



UNIVERSITY OF
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Marine Paint Optimisation: Settling assays with marine periphyton communities and sea lettuce



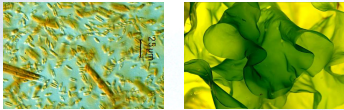
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Optimisation of efficacy vs risk using multi-component mixtures of antifoulants

The Optimisation-Formulation-Performance research route in Marine Paint sets out to identify efficacious biocide combinations with minimal environmental risk.

With the help of specially developed computer algorithms the hundreds of thousands of combinations will be sorted out and ranked in terms of efficacy.

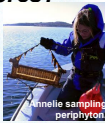
A minor portion of the combinations – those indicating best efficacy at lowest risk – will be transferred to the formulation project within Marine Paint for micro-encapsulation and later to the performance project for fouling testing in a marine environment.



How do we perform settling assays with periphyton and Ulva zoospores?

Marine periphyton or “slime” are microbial communities with high biodiversity and differential sensitivity.

- Microbial communities consist of a multitude of different microbial fouling species and can be sampled from the field using artificial substratum. We used Plexiglas slides that were colonised for 2-4 weeks at 1 m depth.
- We made an inoculum by scraping off the biofilm into filtered sea water with added nutrients, shaking the slide and biofilm vigorously and sieving using a 100 µm mesh.



Sea lettuce belongs to an important group of fouling macroalgae.

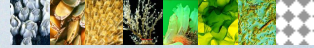
- We collected thallus of *Ulva lactuca* from the field and cultivated these indoors.
- Pieces of *U. lactuca* with mature zoospores were placed in Petri dishes with filtered sea water and algal growth medium and placed under intense light to release mature zoospores that were used as inoculum.



Settling assays

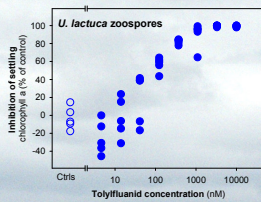
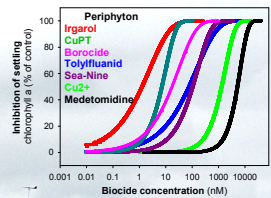
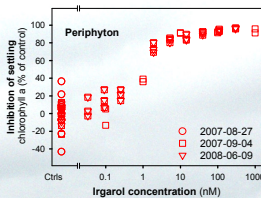
- We used glass vessels (20mL) and distributed inoculum, filtered seawater and biocides in a total volume of 10 mL. Plexiglas settling slides were placed in each vessel.
- The incubation vessels were placed on a shaker and incubated at 15°C and a day-night regime (16:8). For the *Ulva* zoospore assay the vessels were first kept in darkness for three hours to allow an even spore settlement.
- After 24 and 48 hours the slides were moved to new vessels containing fresh solutions.
- After 72 hours of settling/growth a removable piece was broken off the Plexiglas slides and placed in 10 ml of ethanol (96%) and placed on a shaker overnight to elute chlorophyll, measured using a Turner fluorometer. Settling efficacy was thereby estimated as chlorophyll a.

Experimental Approach with Biocides and Foulers in Focus



	Barnacles	Mytilus	Bryozoa	Hydrozoa	Cliona Ulva	Periphyton	?
Borocide®P, KH 101, TPBP (triphenylboronpyridine)							
Cu²⁺, copper							
CopperPyrrithione Copper OMAIDINE™							
Irgarol® 1051							
Medetomidine, catemine							
Sea-Nine®211 N, DCOIT, Kathon™287TN							
Tolyfluand, Preventol ASS							
?							

First results from efficacy testing



All tested biocides inhibit settling and growth of periphyton. The low variance and high reproducibility make the periphyton assay a suitable settling assay for predictive mixture studies such as the Optimisation project.

The work with *Ulva* is in progress with the aim to develop a parallel set of data as for periphyton.

Look out for related presentations 29P-12, 30A-2-4 & 30A-5-2 from Marine Paint!

Marine Paint is a Swedish research programme funded by MISTRA aimed at developing new and effective marine antifouling paints, which are more environmentally friendly than those in use today. www.marinepaint.se

We gratefully acknowledge Areh Chemicals, Rohm&Haas, Cliba Specialty Chemicals GmbH, L-Tech AB and Lanxess for providing biocides and information about their products.

All photos by Ida Wendt except for Ulva (Andreas Nyström), periphyton (Mats Kuylenstierna), barnacles (Alexandra Wattwil), mussels and Cliona (Maria Asplund) and hydrozoa and bryozoa (Judith Fuchs)