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Short communication

The effects of colour and copper on the settlement of the hydroid *Ectopleura larynx* on aquaculture nets in Norway

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ABSTRACT

In an attempt to find more effective and applied solutions to reduce the settlement of the hydroid *Ectopleura larynx* on aquaculture nets in Norway, this study determined the settlement preferences of *E. larynx* with regards to colour and the presence of copper in antifouling coatings. Settlement assays showed that actinulae settled significantly less on white ($67.0 \pm 5.4\%$) than on black surfaces ($87.0 \pm 2.6\%$), whereas there were no significant differences between the remaining colours yellow, red and blue. However, when given a choice between (1) white and black, (2) red and black, and (3) red and white surfaces, actinulae did not preferentially settle on any colour. A field experiment at a salmon farm in Mid-Norway tested the effects of colour (red and white) and the presence of copper (with and without cuprous oxide) in the coating of net panels on the accumulation of fouling. After 10 weeks of immersion, the only macro-fouling species on the nets was *E. larynx*. The presence of cuprous oxide significantly reduced the wet weight of fouling, while colour did not have a significant effect. The mean wet weights of fouling on net panels with cuprous oxide coatings (347 ± 25 g m^{-2} and 330 ± 19 g m^{-2} for red and white nets, respectively) were approximately half of the mean wet weights of fouling on the net panels without cuprous oxide (695 ± 43 g m^{-2} and 695 ± 45 g m^{-2} for red and white nets, respectively). There were no macro-fouling species present on nets with cuprous oxide coatings, and the wet weights of these nets were only due to absorbed water and sediment.

1. Introduction

Biofouling adds considerable weight to farming and culture equipment, threatens the stability of suspended culture systems, and reduces the lifespan of mooring lines (reviewed in Braithwaite and McEvoy, 2005; de Nys and Guenther, 2009). Furthermore, the accumulation of fouling species also decreases the water exchange across nets, causing a reduction in water quality within enclosures (Cronin et al., 1999; Braithwaite et al., 2007). To reduce biofouling and its associated negative impacts, the Norwegian fish farming industry mainly uses red-brown copper-based coatings on nets combined with regular underwater washing (Olafsen, 2006). However, after the coated nets have been immersed for several months, the copper-based coatings lose some of their efficacy, and biofouling starts to develop on the nets. Over the last decade, the hydroid *Ectopleura larynx* (syn. *Tubularia larynx*) has become one of the most common fouling species in the Norwegian fish farming industry, dominating the fouling community on aquaculture nets in Southwest- and Mid-Norway between July and November. During the peak of the biofouling season, the fish farmers need to clean their nets every 2 weeks, which is a resource-demanding task.

The settlement of fouling organisms is influenced by the interaction of abiotic (e.g. hydrodynamics, surface texture, light and gravity) and biotic factors (e.g. microbial films) operating at different temporal and spatial scales (Crisp, 1974; Rodríguez et al., 1993). Surface colour is also an abiotic factor, which affects the settlement of some fouling species. For example, the barnacle *Balanus amphitrite* var. *cirratus*, the polychaetes *Spirorbis* sp. and *Hydroides norvegica* and the bryozoan *Bugula neritina* prefer to settle on black surfaces (Wiseley, 1958; James and Underwood, 1994; Swain et al., 2006), whereas the algae *Ulva rigida* and *Ulva* sp. prefer to settle, have stronger adhesion and grow more quickly on lighter coloured surfaces (Hodson et al., 2000; Finlay et al., 2008). Physical and chemical factors (such as water temperature, light intensity, substrate heterogeneity and chemical extracts of adult specimens) influencing the settlement and recruitment of the hydroid *E. larynx* have also been investigated (Nellis and Bourget, 1996). However, there is little information on the impacts of *E. larynx* on the aquaculture industry and its control, and more specifically, knowledge of the effect of colour and copper in antifouling coatings on the settlement of *E. larynx* is still lacking. In an attempt to find more effective and applied solutions to reduce the settlement of *E. larynx* on aquaculture nets in Norway, the aim of this study was to determine the effects of

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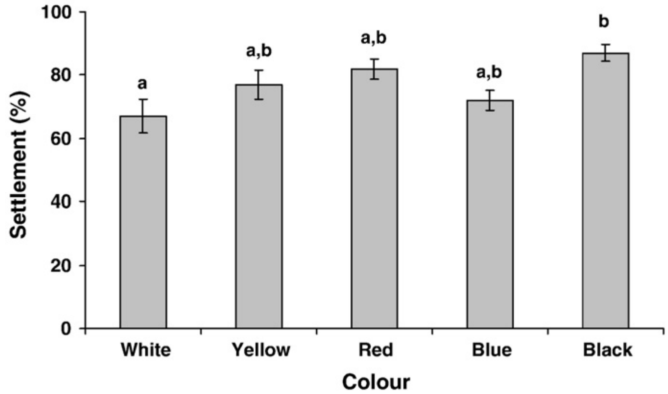

Fig. 1. Effects of colour (white, yellow, red, blue and black) on the settlement (%) of the hydroid *Ectopleura larynx*. Means ± SE are shown ($n=10$). Different superscript letters indicate significant differences ($p<0.05$).

colour independently, and with copper in antifouling coatings, on the settlement of the hydroid *E. larynx*.

2. Materials and methods

2.1. Study site

The field experiments were carried out at a commercial salmon farm located at Sunde (N 63°30.21′; E 09°11.59′), near Hitra, Mid-Norway, from October to December 2008. This salmon farm consisted of 8 net cages with circumferences of 157 m, and used a copper-based coating (NetKem, Netwax NI Gold) on nylon nets combined with underwater washing to control the accumulation of biofouling on the nets.

2.2. No-choice and choice settlement assays with Ectopleura larynx

To determine the effects of colour on the settlement of *E. larynx*, colonies of *E. larynx* were collected from cage nets of the salmon farm located at Sunde in September 2008. The colonies were transported to SINTEF Sealab, Trondheim and kept in recirculating sand-filtered seawater until they released larvae. The larvae of *E. larynx* are termed actinulae; they do not swim, but once they are in contact with a substratum, they can move on the tips of the aboral tentacles pointing towards the substratum at a speed of up to 0.3 cm h^{-1} (Pyefinch and Downing, 1949). The majority of the actinulae settle within the immediate vicinity of the adult colony if substrate is available and suitable (Pyefinch and Downing, 1949; Nellis and Bourget, 1996). First, to run no-choice settlement assays with *E. larynx* actinulae, clear polystyrene Petri dishes (diameter: 5.2 cm, surface area: 21.2 cm^2) were placed on white, yellow, red, blue and black surfaces ($n=10$). Copy paper (Logic™ 300 and Niceday coloured copy paper with the following colour tones: "intense yellow", "intense red" and "intense blue") and a black PVC sheet were used. The Petri dishes were filled with 10 mL of 0.45 µm filtered seawater (FSW), and 10 actinulae were added to each dish. The Petri dishes were kept at a constant temperature of 12 °C and under continuous light (light intensity of approximately 100 µmol m^{-2} s^{-1}) for 72 h. Actinulae were considered as settled, if they were irreversibly attached to the surface. Settled and unsettled actinulae were counted using a dissecting microscope. Second, to run choice settlement assays with *E. larynx* actinulae, clear polystyrene Petri dishes (diameter: 3.5 cm, surface area: 9.6 cm^2) were placed on differently coloured papers, so that the Petri dish bottom was placed on one colour, with one half on the other colour. The following colour combinations were tested: (1) white and black, (2) red and black, and (3) red and white. The Petri dishes were filled with 4 mL of 0.45 µm FSW. Single actinulae ($n=40$) were placed on the line between the two colours. The Petri dishes with actinulae were kept in the same room under continuous light for 72 h. Subsequently, the numbers of settled and unsettled actinulae were counted, and the preferred colours were recorded.

2.3. Accumulation of fouling on nets

To test the effects of colour and the presence of copper in coatings on the accumulation of fouling on nets, a 2×2 factorial design based on colour (red and white) and presence of copper (with and without cuprous oxide) was employed ($n=6$). The colours red and white were chosen for this experiment, because the copper-based coatings used in the Norwegian fish farming industry are mainly red-brown and the no-choice settlement assays showed that *E. larynx* actinulae settled significantly less on white than on black surfaces. The coatings were specifically made for this study by Steen–Hansen Maling. Both red and white copper-based coatings contained approximately 24% cuprous oxide, with the white copper-based coating also containing titandioxide to achieve the white colour. The red and white coatings without copper contained iron oxide and titandioxide, respectively. The net panels (25×25 cm, 25 mm half-mesh) were randomly attached to a PVC frame (6×4 net panels) and deployed at 2 m depth on the outside of a cage at the salmon farm. The wet weights of the net panels (in g) were taken after 6 and 10 weeks of immersion. Due to the differences in the mean initial weights of net panels (red with cuprous oxide: 35.5 ± 0.1 g; white with cuprous oxide: 39.5 ± 0.1 g; red without cuprous oxide: 29.1 ± 0.1 g; white without cuprous oxide: 30.4 ± 0.1 g), the wet weight of the fouling of the net panels was calculated by subtracting the initial weight of the net panel from the wet weight of the net panel with fouling.

2.4. Statistical analysis

All statistical analyses were performed with SPSS version 15. The results of the no-choice settlement assays were analysed with one-factor analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) multiple comparison test (Quinn and Keough, 2002). The results of the choice settlement assays were analysed with Chi-Square tests. Differences in the wet weight of fouling on nets were analysed with two-factor ANOVA (factors: (1) colour; (2) copper) (Quinn and Keough, 2002). The assumptions of homogeneity and normality of variance were checked with the Levene's test and *Q–Q* plots of residuals, respectively.

3. Results

3.1. No-choice and choice settlement assays with Ectopleura larynx

For the no-choice settlement assays, there was a significant difference in the settlement of actinulae between colours (one-factor ANOVA: $F_{4, 45}=3.934$, $p=0.008$). Actinulae settled significantly less on white (67.0 ± 5.4%) than on black surfaces (87.0 ± 2.6%) (Tukey's HSD multiple comparison test, $p=0.008$), whereas there were no significant differences between the remaining colours yellow, red and blue (Fig. 1). Although there was a significant difference between white and black surfaces in the no-choice settlement assays, actinulae

Table 1
Results of the multiple choice settlement assays testing the effects of two colour combinations on the preferential settlement of the hydroid *Ectopleura larynx*.

Colour combination	Preferred colour		No preference	No settlement
White (W)–Black (B)	13 (W)	14 (B)	8	5
Red (R)–Black (B)	17 (R)	14 (B)	6	3
Red (R)–White (W)	12 (R)	16 (W)	9	3

Number of actinulae are shown ($n=40$).