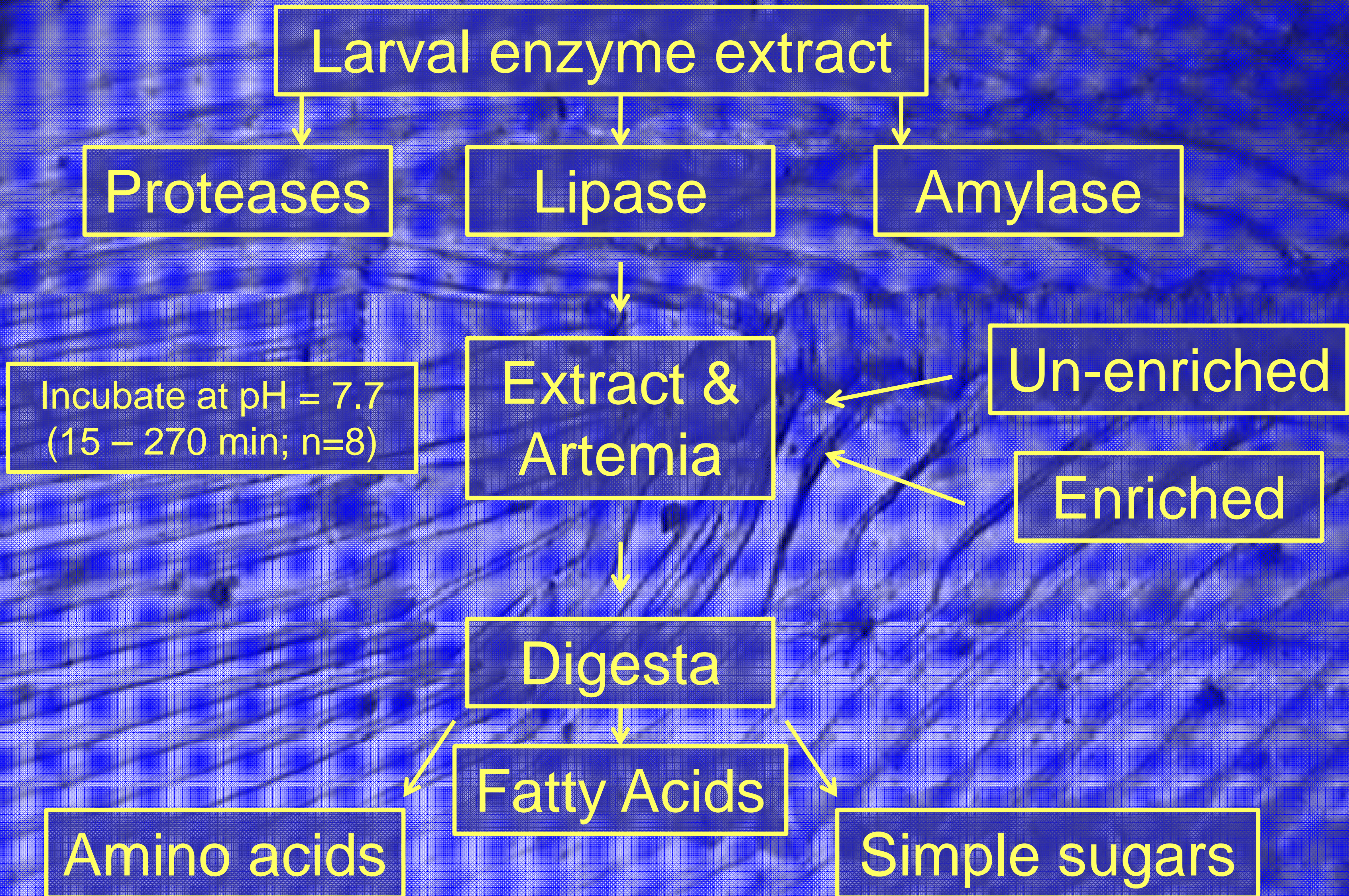
- Opt. temp for enzyme activity > 50 ºC – no biological sign (20 ºC)

Results - Characterisation

Model selection and the predicted optimal pH for enzyme activity

Enzyme	Model	Optimal pH
Alkaline proteases	Normal plot	7.67
Lipase	Skewed normal plot	8.03
Amylase	Gamma plot	7.69

Methods - In vitro



In vitro

- Analysed and modelled with GLM
- Interactions between enzymes
- Tested with *D. marginatus* (soleid)
- Contribution of exogenous enzyme based on degree of digestion (the blank) not calculation of specific enzyme activity

Results – Lipids In vitro

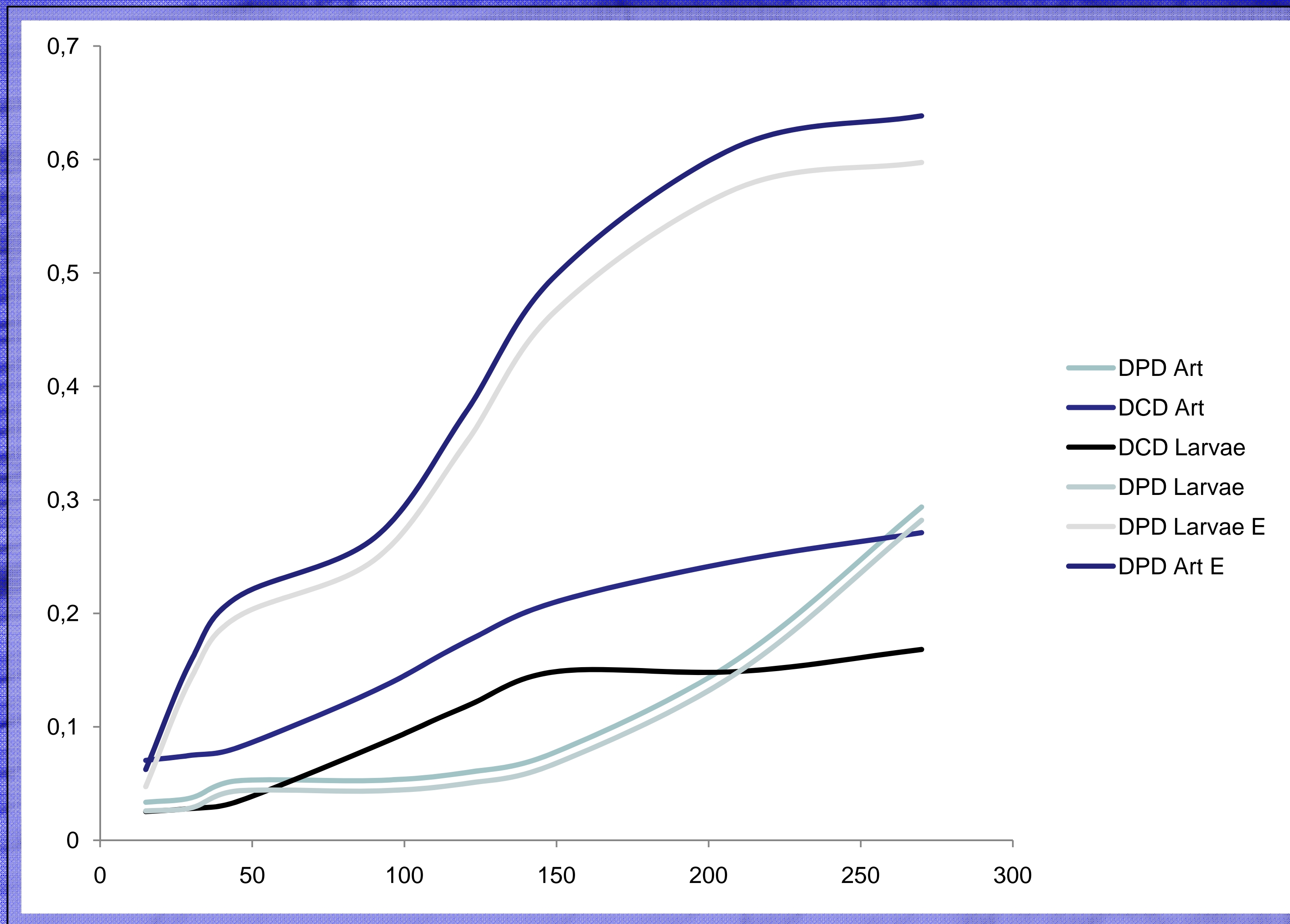
- Korn method - absence of glycerol
- Lack of triglycerides in *Artemia* (enrichment)
- Copepods store fats as wax esters – small amount of triglycerides for energy
- More suitable method required

GLM Models that predicts protein and carbohydrate digestion

DPD = 0.001 x time (min) + 0.592 x Alkaline protease activity – 1.323 x Amylase activity – 0.099 x *Artemia* preparation method + c (AIC = - 136.7, p<001)

DCD = 0.0008 × time (min) – 1.632 × Amylase activity – 0.123 × *Artemia preparation method + c (AIC – -316.1, p<0.001)*

Results – Degree of Digestion



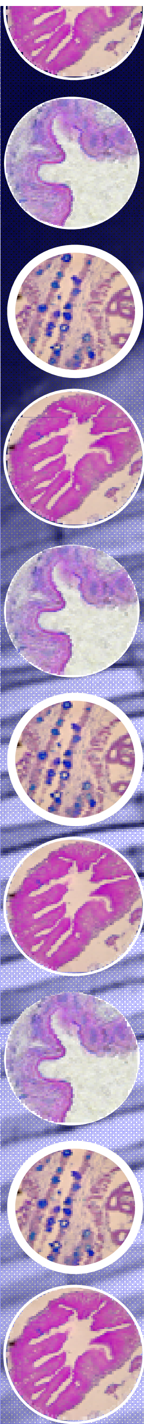
Protein digestion

- Exogenous protease activity (14.6 %) – drop from 22 – 4 % over time
- Enrichment double protein digestion (not due to exogenous protease)
- Little protein digestion in first 150 min – free amino acids
- Incidental due to high exogenous amylase activity
- High levels of protein digestion between 150 – 210 min
- Correspond to the drop in carb digestion

Results – Carb digestion

- Exogenous amylase activity – 40 % (max 62%) of total activity
- Enrichment very little effect
- Complete carb digestion in 150 – 210 min (± 90 % of total)

Conclusion



Confirms the usefulness of in vitro studies to complement or possibly even replace in vivo digestibility studies

Acknowledgements

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Any Questions ?