Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gche20

Toxicological response of *Amphibalanus amphitrite* larvae as an indirect evaluation of antifouling paints' efficacy

Veronica Piazza [a], Christian Corrà [a], Francesca Garaventa [a], Anna Jordan [b], Pietro Dramisino [b] & Marco Faimali [a]
[a] Institute of Marine Science (ISMAR) CNR, Genoa, Italy
[b] Lechler S.p.A., Como, Italy

Available online: 02 Dec 2011

To cite this article: Veronica Piazza, Christian Corrà, Francesca Garaventa, Anna Jordan, Pietro Dramisino & Marco Faimali (2011): Toxicological response of *Amphibalanus amphitrite* larvae as an indirect evaluation of antifouling paints' efficacy, Chemistry and Ecology, 27:sup2, 87-95

To link to this article: http://dx.doi.org/10.1080/02757540.2011.625934

Chemistry and Ecology
Vol. 27, No. S2, December 2011, 87–95

Taylor & Francis
Taylor & Francis Group

Toxicological response of *Amphibalanus amphitrite* larvae as an indirect evaluation of antifouling paints' efficacy

Veronica Piazza[a]*, Christian Corrà[a], Francesca Garaventa[a], Anna Jordan[b], Pietro Dramisino[b] and Marco Faimali[a]

[a]*Institute of Marine Science (ISMAR) CNR, Genoa, Italy;* [b]*Lechler S.p.A., Como, Italy*

(*Received 27 January 2011; final version received 16 September 2011*)

The evaluation of new antifouling paints is usually performed through long and expensive field tests (raft-tests), often depending on the season of exposure. Particularly in the Mediterranean Sea, these tests are representative only if carried out during spring or summer. Therefore, it is strategically important to develop laboratory methods that are able to test and select, during winter, the best formulations to submit to raft-test during the summer. For this reason, an acute toxicity bioassay using larvae (Stage II nauplii) of *Amphibalanus amphitrite* on paint leaching products obtained with an accelerated ageing system was tested as a tool for the indirect evaluation of antifouling efficacy. Seven experimental paint formulations were selected on the basis of previous obtained field efficacy performances, and were subjected to both laboratory bioassays and to a 12-month raft-test. The data show how paint behaviour (expressed as fouling coverage percentage) after 12 months of immersion in the field could be predicted by the results of laboratory bioassays, expressed as immobilisation percentage of larvae exposed for 48 h to leaching products of artificially aged paints.

Keywords: antifouling paints; barnacle; leaching rate; toxicity; efficacy

1. Introduction

In the nautical field, biofouling protection systems are mostly represented by antifouling (AF) paints applied to the ship's hull. There are different types of AF paints, which can generally be divided into soluble matrix, insoluble matrix and self-polishing, depending on the chemical properties of the paint matrix and on the mechanism by which active substances are released.

At present, the market is mainly made up of AF paints containing toxic substances (biocides). During formulation, the toxic molecule is included together with substances forming the paint matrix (bindings, pigments, thickenings, solvents), and is slowly released on contact with seawater. Efficacy length therefore depends on the mechanism of release of the toxic substance (leaching) over time, preventing fouling by generating a chemical barrier at the surface–water interface.

*Corresponding author. Email: veronica.piazza@ge.ismar.cnr.it

ISSN 0275-7540 print/ISSN 1029-0370 online

http://dx.doi.org/10.1080/02757540.2011.625934
http://www.tandfonline.com

In AF paints, biocide release is of fundamental importance and is quantified through leaching rate, which shows the amount of biocide released by the paint. When this value decreases and goes below the fouling settlement inhibition limit, it means that the paint has lost its efficacy. The leaching rate of toxic substances depends upon the paint formulation and on ambient conditions during use [1,2]. Many ambient factors such as temperature, salinity and pH influence biocide activation and release processes [3–5].

At present, due to limitations on the use of organostannic compounds [6–8], formulation of new AF paints has become a complex and expensive process. Evaluation of a biocide's antifouling efficacy and of new paint prototypes is usually performed using field and laboratory methods [9–11].

Laboratory screening is generally aimed at checking a biocide's antifouling efficacy by the use of larval settlement tests [10,12,13] and a series of ecotoxicological bioassays for the characterisation of their environmental compatibility.

The results of laboratory tests on biocides allow a preliminary evaluation of the efficacy and potential toxicity of an antifouling molecule, but it is difficult to foresee the real performance of the finished product once the biocide is included into a paint matrix. Indeed, laboratory tests on experimental formulations consist of a simulation or acceleration of the biocide leaching process to obtain a leaching rate over a stated time. Evaluation of the leaching rate can be performed both analytically [13], by measuring the quantity and chemical composition of the leachate, and ecotoxicologically, through evaluation of the effect of the leachate on non-target organisms (bioassays) in order to evaluate the potential impact on the marine ecosystem [14–16].

Field tests play a fundamental role in analysing the efficacy of new AF paints. The classic raft-test [17,18] consists of the static exposition of painted samples into seawater. Organism settlement is monitored at regular intervals and compared with settlement found on non-painted samples (control samples) usually made of inert material [19,20]. However, this method has some limits becauase of a lower leaching rate with respect to the real rate that would be found on a moving boat, due to environmental conditions of exposure place and mainly to prolonged exposure length (months/years) depending on place periodicity. In the Mediterranean Sea, the verification of a paint prototype's antifouling efficacy is representative only if performed during spring and summer. To select paints with the best antifouling performances, many efficacy raft-tests repeated in different years are often necessary to improve paint formulation. It is therefore important to develop innovative laboratory methods that are able to test and select, during the winter months, the best formulations to submit to raft-tests during the summer season, thus optimising the times and costs of efficacy screening.

The aim of this study was to use a toxic bioassay with Stage II nauplii of *Amphibalanus amphitrite* on paint leaching products as a tool for the indirect evaluation of leaching rate (antifouling efficacy) using a laboratory paint sample accelerated ageing system.

Amphibalanus (=Balanus) amphitrite was selected as a model organism; this sessile crustacean is one of the main species of fouling organism and is cosmopolitan [21]; it is reared and used mainly as a model organism for testing the efficacy of laboratory tests (settlement tests) in antifouling technologies [22–25]. The ease of obtaining and rearing its larval stages has extended its use as a model for studies dedicated to the standardisation of new marine ecotoxicological tests [26–29].

2. Materials and methods

2.1. *Antifouling field efficacy test (raft-test)*

Seven experimental AF paints from 30 available formulations examined in a previous field test were applied on 10 × 30 cm steel panels and plunged into seawater under static conditions for

1 year. In particular, among hard and self-polishing matrix formulations, the two best (3 and 4) and two worst (1 and 2) performing paints were selected. Moreover, two matrices without biocides (5 and 6) and an epossidic finishing product (7) were also selected as reference samples without biocides.

Two distinct series, characterised by different exposures, were prepared: one series (FL) was plunged such that half of the sample was exposed to air to simulate the floating line of the bottom, and the second series (CP) was plunged completely. Experimental samples were painted on both sides to allow exposure in both direct and half-light conditions. Each month, macro pictures of both sides were acquired, and an analysis of fouling was performed using the Dethier method [30], opportunely modified for image analysis. In addition to total surface coverage, fouling biodiversity was also quantified and analysed, on the basis of the percentage of each taxon found relative to total coverage. Thanks to this analysis, it was possible to carry out a characterisation of fouling typology by dividing organisms found on the panels into three categories (slime, soft and hard-fouling), allowing for organisms with different characteristics. The term slime indicates a thin coat, of biological origin, that can be observed on every surface after a short period of immersion in seawater. Soft-fouling is represented by macrofouling organisms without calcareous structures (algae, hydrozoans, ascidians). Hard-fouling, by contrast, is represented by macrofouling organisms with calcareous structures, such as barnacles, serpulids, briozoans and bivalves; these organisms are those mainly problematic in hull biodeterioration.

2.2. *Antifouling laboratory efficacy test*

A toxicity bioassay on *A. amphitrite* larvae (Stage II nauplii) was performed as a tool for the indirect evaluation of leaching rate (antifouling efficacy). A laboratory paint samples accelerated ageing system was used to simulate boat movement.

2.2.1. *Paints accelerated ageing*

A series of samples, 10×5 cm (treated surface 50 cm^2), were painted onto one side. The free surface of the samples was isolated from water using a non-toxic protective product. To simulate field exposure, samples were plunged into a 150-L tank permanently fed with natural seawater (constant flow 2.5 $L \cdot min^{-1}$) at ambient temperature; inside the tank, a current was artificially created to simulate movement of a boat. Samples were located in the tank with the painted side turned to the wall. In the middle of the tank, a rotor, moving with a controlled speed of 500 rpm, was used to produce an upward current along the walls [31]. In this way, water runs on painted surface with a speed of 0.2 $m \cdot s^{-1}$, thus simulating movement of a boat. The ageing process started during winter months (January) and continued to the end of spring (May), it lasted 5 months and was subjected to natural variations in seawater temperature ($11.5°C \leq T \geq 25°C$).

2.2.2. *Obtaining leaching products*

In order to obtain leaching products at fixed ageing times, samples were periodically collected from the tank and placed into 600-mL Pyrex borosilicate beakers containing natural seawater at 37‰ salinity, pH 8 and filtered with a 0.22 μm filter (0.22 FNSW). Beakers were kept at 25°C and aerated. The leaching process was carried out for 24 h and leachates were collected and kept in the dark at 4°C for 48 h before performing toxicity bioassays. For all paints, seven ageing times (T) were selected: T0 = 24 h ; T1 = 7 days; T2 = 14 days; T3 = 30 days; T4 = 2 months; T5 = 3 months; T6 = 4 months; T7 = 5 months.

2.2.3. *Leachate toxicity bioassay*

Toxicity tests were prepared by using leaching products obtained at different ageing times. Tests were performed using Stage II nauplii of *A. amphitrite* obtained 2–4 h before starting the test. Nauplii were obtained from an adult culture according to the method described by Faimali and Garaventa [32]. Toxicity bioassays were performed by adding 15–20 nauplii into multiwell plates containing 2 mL of non-diluted (100%) paint leaching products. Controls were obtained by adding nauplii to 2 mL of 0.22 FNSW, plates were kept in the dark at 20°C. After 24 and 48 h, plates were observed under a stereo-microscope, and larvae that were completely motionless for 10 s were counted as dead. Larvae that presented appendage movement without shifting their own barycentre were counted as 'not-swimming'. The number of dead and 'not-swimming' larvae constitutes 'immobile' larvae, and the percentage of immobilisation was calculated compared with control.

3. Results

3.1. *Antifouling field efficacy test (raft-test)*

In order to quantify and qualify biological covering due to hard-fouling, only the results of half-light-exposed sides are shown; in fact, on the light-exposed sides, significant percentages of hard-fouling covering were not found. On the half-light-exposed side, all paints showed a fouling composition much more heterogeneous in correspondence with the floating line (FL), where typical Mediterranean biofouling organisms were found, for this reason only data regarding this series of samples are shown.

Figure 1 shows the biological coverage percentage composition obtained after 12 months; paint formulations 1 and 2 show the presence of both soft-fouling (hydrozoans, ascidians, algae) and hard-fouling (barnacles, serpulids, briozoans) organisms, thus showing bad antifouling efficacy performances. By contrast, paint formulations 3 and 4, also after 12 months of static immersion,

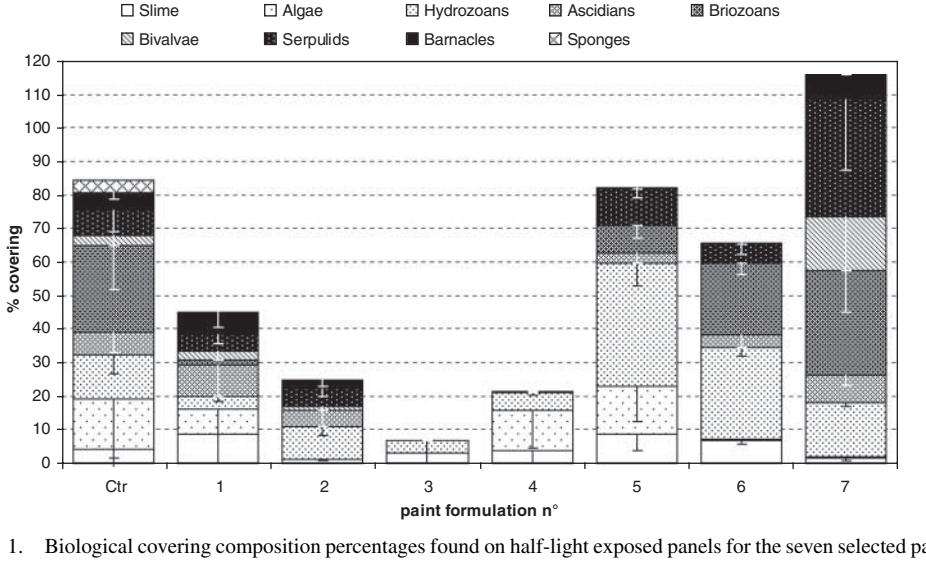

Figure 1. Biological covering composition percentages found on half-light exposed panels for the seven selected paints after 12 months in correspondence to the floating line (FL). Histograms represent mean results of the two sample replicates (mean ± SE; $n = 2$).

show excellent antifouling efficacy: biological coverage composition is small ($\leq$20%) and mainly composed of slime, algae and hydrozoans. Both matrices without biocide (5, 6) show total coverage percentages and fouling composition comparable with those the control (>60%).

It is interesting to observe how samples painted with the epossidic undercoat (7), not containing an antifouling product, show coverage percentages (>100%) higher than those observed for control samples. Moreover, from a more detailed analysis of fouling composition and typology, it is evident that this series of samples is characterised by a higher settlement percentage of calcareous organisms (barnacles and serpulids) belonging to hard-fouling.

3.2. *Antifouling laboratory efficacy test*

Results of *A. amphitrite* larvae immobilisation after 48 h of contact with leaching products of the seven selected paints are reported in Figure 2. An end-point of 48 h was selected as most representative; seawater temperature values are also reported.

The results show how the matrices (5, 6) and the epossidic undercoat (7), not containing biocides, do not have any toxicity towards nauplii, as immobilisation percentages for all ageing times are comparable with those obtained for the control. However, leachates obtained from paints with biocides (1, 2, 3 and 4) show higher immobilisation percentages. In particular, after 14 days of accelerated ageing (T1), paints 3 and 4 show immobilisation percentages >80%. This might mean that after a first short paint-conditioning period, necessary to start surface hydrolysis, there is a correct release of leaching products that has an evident effect on larval immobilisation.

The figure also shows how, starting from the first month of ageing (T3), there is a constant reduction in immobilisation percentages, and, after the fourth month of ageing (T5), an increase occurs again. This trend might have been caused by seawater temperature fluctuations during the ageing process, as it started during winter and ended during late spring, in conjunction with the increase in temperature, which for these paints probably has a positive effect on the hydrolysis process [4,33].

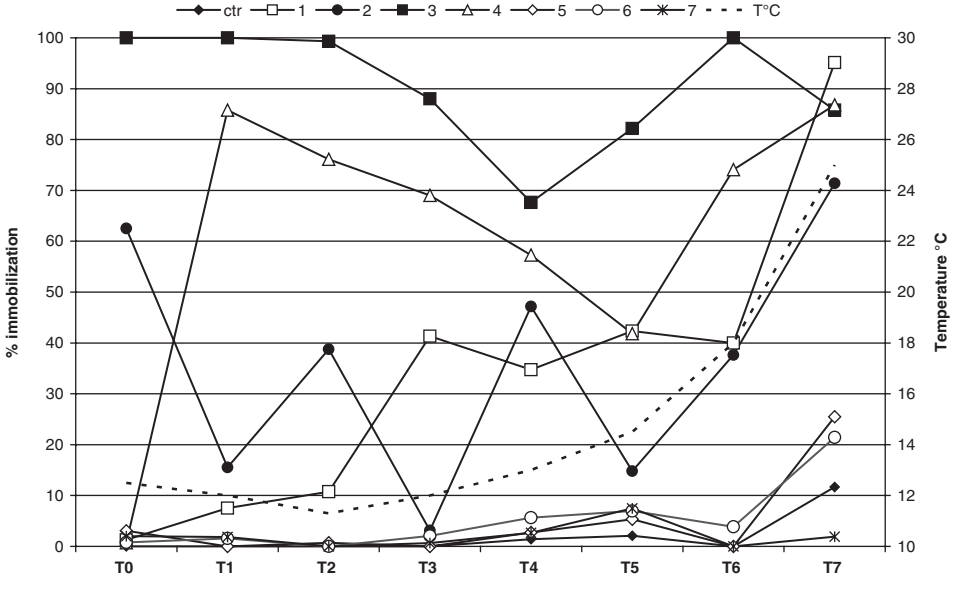

Figure 2. Immobilization percentages of *A. amphitrite* larvae exposed to leaching products obtained after different ageing times (T0…T7). Seawater temperature fluctuations during the experiment are also reported.

The larval immobilisation effect of paint 1 increases constantly with ageing time, whereas paint 2, even though it also shows an increasing trend, seems to be less stable and constant over time.

Moreover, it is interesting to observe how paint 3 shows, in correspondence to T0, a high release of toxic substances (immobilisation percentage = 100%) probably because of its self-polishing matrix, whereas paint 4, with a hard matrix, despite having an excellent leaching rate, required a longer period of activation.

Paints 1 and 2 exhibit lower immobilisation percentages ($\leq$60%) than paints containing biocides (3 and 4), the only exception being the last month (T7). In this case, the increase in the toxic effect for paints 1 and 2 might have been caused by the increase in seawater temperature, causing a greater release of leaching products.

4. Discussion

The sequential execution of a field antifouling efficacy test (raft-test) and the laboratory bioassay on two series of the same experimental AF paints allowed us to check the reliability of the proposed bioassay as a predictive tool for field performances.

Comparison of raft-test and ecotoxicological results showed how paints with a high efficacy towards hard-fouling organisms (barnacles, serpulids) also exhibit high immobilisation percentages towards *A. amphitrite* larvae. By contrast, paints with a low antifouling efficacy during the first months of field immersion, also have a lower ecotoxicological response. It is clear that formulations not containing biocides, which do not exhibit any toxic effect in bioassays, give coverage percentages and a fouling community biodiversity comparable with those obtained from control samples.

The proposed laboratory bioassay was shown to be able to characterise and discriminate, by using the toxicological response of *A. amphitrite* larvae, the different toxic substance release performances of the experimental formulations. The possibility of observing in a laboratory, over a short period (few months), differences in performances, also due to some environmental parameters, confirms the possibility of characterising the potential efficacy of new AF paints. This laboratory method allows us to execute efficacy pre-screening on new paints all year round. In this study, we carried out a field efficacy test (raft-test) and a parallel laboratory bioassay to obtain an indirect measure of leaching rate. Application of the two test typologies on the same series of experimental AF paints allowed us to validate the laboratory test as a predictive tool for paint field performances.

The aim of this study was to check whether toxicological data obtained in laboratory bioassays (larval immobilisation percentage) representing an indirect measure of paint efficacy, were able to provide information on the field behaviour of the paint itself. The results mean that, if a paint leachate tested in laboratory shows low larval immobilisation (= low toxicity), a high coverage percentage of panels will be expected for this formulation in a field test. By contrast, if a paint leachate shows high naupliar immobilisation percentages in laboratory bioassays (= high toxicity), panels exposed in a raft-test will probably show a low coverage percentage.

In order to make comparisons between field efficacy and laboratory tests, two graphs (Figures 3 and 4) reporting both total coverage percentages after 4 and 12 months of field immersion of panels painted with selected formulations and larval immobilisation percentages obtained in laboratory bioassay using leaching products collected respectively after 7 days and 5 months of panels ageing (T1 and T7) are reported.

It is evident both from Figure 3 and Figure 4 that high covering percentages correspond to low larval immobilisation percentages (that indicate a low toxicity of leaching products) and vice versa.

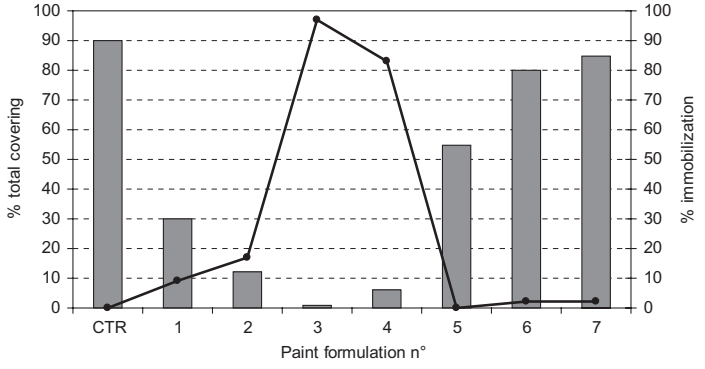

Figure 3. Total coverage percentages (grey bars) after 4 months of panel immersion in seawater and immobilization percentages (black line) obtained by testing paint leaching products collected after 7 days (T1) of accelerated panel ageing with stage II nauplii of *A. amphitrite*.

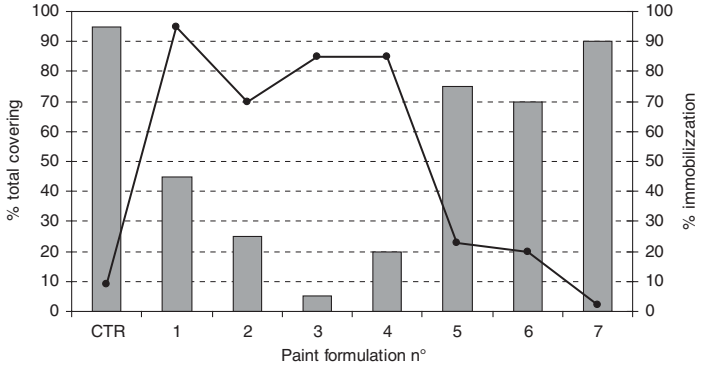

Figure 4. Total coverage percentages (grey bars) after 12 months of panel immersion in seawater and immobilization percentages (black line) obtained by testing paint leaching products collected after 5 months (T7) of accelerated panel ageing with stage II nauplii of *A. amphitrite*.

From Figure 3 it can be seen that percentage coverage after 4 months of field immersion of panels corresponds closely with larval immobilisation percentages obtained in the laboratory bioassay using leaching products collected after 7 days of panels ageing (T1). This correspondence is confirmed by data reported in Figure 4.

This means that the behaviour of field-exposed panels after four months (or even one year) of immersion could be predicted from data (larval immobilisation percentages) obtained in laboratory by using leaching products collected from 7 days (or 5 months) aged panels. This is an excellent result, as the performance of this bioassay during winter months, before carrying out the raft-test, would allow an early selection of most performing paints, thus reducing the number of prototypes exposed in field, saving time and money. In the literature, a number of laboratory bioassays has been designed to search for antifouling compounds. However, there is no evidence to date that these assays provide results reproducible in ecologically realistic field experiments. Comparison of ecotoxicological responses obtained in the laboratory with settlement rates observed in field experiments has been analysed previously [34–37]. Rittschof [34] compared settlement inhibition and toxicity data obtained with *B. amphitrite* larvae to define the antifouling mechanism of some substances. Also Löschau and Krätke [35] showed how antifouling effects may be related to the toxicity of released products on *B. amphitrite* larvae. In his article, Da Gama et al. [36] tested natural extracts from the Brazilian seaweeds *Laurencia obtusa* and *Stypopodium zonale*

in the laboratory through the 'mussel test' and in the field through the 'phytagel method' in order to compare the efficiency of these methods in assessing antifouling activity; their findings suggested that the 'mussel test' is a reliable time- and cost-saving screening method for antifouling substances, although field assays are more sensitive for the detection of their activity spectrum. Feng et al. [37] evaluated the antifouling activity of pyrethroids in both laboratory experiments (settlement test on *B. albicostatus* cyprids) and field experiments and found that the laboratory results were confirmed by field test.

Studies correlating results of laboratory assays with effects in the field are scarce [38–40] but, like our study, they indicate relatively good agreement. Although the power of laboratory assays lies in the rapid, highly sensitive screening of potential antifouling compounds for antifouling effectiveness and toxicity [10], they have weaknesses. These include, for example, the use of a single fouling organism, making it impossible to determine the activity spectrum of compounds and the lack of standardised testing [41]. Although the screening for new antifouling products using laboratory based bioassays can be a useful and quite reliable method, the ecological significance of laboratory bioassays appears to be very limited and should be confirmed by subsequent field experiments. Further work is needed to confirm the validity of the proposed predictive laboratory bioassay.

Acknowledgements

This work was financed by the contribution of the Italian company Lechler S.p.A.

References

[1] K. Schiff, D. Diehl, and A. Valkirs, *Copper emission from antifouling paint on recreational vessels*, Mar. Pollut. Bull. 48 (2004), pp. 371–377.
[2] S.M. Olsen, L.T. Pedersen, M.H. Hermann, S. Kiil, and K. Dam-Johansen, *Inorganic precursor peroxides for antifouling coatings*, J. Coat. Technol. Res. 6(2) (2009), pp. 187–199.
[3] S. Kiil, C.E. Weinell, M.S. Pedersen, and K. Dam-Johansen, *Mathematical modelling of a selfpolishing antifouling paint exposed to seawater: A parameter study*, Trans. Inst. Chem. Eng. 80 (2002), pp. 45–52.
[4] A.O. Valkirs, P.F. Seligman, E. Haslbeck, and J.S. Caso, *Measurement of copper release rates from antifouling paint under laboratory and in situ conditions: Implications for loading estimation to marine water bodies*, Mar. Pollut. Bull. 46 (2003), pp. 763–779.
[5] N. Singh and A. Turner, *Leaching of copper and zinc from spent antifouling paint particles*, Environ. Pollut. 157 (2009), pp. 371–376.
[6] International Maritime Organization (IMO), *International Conference on the Control of Harmful Anti-Fouling Systems on Ships, Adoption of the Final Act of the Conference and Any Instruments, Recommendations and Resolutions Resulting From the Work of the Conference*, 18 October 2001, IMO Headquarters, London, UK, 2001.
[7] K.V. Thomas, M. McHugh, and M. Waldock, *Antifouling paint booster biocides in UK coastal waters: Inputs, occurrence and environmental fate*, Sci. Total. Environ. 293 (2002), pp. 117–127.
[8] T. Cresswell, J.P. Richards, G.A. Glegg, and J.W. Readman, *The impact of legislation on the usage and environmental concentrations of Irgarol 1051 in UK coastal waters*, Mar. Pollut. Bull. 52 (2006), pp. 1169–1175.
[9] F.H. De La Court and H.J. De Vries, *Advances in fouling prevention*, Prog. Org. Coat. 1 (1973), pp. 375–404.
[10] D. Rittschof, A.S. Clare, D.J. Gerhart, A.M. Sister, and J. Bonaventura, *Barnacle in vitro assay for biologically active substace: Toxicity and settlement inhibition assay using mass cultured* Balanus amphitrite *amphitrite Darwin*, Biofouling 6 (1992), pp. 115–122.
[11] A.H. Jacobson and G.L. Willingham, *Sea-nine antifoulant: An environmental acceptable alternative to organotin antifoulant*, Sci. Total. Environ. 258 (2000), pp. 103–110.
[12] A.S. Clare, *Marine natural product antifoulants: Status and potential*, Biofouling 9 (1996), pp. 211–229.
[13] B.T. Watermann, B. Daehne, S. Sievers, R. Dannenberg, J.C. Overbeke, J.W. Klijnstra, and O. Heemken, *Bioassays and selected chemical analysis of biocide-free antifouling coatings*, Chemosphere 60 (2005), pp. 1530–1541.
[14] N. Voulvoulis, M.D. Scrimshaw, and J.N. Lester, *Comparative environmental assessment of biocides used in antifouling paints*, Chemosphere 47 (2002), pp. 789–795.
[15] E. Ytreberg, J. Karlsson, and B. Eklund, *Comparison of toxicity and release rates of Cu and Zn from anti-fouling paints*, Sci. Total. Environ. 408 (2010), pp. 2459–2466.
[16] J., Karlsson, E. Ytreberg, and B. Eklund *Toxicity of anti-fouling paints for use on ships and leisure boats to non-target organisms representing three trophic levels*, Environ. Pollut. 158 (2010), pp. 681–687.

[17] J.E. Hunter and L.V. Evans, *Raft trial experiments on antifouling paints coating Zineb and copper*, Biofouling 3 (1991), pp. 113–137.
[18] N. Clarkson and L.V. Evans, *Raft trial experiments to investigate the antifouling potential of silicon elastomer polymers with added biocide*, Biofouling 9 (1995), pp. 129–143.
[19] G. Relini and M. Faimali, *Il biofouling*, in *Manuale di Metodologie di Campionamento e Studio del Benthos Marino Mediterraneo*, M.C. Gambi and M. Dappiano eds., Biol. Mar. Mediterr. 10 (2003), pp. 285–326.
[20] A. Terlizzi and M. Faimali, *Fouling on artificial substrata*, in *Biofouling*, S. Dürr, and J. Thomason, eds., Wiley-Blackwell, Ames, IA, 2010, pp. 170–184.
[21] D.V. Desai, A.C. Anil, and K. Venkat, *Reproduction in* Balanus amphitrite *Darwin (Cirripedia: Thoracica): Influence of temperature and food concentration*, Mar. Biol. 149 (2006), pp. 1431–1441.
[22] A. Terlizzi, S. Fraschetti, P. Gianguzza, M. Faimali, and F. Boero, *Environmental impact of antifouling technologies: State of art and perspectives*, Aquat. Conserv. Mar. Freshwater Ecosyst. 11 (2001), pp. 311–317.
[23] D.M. Yebra, S. Kiil, and K. Dam-Johansen, *Antifouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings*, Prog. Org. Coat. 50 (2004), pp. 75–104.
[24] A.I. Railkin, *Marine Biofouling: Colonization Processes and Defenses*, CRC Press, Boca Raton, FL, 2004, pp. 320.
[25] L.D. Chambers, K.R. Stokes, F.C. Walsh, and R.J.K. Wood, *Modern approaches to marine antifouling coatings*, Surf. Coat. Technol. 201 (2006), pp. 3642–3652.
[26] M. Faimali, F. Garaventa, V. Piazza, F. Magillo, G. Greco, C. Corrà, E. Giacco, L. Gallus, and C. Falugi, *Swimming speed alteration of larvae of* Balanus amphitrite *as behavioural end-point for laboratory toxicological bioassays*, Mar. Biol. 149 (2006), pp. 87–96.
[27] M. Faimali, F. Garaventa, V. Piazza, G. Greco, C. Corrà, and G. D'amico, *Mortality, settlement inhibition and swimming speed alteration of larvae of* Balanus amphitrite *as acute, chronic and behavioural end-point for laboratory toxicological bioassays*, Biol. Mar. Mediterr. 14 (2007), pp. 114–116.
[28] G. Greco, C. Corra', F. Garaventa, E. Chelossi, and M. Faimali, *Standardization of laboratory bioassays with* Balanus amphitrite *larvae for preliminary oil dispersants toxicological characterization*, Chem. Ecol. 22(1) (2006), pp. 163–172.
[29] A.M.F. Choong, S.L.M. Teo, J.L. Leow, H.L. Koh, and P.C.L. Ho, *A preliminary ecotoxicity study of pharmaceuticals in the marine environment*, J. Toxicol. Environ. Health A 69 (2006), pp. 1959–1970.
[30] M.N. Dethier, E.S. Graham, S. Gohen, and L.M. Tear, *Visual versus random-point percent cover estimations: 'Objective' is not always better*, Mar. Ecol. Prog. 96 (1993), pp. 93–100.
[31] I. Trentin, V. Romairone, G. Marcenaro, and G. De Carolis, *Quick test methods for marine antifouling paints*, XXV Fatipec Congress, Congress Proceedings, Torino, 3 (2000), pp. 55–65.
[32] M. Faimali and F. Garaventa, *Produzione ed allevamento di larve di Cirripedi:* Balanus amphitrite *come specie modello*, in *Metodologie di studio del plancton marino, Manuali e Linee Guida 56/2010 – ISPRA e SIBM*, G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, and C. Totti, eds., ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale), 2010, pp. 562–574.
[33] K. Thomas, K. Raymond, J. Chadwick, and M. Waldock, *The effects of short term changes in environmental parameters on the release of biocides from antifouling coatings: Cuprous oxide and tributyltin,* Appl. Organimetal. Chem. 13 (1999), pp. 453–460.
[34] D. Rittschof, A.S. Clare, D.S. Gerhart, J. Bonaventura, C. Smith, and M. Hadfield, *Rapid field assessment of antifouling and foul-release coatings*, Biofouling 6 (1992), pp. 181–192.
[35] M. Löschau and R. Krätke, *Efficacy and toxicity of self-polishing biocide-free antifouling paints*, Environ. Pollut. 138 (2005), pp. 260–267.
[36] B. Da Gama, R.C. Pereira, A. Soares, V. Teixeira, and Y. Yoneshigue-Valentin, *Is the mussel test a good indicator of antifouling activity?A comparison between laboratory and field assays*, Biofouling 19 (2003), pp. 161–169.
[37] D.Q. Feng, C.H. Ke, S.J. Li, C.Y. Lu, and F. Guo, *Pyrethroids as promising marine antiofoulants: Laboratory and field studies*, Mar. Biotechnol. 11(2) (2009), pp. 153–160.
[38] G.J. Bakus, B. Schulte, S. Jhu, M. Wright, M. Green, and P. Gomez, *Antibiosis and antifouling in marine sponges: Laboratory versus field studies*, in *New Perspectives in Sponge Biology*, Proceeding of the 3rd International Sponge Conference, K. Rutzler, ed., Smithsonian Institution, Washington, DC, 1985, pp. 102–108.
[39] D. Rittschof and J.D. Costlow, *Bryozoan and barnacle settlement in relation to initial surface wettability: A comparison of laboratory and field studies*, in *Topics in Marine Biology*, *Proceedings of the 22nd European Marine Biology Symposium*, J.D. Ros, ed., Instituto de Cienciar del Mar, Barcelona, Spain, 1989, pp. 411–416.
[40] E. Delort, N. Watanabe, H. Etoh, K. Sakata, and H.J. Ceccaldi, *Analysis of initial fouling process in coastal environment: Effects of settlement, attachment, and metamorphosis promoters*, Mar. Biotechnol. 2 (2000), pp. 224–230.
[41] R. de Nys, T. Leya, R. Maximilien, A. Afsar, P.S.R. Nair, and P.D. Steinberg, *The need for standardised broad scale bioassay testing: a case study using the red alga* Laurencia rigida, Biofouling 10 (1996), pp. 213–224.