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Report

Evaluating the efficacy of alternative methods for biofouling control in aquaculture (ALLEGRO)

Prosjektrapport
"Dokumentasjon av effektivitet av alternative metoder for begroingskontroll"

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ABSTRACT

Abstract heading

Biofouling is a challenge in Norwegian salmon farming, especially on nets, but also on equipment such as sensors. Todays' antifouling coatings rely mainly on copper, which is an environmental hazard, and alternative solutions are desired. This project compared the efficacy of 6 alternative antifouling coatings for nets (two with reduced copper content, three with alternative biocides and one biocide free coating) against a commercial copper coating and an uncoated control. For the sensors, two alternative adhesive antifouling films with improved copper leaching control were tested against a copper tape and an uncoated control. Field test results showed that one of the coatings with lower copper content performed comparable to the copper control while the rest was colonised by biofouling faster and/or at higher abundances. The novel antifouling films for sensor performed equally well as the copper control, while indicating higher durability. None of the tested products was able to prevent biofouling entirely, underlining the importance of the search for alternative and improved antifouling technologies.

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1 Norsk sammendrag

Begroing er et begrep for uønsket vekst av marine organismer på strukturer i sjøen, og er en av de største utfordringene i dagens laksenæring. Begroing kan medføre både strukturelle (økte krefter på anlegg og deformasjon av not) og biologiske (f.eks. redusert effektivitet av rensefisk og redusert oksygennivå) konsekvenser for produksjon av fisk. En mye brukt metode for å hindre begroing er å dekke not og andre komponenter med kopperbasert impregnering som er giftig og dermed virker frastøtende på organismer som søker å etablere seg. Konvensjonelle impregneringsprodukter har imidlertid begrenset varighet, og har dermed ikke evnen til å yte tilstrekkelig beskyttelse gjennom en hel produksjonssyklus. Dagens praksis innen næringen har derfor utviklet seg mot at en bruker en kombinasjon av kopperbasert impregnering og *in-situ* notspyling med høytrykk. Rengjøring av nøter er imidlertid kostbart og medfører økt slitasje på nøter og impregnering, samt utslipp av begroingsmateriale som kan virke negativt på fiskens helse. I tillegg er det et økende press fra forbrukergrupper om at kopperutslipp fra oppdrettsanlegg må begrenses av hensyn til miljøet.

I likhet med for nøter, kan begroing også forårsake utfordringer for instrumenter og sensorer som brukes i oppdrett. Slike enheter må derfor rengjøres regelmessig for å sikre at begroingen ikke hindrer korrekt funksjon. Et tilleggstiltak til dette kan være å dekke instrumentenes overflater med koppertape som har til hensikt å hindre etablering. Slik tape har imidlertid begrenset levetid og er vanskelig å bytte ut. Det er derfor også behov for bedre antigroemidler for overflater.

Hovedmålet i ALLEGRO var å evaluere effektiviteten av alternative impregneringer for begroingskontroll på nøter og andre overflatestrukturer

Prosjektet testet seks alternative impregneringstyper for nøter: to med redusert kopperinnhold, tre med andre biocider enn kopper, og én uten biocider. Prøvene ble sammenliknet med to kontrollgrupper med hhv. konvensjonell kopperimpregnering og uten impregnering. Prøvene ble satt ut på en kommersiell oppdrettslokalitet i to perioder på seks (mars-oktober) og tre (juni-oktober) mnd. Gjennom begge forsøksperiodene ble parametere som begroingens blokkering av maskeåpningen i nota, dekningsgrad på notmaterialet og artsrikhet jevnlig registrert via bildeanalyse, mens dens våtvekt ble registrert ved slutten av perioden. Resultatene fra testene viste at én av impregneringene med lavt kopperinnhold hadde en effektivitet sammenliknbar med effektiviteten til den konvensjonelle kopperbaserte impregneringen. De andre alternativene ble tidligere kolonisert av begroingsorganismer enn den impregnerte kontrollprøven og/eller hadde større mengder begroing enn denne. Ingen av de alternative metodene virket derfor å være bedre enn dagens standardløsninger sett fra et rent begroingsperspektiv. Her må det også bemerkes at selv de beste impregneringene ikke klarte å hindre begroing i hele forsøksperioden, noe som understreker behovet for nye løsninger som kan holde en hel produksjonssyklus.

For andre overflatestrukturer ble to nye typer selvklebende film mettet med forskjellige konsentrasjoner av kopperpartikler testet. Disse ble sammenliknet med kommersielt tilgjengelig koppertape designet for begroingshindring og en ubehandlet overflate, og ble satt ut i en periode på 10 mnd. (mars-januar). Også her ble faktorer som dekningsgrad, artsrikhet og våtvekt vurdert månedlig. Resultatene fra testene indikerer at begge kopperfilmene var like effektive i å hindre begroing som koppertapen. I tillegg viste kobbertapen viste tegn til større slitasje på grunn av utslipp av kobber, noe som indikerte at filmene kan antas å være effektive i en lengre periode enn tapen. Til sammen gjør dette filmene til attraktive alternativer.

2 Introduction

Biofouling is the undesired growth of marine organisms on surfaces submerged at sea, and is one of the major challenges in today's salmon farming. Biofouling can cover up to 100% of the mesh openings in cage nets and growth of organisms such as algae, bivalves and hydroids can lead to net deformation and volume reduction (Cronin et al. 1999, Braithwaite et al. 2007, Lader et al. 2008, Guenther et al. 2010). The presence of biofouling can further reduce the effectiveness of cleaner fish (employed to feed on salmon lice), by providing a convenient food source (Kvenseth 1996). Biofouling on farm nets may also affect fish health, by reducing oxygen levels, limiting the removal of waste and facilitating the exposure to pathogens associated with biofouling organisms (de Nys and Guenther 2009, Fitridge et al. 2012).

To limit the impacts of biofouling, farmers commonly use antifouling (AF) coatings on their nets. Most current antifouling coatings contain cupreous oxide as a biocidal agent due to its toxicity to biofouling organisms. The problem related to the use of such antifouling coatings is that, in addition to the copper accumulating in the sediment beneath farms (Loucks et al. 2012, Nikolaou et al. 2014), negative effects relating to embryonic development, movement, enzymatic activity and gill health have been reported from non-target organisms including fishes, invertebrates and algae (reviewed in Burridge et al. 2010).

Today's copper-based coatings are unfortunately not able to prevent biofouling for the entire production cycle. Nets are reported to be fouled after an average of 3 months, and sometimes as fast as after just 4 weeks (SINTEF, unpublished data from NFR Project No. 245480). This forces the farmers to combine copper coatings with the cleaning of the nets using *in situ* high pressure washers. High pressure cleaning is demanding and expensive and poses a variety of risks. For example, mechanical damage to nets caused by abrasive cleaning could lead to the escape of fish, while contact with biofouling removed from the net can irritate the gills or transfer pathogens from the biofouling to the fish (reviewed in Floerl et al. 2016).

In recent times, increasing demand from customers that conduct organic salmon farming, and the increased uptake of certification standards such as the Aquaculture Stewardship Council (ASC) Salmon Standard, which aims to control the discharge of copper associated with salmon farming (Aquaculture Stewardship Council 2012), has led to increased interest in alternatives to copper-coatings for production nets. Available alternatives on the market today include biocide-free coatings, whose smooth, durable surface is intended to facilitate regular washing. Net variations made of plastic (eg EcoNet from Akvagroup) are based on a similar (biocide-free) principle. All of these, however, result in increased cleaning requirements, with the associated risks on net integrity and fish health.

Although the project was mainly aimed at biofouling of nets, biofouling is also a challenge for all other objects at sea in connection to fish farms. This includes structures such as cage collars or mooring equipment (Bloecher et al., 2015), but is especially true for optical sensors such as those used to monitor feeding (Manov et al. 2004, Delauney et al. 2010). Maintenance on sensors must be carried out regularly and carefully to ensure that the sensors deliver the correct data (Delauney et al. 2010), which is essential for a farming industry that depends increasingly on automated operations in order to cope with increasing farm size and expansion to more remote and exposed locations. Currently, solutions for biofouling protection of sensors are scarce. The project therefore included testing of a novel antifouling approach for static surfaces – adhesive antifouling films.

The main objective of the ALLEGRO project was to evaluate the effectiveness of alternative methods for biofouling control on cage nets and other aquaculture substrates. In the project, we tested the antifouling performance of two coatings with reduced copper concentrations (relative to a commercial control), three coatings based on alternative (non-copper) biocides and one biocide-free coating. As novel antifouling technology for sensors, the project evaluated two variations of a copper-embedded film.

3 Material and Methods

3.1 Net sample preparation and placement

A total of six antifouling (AF) coatings were tested: two coatings with a copper content below 5 % (Low 1 and 2), three coatings free of copper, based on alternative biocides (Alt. 1 – 3), and a biocide-free coating (Free) (Table 1). These six novel coatings were compared to two controls: uncoated net samples, and net samples coated with a classic copper-based antifouling coating featuring a cuprous oxide (CuO) concentration of ~20 %).

The test was conducted in two trials, aligned with the periods when hatchery-reared fish are transferred to sea in Norway. The samples of Trial 1 were deployed on 31.3.2017 (Spring trial), and the samples of Trial 2 were deployed on 28.06.2017 (autumn trial). All samples were retrieved on 3.10.2017 after 6 and 3 months at sea, respectively.

Net samples (20 x 20 cm) were cut from a single piece of net material (Raschel knitt nylon smolt netting, Egersund Net) and mailed to each producer, where they were coated (n = 12 per coating and trial). Each sample was given an individually numbered tag (micro ear tags, OS ID), before they were attached to four metal frames (24 samples per frame) in random order using cable ties (Fig. 1). Each individually marked frame held three replicates of each coating (including controls), with samples placed one sample size apart. The frames were distributed to four locations at SINTEF's research farm site Rataren, Frøya (Fig. 2).

Table 1: Overview of the coatings tested

ID	Working principle	Name	Producer
Blank	Uncoated net	Uncoated control	Egersund net
Cu Ctrl	Cuprous oxide >20 %	"Classic copper" control	NetKem
Low 1	Low copper content (CuO = 0.6 %) + copper pyrithione	Brynsløkken I	Brynsløkken
Low 2	Low copper content (CuO <5%) + zinc	EX15-E25 (NetKem II)	NetKem
Alt 1	Algaecide and organic biocide	Brynsløkken II	Brynsløkken
Alt 2	Alternative biocide (2.9 % Econea)	EX16-S15 (NetKem I)	NetKem
Alt 3	Boron compound	Nitto Boron Paint (NBP)	Nitto Seimo
Free	Biocide-free, based on alternative substance	EX16-NO5 (NetKem III)	NetKem

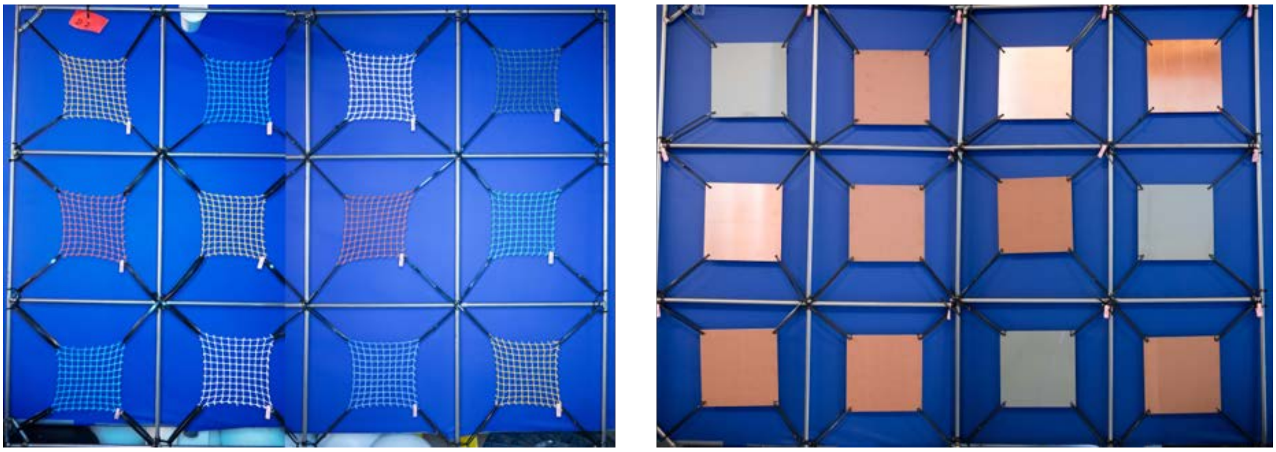


Fig. 1: Half of a frame holding coated net samples (left) and a frame with antifouling films on PVC panels (right).

3.2 Adhesive antifouling film preparation and placement

Two types of self-adhering ‘antifouling films’ embedded with a loading of either 586 g m^{-2} (high) or 306 g m^{-2} (low) of copper particles were compared to two controls: untreated films (no copper) and a commercially available copper shim tape (367 g m^{-2}, McMaster-Carr Supply Co, USA; Table 2). The novelty of the adhesive films was that copper ions had been embedded in the film using cold-spray technology, providing a biocidal surface with controlled release rates (Vucko et al. 2012). Furthermore, the films can be manufactured to cover any surface shape and are comparatively easy to remove when an exchange is desired. In contrast, the copper shim tape consisted of a thin layer of copper with an adhesive backside that leaches copper similar to a coating. These tapes are difficult to remove or exchange when spent.

The experimental antifouling films were manufactured by CSIRO (Australia) and, along with the tape, attached to PVC panels (n = 12) before shipping to Trondheim. Panels were mounted to four metal frames in random order using cable ties (Fig. 1). Individually numbered tags (micro ear tags, OS ID) were attached to the metal frame above each sample. Each individually marked frame held three replicates of each surface protection, with samples placed one sample size apart. The frames were distributed to four locations at SINTEF's research farm site Rataren, Frøya, along with the net samples (Fig. 2). Since preliminary performance tests of the films indicated a longer AF activity than the nets (CSIRO, pers. comm.), these samples were deployed as a single series on 31.3.2017 and recovered on 18.1.2018 after 10 months of immersion.

Table 2: Overview of the antifouling films tested

ID	Working principle	Producer
Blank	Control, untreated film	CSIRO
High Cu	PU film embedded with copper particles (586 g Cu m^{-2})	CSIRO
Low Cu	PU film embedded with copper particles (306 g Cu m^{-2})	CSIRO
Cu Tape	Copper shim tape (367 g Cu m^{-2})	McMaster-Carr Supply Co, USA

Fig. 1: Yellow markers indicate the four locations of frames holding net and antifouling film samples at the Rataren farm site.

3.3 Sampling and analysis

Biofouling growth on net and adhesive film samples was monitored monthly via high-resolution photographs. Before photographing, the frames were cleared of biofouling and the samples were gently rinsed with saltwater from a spray hose to remove silt and entangled debris. The samples were then photographed vertically against a blue background under artificial light to optimise contrast. The experimental trials were terminated once biofouling accumulation in the copper control treatments had reached high abundances. For the spring and autumn trial involving net samples, this resulted in test durations of 6 and 3 months, respectively. The test of the adhesive antifouling films on PVS panels was extended to 10 months due to very slow biofouling development on all coated samples. Due to adverse weather conditions and logistic challenges it was not possible to sample in June and December 2017.

The monthly pictures of the net samples were analysed using two methods: (i) Percentage Net-aperture Occlusion (PNO), which calculates the total contribution of biofouling to the occlusion of the net aperture, was measured using a method described in Guenther et al. (2010). Using this scale, a PNO value of 50 applies to net samples where 50 % of the net aperture is obstructed by biofouling, potentially hindering water flow through. (ii) Fouling Resistance (FR) was measured according to the ASTM standard (American Society for Testing and Materials ASTM 1998), modified to evaluate nets. Using this scale to evaluate the biofouling organisms found on the actual net, a FR value of 100 describes a surface free of fouling, whereas FR=50 applies to surfaces half covered in biofouling. The analysis was based on the identification of taxonomic groups at 66 points on the net, defined by the intersection of the net with 3 randomly placed vertical and horizontal lines. Slimes and algal spores <3 mm were not counted as fouling (FR score = 100 even in their presence), while the presence of larger fouling organisms resulted in a default reduction of the FR score to 95, or lower depending on fouling abundance (American Society for Testing and Materials ASTM 1998). Clearly identifiable dead algal material that had drifted into the net was excluded from the FR analysis. It was not possible to exclude this material from the PNO analysis (Fig. 3).

While PNO is a measure often used for the assessment of nets and AF coatings, concentrating on the obstruction of the net openings and the reduction of its functionality, FR was adapted from a standard used for the assessment of AF coatings on hard substrates and focuses on the functionality of an AF coating (American Society for Testing and Materials ASTM 1998).

For the antifouling film samples on PVC panels, biofouling accumulation was assessed (to broad taxon level) through evaluation of fouling resistance based on 66 random sample points (American Society for Testing and Materials ASTM 1998). The outer 1 cm of the sample panel was not included in the analysis to accommodate for the potential impact of biofouling growing on the outer, unprotected panel edge or backside, obscuring the sample.

Based on the abundance of taxonomic groups identified on the samples in the process of the fouling resistance assessment, Species Richness (S; the number of taxa found on a given sample) and community composition (contribution of individual taxonomic groups to the biofouling community of individual samples) were calculated for both net and antifouling film samples.

At the end of the immersion period, the total weight of accumulated biofouling on nets and panels was measured by subtracting the wet weight of samples before immersion from the total weight after the last sampling event. Since the back and sides of the PVC panels were uncoated, biofouling was removed from these surfaces before the final weight was taken. The weight of the coated samples was standardised relative to the individual uncoated control (calculated per frame). This allowed the presentation of the data as relative biofouling wet weight reduction compared to the uncoated control.

Quantification of the copper leaching from the copper shim tape was accomplished, similar to the analysis of PNO, through a dedicated software developed in LabVIEW. The original image was digitised and segmented (thresholding) based on a colour model focusing on hue, saturation and value, identifying the remaining copper on the sample panel against the background of the PVC. The model best representing the remaining copper was chosen manually. The number of pixels representing the remaining copper was compared to the number of pixels representing a full panel size, allowing the calculation of the remaining percentage of copper cover.

The performance (FR, PNO and relative WW) of the individual coatings was compared separately for the net samples and antifouling films using permutational analysis of variance (PERMANOVA, PRIMER v.7.0). Analyses were based on Euclidian distance with 9999 unrestricted permutations of residuals under a reduced model. A significance level of 5 % was used. Where the number of unique permutations was ≤100, the Monte-Carlo asymptotic pMC-value was consulted (Anderson et al. 2008). The analysis of the net samples was performed only for the month of August, when surfaces associated with the spring and autumn trial had been immersed for a period of 5 and 2 months, respectively. This timeframe was chosen deliberately, because in August the copper control treatment still had visible antifouling ability, but the blank control treatment was already fully fouled. The analyses included the factors "Time" (spring vs. autumn samples; fixed), "Location" (Location of the frame with samples on the farm site (4 locations, random), and "Coating" (Coating type, fixed).

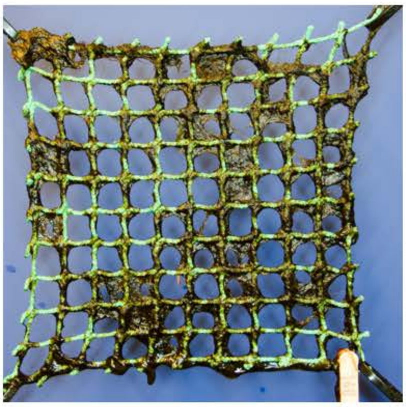
The analyses of the antifouling film samples were limited to the three active antifouling films. The control was excluded from the analysis as no statistics were required to show that the biocidal treatments were effective. Furthermore, the analysis was limited to the months were biofouling was encountered on the panels. The three antifouling films were compared (FR, relative WW) using a repeated measures model including the factors "Time" (length of immersion, 7, 8 or 10 months, fixed), "Location" (4 locations, random) and "Coating" (film type, fixed). Where PERMANOVA indicated no significant differences between locations (significance level of 25 %) this term was pooled to increase power (Anderson et al. 2008).

Based on the performance of the coatings assessed with different measurements, a summary table was created to show the major trends and give an indication of a performance ranking in major groups.

All values presented in the text are means if not indicated otherwise.

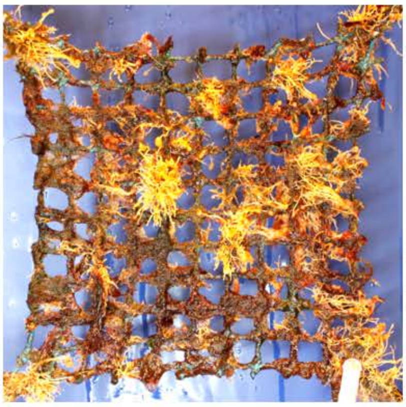
←
Free
3 months
FR: 90
PNO: 6

←
Alt 3
3 months
FR: 100*
PNO: 22*

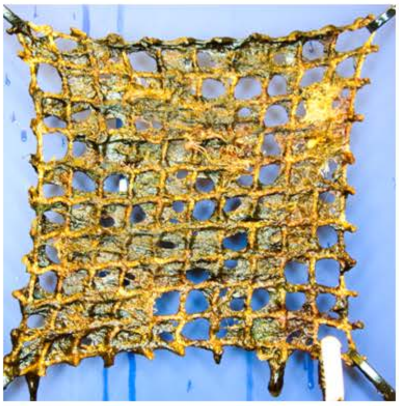


←
Lav 2
3 months
FR: 100*
PNO: 36*

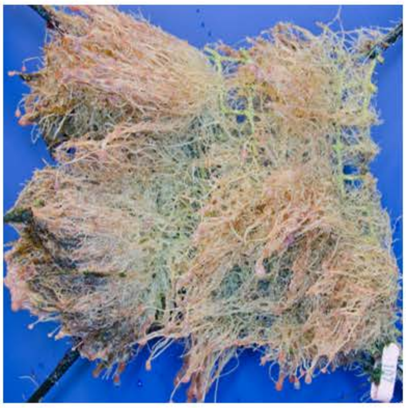
←
Lav 1
4 months
FR: 47
PNO: 51



←
Alt 2
5 months
FR: 0
PNO: 66



←
Blank
3 months
FR: 0
PNO: 79



←
Alt 2
4 months
FR: 0
PNO: 99

Fig. 3: Examples of biofouling on net samples, with information on Fouling resistance (FR) and Percentage Net-aperture occlusion (PNO). An asterisk indicates samples where discrepancy between FR and PNO occurred due to the presence of dead material that was excluded in the FR measurement.

4 Results

4.1 Net coatings

Over the course of the two trials, 21 taxonomic groups were identified on the net panels. The hydroid *Ectopleura larynx*, together with few individuals of one other hydroid species, was the most common biofouling organism on the net panels, accounting for 51% of biota observed. Hydroids were found on the samples as early as May, 2 months after immersion. Algae, comprised of 5 taxonomic groups, were the second most frequent taxonomic group (35 %), and present from May alongside the hydroids. Ascidians, including *Ciona intestinalis*, were found on the coated samples only in September, the final month of both trials, but at very high abundances, making them the third most frequent fouling organisms. Other invertebrates were present at lower numbers. This included erect and encrusting bryozoans, which also settled mainly in September, the final month of the trials. Caprellid amphipods, in contrast, arrived together with the hydroids and were present throughout the experimental period. In addition, low numbers of the tube-building amphipod *Jassa* sp., bivalve species such as *Mytilus edulis* and *Hiatella arctica*, one anemone species, and one stauromedusa species were found.

Biofouling development was slower in the spring trial than in the autumn trial, but generally reached higher total abundance on experimental surfaces. Initial biofouling development began on the **uncoated control (Blank)** samples in May, 2 months after immersion, and was found on 60% of the sample panels (Fig. 4). However, since it consisted mainly of very young hydroids, it had little impact on the fouling resistance (mean FR = 95; Fig. 5) and Percentage Net-aperture Occlusion (mean PNO = 14 Fig. 6). From the next month onwards, biofouling was found in abundance on all samples of the uncoated control, resulting in a very low fouling resistance (9) and high PNO values (76) in June, 3 months after immersion. Fouling resistance was lowest after 6 months of immersion in September (0), and PNO peaked in August (95), 5 months after immersion.

In the autumn trial, all control samples were heavily fouled already after one month's immersion with correspondingly low FR (Aug & Sep: 0) and high PNO (Aug.:97, Sep.: 94) values. The uncoated control samples accumulated the highest biofouling wet weight in both trials (mean: spring: 188 g, autumn: 135 g). The samples were dominated by hydroids for most of the time, with exception of June when algae were most abundant, and September in the spring trial, when ascidians dominated the samples, followed by bryozoans (Fig. 7). The uncoated samples had the highest species richness (S) measured during the trial (S = 12, Fig. 8).

The samples coated with the **commercial copper coating (Cu Ctrl)** were more distinct from the uncoated control than any of the novel antifouling coatings tested. The copper control was free of fouling for longest, and remained clean on all replicates before August. In August and September of both trials, biofouling was found on 100 % of the copper control panels (Fig. 4). The copper control had the highest fouling resistance values of all coatings in August in both trials (spring: 68; autumn: 72; Fig. 5). In September, however, FR dropped dramatically for copper control surfaces in both trials (spring: 8; autumn: 2). A similar development could be observed in the PNO data (Fig. 6). The copper control had low PNO values in August (spring: 31; autumn: 24), but they increased strongly in September in both trials (spring: 80; autumn: 58). This sudden increase in biofouling abundance was caused mainly by strong growth of the hydroid *E. larynx* in August and September. In the spring trials, relative wet weight reduction by the copper control samples did not differ much from the other coatings (Fig. 7). In the autumn trial, however, the copper control accumulated more biofouling than most other coatings, with exception of the Alt 1 and biocide free coating (Fig. 7). Through most of both trials, the copper control samples had the lowest species richness (Fig. 8) and were strongly dominated by hydroids in both trials. The only other species on the copper coated samples were algae and caprellid amphipods (Figs. 9 and 10).

The data indicated that August was the key time point where the blank control was fully fouled while the copper control still showed considerable antifouling ability before performance of all coatings in the test decreased considerably. Therefore, the comparison of the novel coatings to the copper and blank control was undertaken using the August data.

In that month, Fouling Resistance of the samples differed between the two trials and between coatings, and was not always consistent between locations (Time x Location x Coating: $F_{21;115}$= 2.279, p = 0.006). The same relationship was found for the Percentage Net-aperture Occlusion of the samples (Time x Location x Coating: $F_{21;115}$= 3.22, $p < 0.001$). The relative biofouling wet weight accumulation also differed between the two trials and between coatings, but was consistent over the various locations (Time x Coating: $F_{6;137}$= 9.208, $p < 0.001$).

The Low 1 coating of the two **coatings with low copper content** performed best relative to the copper control, while the Low 2 coating performed less consistent. Biofouling started to accumulate on the panels in June of the spring trial, 3 months after immersion, later than on most other coatings, and with relatively low prevalence (27 and 20 % of the panels of Low 1 and 2, respectively; Fig. 4). In August, after 5 months of immersion (spring trial), fouling resistance of both coatings was below 50% (Low 1: 43, Low 2: 24). While not as good as the copper control, the Low 1 coating performed significantly better than all other coatings (pairwise comparisons, $p < 0.05$; Fig. 5). In August of the autumn trial, the Low 1 coating had FR values similar to the copper control and higher than all other coatings (pairwise comparison, $p < 0.05$). In September, its FR did not decrease as rapidly as that of the other coatings. The Low 2 coating did not differ from the blank control in August of both trials.

PNO measurements in August of the spring trial, 5 months after immersion, showed that the Low 1 coating was similar to the copper control and had significantly less net-aperture occlusion than the other tested coatings (pairwise comparison, $p < 0.05$). The Low 2 coating did not differ from the blank control in August (pairwise comparison, $p < 0.05$), but had considerably lower PNO values in September, together with the Low 1 coating. In August of the autumn trial, 2 months after immersion, Low 1 and 2 did not differ from the copper control. In September, 3 months after immersion, PNO values of the Low 1 coating were the only ones to stay below 20 %.

The Low 1 coating accumulated significantly less wet weight than the copper control in both trials (pairwise comparison, $p < 0.05$), while the Low 2 coating accumulated less weight only in the second trial (pairwise comparison, $p < 0.05$, Fig. 7). Although initial settlement on the Low 1 samples in June consisted of hydroids and caprellid amphipods, it was dominated by algae in both trials (Figs. 9 and 10) and had average species richness numbers (Fig. 8). In the spring trial the first settler on the Low 2 coating were hydroids, but they were displaced by ascidians and algae by the end of the trial after 6 months at sea. In the autumn trial, the biofouling on the Low 2 coating consisted mainly of algae, similar to the Low 1 coating. Species richness was generally higher for this coating.

The performance of the coatings containing **alternative biocides (Alt 1 – 3)** was in general inferior to the ones containing copper (Copper Ctrl, Low 1 and 2). Alt 1 and 2 started accumulating biofouling in May, 2 months after immersion, though at lower levels than the uncoated control (Fig. 4). Alt 3 was fouling free until June, 3 months after immersion. During the spring trial, fouling resistance started to decrease in June, and was 0 for all three coatings in August, 5 months after immersion (Fig. 5). In August of the autumn trial, Alt 2 had a large variability in fouling resistance and did not differ from both copper control and blank control. In contrast, Alt 3 performed better than the blank control, yet not as good as the copper control or Low 1 coating (pairwise comparison, $p < 0.05$). Alt 1 had an average fouling resistance of 0 (Fig. 6).

During August of the spring trial, PNO values of the alternative coatings did not differ from the blank control. During the autumn trial, PNO values were similar to the copper coated samples for Alt 2 and 3, while Alt 1 accumulated increased amounts of fouling – similar to the uncoated control (pairwise comparison, $p < 0.05$).
Relative wet weight accumulation did not differ from the copper control in the spring trial (Fig. 7). In the autumn trial, Alt 2 and 3 did significantly better than the copper control, while Alt 1 accumulated significantly more biofouling than the copper control (pairwise comparison, $p < 0.05$). Species richness was relatively low on Alt 1 and 3 in the spring trial, and close to the average in the autumn trial (Fig. 8). Alt 2 had high species richness in the spring trial and reached the highest species richness of all coatings in the autumn trial.
The species composition of Alt 1 coated samples was similar to the uncoated control and was dominated by hydroids for most of the time (Figs. 8 and 9). The only exception was the community on the September samples of the spring trial, where in addition to hydroids also larger numbers of bryozoans and some ascidians were found. Although initial biofouling on the Alt 2 and 3 in the spring trial was dominated by hydroids (and caprellid amphipods for Alt 2), the August and September months of both trials were dominated by algal biofouling.

The **biocide free coating (Free)** behaved generally very similar to the uncoated control throughout both trials. It was the first coating to reach high prevalence of biofouling on the samples (82 %) in May after 2 months of immersion (Fig. 4) and fouling resistance was among the lowest of all tested novel coatings in both trials (Fig 4). Although PNO values were low in May and June of the spring trial, they increased dramatically in August, 5 months after immersion (99; Fig. 6). In the autumn trial, the biocide free coating also had high PNO values (94) similar to the uncoated control. In the spring trial, relative wet weight accumulation did not differ from the copper control, but in the autumn trial the biocide free coating accumulated more biofouling wet weight than the copper control (Fig. 7).
Species richness was low on the biocide free coating in both trials for most of the time, but increased towards the last moth of both trials (Fig. 8). The main biofouling species on the biocide free coating were hydroids, with exception of the September samples of the spring trial, where bryozoans made up almost 50 % of the fouling species, in addition to ascidians, caprellid amphipods and algae (Figs. 9 and 10).

To **summarise** the results, the following pattern can be described: The copper control coating performed best of all coatings tested, closely followed by the Low 1 coating (Table 3). These two coatings were able to prevent the settlement and accumulation of biofouling organisms longest. The second low-copper coating Low 2 and the alternative coatings Alt 2 and 3, made up the next group of coatings in the ranking. They were able to delay biofouling considerably but failed earlier than the copper control and the Low 1 coating. The Alt 1 and the biocide free coating performed better than the blank control yet could not compete with the other coatings.

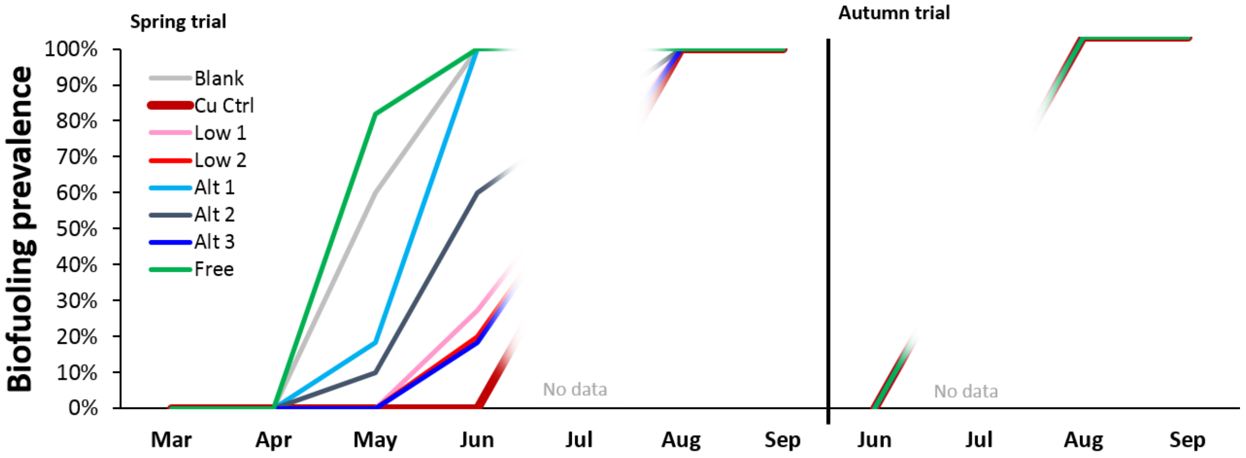


Fig. 4: Prevalence of biofouling on net samples treated with 8 different coatings in the spring (6 months duration) and an autumn (3 moths duration) trial.

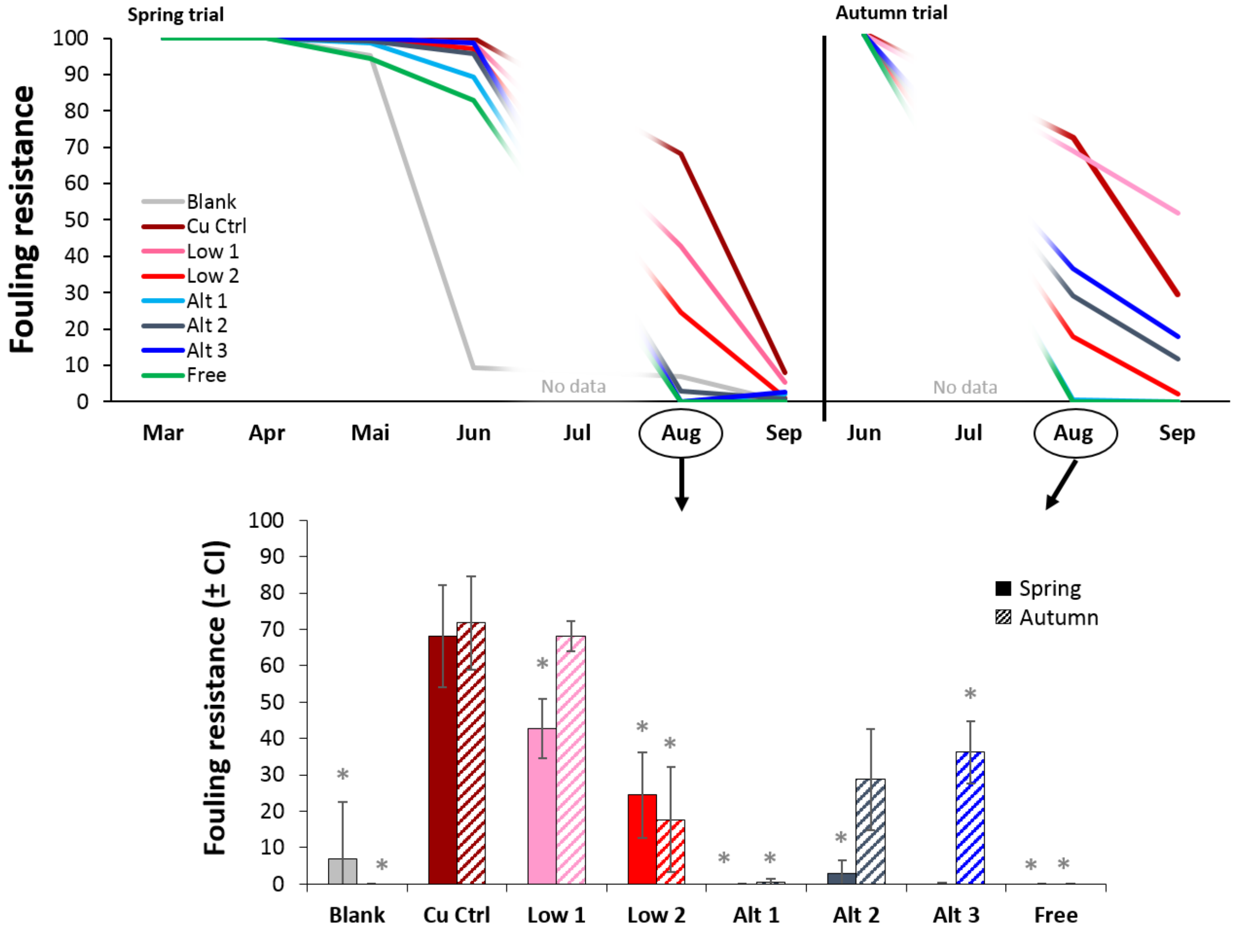


Fig. 5: Fouling Resistance of 8 different coatings of net samples in the spring (6 months duration) and an autumn (3 moths duration) trial, including a detailed depiction of the situation in August of both trials. Coatings with significantly lower FR values than the copper control are indicated by an asterisk

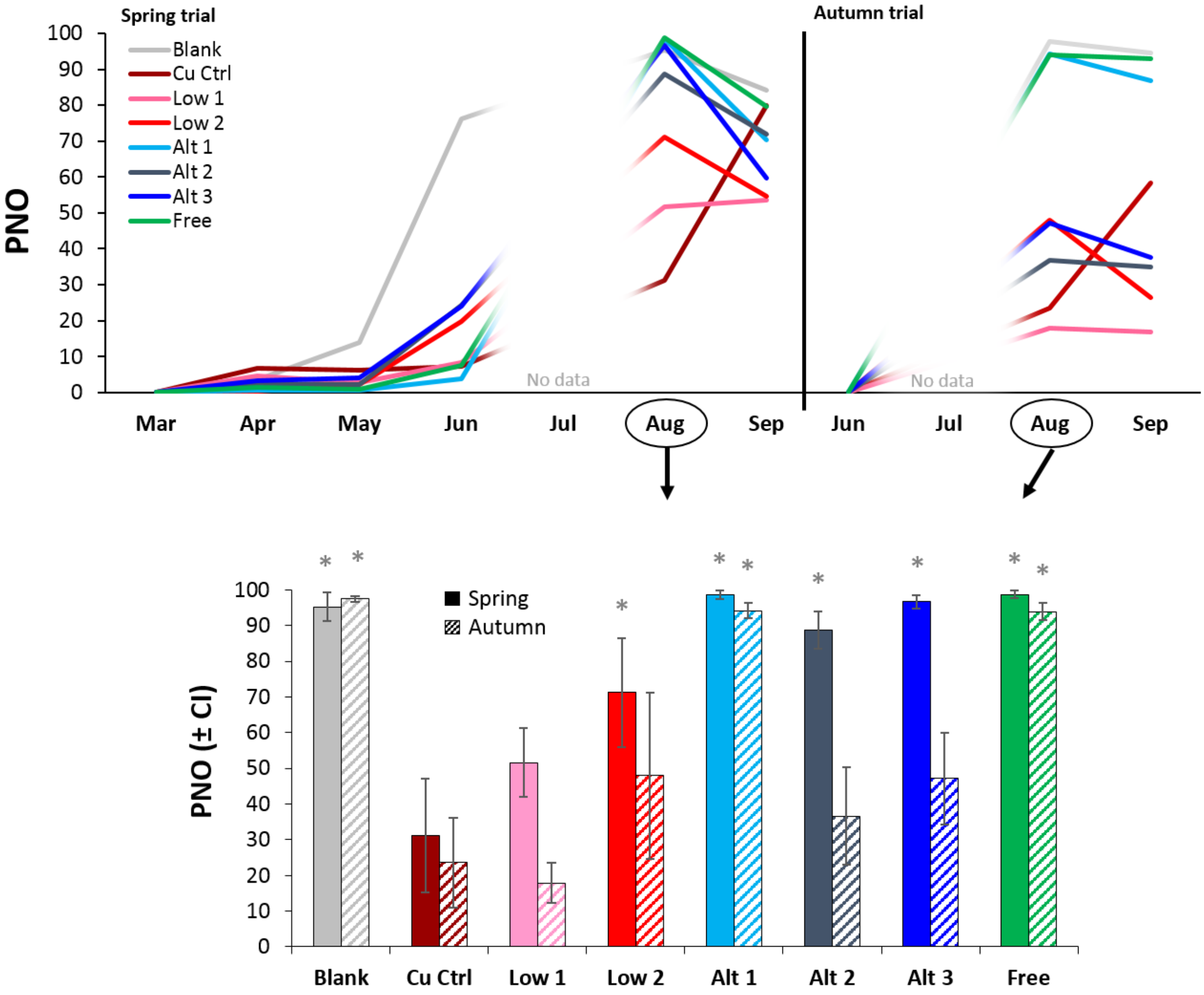


Fig. 6: Percentage Net-aperture Occlusion (PNO) of net samples with 8 different coatings in the spring (6 months duration) and an autumn (3 moths duration) trial, including a detailed depiction of the situation in August of both trials. Coatings with significantly higher PNO values than the copper control are indicated by an asterisk.

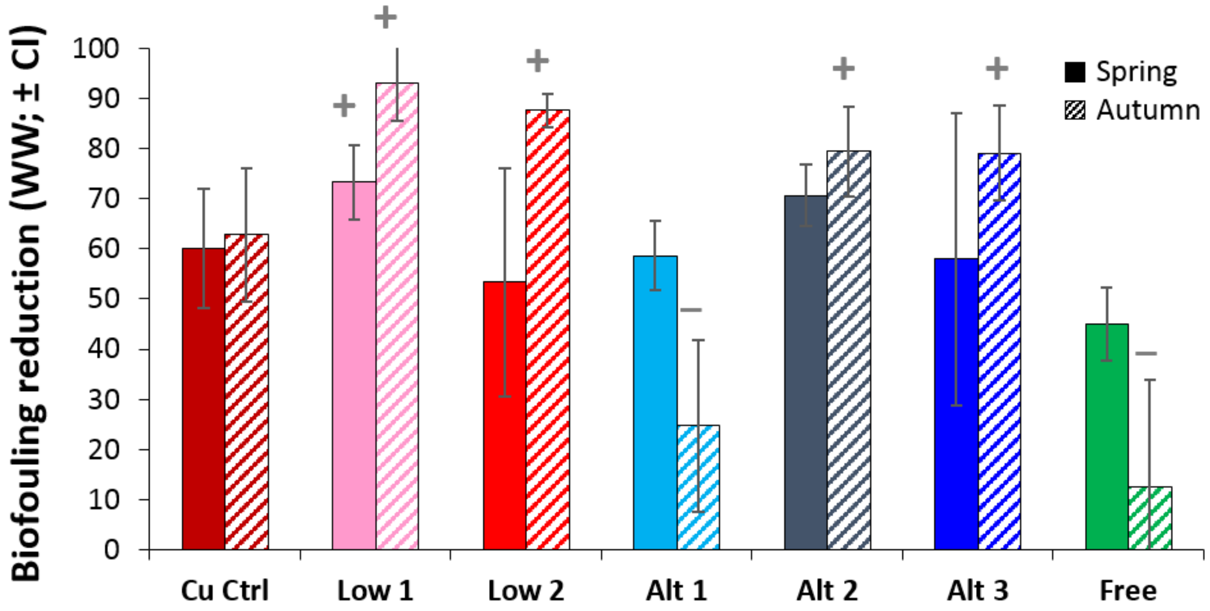


Fig. 7: Biofouling wet weight (WW) reduction (± CI) relative to the uncoated control nets, measured on nets with 7 different coatings after 6 months (spring trial) and 3 moths (autumn trial) at sea. Significant differences compared to the copper control are indicated by + (more BF reduction) and – (less BF reduction) signs.

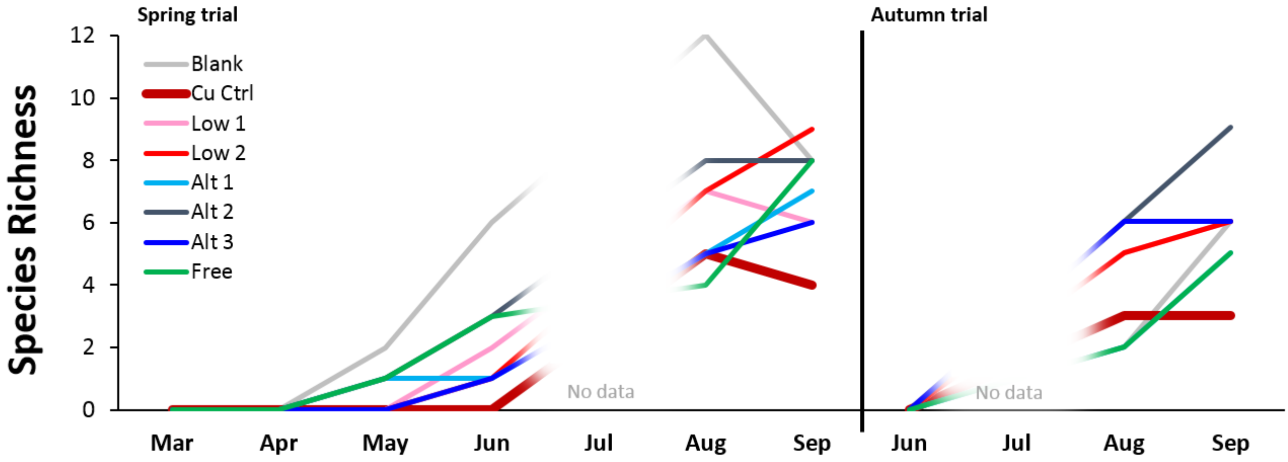


Fig. 8: Species richness measured on nets with 8 different coatings in the spring (6 months duration) and an autumn (3 moths duration) trial.

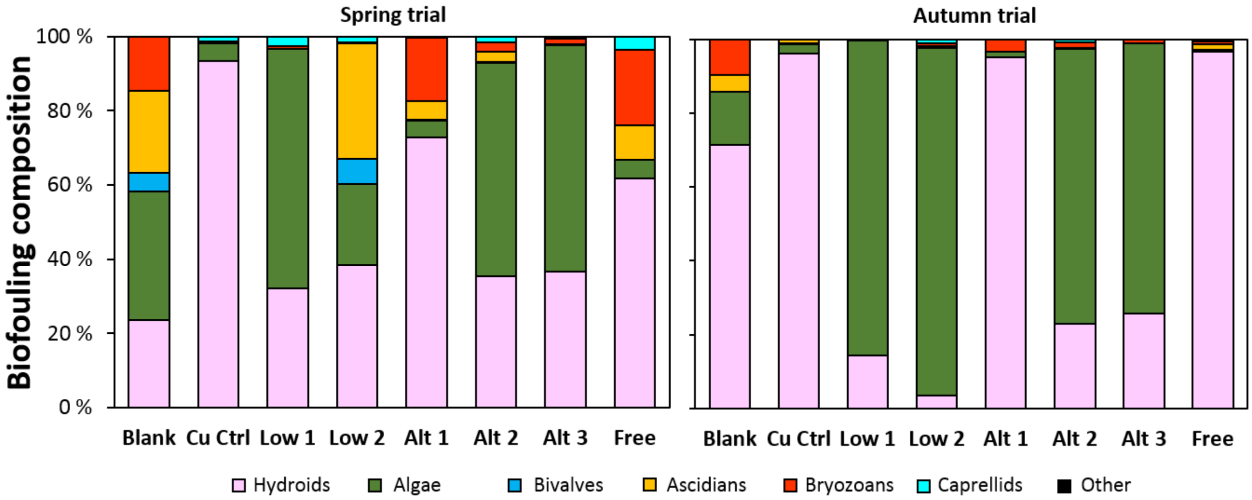


Fig. 9: Total biofouling composition on nets with 8 different coatings after 6 months (spring trial) and 3 moths (autumn trial) at sea, depicting percentage cover of hydroids, algae, bivalves, ascidians, bryozoans and caprellids.

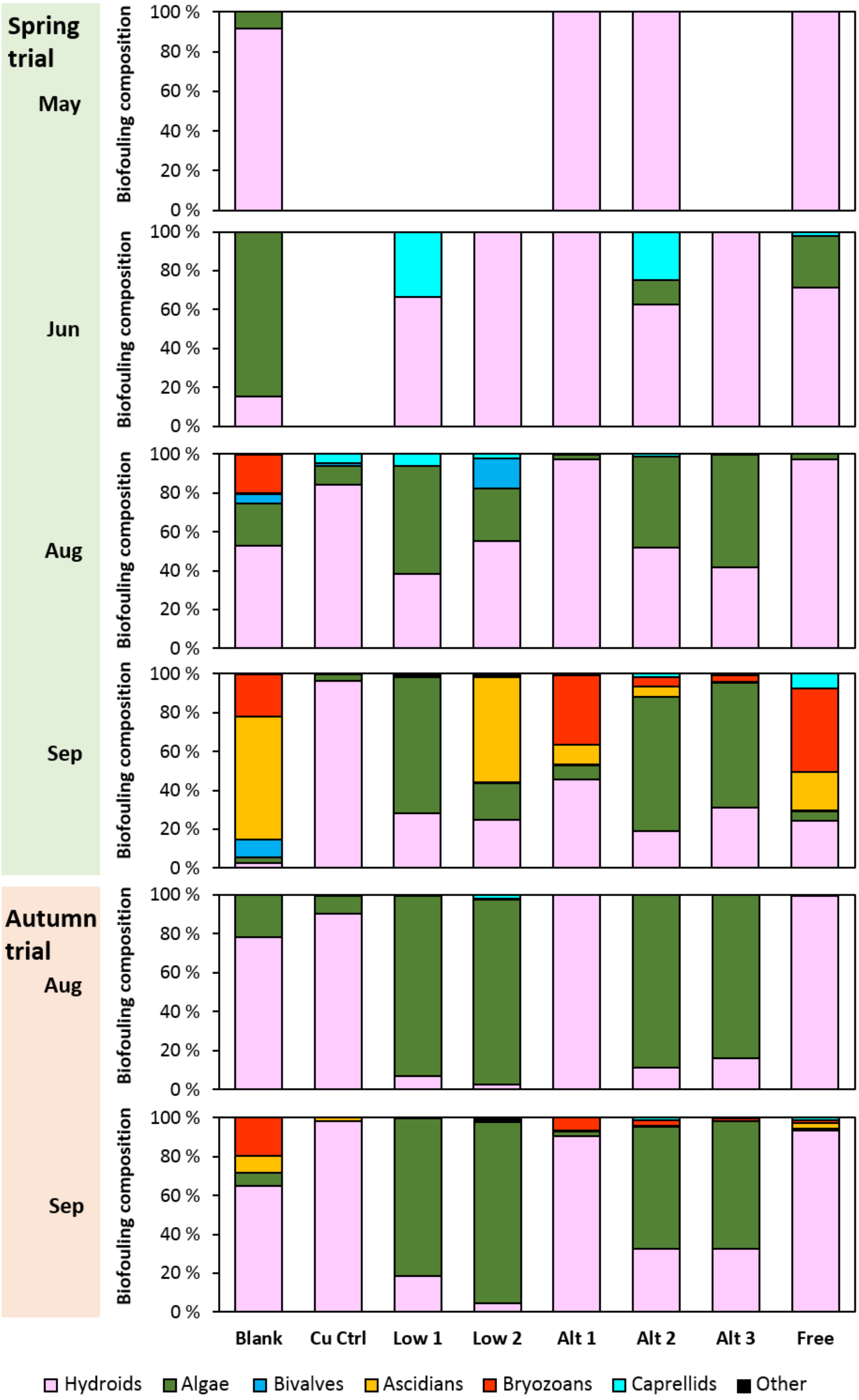


Fig. 10: Monthly biofouling composition on nets with 8 different coatings immersed for 6 months (spring trial) and 3 moths (autumn trial), depicting percentage cover of hydroids, algae, bivalves, ascidians, bryozoans and caprellids. There was no biofouling present in April.

Table 3: Summarising overview of the performance of the coatings measured in Biofouling (BF) prevalence, Fouling resistance, Percentage Net-aperture Occlusion (PNO), relative biofouling wet weight reduction (re. WW red.) and Species richness (best performance indicated by ++++).

	BF prevalence		Fouling resistance		PNO		rel. WW red.		Species richness			
	Spr.	Aut.	Spr.	Aut.	Spr.	Aut.	Spr.	Aut.	Spr.	Aut.	+ sum	Group
Cu Ctrl	++++	+	++++	++++	++++	+++	+++	+++	++++	++++	**34**	**A**
Low 1	+++	+	+++	++++	++++	++++	++++	++++	++	++	**31**	**A**
Low 2	+++	+	+	++	+	++++	+++	++++	++	++	**23**	**B**
Alt 1	++	+	+	+	+	+	++	++	+++	+++	**17**	**C**
Alt 2	++	+	+	+++	+	++++	+++	++++	++	+	**22**	**B**
Alt 3	+++	+	+	+++	+	++++	+++	++++	+++	++	**25**	**B**
Free	+	+	+	+	+	+	++	++	+++	+++	**16**	**C**
Blank	+	+	+	+	+	+	+	+	+	+++	**12**	**D**

4.2 Adhesive antifouling films

Over the course of the 10 months at sea, 20 taxonomic groups were identified on the PVC panel samples. They were the same species as those found on the net samples but lacked the second hydroid species. Algae were the most common biofouling organisms, accounting for 36 % of specimens encountered. The hydroid *Ectopleura larynx* was the next common organism (27 %), followed by ascidians (20 %).
On the panels coated with antifouling films containing copper or copper shim tape (= copper control), only algae, hydroids and, in small numbers, the tube-building amphipod *Jassa* sp. were found. On the blank control panels, the species number was much higher, with a maximum of 11 taxa on the September, October and November samples each.

All blank panels were heavily fouled 2 months after immersion, while it took much longer for the antifouling samples to accumulate biofouling. In October, 7 months after immersion, the first fouling organisms were found on the copper films and tape. While 100 % of the high-load samples were fouled, biofouling was less prevalent on the low-load (67 %) and the tape samples (50 %; Fig. 11). The prevalence of biofouling peaked in October and November and decreased until the last sampling in January.
Fouling resistance of the blank samples was 0 after one month at sea and varied slightly after (Fig. 12). Fouling resistance of the antifouling samples was 100 until October (7 months after immersion), from where it decreased. There was no significant difference in the performance of three antifouling films. Fouling resistance did, however, vary to some degree between immersion time and locations (Time x Location: $F_{6;83}= 2.78$; $p = 0.0173$).
After 10 months of immersion, the blank control panels had gained most weight with an average of 59 g. Although the differences were not very large, the copper shim tape prevented significantly more weight accumulation than the embedded copper films (Coating: F2;30= 14.60, $p < 0.001$; pairwise comparison, $p < 0.05$; Fig. 13).
On the copper shim tape, the leaching of copper was visible through the loss of colour of the tape, showing the PVC panel below the transparent layer of adhesive (Fig. 14). On average, 16 % of the visible copper surface was lost from the tape panels by the end of the 10-month trial. No copper loss was visible from the films embedded with copper particles.

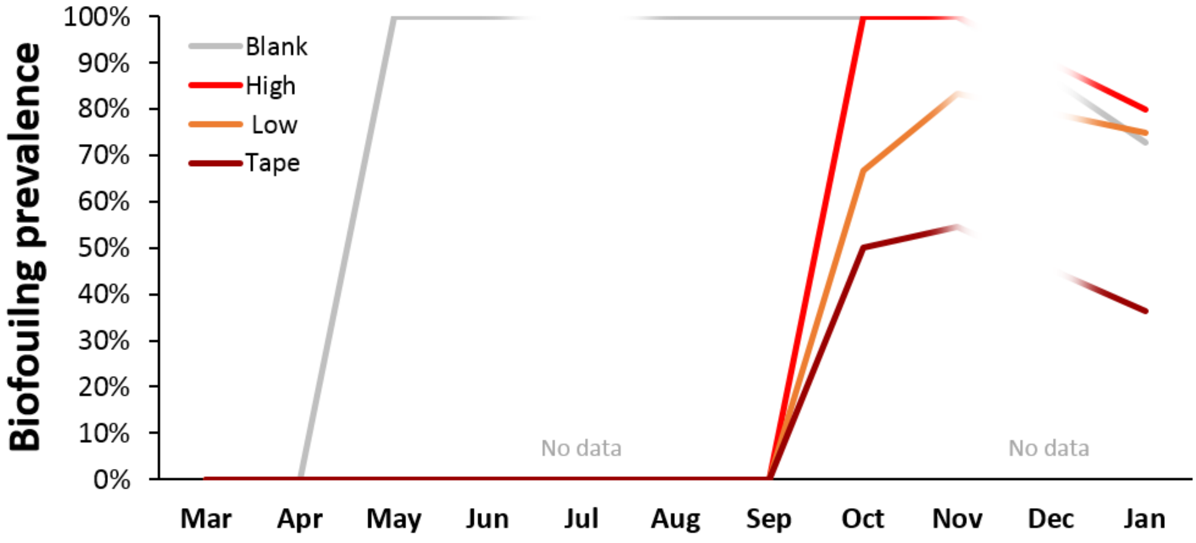


Fig. 11: Prevalence of biofouling on 4 tested surfaces during a 10 months trial.

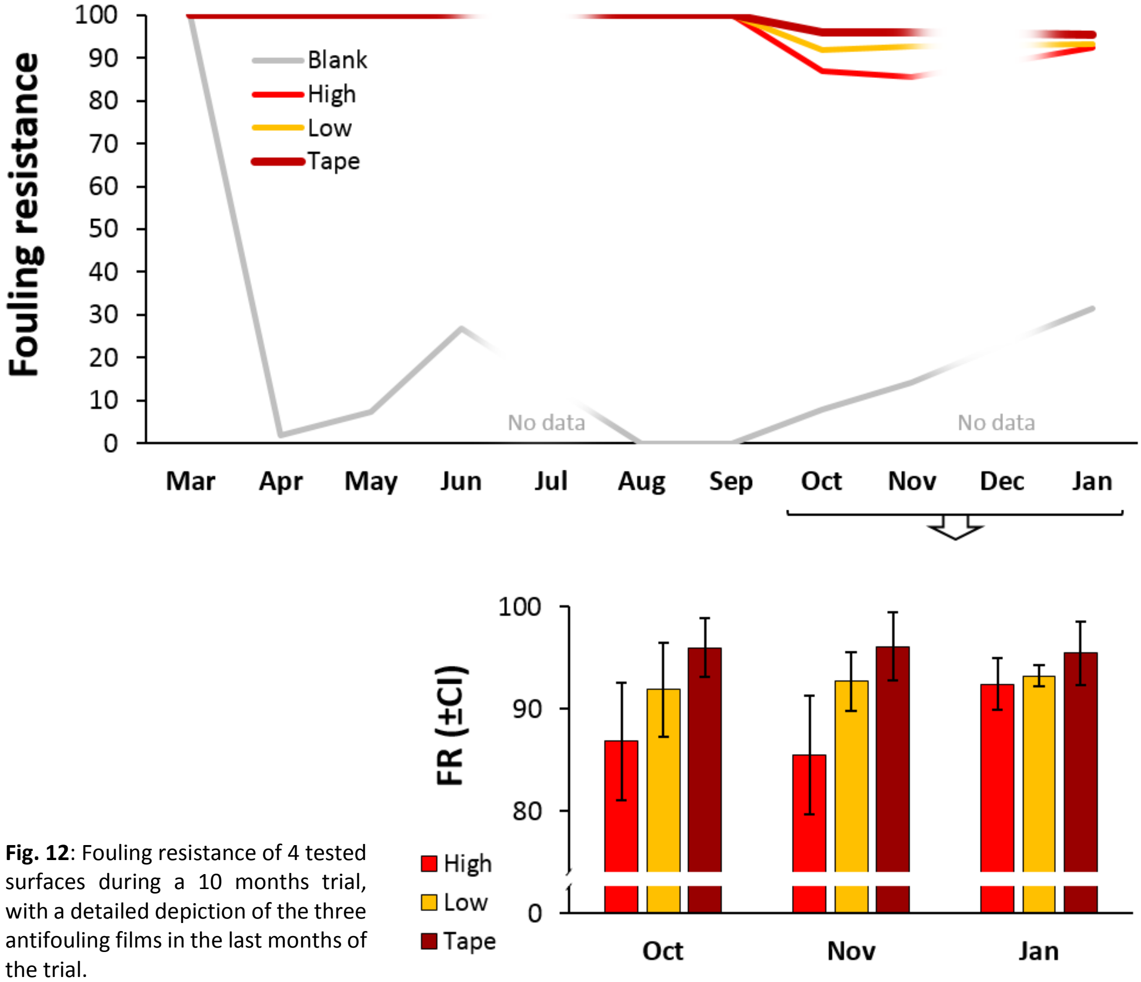


Fig. 12: Fouling resistance of 4 tested surfaces during a 10 months trial, with a detailed depiction of the three antifouling films in the last months of the trial.

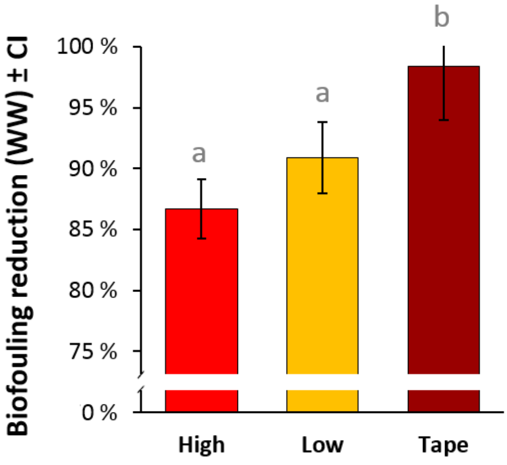


Figure 13: Biofouling wet weight (WW) reduction relative to the uncoated control samples (average ± CI), measured on panels with 3 different coatings. Lower case letters indicate statistically significant differences.

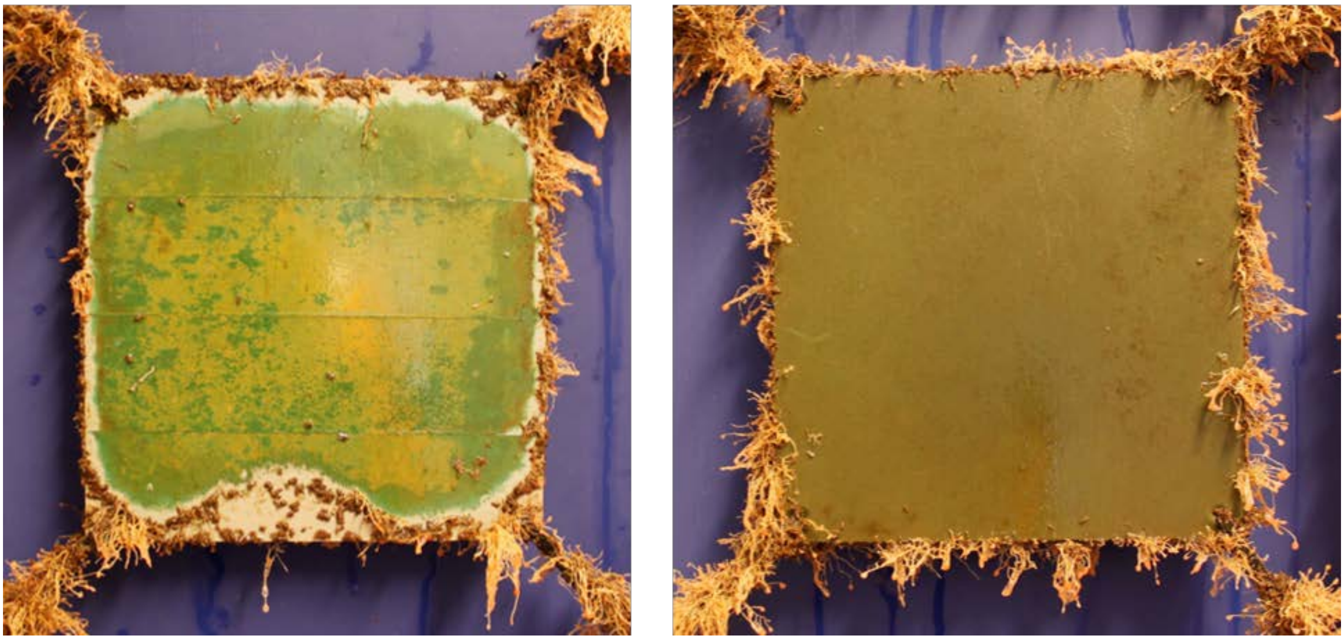


Figure 14: Left: Example of a PVC panel coated with copper shim tape (green/yellow). Where the copper has leached out, the grey PVC base plate can be seen. Right: In comparison, no patchy leaching is visible from the copper-embedded antifouling film.

5 Discussion

5.1 Net coatings

None of the new coatings tested was able to prevent biofouling for the entire duration of the experiment, or performed better than the established copper control coating. However, the coatings were able to delay the onset and abundance of biofouling for some time. Although the general trend in biofouling accumulation and community composition on the coatings was similar over the two trials, biofouling accumulation was much faster in the autumn trial, when samples were immersed during the peak of the biofouling season. Compared to the spring trial, however, the autumn trial had a lower species richness, where the fastest-growing species were able to monopolise the available space in the young community quickly, a common pattern in biofouling community development (Bloecher et al. 2013).

None of the experimental coatings performed as well or better than the copper control coating, which only few organisms, such as hydroid *Ectopleura larynx* and some algal species, were able to settle on. However, since the copper content of the Low 1 coating, which performed comparably, was substantially lower (0.6 % CuO compared to > 20 % in the copper control), it is likely that the observed antifouling activity was not caused only by copper. The second component in the Low 1 coating, an algaecide (copper pyrithione), likely contributed to this success. In contrast, the second low-copper copper coating (Low 2), with a copper content > 5 %, mixed with zinc, performed similar to two of the coatings based on alternative biocides (Alt 2 contained 2.9 % Econea, Alt 3 contained boron), but not as good as the copper control and Low 1 coatings.

The experiments also showed that even biocide-free coatings were still able to influence settlement to some extent compared to the uncoated control and may therefore offer a temporary advantage to leaving nets uncoated. This effect may be due to the ability of a coating to alter the naturally variable surface of a raschel knit nylon net, sealing and smoothing the surface, and thereby potentially making it less attractive to settling organisms (Baum et al. 2017).

The relative performance of the coatings on the basis of relative biofouling wet weight reduction differed to some extent from results obtained via the other methods of assessment (i.e. FR, PNO). A possible explanation may be that measurements based on wet weight are strongly affected by the species composition on the samples (Gansel et al. 2016). Some biofouling species may be heavier or may retain more water than others, with increased effects on comparisons of diverse biofouling communities. Since differences in composition between communities were quite pronounced at the end of the trials, this may have led to the deviating results.

The net coatings varied in colour from white to black (cover figure, Fig. 1). This colour variability between individual coatings may have influenced the species composition on the net samples, attracting specific species while repelling others (Hodson et al. 2000, Satheesh and Wesley 2010). However, it is unlikely that the colour has influenced fouling resistance for an extended period of time, since other, less colour sensitive species would likely have settled indiscriminately. Specifically, the hydroid *E. larynx*, one of the main biofouling organisms in this study that settled from early on, has been shown to settle indifferently to coating colour (Guenther et al. 2009). Thus, although coating colour may have influences initial settlement and community composition, it is unlikely to have impacted the overall biofouling prevention assessment.

In the first two months of the spring trial, considerable amounts of dead algal material drifted into the samples via local current regimes. Not all the material could be removed by gentle rinsing of the samples prior to photographing, and consequently complicated the analysis of the first two sampling events. While it was possible to identify the dead material in most cases in the manual analysis for Fouling Resistance, the accumulated dead material did influence the PNO values in the first two months of the Spring trial. However, the random arrangement of treatment replicates on the experimental frames ensured an 'even' distribution of any effects caused by drifting material and, fortunately, there was little drifting material in the successive months of the trial.

Interestingly, the producers of the individual coatings reported surprise upon seeing the results, as they did not concur with their own observations for some of the coatings. It appears that the Low 1 coating had not previously performed as well as a classic copper coating, while the performance of the Alt 1 coating was below expectation. Part of the explanation for this discrepancy in test outcome may be the temporal and spatial variation in biofouling species ready for settlement that is typical for marine waters (Fitridge et al. 2012, Bloecher et al. 2013). Furthermore, according to the producer, the Alt 3 coating is usually used on nets other than raschel knit nylon nets and is reported to perform much better then. This indicates the interaction between the coating and the net material as another factor that may have influenced its performance.

Conclusion

The tests conducted in the ALLEGRO project show that there is potentially one product (Low 1) whose performance was comparable to the common copper coating. All other new coatings performed worse than the copper control coating, in that they either became colonised sooner (all coatings were fouled after 5 months or earlier), attracted more biofouling, or both. As such, none of the new coatings presents a better alternative to the *status quo*, from an antifouling perspective and without the added use of net washing approaches. Since the test was conducted at a single location, the generality of the results is uncertain, and outcomes may differ if tests were repeated in a different biofouling environment. Unfortunately, this study was unable to confirm the existence of a new coating that can protect nets for the duration of the entire salmon grow-out phase at sea. This again underlines the importance of the search for alternative and improved antifouling technologies.

5.2 Adhesive antifouling films

Both tested novel adhesive antifouling films performed equally well as the copper shim tape, todays standard method for protection of hard surface structures such as on sensors. Although no significant differences in Fouling Resistance could be found, indications for differences in functionality became evident during this 10-month assessment. When biofouling organisms finally began to settle on the antifouling surfaces, this happened with relatively equal distribution of organisms on the copper-loaded films, indicating a uniform loss of antifouling function from the surface. in contrast, the copper shim tape leached ions following a distinct pattern from the outside towards the inside. Biofouling organisms settled heavily following this gradient from the outside (sooner) towards the inside (later), with few organisms settling on intact copper tape. For a long-term use a trend like this indicates a patchier protection of the surface where areas of heavy fouling alternate with areas relatively free of fouling. In comparison, biofouling on the antifouling films is likely to accumulate uniformly, also during longer exposure, making colonisation more predictable and enabling optimised maintenance regimes.

This loss of copper may furthermore have influenced the wet weight measurements of the panels. The shim tape samples lost on average 16 % of the copper, accounted for a weight loss of ~2,3 g. With an average biofouling accumulation of less than 10 g per panel, this potentially explained the lack of an increase in weight despite visible fouling.

The higher concentration of copper particles in the High load copper films compared to the Low load films (586 g m^{-2} vs. 306 g m^{-2}) did not result in better performance. A possible explanation is based on the experience of the producer that depth the particles are embedded during production of the films plays a crucial role in the availability of the copper and thus the antifouling activity of the sample. If the copper particles are embedded to deep, leaching rates will be too low to be effective. At the same time this also influences long-term activity of the sample with reduced longevity of leaching rated are too high. It is possible that the High-load samples had no higher leaching rates and subsequent antifouling activity than the Low-load samples due to a deeper embedding of the copper particles.

On the blank control samples 0 fouling resistance was measured after just 2 months at sea, but thereafter some variation occurred in the FR values over the period of the experiment. This was likely because some fouling organisms developed to a size where they were easily removed by stronger currents in severe weather.

Conclusion

The test conducted in the ALLEGRO project show that adhesive antifouling foils with embedded copper particles offer an equal alternative to copper shim tape and may in fact possess an advantage with regard to longevity of the protective film.

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