PROJECT FINAL REPORT

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Executive summary

Marine biofouling, the unwanted growth of marine organisms on the surfaces of boat hulls and other structures submerged into sea water, is a major global problem. It leads to substantially increased fuel consumption and CO2 emissions in the marine transport sector. In addition it can lead to loss of function of protective coatings, in turn decreasing service life and increasing maintenance costs. Marine Biofouling is also a vehicle for spreading of foreign invasive between different ecosystems globally.

Traditional antifouling (AF) technologies are based on coatings that continuously release biocides. The dominating AF solution on the market today is to use copper compounds as the active biocide in coatings that slowly erode with time in water. One alternative AF solution for ships is so called fouling release (FR) coatings, which are based on achieving low adhesion between the fouling organisms and the surface, so that the biofouling films detaches when the ship reaches a certain speed. Neither of these two approaches can be regarded as sustainable. Although being efficient, there are serious concerns about the ecological effects of the continuous release of copper into the oceans, whereas the FR coatings have issues with durability and cost.

Low emission antifouling (LEAF) is new innovative concept for AF coatings. The antifouling effect makes use of the behaviour of settling barnacles. When barnacles – which are among the most severe biofoulers in many waters- settle on a surface they penetrate into the material. In LEAF the biocide is incorporated and immobilized within the coating which means that the barnacles are exposed to the biocide only after settlement. The antifouling effect is thus not relying on a continuous release biocide and also opens up for the use of non-erodible coatings with potential for long service life. Prior to the start of the project the LEAF concept had been shown to be efficient towards barnacle fouling for a three year period, using ivermectin as the biocide in model polymers.

The aim of the LEAF project has been to further develop and optimize the concept, to demonstrate its industrial feasibility, and to perform a sustainability assessment. RTD activities included: (i) screening and optimization the main biocide and complementary biocides for achieving a more holistic AF efficacy, (ii) screening and optimization of paint ingredients for minimizing biocide emission and maximizing service life, (iii) formulation, characterization and field testing of antifouling paints, (iv) life cycle assessment and assessment of ecotoxicology and human safety aspects, and (v) scale up of prototype paint production and demonstration in boat test.

The project has developed and demonstrated an antifouling paint that shows excellent efficacy and very high user satisfaction in the field, as tested by 55 boat owners in Scandinavia, Mediterranean, and Caribbean waters. Furthermore, the paint is significantly cheaper to produce and has a lower CO2 footprint than current benchmark antifouling paints. Based on the positive results, both in terms of performance in the field, industrial feasibility and sustainability assessment, the project has taken measures to facilitate future product registration according to European regulations, which will be a critical step for introducing the LEAF paint on the market. Dissemination and communication activities have resulted in a high profile and great interest for the project in the scientific, industrial and end-user communities.

In summary, the project has successfully developed and demonstrated a new sustainable marine antifouling coating technology that is now on the verge of becoming an innovative product on the market.

Background and objectives

The problem

Marine biofouling is the unwanted attachment and growth of marine organisms on immersed artificial structures in the marine environment. The most obvious penalty of colonization of marine biofilms, plants and animals on ship hulls is the increased fuel consumption and associated increases in CO_2 emissions. Crustacean fouling of ships with no antifouling protection may increase the hydrodynamic drag by up to 40%. Consequentially, fuel consumption can rise by 30 % in certain weight classes. In addition, the unwanted growth of marine organisms on surfaces is often associated with material degradation, via biocorrosion or loss of integrity of corrosion protective coatings due to hard fouler penetration. Another unwanted aspect of the marine fouling is the spreading of alien species transported on ship hulls to different part of the globe. Sensitive marine ecosystems, for example the coastal waters of Australia, have been invaded by foreign species that have irreversibly altered the biological balance causing permanent harm to the local ecosystem. Novel research has revealed that biofouling is, alongside the handling of ballast water, the major contributing factor to this global problem. Outside the transport sector, marine biofouling is also a significant problem in aquaculture, desalinization plants and power plant cooling systems around the world, where it constricts water flow. Due to the considerable negative effects, biofouling of surfaces in marine environments is a widespread problem that needs to be prevented by different solutions.

The dominating anti-fouling methods for ship and static marine constructions are based on coatings that release different kinds of biocides. In order for achieve antifouling efficacy, the release rate of the biocide must be maintained over a certain value to reach an effective concentration of biocide at the coating/water interface. Paints that are slowly erodible in water are usually used, and the erosion process is one of the factors regulating the release rate to maintain a necessary interface concentration.

Much of the public and scientific debate about marine antifouling has been focused on the type of biocides included in the paint layers to prevent biofouling. One major concern is that the use of leaching biocides in marine antifouling paints also may result in irreversible changes of the biological life in the marine environment, especially the marine sediments. The earlier use of tributyl tin (TBT) in marine antifouling paint was very effective for preventing marine biofouling, but leaching of TBT also made molluscs like oysters sterile. Due to these and other negative ecological effects, TBT has been banned in most countries. Today, the dominating marine antifouling technology is based on using copper compounds as the biocide in an erodible paint formulation. Although efficient, this approach has some drawbacks. Since they are based on a continuous release of copper and paint erosion, it is difficult to achieve coatings with a long service life with this technology, which in turn influences maintenance costs. In addition, some concerns are starting to be raised regarding the fate and possible ecological effects of copper compounds in the marine environment. The problem can become especially severe in isolated water bodies such as enclosed marinas and harbors that experience little water exchange combined with high levels of boating activity. Alternative, copper-free antifouling solutions are therefore much sought for.

One alternative approach, developed in the past decades and growing in importance in the scientific community, is non-biocide based antifouling techniques working on physic-chemical principles aimed at preventing or minimizing the adhesion of the biofoulers to the surface. A surface with low friction, low surface energy or high hydrophobicity results in a lower adhesion of the fouling organisms. This in turn can enhance the release of fouling attached to the surface if the cruising speed reaches a sufficient velocity. This approach can be termed fouling release (FR) coatings. An alternative approach is to apply a controlled microtopography to the surface. Micro-structured surfaces have also showed promising results in preventing settlement of hard fouling such as barnacles as well as microalgae and other slime forming organisms. Silicone based coatings are commonly used in the FR approach. These coatings are ecologically inert, but have problems with mechanical strength and long term stability. Some of these coatings are commercially available but market penetration is limited due to high material and maintenance costs. For micro-structured surfaces, high production costs are a major disadvantage.

The LEAF concept

The low emission antifouling concept behind the LEAF project is based on previous research efforts focusing on the barnacle, which is regarded as the most serious biofouling organism in marine waters. Adult barnacles on a ship hull can increase the water friction and fuel consumption by up to 40%. The adult barnacles can also penetrate and destroy coatings, triggering the corrosion process on stainless steel structures. Instead of relying on a continuous release of biocides as in conventional antifouling strategies, the LEAF concept makes use of the settling and penetration behavior of barnacles. The biocide is incorporated and immobilized in the coating and only when the barnacles settle and start to penetrate the coating they are exposed to the biocide. Before the start of the LEAF project, proof of the concept had been shown using a specific class of biocides and model polymer coatings. The active substances used belonged to the family macrocyclic lactones, which are produced by a soil bacterium living also in the sea sediment.

Investigations with one substance of the avermectin family, ivermectin, had shown effective prevention of barnacle colonization even for concentrations as low as 0.1 weight % added to the paint (see Fig 1). Moreover, the antibarnacle efficacy persisted for at least three years of continuous exposure to water, despite the low loading in the paint at initial formulation. Other experiments showed that an appropriate hardness allowing barnacles to penetrate the coating, was an important factor for achieving an anti-barnacle effect. The ivermectin addition in the paint did, however, not inhibit other biofouling organisms such as algae or bryozoan.

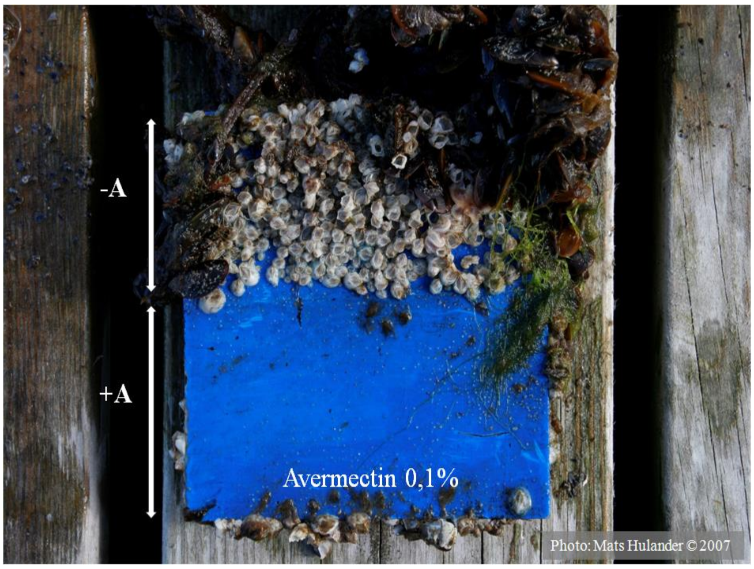


Fig 1. Photograph of a field test panel immersed in a harbor for three month at the Swedish west coast. The panel was painted at the upper half (-A) with a copper free paint. The lower part (+A) was coated with similar paint with the addition of 0.1 % ivermectin. Note dense density of barnacles at the upper part and the relative absence of barnacle growth at the lower, ivermectin-containing, part.

Objectives of the project

The main overall goal of the LEAF project has been to develop and demonstrate an innovative marine antifouling coating based on the very promising concept described above. The overall criteria and performance indicators for the coatings to be developed were the following:

- Antifouling performance equal or better than currently used technologies on the market
- Lower environmental impact of biocides, by zero or very low emission of biocide
- Increased lifetime and lower maintenance costs, compared to currently used technologies

To reach these criteria, the LEAF project focused on the following specific goals addressed in five technical work packages (see project structure in Fig. 2):

1. Optimized selection of biocide, for high efficacy against barnacles combined with low diffusivity in paint system.
2. Optimization of paint components, to minimize biocide emission rates and maintaining high stability to achieve long service life.
3. Development and optimization of co-biocide addition to achieve antifouling efficacy against a broader spectrum of fouling organisms.
4. Demonstration of industrial feasibility by scaling up production of a prototype paint to be used in full scale efficacy test on boats in different fouling conditions.
5. Sustainability assessment, including life cycle assessment, ecological and human safety aspects, in preparation for future registration and exploitation.

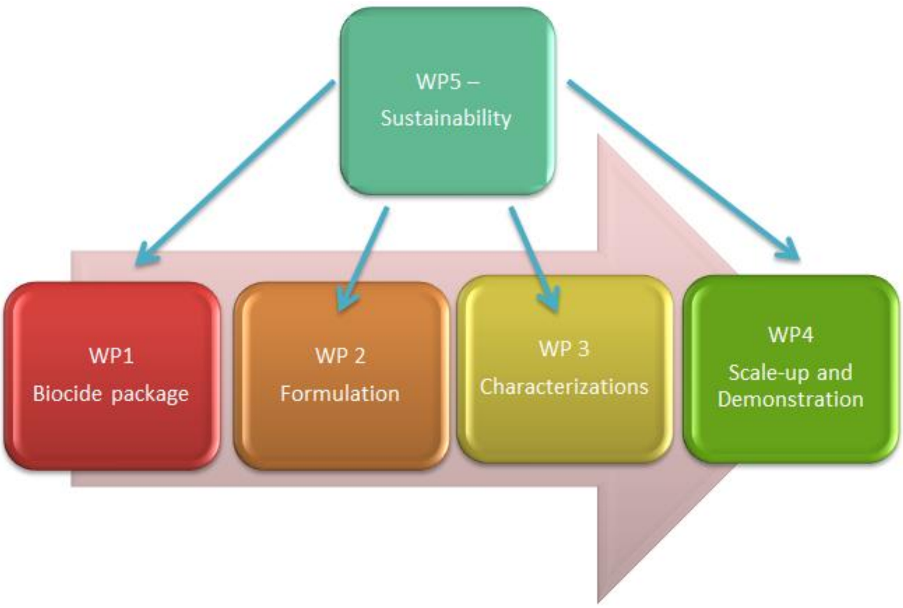


Fig 2. Work plan of the LEAF project describing the different work packages.

Main S & T results

This section provides a description of the main scientific and technological results achieved in the different work packages.

WP1 – Optimization of Biocide package

In order to screen for new environmentally benign biocides several bioassays were developed and implemented in the project. Bioassays targeting main antifouling species such as barnacles, algae and bugula were developed.

Enzyme based bioassay targeting macroalgae adhesion

It has been proposed that oxidases enzyme mediated polyphenol–carbohydrate (alginate) adhesive is involved in the adhesion process of marine algae from diverse taxa. Without good adhesion to substratum the establishment of an algae biofouling community is hampered. Thus, an enzyme based bioassay aimed of investigating eventual effects on inhibition of key enzyme involved in adhesion was developed. The aim was screening for new antifouling molecules with this novel mode of action. The research effort was focused on three important fouling macroalgae species from three taxonomic classes: Phaeophyceae (Undaria pinnatifida), Rhodophyceae (Porphyra sp.) and Chlorophyceae (Ulva sp.)

Initial investigation demonstrated the presence of key oxidase enzyme in all three species indicating that that the chosen enzyme could be used as target for new anti-algal compounds.

The bioassay was validated using commercial well known oxidase inhibitors. Note, these molecules demonstrate environmentally unacceptable effects and thus not suitable for antifouling purposes. Correlated results using a commercially available enzyme indicated that there was no need for extracting enzyme from algae for assessing the anti-algal effect of new compounds.

Three well known commercial co-biocides (Iragrol, Sea-nine-211 and copper tiocyanate) was analysed but none of the molecules showed any effect onthe chosen enzyme as anticipated. This result was stressing the possibility to screen for new anti-algal compounds displaying inhibiting activity of oxidase enzyme involved in bioadhesives synthesis.

Summary:

- The specific oxidase activity is conserved amongst the three classes of macroalgae: Phaeophyceae, Rhodophyceae and Chlorophyceae.
- This enzyme-based bioassay could provide a fast, non-specific and season-constraints free assessment of the adhesion process of macroalgae spores.
- The bioassay could allow the discovery of anti-algal products with non-toxic mode of action.
- This new developed bio-assay is fast, reliable and not subjected to seasonal or geographical variations.

Microalgae and bacterial bioassay

A rapid method for investigating antimicrofouling activity of antifouling compounds was developed based on a 96-well microplate. The inhibition of growth of 15 bacterial strains and 5 algal strains was evaluated. Both new natural antifouling and commercially available compounds were evaluated.

The method was used to demonstrate inhibitory effect on biofilm forming bacteria as well as inhibiting adhesion and growth of microalgae. Of the commercially available substances tested Thiram and Zink pyrithione generally showed low MIC values. Some of the natural antifouling products (NAP) screened showed similar inhibiting activity as commercial co-biocides and could be further explored in antifouling coatings.

As we approached the limit of detection of many of the analytical techniques biological assays were introduced to qualitatively assess the coating performance during formulation development. By applying thin model coatings on the inside of the wells of the 96-well plate the release of compounds could be evaluated. In this setting the method was used to validate the effect of biocide-paint component affinity pairs. For example, the release of Zink pyrithione could be controlled by addition of SiO_2 nanoparticles to paint formulations, see Fig 3.

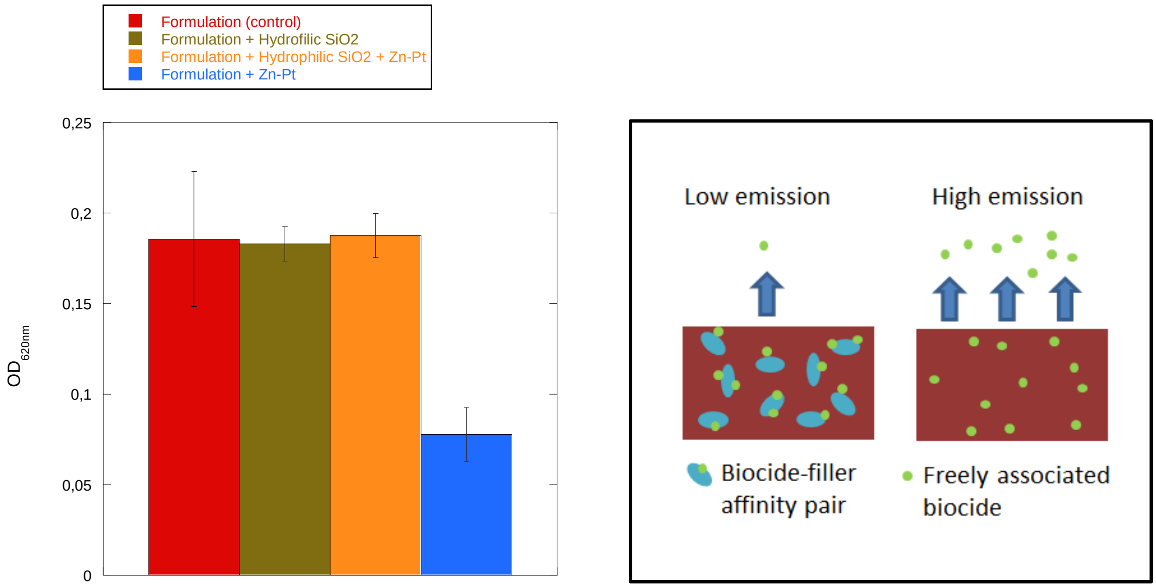


Fig 3. (Left) Absorbance measured at 620 nm demonstrated effect on micoalgae attachment upon addition of SiO_2 nanoparticles to the formulation. (Right) Schematic illustration of the proposed biocide-filler affinity pair model resulting in decreased emission.

Summary:

- This microfouling bioassay could provide a fast, non-specific and season-constraints free assessment of the inhibiting activity of compounds targeted against microfouling including biofilm forming bacteria and microalgae spores.
- The bioassay could allow the discovery of anti-microfouling compounds with non-toxic mode of action.
- This new developed bio-assay is fast, reliable and not subjected to seasonal or geographical variations.
- The method could also be used as a bioassay to qualitatively assess performance of model formulations

Barnacle bioassays

Bulk Bioassay

For screening of bioactive compounds targeted against barnacles a bulk barnacle bioassay was developed and used through the project. The bioassay uses the nauplii and cypris larvae of the notorious fouler, *Amphibalanus Amphitrite* as model organism. Parameters studies were the mortality of the nauplii larvae and inhibition of cypris settling upon addition of the compounds The antifouling potential of a natural antifouling products (NAP) analogue, 1-hydroxy-2-o-acyl-sn-glycero-3-phosphocholine, was shown to be effective against this barnacle species, see Fig 4.

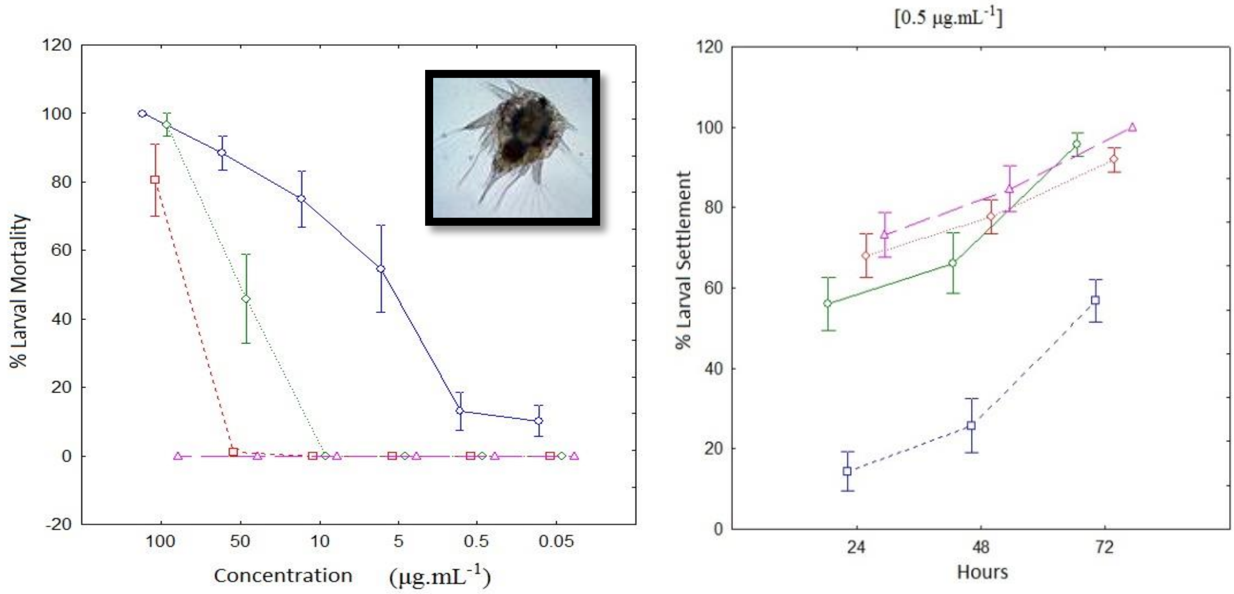
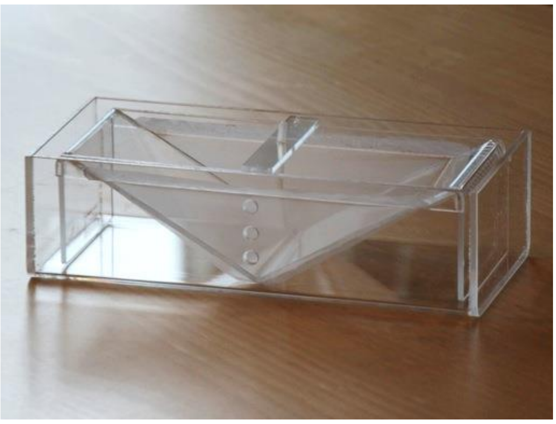


*Fig 4. (Left) Percentage (±SE) larval mortality of A. amphitrite nauplii after 24 hours in different concentrations of 1-hydroxy-2-o-acyl-sn-glycero-3-phosphocholine. (Right) Percentage of larval settlement of A. amphitrite cypris after 24, 48, 72 hours after incubation in 0.5 µg*mL^{-1} 1-hydroxy-2-o-acyl-sn-glycero-3-phosphocholine.Blueline: $CuSO_4$; Redline: Solvent; Green line: Compound; Purple line: Seawater control*

Post-settlement bioassay

In order to speed up the screening of potential low emission antifouling agents targeted against barnacles a post-settlement bioassay was developed. A novel settlement flow cell (Fig. 5) was developed and an all year round bioassay was established. The bioassay is designed to test inhibition of settlement of barnacle larvae (cyprids). In this work the species *Balanus improvisus* was used, which is available at Tjärnö Marine Laboratory from a year-round culture facility. This bioassay has specific characteristics that allowed the testing of a variety of newly developed paint formulations and biocides within LEAF project. (1) the constant flow of water through the cell prevents accumulation of biocides or other substances within the bulk water and it is possible to discriminate effects at the coating surface from effects by biocides in the bulk, (2) the assay can be run in choice and no-choice variants, (3) the design of the flow cell prevents any cyprids from settling on other surfaces than the exposed test panels, and (4) it allows adjustment of the water level within the flow cells.

The method was used to screen for post-settlement activity among several macrocyclic lactones as well as finding the optimal loading in the formulation as shown in Fig 6. Abamectin demonstrated similar activity as the Ivermectin. Abamectin is already used in crop protection, which facilitates registration as use in antifouling.



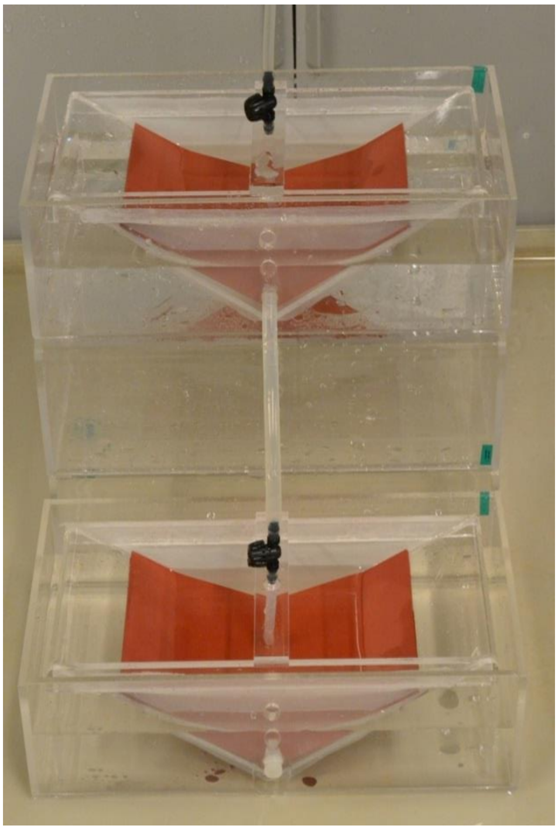


Fig. 5. (Left) Design of one flow-through cell placed in a transparent aquarium. The inflow is visible at the mid top and the outflow is shown at three alternative heights. The triangular sides of the flow cell are covered by 90-µm plankton net. (Right) Two flow-through cells with painted panels coupled in series to allow control for effects caused by eventual biocide released into the bulk water. Bulk water effects are recorded as settlement on control panels in the downstream flow cell.

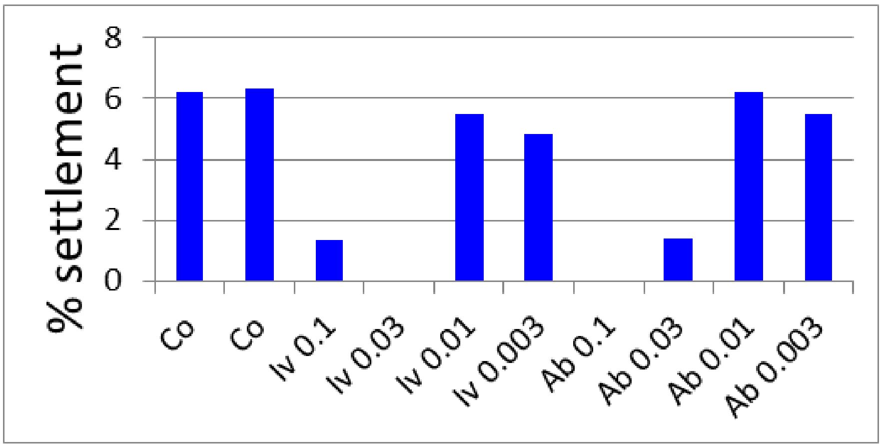


Fig 6. Results from a test with a low-emission LEAF paint with settlement in the downstream flow-through cell. Co is control panels with paint without biocide, Iv is paint with ivermectin and Ab is paint with abamectin. The numbers indicate the percentage of biocide in the paint.

Summary:

- The new flow-through cell for bioassay of barnacle cyprid settlement successfully addresses the problem of accumulation of toxin in the bulk water when using static tests.
- With the flow-through cells released toxin from coated panels can be washed out of the system, which was shown to be efficient in the test of a traditional copper paint.
- With biocides having strong surface affinity and low solubility in water there may be the potential problem of released toxin "creeping" onto all surfaces with a flow cell thus exerting a toxic effect also on control surfaces. This problem is solved by coupling two flow cells in series where the downstream flow cell contains control panels testing for any toxin released from test panels in the upstream flow cell. This was successfully demonstrated in a test with one of the versions of LEAF low-emission paint formulations.
- The new flow through bioassay adds a new tool to rapid laboratory-based first stage screening of candidate compounds for use in AF coatings.

WP2 - Development and formulation of antifouling coating system

The objective of this work package was to formulate the biocides selected in WP 1 into functional antifouling coatings with the desired LEAF characteristics, i.e. low emission. To reach objective novel screening assays were developed and implemented. One aim was to find high affinity biocide-matrix component pairs to prevent diffusion of low molecular mass substances resulting in high release to the surrounding water. The coatings should also display high durability and water stability to be able to withstand several fouling seasons. The general strategy was to screen commercially available matrix components already used in paint manufacturing. This to reach the performance indicator that up-scaling should be possible.

Another objective is to explore the possibility to produce an antifouling coating containing both the post-settlement anti-barnacles strategy and fouling release strategy.

Affinity between biocide-matrix components

Suitable matrix components such as fillers with affinity towards bioactive components were sought for. The filler particle could be used "as is" or chemically modified as shown in Fig 7. To reach objective two different methods were developed to screen for affinity between particles and bioactive substances. One was based on thin film chromatography (TLC) and the other was based on depletion and UV/Vis analysis. To measure affinity between binder and bioactive substances surface sensitive methods such as QCM-D, RESI and SPR was employed.

In Fig 8 the migration distance measured with the TLC method is plotted against the partition coefficient octanol/water (log P) of some selected antifouling substances. The short migration distance of Zink pyrithione (Zn-Pt) indicates high affinity towards non-modified SiO_2 particles. Zn-Pt is a co-biocide that could complement the activity of the macrocyclic lactones and was therefore selected as one lead substance for further development of antifouling coatings.

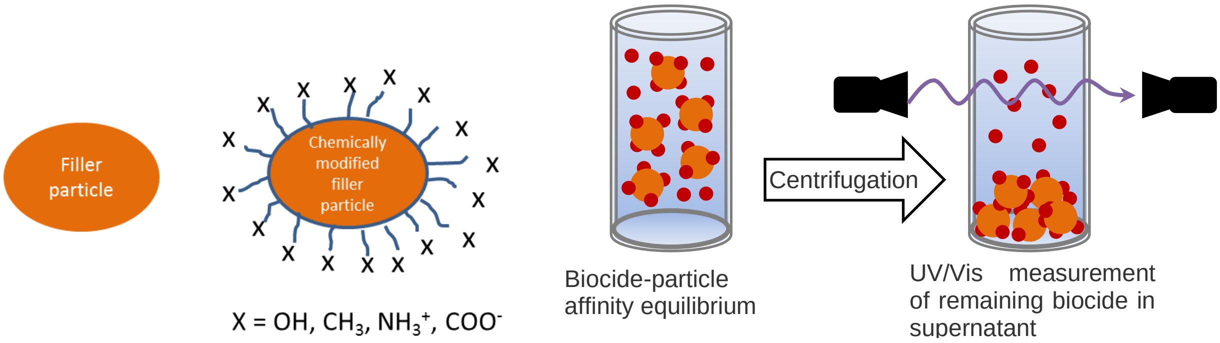


Fig 7. (Left) Schematic illustration highlighting the difference between a non-modified and chemically modified filler particle. (Right) Overview of the experimental setup for UV/Vis detection of affinity

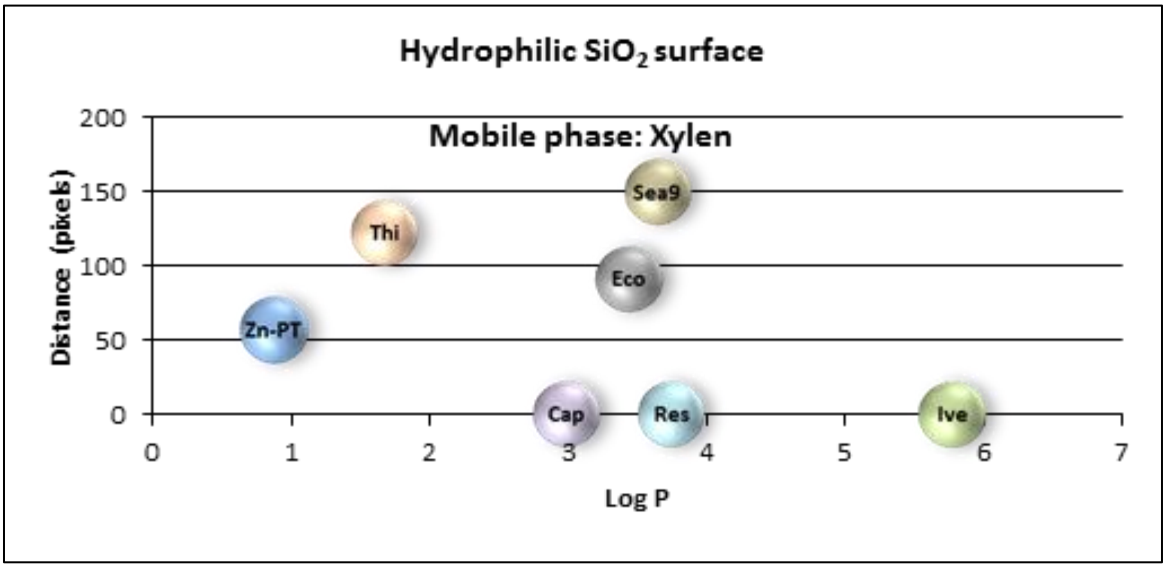


Fig 8. Distance travelled (pixels) of selected biocides on a hydrophilic TLC surface as a function of their individual Log P value.

Summary:

- A range of novel methods were developed in that could be used as screening tool during paint formulations.
- Biocide-particle pairs were found and further explored in the project

Mechanical properties with emphasis on erosion

Erosion or mass loss is important to control when designing coatings with extended life time. High erosion will result in high release of biocide to the environment based on mass balance and result in short service life of the coating.

It was demonstrated that a hydrogenation process (Fig 9) of the abietic acid present in the rosin binder significantly increased the water stability (Fig 10). By replacing the carbon-carbon double bonds present in abietic acid hydrogenated the molecule is less prone for oxidation. Oxidation results in increased water solubility and by this increased erosion. Hydrogenated rosin was therefore used in formulation development when rosin was the binder of choice.

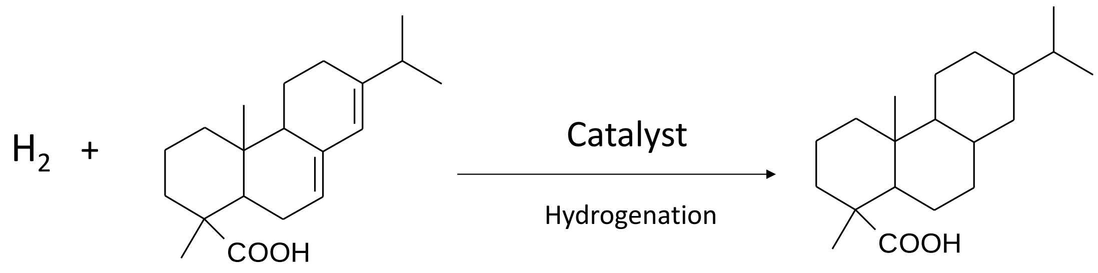


Fig 9. Chemical structure of abietic acid (left) and hydrogenated abietic acid (right). Abietic acid is the main component found in rosin binder.

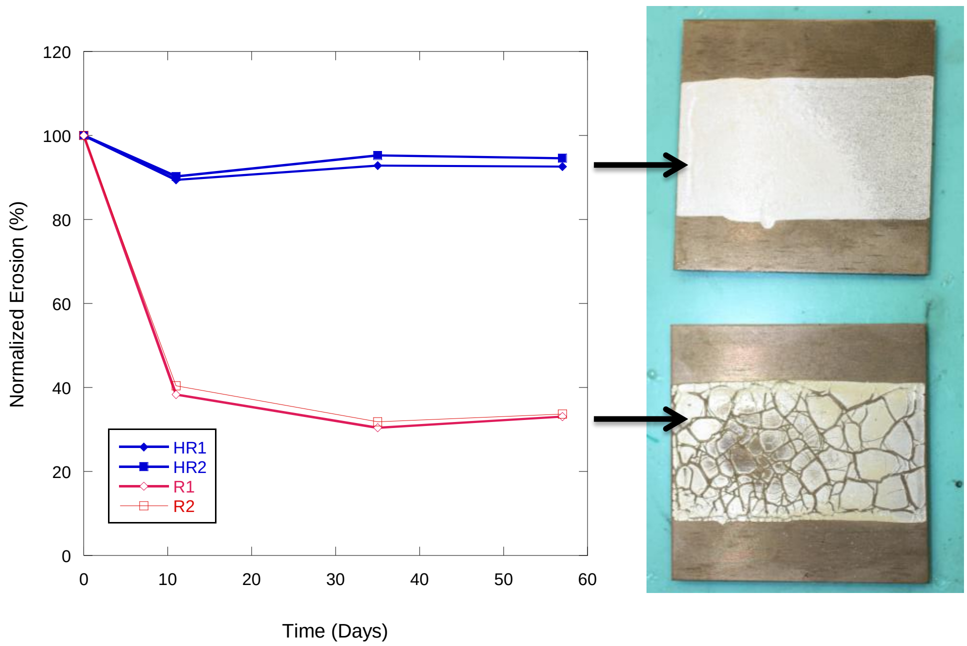


Fig 10. Erosion data in artificial seawater for rosin (no hydrogenation, R1 and R2) compared with hydrogenated rosin (HR1 and HR2). Also shown is a photograph of the two binders after 2 months of immersion displaying the difference in stability between the two binders.

Summary:

- The low emission and post-settlement mode of action required new screening methods before selection of matrix components.
- Binders displaying high water stability and Buchholz hardness below the threshold were selected for further use in the formulation development

Formulation development

Several aspects needed to be taken into consideration during formulation development. Filler particles were carefully selected to give not only affinity to bioactive substances but also control, for example, viscosity. Binder selection was important. Rosin, which is the main binder system used for amateur use, was evaluated but formulations for professional use in which other binder systems were explored were simultaneously developed and evaluated. In addition to binder selection we needed to include film former while keeping the hardness of the formulation under a given value. Not directly in objectives but also carried out during the project was to search for environmentally benign plasticizers. A large effort during the project was to develop a water-based formulation. To reach this objective a stable dispersion containing abamectin was developed.

As formulations were produced they were continuously characterized (WP3). The results were used in an iteration process in order to improve performance and antifouling efficacy. In Table 2 some of the data regarding formulations produced within the LEAF project is summarized. One of the performance criteria within the project was that the formulations developed should be feasible to produce in large quantities at commercially viable cost. It is therefore interesting to note that the rosin based LEAF formulations are actually significantly cheaper to produce than a commercial copper based reference formulation. Even, when using a high performing polymer binder the LEAF formulation can match the production cost of the rosin based commercial formulation. The main reason for the cost reduction is that the LEAF formulations do not contain any copper.

Table 2; List of selected formulations produced within the LEAF project

Formulation	Binder	Amateur (A) or Professional (P) use	Cost, relative to ablative antifouling paint on the market
LEAF 5.2	Rosin, ablative	A	60% cheaper
LEAF 6.0	Rosin, ablative	P	60 % cheaper
LEAF Prototype	Rosin, ablative	A	40% cheaper
LEAF 7.0	Polymer, SPC	P	Same cost
LEAF WB	Polymer, water borne	A	*

*Knowledge partly outside the LEAF consortium

Summary:

- Antifouling formulations based on the post-settlement mode of action could be produced at a commercially acceptable level.

Feasibility study of LEAF/FR

Silicone (PDMS) systems embedded with 0.1% macrocyclic lactone (ivermectin or abamectin) were prepared and extensive surface characterizations was performed followed by field studies.

Ivermectin embedded in a vinyl cured silicone system demonstrated good performance for two fouling seasons in Sweden, proving the feasibility of the concept. Barnacles were able to settle and metamorphose on the PDMS surface but they were not able to grow to adult barnacles. On the PDMS control panels with no ivermectin the surface rapidly got covered in an adult barnacle community.

By incorporation of PEG via the cross-linker the surface properties was altered (more hydrophilic). Low amount of PEG (2%) showed best performance and was denoted "god" to "acceptable" in the field study in Italy. Increasing the PEG content (4% and 10% respectively) reduced the performance of the PDMS system.

Summary

- Post-settlement antifouling activity could be achieved in silicone fouling-release coatings.
- Amphiphilic coatings by incorporation of PEG improved the efficacy of silicone fouling-release coatings

WP3 – Testing of antifouling coating system

The overall objective of this work package was to investigate important properties of antifouling coating systems with emphasis on LEAF characteristics, Fig 11. The coatings should display low biocide emission. They should be sufficiently soft to facilitate biocide bioavailability. They should be durable in harsh marine water environment to withstand several fouling seasons. And finally, display low toxicology towards non-target organisms. These properties are not totally independent from each other and were addressed simultaneously during the development of formulations. The formulations were evaluated in Field studies carried out in Sweden, UK, Italy and Brazil.

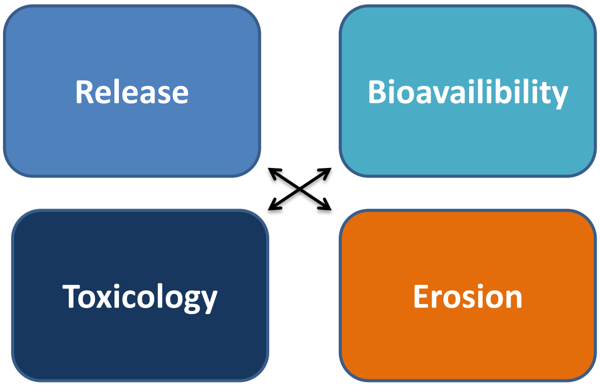


Fig 11. Coating properties investigated important to control in order to reach the proposed performance indicators.

Erosion

The erosion could be controlled by varying the ratio between the rosin binder and the acrylic film former as shown in Fig. 12. A ratio between rosin binder/acrylic resin of 0.9 was found to be the optimal. Further decreasing the ratio to 0.3 resulted in harder coatings, and by this reduced bioavailability.

Model formulation
Rosin binder
Acrylic resin
Mineral fillers
Pigment
Plasticizer
Wetting agent

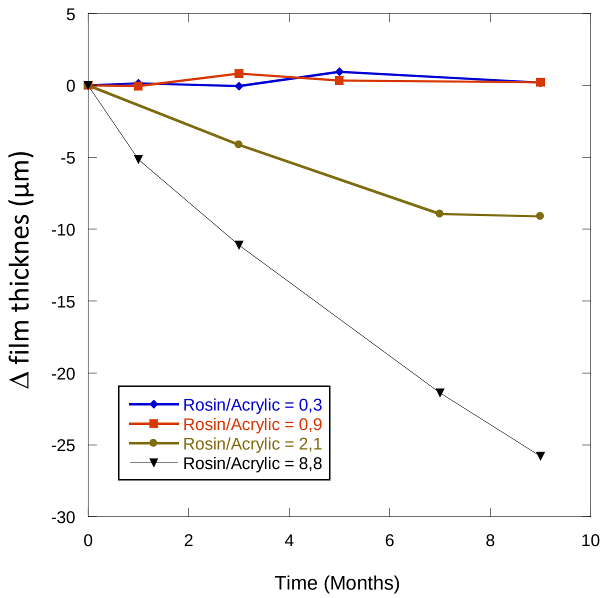


Fig 12. The change in film thickness (µm) of coatings exposed in circulating artificial seawater. The ratio between the rosin binder and acrylic resin was varied while other paint components were kept constant.

Biocide Release

Minimizing the unintended release was one main aim of the LEAF project. This was accomplished by fine tuning paint composition such as ratio of rosin binder to acrylic resin as well as controlling the type and volume fraction of mineral fillers. The optimized formulations both showed a decreased initial burst effect as well as decreased steady-state release. This is exemplified with the release of the lead molecule abamectin shown in Fig 13.

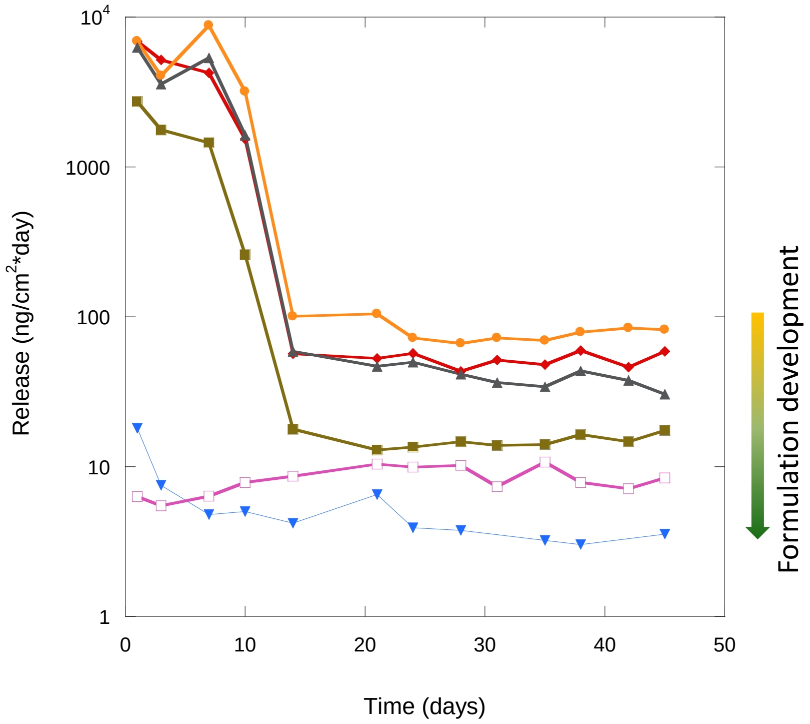


Fig 13. The release of abamectin as measured using the ISO 15181 methodology.

Toxicology

The toxicology was assessed by exposing non-target organisms, including red algae (*Ceramium tenuicorne),* crustacean *(Nitocra spinipes),* Pacific oyster *(Crassostrea gigas)* and coral reef *(Symbiodinum sp.),* for spiked biocide solutions as well as leachate water from LEAF formulations. Comparisons with commercial Cu-based formulations were performed.

Growth inhibition of *Ceramium tenuicorne* differed between the LEAF paint formulations and decreased as a result of the progress of development.

The sensitivity of *Nitocra spinipes* to leachate water from LEAF paint formulations was reflected both in the type of biocide used, as well as progress of formulation development, similarly to the results obtained using *Ceramium.* It was also found that other paint components used in the formulations displayed toxicity reflecting the importance to carefully select not only biocides but also other paint components.

Abamectin was not or weakly toxic to the fertilization and sperm motility in the Pacific oyster. Thus, abamectin is more active towards the target organism barnacle than to the non-target organism Pacific oyster.

Field tests

Antifouling efficacy was evaluated in field studies at four different locations varying in fouling pressure; Tjärnö on the west coast of Sweden (low pressure), La Spezia in the northern Mediterranean sea (average pressure), Portsmouth in UK (average pressure) and Guanabara Bay in Brazil (high pressure).

Optimized coating formulations showed good efficacy against hard foulers at all locations. Example of a static field panels exposed for one fouling season in Italy and in Sweden are shown in Fig 14.

Fig 14. (Left) Photographs of panels (Control and LEAF 5.2 formulation) exposed in Sweden (Right) Photographs of panels (Control and LEAF 5.2 formulation) exposed in Sweden. The control formulation contains the same components as in LEAF 5.2 but without the biocides.

Summary

- Release of abamectin from optimized LEAF formulations including LEAF prototype is in the order of 10 ng/cm2*day.
- Erosion of optimized formulations is well below 5 µm/year.
- Coating hardness of optimized formulations is below the stated limit of Buchholtz 75.
- The formulations show multi-season antifouling efficacy on static field panels and boat tests

WP4 – Technology demonstration and validation

In order to validate industrial feasibility and performance under real use conditions, the LEAF prototype paint was used in a demonstration activity including scale-up of production and use in a boat test in different geographical locations. In terms of technology readiness level (TRL), the demonstration test represented the necessary step from TRL6 to bring the LEAF prototype to TRL7, i.e., tested and demonstrated in industrial environment. In this particular case "industrial environment" is not only sea-water, but the conditions of production, distribution, and usage close to real life.

Scale-up of production

Based on the optimized formulation developed in WP3, 500 litres of LEAF prototype paint was manufactured in the facilities of industrial partner Boero Bartolomeo. The production process and quality control were essentially the same as those used for other antifouling paints. One interesting observation from the production was that the raw material cost for the LEAF prototype paint was lower than for standard antifouling paints.

Storage testing of the LEAF prototype paint according to standard methods indicated no changes in physico-chemical properties Furthermore, no sediments, no increasing of viscosity or gelling formation were discovered in the product.

The product was packaged in 2.5 litre standard cans for antifouling paints, which were labeled and shipped to the users in the boat test together with Technical Data Sheets and Material and Safety data Sheets. A can of LEAF paint is depicted in (Fig. 15).

Summary:

- 500 litres of LEAF prototype paint was successfully produced. The raw material cost was lower than for standard antifouling paint.

Fig 15. Can of LEAF prototype paint

Boat test

The boat test aimed at validating the performance and user satisfaction of the prototype paint. This demonstration test was not primarily designed as a scientific field test of an experimental formulation on boats. Instead the main priority was to obtain direct end-user (boat owners) feedback on the general performance and antifouling efficacy of the prototype paint, in order to assess the market acceptance.

A call for volunteer boat owners was issued, and by using traditional and digital information channels such as web pages, sector specific magazines, Facebook groups and many other distribution channels, the LEAF consortium managed to reach a very high number of boat owners. The announcement of the boat test in summer 2015 was announced early in 2014. When the online registration closed in March 2015, 133 volunteer boat owners had registered as interested in participating in the boat test. From these, a total of 55 volunteer from nine countries were selected, taking in consideration the parameters needed to be covered in the demonstration process. These parameters were geographical distribution, predicted usage rate of the vessel, type of vessel, type of hull (aluminium, glass fibre, wood, etc.) and many other, not forgetting the logistic feasibility. Some of the paint was distributed by surface transport, whereas many of the Swedish participants received their paint directly at SP's facilities in Borås, Sweden (fig. 16), where they were also invited to a short information seminar about the LEAF project.

The boat owners applied the LEAF-Prototype paint and launched their boats in seawater for the sailing season. The test for all 55 boats started between April/May 2015. Geographical locations for the testing included Scandinavian, Mediterranean, and Caribbean waters.

In September 2015, after 4 to 5 months testing, an online survey was distributed to all the 55 participants, who were asked to provide feedback on various aspects of the performance of the paint such as ease of mixing, applicability, ease of cleaning, antifouling efficacy, as well as general satisfaction and fulfillment of expectations. By February 2016, in total 38 of the testers had completed the questionnaire, and many of them had also provided photographs showing the appearance of the hull after the boating season. One example of a boat hull after the testing period is shown in Fig 17.

Fig 16. Distribution of LEAF prototype at information event at SP, Borås Sweden, 8 April 2015 (Source: Local Newspaper "Borås Tidning").

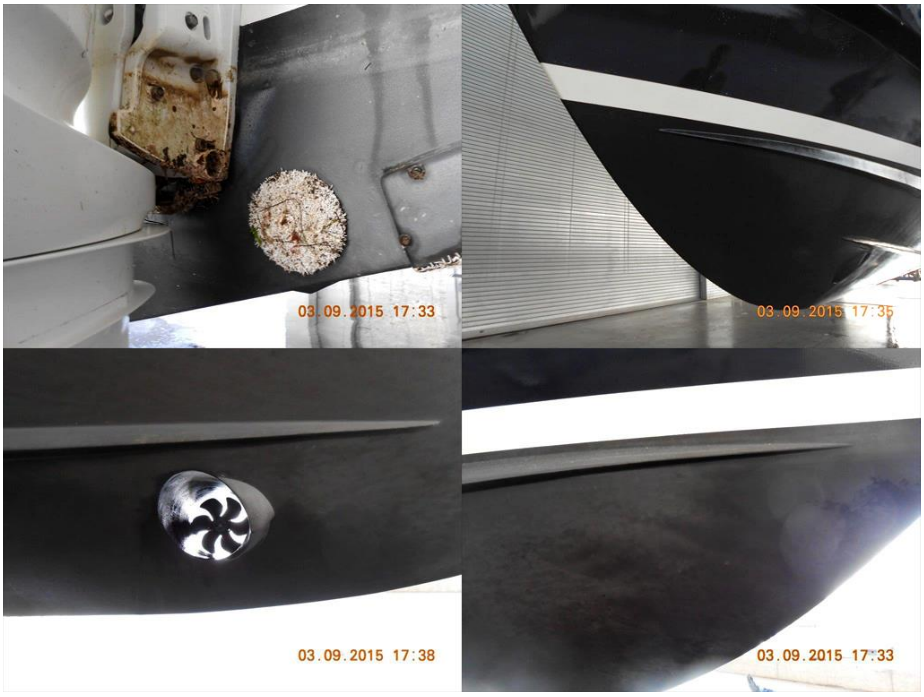


Fig 17. Example of boat hull after completed test for 4 months. No visible fouling can be observed, except on the circular cathodic protection area which had not been painted with LEAF paint.

The results from the test, according to responses in the questionnaire, showed an excellent antifouling efficacy. All (100%) respondent reported no or low presence of had fouling organisms (tube worms or barnacles). 75% reported no or low fouling by soft foulers (algae or slime). 44% of the respondents stated that the paint exceeded their expectations, while 52% stated that the LEAF prototype met their expectations extremely or very well. 95% of the interviewed boat owners report that the "*likelihood for them to buy LEAF-Prototype paint if this was on the market today*" is between "*extremely*" to "*very likely*" while the remaining 5% consider this as "*somewhat likely*" (see Fig 18).

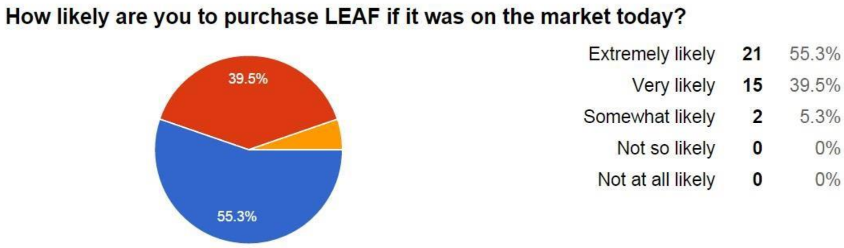


Fig 18: Market acceptance question.

Summary:

- The performance validation of the LEAF prototype paint, conducted with 55 boats in different geographical locations during the 2015 boating season, showed excellent overall performance and antifouling efficacy in the field, high user satisfaction, and very high market acceptance.

WP5 – Sustainability assessment

Since the LEAF project aimed at developing an antifouling coating with less negative impact on the environment than the current solutions on the market, a sustainability assessment was an important ingredient in the project. The sustainability assessment addressed several aspects. First of all, the impact of the whole lifecycle of the product on several important environmental impact categories was assess by a Life Cycle Assessment (LCA). To assess potential ecotoxicological effects, an aquatic risk assessment using established worst case scenarios. The risk assessment also addressed human safety aspects.

Life Cycle Assessment

The Life Cycle Assessment included all the different phases of the paint life cycles, from extraction and production of raw materials used in the paint or the paint packaging and the fuels used for energy, via transport and logistics, to removal of the paint after finished service life. The functional unit used in the study was 1 square meter hull painted with an efficient anti-fouling paint for 1 year. The result for the LEAF paints were benchmarked against a representative copper-based antifouling paint on the market.

The LCA results for global warming potential (see Fig 19), acidification potential and eutrophication potential showed less impact for the LEAF prototype and the other LEAF paints assessed in the project, when compared to the reference paint. The differences are relatively small and mostly due to the presence of copper in the reference.

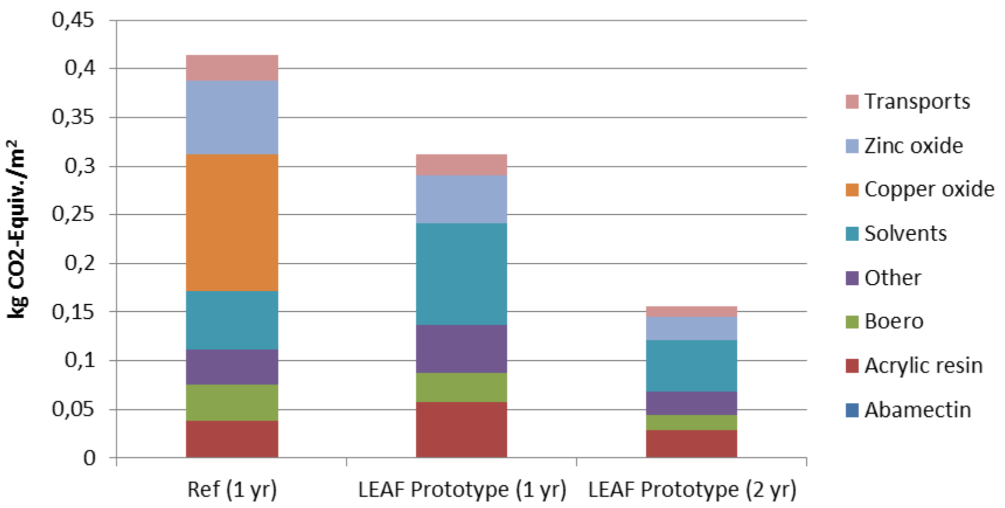


Fig 19. Global warming potential from the reference paint and Leaf prototype.

In a preliminary Life Cycle Cost (LCC) analysis, it was also shown that the LEAF paints have significantly lower life cycle costs than the reference paint. Since the goal of the project was to develop an efficient antifouling paint without increased environmental impact and cost, the LCA and LCC results are satisfying.

Summary:

- Life Cycle Assessment and a preliminary Life Cycle Cost assessment show that the LEAF paint has a lower environmental impact and lower life cycle cost than a representative copper-based reference paint.

Aquatic Risk and Human Safety

A preliminary risk assessment was conducted for standard OECD-EU marina and harbour scenarios, including in-life service and on-shore application/removal activity driven exposure scenarios. It was assumed that the “LEAF Prototype” paint containing abamectin (1 g/L) is applied on all vessels (i.e. 100% market share) and on the 20% of vessels. It was also assumed that all maintenance activities related to removal of paint from pleasure crafts (non-professional and professional) would have a worst-case emission to the marina surface water of 100%. A leaching rate of 4 $ng/cm^2/day$ (measured for the current LEAF prototype) and a future refined leaching rate of 1.4 $ng/cm^2/day$ were used for the in-life service scenarios.

A worst-case first tier risk assessment using a limited regulatory acceptable aquatic ecotoxicity dataset showed that for some marina and harbour in-life service emission scenarios the risks were initially found unacceptable. For the worst-case scenarios related to both application/removal in marinas and harbours risks were also found unacceptable but for the typical application/removal scenarios the risks were found acceptable. The risks to sediment dwelling organisms were found acceptable for all harbour and marina scenarios except for in-life service marina scenarios when a worst-case 100% application factor was assumed. When the more realistic 20% application factor was assumed the risk to sediment dwelling organisms were found acceptable for all scenarios.

As a first step, the risk assessment was refined by considering further chronic toxicity endpoints on marine species found in the open literature and non-GLP laboratory work conducted under the LEAF project. With this first refinement step the assessment factors for the derivation of the PNEC values were lowered and the risks were found acceptable for all harbour scenarios and for all application/removal marina scenarios. For marina in-life service scenarios the risks were found marginally unacceptable using the refined leaching rate only when a worst-case 100% application factor was assumed. When the more realistic 20% application factor was assumed risks to aquatic organisms were found acceptable for all scenarios. Risks to sediment organisms were found acceptable for all scenarios for both application factors assumed.

Additional ecotoxicity testing on more aquatic species can be used to further refine the risk assessment.

As LEAF paint’s key feature is the effective antifouling activity against barnacles by intoxication without the need to release the biocidal active in the water environment (i.e. not functioning as a standard biocide antifouling paint with slow release of the main and booster biocides) it is considered critical that for the demonstration of acceptable risks to the marine environment, evidence of the actual very low leaching rates is provided based on both standardised leaching rates tests and also by measurements of the biocide release from the paint film after the life time of the paint.

Summary:

- The risk assessment showed unacceptable risks for some of the worst case scenarios, while for more realistic scenarios the aquatic and human risks are acceptable. Additional ecotoxicity testing can be used for further refining of the risk assessment.
- The leaching rate is a critical parameter in determining the risks, and measurements of leaching rates in lab should be complemented by measurements after the life time of the paint.

Dissemination activities

The main objective of the disseminations activities in the LEAF project has been to raise the awareness of the feasibility and the benefits of the proposed novel approach in marine fouling control as an alternative to heavy metal leaching paints to prevent marine fouling on ship hulls. Beside the reporting of scientific results in scientific journals and at scientific conference, channels, such as public media and social media have been used raise the awareness of project and its achievements. Prioritized target groups of the dissemination activities have been, ship building, ship repair and ship painting industry¸ decision makers, and commercial as well as private ship and boat owners. A list of dissemination activities is provided at the end of this report.

LEAF Web page

The LEAF project website www.leaf-antifouling.eu was launched November 2012, and has since then been significantly revised twice. In the last revision, early 2015, a change of platform was made to the Wordpress platform, which facilitated continuous updating of the contents. This update also included linking to the LEAF Facebook page which is a more interactive communication channel. Using search engine optimization and successful backlinking from the early communication activities in public media, the LEAF web site has obtained high visibility; when searching Google on the search term “antifouling project” the LEAF web site is among the first hits.

Facebook page

The LEAF Facebook page https://www.facebook.com/LEAFantifouling/ was launched in November 2014. The main purpose was to provide an interactive forum for sharing information about the project in general, and especially the demonstration activity. In the latter, different boat owners where provided with a LEAF prototype to be tested under realistic conditions during the 2015 boating season. Reports and images from the users of the prototype paint have continuously been submitted on the Facebook page, and still continue to do so.

Public outreach

The LEAF project has been very successful in attracting the attention of public media. The project has appeared several times on Swedish TV and radio news, and in ca 10 articles in public press. Together with presentations at public and professional fairs, articles in SP’s news magazine which reaches ca 10,000 readers, and the activity at the LEAF Facebook page, this means that news of the project has reached a wide audience, even beyond the relevant scientific and professional communities.

Scientific communication

From the start, the LEAF project has had ambitious goals when it comes to scientific publications. Two articles in scientific journal, one PhD thesis, and 19 conference contributions have been published. Additional scientific publications from the project will appear after the end of the project.

Final conference

The LEAF Final Conference, entitled “Antifouling - New Perspectives” was organized in Gothenburg 29 September 2015. The conference gathered ca 70 attendees from 13 countries and representing industry, academia and other stakeholders. In connection with the Final Conference, a special symposium, “Combatting Biofouling”, was co-organized with the European Society for Marine Biotechnology.

Summary and concluding remarks

In summary, the LEAF project has fully achieved its objectives and developed and demonstrated new low emission antifouling coatings, with the following features:

- Antifouling efficiency towards a wide range of organisms, in parity with today's state-of-the-art, copper-based coatings currently on the market.
- Low biocide release rates, not relying on erosion of the coating, which provides a basis for increased service life.
- Lower environmental impact in terms of global warming potential, acidification potential and eutrophication potential
- Industrial feasibility, including lower production cost than standard antifouling paint on the market

The efforts done towards any innovation in the field of biocide based antifouling always face the hinder represented by the strict and necessary regulations for the introduction of biocide molecules and biocide products. The LEAF team was aware of this challenge from day one of the project, and this is why the LEAF consortium chose to start from the molecule abamectin. This molecule is already registered in the European Biocide Register for another use. Abamectin is registered and allowed for agricultural use in PT18 of the Biocide Pruduct Regulation. In a study done in the frame of the LEAF project, by a subcontractor, the identified data gap for possible registration of abamectin in PT12 (antifouling) was found to be relatively small; 2-3 additional toxicology studies need to be produced for registering the molecule itself. Other studies will be necessary when a paint company will register a paint based on this technology.

Regarding the registration of the molecule, a safe use will need to be shown to the authorities. For this reason LEAF produced also an Aquatic Risk Assessment showing good risk quotient in almost all the standard scenarios. This first assessment can be refined with more studies in the future when and if registration becomes actual.

So in conclusion, the LEAF consortium has not only demonstrated the efficacy, the feasibility and the environmental sustainability of an alternative antifouling paint, but also paved the way for future exploitation. In fact, contact with a chemical company has already been established and the next steps towards introducing a LEAF paint on the market have thus already been taken.

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List of dissemination activities

Publications in professional and scientific media

1. Emiliano Pinori, Low biocide emission antifouling based on a novel route of barnacle intoxication, *PhD Thesis*, University of Gothenburg, 2013-06-07 https://gupea.ub.gu.se/handle/2077/32814
2. Pinori E, Elwing H, Berglin M: The impact of coating hardness on the anti-barnacle efficacy of an embedded antifouling biocide. *Biofouling* **2013**, 29(7):763-773.
3. Trepos R, Pinori E, Jonsson PR, Berglin M, Svenson J, Coutinho R, Lausmaa J, Hellio C. Innovative approaches for the development of new copper-free marine antifouling paints. *Journal of Ocean Technology* **2014,** 9:7-18.

Scientific conferences, workshops and professional fairs

1. Mattias Berglin, Oral presentation at *Scandinavian Coating 2013*, Gothenburg, Sweden, 2013-05-29
2. Emiliano Pinori, Oral presentation at *Xth BIOINC 10th meeting on Bioufouling, benthic ecology and biocorrosion*, Arraial do Cabo, Brazil, 2013-07-29
3. Mattias Berglin, Oral presentation at *Smart & Functional Coatings*, Torino, Italy, 2013-09-26
4. Mattias Berglin, Oral presentation at *Green and Sustainable Materials and Surface Chemistry*, Stockholm, Sweden, 2013-10-23
5. Hans-Björne Elwing, Oral presentation at national meeting at *College of Shipping*, Kalmar, Sweden, 2013-11-26
6. Mauro Legrottaglie, Oral presentation at *Genova International Boat Show 2013*, Genova, Italy, 2013-10-(02-06)
7. Mauro Legrottaglie, Oral presentation at *Mets International Boat Show 2013*, Amsterdam, Netherlands, 2013-11-(19-21)
8. Emiliano Pinori , Oral presentation at *Aquaculture* 2013, Nashville, USA, 2013-02-(21-25)
9. E. Pinori, “Overview and Status of LEAF Project”, *17th ICMCF International Congress on Marine Corrosion and Fouling*, National University of Singapore, Singapore, 2014-07-07
10. C. Pansch, “Lab-based all year round anti-fouling bioassay to screen for pre- and post-settlement biocide activity against barnacle”, *17th ICMCF International Congress on Marine Corrosion and Fouling*, National University of Singapore, Singapore, 2014-07-09
11. R. Coutinho, “Antifouling tests of LEAF project in tropical waters” *17th ICMCF International Congress on Marine Corrosion and Fouling*, NUS National University of Singapore, Singapore, 2014-07-09
12. S. Garofoli, “New characterization methods in Low Emission AntiFouling coatings development” *17th ICMCF International Congress on Marine Corrosion and Fouling*, NUS National University of Singapore, Singapore, 2014-07-09
13. M. Berglin, "Non-covalent affinity between biocides and matrix components – Friend or Foe?" *17th ICMCF International Congress on Marine Corrosion and Fouling*, National University of Singapore, Singapore, 2014-07-07

14. C. Hellio, "Low Emission AntiFouling (LEAF) Project: Development of an antimacroalgal assay based on enzyme inhibition", *17th ICMCF International Congress on Marine Corrosion and Fouling*, National University of Singapore, Singapore, 2014-07-08
15. E. Pinori, "Fate of barnacles on paint containing 0.1% Ivermectin" – 2nd International *Symposium "Current Topics on Barnacle Biology"*, Tropical Marine Science Institute, NUS National University of Singapore, 2014-07-11
16. C. Hellio, "Biomimetics approches for the development of new antifouling and anticorrosion solution", *XIth BIOINC 11th meeting on Bioufouling, benthic ecology and biocorrosion*, Arraial do Cabo, Brazil, 2015-07-27
17. M. Berglin, "Galenic antifouling", *XIth BIOINC 11th meeting on Bioufouling, benthic ecology and biocorrosion*, Arraial do Cabo, Brazil, 2015-07-28
18. E. Pinori, "From research to innovation – the LEAF project", *XIth BIOINC 11th meeting on Bioufouling, benthic ecology and biocorrosion*, Arraial do Cabo, Brazil, 2015-07-28
19. M. Legrottaglie, "Antifouling paints – from a paintmaker's perspective", *XIth BIOINC 11th meeting on Bioufouling, benthic ecology and biocorrosion*, Arraial do Cabo, Brazil, 2015-07-28

Public outreach (Popular presentations, public media)

1. Emiliano Pinori, "New methods to combat barnacles", *Technology and Research* (customer magazine of the SP Group), Nov 2012, p 8-10 http://www.mypaper.se/show/sp/show.asp?pid=345355910866694
2. Mattias Berglin, Popular presentation on Marine antifouling at the *SP Day*, Stockholm, 2013-04-18. 450 delegates including SP people and industry
3. Mattias Berglin, Popular presentation on Marine antifouling at *Gothenburg International Science Festival*, Gothenburg, 2013-04-27
4. Emiliano Pinori, *Båtliv* (major Swedish boating magazine) , 2013-07-03,
5. Emiliano Pinori, "Barnacles dig their own grave", *European Coating*, 2013-07-04, http://www.european-coatings.com/Raw-Materials-Technologies/Applications/Protective-Marine/Barnacles-dig-their-own-grave
6. "Båtägare kan slippa havstulpaner med ny miljövänlig metod", Press release about Emiliano Pinori's PhD Thesis (SWE); 2013-06-25, national and international (Scandinavian) level of dissemination reached http://www.science.gu.se/aktuellt/nyheter/Nyheter+Detalj/?languageId=100000&contentId=1175568
7. "Boat Owners Can Fight Barnacles with New Eco-Friendly Method", Press release about Emiliano Pinori's PhD Thesis (ENG); 2013-06-25, international level of dissemination reached, http://www.science.gu.se/english/News/News_detail/?languageId=100001&contentId=1175568
8. Article in daily news journal *Metro*, Gothenburg, 2013-06-30
9. Article in daily news journal *GP -Göteborgs Posten*, 2013-07-01
10. Article in daily news journal *BT - Borås Tidning*, 2013-07-02
11. Article in daily news journal *SN – Södermansläns Nytt*, 2013-07-05

12. ”Ny miljövänlig metod ska ge rena båtbottnar”, *Ny teknik*, 2013-07-06 http://www.nyteknik.se/nyheter/innovation/forskning_utveckling/article3720339.ece
13. Emiliano Pinori (interviewed), *TV4 Västnytt* (Swedish regional TV news), 2013-07-12, http://www.svt.se/nyheter/regionalt/vastnytt/genombrott-i-kampen-mot-havstulpanen
14. Emiliano Pinori (interviewed), *TV4 Aktuellt* (Swedish national TV news), 2013-07-12
15. Emiliano Pinori (interviewed), *Radio P4 Gothenburg* (one of the four Swedish public radio channels), 2013-06-26
16. Emiliano Pinori (interviewed), *Radio P4 Blekinge* (one of the four Swedish public radio channels), 2013-06-27
17. Jukka Lausmaa, Seminar at *Arctic University*, Tromsö, Norway, 2014-06-03
18. Emiliano Pinori (interviewed), *Borås Tidning*, 2015-04-09
19. Mia Dahlström and Emiliano Pinori, “Marine antifouling for all applications”, *SP-INFO* 2014:22
20. Emiliano Pinori (interviewed) *Swedish Radio P1*, 2015-04-10
21. Emiliano Pinori (interviewed), *SEIL Magasinet* (Norwegian sailing magazine), 2015-04-14
22. *LEAF Final Conference*, including 11 oral presentations from the LEAF project, Gothenburg, Sweden, 2015-09-29

Web sites

1. LEAF internal Website; An intranet web-page/share point accessible only by login for project partners, launched 2012-10-17, http://team.splogin.se/sites/leaf/SitePages/Home.aspx
2. LEAF public website; An internet web site with non confidential (or public dissemination level) project informations, first published 2012-10-18, www.leaf-antifouling.eu
3. LEAF Facebook page; an interactive web page, first published 2014-11-24, www.facebook.com/LEAFantifouling/