LOW SENSITIVITY OF REPRODUCTIVE LIFE-STAGES IN THE PACIFIC OYSTER

(*CRASSOSTREA GIGAS*) TO ABAMECTIN

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Abstract

Hard surfaces submerged in the marine environment often become colonised by macro-organisms unless the surfaces have some form of biofouling protection. While protective paints that contain tributyltin or copper work well to prevent biofouling, release of these materials into the environment has been shown to have wider negative impacts. Consequently, new low-release antifouling paints are being developed with alternative active ingredients, such as avermectins, yet little is known about their potential effects on non-target organisms in marine environments. Here we investigated the toxicity of a key avermectin, specifically abamectin, on several aspects of reproduction (sperm motility, fertilisation success, early larval development) in the Pacific oyster, *Crassostrea gigas*. Oyster reproduction was generally insensitive to the low concentrations of abamectin, although greater concentrations of abamectin did negatively affect all three endpoints – LOECs were 1000 µg.l^{-1}, 500 µg.l^{-1}, and 100 µg.l^{-1} abamectin for sperm motility, fertilisation success, and larval development, respectively. A similar pattern was found in the EC$_{50}$s of the three endpoints (mean ± SE) 934 ± 59 µg.l^{-1}, 1076.26 ± 725.61 µg.l^{-1}, and 140 ± 78 µg.l^{-1} abamectin (sperm motility, fertilisation success, and larval development, respectively). Together, these results clearly indicate that of the three endpoints considered, larval development was more sensitive to abamectin (lower LOEC, EC$_{50}$) than fertilisation success and sperm motility. Although more data are needed from a wider range of marine species and environments to fully assess potential toxicity effects on non-target organisms, our results highlight the potential utility of abamectin in low-release antifouling paints.

1. Introduction

Untreated exposed surfaces placed in the marine environment are usually quickly colonised by a diverse range of marine macro-organisms, which can grow rapidly to create a substantial layer of biofouling (Flemming 2002, Chambers et al. 2006, Fitridge et al. 2012). Biofouling can have wide-ranging, detrimental, and costly effects: on ships it can reduce speed, increase fuel costs, and constrain manoeuvrability; on static structures like platforms and jetties it can compromise safety by reducing stability and concealing structural defects; and on aquaculture infrastructure biofouling can restrict water exchange, increase disease risk, and cause damage to enclosures (Turner 2010, Schultz et al. 2011, Fitridge et al. 2012). Consequently, it is a common practice to protect surfaces from biofouling by applying antifouling paints that slowly release biocides and prevent the initial settlement of organisms (reviewed in Chambers et al. 2006).

While often effective in preventing biofouling, the release of biocides from antifouling coatings into the wider marine environment can also have widespread and unintended consequences for non-target organisms, ecosystems, and human societies (Terlizzi et al. 2001). For example, tributyltin (TBT) was a very effective antifouling additive in paints, but TBT leakage and contamination adversely affected growth and reproduction in a range of non-target organisms including oysters which, in turn, caused large impacts to the associated fishing and aquaculture sectors (Alzieu 1991, 2000). As a result, TBT was phased out of antifouling coatings (Champ 2003, Gipperth 2009) and replaced by alternative substances including copper and zinc (Chambers et al. 2006, Turner 2010). These alternatives also, however, have negative effects on non-target organisms, including oysters (Evans et al. 2000, Guardiola et al. 2012). Consequently, novel, more environmentally acceptable alternatives are being sought (Callow & Callow 2011, Gorman 2014).

One group of chemicals gaining attention for their potential use in antifouling coatings are the avermectins (e.g. Pinori et al. 2011, Pinori et al. 2013, Trepos et al. 2014), a group of macrocyclic lactones that include abamectin, ivermectin, and dormectin (Tišler & Eržen 2006). Derived from soil microorganisms, these chemicals are often highly toxic to insects and can also be toxic to mammals (Fisher & Mrozik 1989, Lankas & Gordon 1989, Wislocki et al. 1989). Typically, avermectins have been used as pesticides to control mites, other crop pests, and parasites of livestock, as well as in antifilarial chemotherapy in humans (Lumaret et al. 2012, Bai & Ogbourne 2016). Of the avermectins, abamectin is potentially the most suitable candidate for incorporation in antifouling paints from a regulatory standpoint; abamectin has already been approved and registered in the Biocide Products Regulation (European Chemicals Agency) under PT18 (as an insecticide in agricultural/for terrestrial use), and therefore many of the requirements for registration under PT21 (antifouling purposes) have been complied with (EuropeanCommission 2011). There remains, however, a lack of understanding regarding effects of abamectin specific to aquatic systems, which must be addressed before abamectin can be approved for use in commercial antifouling products.

To date, most focus on the impacts of avermectins in aquatic systems relates to the toxicity of run-off from terrestrial pesticide use into freshwater habitats (see Lumaret et al. 2012 for a comprehensive summary of the ecotoxicity of macrocylic lactones). Relatively little is known about the toxicity of abamectin released into the marine environment, and there are few ecotoxicological assessments involving marine or estuarine organisms (Table 1). Only two studies tested the effects of abamectin on sensitive early life history stages of reproduction (Tamparon et al. 2015) or larval development (Wislocki et al. 1989) (results reported in Table 1). Broadcast spawning organisms are of particular interest in this context as their

external reproduction may make them particularly sensitive to antifoulants (Gazeau et al. 2013). For example, reproduction in the Pacific oyster, *Crassostrea gigas*, which releases gametes freely to the water column where external fertilisation and subsequent larval development take place (Yonge 1960, Park et al. 1988), has been shown to be affected by biocides (e.g. Alzieu 1991, Alzieu 2000). Moreover, species that reside in shallow coastal areas may experience particularly high concentrations of biocides (Thomas 2001). Thus there is a clear need for more information on toxicity of abamectin to broadcast-spawning species inhabiting shallow coastal marine environments where biocide concentrations from antifouling coatings are likely to be greatest. We chose to address these issues in the common, invasive, and free-spawning bivalve *C. gigas*. Investigating the impacts of abamectin on *C. gigas* is of particular value given the diverse, and valuable, ecosystem services provided by this species (Grabowski et al. 2012, Lemasson et al. 2017). Consequently, here we investigated the effects of abamectin on multiple reproductive endpoints (sperm motility, fertilisation success, early larval development) in the Pacific oyster, *Crassostrea gigas*.

Table 1. Ecotoxicological data available for abamectin exposure in marine and estuarine species

Species	Common name	Phylum	Duration, method, LC/EC$_{50}$	Reference
Panaeus duorarum	Pink shrimp	Crustacea	96 h exposure of adults: LC$_{50}$ = 1.6 µg.l^{-1}	Wislocki et al. (1989)
Americamysis bahia	Mysid (estuarine)	Crustacea	96 h exposure of adults: LC$_{50}$ = 0.022 µg.l^{-1}	Wislocki et al. (1989)
Callinectes sapidus	Blue crab	Crustacea	96 h exposure of adults: LC$_{50}$= 153 µg.l^{-1}	Wislocki et al. (1989)
Strongylocentrotus sp.	Sea urchin	Echinodermata	10 h exposure of fertilising eggs/developing larvae: LC$_{50}$ = ~ 1 µg.l^{-1}	Tamparon et al. (2015)
Crassostrea virginica	Eastern oyster	Mollusca	96 h exposure of fertilising eggs/developing larvae: LC$_{50}$ = 430 µg.l^{-1}	Wislocki et al. (1989)
Crassostrea gigas	Pacific oyster	Mollusca	20 min exposure sperm motility: EC$_{50}$ = 1017 µg.l^{-1}	This study
Crassostrea gigas	Pacific oyster	Mollusca	2 h exposure of fertilising eggs: EC$_{50}$ = 4202 µg.l^{-1}	This study
Crassostrea gigas	Pacific oyster	Mollusca	26 h exposure of developing larvae: EC$_{50}$ = 72 µg.l^{-1}	This study

2. Materials and Methods

2.1 Collection of animals and gametes

Adult Pacific oysters, *Crassostrea gigas*, were collected from Tjärnö, Sweden in December of 2014 and transferred to Ostrea Svergie AB (Koster, Sweden) where they were conditioned to maturity under laboratory conditions. Experiments were conducted at the Sven Lovén Centre for Marine Sciences (Tjärnö, Sweden) in July of 2015. All experiments were run at 20 $\pm$ 1°C (the typical ambient seawater temperature in this region during the spawning season).

Concentrated gametes were extracted from each individual using a Pasteur pipette inserted through a hole drilled in the shell above the gonad (Havenhand & Schlegel 2009). Sperm from each male were stored in separate Eppendorf tubes on ice to maximise longevity, eggs from each female were stored separately in 0.22 µm filtered seawater (FSW) at ambient temperature. For each male, sperm concentrations were determined from hemocytometer counts of samples stained and immobilised with Lugol's solution. Sperm from three males were then pooled to obtain a concentrated mixed batch with equal (1:1:1) representation from each male. Extracted eggs were left in FSW for one hour to allow their hydration, during which time concentrations were adjusted such that eggs of three females could be combined to form a pooled batch with equal (1:1:1) representation. Gametes were pooled to reduce subsequent variation in response variables caused by individual differences in male:female compatibility. These pooled batches of gametes were used for all experiments.

2.2 Experimental treatment: abamectin addition

Abamectin (CAS 71751-41-2) is a mixture of $\geq$ 80% avermectin B_{1a} ($C_{47}H_{70}O_{14}$ CAS 65195-55-3) and $\leq$ 20% avermectin B_{1b} ($C_{48}H_{72}O_{14}$ CAS 65195-56-4). Experimental treatments were prepared by diluting an abamectin stock solution (Wuhan Yuancheng Technology

Development Co., China) with FSW to yield five effective final abamectin concentrations (1000, 500, 100, 10 and 1 $\mu g.l^{-1}$), plus a FSW control (0 $\mu g.l^{-1}$).

2.3 Sperm motility, fertilisation success, larval development

Sperm motility was quantified using the Sperm Accumulated Against Surface (SAAS) technique (Falkenberg et al. 2016). Sperm were diluted with seawater containing the appropriate abamectin treatment (0, 1, 10, 100, 500, or 1000 $\mu g.l^{-1}$) to a concentration of 2×10^6 $sperm.ml^{-1}$ in each of six replicate wells in a multi-well plate. Sperm suspensions were left for 10 min before pipetting 1.5 ml into new wells. The bottom surface of each new well was then observed using a phase-contrast inverted microscope (Leica, DMIL, Germany) equipped with a digital video camera (PixeLINK, PL-D725CU, Canada). Sperm accumulation after a further 10 min (i.e. 20 min after initial exposure to the treatments) was determined by counts from still images of different central areas of the lower surface of the wells ($n = 3$ images per well). This procedure was repeated for "dead" sperm diluted with FSW (0 $\mu g.l^{-1}$; sperm were killed by warming to ~50 °C for 5 min, after which time no motile sperm were observed).

Fertilisation success was quantified using eggs mixed with the same sperm as used in sperm motility trials. Final concentrations of gametes were adjusted to yield 5×10^8 $sperm.ml^{-1}$ and 300 $eggs.ml^{-1}$ in the treatments (previous work had shown that these concentrations resulted in ~50% fertilisation success in controls, which maximises sensitivity of the assay and allows for positive responses to the treatments; Marshall 2006). Gametes were mixed with seawater containing the appropriate abamectin treatment (0, 1, 10, 100, 500, or 1000 $\mu g.l^{-1}$) and left to fertilise for 12 min. Fertilisations were then halted by separating sperm and eggs via centrifugation for 5 min at 2000 g (Eppendorf, Centrifuge 5810R, Germany). The supernatant

containing the sperm was discarded and the remaining 1 ml of lightly pelleted eggs were resuspended in 6.5 ml of the appropriate treatment water, 2.5 ml of which was distributed to each of three 35 mm Petri dishes. Each dish was photographed (Nikon D810, Japan, DSLR camera mounted on an Olympus SZX16, Japan microscope) and total egg count determined using the 'Colony Counter' plugin in ImageJ64 (Schneider et al. 2012). Eggs were left to develop for 2 h after fertilisation, after which time each replicate dish was photographed again. Fertilisation success was determined by recording the proportion of cleaving eggs in each of the three replicate dishes.

To quantify larval development, eggs/embryos used in fertilisation trials were maintained in their treatments until 26 h post-fertilisation (the point at which larvae of this species reach the D-stage; Park et al. 1988), after which they were euthanised with 95 % ethanol to stop development. The total number of D-stage larvae in each well was subsequently counted manually under a microscope (Olympus IX71) and compared to the total egg count obtained earlier to provide larval development success.

2.4 Statistical analysis

Each response variable (i.e. sperm motility, fertilisation success, and larval development) was analysed with a separate one-way ANOVA with 'abamectin concentration' as a fixed factor (6 levels) and $n = 6$ replicate wells for sperm motility or $n = 3$ dishes for fertilisation success and larval development. Prior to analysis, sperm motility data were square root transformed, fertilisation success and larval development data were arc-sine transformed, and model estimates/residuals inspected visually to ensure normality (following Quinn & Keough 2002, Zuur et al. 2010). When relevant, *post-hoc* Tukey's tests were used to identify which

treatment levels of abamectin concentration differed. Analyses were done in SPSS V.24 (IBMCorp 2016).

The resulting data were modelled using the R package DRC to produce dose-response curves and calculate toxicity estimates (Figure A1) (Ritz & Streibig 2005). Regression models tested included log-logistic and Weibull models with different levels of parameterization. For sperm motility models, a lower limit was set at 15 as this was the average sperm accumulated at surface (SAAS) in the dead controls. Akaike's Information Criterion (AIC) was used to compare models. The concentrations of abamectin that produced a 50% inhibition between the maximal and baseline in each of the specified endpoints (EC_{50}) were determined from the best-fit models. Confidence limits (95%) were estimated using the delta method. For larval development, the EC_{50} was lower than the LOEC and therefore we used the same fitted dose-response model to also calculate an EC_{10} (the concentration of abamectin that produced a 10% inhibition) as this provides a more reliable measure of a concentration where small biological effects were predicted (Warne & van Dam 2008).

3. Results

All three measures of oyster reproduction showed a statistically significant, negative dose-dependent response to abamectin (Figure 1; sperm motility: $F_{(5, 12)} = 5.787$, $p = 0.001$; fertilisation success: $F_{(5, 12)} = 12.513$, $p < 0.001$; larval development: $F_{(5, 12)} = 25.011$, $p < 0.001$). The sensitivity of this dose-response increased with developmental stage (Figure 1) such that sperm motility was significantly reduced (relative to controls) only at the greatest concentration of abamectin (Figure 1A, 1000 µg.l^{-1}; *post-hoc* tests), whereas fertilisation success was significantly reduced at the two greatest abamectin concentrations (Figure 1B; 500, 1000 µg.l^{-1}; *post-hoc* tests), and larval development was significantly

reduced relative to controls at the three greatest abamectin concentrations (Figure 1C; reduced at 100 µg.l^{-1}, and completely inhibited at 500 and 1000 µg.l^{-1}; *post-hoc* tests). Consequently, the Lowest Observable Effect Concentrations (LOECs) were 1000 µg.l^{-1}, 500 µg.l^{-1}, and 100 µg.l^{-1} for sperm motility, fertilisation success, and larval development respectively.

Dose-response curves produced EC$_{50}$ estimates for both sperm motility and fertilisation success that were close to the greatest abamectin concentration tested (i.e. ~ 1000 µg.l^{-1}), whereas the EC$_{50}$ estimate for larval development was much lower and well within the range of concentrations tested (i.e. < 1000 µg.l^{-1}). For sperm motility, the best fit dose response model was a Weibull (type 1) four parameter model (Table A1), EC$_{50}$ (mean ± SE) = 934 ± 59 µg.l^{-1}. For fertilisation success the best fit model was a Weibull (type 2) three parameter model (Table A1), EC$_{50}$ = 1076.26 ± 725.61 µg.l^{-1}. In contrast, the fitted larval development EC$_{50}$ estimate was much lower (and within the range of abamectin concentrations tested); the best fit model was a Weibull (type 1) three parameter model (Table A1), EC$_{50}$ = 140 ± 78 µg.l^{-1}. Because the EC$_{50}$ was similar to the LOEC for this endpoint, we used the same fitted dose-response model to calculate an EC$_{10}$ which was 24 ± 52 µg.l^{-1}.
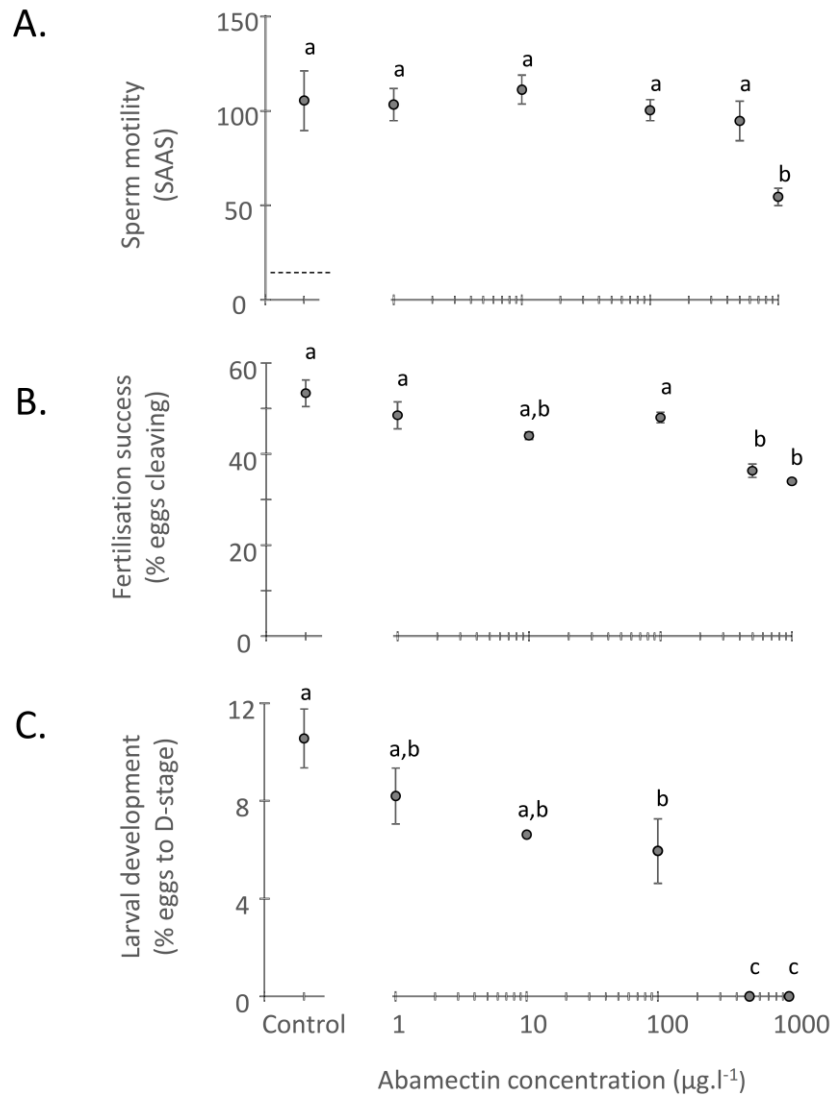
Figure 1. Response of oyster, *Crassostrea gigas*, sperm motility (A), fertilisation success (B), and larval development (C) to abamectin. Data points are means ± SE (*n* = 6 for sperm motility, *n* = 3 for fertilisation and larval development); letters represent groups that were significantly different in *post-hoc* tests; dashed line in (A) represents sperm accumulation after 20 min for a dead sperm (control) treatment in seawater.

4. Discussion

We found that sperm motility, fertilisation success, and early larval development in the Pacific oyster were relatively robust to short-term exposure to abamectin. This robustness did, however, vary among the response variables. That is, sensitivity to abamectin increased with developmental stage and exposure time (i.e. sperm motility and fertilisation success were less sensitive than larval development). This finding was reflected both in the lowest concentrations at which significantly reduced responses were detected (i.e. LOEC) and estimated concentrations that would produce 50% inhibition between maximal and baseline measures (i.e. EC_{50}). Similar patterns of increasing sensitivity in sequential developmental stages (e.g. sperm motility < fertilisation success < larval development) have been identified for a range of organisms with other additives (e.g. Ringwood 1992, Bellas et al. 2001, Geffard et al. 2001, Fitzpatrick et al. 2008). Such effects are perhaps unsurprising given the dependence of higher developmental stages on lower ones. While sperm motility would only be affected by direct effects on this stage, fertilisation success would be influenced by any direct effect on this process and the effect on sperm motility, while larval development would be determined by direct effects on this process, fertilisation success and sperm motility (Fitzpatrick et al. 2008). Moreover, later developmental stages were exposed to the treatments for longer periods, creating the possibility for expression of more chronic responses. Consequently, despite the potential for all the reproductive endpoints considered here to be used as ecotoxicological endpoints (e.g. Lewis & Watson 2012, Falkenberg et al. 2016), larval development may be the most sensitive.

Our results are broadly comparable to the responses of other aquatic organisms to avermectins (responses to abamectins summarised in Table 1). In terms of larval development, the EC_{50} we observed here (140 ± 78 μg.l^{-1}) was lower than – but within an

order of magnitude of – that reported in Wislocki et al. (1989) for fertilising/early developing larvae in the eastern oyster, *Crassostrea virginica* (LC_{50}: 430 µg.l^{-1}). It is important to note, however, that the Wislocki et al. (1989) test involved 96 h continuous exposure to elevated abamectin concentrations, whereas our exposure time for the larval development test was 26 h. Clearly such differences in exposure time preclude direct comparisons of sensitivity of larval development to abamectin (Wislocki et al. 1989 also report few details of their tests, which further precludes relevant comparison). It does appear, however, that other organisms exposed to abamectin for long periods (96 h) were much more sensitive to abamectin than oysters (e.g. mysid, *Americamysis bahia*, LC_{50} = 0.022µg.l^{-1}; Table 1). Similar differences in sensitivity are also apparent for shorter exposure times: the only short duration (10 h) test of abamectin on a marine invertebrate (fertilisation/larval development in the sea urchin, *Strongylocentrotus* sp., LC_{50} = ~ 1 µg.l^{-1}; Tamparon et al., 2015) identified greater sensitivity than we detected for oysters (2 h exposure of fertilising eggs EC_{50} = 4202 µg.l^{-1}, 26 h exposure of developing larvae EC_{50} = 72 µg.l^{-1}; Table 1). In freshwater species, sensitivity to abamectin can be even more variable, with EC_{50} ranging over six orders of magnitude (from 4000 µg.l^{-1} for the rotifer *Brachionus caclcyflorus*, to 0.0051 µg.l^{-1} for the cladoceran *Daphnia similis*; summarised in Casali-Pereira et al. 2015). If comparisons are extended to another avermectin, specifically ivermectin, similar results and variability in sensitivity are also found (summarised in Davies et al. 1997, Lumaret et al. 2012, Bai & Ogbourne 2016). Together, these results clearly indicate that more data are required before the toxicity of abamectin can be generalised for aquatic species.

Comparisons of assessments for sensitivity to a biocide need to consider not only the endpoint and species tested, but also the likely relevance of exposure duration and concentration to those found in the field. The only previous assessment of oyster responses to

abamectin involved static tests over relatively long periods of time (96 h; Wislocki et al

1989). We purposefully chose to run much shorter exposures ($\leq$ 26 hrs) that corresponded to

clear developmental end-points (second cleavage is visible 2 h after fertilisation and larvae

reach D-stage 15-28 h after fertilisation; Park et al. 1988). In terms of concentration,

available data confirm that concentrations of abamectin in seawater near treated surfaces are

likely to be much lower than the LOECs and EC_{50}s we observed. For example, Pinori et al.

(2013) measured a steady-state release rate for a formulation containing ivermectin (0.1%

w/v) at $34 - 70$ $ng.cm^{-2}.d^{-1}$. Assuming similar formulations and release rates for abamectin,

this would suggest that concentrations high enough to have biological effects may only

accumulate in very small volumes of water in boundary layers immediately adjacent to

painted surfaces. However, in small or isolated bodies of water with many coated surfaces

(e.g. a marina) the concentrations of abamectin to which sperm/eggs/developing larvae are

exposed might be greater, or, as with other avermectins, the biocide might accumulate and

persist in the sediment. In the latter case, organisms may be affected over longer time periods

and effects (both lethal and sub-lethal) on adult/benthic life stages would also be relevant

endpoints (Davies et al. 1997, Grant & Briggs 1998, Sanderson et al. 2007).

In the marine environment more generally, background concentrations of avermectins are

likely to be much lower than those that had an effect here. Non-antifouling sources of

avermectins in the marine environment can include run-off from agricultural spraying, direct

pharmaceutical use in aquaculture and, rarely, spillage of concentrated products during

transport (Tišler & Eržen 2006, Bai & Ogbourne 2016). While these sources may drive local

elevations in avermectin concentration over brief periods, it has been argued that the

concentrations of these chemicals are likely to be low in marine waters as they generally bind

readily to soils and sediments and have short half-lives in aquatic environments (Wislocki et

al. 1989, Sanderson et al. 2007). It should be noted, however, that concentrations and toxicities of avermectins (and their breakdown products) in aquatic settings are poorly known (Bai & Ogbourne 2016). Nonetheless, it seems likely that the additional risk from abamectin-based antifouling paints to reproduction in oysters is negligible.

Increasingly, there is a need to study and understand the potential effects of alternatives to traditional antifouling substances – in this case abamectin. Here, we identified that although acute exposure of oyster sperm and eggs impaired sperm motility, fertilisation success, and larval development, these responses were only observed at high concentrations of abamectin. While our results are an indication that abamectin use in low-release antifouling paints is unlikely to have unintended environmental consequences for early life-stages of Pacific oysters, more work is needed to draw a general conclusion. Of particular priority for consideration should be the large variation found in sensitivity among different species and life stages (noted above) and whether a sufficient diversity of species has been tested to characterise the general toxicity of abamectin in the marine environment. In addition, environmentally relevant exposure times should be considered; for example, longer-term chronic exposure of adults to abamectin may be relevant if this substance accumulates in benthic sediments, potentially resulting in sub-lethal effects that modify reproductive stages (as observed in terrestrial studies Elbetieha & Da'as 2003, Celik-Ozenci et al. 2011, Celik-Ozenci et al. 2012). When combined with the understanding gained from future studies addressing these issues, our findings might then better inform decision-making regarding the use of abamectin in marine antifouling products, enabling the prevention of biofouling while also limiting unintended impacts in the marine environment.

Acknowledgements

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Appendix

Table A1. Dose-response models fitted to the data for oyster, *Crassostrea gigas*, sperm motility, fertilisation success, and larval development to abamecin. Best-fit models (bold) were selected using the Akaike Information Criterion (AIC). Lack-of-fit test results and model parameter estimates are provided for each model

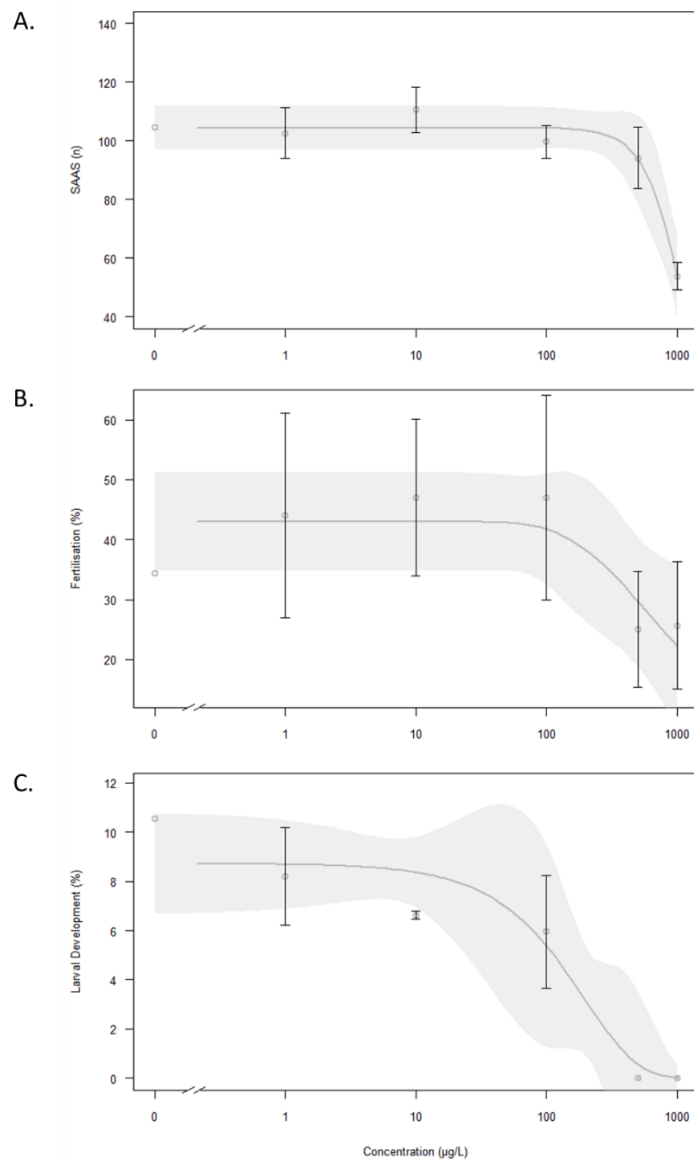|		Sperm motility	Fertilisation success	Larval development
Model	**AIC**			
3 Parameter Log-Logistic		-	147.8919	77.69906
4 Parameter Log-Logistic		991.6713	148.3451	77.69906
5 Parameter Log-Logistic		993.6888	150.3454	79.33248
3 Parameter Weibull type I		-	148.0950	**77.14799**
4 Parameter Weibull type I		**991.6466**	148.3421	77.59704
3 Parameter Weibull type II		-	**147.3710**	83.03142
4 Parameter Weibull type II		1056.6576	148.3422	79.72869
Lack of Fit test results				
		$F_{(102,105)} = 0.6816$, $p = 0.5653$	$F_{(12,15)} = 0.9341$, $p = 0.4543$	$F_{(12,15)} = 3.2529$, $p = 0.0598$
Model Parameters (estimate [SE], significance)				
Hillslope		2.72 [0.92], $p = 0.004$	-0.69 [0.43], $p = 0.125$	1.07 [1.03], $p = 0.311$
Lower limit		fixed at 15	fixed at 0	fixed at 0
Upper Limit		104.51 [2.79], $p = \leq 0.001$	42.97 [3.83], $p = \leq 0.001$	8.71 [0.97], $p = \leq 0.001$
Inflection Point		1069.04 [76.67], $p = \leq 0.001$	633.81 [338.11], $p = 0.081$	196.82 [67.09], $p = 0.01$
Asymmetry factor		NA	NA	NA
Residual SE		23.31 (df = 105)	12.72 (df = 15)	1.81 (df = 15)

Figure A1. Best fit dose-response models (Table A1) of oyster, *Crassostrea gigas*, sperm motility
(A), fertilisation success (B), and larval development (C) to abamectin. Data points are means ± SE
($n = 6$ for sperm motility, $n = 3$ for fertilisation and larval development); the solid grey line is the
modelled dose response curve used to derive EC_{50} and EC_{10}; the shaded region represents the 95%
confidence limits provided by the model