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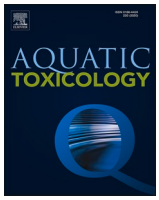

Effects of simulated environmental discharges of the salmon lice pesticides deltamethrin and azamethiphos on the swimming behaviour and survival of adult Northern shrimp (*Pandalus borealis*)

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ABSTRACT

Swimming behaviour was investigated in adult egg-carrying northern shrimp (*Pandalus borealis*) exposed to dilute concentrations of the pesticides Alpha Max® (active ingredient deltamethrin) and Salmosan® (active ingredient azamethiphos) used to control parasitic copepods in salmon aquaculture. These treatments are applied topically within fish nets or well boats. Following a short treatment period, the pesticides are directly discharged to sea, exposing non-target organisms such as *P. borealis* to diluted concentrations of these chemicals. Locomotor activity was measured continuously in individual shrimp over several days within which they were exposed to treatments of diluted AlphaMax® or Salmosan®. Dilutions were based on modelling and dispersion studies from the literature and were considered environmentally realistic for greater than 1 km from point of discharge. 24 h continuous flow treatments were delivered within a 3.5-day monitoring period to observe the timeline of events following the release of treatment water, addressing questions of temporal responses in locomotor activity, recognising key time points of significant events and assessing the survival capacity of the shrimp. Exposure of shrimp to 1 ng l^{-1} deltamethrin triggered an immediate increase in swimming activity which reduced in intensity over the following 22 h leaving all shrimp either moribund or dead. A further exposure trial exposing shrimp to 0.2 ng l^{-1} deltamethrin (nominal) showed an increase in activity at the start of exposure that continued throughout the 24 h delivery, returning to previous levels by the end of the 3.5-day monitoring period. All these shrimps survived for at least four weeks after exposure, putting the threshold concentration of deltamethrin leading to immobility or death in adult *P. borealis* within this study at greater than 0.2 ng l^{-1} (nominal) and less than 1 ng l^{-1} (measured). Exposure of *P. borealis* to azamethiphos at 30 ng l^{-1} induced several periods of significantly increased activity within the first 10 h of exposure and an extended period of reduced activity during post exposure, though no morbidity was observed with this treatment. No significant increase in activity or morbidity was observed in shrimp during a water vehicle control assessment. Shrimps exposed to a combination of 30 ng l^{-1} azamethiphos and 1 ng l^{-1} deltamethrin broadly followed the response pattern shown by shrimp exposed to 1 ng l^{-1} deltamethrin alone. Pesticide residues were not detected in post exposure tissue analyses for either chemical. The potential ecological significance of increased swimming activity at the start of pesticide exposures is discussed.

1. Introduction

Salmon lice remain a major challenge for open water pen salmon aquaculture. These ectoparasitic copepods cause damage to the skin of their hosts that can lead to imbalances in physiological processes and provide a gateway for disease with the result that both fish welfare and productivity of the aquaculture facility are reduced (Thorstad and Finstad, 2018; Bowers et al., 2000). Furthermore, wild populations of salmon are thought to be affected by lice spreading into the wider environment from high density aquaculture operations (Thorstad and Finstad, 2018; Torrissen et al., 2013). Aquaculture regulations in Norway demand regular counts of salmon lice and when limits are exceeded

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a variety of methods are employed to reduce the number of lice. Despite increasing development and use of non-medicinal methods, several chemical pesticides are still widely used (Aaen et al., 2015; Burridge and van Geest, 2014; Lillicrap et al., 2015; Overton et al., 2019). Chemical treatments for lice are delivered to salmon either within medicated feed or dispersed in water as a topical treatment to thousands of fish simultaneously, either within their growing pens, using a tarpaulin to surround the net enclosure to hold the fish and chemical treatment within a fixed water mass, or following the transfer of fish into a well boat. At the end of the treatment period these pesticides are normally discharged directly into the sea (Langford et al., 2014). Operational constraints on the exposure time for treating salmon with topical pesticides demands that relatively high concentrations of these chemicals are used to ensure their efficacy against the parasitic copepod target. Chemical treatments can be repeated over time and although it is now recommended to avoid the use of combinations of pesticides for single pen treatments (Grefsrud et al., 2018), discharges from farms within the same region applying different chemical treatments can potentially generate combinations of pesticides within the surrounding water masses (Grefsrud et al., 2018).

Laboratory studies have found non-target marine organisms, particularly crustaceans, to be sensitive to low concentrations of these salmon lice treatment chemicals (Burridge et al., 2014; Burridge and Van Geest, 2014, 2008; Urbina et al., 2019; Bechmann et al., 2020). Coastal fjords are popular locations for open water salmon farming in Norway and these areas are often also the habitat of the northern shrimp (*Pandalus borealis*), a species with both ecological and commercial value (Bergström, 2000). This present study has examined the responses of adult egg carrying *P. borealis* in the laboratory following their exposure to dilute concentrations of two neurotoxic pesticides used in salmon lice control, AlphaMax® (active ingredient deltamethrin) and Salmosan® (active ingredient azamethiphos). Deltamethrin causes paralysis in salmon lice through interference of neuron signal transmission by disruption of the sodium ion channel, while azamethiphos is an acetylcholinesterase inhibitor that also causes paralysis in this copepod, following accumulation of the neurotransmitter acetylcholine (Urbina et al., 2019). Locomotor activity therefore presents itself as an ideal endpoint with which to measure the effects of exposure of the shrimp to dilute solutions of these neurotoxic pesticides.

An important objective of this study was to use environmentally realistic test concentrations within exposure trials. To this end, results from dispersion modelling and dye plume field measurements reported in published literature were used to indicate likely concentrations of pesticide chemicals found in the environment following their discharge from net pens or well boats. The results of these studies indicated that pesticides diluted 100–1000 times from the original treatment concentration could persist for some hours at distances up to 2 km from the original point of discharge (Brokke, 2015; Ernst et al., 2001; Page et al., 2015; Page and Burridge, 2014; Refseth and Nøst, 2018). Dilution and persistence parameters will of course be modified by specific physical conditions such as depth and current speed found at different sites. Most test exposures within the present study used 1000 - fold dilutions of the pesticide manufacturers recommended concentration for salmon lice treatment. A further exposure using a 10,000-fold dilution of deltamethrin is also reported here.

Activity of individual shrimps was recorded continuously over the entire course of the exposures. Each exposure sequence included a 30-h baseline pre-exposure period, a 24-h continuous flow exposure and a 30-h post exposure period. This approach was adopted to address questions of temporal responses in activity patterns, recognising key time points of significant events and assessing the survival capacity of the shrimp. Data generated from continuous monitoring of activity over several days provides valuable information for estimating the degree of susceptibility of wild shrimp populations to pesticide discharges and has direct relevance in situations where tidal conditions and multiple sequential salmon cage treatments could expose shrimp in the field to low concentrations of pesticide chemicals for extended periods of time (Crane et al., 2011). Continuous recording of swimming and walking activity of individual shrimp was complemented with direct observations on their condition during and at the completion of the trials, focusing on posture and morbidity. Tissues from shrimp exposed during the trials were subsequently analysed for the presence of the test pesticides.

The questions addressed by this study were:

1) Is locomotor behaviour of adult egg carrying P. borealis modified by exposure to environmentally relevant dilute salmon lice pesticide treatments and what if any is the temporal relationship between exposure and effect?
2) Do environmentally realistic dilute concentrations of the selected salmon lice pesticide treatments affect shrimp survival?
3) Are there measurable traces of pesticides detected in adult shrimp tissue 30 h after exposure?

2. Materials and methods

2.1. Animal collection and maintenance

Northern shrimp (*Pandalus borealis*) were collected by trawl from Hillefjord (North of Åmøy, Rogaland County, Norway (59° 04′ 00′’ N, 5° 45′ 00′ E) in January 2018 using a net with a modified cod end to minimise damage to the shrimp. Trawling period was 40 mins at a depth of 100 m. On board the boat, shrimp were transferred from the trawl to aerated seawater holding tanks. On arrival at the laboratory, within 2 h of capture, shrimp were randomly distributed amongst eight independent 500 l tanks, each supplied with flow through seawater pumped from a depth of 75 m from the fjord adjacent to the laboratory and passed through a sand filter prior to delivery to the tanks. Seawater temperature in the holding tanks was controlled at 7 ± 0.5 °C using a heat exchange system and salinity was recorded at 34 ± 0.5 PSU. Shrimp were acclimated to laboratory conditions for 2 weeks and fed daily *ad libitum* on a diet of 3 mm fish feed pellets (Spirit supreme, Skretting, Norway). Daily inspections of the shrimp were carried out and any dead or moribund individuals found in the tanks were removed. All experimental work undertaken was approved by the Norwegian Animal Research Authority (FOTS).

2.2. Continuous recording of activity

Following completion of the acclimation period, individual adult egg bearing shrimp (size range 10 −12.5 cm length) were selected at random from the holding tanks and placed into a smaller test tank. Each of four identical test tanks was fed a constant flow of filtered seawater via a header tank at a rate of 680 ml min^{-1} to give a standing volume of 6.3 l (Fig. 1). Seawater temperature was controlled at 7 ± 0.5 °C (approximate water temperature at trawling depth). Activity in shrimp was logged using an infrared light beam system that allowed simultaneous continuous recording of individual shrimp held under low-light conditions over several days. Full details of the system can be found within Supplemental materials -A. The room housing the test tanks and recording equipment was held at a constant low light level with an average intensity of two lux above the tanks. Disturbance of the animals during the monitoring period was limited to short daily system checks, using low intensity torch light. One pellet of commercial fish feed was added each day to those tanks where the previous pellet had been consumed.

2.3. Test chemicals

The commercial products Alpha Max® and Salmosan® were used to prepare the test treatments. Adult shrimp were exposed to 1000-fold dilutions of the manufacturers recommended doses for use as treatments against salmon lice. Thus, 2 ng l^{-1} deltamethrin, the active ingredient of Alpha Max (10 mg/ml), and 100 ng l^{-1} azamethiphos, the

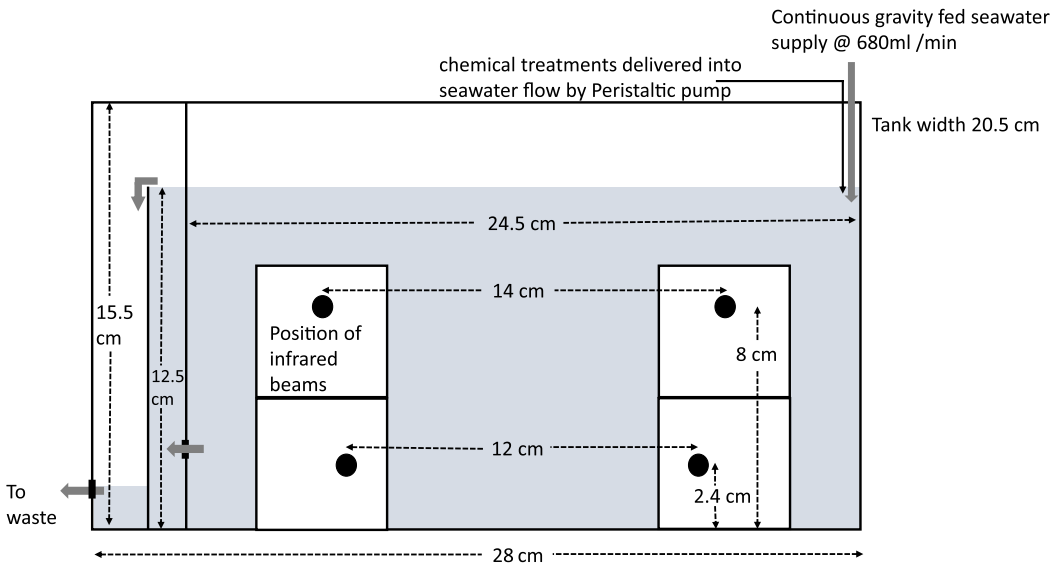

Fig. 1. Test tank used to house individual shrimp, showing dimensions and positioning of the infrared light beams used to monitor and record activity.

active ingredient of Salmosan (500 mg/g) were the target nominal concentrations. Formulations for these commercial products contain chemicals to assist in processes such as emulsification and stabilisation of their respective active ingredients. They would have been present at very low concentrations in the treatments used within the present study and were considered unlikely to exert any influence on the toxicity of the treatment (Bechmann et al., 2020). A combination of 2 ng l^{-1} deltamethrin and 100 ng l^{-1} azamethiphos (target nominal concentrations) was used as a third treatment. Chemical treatments were diluted in distilled water to create 10 l volume stock solutions. To achieve the desired dilutions, treatments were delivered by peristaltic pump into the individual test tanks at a rate of 2.5 ml min^{-1} into a continuous seawater flow of 680 ml min^{-1} with the ends of both input tubes fixed alongside one another to maximise mixing and distribution within the test tanks. The negligible reduction in salinity expected from the distilled water was the same for all treatments. A vehicle control treatment using distilled water alone was also tested. A final treatment exposed shrimp to deltamethrin at a 10,000-fold dilution of salmon treatment concentration at 0.2 ng l^{-1} (nominal target concentration).

Measured concentrations of test chemicals obtained from water samples taken from test tanks were used to describe the various treatments within the results and discussion sections (with the exception of the 0.2 ng l^{-1} deltamethrin treatment where no measurement was made). Seawater temperature was logged every hour in one tank from each treatment group (DST CTD, Star-Oddi, Gardabær, Iceland). The salinity of the intake water was recorded every 5 min throughout the duration of the experiments using a CT-probe (Aqua TROLL 100®, In-Situ Inc., Collins, USA) with Win-Situ 5 data acquisition software (In-Situ Inc., Collins, USA).

2.4. Exposure sequence

Each exposure trial lasted 4.5 days, with shrimp given the initial 24 h to acclimate to test tank conditions. Thereafter continuous recording of their activity commenced. After 30 h of recording, individual chemical treatments were delivered for 24 h via a peristaltic pump (Model 520S Watson and Marlow, Cornwall, UK) at the nominal concentrations described above, with recording of activity continuing for a further 30 h following the end of treatment delivery. Four exposure tanks, each containing a single shrimp were used for each treatment exposure run. To increase the number of replicates for each chemical treatment, each exposure trial was repeated. Vehicle control treatments were run within the sequence of pesticide exposure trials.

2.5. Preparation of water and tissue samples for chemical analyses

Water samples (680 ml individual sample volume) were obtained by feeding seawater from exposure and control tanks directly into pre-baked glass flasks that were immediately sealed. Samples were taken from 3 azamethiphos exposure tanks, 3 deltamethrin exposure tanks, 3 combined azamethiphos and deltamethrin exposure tanks and 3 control tanks. Azamethiphos samples were stored frozen at −20 C prior to analysis, while deltamethrin and seawater samples were taken and despatched for analysis the following day without freezing.

Shrimp used for tissue analysis for accumulated deltamethrin and azamethiphos, together with vehicle control shrimp, were sacrificed on completion of the exposures and stored at −80 C prior to shipping under dry ice to the Norwegian Institute for Water Research (NIVA), Oslo, Norway, for analysis.

2.6. Chemical analyses of water and shrimp tissue

2.6.1. Reagents and chemicals

Standards of azamethiphos, deltamethrin and d5-atrazine as well as HPLC grade, acetonitrile, acetic acid, formic acid, sodium sulphate, sodium acetate, ammonium acetate, zinc chloride, Supelclean PSA sorbent florisil (SPE-FL) column and Costar nylon spin-X filters from Corning (Salt Lake City USA) were purchased from Sigma-Aldrich (Steinheim Germany). HPLC grade diethylether, isohexane, dichloromethane (DCM) and acetone were obtained from Rathburn Chemicals (Walkerburn Scotland). The d6-cyfluthrin was obtained from LGC Standards (Wesel, Germany), and QuEChERS (1,5 gNaAc+6 g MgSO4) from Waters. Standard stock solutions were prepared in acetone and diluted further to appropriate concentrations with acetonitrile or cyclohexane and kept in the dark at +4 °C.

2.6.2. Deltamethrin analysis (full method description provided in supplemental materials - B)

Internal standard, d6-cyfluthrin, was added to 680 mL seawater samples and extracted with 75 mL of dichloromethane for one hour under magnetic stirring. Sodium sulphate was added to the extracts to remove water and the extracts were then concentrated using nitrogen and transferred to 0.25 mL cyclohexane prior to the gas chromatography – mass spectrometer (GC/QQQ) analysis. For tissues, internal standard, d6-cyfluthrin, (and d5-atrazine for samples also exposed to azamethiphos) and 0.5 ml saturated zinc chloride solution was added to 2.5 g of pooled homogenised tissue and extracted with 5 mL acetonitrile

acidified with 1% acetic acid for one hour in an ultrasonic bath. The extraction was repeated with another 5 ml of solvent. The extracts from the two extractions were combined and added to 3 g QuEChERS. Two mL extract was recovered and for the samples exposed to both deltamethrin and azamethiphos 10% of the extract was transferred to vials for analysis of azamethiphos. The remaining extract was evaporated to near dryness and resolved in 1 mL of cyclohexane. The analysis was performed using an Agilent 7890BN GC system. The recovery of the two spiked seawater samples (5 ng/l) was 168% and 125%. The average recovery of the three spiked shrimp samples (40 ng/g) was 128%, with RSD of 18%. The limit of detection for water samples was 0.1 ng l^{-1}.

2.6.3. Azamethiphos analysis (full method description provided in supplemental materials - C)

A 200 mL sample of seawater was spiked with 10 ng d5-atrazine and shaken with 50 mL DCM. The DCM extract was evaporated to dryness and resolved in 1 mL of 1:1 ACN and water and filtered. A 2 g sample of pooled homogenised tissue was spiked with 20 ng of d5-atrazine and extracted twice with 5 mL acetonitrile (ACN). After centrifugation the extracts were combined. The water was salted out by adding 1 g of NaCl and the final ACN extract was concentrated to 1 mL of ACN and further diluted to 2 ml with water followed by filtration (0,22 um nylon Spin-X filter (Corning, US)). Azamethiphos was analysed on a Waters Acquity UPLC system connected to a Quattro Ultima triple quadrupole mass spectrometer. The average recovery of three spiked seawater samples was 95% with RSD of 2.8% for azamethiphos, while the average recovery of three tissue samples was 92% with RSD of 3.1%.

2.7. Analysis of shrimp activity data

The series of exposures was conducted over several weeks and with some variation in pre-exposure basal activity observed amongst the treatments it was considered optimal to analyse each treatment independently using a cohort approach. This involved initially recording activity in individual shrimp from each treatment group over 30-h to establish a baseline of mean hourly activity under control conditions against which to compare their hourly activity levels in the periods during treatment delivery and the subsequent post-exposure period. Some data sets failed normality criteria (Shapiro-Wilk) and so all statistical analyses were carried out using the non-parametric Wilcoxon matched-pairs test within each treatment group with a 5% probability level ($P < 0.05$) used to indicate significant differences between post exposure hourly activity levels and the average hourly rates of the shrimp prior to exposure. All statistical analyses were carried out in R Studio (4.0.3).

3. Results

3.1. Chemical analyses

3.1.1. Deltamethrin

Water sample analyses from 3 individual combined exposure deltamethrin/azamethiphos exposure tanks gave concentrations of 1.3, 1.3 and 1.14 ng l^{-1} against a nominal concentration of 2 ng l^{-1}. Water samples from 3 individual tanks taken during the deltamethrin exposure gave concentrations of 0.6, 1.0 and 0.74 ng l^{-1} against a nominal concentration of 2 ng l^{-1}. The two deltamethrin isomers measured in each sample were combined to give the final concentration. The mean measured concentration of deltamethrin taken from tanks supplied with a nominal concentration of 2 ng l^{-1} was therefore very close to 1 ng l^{-1}. Water samples from the lower concentration deltamethrin exposure (nominal 0.2 ng l^{-1}) were not analysed as these were expected to be close to or below the level of detection. Analysis of shrimp tissue samples taken at the end of the 1 ng l^{-1} (measured) exposure did not detect the presence of deltamethrin.

3.1.2. Azamethiphos

Azamethiphos Samples from 3 individual tanks gave concentrations of 23, 33 and 30 ng l^{-1} against a nominal concentration of 100 ng l^{-1}. Water sample analyses from 3 individual combined exposure deltamethrin/azamethiphos exposure tanks gave concentrations of 29, 0.7 and 34 ng l^{-1} against a nominal concentration of 100 ng l^{-1}. Measured concentrations therefore provided an average concentration for azamethiphos (omitting the 0.7 ng l^{-1} outlier at) at close to 30 ng l^{-1}. Analysis of shrimp tissue samples taken at the end of the exposures did not detect the presence of azamethiphos.

Volumetric measures using mass loss over time for treatment solutions indicated accurate delivery of all stock solution volumes in all the tested treatments and controls.

3.2. Exposure trials

3.2.1. Deltamethrin 1 ng l^{-1} (mean measured concentration)

Swimming behaviour was variable during the pre-exposure period with a lull in activity after 20 h that continued for approximately 6 h. In general, the majority of shrimp were active and mobile throughout the 30 hrs before exposure (Fig. 2). With the addition of the 1 ng l^{-1} deltamethrin treatment, activity increased significantly during the first 3 h of the exposure, before returning to close to the pre-exposure level for the following 7 h. Thereafter activity declined significantly with an eventual cessation of all activity 22 h after the start of exposure. Inspection of the shrimp at the end of the trial found them to be either dead or moribund (lying on side with occasional twitching of limbs) (see Table 1). Moribund shrimp were maintained for 30 h in a clean seawater flow following delivery of the treatment to give them the opportunity to recover but no further activity was observed.

3.2.2. Deltamethrin 0.2 ng l^{-1} (nominal concentration)

With a dilution of the deltamethrin treatment from 1 ng l^{-1} to 0.2 ng l^{-1} (nominal) a similar increase in activity was once again observed after the onset of treatment delivery (Fig. 3). Elevated activity levels continued throughout the exposure and beyond its cessation, eventually returning to levels seen prior to exposure. There was no mortality or incapacity observed at this concentration and the shrimp remained alive and apparently healthy for several weeks after the conclusion of the exposure trial. These results indicate the critical concentration of deltamethrin that leads to mortality or a moribund state lies between 0.2 (nominal) and 1 ng l^{-1} under the conditions tested here.

3.2.3. Azamethiphos 30 ng l^{-1} (mean measured concentration)

During the first 11 h of azamethiphos delivery there were several hours where a significant increase in activity occurred (Fig. 4). As the exposure continued hourly activity reduced significantly for two hours, then returned to levels seen in the pre-exposure period, with a single hour registering a significant increase several hours after the cessation of the azamethiphos delivery. Unlike shrimp exposed to deltamethrin, these shrimps maintained normal posture through to the end of the exposure and recovery period (longer term health could not be evaluated as all shrimp were sacrificed and frozen at the end of the procedure for chemical analyses). An extended period of reduced activity not observed in the shrimp during the pre-exposure period was observed beyond day 3 of the recording sequence. A similar period of reduced activity was, however, also observed in the vehicle control animals.

3.2.4. Combined exposure of 1 ng l^{-1} deltamethrin and 30 ng l^{-1} azamethiphos (mean measured concentrations)

The combination of deltamethrin and azamethiphos within a single exposure treatment resulted in an immediate significant increase in swimming activity, with this response continuing for 3 h before falling back towards the pre-exposure level for several hours, followed by a cessation of all activity (Fig. 5) in a pattern similar to that observed during the 1 ng l^{-1} deltamethrin treatment. All shrimp were either

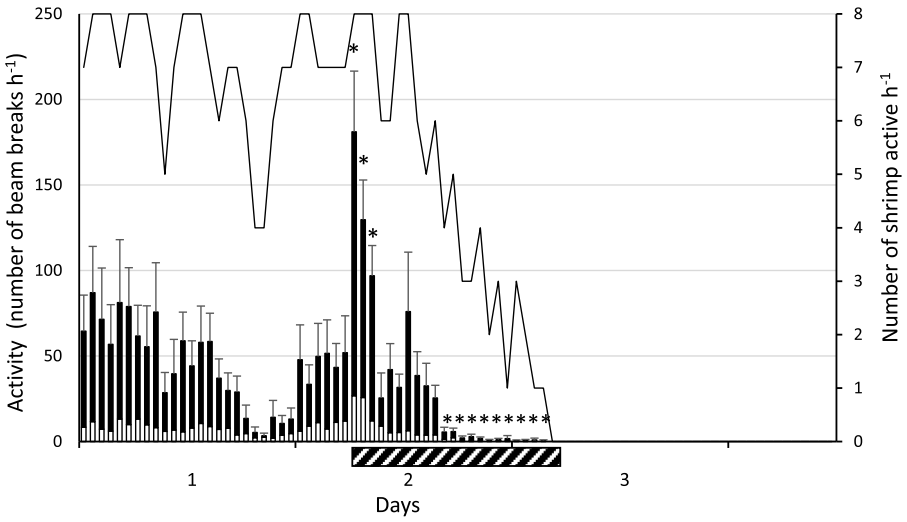

Fig. 2. Mean hourly activity (+ SEM) of shrimp exposed to 1 ng l^{-1} deltamethrin. Dark regions of columns represent swimming and pale areas represent walking activity. Significant differences in hourly activity rates, during and post exposure, from the average calculated from the 30-hour period prior to exposure are indicated by a * (Wilcoxon matched-pairs test, $t < 0.05$, $n = 8$). The solid line plots the total number of shrimp active within each hourly recording period and the diagonally striped rectangle indicates the 24-hour period of exposure.

Table 1
Condition of shrimp 14 h after the start of delivery and 30 h after the cessation of each treatment. Upright/typical describes a normal standing pose, lying on side indicates shrimp are no longer upright but are showing small movements in appendages and lifeless indicates motionless shrimp lying on their side. (* 14 h post exposure start data only available for 4 shrimp from combined treatment).

Treatment	Posture/condition of shrimp ($n = 8$) (14 h) after start of exposure and 30 h after its end		
	Upright/ typical	Lying on side	Lifeless
1 ng l^{-1} deltamethrin	(0) 0	(6) 5	(2) 3
0.2 ng l^{-1} deltamethrin (nominal)	(8) 8	(0) 0	(0) 0
25 ng l^{-1} azamethiphos	(8) 8	(0) 0	(0) 0
Combined 1 ng l^{-1} deltamethrin and 25 ng l^{-1} azamethiphos *	(3) 2	(1) 3	(0) 3
Water vehicle control	(8) 8	(0) 0	(0) 0

inactive, moribund, or dead at the end of the exposure trial.

3.2.5. *Vehicle control (distilled water)*

An increase in activity was recorded as the distilled water vehicle control was delivered, though this was not statistically significant (Fig. 6). It is possible that some of the shrimp detected and responded to the negligible reduction in salinity introduced by the vehicle control delivery. There were several hours within the post exposure 30-hour period where activity dropped below the minimum levels observed during the pre-exposure period, though similar periods of low activity were observed within other trials.

3.3. *Observations on shrimp during and on completion of the exposure treatments*

Posture and morbidity changes were observed in shrimp exposed to 1 ng l^{-1} deltamethrin, either singularly or in combination with 30 ng l^{-1} azamethiphos, but not in shrimp exposed to the other treatments (Table 1). Even though two of the shrimp exposed to the combined treatment maintained an upright posture until the end of the exposure,

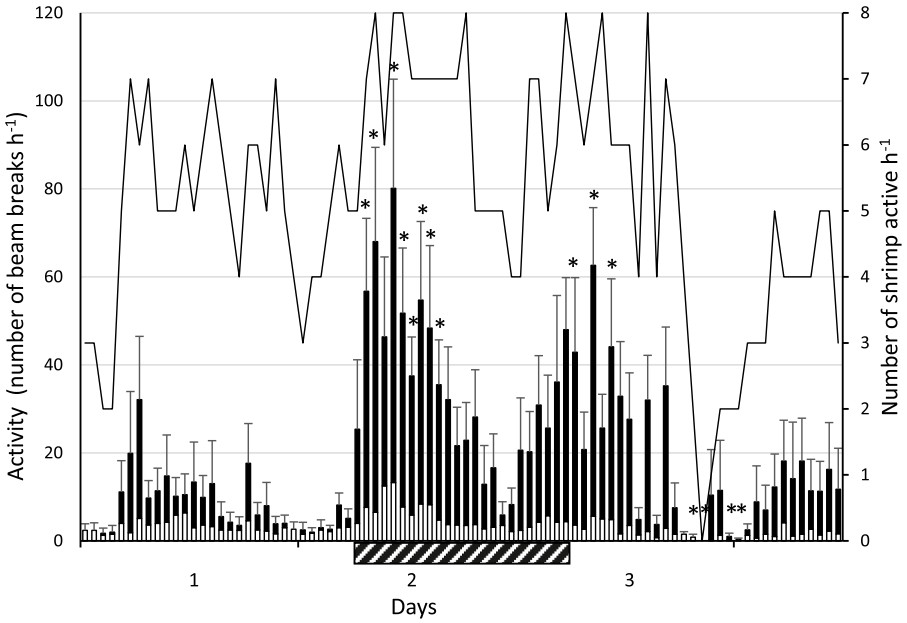

Fig. 3. Mean hourly activity (+ SEM) of shrimp exposed to 0.2 ng l^{-1} deltamethrin (nominal). Dark regions of columns represent swimming and pale areas represent walking activity. Significant differences in hourly activity rates, during and post exposure, from the average calculated from the 30-hour period prior to exposure are indicated by a * (Wilcoxon matched-pairs test, $t < 0.05$, $n = 8$). The solid line plots the total number of shrimp active within each hourly recording period and the diagonally striped rectangle indicates the 24-hour period of exposure.

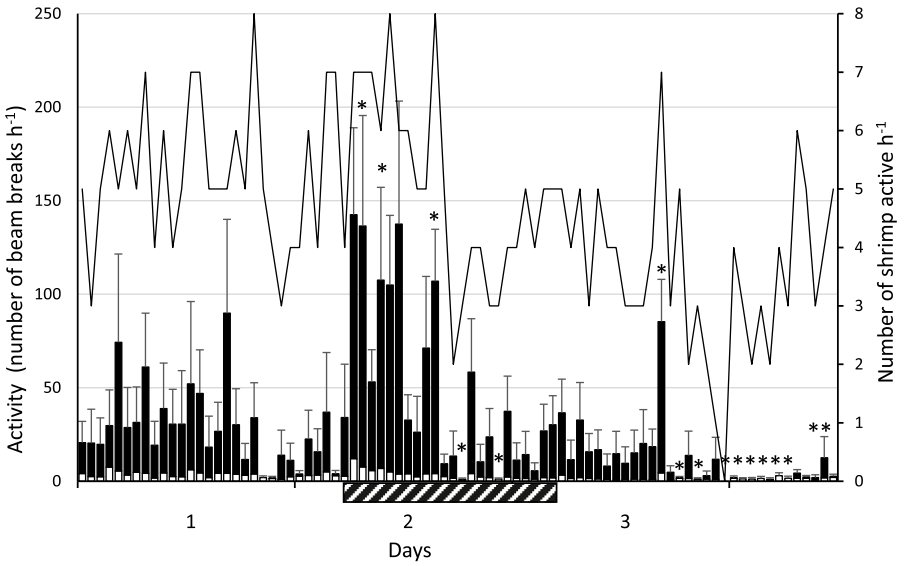

Fig. 4. Mean hourly activity (+ SEM) of shrimp exposed to 30 ng l^{-1} azamethiphos. Dark regions of columns represent swimming and pale areas represent walking activity. Significant differences in hourly activity rates, during and post exposure, from the average calculated from the 30-hour period prior to exposure are indicated by a * (Wilcoxon matched-pairs test, $t < 0.05$, $n = 8$). The solid line plots the total number of shrimp active within each hourly recording period and the diagonally striped rectangle indicates the 24-hour period of exposure.

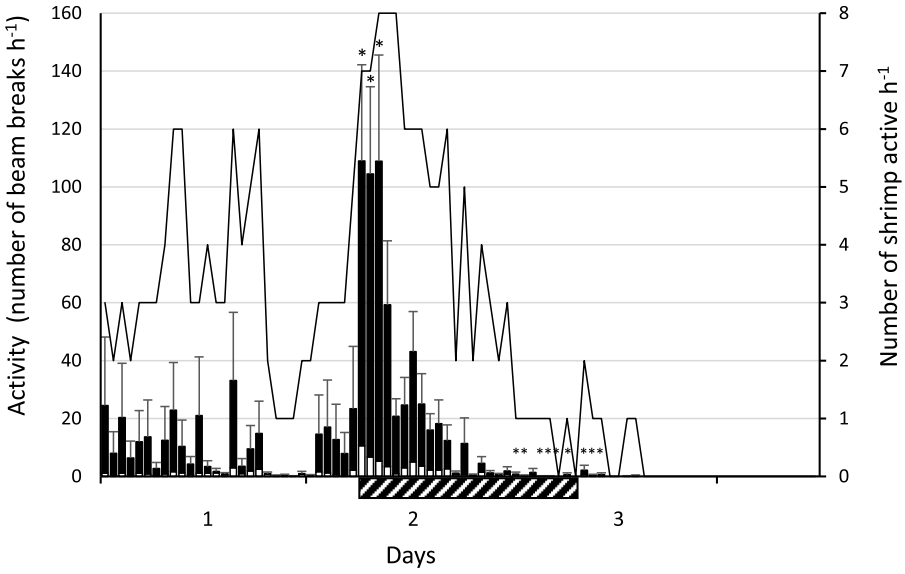

Fig. 5. Mean hourly activity (+ SEM) of shrimp exposed to a combination of 1 ng l^{-1} deltamethrin and 30 ng l^{-1} azamethiphos. Dark regions of columns represent swimming activity and pale areas represent walking activity across the base of the tank. Significant differences in hourly activity rates, during and post exposure, from the average calculated from the 30-hour period prior to exposure are indicated by a * (Wilcoxon matched-pairs test, $t < 0.05$, $n = 8$). The solid line plots the total number of shrimp active within each hourly recording period and the diagonally striped rectangle indicates the 24-hour period of exposure.

the activity plot for these animals showed little or no locomotory movement after treatment delivery was completed.

4. Discussion

4.1. Chemical analysis of exposure treatments

Deltamethrin concentration was measured in the exposure tanks at between 30 and 65% of the nominal concentration of 2 ng l^{-1}. Azamethiphos concentration was measured at between 0.7–33% of the nominal concentration of 100 ng l^{-1}. Lower measured concentrations are likely due to a combination of binding and retention of chemicals to organic matter within the tank, tank surfaces and losses during analysis. Similar discrepancies between measured and nominal concentrations of deltamethrin have been reported and discussed elsewhere (Burridge et al., 2014; Ernst et al., 2014). The lack of any detectable trace of deltamethrin in tissue samples taken from shrimp exposed to this pesticide within the present study is in agreement with the findings of Langford and co-workers (2014) who similarly did not detect the chemical in tissues taken from biota sampled in the vicinity of aquaculture locations where deltamethrin had been used. Based on these findings, depuration of deltamethrin appears to be rapid in this species and although no reports of this process in shrimp could be found in the scientific literature, an investigation of deltamethrin depuration in blue mussels reported a rapid post exposure removal of the pesticide from their tissues (Brooks et al., 2019). As with deltamethrin, depuration of azamethiphos appears to be rapid with no evidence of the pesticide found in the tissues of exposed shrimp when analysed 30 h after the end of exposure. Taken together these findings and previous evidence suggest that deltamethrin and azamethiphos have a low accumulation potential in marine invertebrates and would not provide any useful measure of the degree of exposure to these pesticides when measured in natural invertebrate populations in the field.

4.2. Effects from deltamethrin exposure

Sensitivity to deltamethrin (Alpha Max®) has been documented previously in a number of crustaceans (Brokke, 2015; Burridge et al., 2014; Fairchild et al., 2010; Gebauer et al., 2017; Van Geest et al., 2014a, 2014b), in both larval and adult life stages. Sensitivity of larval

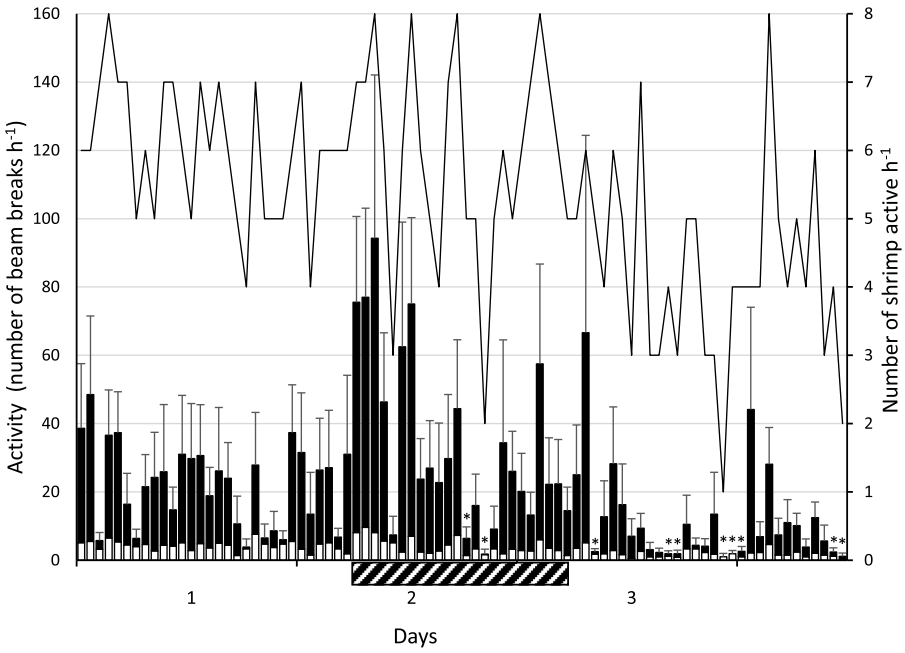

Fig. 6. Mean hourly activity (+ SEM) of shrimp exposed to vehicle control (distilled water). Dark regions of columns represent swimming and pale areas represent walking activity. Significant differences in hourly activity rates, during and post exposure, from the average calculated from the 30-hour period prior to exposure are indicated by a * (Wilcoxon matched-pairs test, $t < 0.05$, $n = 8$). The solid line plots the total number of shrimp active within each hourly recording period and the diagonally striped rectangle indicates the 24-hour period of exposure.

stages of *P. borealis* to deltamethrin and azamethiphos has recently been described by Bechmann et al. (2020) and a general review of crustacean sensitivity to pesticides has been provided by Urbina et al. (2019). The focus here was on the reported effects of deltamethrin on behavioural changes in adult decapod crustaceans. Changes in behaviour in animals resulting from exposure to toxic chemicals can lead to serious consequences if, for example, their ability to feed or seek shelter from predators is affected.

In addressing the question of a temporal relationship between exposure to dilute concentrations of deltamethrin and swimming behaviour set at the start of this study, the rapid and significant increase in swimming activity in shrimp exposed to both concentrations tested could indicate the detection of very low concentrations of deltamethrin and the initiation of an avoidance response that could serve to move adult shrimp away from a dispersing plume of dilute pesticide. This has important implications for natural populations in the field. If the swim response is directional and away from the stimulus then adult shrimp could escape the toxic effects of prolonged exposure to an advancing plume. If, alternatively, the increased activity represents triggering of random non-directional swimming, then it is likely exposed shrimp will succumb to the pesticide exposure as the plume advances, experiencing neurotoxicity and potential morbidity or death. In either event, affected shrimp populations could either suffer directly from neurotoxic mortality, or displacement from their original habitat. Repeated discharges of pesticides within any given area could lead to loss of shrimp populations from their established grounds. Further research will be required to discover if the swimming response is in fact a directional avoidance response or the triggering of random swimming behaviour. Exposure of shrimp to the lower concentration of deltamethrin (0.2 ng l^{-1}, nominal) increased swimming activity consistently throughout the 24 h exposure period and for some hours beyond, without mortality or morbidity. It appears that at this concentration deltamethrin does not lead to neurotoxic paralysis in the shrimp but does trigger an increased activity level that subsequently returns to its original level once the exposure has ceased. These shrimp showed no obvious post exposure ill effects. Discovering the biochemical and physiological processes driving this response requires further investigation. By comparison, the vehicle control shrimp also showed an initial increase in mean swimming activity as the water was delivered, though in this case the increase was not statistically significant and may represent a response of some shrimp to the slight reduction in salinity.

Studies examining the effects of neurotoxic pesticides such as deltamethrin and azamethiphos on adult marine decapod crustaceans are relatively scarce, not least perhaps because these compounds are manufactured to be lethal to crustaceans under use and therefore it is primarily LC50 tests that are carried out on individual species to discover their susceptibility to exposure. Morbidity or death was recorded in all *P. borealis* exposed to deltamethrin at a concentration of 1 ng l^{-1} for 24 h. This makes them more sensitive than some other adult decapod crustaceans where 24 h LC50 tests have reported values for adult lobsters at 15 ng l^{-1} and shrimp (*Crangon septemspinosa*) at 27 ng l^{-1} respectively (Burridge et al., 2014) and the prawn *Palaemon serratus* at 50 ng l^{-1} following a 96-hour LC50 test (Oliveira et al., 2012). It should however be noted that the exposure regime in the present study, where a continuous flow exposure system was used, differs markedly from the standard LC50 static test protocol. However, while conducting the tests described above, Burridge et al. (2014) noted behavioural changes in adult lobsters and shrimp exposed to low concentrations of deltamethrin that included altered posture and twitching in appendages. Similarly, swimming behaviour in the prawn (*P. serratus*) showed reduced velocity in shrimp previously exposed to concentrations of deltamethrin as low as 0.6 ng l^{-1} (Oliveira et al., 2012). More recent research investigating impacts of deltamethrin on adult *P. borealis* reported abnormal behaviour (stress swimming and posture) in shrimp exposed to 6 ng l^{-1} deltamethrin for 2 h (Frantzen et al., 2020). From the available evidence it is clear that low concentrations of deltamethrin can trigger changes in swimming behaviour, posture control and morbidity in adult decapods and that direct discharges of this chemical into the marine environment pose a serious risk to non-target crustaceans.

4.3. Effects from azamethiphos exposure

Several periods of significantly increased swimming activity recorded in shrimp during the first 10 h of exposure to azamethiphos at 30 ng l^{-1} could signal detection of the pesticide and initiation of an avoidance response, similar to that observed with deltamethrin. Unlike deltamethrin, swimming activity returned to pre-exposure levels for some hours after the treatment delivery. This was followed by an extended period of very low activity through to the end of the trial, though none of the shrimp subsequently showed morbidity or altered posture. The lack

of morbidity following the azamethiphos exposure is consistent with results reported following exposure of various larval stages and adult lobsters *(H. americanus)* to a range of concentrations of azamethiphos, including a one hour exposure at concentrations as high as 85 µg/L that did not record any mortality (Burridge et al., 2014). A long-term exposure of adult lobsters to a low concentration of azamethiphos (61 ng l^{-1}), closer to the concentration used in the present study, did not result in significant mortality compared with controls but a subsequent live transport simulation did show significant increase in deaths in the azamethiphos treated group suggesting a physiological impairment reducing tolerance of the additional physical stress (Couillard and Burridge, 2015). Field collected copepods exposed to 5 times the salmon treatment concentration of azamethiphos showed no change in mobility, feeding rate or survival (Van Geest et al., 2014a). The available evidence suggests that azamethiphos at environmental concentrations predicted at 1–2 km from point source of salmon treatment discharge poses a limited immediate threat of morbidity to adult crustaceans, though the initiation of a possible avoidance response could disrupt shrimp populations living on established grounds close to pesticide discharge points.

4.4. Effects of combined deltamethrin and azamethiphos

Synergistic effects of azamethiphos and deltamethrin have been documented for chameleon shrimp *(Praunus flexuosus)* and grass prawns *(Palaemon elegans)* (Brokke, 2015). In the current study, however, mortality appeared to be driven primarily by the presence of deltamethrin, with both the changes in activity and mortality similar to that obtained from the deltamethrin exposure alone, with no obvious synergism or antagonism from combining the treatments indicated. Certainly, of the two treatments tested in the present study deltamethrin clearly exerted significant effects on *P. borealis* behaviour and mortality whereas azamethiphos had a more limited impact, causing changes only in activity patterns.

5. Conclusion

Following treatment with environmentally realistic concentrations of deltamethrin and azamethiphos a significant increase in swimming activity intensity was observed in shrimp during the initial hours of delivery. These actions suggest an avoidance response that, if swimming activity was directional rather than random, could distance the shrimp from pesticide discharges and reduce potential toxic effects. However, even if such avoidance responses were effective, there is a danger that repeated pesticide discharges over time would lead to the displacement of shrimp populations from established grounds.

Comparison of shrimp survival through the timeline of treatment delivery and subsequent post-delivery period highlighted a clear difference between the two pesticides. Treatments that included deltamethrin at 1 ng l^{-1} resulted in morbidity after 12 –14 h of exposure, whereas in shrimp exposed to azamethiphos (30 ng l^{-1}) no morbidity was recorded within the timeframe of the exposure trial. The possibility remains, however, that delayed morbidity could occur in these shrimp, some days after exposure. For deltamethrin, the threshold concentration for morbidity in *P. borealis* was found to lie between 0.2 (nominal) and 1 ng l^{-1} (measured) under the experimental regime used.

Under the conditions tested within the present study neither pesticide was detected in shrimp tissues 30 h after the end of the exposure. These findings suggest that deltamethrin and azamethiphos have a low accumulation potential in *P. borealis* and would therefore not provide a useful measure of the degree of exposure of natural shrimp populations in the field to these pesticides.

The sensitivity of *P. borealis* to low concentrations of the salmon lice pesticide deltamethrin deserves further investigation into the impacts its discharge has on the survival and possible migration of shrimp populations within established habitats in the vicinity of aquaculture operations. Continuing the use of this pesticide to support the expansion of coastal salmon cage farming in areas associated with shrimp grounds could have serious consequences for this ecologically and commercially important shrimp, together with many other crustacean species.

Contributors

Shaw Bamber: contributed to the design of the experiments, performed the practical experimental work and data analyses was the major contributor in the preparation of the manuscript. **Jan Thomas Rundberget:** performed the chemical analyses of azamethiphos in the water and tissue samples and contributed towards the preparation of the manuscript. **Alfhild Kringstad:** performed the chemical analyses of deltamethrin in the water and tissue samples and contributed towards the preparation of the manuscript. **Renée Katrin Bechmann:** contributed to the design of the study and preparation of the manuscript and was leader of the PestPuls project of which this research was a component.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.105966.

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