Guidelines for efficacy testing of antifouling coatings for nets in field tests[1]

Developed by SINTEF Ocean on behalf of the Norwegian Environment Agency

Scope

This document describes the methodology for field-based efficacy testing of antifouling coatings for aquaculture nets. Efficacy is assessed in comparison to an uncoated control surface and demonstrated in relation to a defined product claim. Results are reported in connection with stated test conditions. *The test is designed to satisfy the requirements for applications for approval of biocide products described in REGULATION (EU) No 528/2012 (BPR).*

Safety

This test methodology does not address possible safety, health and environmental concerns associated with its use. All operations should be performed in accordance with all relevant local and national regulations. Any experiments, including efficacy field trials with new antifouling products or new active substances, should be in compliance with the requirements of article 56 of the Biocidal Products Regulation.

Accuracy and validity of the test

Results are unique to each test due to the variability of biofouling species composition, settlement pressure and growth related to factors such as time and geographic area or environmental parameters (e.g., salinity, water temperature). It is therefore not possible to directly compare results of individual samples between different tests. A direct comparison of results (ranks or % cover values) is only possible within one test conducted at a single site and time.
When translating test results to claims about expected performance one should be aware that efficacy tests with net panels submerged outside a commercial finfish cage mimic realistic conditions only to a certain degree as they are not subject to contact with farmed fish or exposure to farming operations (e.g., net cleaning, chemical bath treatments).

Definitions used in this guide

Treatment: net panels treated with one of the coatings to be tested. A test includes at least two treatments: a coated test product and an uncoated control.

Efficacy test: comparison of biofouling accumulation on net samples with different treatments (e.g., antifouling coatings, control) conducted at one place at one time.

Efficacy test series: repetition of an efficacy test at different sites and/or times.

Sample: a single net panel used in a test.

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[1] Bloecher, N, Floerl, O (2018) Guidelines for efficacy testing of antifouling coatings for nets in field tests. Technical paper, Norwegian Environment Agency, Oslo, 18 p.

Replicate: one sample within a group of samples that are subjected to the same treatment. Each treatment should include at least 3 replicates.

Appendix A includes background information on the parameters and methods described in this guideline and a discussion of potential strengths and weaknesses.

Required equipment & set-up

Samples: Net samples must have a size of at least 625 cm^2 (e.g., 25 x 25 cm).

Net material: If not explicitly stated otherwise in the product claim, samples must be a commercially available and commonly used white nylon product. Within one test series, all treatments (i.e. coatings and control) must be tested on the same material.

Frame: Frames should be made from steel or other suitable and durable materials and should not be coated with antifouling products. If coated frames are used, this has to be described in the product evaluation report. Size/orientation of the frames must be the same for all samples in one test series. Frames should be marked and numbered so that photographs are always taken from the same direction.

Replication: Each coating (and control) treatment in a test must have at least 3 replicates per site. For a full test series, a test must be replicated over at least 3 sites. These 3 tests within a series do not need to be conducted at the same time, but can be conducted in consecutive years.

Sample attachment: Sample panels must be attached to holding frames at randomly chosen positions rather than organised by treatment. Panels must be attached one sample-width apart and must not be in direct contact with the frame.

Sample marking: Each sample must have a unique identifier. A record of sample positions should be kept in case identifiers are lost. Plastic sheep tags are one example of suitably durable identifiers.

Placement of frames: Frames must be placed such that sample panels have a vertical orientation, within 15 m of a cage containing fish and in a depth between 2 m and 5 m. All frames within a series must be placed at the same depth.

Test site: The test must be conducted at 3 (or more) active farm sites within those environments the product is claimed to be designed for. Preferably the location of the test sites should include various environments (e.g., northern vs. southern location / coastal vs. fjord location).

Reference products: Blank/uncoated control panels must be included in the test. When control panels at one site have reached 100 % biofouling cover, they must be photographed and exchanged for new control samples, to make sure new settlement can still be detected. Inclusion of a reference product (i.e. existing antifouling coating of tested performance) is optional but recommended.

Duration and timing of the test: A test must run for at least 6 months, and must include the main biofouling season at the test location (e.g., Mid-Norway: May – October) to ensure maximum biofouling pressure. Exception: if the product claims to be designed for a specific period outside the main biofouling season (e.g. – "winter growth").

Sample maintenance: Rinsing of samples does not simulate in-situ net cleaning with high- or low-pressure water conducted on site. Products that are designed for use in connection with regular in-situ net cleaning need to prove the stability of their product against this potentially abrasive treatment in a separate test. If rinsing is carried out to remove debris or other material entangled in the net, this has to be conducted using dedicated equipment that dispenses seawater in a fine spray at a maximum rate

equalling 7.5 L min^{-1} per 0.0625 m^2. Rinsing must not remove any living, attached biofouling organism. All samples have to be treated equal (incl. control treatments) on all sites.
Where rinsing is carried out for any treatment/s, high-resolution photographs need to be taken before and after rinsing so that changes to these samples are documented.

Sample analysis

Personnel: Personnel responsible for sample analysis must have received an introduction to product testing based on these guidelines and a protocol to follow. They need to be able to identify common biofouling species and groups in their various life-history stages.

Analysis interval: Samples must be analysed at least every 4 weeks. Reasons for delay must be explained in the sample evaluation report.

Preparation of sample analysis: Sample tags and frames must be cleaned from biofouling prior to analysis. Care must be taken that the cleaning process does not affect sample panels present on the frame (via physical interference or the risk of desiccation due to prolonged exposure to air; also, the samples should not be subject to contact with freshwater from deck hoses or severe rainfall).

Photographing the samples: Samples must be photographed outside the water, against a neutral background (e.g., blue or white) with sufficient light and camera resolution to allow cropping and zooming during analysis. The sample frames should be raised vertically and individual samples should not rest on the background to avoid shading. The sample identifier needs to be clearly visible. The photo must show the entire sample panel and be taken at a 90-degree angle to the panel surface to avoid distortion.
High-resolution photographs depicting the entire frame need to be taken before and after cleaning of the frame to ensure that any damage to samples caused by cleaning of the frames can be traced.

Analysis of sample panels: Depending on the product claim, samples may be analysed for (**A**) total biofouling load or (**B**) % cover of individual biofouling groups.

> **Analysis of type A**: *Biofouling load* of a sample can be quantified using a nominal rank scale ranging from 0 (free of biofouling) to 5 (entirely covered with biofouling organisms). The allocation of biofouling ranks must follow formal definitions and reference images of the ranks. All biofouling on the nets must be taken into account for this analysis. It is not possible to exclude fouling species of lesser relevance for aquaculture nets (e.g., slime). For detailed instructions on how to conduct this analysis, see Appendix B.
>
> **Analysis of type B:** *% biofouling cover* is determined for the following, mandatory categories:
>
> - Slime (slime, diatoms, algae < 5mm)
> - Macroalgae
> - Invertebrates ('animals')
>
> In addition, it is recommended to identify main invertebrate biofouling groups (e.g. hydroids, mussels, ghost shrimps) that are relevant for aquaculture nets.
> The analysis enables the calculation of a **Macrofouling Score** (MS) that takes into account the presence of macroalgae and invertebrates, but ignores slime since it is not of relevance for nets.
> For detailed instructions on how to conduct this analysis, see Appendix C.

Evaluation

The efficacy of a coating is demonstrated if the coated samples accumulated considerably less biofouling than the uncoated control samples.

Control samples should show an increase of biofouling rank / cover with time, to ensure that biofouling pressure was present during the entire experimental period. If the control panels reach 100 % cover and are exchanged during the test period, the control will still be considered as 100 % fouled, even if the exchange control panels are not fully covered. If control panels no longer show an increase in biofouling rank/cover over a 4-week period, the analysis of the experiment beyond this point in time should not be included in the evaluation since the performance of test coatings can then not be distinguished from a lack of biofouling activity.

Results of the 3 (or more) replicates of a treatment within one test (same time, same site) should be averaged and the data presented as proportion of the control treatment. In a following step, the data averages for each treatment may then be averaged across all tests in a test series to compare the overall efficacy of single treatments. See Appendices B and C for details.

Reporting

The test report must contain the following:

- Introduction
- Materials and methods
- Results and raw data (photographs and raw sample ranks or counts)
- Conclusion/discussion

In detail, this includes:

- Name of the reporting company
- Description of the tested products (incl. e.g., protocol of coating application)
- Dates of immersion of sample panels, and dates and duration of subsequent retrievals (regular census) and of any other treatments (e.g. rinsing of samples) or interferences (e.g. inspection following storm events)
- Site description (incl. coordinates, maximum and minimum water temperature and salinity for the duration of the test)
- Site map indicating the placement of the samples at the cages, incl. the main current directions
- Dimensions and material type of the net panels used
- Number of samples per treatment
- Description of the frames used in the test
- Orientation and exposure depth of the samples
- A discussion of any special conditions or treatments that may have arisen during the test (e.g., sample maintenance routines or the occurrence of severe storms or rainfall events)
- Raw data (picture + biofouling rank / percent cover) for each sample
- Fouling load/Macrofouling-Score per sample at each inspection time relative to the control sample
- A discussion on the validity and acceptability of the test result relative to the intended product claim for the product tested when commercialised (e.g. recommended use area, protection time/re-coating interval, fouling conditions in targeted markets, etc.).
- An interpretation of the test data generated and a conclusion on the efficacy of the coating under test.

Appendix A

Explanations and justifications of principles and methods contained in the Guidelines for efficacy testing of antifouling coatings for nets in field test

This document provides background information on the parameters and methods described in the guidelines and a discussion of potential strengths and weaknesses.
Original text paragraphs of the guidelines are shaded in grey.

Discussion of the required equipment & set-up

Samples: Net samples must have a size of at least 625 cm^2 (e.g., 25 x 25 cm).

➔ It is important that tests are based on net sample dimensions that are in proportion to the size of biofouling organisms and communities that may develop on them. Several years of biofouling research at SINTEF indicate that test panels of 25x25cm (or larger) attract biofouling communities that are representative of general patterns observed on production cages (Guenther et al. 2009).

Net material: If not explicitly stated otherwise in the product claim, samples must be a commercially available and commonly used white nylon product. Within one test series, all treatments (i.e. coatings and control) must be tested on the same material.

➔ The use of a commonly used product assures the relevance of the test results for potential users of coatings. The utilisation of identical net materials in all treatments is a requirement for comparability of results between treatments.

Frame: Frames should be made from steel or other suitable and durable materials and should not be coated with antifouling products. If coated frames are used, this has to be described in the product evaluation report. Size/orientation of the frames must be the same for all samples in one test series. Frames should be marked and numbered so that photographs are always taken from the same direction.

➔ Antifouling coatings on the frames can influence the settlement and growth of organisms on adjacent net samples due to leaching of chemicals. This should be avoided as it might skew the test results.
Equal size and positioning of all samples is a requirement for comparability of results between samples and treatments.

Replication: Each coating (and control) treatment in a test must have at least 3 replicates per site. For a full test series, a test must be replicated over at least 3 sites. These 3 tests within a series do not need to be conducted at the same time, but can be conducted in consecutive years.

➔ The settlement of biofouling larvae and spores is naturally variable, even on scales as small as a salmon farm site (Guenther et al. 2010, Bloecher et al. 2013). Variability between farming sites is usually even larger and dependent on many factors (e.g., geography, salinity, temperature; Fitridge et al. 2012). By deploying samples at at least 3 farm sites with at least 3 replicates at each farm site, this natural variability can be captured and accounted for in the interpretation of

results. Since variation between years is only one factor among many, and does not necessarily contribute the largest variation, tests do not need to be conducted in the same year.

Sample attachment: Sample panels must be attached to holding frames at randomly chosen positions rather than organised by treatment. Panels must be attached one sample-width apart and must not be in direct contact with the frame.

➔ Settlement of biofouling organisms can be influenced by their direct surroundings. In addition, some organisms, such as blue mussels, have life-history stages in which they are able to re-settle on nearby substrates. To prevent any interference between panels, a distance of one panel width has become a standard in scientific biofouling tests. Random positioning of samples within the holding frame (e.g. using alpha-numeric positions based on rows and columns – A1, A2, B1, etc.) prevents 'sampling bias' due to differences in environmental parameters or biofouling patterns within frames.

Placement of frames: Frames must be placed such that sample panels have a vertical orientation, within 15 m of a cage containing fish and in a depth between 2 m and 5 m. All frames within a series must be placed at the same depth.

➔ The sample orientation should reflect the vertical orientation of the main part of the cage net, ensuring similar exposure to light and water currents. Direct vicinity to the net cages with fish guarantees higher biofouling pressure and composition (Bloecher et al. 2015). In contrast, positioning of samples close to other structures such as, for example, a feed barge covered by hard-substrate biofouling can result in biofouling composition less or not at all representative of net cages.

The recommended depth range allows growth of both algae and invertebrate biofouling organisms since it offers sufficient light (Guenther et al. 2010). It also represents the area of the cage most susceptible to biofouling according to farmers and net cleaning service personnel in Norway.

This parameter may need to be adapted when efficacy is tested in geographic regions where conditions differ.

Test site: The test must be conducted at 3 (or more) active farm sites within those environments the product is claimed to be designed for. Preferably the location of the test sites should include various environments (e.g., northern vs. southern location / coastal vs. fjord location).

➔ Since the types, abundance and growth rates of biofouling organisms vary between coastal environments, and can drastically change with latitude, the performance of a product may not be directly transferable from one geographic region to another. Therefore, a test should include the regions referred to in the product claim.

(See also discussion of 'Replication')

Reference products: Blank/uncoated control panels must be included in the test. When control panels at one site have reached 100 % biofouling cover, they must be photographed and exchanged for new control samples, to make sure new settlement can still be detected. Inclusion of a reference product (i.e. existing antifouling coating of tested performance) is optional but recommended.

➔ The inclusion of a control sample is necessary to enable the standardisation of the abundance of biofouling on test panels exposed at individual sites with differences in biofouling pressure.

Sample maintenance: Rinsing of samples does not simulate in-situ net cleaning with high- or low-pressure water conducted on site. Products that are designed for use in connection with regular in-situ net cleaning need to prove the stability of their product against this potentially abrasive treatment in a

separate test. If rinsing is carried out to remove debris or other material entangled in the net, this has to be conducted using dedicated equipment that dispenses seawater in a fine spray at a maximum rate equalling 7.5 L min^{-1} per 0.0625 m^2. Rinsing must not remove any living, attached biofouling organism. All samples have to be treated equal (incl. control treatments) on all sites.
Where rinsing is carried out for any treatment/s, high-resolution photographs need to be taken before and after rinsing so that changes to these samples are documented.

➔ Rinsing may be undertaken to remove silt and entangled/entrained debris and algae from sample panels. If excessive water pressure is used, rinsing may remove attached biofouling organisms or damage the coating. By restricting the water pressure, this risk will be minimised, though not removed. Therefore, if rinsing is undertaken then all samples - including the control samples - need to be treated equally. Saltwater is to be used to prevent the destruction of organisms with sensitive osmoregulation.

Discussion of the sample analysis

Analysis interval: Samples must be analysed at least every 4 weeks. Reasons for delay must be explained in the sample evaluation report.

➔ Although biofouling growth can be excessive within 4 weeks, experience shows that broad differences in the effectiveness of antifouling coatings are usually discernible at this census interval.
The interval may need to be adapted if the guideline is to be used in regions with very high rates of biofouling development.

Preparation of sample analysis: Sample tags and frames must be cleaned from biofouling prior to analysis. Care must be taken that the cleaning process does not affect sample panels present on the frame (via physical interference or the risk of desiccation due to prolonged exposure to air; also, the samples should not be subject to contact with freshwater from deck hoses or severe rainfall).

➔ Cleaning of frames and tags ensures the correct identification of the samples. Furthermore, cleaning of the frames prevents growth from the frame from affecting the samples (e.g., long algae may brush off/hinder the growth of organisms on samples, or excessive biofouling on frames might spread to adjacent samples). In addition, it reduces strain on the frame due to excessive weight or drag.

Analysis of sample panels: Depending on the product claim, samples may be analysed for (**A**) total biofouling load or (**B**) % cover of individual biofouling groups.

Analysis of type A: *Biofouling load* of a sample can be quantified using a nominal rank scale ranging from 0 (free of biofouling) to 5 (entirely covered with biofouling organisms). The allocation of biofouling ranks must follow formal definitions and reference images of the ranks. All biofouling on the nets must be taken into account for this analysis. It is not possible to exclude fouling species of lesser relevance for aquaculture nets (e.g., slime). For detailed instructions on how to conduct this analysis, see Appendix B.

➔ This method ranks the total biofouling load on a net sample into categories, independent of its composition. It was included in the guideline in order to describe a method that is less time consuming and can be conducted by personnel without specific biofouling expertise.
The nominal scale included in this guideline comprises evenly spaced ranks that reflect successive biofouling accumulation. This allows the calculation of average ranks for multiple samples and ultimately the comparison between treatments from different tests within a series.

This linear scale, however, has the disadvantage that it does not fully reflect the disproportionate importance of the early stages of biofouling accumulation observed by many producers and customers of antifouling coatings in Norway. They are generally of the opinion that the initial growth of biofouling consisting of macroalgae or invertebrates on a net indicates the first sign of failure of a coating (the presence of slime fouling on the net sample is considered of lesser importance since it has little impact on water quality in the cage and does not attract cleaner fish). This concept may be better represented by a non-linear rank scale with finer-scale steps in the early stages of biofouling development. Higher biofouling loads may be represented by larger steps since an increase of 10 or 20 % biofouling cover is no longer as critical once the accumulation on the net has exceeded the initial attachment of individual organisms.
However, although the linear rank scale included in this guideline may be less sensitive to initial stages of biofouling accumulation, we do not see this as a disadvantage. Differences between uncoated controls and coated samples are expected to be considerable before the performance of a product is regarded as acceptable.

Analysis of type B: *% biofouling cover* is determined for the following, mandatory categories:

- Slime (slime, diatoms, algae < 5mm)
- Macroalgae
- Invertebrates ('animals')

In addition, it is recommended to identify main invertebrate biofouling groups (e.g. hydroids, mussels, ghost shrimps) that are relevant for aquaculture nets.
The analysis enables the calculation of a **Macrofouling Score** (MS) that takes into account the presence of macroalgae and invertebrates, but ignores slime since it is not of relevance for nets.
For detailed instructions on how to conduct this analysis, see Appendix C.

➔ This method follows the recommendations in the present Guidance on the Biocidal Products Regulation: Volume II Parts B+C Version 1.0 February 2017 by estimating the contribution of the following three groups to the overall biofouling cover on a net sample: slime, macroalgae, and invertebrates ('animals'). The analysis is based on a system of ≥60 randomly located ‘spots’ on the net sample that results in a robust and objective analysis of the sample. If desired, it is easily possible to include a finer resolution in the analysis without modification to the method, for example to further refine the group of 'invertebrates' into biofouling taxa that are especially relevant in salmon aquaculture, e.g., hydroids, mussels or ghost shrimps. This allows the testing of product claims with specific reference to individual taxa or species. The data resulting from this method can easily be analysed and compared between treatments.
The main disadvantage of this method compared to the alternative rank method is the fact that it is more time consuming and needs some experience regarding the identification of organisms on the net samples.

References

Bloecher N, Floerl O, Sunde LM (2015) Amplified recruitment pressure of biofouling organisms in commercial salmon farms: potential causes and implications for farm management. Biofouling 31:163-172

Bloecher N, Olsen Y, Guenther J (2013) Variability of biofouling communities on fish cage nets: A 1-year field study at a Norwegian salmon farm. Aquaculture 416–417:302-309

Fitridge I, Dempster T, Guenther J, de Nys R (2012) The impact and control of biofouling in marine aquaculture: a review. Biofouling 28:649-669

Guenther J, Carl C, Sunde LM (2009) The effects of colour and copper on the settlement of the hydroid *Ectopleura larynx* on aquaculture nets in Norway. Aquaculture 292:252-255

Guenther J, Misimi E, Sunde LM (2010) The development of biofouling, particularly the hydroid *Ectopleura larynx*, on commercial salmon cage nets in Mid-Norway. Aquaculture 300:120-127

Appendix B

Analysis of total biofouling load on aquaculture net panels

Biofouling, consisting of slime (silt, microalgae such as diatoms, algae < 5mm) and larger organisms (macroalgae and invertebrates ('animals')) on net samples is ranked according to the following 6 categories, ranging from 0 (no fouling) to 5 (very heavy fouling), described in *Table B1*:

Table B1: Ranks describing the biofouling accumulation on net panels

Rank	Description	Visual estimate of biofouling cover
0	*No fouling or just light slime fouling*: Light slime fouling only, no macrofouling organisms present	Nil
1	*Light fouling*: Distinct slime and/or initial biofouling organisms present in small patches	< 20 % of the surface area of the net sample
2	*Moderate fouling*: Biofouling organisms present in larger patches	20 – 39 % of the surface area of the net sample
3	*Considerable fouling*: biofouling organisms present on approximately half of the net	40 – 59 % of the surface area of the net sample
4	*Heavy fouling*: biofouling organisms cover most of the net	60 – 80 % of the surface area of the net sample
5	*Very heavy fouling*: biofouling organisms cover almost or all of the net and occlude part of the mesh openings	> 80 % of the surface area of the net sample

For each rank, example pictures are provided to support the analysis (*Figures B1* and *2*).

To enable a comparison between treatments within a test series consisting of several tests, relative ranks have to be calculated that express biofouling cover relative to the control (*Figure B3*).

(1) Average the rank values of the replicates of one treatment within one test.

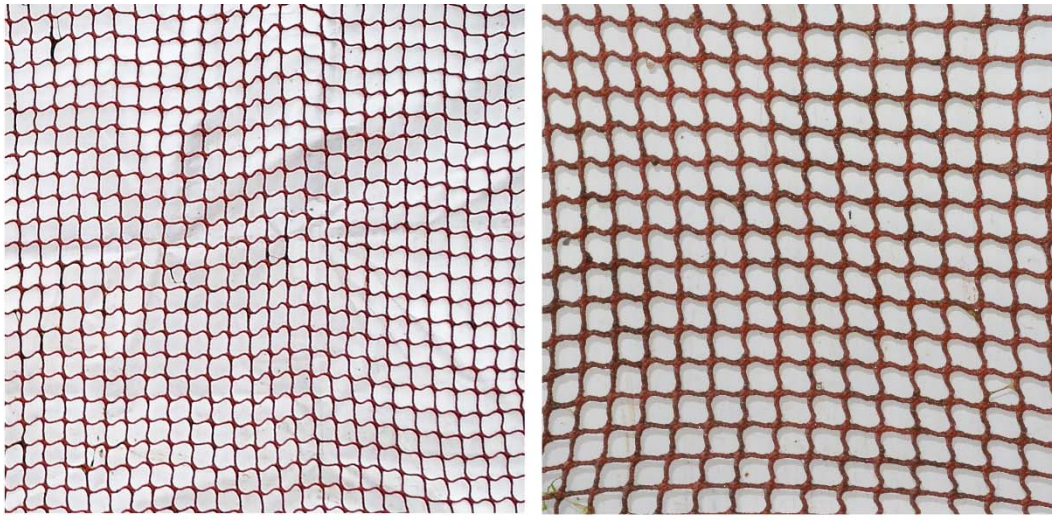
(2) Calculate the *Biofouling reduction* (rank) for each treatment in each test using the following formula:

Biofouling reduction (rank) = Average rank Control – Average rank Treatment X

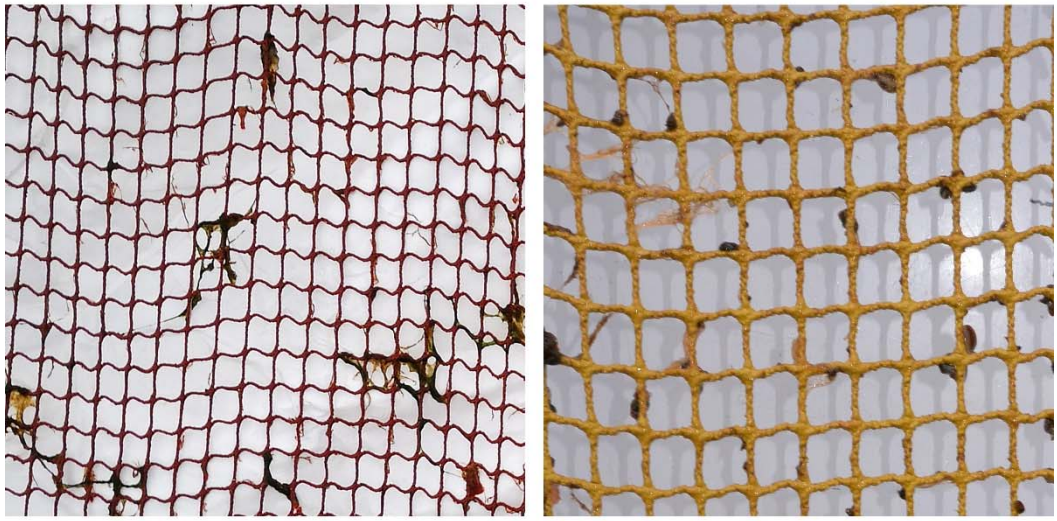
(3) Average the calculated Biofouling reduction rank for each treatment over all tests.

This *Overall average biofouling reduction* rank can be compared between treatments and can be used to evaluate the efficacy of the treatment, using appropriate statistical methods.

Rank 0:
No fouling or just light slime fouling
(Biofouling cover = 0 % of the surface area of the net sample)



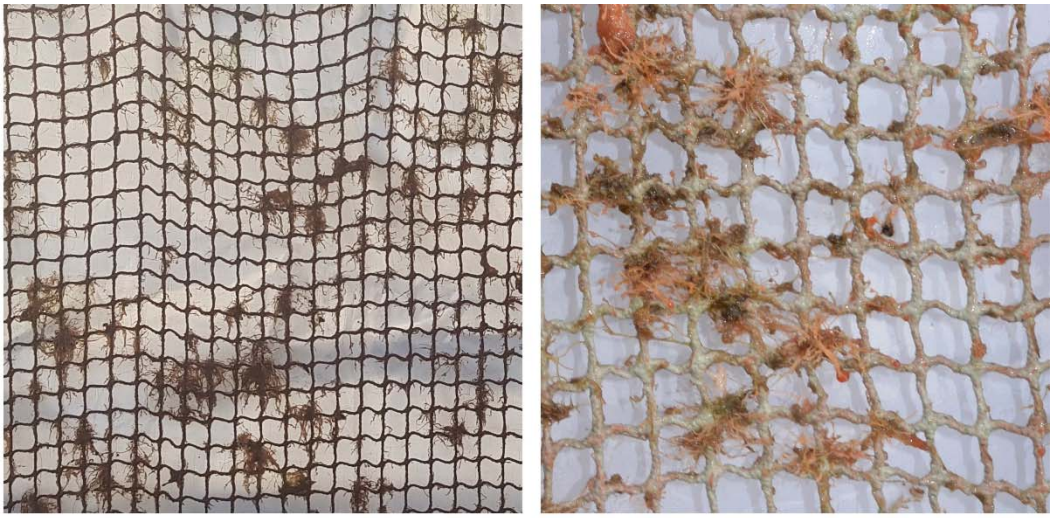
Rank 1:
Light fouling
(Biofouling cover < 20 % of the surface area of the net sample)



Rank 2:
Moderate fouling
(Biofouling cover = 20 – 39 % of the surface area of the net sample)

Figure B1: Example pictures of biofouled nets ranked 0 to 2 (Images curtesy of Brynsløkken AS and Steen Hansen AS)

Rank 3:
Considerable fouling
(Biofouling cover = 40 – 59 % of the surface area of the net sample)



Rank 4:
Heavy fouling
(Biofouling cover = 60 – 80 % of the surface area of the net sample)

Rank 5:
Very heavy fouling
(Biofouling cover > 80 % of the surface area of the net sample)

Figure B2: Example pictures of biofouled nets ranked 3 to 5 (Images curtesy of Brynsløkken AS and Steen Hansen AS)

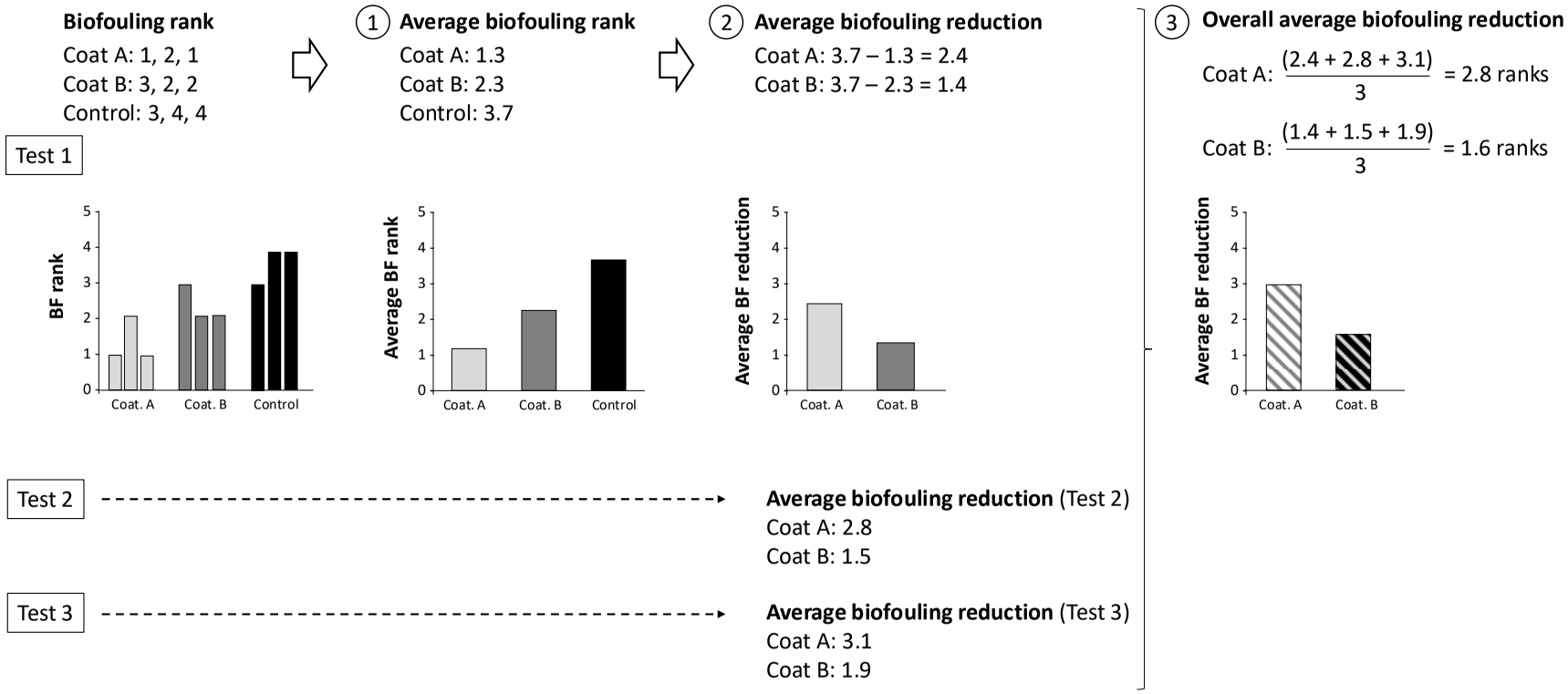


Figure B3: Example of how to compare treatments and assess efficacy in an efficacy test series consisting of 3 tests and 3 treatments (Coating A, Coating B and Control), e.g. tests conducted in different geographical locations and/or at different times

Appendix C

Analysis of % biofouling cover on aquaculture net panels

Step-by-step guide

1) Overlay the net panel randomly with at least 3 vertical and 3 horizontal lines that intersect the net strands (lines may also intersect knots in the net; *Image A*). The number of intersecting points (white arrow) should be at least 60.

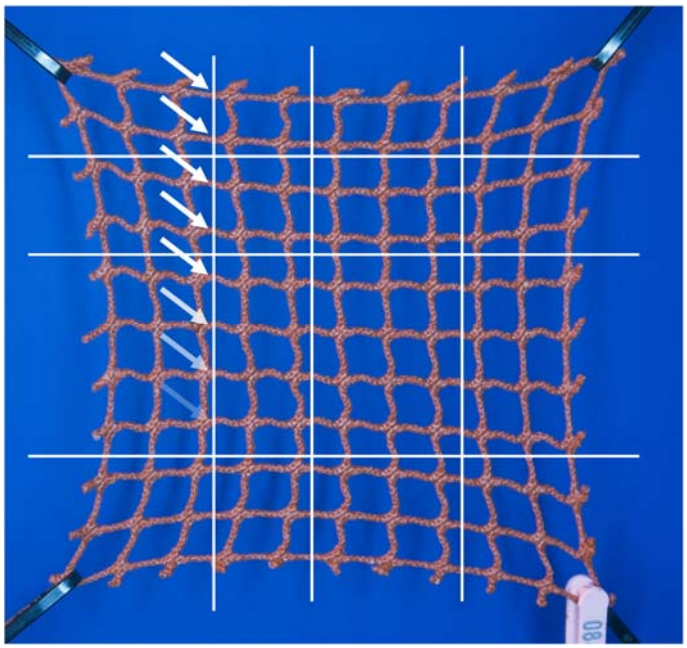


Image A

2) Follow each line and identify the organism at the intersection of the line with the net (= counting point, *Image B*).

Image B

3) Sort the organisms into the following categories:
   - Slime (slime, diatoms, algae < 5mm)
   - Macroalgae
   - Invertebrates ('animals') – the important species (e.g., hydroids, caprellids) should be identified individually, others may be counted as 'other'

4) For an organism to count, the **attachment point** should be at the counting point (*Image C*). It does not count if just a part of the organism (algal "blade", hydroid polyp head) is visible at the counting point (*Image D*)

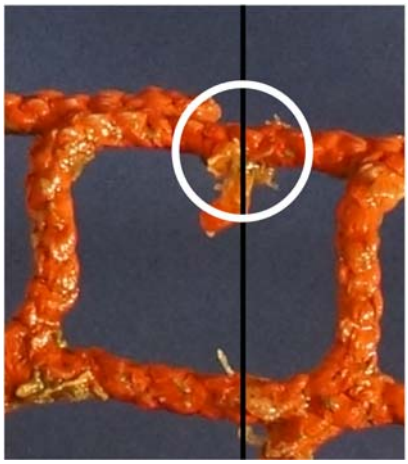


Image C

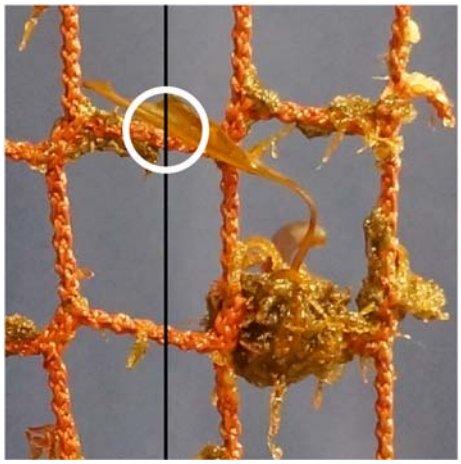


Image D

5) Sometimes, the line will cut not only though the section of the net that is perpendicular to the line, but also through other parts of the net. These sections are disregarded, organisms are only counted where the line crosses the net thread (*Image E*)

Image E

6) If more than one organism is found at a counting point, consider only the dominant organism (*Image F*).

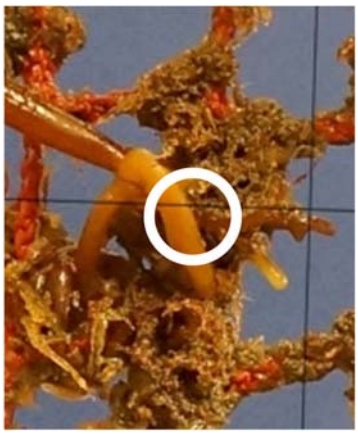


Image F

7) Sometimes larger organisms will cover parts of the net although it is clear that they are not attached at the intersection point (e.g., large algae, *Image G*). Disregard these points and subtract them from the total point count (see *Table C1*).

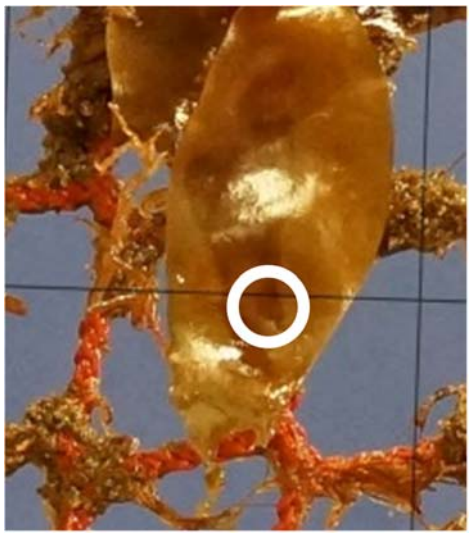


Image G

8) Infrequent species are sometimes not captured by the counting lines. If the species is relevant to aquaculture (e.g., hydroids, indicating first settlement and the beginning failure of the coating) and this test, a single account of this species is included in the list of present species, and the number of total points counted on the net is increased by 1 point.

9) Count the total number of occurrences per category and note them in the table (*Table C1*). Also, add the total number of points per sample. This should be a minimum of 60.

Table C1: Example of a counting table where % cover of relevant fouling organisms is calculated based on the total number of counted points (Points on net – points not visible)

Sample ID	Points on net	Points not visible	Points covered with biofouling					% cover
					Invertebrates			
			Slime	Macroalgae	Hydroids	Caprellids	other invertebrates	
A1.1	60	1	3	10	20	2	1	**61 %**

The % cover of biofouling is calculated using the following formula:

$$\text{Biofouling cover (\%)} = \left(\frac{\text{Slime + Macroalgae + Invertebrates}}{\text{Points on net – Points not visible}} \right) \text{x } 100$$

In this example, biofouling organisms occur on 36 points in sample A1.1 (slime [3] + macroalgae [10] + hydroids [20] + caprellids [2] + other invertebrates [1]). This value is divided by the number of counted points (i.e., the number of points on the net [60] – the number of points that were not visible [1] = 59 counted points), and then multiplied by 100. The percent cover of biofouling in sample A1.1 is 61 %.

10) To calculate the Macrofouling Score (MS), only include the % cover that is made up of 'Macroalgae' and 'Invertebrates' in the calculation,

$$\text{Macrofouling Score} = \left(\frac{\text{Macroalgae + Invertebrates}}{\text{Points on the net – Points not visible}} \right) \text{x } 100$$

In this example, 'macrofouling organisms' (excluding slime) occur on 33 points in sample A1.1 (macroalgae [10] + invertebrates [23]). This value is divided by the number of counted points (i.e., the number of points on the net [60] – the number of points that were not visible [1] = 59 counted points), and then multiplied by 100. The Macrofouling Score of sample A1.1 is 56 %

11) To enable a comparison between treatments within a test series consisting of several tests, the reduction of biofouling cover has to be calculated relative to the individual control (*Figure C1*).

(1) Average the % cover values of the replicates of one treatment within one test.

(2) Calculate the *Biofouling reduction* (%) for each treatment in each test using the following formula:

$$\text{Biofouling reduction (\%)} = (1 - \left(\frac{\text{Average BF cover Treatment X}}{\text{Average BF cover Control}} \right)) \text{ x } 100$$

(3) Average the calculated Biofouling reduction for each treatment over all tests.

This *Overall average biofouling reduction* value can be compared between treatments and can be used to evaluate the efficacy of the treatment, using appropriate statistical methods.

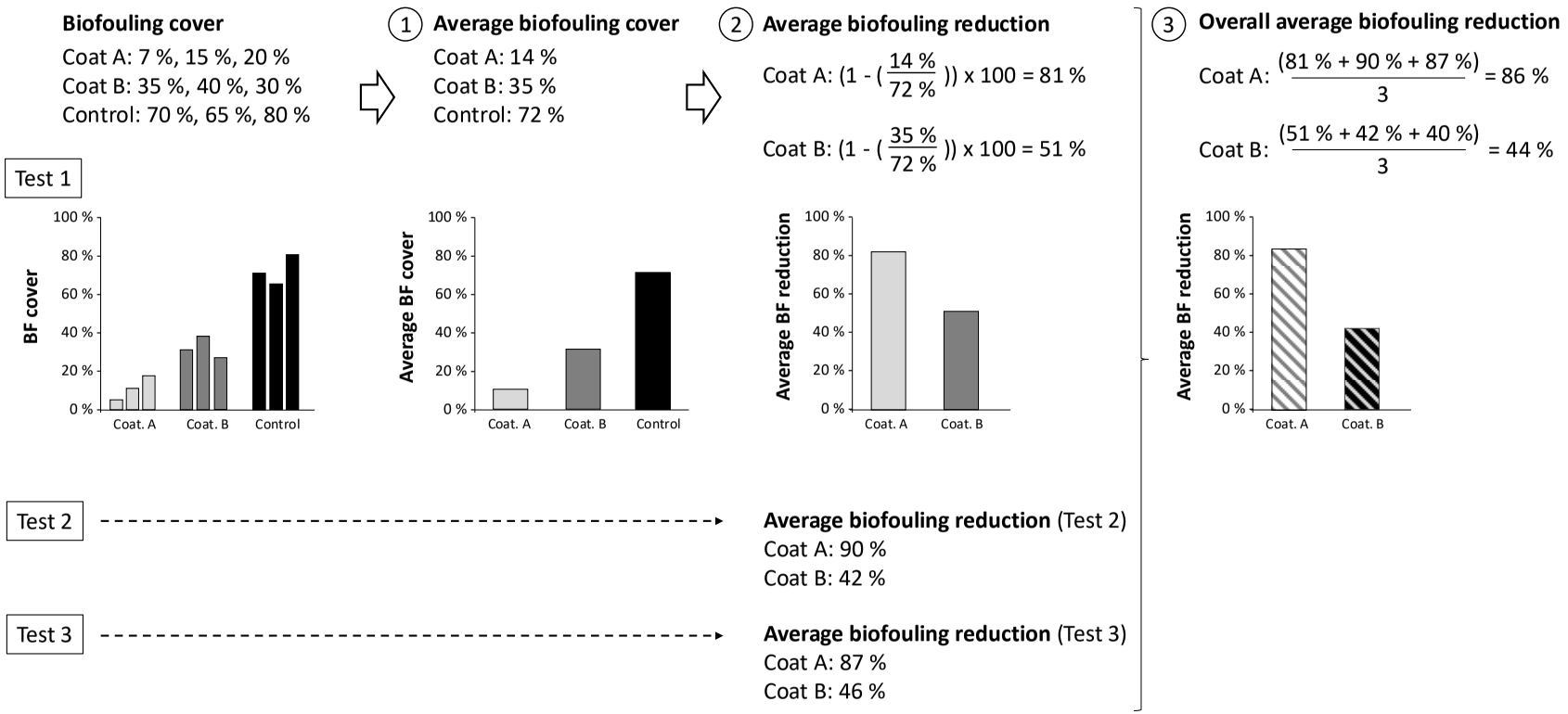


Figure C1: Example of how to compare treatments and assess efficacy in an efficacy test series consisting of 3 tests and 3 treatments (Coating A, Coating B and Control), e.g. tests conducted in different geographical locations and/or at different times