

Utilization potential of microalgae cultivated in processing water from shrimp recirculating aquaculture systems (RAS)

AquaLoop - International Conference

SESSION 1: Closing the Nutrient Loop & Byproduct Valorization

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Background

- ✓ penaeid shrimps account for more than 60% of global crustacean production (FAO, 2024);
- ✓ production of whiteleg shrimp in most of Europe is practically only feasible in land-based recirculating aquaculture systems (RAS);
- ✓ RAS systems are not 100% efficient, especially at high stocking densities (= partial water exchange required);
- ✓ searching for more effective circular economy solutions for RAS systems, which help not only to minimize waste/byproduct but also, to generate additional value;

- ✓ microalgae - purify nutrient-rich processing water from RAS during growth and, in addition, produce a biomass rich in lipids that can serve as a dietary supplement for shrimp or can be utilised in other branches of the economy;



Photo credit: Piotr Kendzierski



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Aim

To identify Baltic microalgae strains able to grow in processing water from whiteleg shrimp RAS aquaculture, while producing a high yield of biomass.

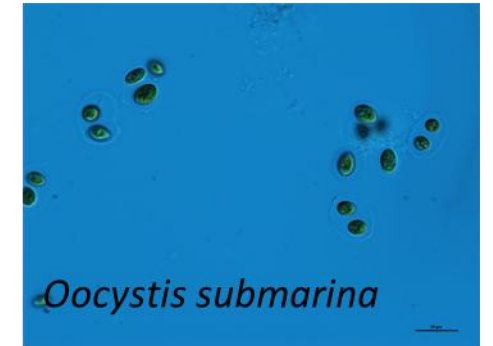
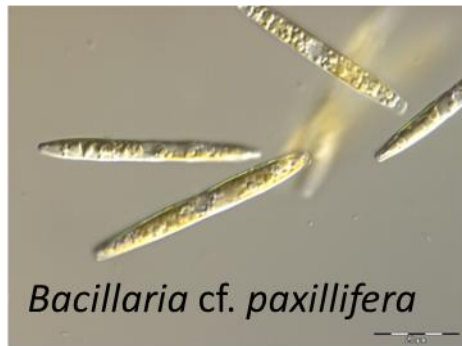


Photo credit: F. Pniewski

microalgae strains from the Baltic Sea

Materials & Methods (shrimp cultivation)

- ✓ a small-scale laboratory RAS system was developed, consisting of a main unit and four shrimp water tanks with a total capacity of 1500 L (4 separate tanks ca. 300l of capacity each + main unit)
- ✓ *P. vannamei* post-larvae (n=1000) were imported from a hatchery in Austria (White Panther Produktion GmbH)

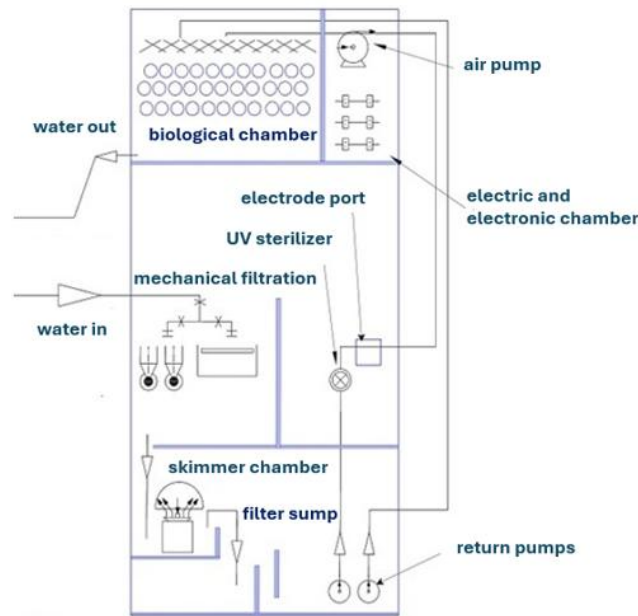


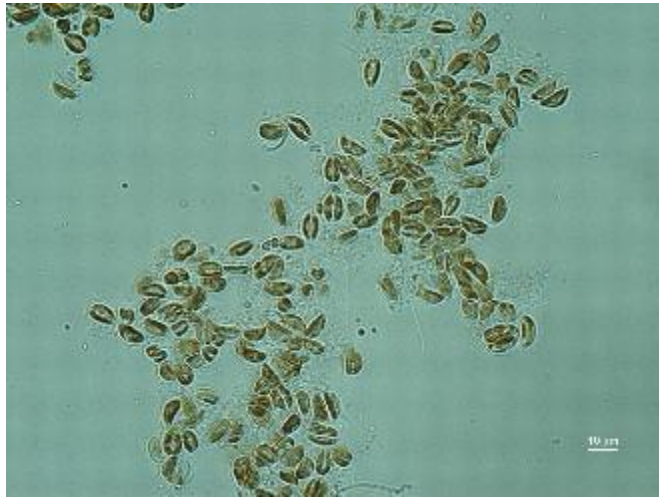
Photo credit: J. Hegele-Drywa



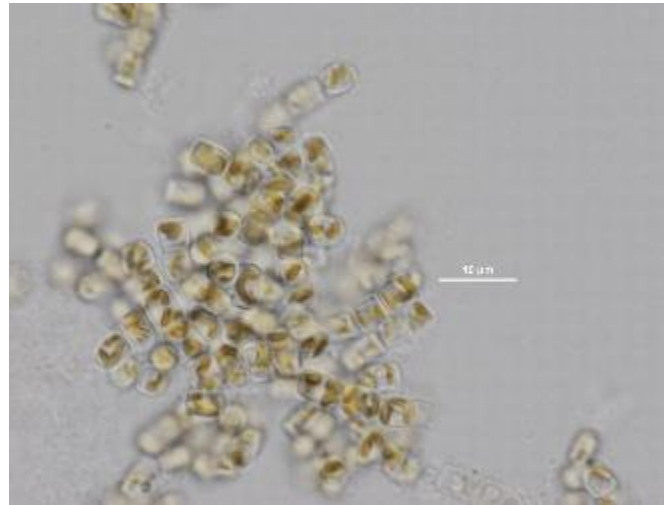
- ✓ shrimps reared under controlled conditions: T=27°C, S=30 PSU, pH ~7.8, photoperiod 15 h L: 9 h D.
- ✓ shrimps fed 5 times a day, with commercial feed

Materials & Methods (microalgae cultivation)

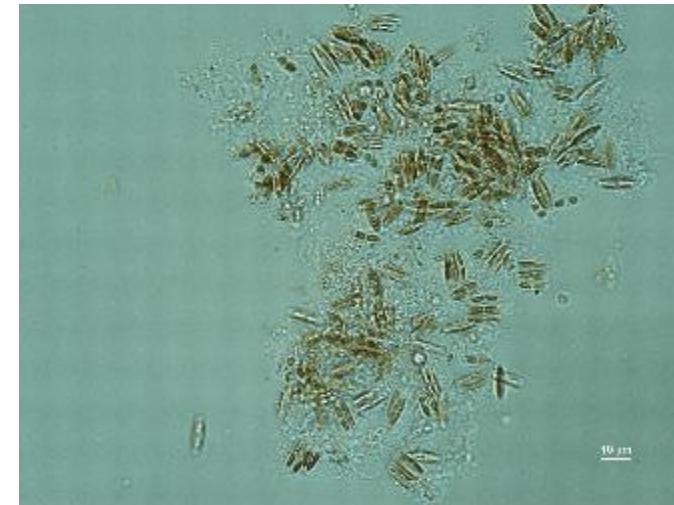
- ✓ processing water collection (10 l) from the RAS at week 13 of shrimp culture;
- ✓ determination of nutrient concentrations in RAS processing water using colorimetric cuvette tests (Hach Lange GmbH, Germany) - (1 mg L⁻¹ for ammonium nitrogen, 122 mg L⁻¹ for nitrate, 3 mg L⁻¹ for phosphate, and 1 mg L⁻¹ for silica);
- ✓ selection of three fast-growing diatom strains (growth requirements):



Amphora sp. CP3



Gedaniella sp. PL1.21



Nitzschia cf. *aurariae* BA158

Photo credit: F. Pniewski

Materials & Methods (microalgae cultivation)

- ✓ preparation of media for microalgae culture - eight variants (4 replicates for each one) using two salinities;
- ✓ start of microalgae culture (T = 20 °C, light intensity 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, t=12 days);
- ✓ monitoring of growth - daily measurements of optical density (at 750 nm) and photosynthetic pigments (chlorophyll a and fucoxanthin);

- ✓ determination of lipid concentration in the biomass;
- ✓ determination of nutrient concentrations in all microalgal cultures;



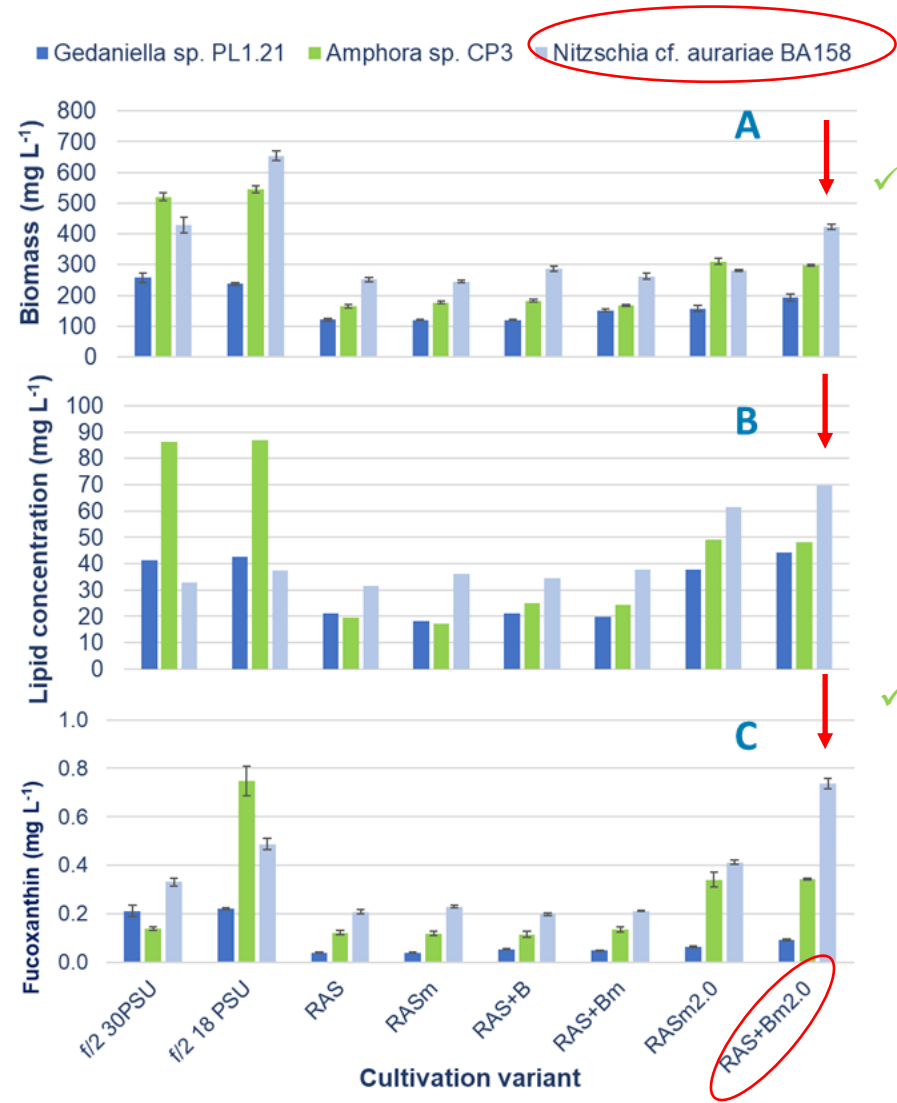
Photo credit: F. Pniewski

Characteristics of eight medium variants used for microalgae cultivation (ASW – artificial salt water).

| No | Variant | Characteristics |
|----|-------------|--|
| 1 | f/2 30 PSU | f/2 medium prepared using ASW |
| 2 | f/2 18 PSU | f/2 medium prepared using ASW |
| 3 | RAS | Unchanged RAS water |
| 4 | RAS+BAL | RAS and Baltic water mixed in the ratio of 1:1 to reach a salinity of 18 PSU |
| 5 | RASm | RAS water with the increased P concentration to obtain N:P ratio of 24:1 (as in the f/2 medium) |
| 6 | RAS+BALm | RAS and Baltic water mixed in the ratio of 1:1 to reach a salinity of 18 PSU with the increased P concentration to obtain N:P ratio of 24:1 (as in the f/2 medium) |
| 7 | RASm2.0 | RAS water with the addition of silica and microelements (including Fe) |
| 8 | RAS+BALm2.0 | RAS and Baltic water mixed in the ratio of 1:1 to reach a salinity of 18 PSU with addition of silica and microelements (including Fe) |

Results

- ✓ all of the tested strains grew in the RAS processing water (Fig. A);
- ✓ the highest biomass ($423 \pm 10 \text{ mg L}^{-1}$) was observed for *N. cf. aurariae* BA158 in the mixed RAS and Baltic water supplemented with increased silica and iron amounts;



- ✓ for *N. cf. aurariae* BA158 cultured in the mixed RAS and Baltic water supplemented with increased silica and iron, also the highest concentrations of lipids (69.5 mg L^{-1} , Fig. B), fucoxanthin ($0.74 \pm 0.01 \text{ mg L}^{-1}$, Fig. C), and chlorophyll a ($1.95 \pm 0.05 \text{ mg L}^{-1}$) were recorded;
- ✓ *Gedaniella* sp. PL1.21 was characterized by the lowest biomass, content of pigments and lipids, regardless of the medium in which it was cultivated;

Results

- ✓ all strains removed a significant portion of nutrients from processing water, but the most effective was *Amphora* sp., which removed as much as 85% of nitrates (variant RAS+BAL) and 80% of phosphates (variant RAS+BALm2.0);

Percentage of nitrates ($\Delta\text{N-NO}_3^-$) and phosphates (ΔP) removed by three diatom strains in six medium variants containing processing water from RAS.

| Variant | <i>Gedaniella</i> sp. PL1.21 | | <i>Amphora</i> sp. CP3 | | <i>Nitzschia</i> cf. <i>aurariae</i> BA158 | |
|-------------|------------------------------|------------------|-------------------------|------------------|--|------------------|
| | $\Delta\text{N-NO}_3^-$ | ΔP | $\Delta\text{N-NO}_3^-$ | ΔP | $\Delta\text{N-NO}_3^-$ | ΔP |
| RAS | 34 | 66 | 77 | 3 | 10 | 10 |
| RAS+BAL | 69 | 31 | 85 | 51 | 56 | 51 |
| RASm | 52 | 48 | 77 | 34 | 24 | 37 |
| RAS+BALm | 61 | 39 | 77 | 59 | 53 | 56 |
| RASm2.0 | 50 | 50 | 41 | 48 | 34 | 39 |
| RAS+BALm2.0 | 68 | 32 | 57 | 80 | 45 | 70 |

- ✓ *Amphora* sp. removed over 50% of nitrates and phosphates at the same time in three, and *N. cf. aurariae* in two out of six medium variants, which indicates their high efficiency;

Conclusions

- ✓ Processing water from shrimp farming in RAS system can be used as a medium for the efficient growth of Baltic Sea microalgae to remove excess nutrient concentrations;
- ✓ Success of this approach depends on appropriate modification of the processing water to achieve the correct N:P ratio and, if necessary, to adjust silica and iron concentrations;
- ✓ Since all of the strains efficiently removed nutrients, the choice of the strain for cultivation should be based on its ecophysiological and biochemical traits to produce high-quality and commercially viable biomass, thereby adding value to RAS systems;

Future plans...

- ✓ To examine the potential of mixed-strain microalgae cultivation for removing nutrients and producing lipid-rich biomass;
- ✓ Determine the utility and potential of the cultivation medium based on raw/non-filtered processing water from RAS systems for increasing biomass production of microalgae;



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Thank You!

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