Evaluating the use of biofloc produced in a RAS as a feed ingredient using *Artemia* as a model

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Background

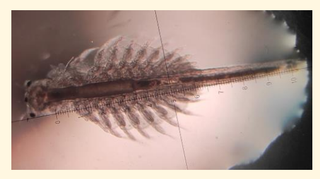
Aquaculture, one of the fastest-growing agricultural sectors, aims to meet the increasing demand for fish. The development of aquaculture highly depends on feed that is sustainable, nutritious and cost-effective solutions (with rising feed costs).

Biofloc is a microbial biomass produced through the assimilation of fish excretions, with the addition of an external carbon source. It contains about 40% protein and its potential as a fish feed ingredient was suggested (Nayak et al., 2023).

Fish feeding trials are lengthy, complex and require animal ethics permits. *Artemia* are non-selective filter-feeders with a short generation time. Their potential use in small-scale, short-term experiments, with high control over rearing conditions and no need of animal ethics approvals, makes *Artemia* a good candidate as a model organism.

Our Aim: To investigate the use of *Artemia* as a model in feeding trials for aquaculture.

Methodology



Day 0 **Hatching *Artemia***

Day 1 **Feeding start**

Daily analyses: *Artemia* length & survival

Day 7 **Sampling**

Feeding with biofloc in commercial fish feed at inclusion levels of: **15, 25, 50 % and 0% (as control)**

Stocking Density: 500 artemia / L

Sampling: whole *Artemia* for immune gene expression (qPCR)

Day 4 **Sampling & challenge**

2 separate trials were conducted:

- Trial 1: 0, 15, 25 % biofloc
- Trial 2: 0, 50, 100 % biofloc

Sampling: whole *Artemia* for immune gene expression (qPCR)

Bacterial challenge: immersion in *Shewanella* sp.

Results

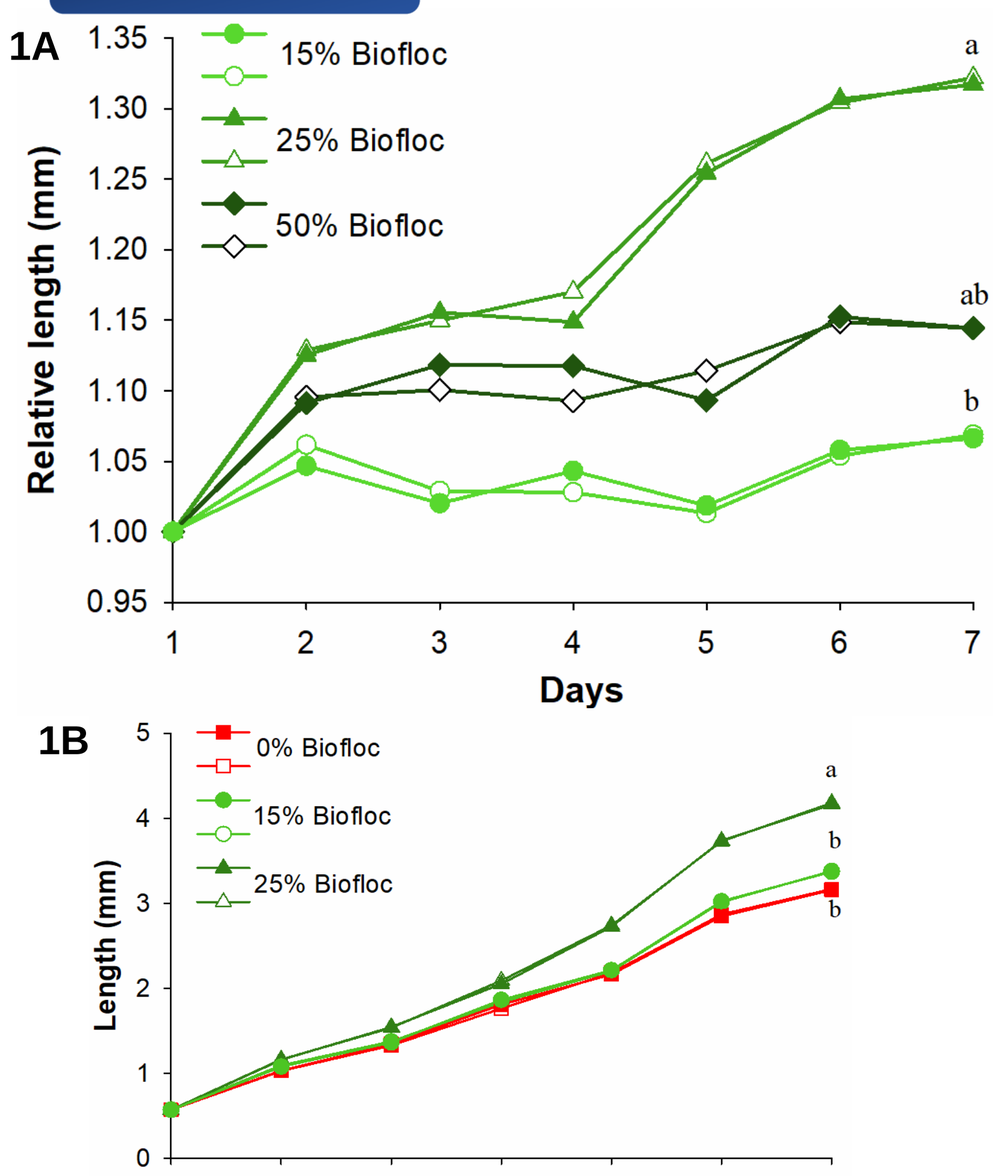


Fig 1: **Growth of *Artemia*** fed diets supplemented with different amounts of biofloc (15, 25 and 50%) and 0% (un-supplemented control)
(A) Growth relative to control
(B) Average growth in Trial 1 ($p < 0.05$)

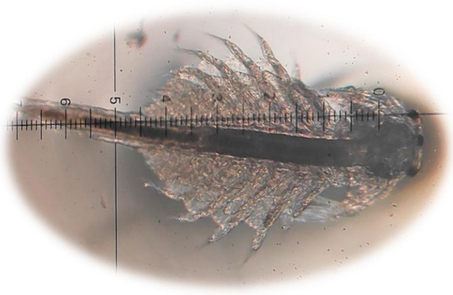
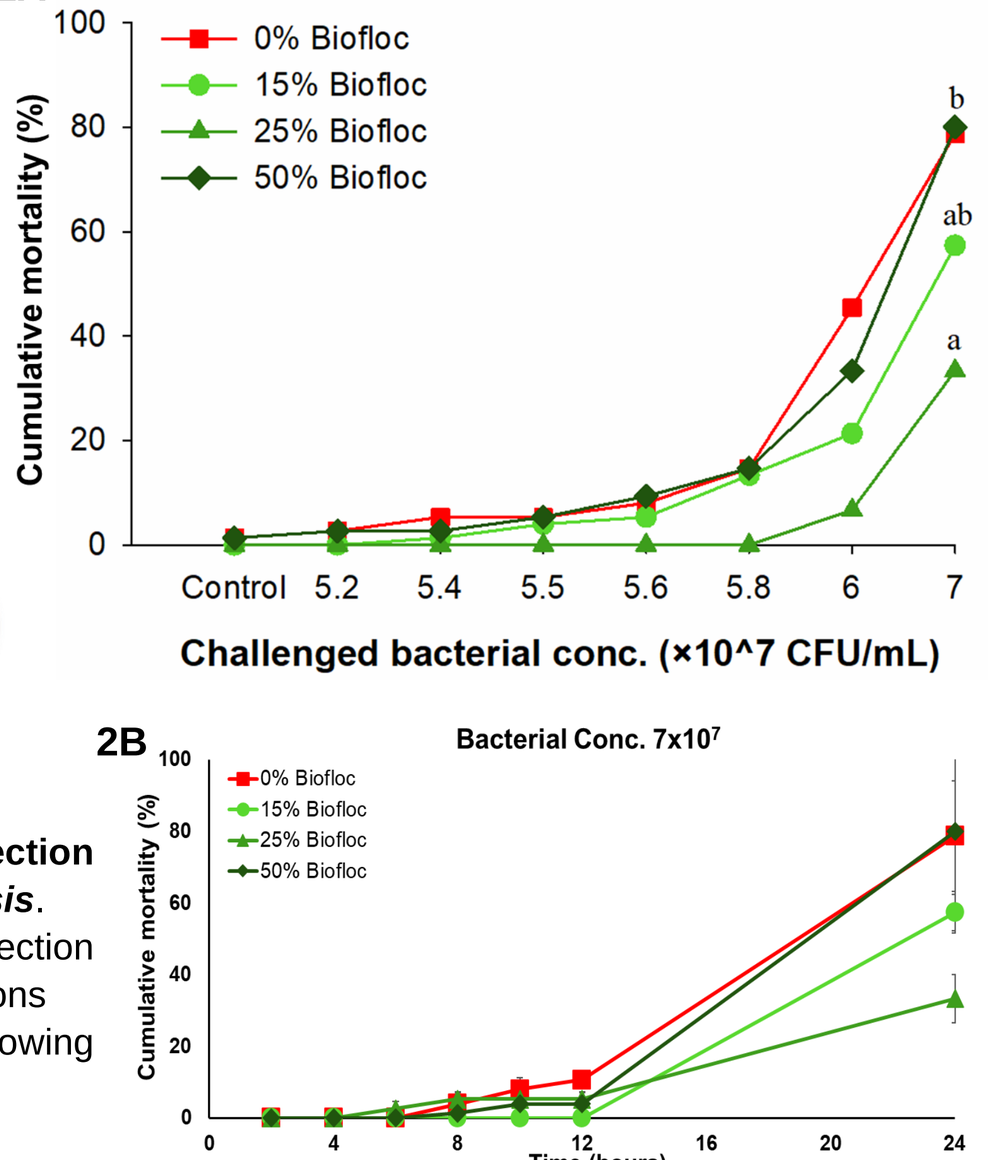


Fig 2: **Mortality following infection with *Shewanella jiangmenensis*.**
(A) Mortality after 24 hrs of infection at different bacterial concentrations
(B) Mortality over time following infection with $7*10^7$ CFU/ml
($p < 0.05$).



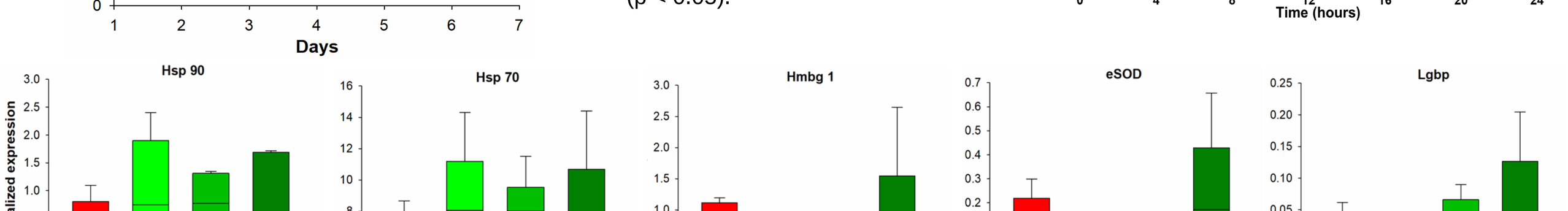


Fig 3: Expression of genes encoding innate immune factors in *Artemia* (a total of 9 genes were analyzed). Data are presented as box plots expressing means ± SD and ranges (whiskers) (n=3; 20-30 *artemia* per replicate). Expression levels are shown as $2^{-\Delta CT}$ (ΔCT, difference in cycle threshold). Expression is normalized to three internal control genes: Ef1-α, βAct, and GADPH.

Discussion & Conclusions

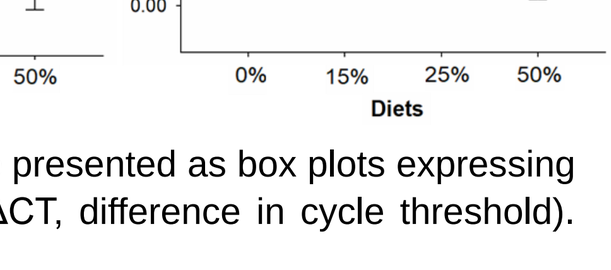
- Feeding with 25% biofloc improved *Artemia* growth and their survival following bacterial challenge, compared to control and to diets containing higher (50%) and lower (15%) biofloc inclusion levels.

Comparing to a similar study done with fish (Nayak et al., 2023**) :**

- In contrast to the higher growth observed in biofloc-fed *Artemia*, fish that were fed with 20% biofloc diet showed reduced growth compared to the control group (Fig. 4 A), .
- However, both biofloc-fed *Artemia* and fish were better protected from bacterial challenge (Fig 4 B). Both appeared to be immunostimulated as evident by elevated immune-gene expression.

Fig 4 A

Fig 4 B



References

Nayak S, Yogev U, Kpordzaxor Y, et al (2023) From fish excretions to high-protein dietary ingredient: Feeding intensively cultured barramundi (*Lates calcarifer*) a diet containing microbial biomass (biofloc) from effluent of an aquaculture system. Aquaculture 562:738780.

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