Fouling-release and chemical activity effects of a siloxane-based material on tunicates

Natalia Filip* [1,3], Amanda Pustam[2,3], Veronica Ells[1,3], Kathleen M. T. Grosicki[1,3], Jin Yang [2,3], Ikenna Oguejiofor [2,3], Cory D. Bishop[1,3] , M. Edwin DeMont[1,3] , Truis Smith-Palmer[2,3], and Russell C. Wyeth[1,3]

Biology[1] and Chemistry[2] Departments and Centre for Biofouling Research[3]

St Francis Xavier University

2321 Notre Dame Ave, Antigonish, Nova Scotia, Canada, B2G 2W5

*corresponding author

Further contact details:

Natalia Filip: nfilip@stfx.ca; 1 778 677 4210

Amanda Pustam: apustam@gmail.com; 1 902 867 2270

Veronica Ells: x2012ajv@stfx.ca; 1 902 867 3985

Kathleen M. T. Grosicki: x2010pae@stfx.ca;1 902 867 3886

Jin Yang: yangjin@uvic.ca; 1902 867 2270

Ikenna Oguejiofor: x2011sbs@stfx.ca; 1 902 867 2270

Cory D. Bishop: cbishop@stfx.ca; 1 902 867 4944

M. Edwin DeMont: edemont@stfx.ca; 1 902 867 5116

Truis Smith-Palmer: tsmithpa@stfx.ca; 1 902 867 2270

Russell C. Wyeth: rwyeth@stfx.ca; 1 902 867 3886

This work was supported by the Natural Sciences and Engineering Research Council Collaborative Research and Development program under Grant CRDPJ 411942-10 to TS-P, CDB, MED, and RCW; Encana Corporation's Deep Panuke E&T and R&D Fund under Grant DP-Eng-04 to TS-P, CDB, MED, and RCW; and the Canadian Foundation for Innovation under Grant 25196 to CDB and Grant 19286 to RCW.

Abstract

The antifouling performance of a siloxane-based elastomeric impression material (EIM) was compared to that of two silicone fouling-release coatings, Intersleek 757 and RTV-11. In field immersion trials, the EIM caused the greatest reduction in fouling by the solitary tunicate *Ciona intestinalis* and caused the longest delay in the progression of fouling by two species of colonial tunicate. However, in pseudobarnacle adhesion tests, the EIM had higher attachment strengths. Further laboratory analyses showed that the EIM leached alkylphenol ethoxylates (APEs) that were toxic to *C. intestinalis* larvae. The EIM thus showed the longest duration of chemical activity measured to date for a siloxane-based coating (4 months), supporting investigations of fouling-release coatings that release targeted biocides. However, due to potential widespread effects of APEs, the current EIM formulation should not be considered as an environmentally-safe antifoulant. Thus, the data also emphasize consideration of both immediate and long-term effects of potentially toxic constituents released from fouling-release coatings.

Keywords: alkylphenol ethoxylates; antifouling; fouling-release; silicone coatings; *Ciona intestinalis*, surfactants.

1. Introduction

Antifouling coatings can be divided into two broad categories: chemically-active coatings that release active compounds that kill or otherwise deter organisms from settling on a surface, and non-toxic fouling-release coatings that weaken adhesion of organisms and facilitate their release. To date, the most effective chemically active coatings have been based on heavy metals, many of which were banned due to their negative effects on the environment (reviewed by Yebra et al., 2004; Almeida et al., 2007). Consequently, numerous alternatives have been tested, including chemically-active coatings using enzymes or other natural products (eg Dobretsov et al. 2006, 2013; Fusetani, 2011; Quian et al. et al 2010), as well as a range of fouling-release coatings (reviewed by Almeida et al., 2007; Lejars et al., 2012). The most effective fouling-release coatings have been those based on polydimethyl siloxane (PDMS). The low surface energy and elastic modulus of PDMS elastomers decreases the strength of attachment of marine organisms. A large number of these siloxane-based fouling-release coatings have been tested (eg Holm et al., 2000; Swain et al., 2000; Terlizzi et al., 2000), together with additional studies of composition variants intended to enhance antifouling performance (eg Carl et al., 2012; reviewed by Lejars et al., 2012; Martinelli et al., 2012; Rahman et al., 2011).

Although siloxane-based coatings tend to have lower toxicity than chemically-active coatings that deliberately release biocides, there is still the possibility that these nominally fouling-release coatings leach chemicals that can either directly affect fouling organisms or have more downstream effects in the environment. A few of studies have considered this possibility (Nendza, 2007), and most have found acute toxic effects of

leachates (Feng, 2012; Rittschof & Holm, 1997; Truby, 2000; Watermann, 1997). Thus it is important that tests of anti-fouling performance for siloxane-based coatings consider both physical and chemical mechanisms.

The problematic fouling organisms that motivated this study are tunicates. Tunicates are prominent members of marine fouling communities that have caused considerable ecological and socioeconomical impacts in some geographic locations (Reviewed by Aldred & Clare, 2014; Bullard et al., 2010; Lambert 2001; Lambert & Lambert, 2003; Simkanin et al., 2013). Tunicate fouling has been particularly troublesome for some aquaculture operations worldwide, creating increased operation costs and decreased yield and product quality (Adams et al., 2011; Carman et al., 2010; Fitridge et al., 2012). Eradication techniques used in shellfish aquaculture have focused on chemical or physical treatments to remove tunicates after fouling has occurred (Arens et al., 2011; Carver et al., 2003; LeBlanc et al., 2007; Parent et al., 2011; Piola et al., 2010) Thus, the overall goal of this study was to test the performance of a silicone-based coating at limiting tunicate fouling.

The coating tested was chosen based on a serendipitous discovery of its antifouling properties. Vinyl siloxane polymers (VSP) are vinyl terminated PDMS elastomers that have outstanding impression accuracy, and are widely used as dental molds (Mandikos, 1998). VSPs have also been employed for biofouling studies, including testing their fouling-release characteristics (Kim et al., 2008), using them as matrices for dual-function biocidal and fouling-release coatings (Choi et al., 2007), and using them as molding materials for testing natural surface topographies that resist fouling (Scardino & de Nys, 2004). In a preliminary study, an elastomeric impression

material (EIM, a commercial dental mold formulation of VSP) was used to create molds with different topographies that were then submerged at a site in Nova Scotia, Canada with a non-indigenous tunicate infestation. Although no effect of topography was found, little tunicate fouling accumulated on the EIM molds, and those organisms that did attach were easy to remove (Phillips, Bishop and Wyeth, 2013, unpublished observations). Based on this preliminary result, surface topography was eliminated from subsequent experimentation, and, instead, it was hypothesized that fouling-release characteristics exhibited by the EIM caused the reduced tunicate fouling. The first specific aim of this study was to test the fouling-release hypothesis, assessing the performance of the EIM relative to other silicone-based coatings in field immersion trials and pseudobarnacle adhesion tests. The second aim was to test the alternate possibility of chemical activity, using larval toxicity assays and chemical analyses to gather evidence regarding any leachates from the material.

2. Materials and Methods

2.1 Coating Treatments

The antifouling performance of an EIM was compared to that of uncoated polyvinyl chloride (PVC) controls and two commercially available coatings acting as performance references. Medical-grade EIM (Kerr Corporation, Part 29036) was prepared according to package instructions. The two reference treatments were Intersleek 757 (INT, International Coatings Ltd.), a silicone-based fouling-release coating system, and RTV-11 (RTV; Momentive Performance Materials Holdings LLC), a commercially available silicone known to have fouling-release properties (Holm et al., 2000; 2006; Kavanagh et al., 2003; Truby et al., 2000; Wynne et al., 2000). Both INT and RTV were

prepared in accordance with manufacturer instructions and applied using paintbrushes. The INT coating was comprised of Parts BXA757, BXA758, and BXA759 and the back plates were first coated with Intershield 300 primer (Parts ENA301 and ENA303) and Intersleek 731 tiecoat formula (Parts BXA730 and BXA381). All coatings were applied to PVC back plates (25 cm x 15 cm), with controls left as bare PVC (see Table 1 for physical characteristics of the coatings). Nylon bolts were required to secure the EIM to the back plate, since no attachment system is available for this material. For consistency, nylon bolts were also placed on all other treatments. No mechanical damage was observed on any of the coatings during the study, with the exception of one RTV-coated plate that sustained partial detachment of a 6 cm^2 area that was still possible to include in the analysis.

2.2 Field immersion trials

2.2.1. Sites

Field immersion tests were conducted at two sites in Nova Scotia, Canada where previous observations indicated invasive tunicates were prevalent: Strait of Canso Yacht Club (decimal degrees of latitude and longitude: 45.6134, -61.3653), in Port Hawkesbury, infested with *Ciona intestinalis*, and Cribbons Point Marina (45.7558, -61.8971), on the Northumberland Strait, where both *Botryllus schlosseri* and *Botrylloides violaceous* are abundant.

Sets of four test plates, one for each treatment, were attached with nylon cable-ties to rectangular frames constructed from standard household acrylonitrile butadiene styrene pipe. Ten replicate frames were submerged along a dock at each test site, with frames (separated by 0.5 m to 3 m) suspended vertically 1 m below the water surface and

oriented away from direct sunlight. Within frames, treatment plate order was varied systematically, such that each treatment was adjacent to the other treatments an equal number of times.

2.2.2. Fouling Measurement

Fouling accumulation, as measured by cover area, was quantified based on a modification of ASTM D3623 (2012). A time-series of plate images was used, allowing non-destructive quantification of temporal changes of fouling. Plates were submerged on the 26 and 27 of June 2013, and temporarily raised out of the water and photographed 14 times (at weekly intervals over 12 weeks, and then after 14 and 17 weeks). The camera (Nikon Coolpix S5100) was mounted on a box fitted on the inside with a perimeter of LED lights to ensure similar lighting conditions among photographs. To reduce interference from organisms attached to the uncoated sides of the back plates, images were cropped to the 22.0 cm x 11.7 cm rectangular area inside the bolts (257 cm^2 total area, final resolution: 3400 x 1750 pixels). Seascape software (Teixidó et al., 2011) was used to segment downgraded images (850 x 437 pixels) into pixel regions based on colour. Segmented regions were categorized manually as background (bare surface), *C. intestinalis*, colonial tunicates (*B. schlosseri* and *B. violaceous*), bryozoans (*Bugula* sp), colonial hydroids (species not identified), other fouling organisms, or unknown (when obscured by growth from outside the plate).

2.2.3. Statistical Analyses

All statistical analyses for the field immersion trial and subsequent experiments were performed using SPSS v20 or v22 (IBM), with $\alpha = 0.05$. At both sites, fouling was dominated by tunicates, with no other fouling organisms exceeding 11% cover on any of

the treatments (even on plates without tunicate cover). Thus, we were able to exclude other fouling organisms from our analyses, exclusively comparing cover by tunicates (either *C. intestinalis* or the two colonial tunicates) on the different coating treatments. Repeated measures multivariate analyses of variance (rmMANOVA), followed by Tukey's post-hoc comparisons, were used to test for statistical differences in cover resulting from treatment (between-subject factor), time (number of weeks after deployment: within-subject factor), and the interaction of treatment with time (O'Brien & Kaiser, 1985; Ghosh et al., 1999). Percent cover data were arcsine-transformed prior to analysis, and we relied on the robustness of the rmMANOVA procedure (O'Brien & Kaiser, 1985) to accommodate departures from normality and homoscedasticity in our data. Since in both cases the effects of treatment did depend on time, we subsequently used separate univariate tests of the treatment effects on weeks 5, 7 or 8 (for *C. intestinalis* and the colonial tunicates, respectively), 11, 14 and 17 to establish at what time points there were significant differences among the coatings. These tests did not include repeated measures, and thus non-parametric methods could be used to better accommodate the significantly non-normal data in most weeks. Kruskal-Wallis tests (with pairwise comparisons) were used to compare cover on the different coatings in each of the 5 weeks, and Bonferroni's correction was used for the 5 tests required for each site (setting $\alpha = 0.01$).

2.3. Adhesion tests

To assess fouling-release performance of all four coating treatments (EIM, RTV, INT, and controls), pseudobarnacle adhesion measurements were performed based on ASTM D5618 (2011). Two versions of each coating (including controls) were tested,

totaling 8 treatments with 3 replicate plates each. Unexposed versions of each coating were freshly prepared, while previously exposed versions were immersed at the Strait of Canso Yacht Club for 62 weeks (and subsequently cleaned of all fouling, exposing the original coating material). Ten pseudobarnacles (aluminum studs of 9 mm diameter) were glued with a two-component epoxy adhesive (Loctite Hysol 1C) onto the surface of each plate. The adhesive was allowed to cure for 7 days at room temperature before testing. The shear force (N) required to detach the studs was measured with a handheld force gauge (Lutron FG-20KG) that was positioned parallel to the surface of the plates, and the forces registered were converted into measurements of adhesion strength (MPa). Adhesion measurements did not differ significantly among replicate plates (separate Kruskal-Wallis tests for each treatment, testing the effect of plate), and thus were pooled within coating treatments (n=30 per treatment). A Kruskal-Wallis test followed by pairwise comparisons, was used to test for statistical differences in the strength of adhesion. Adhesion strength on control plates exceeded 1 MPa for all replicates, but could not be measured exactly, since studs could not be detached from the plates. Consequently, measurements for control plates were not included in the statistical analysis.

2.4. Larval toxicity assays and chemical leachate identification

To assess any chemical activity effects of the EIM, we used three successive assays to test the effect of EIM on *C. intestinalis* larvae. All assays were conducted in accordance with Canada Council for Animal Care regulations. *C. intestinalis* larvae were reared in the laboratory (see supplementary material) and subsequently exposed to various experimental treatments in artificial seawater (ASW), as well as ASW alone as

controls. All assays were conducted in sterile 12-well tissue culture plates with 10 larvae per well (0.5 mL of culture suspension added to 1.5 or 2 mL of experimental solution, depending on the experiment) and incubated at 18 °C. Using an Olympus SZX16 stereomicroscope, larval morphology, mortality, and developmental stage were scored periodically for up to 48 h (when 80 % of control larvae had completed metamorphosis). The exact timing of scoring varied among the three assays, depending on the timing of any effects of the treatments (see below and Results). Mortality was assessed primarily by larval movements, as well as presence (dead) or absence (living) of a haze around the larvae, presumably due to cellular breakdown or microbial growth. To validate this approach, Sytox Green live/dead stain (Life Technologies) was used to confirm that a subsample of larvae scored as dead did indeed contain dead larvae. Developmental progression was divided into 4 stages: pre-metamorphosis, tail resorption, ampullar extension, and siphon opening (see supplemental Figure 1). A larval progress score, defined as (% tail resorption × 1) + (% ampulla extension × 2) + (% siphon opening × 3), was used to measure the capability of larvae to metamorphose and develop, ranging from 0 (no progress towards metamorphosis by any individual in a well) to 300 (complete metamorphosis of all individuals).

2.4.1. Assay 1: Effects of EIM on Larvae

Larval assays were conducted with EIM discs, leachates of the EIM, and leachates of EIM components, to test for biocidal effects of EIM on *C. intestinalis* larvae. EIM treatments consisted of Kerr Extrude discs (20 mm diameter) that were placed at the bottom of wells in the culture plates. For EIM leachate treatments, EIM discs were each placed at the bottom of the wells and incubated in ASW at room temperature for 24 h,

and then removed before the addition of larvae. For EIM component leachate treatments, the two pastes provided by the commercial suppliers, as well as the two major siloxane components (listed on the Material Data Safety Sheets), polymethylhydrosiloxane (PMHS) and vinyl terminated polydimethylsiloxane (PDMS), purchased from Sigma-Aldrich (Oakville, Canada), were tested. For all components, a 3 g sample of the material being tested was added to 10 mL ASW. After 24 h, the ASW was aliquoted and mixed with larval culture aliquots (2.5 mL total volume per culture plate well). In all cases, trials with three or six replicate wells for each treatment were conducted over 24 h. Due to the dichotomous effects of the various treatments (either 100% or 0% mortality, with no variability and no mortality in control wells), statistical analyses were not required.

2.4.2. Identification of APEs in EIM leachates

Since Assay 1 indicated that a leachable component of the EIM was toxic to larvae and the manufacturer refused to disclose the full recipe for the commercial EIM, mass spectrometry and UV absorption spectroscopy were used to characterize the EIM leachates. Leachates of the EIM, as well as both component pastes, were prepared by placing 2 g of each in 10 mL of distilled water for 48 h. Since previous reports indicated Igepal CO-630 could be an additive for dental EIMs (Bryan & Anderson, 1987; Chai & Yeung, 1991; Mandikos, 1998) leachates were compared to an Igepal CO-630 standard using low-resolution mass spectrometry. Measurements were made using a micrOTOF from Bruker Daltonics (Bremen, Germany) with positive electrospray ionization (4500 V), a sample flow rate of 2 µL per minute, and drying temperature of 180 ^{o}C. Leachates from discs in ASW or in distilled water were also analyzed on a Cary100 UV-VIS spectrometer. Absorbance was measured at 221 nm to estimate alkylphenol ethoxylate

(APEs) concentration in the leachate samples, using a standard curve for absorbance vs concentration derived for Igepal CO-630.

2.4.3. Assay 2: Effects of Igepal CO-630 and EIM leachate on larvae

Since Assay 1 along with the mass spectrometry and UV spectroscopy results suggested a surfactant similar to Igepal CO-630 as the likely toxic component of EIM leachates, the effects of this surfactant and the leachate from EIM were compared in larval assays. Stock solutions of Igepal CO-630 (1000 mg L^{-1}) were prepared by dissolving the surfactant in ASW, then diluting and mixing with larval culture aliquots to achieve a final concentration of 10 mg L^{-1} (in a total volume of 2 mL per plate well). This concentration was chosen to allow monitoring of developmental effects over 48 h, since the higher concentrations of surfactant that leached from EIM discs in the previous assay were lethal within 30 min (see results). The leachate treatment was prepared by shaking an EIM disc in 50 mL ASW, with concentration estimated by absorption spectroscopy (see above). Trials with six replicate plate wells for each treatment were conducted over 48 h. Again, dichotomous effects made statistical analyses unnecessary.

2.4.4. Assay 3: Effect of EIM leachate concentration on larvae

To explore potential concentration-dependent effects of EIM leachates, larval assays measuring progress through metamorphosis were performed using three concentrations of EIM leachates. EIM leachate concentrations were determined by UV absorption spectroscopy. Leachates were then diluted as needed and added to larval suspensions in plate wells, to achieve final APE concentrations of 3 mg L^{-1}, 7 mg L^{-1} and 10 mg L^{-1} respectively (in a total volume of 2 mL per plate well). Trials with six replicate plate wells for each treatment were conducted over 18 h. Differences in metamorphosis

progress and mortality among concentration treatments were tested for statistical significance at 5 h and 18 h with either a Kruskal-Wallis test (with pairwise comparisons among the treatments) or a Mann-Whitney test (depending on the number of treatments with surviving larvae).

2.4.5 Time course of APEs leached from EIM discs.

Leached APE concentrations from different EIM samples previously immersed in large volumes of seawater for different durations were measured to characterize how APEs leached over time. EIM samples were either immersed in seawater tanks or at a local marina (as part of the initial pilot study noted in the introduction) for 7, 13 or 22 weeks, and compared to freshly prepared EIM samples. EIM discs (7 mm diameter) from all samples were placed in 2 mL of ASW and stirred for 8 h, to allow release of insoluble material. Leached APE concentrations were then measured at hourly intervals by absorption spectroscopy (see above) over a further 8 h while the disc was immersed in 2 mL of newly replaced ASW (stirred except during absorption measurements). Three replicate discs were used per treatment, and the APE concentrations for all 4 treatments were compared with a Kruskal-Wallis test at the half-way point of the measurements (4 h).

3. Results

3.1. Field immersion trials

3.1.1. Cover by Ciona intestinalis

At Strait of Canso, plate cover was driven almost exclusively by *C. intestinalis* recruitment and growth, which varied over time and among treatments (Figures 1 & 2). Other common fouling organisms included the colonial tunicate species *B. schlosseri* and

B. violaceous, as well as colonial hydroids (species not identified), which were observed on all three silicone coating treatments but were absent on control plates. Erect bryozoans (*Bugula* sp.) were also present on all treatments during weeks 8 to 14, however, they accounted for a small proportion of fouling overall (< 4%) and were therefore not included in the figures presented here. Statistical analyses for this site were limited to *C. intestinalis*, given its high proportion in total fouling cover among treatments (Figure 2a).

Among the coating treatments, control plates experienced the highest *C. intestinalis* cover, while EIM experienced the lowest. The two performance references showed intermediate cover (Figure 3a). On control plates, *C. intestinalis* recruits were first measured on week 3 of the experiment. Cover then increased rapidly, reaching saturation at approximately 90% cover after a 6-week immersion period, and plateauing during the remaining weeks of the experiment. Compared to controls, cover on other treatments was lower and accumulated more slowly (Figure 3a). Statistical analysis confirmed that *C. intestinalis* cover was strongly affected by coating treatment (rmMANOVA, $F_{3, 32} = 17.426$, $p < 0.001$), and by time (same analysis, $F_{13, 20} = 34.721$ $p < 0.001$), and that the effect of treatment depended on time (same analysis, interaction effect, $F_{39, 66} = 2.671$, $p < 0.001$). Overall, although cover on EIM increased over time, it was significantly lower than cover on all other treatments (rmMANOVA Tukey's post-hoc comparisons for the main effect of treatment; also confirmed by comparing cover averaged over time, Kruskal-Wallis $H_3 = 26.4$, $p < 0.001$ with post-hoc comparisons $p < 0.05$). Maximum cover on EIM was observed on week 17, when *C. intestinalis* and colonial hydroids respectively covered approximately 15% and 11% of the surfaces of the plates (Figure 2b). It is important to note that *C. intestinalis* recruitment occurred almost

exclusively on the nylon bolts used to secure the EIM, with subsequent growth of these recruits resulting in the measured cover. Similarly, hydroid colonies appeared to originate from the uncoated plate edges, and encroach over the EIM surface. In the analyses of specific weeks, EIM was the only treatment with significantly lower cover than controls on a consistent basis (Table 2; Figure 3a). Differences between EIM and both RTV and INT varied over time. Cover peaked at 60% on RTV and 70% on INT, after an 8-week exposure period. After this period, cover on INT declined to approximately 30%, as clumps of *C. intestinalis* detached under their own weight (Filip, pers. obs.), whereas on RTV, the cover was maintained at approximately 50%. Differences in cover patterns between RTV and INT were not statistically significant. Compared to EIM, cover on RTV was not significantly different up to week 11, but significantly higher thereafter. In contrast, INT plates had significantly higher cover than EIM up to week 8, but did not in the following weeks. The overall pattern was thus for EIM to have significantly lower cover than controls, RTV and INT, and then later convergence with cover on INT.

3.1.2. Cover by colonial tunicates

At Cribbons Point, cover varied among treatments to a far lesser degree than at Strait of Canso (Figure 1). Cover was almost exclusively driven by colonial tunicates (*B. violaceous* and *B. schlosseri*). Other organisms that occasionally attached to the coating surfaces (eg bivalves and encrusting bryozoans) contributed < 1% of the total cover and were therefore not included in statistical analyses. We also noted mussels in small gaps between the EIM and the back plate.

Although cover patterns were similar across all treatments, there were differences in the rate of accumulation (Figure 3b). In all treatments, plate cover initially increased

until saturation (90-100% surface cover), but then decreased as tunicate colonies died-off. Differences amongst the treatments were apparent in the timing of both the initial increase and subsequent decrease in cover. Statistical analyses confirmed that tunicate cover was influenced by coating treatment (rmMANOVA, $F_{3, 36} = 11.435$, $p < 0.001$) and by time (same analysis, $F_{13, 24} = 1137.126$, $p < 0.001$), and the effect of treatment depended on time (same analysis interaction effect, $F_{39, 78} = 7.697$, $p < 0.001$). The Tukey's post-hoc comparisons for the main effect of treatment in the rmMANOVA identified significant differences between EIM and all the other treatments, while comparing averaged cover over time indicated that the only significant difference was between EIM and CTL (Kruskal-Wallis $H_3 = 16.7$, $p < 0.001$; post-hoc comparison $p < 0.001$). All together, these results suggest any differences between treatments were largely driven by their differing interactions with time, which was also evident in the analyses of specific weeks. Initial growth of cover on controls and INT was similar, with no significant differences in weeks 5 or 7 (Table 2), and reaching saturation in week 6 (Figure 3b). Cover on RTV was delayed by one week, resulting in significantly lower cover than on either controls or INT in week 5 but not week 7, when cover saturated. EIM cover was also delayed, with significantly lower cover than controls and INT in both weeks 5 and 7, and not reaching saturation until week 8. However, difference in cover resulting from the one-week delay between EIM and RTV was not significant in either week 5 or 7. The lag between treatments continued through the subsequent cover decreases, which occurred first for INT and controls, resulting in significantly less cover than both EIM and RTV in week 11. INT was unique in that it experienced a partial recovery of tunicate cover due to colony re-growth. EIM and RTV plates experienced an

almost identical rate of cover decrease. At the end of the experiment, cover differences among treatments were negligible. Thus, the overall pattern was that none of the siloxane-based coatings ultimately reduced fouling by colonial tunicates compared to controls, but that EIM and RTV delayed progression of the fouling cover.

3.2. Adhesion tests

In the test of fouling-release performance, EIM, RTV and INT treatments all substantially reduced adhesion compared to controls (Figure 4). Among the silicone-based coating treatments, statistical analysis confirmed that the adhesion strength of pseudobarnacles was strongly affected by coating treatment and by previous exposure (Kruskal-Wallis $H_5 = 139$, $p < 0.001$; see Figure 4 for pairwise comparisons). For the unexposed treatments, the INT treatment was the most effective at decreasing adhesion, followed by RTV and EIM, which were similarly effective. The 62-week exposure to ocean conditions affected adhesion strength to varying degrees on the different treatments. Adhesion on previously exposed INT plates showed no significant change following exposure, while exposure caused a substantial and significant increase in adhesion strength for EIM, and an intermediate (but still significant) increase for RTV.

3.3. Larval toxicity assays

3.3.1. Assay 1: Effects of EIM on larvae

When larval assays were carried out with a disc of Kerr EIM in each well, 100 % of larvae had deformed tails (Figure S2 in supplementary material) and were dead within 30 min of exposure. The larvae did not exhibit any swimming or other activity, while control larvae had 0% mortality, straight, long tails, and exhibited swimming or other movements. When an EIM disc was left in ASW in a well for 24 h, and then removed

before addition of larvae to the well, the same toxic effect on larvae was observed. In an additional test, when the EIM had been immersed in seawater at a local marina for 7 weeks prior to the assay, 100% of larvae had deformed tails after 15 ± 12 h (mean ± SD, n = 6). Since these results indicated that a leachable component of the EIM was causing larval mortality, larval assays were performed with various components of the EIM, including leachates of the base and accelerator pastes as well as the isolated siloxane polymers used in EIM preparation (PDMS and PMHS). Only leachates from the base paste produced similar effects to those of EIM discs (included as a positive control), with exposed larvae showing deformed tails and 100 % mortality after 24 h. In contrast, leachates of the other components had no effect on larvae during a 24 h exposure, with 0% mortality, and development that was similar to control larvae exposed only to ASW. Subsequent testing of two additional commercial formulations of VSP yielded similar results: leachates caused deformed tails and rapid mortality of larvae.

3.3.2. Identification of APEs in EIM leachates

Mass spectrometry and UV absorption spectroscopy showed that APEs were released from EIM discs on contact with water or ASW. The mass spectra of Kerr EIM leachates showed characteristic ethyleneoxy branch fragments, also present in the Igepal CO-630 standard solutions. The mass to charge ratio (m/z) of the fragmented sodiated ion species in the leachate matched 82 % of those in the standard, which confirmed the structural similarity of leached EIM compounds to Igepal CO-630. A comparison of spectra of leachates of base and accelerator pastes with spectra of disc leachate and Igepal CO-630 confirmed that APEs similar to those in the disc leachate and Igepal CO-630 were present in the base paste component of the 2-part mixture (Figure S3 in

supplementary material). In addition, the UV spectra of Kerr EIM leachate and Igepal CO-630 were comparable, with the most intense peak at a wavelength of 221 nm. These results thus provide evidence that APEs similar to those of Igepal CO-630 were leached from the commercial EIMs.

3.3.3. Assay 2: Effects of Igepal CO-630 on larvae

Since an APE similar to those of Igepal CO-630 appeared to be the cause of EIM-induced mortality, the effects of Igepal CO-630 and EIM leachate on larvae were compared. Larvae exposed to either of these treatments initiated metamorphosis, as indicated by tail resorption, but were dead within 24 h.

Assay 3: Effect of EIM leachate concentration on larvae. A delay in metamorphosis was apparent after 5 h (Figure 5A; Kruskal-Wallis $H_3 = 13.3$, $p = 0.004$) for the 3 mg L^{-1}, 7 mg L^{-1} and 10 mg L^{-1} leachate concentrations tested (respective pairwise comparisons with control ASW: $p = 0.007$, $p = 0.027$, $p = 0.037$), while mortality increased significantly only in the 10 mg L^{-1} concentration (Figure 5A; Kruskal-Wallis $H_3 = 17.2$, $p = 0.001$ and pairwise comparison with control: $p = 0.001$). After 18 h, all larvae in the 7 and 10 mg L^{-1} treatments were dead, with little or no mortality in controls and the lowest 3 mg L^{-1} treatment, although metamorphosis was significantly slowed in the latter treatment (Mann-Whitney test, $n_1 = 6$; $n_2 = 6$; $U = 36$, $p = 0.002$).

3.3.4. Time course of APEs leached from EIM discs

APEs were detectable in the leachates of all EIMs tested, regardless of the length of exposure to seawater. The highest concentration of APEs was produced by EIM material that had not been previously exposed to seawater, while successively lower levels were released from samples immersed in seawater for 7, 13 and 22 weeks,

respectively (Figure 6), with a significant difference between the unexposed and 22 week exposure treatments after 4 h (Kruskal-Wallis test, $H_3 = 10.39$, $p = 0.016$, pairwise comparison $p = 0.013$).

4. Discussion

The results from field immersion trials demonstrate that the EIM formulation performed significantly better as an antifouling surface for tunicates than either fouling-release performance reference coatings or controls. However, in adhesion tests, the EIM material was significantly less effective as a fouling-release surface than the performance references. Furthermore, larval toxicity assays demonstrated that the EIM leached a substance that has biocidal effects on *C. intestinalis* larvae. Thus, we conclude that the antifouling mechanism of the EIM is probably a combination of fouling-release and chemical activity effects, and that its enhanced antifouling performance over the two performance reference coatings is due to leaching of a biocide. However, given the potentially negative environmental effects of the leachate (probably a nonylphenol ethoxylate similar to the commercially available Igepal CO-630), efforts should be directed towards alternate formulations of siloxane-based fouling-release coatings for targeting tunicates.

4.1. Fouling-release performance of EIM

The EIM was intermediately effective at decreasing pseudobarnacle adhesion. Compared to controls, EIM acted as a fouling-releaser, but it was not as effective at reducing adhesion as other fouling-release coatings (RTV and INT), especially after long exposure to seawater. Despite the limited compositional information available for all three commercial siloxane-based products, it is possible to consider mechanisms to

account for the differences in performance. First, the high filler loading of the EIM may have interfered with its fouling-release properties. Stein et al. (2003) showed that antifouling performance decreases when the filler content is augmented from 0 to 29%, regardless of the filler type, and the calcium silicate filler content of the EIM tested in the current study was even higher (30-50%, from the MSDS). Alternatively, the presence of the APE(s) within the siloxane matrix of the EIM may have also affected the fouling-release performance of the EIM by increasing surface hydrophilicity (Oh et al., 2003). However, this hypothesis is inconsistent with the results from the adhesion test and the leachate concentration assays. In these experiments, the fouling-release performance of the EIM declined after exposure to seawater (Figure 4), and the concentration of leachates released from material decreased. Had the presence of APEs in the matrix caused a decrease in the fouling-release performance of the EIM, an increase in performance following immersion (and depletion of APEs) would be expected. Finally, it is also possible that the VSPs used in the EIM may have increased adhesion relative to the other coatings. Although VSPs have been used as matrices for fouling-release coatings (Choi et al., 2007; Kim et al., 2008), they are less prevalent than standard PDMS in coatings studied to date, and to our knowledge there are no systematic comparisons of the consequences of different terminal groups of the siloxane resins. Nonetheless, based on the evidence available to date, we suggest that the higher filler content is the probable cause for the reduced fouling-release performance of the EIM.

4.2. Chemical activity effects of EIM specific to C. intestinalis

Our data indicate that despite the moderate fouling-release characteristics of the EIM, there was a strong antifouling effect observed in the field that was probably the

result of an APE that was leached from the material. In the field, the APE toxicity appeared to be highly local, having little effect on recruitment and growth of *C. intestinalis* that settled only millimeters away from the EIM material. In contrast, EIM only delayed fouling by colonial tunicates, while hydroids, bryozoans, and mussels were all able to grow in close contact with the EIM. Thus the primary antifouling effect of EIM was specific to pre-metamorphic *C. intestinalis* (at least compared to the other taxa observed at the field sites), and was localized to just the surface of the EIM material. Overall, these data show that a VSP formulation with chemical activity can create an antifouling surface that inhibits recruitment of specific fouling organisms.

4.3. APE leachate

Spectroscopic methods and larval assays identified an APE similar to those in the nonionic surfactant, Igepal CO-630, as the likely candidate for the toxic EIM leachate. EIMs previously immersed in seawater for varying durations showed that APE(s) could be released for long periods (at least 22 weeks), and that after 7 weeks of exposure, the leached APE(s) still reached concentrations comparable to the concentrations of Igepal CO-630 that caused larval mortality (compare Figure 5 and 6). Nonylphenol ethoxylates such as Igepal CO-630 can have harmful environmental effects (Cowan-Ellsberry et al., 2014; Lewis, 1991; McWilliams & Payne, 2002; Rebello et al., 2014), particularly via their breakdown products that can act as endocrine disruptors (Meier et al., 2001; Soares et al., 2008). Consequently, the EIM in its current formulation should not be used as an antifouling coating. On the other hand, the observation that antifouling effects were maintained in the field over more than 4 months, suggests that biocides (specific to particular fouling species and without downstream environmental effects) could be

combined with VSPs as a basis for dual-function antifouling coatings with both fouling-release and chemical activity effects (Akuzov et al., 2013; Barrios et al., 2005; Choi et al., 2007; Clarkson & Evans, 1995; Haque et al., 2005; Majumdar et al., 2008).

4.4. Implications for biofouling studies of ephemeral populations

The field results of this study emphasize the importance of adequate replication in biofouling studies of ephemeral populations in natural habitats. Tunicate populations varied considerably in this study and in others (Carver et al., 2006; Sephton et al., 2011). The experimental design included a replicate size of ten, which allowed for significant cover trends to emerge despite such variation. In contrast, a sample size of four (or less), which is common among biofouling studies (eg Bonafede & Brady, 1998; Terlizzi et al., 2000; Truby et al., 2000; Choi et al., 2007; Kristensen et al., 2010; Rahman et al., 2011), would have undermined the ability to draw conclusions, given the amount of variability observed (as has occurred in previous studies; Holm et al., 2000; Pelletier et al., 2009). Consequently, greater replicate numbers will also likely be required for biofouling studies involving other ephemeral populations.

4.5. Implications for antifouling coatings

Although the EIM we tested is not a candidate for an environmentally-safe coating, the results reported here nonetheless have three major implications for future efforts to develop antifouling coatings with low environmental impact. First, the data can be incorporated into ongoing efforts to develop dual-functioning silicone coatings combining fouling-release properties with additional biocidal capabilities (Akuzov et al., 2013; Barrios et al., 2005; Choi et al., 2007; Clarkson & Evans, 1995; Haque et al., 2005; Majumdar et al. 2008). Our results demonstrate the longest duration of effectiveness

(more than 4 months in the field) based on a chemical released from a silicone surface. Given that the measurements were limited by the seasonality of the fouling species, and that the EIM was not specifically designed for an antifouling purpose, it is plausible that future work testing different formulations, including different fillers and other additives (Beigbeder et al., 2008; Choi et al., 2011; Kavanagh et al., 2003; Price et al., 1992), could substantially extend the duration over which silicone coatings release targeted biocides. Second, alternative biocides (targeting tunicates or other taxa) that can be released slowly from VSP elastomers must be investigated. Based on the results reported here, biodegradable surfactants might be considered, although depending on how they are incorporated into the surface their amphipathic nature may diminish the fouling-release characteristics of silicone matrices, which are affected by surface hydrophobicity. In the context of tunicate fouling, recent work has identified two other promising tunicate antifoulants, polygodial and ubiquinone-10, which inhibit tunicate metamorphosis with no apparent adverse effects on non-target organisms (Cahill et al., 2012; 2013). Finally, the possibility that other siloxane-based coatings with additives may release toxic leachates must be considered. Leachates from RTV are toxic to crustaceans (Rittschof & Holm, 1997; Truby, 2000). RTV leachates may also have a relatively minor effect on tunicates, given the delayed accumulation of *C. intestinalis* and colonial tunicate cover on RTV-11 compared to control and INT. Urchin and fish larvae also showed acute effects (including mortality) from siloxane-based coating leachates, including known silicone oil additives and unknown additives from commercial coatings (Feng, 2012). Thus, although the siloxane-based coatings clearly reduce attachment strengths, chemical activity may also add to their antifouling effects. Future assessments of siloxane-based

coatings should therefore consider testing both attachment-strength and chemical activity to properly understand the mechanisms behind any antifouling effects, and also assess the ultimate environmental fate of any leachates.

Acknowledgements

This project was undertaken and completed with the financial assistance of Encana Corporation's Deep Panuke E&T and R&D Fund. We acknowledge the support of a Natural Sciences and Engineering Research Council Industrial Postgraduate Scholarship to NF, with partner support from the Aquaculture Association of Nova Scotia and the Prince Edward Island Aquaculture Alliance, a Leo. P. Chiasson award to VE, and an Irving Mentorship Program Student Summer Research awards to KG and IO. We thank the marina managers who accommodated our sampling, and Steven Macdonald for his invaluable assistance with equipment. We thank International Paint (Akzo Nobel Coatings Ltd) for donating the Intersleek coating.

Author contributions

NF contributed to funding, conception, design and execution of the experiments, and manuscript preparation. AP contributed to conception, design and execution of the experiments and manuscript preparation. VE, KG, JY and IO contributed to execution of the experiments. CDB contributed to funding and conception and design of the experiments. MED contributed to funding and conception of the experiments. TS-P and RCW contributed to funding, conception and design of the experiments, and manuscript preparation.

Competing interests

The authors declare no competing financial interests.

References

Adams, C.M., Shumway, S.E., Whitlatch, R.B., Getchis, T., 2011. Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. J World Aquac Soc. 42:242–252.

Akuzov, D., Brümmer, F., Vladkova, T., 2013. Some possibilities to reduce the biofilm formation on transparent siloxane coatings. Colloids Surf B Biointerfaces. 104:303–310.

Aldred, N., Clare, A.S. 2014., Mini-review: impact and dynamics of surface fouling by solitary and compound ascidians. Biofouling 30:259-70.

Almeida, E., Diamantino, T.C., de Sousa, O., 2007. Marine paints: the particular case of antifouling paints. Prog Org Coat. 59:2–20.

Anonymous., 2011. ASTM D5618 − 94. Test Method for Measurement of Barnacle Adhesion Strength in Shear. Annual Book of ASTM Standards. Vol. 06.02. West Conshohocken, PA: American Society for Testing and Materials.

Anonymous., 2012. ASTM D 3623-78a. Standard test method for testing antifouling panels in shallow submergence. Annual Book of ASTM Standards. Vol. 06.02. West Conshohocken, PA: American Society for Testing and Materials.

Arens, C., Paetzold, C., Ramsay, A,, Davidson, J., 2011. Pressurized seawater as an antifouling treatment against the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* in mussel aquaculture. Aquat Invasions. 6:465–476.

Barrios, C.A., Xu Q., Cutright, T., Newby, B.Z., 2005. Incorporating zosteric acid into silicone coatings to achieve its slow release while reducing fresh water bacterial attachment. Colloids Surf B Biointerfaces. 41:83–93.

Beigbeder, A., Degee, P., Conlan, S.L., Mutton, R.J., Clare, A.S., Pettitt, M.E., Callow, M.E., Callow, J.A., Dubois, P., 2008. Preparation and characterization of silicone-based coatings filled with carbon nanotubes and natural sepiolite and their application as marine fouling-release coatings. Biofouling. 24:291–302.

Bernbom, N., Ng, Y.Y., Olsen, S.M., Gram, L., 2013. *Pseudoalteromonas* spp. serve as initial bacterial attractants in mesocosms of coastal waters but have subsequent antifouling capacity in mesocosms and when embedded in paint. Appl Environ Microbiol. 79:6885–6893.

Bonafede, S.J., Brady, R.F., 1998. Compositional effects on the fouling resistance of fluorourethane coatings. Surf Coat Int. 81:181–185.

Brady, R.F., Singer, I.L., 2000. Mechanical factors favoring release from fouling release coatings. Biofouling. 15:73–81.

Bryan, T.T., Anderson, H.L., 1987. Containing a solubilizing surfactant for improved wettability; dental impressions. US4657959 A.

Bullard, S.G., Shumway, S.E., Davis, C.V., 2010. The use of aeration as a simple and environmentally sound means to prevent biofouling. Biofouling. 26:587–593.

Cahill, P., Heasman, K., Jeffs, A., Kuhajek, J., Mountfort, D., 2012. Preventing ascidian fouling in aquaculture: screening selected allelochemicals for anti-metamorphic properties in ascidian larvae. Biofouling. 28:39–49.

Cahill, P.L., Heasman, K., Hickey, A., Mountfort, D., Jeffs, A., Kuhajek, J., 2013. Screening for negative effects of candidate ascidian antifoulant compounds on a target aquaculture species, Perna canaliculus Gmelin. Biofouling. 29:29–37.

Carl, C., Poole, A.J., Vucko, M.J., Williams, M.R., Whalan, S., de Nys, R., 2012. Enhancing the efficacy of fouling-release coatings against fouling by *Mytilus galloprovincialis* using nanofillers. Biofouling. 28:1077–1091.

Carman, M.R., Morris, J.A., Karney, R.C., Grunden, D.W., 2010. An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast: assessment of native and invasive tunicates in shellfish aquaculture. J Appl Ichthyol. 26:8–11.

Carver, C.E., Chisholm, A., Mallet, A.L., 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production . J Shellfish Res. 22:621–631.

Carver, C.E., Mallet, A.L., Vercaemer, B., 2006. Biological Synopsis of the Solitary Tunicate *Ciona intestinalis*. Canadian Manuscript Report of Fisheries and Aquatic Sciences. 2746: v+ 55 pp.

Chai, Y.Y., Yeung, T.C., 1991. Wettability of nonaqueous elastomeric impression materials. Int J Prosthodont. 4:555–560.

Choi, J-H., Kim, M-K., Woo, H-G., Song, H-J., Park, Y-J., 2011. Modulation of physical properties of polyvinylsiloxane impression materials by filler type combination. J Nanosci Nanotechnol. 11:1547–1550.

Choi, S,. Jepperson, J., Jarabek, L., Thomas, J., Chisholm, B., Boudjouk, P., 2007. Novel approach to anti-fouling and fouling-release marine coatings based on dual-functional siloxanes. Macromol Symp. 249-250:660–667.

Clarkson, N., Evans, L.V., 1995. Raft trial experiments to investigate the antifouling potential of silicone elastomer polymers with added biocide. Biofouling. 9:129–143.

Cowan-Ellsberry, C., Belanger, S., Dorn, P., Dyer, S., McAvoy, D., Sanderson, H., Versteeg, D., Ferrer, D., Stanton, K., 2014. Environmental safety of the use of major surfactant classes in North America. Crit Rev Environ Sci Technol. 44:1893–1993.

Darbyson, E.A., Hanson, J.M., Locke, A., Willison, J.M., 2009. Settlement and potential for transport of clubbed tunicate (*Styela clava*) on boat hulls. Aquat Invasions. 4:95–103.

Dobretsov, S., Dahms, H.-U., Qian, P.-Y., 2006. Inhibition of biofouling by marine microorganisms and their metabolites. Biofouling 22, 43–54.

Dobretsov, S., Abed, R.M.M., Teplitski, M., 2013. Mini-review: Inhibition of biofouling by marine microorganisms. Biofouling 29, 423–441.

Edwards, P.K., Leung, B., 2009. Re-evaluating eradication of nuisance species: invasion of the tunicate, *Ciona intestinalis*. Front Ecol Environ. 7:326–332.

Feng, D., Rittschod, D., Orihuela, B., Kwok, K.W.H., Stafslien, S., Chisholm, B., 2012. The effects of model polysiloxane and fouling-release coatings on embryonic development of a sea urchin (*Arbacia punctulata*) and a fish (*Oryzias latipes*). Aquat Toxicol. 110-111:162-169.

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.

Fusetani, N., 2011. Antifouling marine natural products. Nat. Prod. Rep. 28, 400–410.

Ghosh, D., Deisher, T.A., Ellsworth, J.L., 1999. Statistical methods for analyzing repeated measures. J Pharmacol Toxicol Methods. 42:157–162.

Haque, H., Cutright, T.J, Newby, B-M.Z., 2005. Effectiveness of sodium benzoate as a freshwater low toxicity antifoulant when dispersed in solution and entrapped in silicone coatings. Biofouling. 21:109–119.

Hirota, H., Okino, T., Yoshimura, E., Fusetani, N., 1998. Five new antifouling sesquiterpenes from two marine sponges of the genus *Axinyssa* and the nudibranch *Phyllidia pustulosa*. Tetrahedron. 54:13971–13980.

Holm, E.R., Kavanagh, C.J., Meyer, A.E., Wiebe, D., Nedved, B.T., Wendt, D., Smith C.M., Hadfield, M.G., Swain, G., Wood, C.D., et al., 2006. Interspecific variation in patterns of adhesion of marine fouling to silicone surfaces. Biofouling. 22:233–243.

Holm, E.R., Nedved, B.T., Phillips, N., Deangelis, K.L., Hadfield, M.G., Smith, C.M., 2000. Temporal and spatial variation in the fouling of silicone coatings in Pearl Harbor, Hawaii. Biofouling. 15:95–107.

Holmstrom, C., Egan, S., Franks, A., McCloy, S., Kjelleberg, S., 2002. Antifouling activities expressed by marine surface associated *Pseudoalteromonas* species. FEMS Microbiol Ecol. 41:47–58.

Kavanagh, C.J,, Swain. G.W., Kovach. B.S., Stein, J., Darkangelo-Wood, C., Truby, K., Holm, E., Montemarano, J., Meyer, A., Wiebe, D., 2003. The effects of silicone fluid

additives and silicone elastomer matrices on barnacle adhesion strength. Biofouling. 19:381–390.

Kim, J., Nyren-Erickson, E., Stafslien, S., Daniels, J., Bahr, J., Chisholm, B.J., 2008. Release characteristics of reattached barnacles to non-toxic silicone coatings. Biofouling. 24:313–319.

Kristensen, J.B., Olsen, S.M., Laursen, B.S., Kragh, K.M., Poulsen, C.H., Besenbacher, F., Meyer, R.L., 2010. Enzymatic generation of hydrogen peroxide shows promising antifouling effect. Biofouling. 26:141–153.

Lambert, C.C., Lambert, G., 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Mar Ecol Prog Ser. 259:145–161.

Lambert, G., 2001. A global Overview of ascidian introductions and their possible impact on the endemic fauna. In: The biology of ascidians. H. Sawada, H. Yokosawa, and C.C. Lambert, editors. Japan: Springer-Verlag. p. 249-257.

LeBlanc, N., Davidson, J., Tremblay, R., McNiven, M., Landry, T., 2007. The effect of anti-fouling treatments for the clubbed tunicate on the blue mussel, *Mytilus edulis*. Aquaculture. 264:205–213.

Lejars, M., Margaillan, A., Bressy, C., 2012. Fouling release coatings: a nontoxic alternative to biocidal antifouling coatings. Chem Rev. 112:4347–4390.

Lewis, M.A., 1991. Chronic and subletal toxicities of surfactants to aquatic animals: a review and risk assessment. Water Res. 25:101–103.

Majumdar, P., Lee, E., Patel, N., Stafslien, S.J., Daniels, J., Chisholm, B.J., 2008. Development of environmentally friendly, antifouling coatings based on tethered quaternary ammonium salts in a crosslinked polydimethylsiloxane matrix. J Coat Technol Res. 5:405–417.

Mandikos, M.N., 1998. Polyvinyl siloxane impression materials: an update on clinical use. Aust Dent J. 43:428–434.

Martinelli, E., Sarvothaman, M.K., Galli, G., Pettitt, M.E., Callow, M.E., Callow, J.A., Conlan, S.L., Clare, A.S., Sugiharto, A.B., Davies, C., Williams, D., 2012. Poly(dimethyl siloxane) (PDMS) network blends of amphiphilic acrylic copolymers with poly(ethylene glycol)-fluoroalkyl side chains for fouling-release coatings. II. Laboratory assays and field immersion trials. Biofouling. 28:571–582.

McWilliams, P., Payne, G., 2002. Bioaccumulation potential of surfactants: a review. Spec Publ-R Soc Chem. 280:44–55.

Meier, S., Andersen, T.E., Hasselberg, L., Kjesbu, O.S., Klungsøyr, J., Svardal, A., 2001. Hormonal effects of C4-C7 alkylphenols on cod (Gadus morhua). Enzyme. 3:2.

Nendza, M. Hazard assessment of silicone oils (polymethylsiloxanes, PDMS) used in antifouling-/foul-release-products in the marine environment- Marine Pollution Bulletin 54, 1190-1196.

O'Brien, R.G., Kaiser, M.K., 1985. MANOVA method for analyzing repeated measures designs: an extensive primer. Psychol Bull. 97:316.

Oh, Y-I., Lee, D-Y., Hwang, S-Y., Kim, K-N., Kim, K-M., 2003. Effect of non-ionic surfactants on surface properties of hydrophilic polyvinyl siloxane impression materials. Colloids Surf Physicochem Eng Asp. 229:9–17.

Parent, M., Paetzold, S.C., Quijón, P.A., Davidson, J., 2011. Perforation with and without vinegar injection as a mitigation stratedy against two invasive tunicates, *Ciona intestinalis* and *Styela clava*. Manag Biolog Invasions. 2:27–38.

Pelletier, É., Bonnet, C., Lemarchand, K., 2009. Biofouling growth in cold estuarine waters and evaluation of some chitosan and copper anti-fouling paints. Int J Mol Sci. 10:3209–3223.

Piola, R.F., Dunmore, R.A., Forrest, B.M., 2010. Assessing the efficacy of spray-delivered "eco-friendly" chemicals for the control and eradication of marine fouling pests. Biofouling. 26:187–203.

Price, R.R., Patchan, M., Clare, A., Rittschof, D., Bonaventura, J., 1992. Performance enhancement of natural antifouling compounds and their analogs through microencapsulation and controlled release. Biofouling. 6:207–216.

Qian, P.-Y., Xu, Y., Fusetani, N., 2010. Natural products as antifouling compounds: recent progress and future perspectives. Biofouling 26, 223–234.

Rahman, M.M., Chun, H.H., Park, H., 2011. Waterborne polysiloxane–urethane–urea for potential marine coatings. J Coat Technol Res. 8:389–399.

Rebello, S., Asok, A.K., Mundayoor, S., Jisha, M.S., 2014. Surfactants: toxicity, remediation and green surfactants. Environ Chem Lett. 12:275–287.

Rittschof, D., Holm, E.R., 1997. Antifouling and fouling-release: a primer. In:Fingerman, M., Nagabhushanam, R., Thompson, M, F, M.F. (Eds.), Recent advances in marine biotechnology, endocrinology and reproduction, Vol. I. Oxford & IBH Publishing, New Delhi, pp. 497-512.

Scardino, A., de Nys, R., 2004. Fouling deterrence on the bivalve shell *Mytilus galloprovincialis* : a physical phenomenon? Biofouling. 20:249–257.

Sephton, D., Vercaemer, B., Nicolas, J.M., Keays, J., 2011. Monitoring for invasive tunicates in Nova Scotia, Canada (2006-2009). Aquat Invasions. 6:391–403.

Simkanin, C., Dower, J.F., Filip, N., Jamieson, G., Therriault, T.W., 2013. Biotic resistance to the infiltration of natural benthic habitats: examining the role of predation in the distribution of the invasive ascidian *Botrylloides violaceus*. J Exp Mar Biol Ecol. 439:76–83

Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., Lester, J.N., 2008. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ Int. 34:1033–1049.

Stein, J., Darkangelo-Wood, C., Takemori, M., Vallance, M., Swain, G., Kavanagh, C., Kovach, B., Schultz, M., Wiebe, D., Holm, E., et al., 2003. Structure property relationships of silicone biofouling-release coatings: effect of silicone network architecture on pseudobarnacle attachment strengths. Biofouling. 19:87–94.

Swain, G.W., Anil, A. C., Baier, Robert E, Chia, Fu-Shiang. 2000. Biofouling and barnacle adhesion data for fouling-release coatings subjected to static immersion at seven marine sites. Biofouling. 16:331–344.

Teixidó, N., Albajes-Eizagirre, A., Bolbo, D., Le Hir, E., Demestre, M., Garrabou, J., Guigues, L., Gili, J., Piera, J., Prelot, T., Soria-Frisch, A., 2011. Hierarchical segmentation-based software for cover classification analyses of seabed images (Seascape). Mar Ecol Prog Ser. 431:45–53.

Terlizzi, A., Conte, E., Zupo, V., Mazzella, L., 2000. Biological succession on silicone fouling-release surfaces: long-term exposure tests in the harbour of Ischia, Italy. Biofouling. 15:327–342.

Truby, K., Wood, C., Stein, J., Cella, J., Carpenter, J., Kavanagh, C., Swain, G., Wiebe, D., Lapota, D., Meyer, A., et al., 2000. Evaluation of the performance enhancement of silicone biofouling-release coatings by oil incorporation. Biofouling. 15:141–150.

Watermann, B., Berger, H.D., Sonnichsen, H., Wilemensen, P., 1997. Perfomance and effectiveness of non-stick coatings in seawater. Biofouling 11, 101-118.

Wynne, K., Swain, G., Fox, R., Bullock, S., Uilk, J., 2000. Two silicone nontoxic fouling release coatings: Hydrosilation cured PDMS and $CaCO_3$ filled, ethoxysiloxane cured RTV11. Biofouling. 16:277–288.

Yebra, D.M., Kiil, S., Dam-Johansen, K., 2004. Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. Prog Org Coat. 50:75–104.

Tables

Table 1. Thicknesses and contact angles of the coatings compared in field immersion trials. Mean and standard deviation (n=6) for static contact angles were calculated for the silicone-based coatings and the bare PVC control plates. Since water drops changed shape considerably over the first 2 min of exposure to EIM (presumably due to surfactant leaching), drops were measured twice for this coating: immediately after placing the drop on the surface and again 3 min later. All other coatings showed unchanging contact angles.

Coating	Thickness (mm)	Contact Angle (°)	
		Mean	St. Dev.
PVC Control	-	74.3	3.4
EIM (0 min)	3.0	68.8	6.9
(3 min)		39.9	1.1
RTV	0.5	104.7	1.8
INT	1.0	104.2	1.9

Table 2. Kruskal-Wallis statistics for field immersion trials comparing percent cover of tunicates amongst four treatments (n = 10 replicate plates each): control (CTL), elastomeric impression material (EIM), Intersleek 757 (INT), and RTV-11 (RTV). Cover is compared for selected weeks (since immersion) for both *Ciona intestinalis* (at Straight of Canso, NS, Canada) and colonial tunicates (*Botryllus schlosseri* and *Botrylloides violaceous*; at Cribbons Point, NS, Canada). All test statistics have 3 degrees of freedom. Tukey's post-hoc comparisons were used to separate treatments into homogenous subsets (within a row, treatments with different letters are significantly different at $\alpha = 0.05$).

Tunicate	Week	H	*P*-value	Homogeneous Subsets			
				CTL	EIM	INT	RTV
C. intestinalis	05	30.9	<0.001	c	a	b,c	a,b
C. intestinalis	08	21.4	<0.001	b	a	b	a,b
C. intestinalis	11	15.5	0.001	b	a	a,b	a,b
C. intestinalis	14	17.9	<0.001	b	a	a,b	b
C. intestinalis	17	15.9	0.001	b	a	a,b	b
Colonial tunicates	05	30.7	<0.001	b	a	b	a
Colonial tunicates	07	29.6	<0.001	b	a	b	a,b
Colonial tunicates	11	17.8	<0.001	a,b	a	b	a
Colonial tunicates	14	14.5	0.002	a,b	a	b	a
Colonial tunicates	17	4.94	0.177	a	a	a	a

Figure Captions

Figure 1. Comprehensive view of field immersion trials showing the progression of fouling accumulation (darker regions) across all coating treatments. Contrast-enhanced images of all experimental plates for control (CTL), RTV-11 (RTV), Intersleek 757 (INT), and elastomeric impression material (EIM) treatments are concatenated into montages by replicate (rows) and time (columns), for all sampling dates at the two sites. Fouling at the two sites was dominated by either *Ciona intestinalis* or colonial tunicates (*Botryllus schlosseri* and *Botrylloides violaceous*). Scale: each individual plate image represents 20.0 x 11.7 cm.

Figure 2. Mean percent cover (n = 10) over time (weeks) for *C. intestinalis* (solid line), colonial tunicates (dashed), and hydroids (dot - dashed) on each coating treatment: (a) control, (b) elastomeric impression material (EIM), (c) Intersleek 757 (INT) and (d) RTV-11 (RTV). Plates were submerged at Port Hawkesbury, NS, Canada, on June 26, 2013 (week 0). Error bars indicate standard error of the mean.

Figure 3. Changes in mean percent cover of (a) *C. intestinalis* and (b) colonial tunicates with duration of immersion (weeks) for the four coating treatments (n = 10 plates each): control (dot-dashed line), RTV-11 (dashed), Intersleek 757 (dotted), and elastomeric impression material (solid). Plates were submerged at (a) Port Hawkesbury, and (b) Cribbons Point, NS, Canada on June 26 and 27 of 2013, respectively (week 0), and monitored for 17 weeks. Error bars indicate standard error of the mean.

Figure 4. Mean ´pseudobarnacle´ adhesion strengths for the elastomeric impression material (EIM), Intersleek 757 (INT), and RTV-11 (RTV) coating treatments (MPa; n> 28 per treatment). To compare the coatings before and after any leaching that might occur, both freshly prepared (unexposed) plates and plates that had previously been submerged in the field for 62 weeks and subsequently cleaned of all fouling (previously exposed) were tested. Control (CTL) plate data is included in the figure for comparison to the silicone-based coatings, but was excluded from the statistical analysis as adhesion strengths exceeded the maximum measurement of the force gauge (1.00 MPa). Error bars indicate standard error of the mean. Letters indicate subsets of treatments with adhesion strengths that could not be distinguished based on pairwise comparisons following a Kruskal-Wallis test (i.e. treatments with different letters were significantly different).

Figure 5. Larval toxicity assay 3: Effects of leachate concentrations of 0 mg L^{-1} (ASW, white bars), 3 mg L^{-1}, 7 mg L^{-1}, and 10 mg L^{-1} (increasing concentration corresponds to increasing grey densities) on *C. intestinalis* larvae. Higher concentrations (a) reduced larval progression through metamorphosis (see methods for score calculation), and (b) increased mortality relative to controls. Error bars indicate standard error of the mean.

Figure 6. Estimated mean concentration of APEs leached from a 7 mm EIM disc in 2 ml ASW as a function of time. Tests were performed with EIM material that was freshly prepared (unexposed (×)), or previously immersed in seawater for 7 (□), 13 (△) or 22 weeks (♦). Error bars indicate standard deviation of the mean.

Supplemental Figure Captions

Figure S1. *Ciona intestinalis* larvae in the four scored metamorphosis stages, which progress from: (a) Premetamorphosis, showing head or trunk (arrowhead) and tail (arrow); (b) Tail resorption, showing papilla (arrowhead) and tail sheath from which tail has been resorbed (arrow); (c) Ampulla extension, showing ampulla (arrowhead) and resorbed tail (arrow); (d) Siphon opening, showing attachment point to substrate (arrowhead) and siphon (arrow). Scale bars = 250 μm.

Figure S2. Larval toxicity assay 1: *Ciona intestinalis* larvae in (a) ASW, and (b) ASW with 10 mg L^{-1}APE leachate. Arrowheads in (b) point to the deformed tails of larvae. Larvae in ASW with an EIM disc or in ASW with 10 mg L^{-1} Igepal CO-630, had similar tail features as (b). Scale bar = 500 μm.

Figure S3. Mass spectra of (a) leachate from cured EIM, (b) pure Igepal CO-630 solution, (c) leachate from EIM accelerator paste, and (d) leachate from EIM base paste. Note the different intensity scales in each panel.

Supplementary Material

C. intestinalis larval rearing

C. intestinalis adults were collected from the Strait of Canso Yacht Club, Port Hawkesbury, NS from June to November 2013. Adults were maintained in chilled UV-sterilized seawater (8.5 °C). From June to September, the animals were exposed to light on a natural daylight cycle, while from October to December, light exposure was increased to 16 h to encourage egg production. Tunicates were fed daily with Shellfish Diet 1800® microalgal concentrate (Reed Mariculture Inc., Campbell, California), a three part mix of dried spirulina, kelp and commercial tropical fish flakes crushed together (2 mL, dry measure), and live brine shrimp (hatched from 0.2 mL of eggs). Additionally, 250 mL each of live algae cultures (*Dunaliella tertiolecta* and *Rhodomonas lens*) were provided every other day.

Eggs were collected with a pipette from the oviducts of four to eight adults and washed several times in a glass bowl containing 20 mL artificial seawater (ASW; pH 8.1 ± 0.1, prepared from 34 g L^{-1} Instant Ocean, United Pet Group, Blacksburg, VA, USA) dissolved in distilled water. Sperm (from different adults) were suspended in 10 mL ASW in a Syracuse dish. A 500 µl aliquot of this suspension was added to the eggs, mixed gently, and left for 1 h in an18 °C incubator. The fertilized eggs were then washed in ASW and cultured in the incubator. Larvae hatched after 20 h and those having straight tails and that were swimming were selected for use in larval assays within 2-3 h of hatching. Larvae were exposed to various treatments in sterile Falcon 12-well tissue culture plates (VWR, Mississauga, Canada) with 10 larvae per well (0.5 mL of culture suspension added to 1.5 or 2 mL of experimental solution) and incubated at 18 °C.

Larval morphology, mortality, and developmental stage were scored periodically for up to 48 h using an Olympus SZX16 stereomicroscope. Morphology was scored based on whether tails were straight or deformed (a distinct, morphology, resulting repeatedly from exposure to some treatments).

Figure 1

Click here to download high resolution image

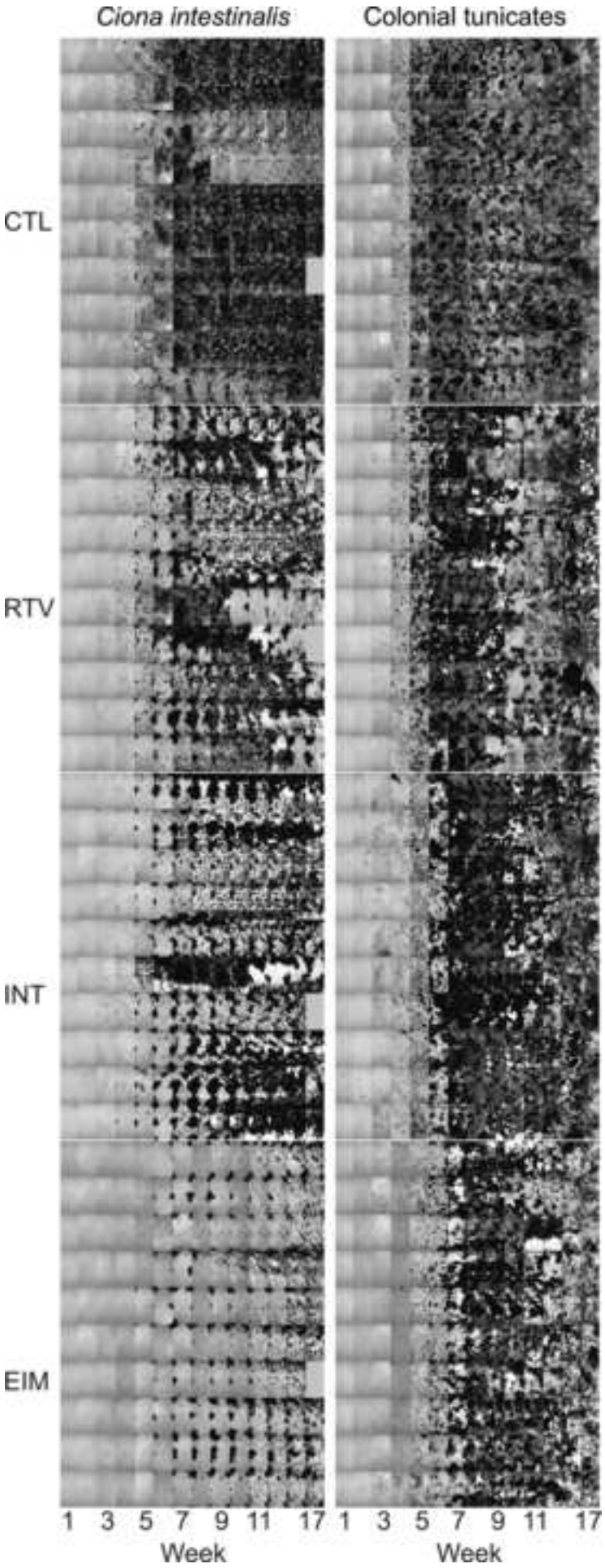


Figure 2

Click here to download high resolution image

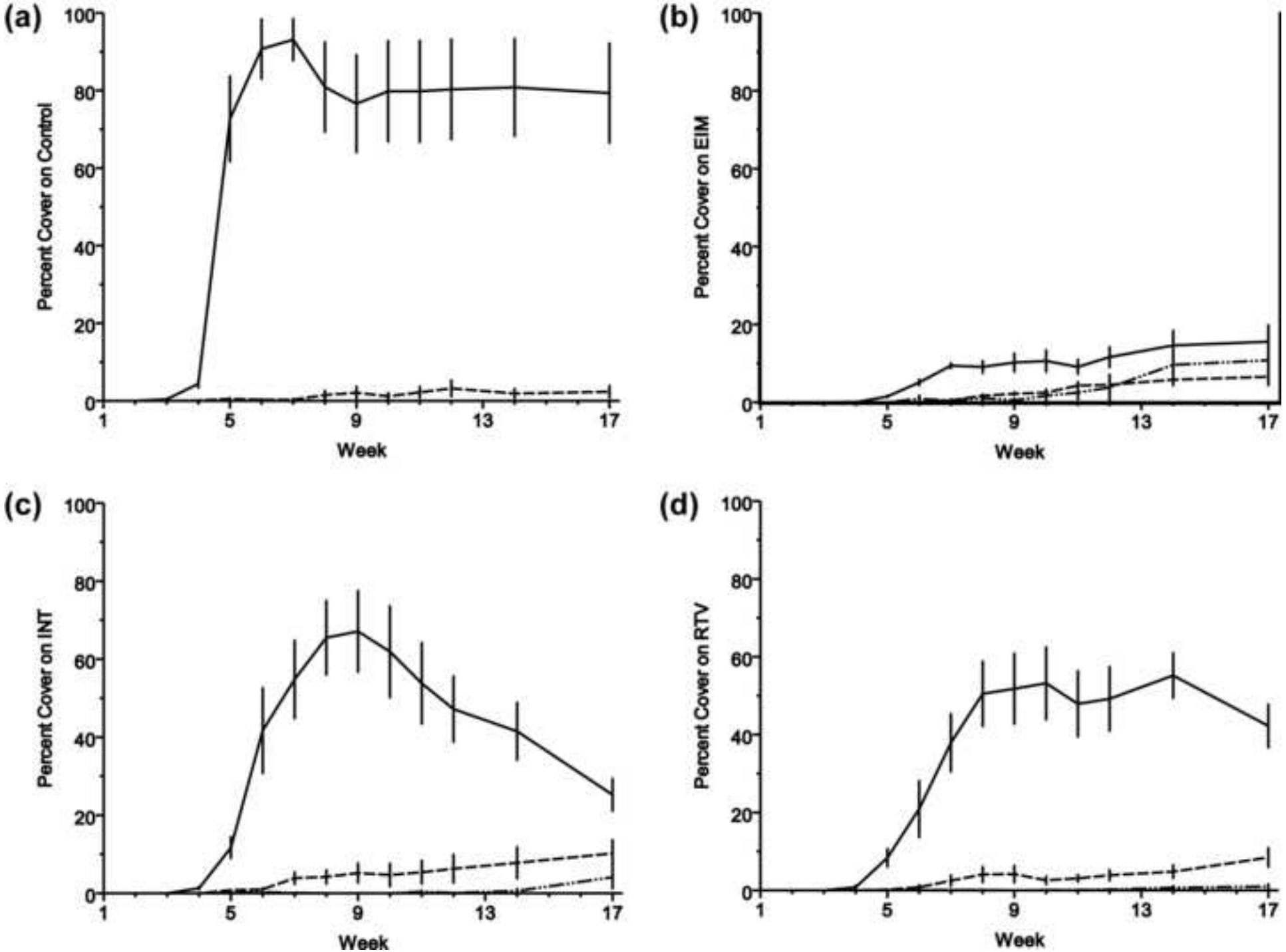


Figure 3

Click here to download high resolution image

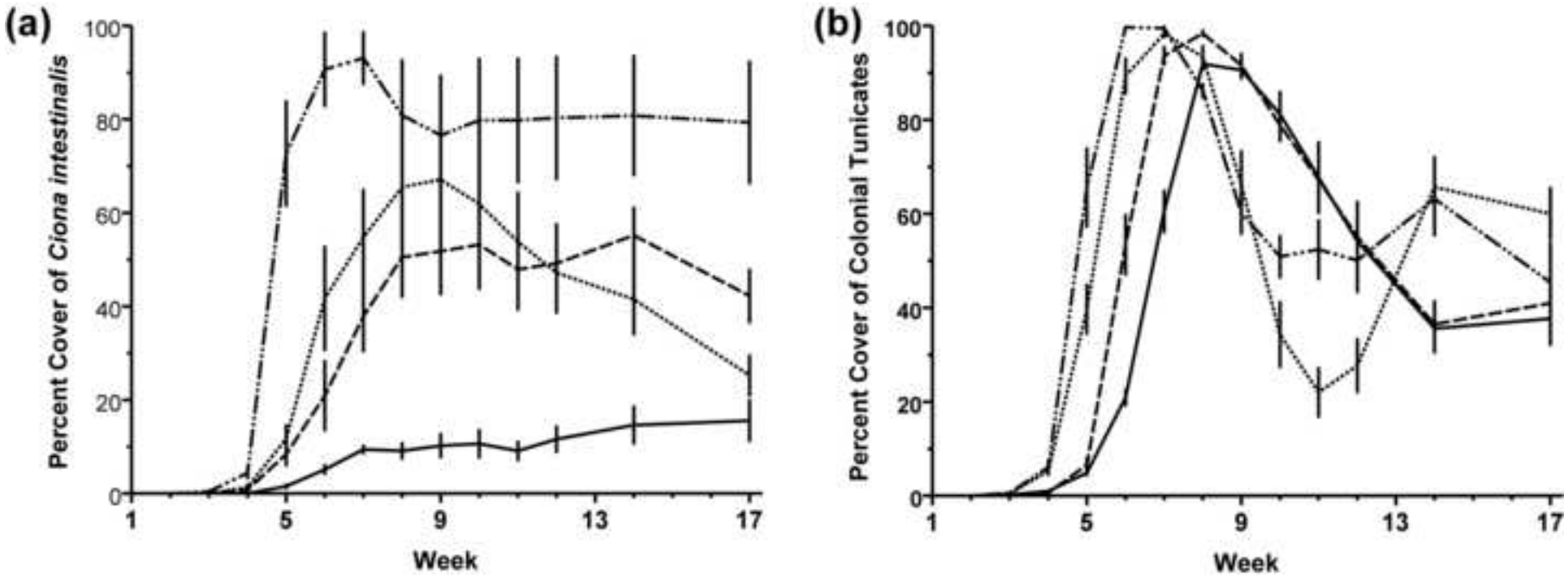


Figure 4

Click here to download high resolution image

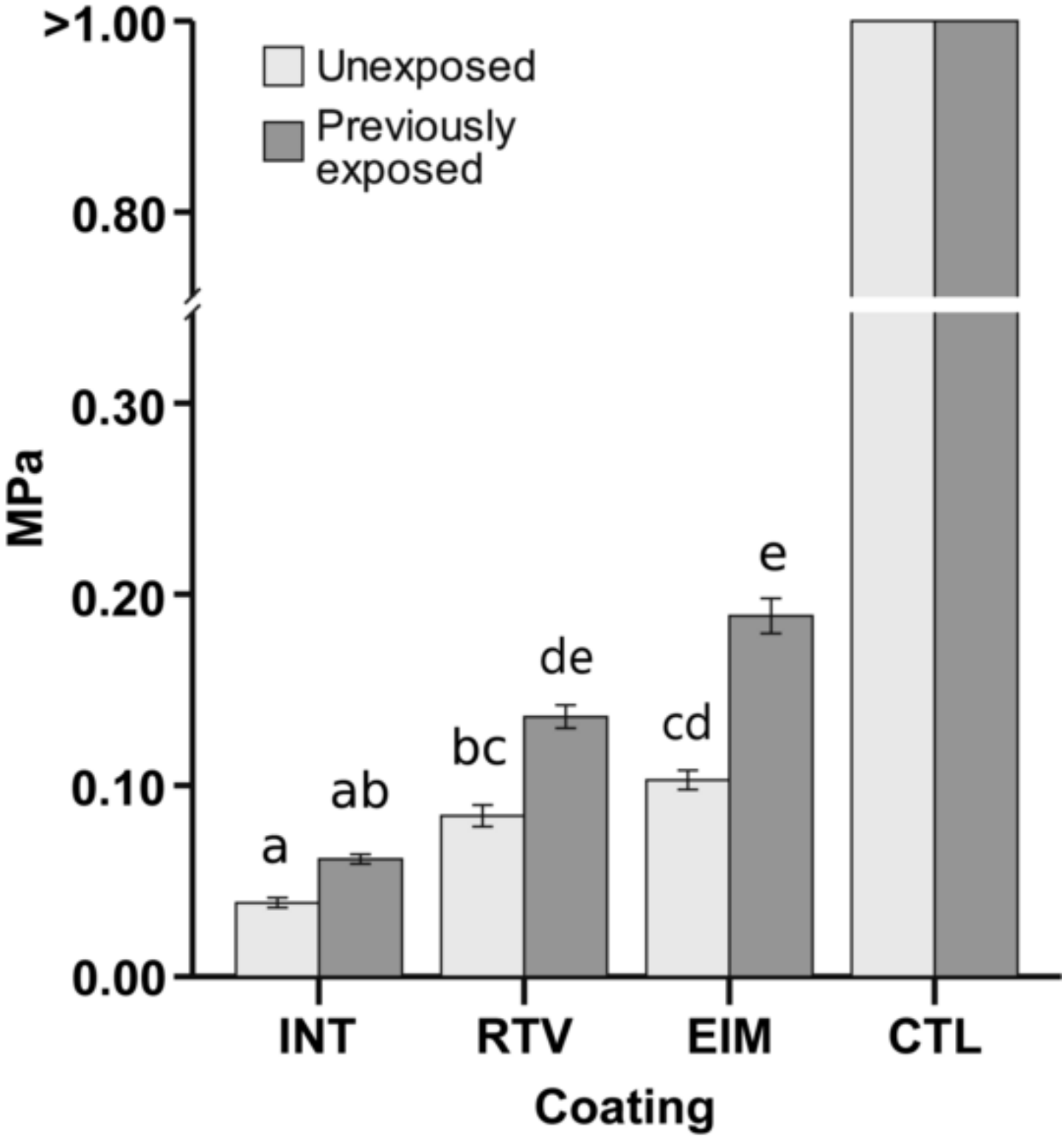


Figure 5

Click here to download high resolution image

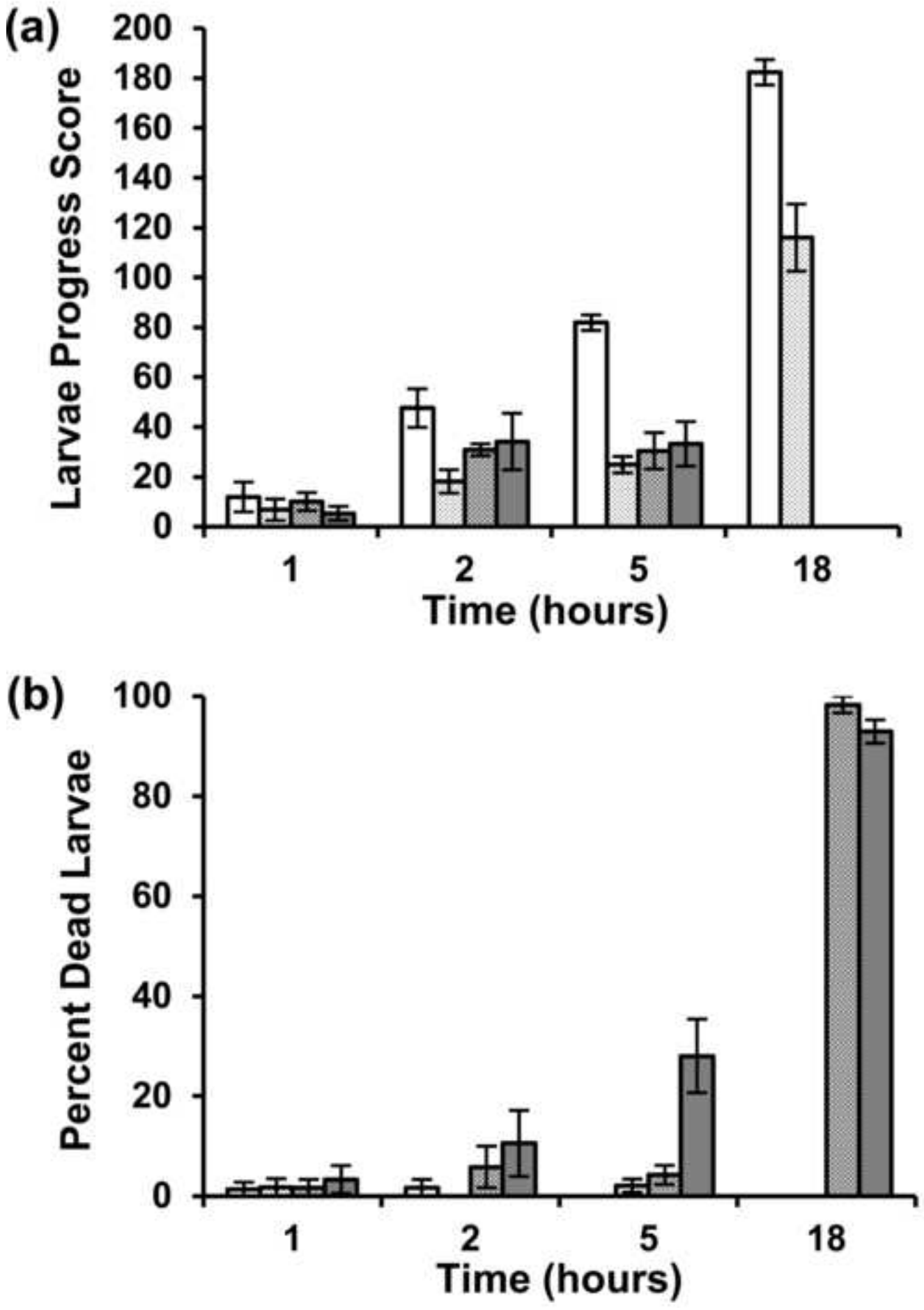
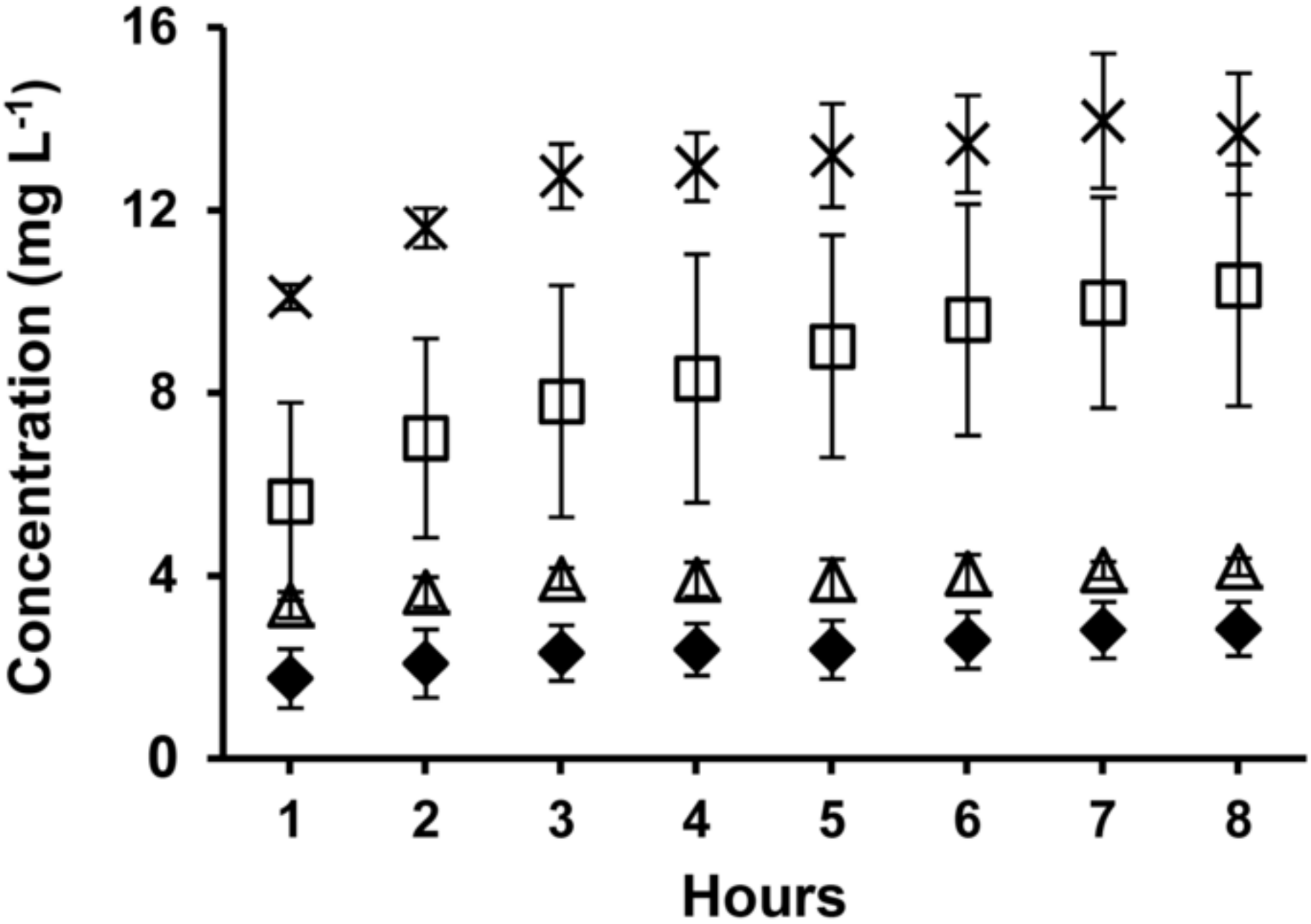
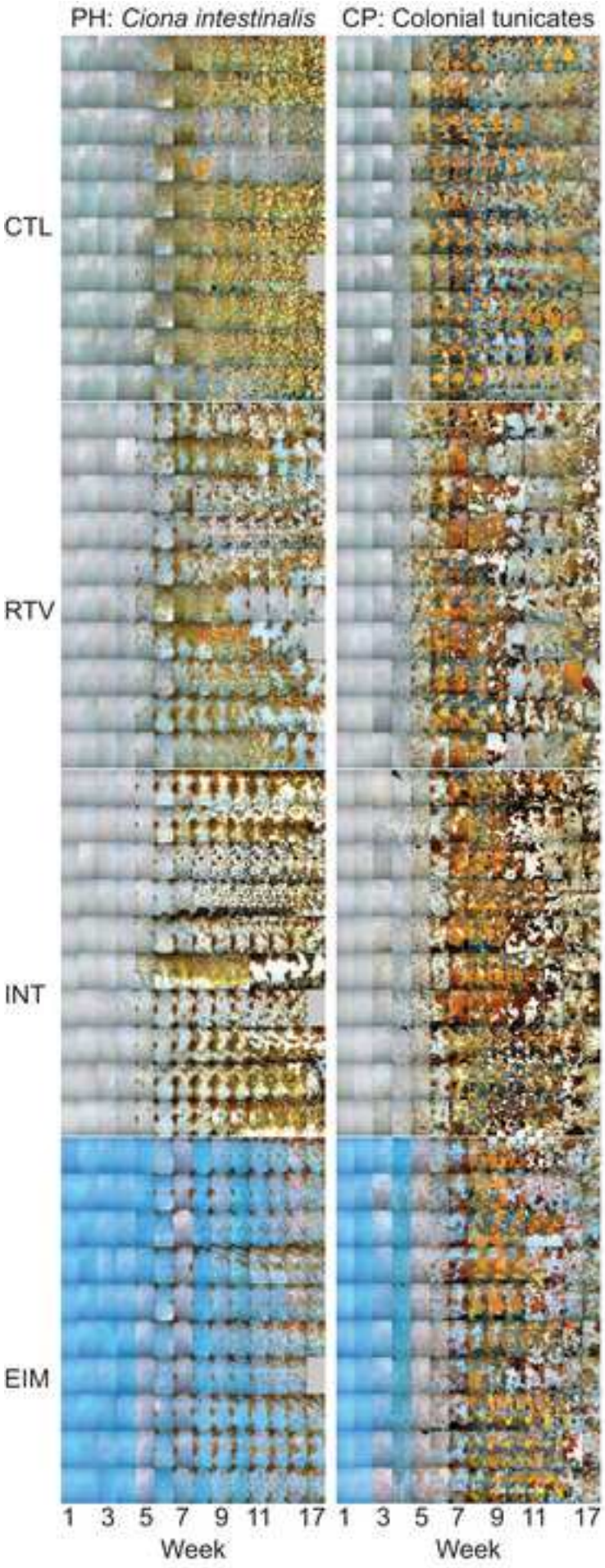


Figure 6

Click here to download high resolution image

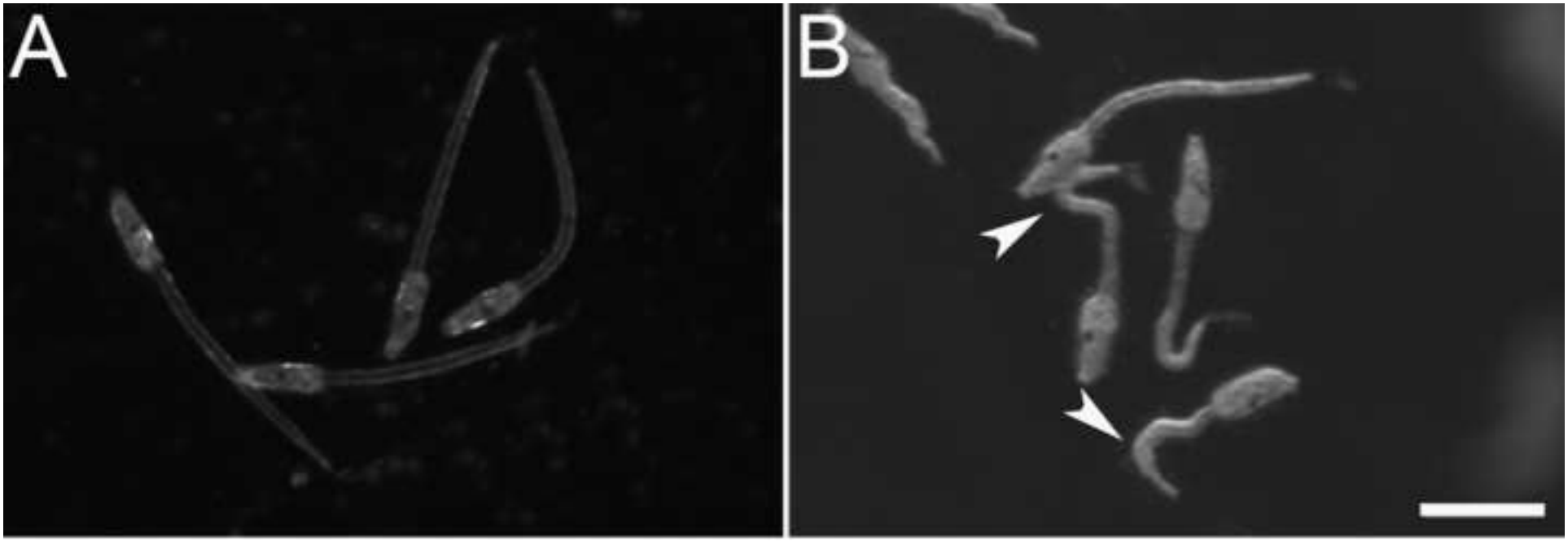


Online colour version of Figure 1
Click here to download high resolution image



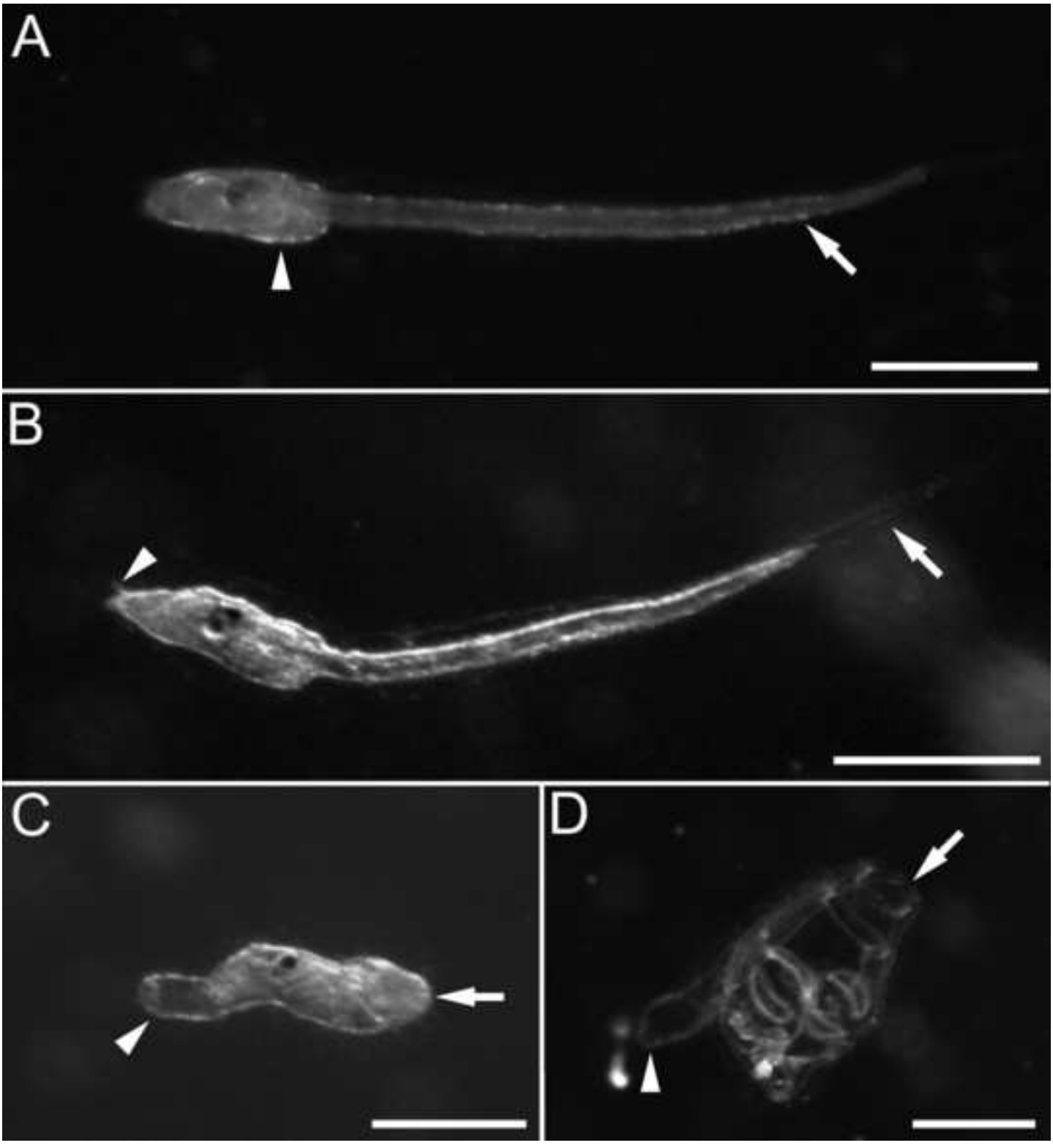
Supplementary material- Figure 1

Click here to download high resolution image



Supplementary material- Figure 2

Click here to download high resolution image



Supplementary material- Figure 3

Click here to download high resolution image