



Lab-based all year round anti-fouling bioassay to screen for pre- and post-settlement biocide activity against barnacles.

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Rationale

- Current antifouling technologies are mainly based on the continuous release of biocides and consequently discharge into the environment.
- Barnacles are important fouling organisms worldwide - largely increasing the hydrodynamic drag of ships and damaging coatings on underwater surfaces.
- Barnacles are extensively used as a model in antifouling research mostly in static, lab-based systems - however, reliable flow-through test assays for the screening of antifouling paints and its incorporated biocides are rare.
- We have developed a test assays to screen diverse low-release biocide paints and their pre- as well as post-settlement activity against barnacles.

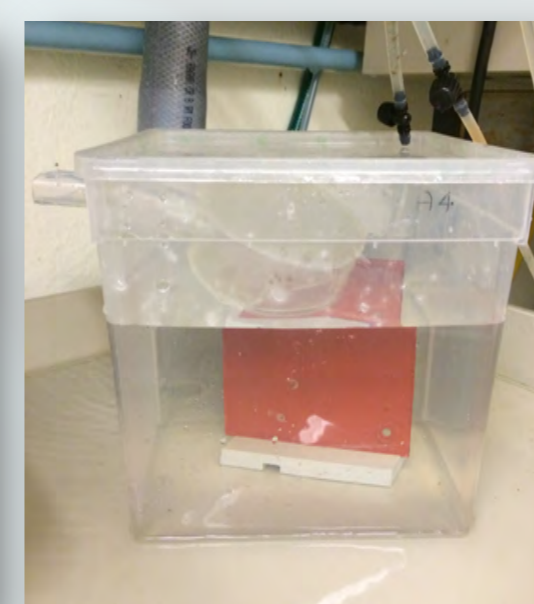
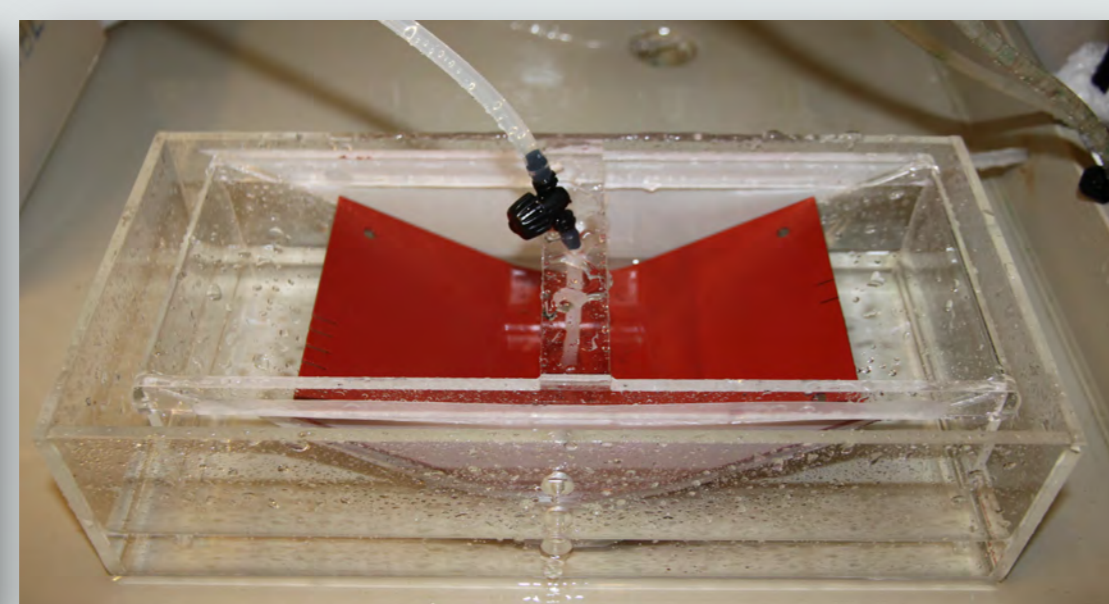
The Flow Cell Assay

- Test assay for coatings where antifouling activity is confined to the paint surface and biocides are bound to the paint matrix - antifouling activity exercised only when the fouling organism makes contact with the paint, e.g. at settlement or post-settlement penetration:
- Open flow cells with continuous flow-through seawater prevent accumulation of biocides
- Design and nylon net ensure the panels to be the only surfaces available for settlement
- Choice or no-choice tests: each flow cell holds two treated or one treated and one control panel (11 x 11 cm)



Settlement tests

Post-settlement mortality tests



- Pre-soaking of panels

- A minimum of 200 cyprids per flow cell
- Flow-through seawater (25 PSU & 20°C)
- Flow rates from 1.5 to 30 l per h
- Water level adjustable from 1/3 (65 ml) to 2/3 (250 ml)

- Feeding with diatom algae (*Skeletonema marinoi*, *Chaetoceros calcitrans*, *Thalassiosira pseudonana*) in 1.5 l beakers

- Feeding with *Artemia salina* in 6 l aquaria

Flow-through seawater (10/30 l per h, 25 PSU & 20°C)

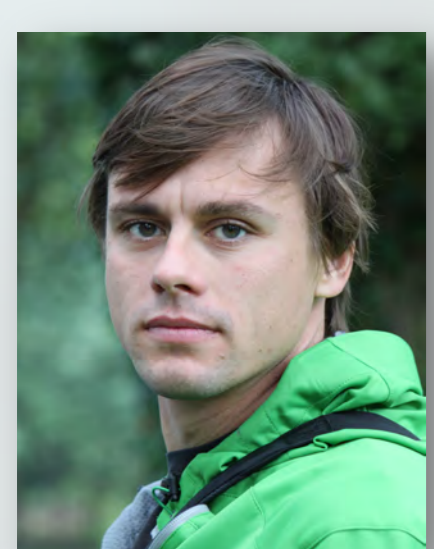
Counts of settlers and survivors every second day

Acknowledgements

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Conclusions

This settlement assay produces all-year round reliable results in order to test for large-scale and more realistic anti-settlement activity of biocides against barnacles.

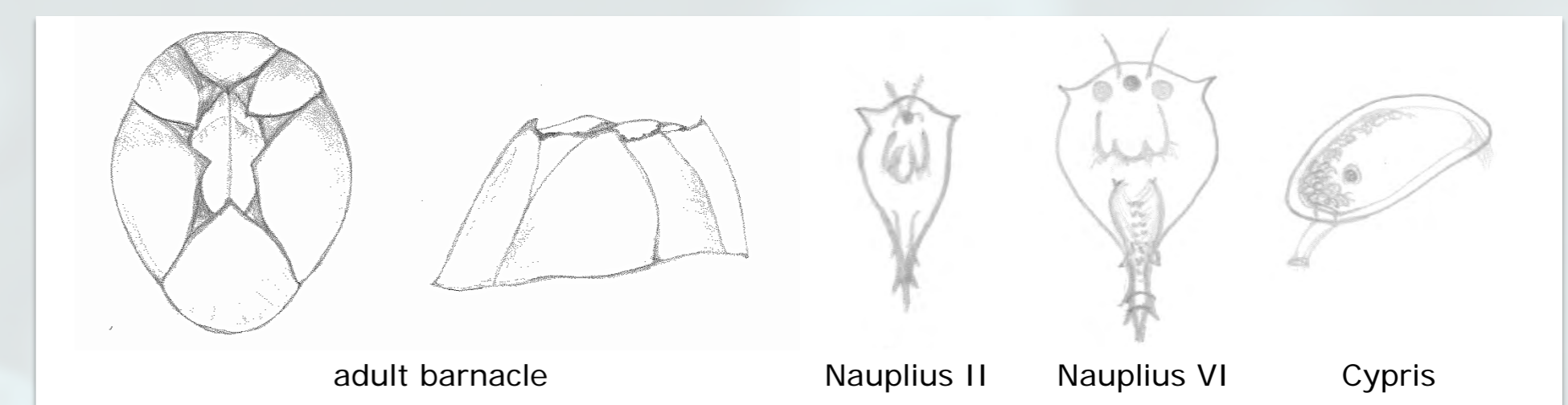
The settlement assay can be easily extended to screen for post-settlement activity of biocides.

It has the potential to be further applied in various other fields of anti-fouling research, in pre- and post-settlement inhibition studies.

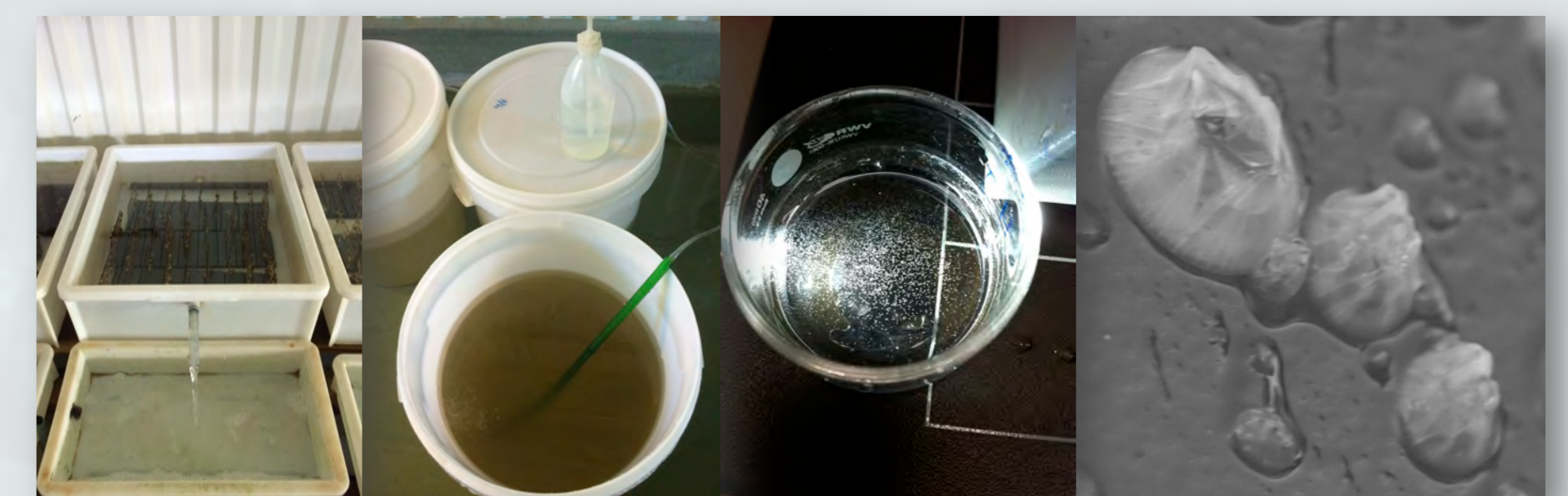
This assays is at the moment applied within the European FP7 Collaborative project "LEAF 1", aiming to develop sustainable antifouling solutions based on a newly discovered "Low Emission AntiFouling" paint concept.

Balanus (Amphibalanus) improvisus in culture

- Can be found worldwide
- By far the most common barnacle species in the brackish Baltic Sea
- All year round culture at the Sven Lovén Centre for Marine Sciences - Tjärnö (University of Gothenburg, Sweden) provides continuous supply of cyprids:



- Adults (collected in July) are kept in flow-through seawater (25 PSU & 20 °C) fed with *Artemia salina* ad libitum
- Larvae reared in 20 l buckets at 26 °C and fed diatom algae (*Skeletonema marinoi*, *Chaetoceros calcitrans*, *Thalassiosira pseudonana*) for 6 days
- Cyprids are collected and used in the diverse assays



Preliminary Results

- Preliminary tests with biocide-free panels show high settlement rates and demonstrate that settled barnacles can be monitored after metamorphosis to explore time-dependent post-settlement effect of biocides.

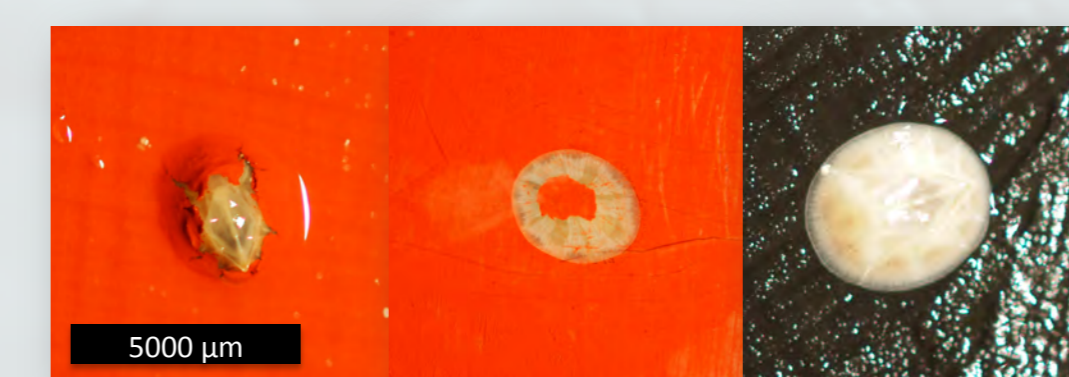
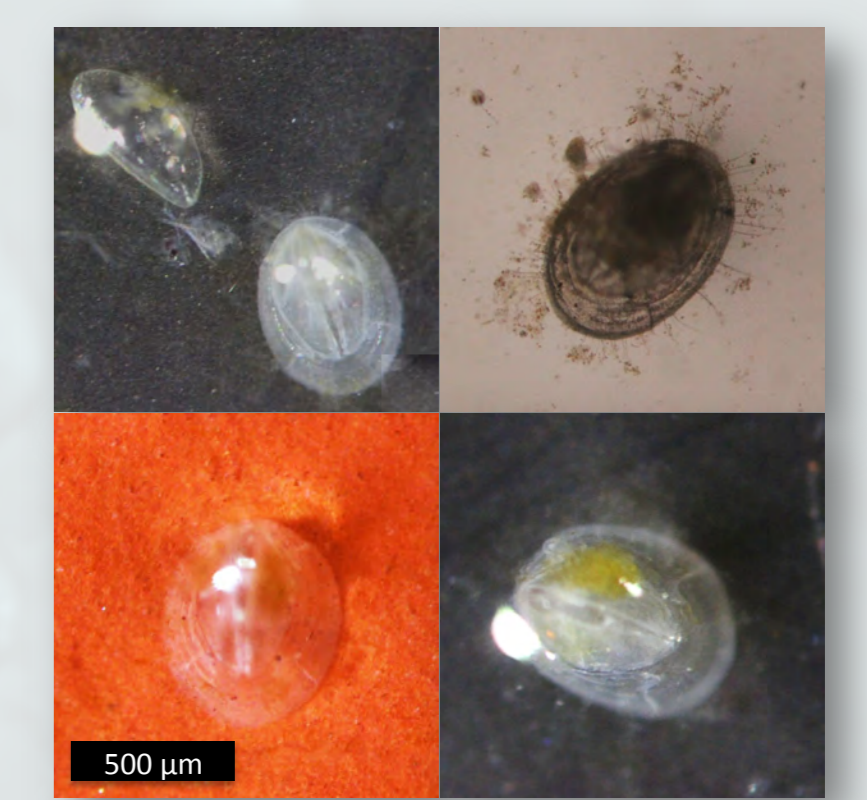
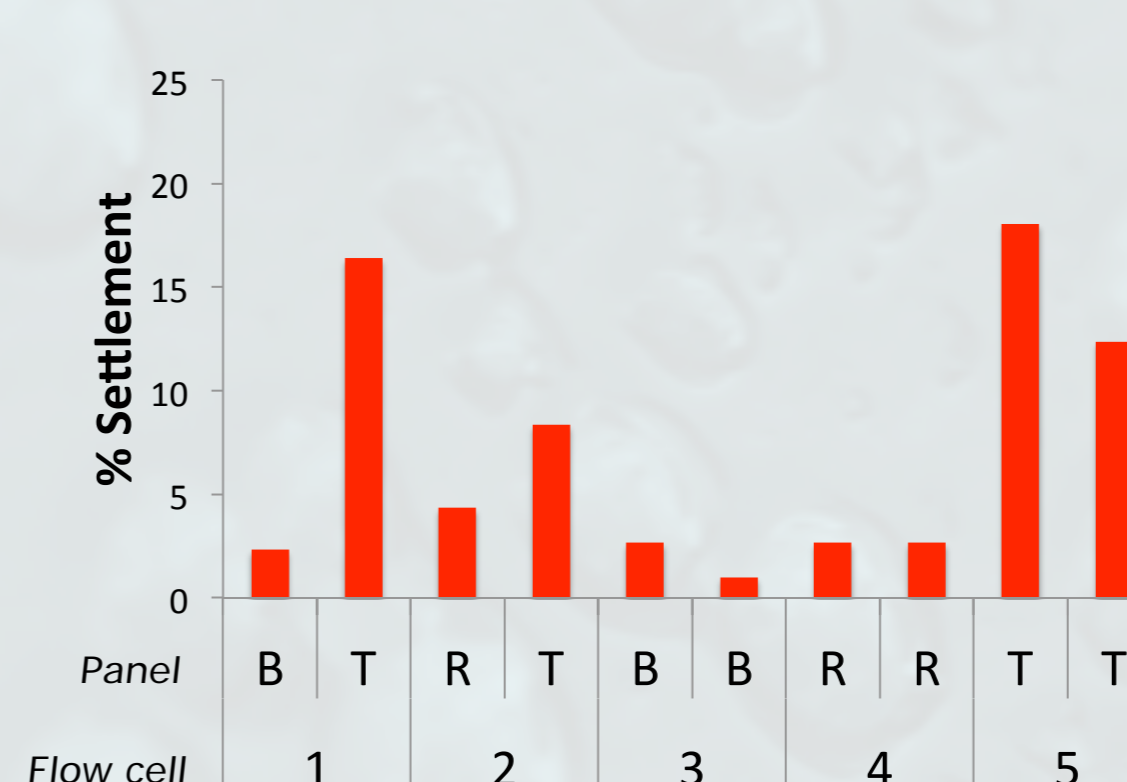


Figure 1 - Settlement and post-settlement growth of *B. improvisus* on PMMA (Plexiglas®; T=transparent), LEAF-4.1 paint (R=red) and LEAF-A paint (B=black) without biocides.

- This novel flow-cell assay is now used to test for antifouling activity against barnacles, where the biocide effect is triggered when post-settlement barnacles penetrate the coating.

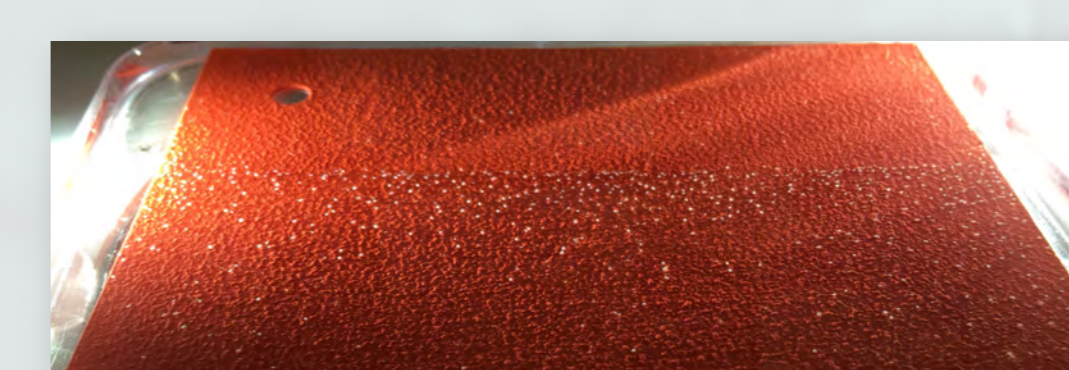


Figure 2 - Settlement of *B. improvisus* on PMMA (Plexiglas®) and LEAF-4.1 paint without (Co) and with biocide (Ab & Iv), photo=LEAF-4.1-Co.

