UNIVERSITY OF GOTHENBURG

Marine Paint

Marine Paint Optimisation: Settling assays with sea squirt and barnacles

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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.

We have selected a number of important fouling groups (barnacles, mussels, bryozoans, hydroids, ascidians, macroalgae and periphyton) for settling tests and a few examples are presented here.

Biocides in Focus

Substance	Main target(s)	Company
Borocide®P, KH 101, TPBP (triphenylboron-pyridine)	Soft and hard foulers, broad-spectrum antimicrobial	Rohm&Haas/ INVISTA
Copper pyrithione, Copper OMADINE™	Algae, bacteria, fungi	Arch Chemicals
Irgarol® 1051	Algae	Ciba Specialty Chemicals
Medetomidine, Catemine	Barnacles	I-Tech AB
Sea-Nine® 211 N, DCOIT (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), Kathon™ 287T N Biocide	Broad spectrum of activity against bacterial slime, algae, barnacles, seaweed, and other marine organisms	Rohm&Haas
Tolylfluanid, Preventol A5S	Effective against a broad range of fouling organisms	Lanxess
Cu^{2+}, Copper	Effective against a broad range of fouling organisms	

How we perform settling assays with sea squirts and barnacles?

Sea squirt belongs to the group "soft foulers" and is a common test species with a well studied genome.

- Adult sea squirts (*Ciona intestinalis*) with mature gametes were collected from the field by divers.
- Eggs from 16 animals and sperm from another 8 animals were dissected out, mixed and allowed to stand for 1 hour to fertilise. The sperm were filtered off and the fertilised eggs were left until the larvae were about to hatch.
- 40-60 larvae were added to each well in 6-well microplates containing 10 ml of filtered seawater and biocides.
- After 48 hours the solutions were exchanged and non-settled larvae removed.
- Settled *Cionas* were left to grow for a subsequent period of 48 hours and the number of settled and normally metamorphosed individuals were then counted.

Newly hatched larvae of *Ciona intestinalis*

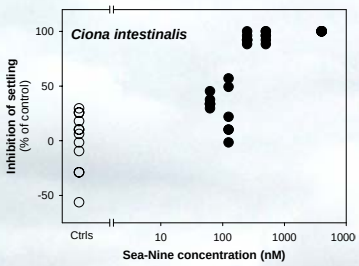
Barnacles are often classified as the worst foulers because of their hard shell which is difficult to remove.

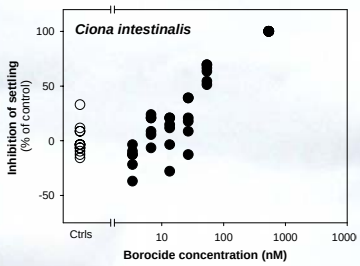
- We used cultivated cyprid larvae (1-4 days old) of *Amphibalanus (Balanus) improvisus*.
- Twenty cyprids were added to each well in 6-well microplates containing 10 ml of filtered seawater and biocides.
- The cyprids were left to settle for 5-7 days and the number of settled and metamorphosed barnacles were then counted.

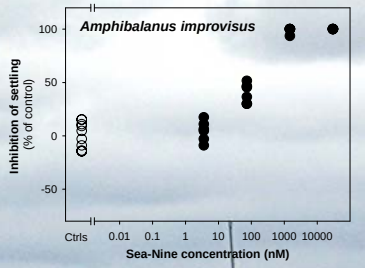
Cyprid larvae of *Amphibalanus improvisus*

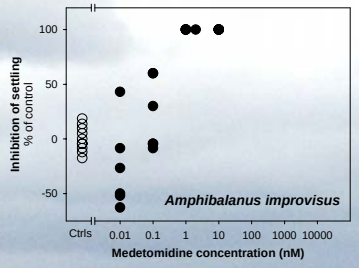
Both assays were incubated at 15°C and with a day-night regime (16:8).

First results from efficacy testing









The **work is in progress** with the aim to develop a full data set for all substances. Additionally, promising settling studies with *Mytilus edulis* are initiated.

Medetomidine inhibits settling of barnacles at low concentrations but no mortality was found, even at the highest concentration tested.

Look out for related presentations 28P-19, 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***

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Photos by Ida Wendt (background), Maria Asplund (*Ciona*), Kent Berntsson/Per Jonsson (cyprids), Sam Dupont (*Ciona* larvae) and Alexandra Wattwil (barnacles).