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Lethal and sublethal effects in Pink salmon (*Oncorhynchus gorbuscha*) following exposure to five aquaculture chemotherapeutants

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Abstract

Early life stages of Pink salmon (*Oncorhynchus gorbuscha*) are at risk of exposure to the active ingredients of chemotherapeutant formulations (hydrogen peroxide [HP], azamethiphos [AZ], emamectin benzoate [EB], cypermethrin [CP] and deltamethrin [DM]) used to control sea lice in salmon aquaculture. LC50 values (95% confidence intervals) for acute 48-h water exposures in order of least to most toxic to seawater-adapted pink salmon fry were: HP (227 [138–418] mg/L), EB (1090 [676–2006] µg/L), AZ (80 [52–161] µg/L), CP (5.1 [3.0–10.5] µg/L), and DM (980 [640–1800] ng/L), and in subchronic 10-d lethality sediment exposure tests: EB (2065 [1384–3720] µg/kg), CP (97 [58–190] µg/kg), and DM (1035 [640–2000] ng/kg). Alterations in behaviour varied between chemicals; no chemical attracted pink salmon fry; fish avoided HP to a limited extent at 50 mg/L), as well as EB (300 µg/L), and AZ (50 µg/L). Significant concentration-dependent decreases in olfactory responsiveness to food extract were seen following AZ, CP and DM exposures that occurred at lower concentrations with longer exposure periods (10 µg/L, 0.5 µg/L and 100 ng/L thresholds at 7 d). Following 10-d sediment exposures, olfaction was only affected by CP exposure at 50 µg/kg. Significant decreases in swimming performance (Ucrit) occured for HP, AZ, CP and DM at concentrations as low as 100 mg/L, 10 µg/L, 2 µg/L and 200 ng/L, respectively. This study provides comprehensive data on the lethal and sublethal effects of aquaculture chemotherapeutant exposure in early life stage pink salmon.

Keywords Chemotherapeutants · Hydrogen peroxide · Azamethiphos · Cypermethrin · Deltamethrin · Emamectin benzoate

Introduction

Farmed salmon in coastal near-shore waters are often densely populated and susceptible to outbreaks of the parasitic sea lice, *Lepeophtheirus salmonis* and *Caligus spp*., that can be a large source of mortality and economic loss at farms. Estimates have put past losses at $500 million annually worldwide, representing 6% of product value (Mustafa et al. 2001; Costello 2009); presently treatments occur before infections become lethal, mitigating such losses. Outbreaks also impact wild fish populations by transfer from farmed to wild fish *via* escapees, or transit of wild fish near infected sites (Heuch et al. 2005; Krkošek et al. 2005). Contributions to declines in wild Pacific salmon stocks have been associated with sea lice outbreaks in open net pens that coincide with the proximal out-migration of vulnerable juvenile smolts (Krkošek et al. 2005; Krkošek et al. 2007). Sea lice outbreaks on farms have also been implicated as a contributing factor towards the collapse of sea trout (*Salmo trutta*) stocks in Norway, Scotland, and Ireland (Heuch et al. 2005).

Sea lice management strategies include improved animal husbandry, site fallowing, infrastructural modifications, and include non-chemical strategies such as feeder fish and warm-water treatments. An important additional management strategy to control outbreaks in many jurisdictions is with anti-sea lice chemotherapeutants (Burridge and Van Geest 2014). The first anti-sea lice chemotherapeutants were used in 1994 in Atlantic Canada in response to sea lice infestations in New Brunswick (Burridge and Van Geest 2014). Salartect® (active ingredient (AI): hydrogen

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peroxide), Salmosan® (AI: azamethiphos), ivermectin, and natural pyrethrin formulations were issued emergency registrations. Excis® (AI: cypermethrin) was registered under a research permit. SLICE® (AI: emamectin benzoate) was introduced in 1999 following issues with resistance and poor efficacy in other products, and became the only product fully registered for use in Canada by 2009. Resistance to SLICE® soon developed in Atlantic Canada leading to major outbreaks in 2009 and 2010. Emergency registrations were consequently issued for Salmosan®, Paramove®50 (AI: hydrogen peroxide), and AlphaMax® (AI: deltamethrin). AlphaMax® was not renewed after 2010, while Paramove®50 and Salmosan® were fully registered in 2016 and 2017, respectively (PMRA 2016; PMRA 2017). Currently, SLICE®, Paramove®50, and Salmosan® are the only products fully registered in Canada.

A non-target species of concern for chemotherapeutant exposure is the pink salmon (*Oncorhyncus gorbuscha*), a species with cultural, ecological, and economic importance (Garibaldi and Turner 2004; Schindler 2003). Post-application release and dispersion of anti-sea lice chemotherapeutants can occur in pink migratory routes (Quinn and Myers 2004; Krkošek et al. 2005; Krkošek et al. 2007). This is particularly concerning for out-migrating juvenile pinks that spend the spring and part of the summer feeding in the protected nearshore waters housing open net pen operations (Godin; 1981; Quinn and Myers 2004). Pink salmon may be exposed to bath treatments (i.e. Salmosan® and Paramove®50) directly in the water column or to in-feed treatments (i.e. SLICE®) indirectly through their diet, which has been demonstrated to be partially epibenthic in origin in some populations (Godin 1981), and through sediment exposure.

The active ingredient in SLICE® is emamectin benzoate (EB), which acts by binding to glutamate-gated chloride channels thereby increasing permeability to chloride ions at inhibitory synapses and leading to paralysis and death (Roy et al. 2000). Due to its hydrophobic nature (Log Kow = 5.0) and long half-life (>120 d in marine sediment), EB is expected to partition into sediment (Roy et al. 2000; Bloodworth et al. 2019). When exposed to fish feed spiked with SLICE®, adult Atlantic salmon, Atlantic salmon smolts, and rainbow trout (*Oncorhynchus mykiss*) tolerated up to 173, 54, and 218 µg/kg/d, respectively, for up to 7 d with no adverse effects (Stone et al. 1999; Roy et al. 2000; Stone et al. 2002). Signs of toxicity appeared in these three species at 356, 272, and 413 µg/kg/d, respectively, and included discolouration, appetite depression, and loss of coordination. The recommended dose in fish farms is 54 µg/kg/d administered *via* spiked fish feed. Although not the recommended treatment method in Canada, when EB was administered through intraperitoneal injection, one dose of 400 µg/kg resulted in decreased growth in Atlantic salmon over 52 d (Skilbrei et al. 2015).

The active ingredient in Paramove®50 is hydrogen peroxide; its mechanism of action is unknown but appears to be mechanical paralysis caused by bubble formation in hemolymph following the peroxidation of cellular membranes (Overton et al. 2018). Hydrogen peroxide is considered to pose a relatively low risk to the marine environment, due to its metabolites being water and oxygen, its relatively short half-life (7 d in seawater), and complete miscibility in seawater (Burridge and Van Geest 2014). Target concentration in net pens is 1.2–1.8 g/L with the final concentrations in the water column depending on dilution factors such as tidal amplitude, current, water depth, and weather (Burridge and Van Geest 2014; Ernst et al. 2014). Toxic effects (gill damage and mortality) were reported after 20-min exposures to 2.4 and 1.5 g/L hydrogen peroxide in Atlantic salmon adults and post-smolts, respectively, with higher mortality observed at higher temperatures (Keimer and Black 1997; Overton et al. 2018). Other fish species appear to be more sensitive, with 1-h LC50 values for rainbow trout and cutthroat trout (*Oncorhynchus clarkii*) fry and fingerlings ranging from 0.32–0.50 g/L (Arndt and Wagner 1997). Other sublethal effects reported in Atlantic salmon include elevated plasma glucose, electrolyte and cortisol levels, indicating stress (Vera and Migaud 2016).

The active ingredient in Salmosan® is azamethiphos, which is an acetylcholinesterase inhibitor that leads to the overstimulation of the nervous system resulting in paralysis and eventual death (Burridge et al. 2014). As with Paramove, Salmosan® is expected to remain in the aqueous phase due to its hydrophilic nature (log Kow = 1.05), short half-life (6–9 d in seawater), and high solubility in seawater (1.1 g/L) (Tomlin 1997; Burridge et al. 2010). The target treatment concentration of Salmosan® is 100 µg/L azamethiphos, providing a large safety margin for Atlantic salmon that have experienced 15% mortality after a 1-h exposure to 1000 µg/L azamethiphos (Sievers et al. 1995). The 96-h LC50 value for stickleback (*Gasterosteus aculeatus*) is 190 µg/L (Ernst et al. 2001). The European eel (*Anguilla anguilla*) and European sea bass (*Dicentrarchus labrax*) survived a 240-min exposure to 100 µg/L azamethiphos in formulation as Salmosan®, while rainbow trout experienced 100% mortality at this concentration (Intorre et al. 2004).

The active ingredient in AlphaMax® is the synthetic pyrethroid deltamethrin. It prevents the closure of sodium channels and leads to nerve depolarization, paralysis, and death (Burridge et al. 2014). Deltamethrin partitions and accumulates in sediments due to its low water solubility (<2 µg/L; Tomlin 1994), moderate hydrophobicity (Log Kow = 4.6; Tomlin 1994), and long half-life in sediment (140 d; Gross et al. 2008). AlphaMax® is applied at a treatment concentration of 2 µg/L deltamethrin; to compare, the 30-min LC50 value for adult Atlantic salmon ranges from 53 to 96 µg/L deltamethrin (Gross et al. 2008) and the 96-h LC50 for juveniles is 0.59 µg/

L deltamethrin (Zitko et al. 1979). Reported 96-h LC50 values range from 1.0 to 1.7 µg/L deltamethrin for juvenile rainbow trout (*Oncorhynchus mykiss*; Ural and Sağlam 2005; Velíšek et al. 2007); 0.06 to 1.65 µg/L for the common carp (*Cyprinus carpio;* Svobodová et al. 2003; Çalta and Ural 2004); 14.5 µg/L for Nile tilapia (*Oreochromis niloticus*; Golow and Godzi 1994), and 0.14 to 0.25 µg/L for Eastern rainbow fish (*Melanotaenia duloulayi*; Thomas et al. 2008). Toxicity values representing shorter exposure times include a 48-h LC50 vlaue of 5.13 µg/L deltamethrin for the guppy (*Poecilia reticulata*; Viran et al. 2003); 24-h LC50s of 13 and 26 µg/L for the iridescent shark (*Pangasius hypophthalmus*; Hedayati et al. 2014) and the freshwater platy (*Xiphophorus maculatus*; Tarkhani and Imanpoor 2012), respectively, and a 1-h LC50 value of 2.5 µg/L for the European catfish (*Silurus glanis*; Köprücü et al. 2006).

The active ingredient in Excis® is another synthetic pyrethroid cypermethrin and, like deltamethrin, acts on the central nervous system through interference of sodium channel functioning. Cypermethrin has a similar environmental fate as deltamethrin, characterized by low water solubility (4 µg/L), moderate hydrophobicity (Log Kow = 4.5; Tomlin 1994), and a long half-life in sediment (35–80 d; SEPA 1998). Excis® is applied at a treatment concentration of 5 µg/L which provides a narrow therapeutic threshold for Atlantic salmon with a 96-h LC50 of 2 µg/L (Mcleese et al. 1980). Reported 96-h LC50s range from 0.9 to 2.6 µg/L cypermethrin for the common carp (Stephenson 1982; Saha and Kaviraj 2008); 1.2 µg/L for the brown trout (*Salmo trutta*; Stephenson 1982); 0.5 µg/L for rainbow trout (Stephenson 1982); 2.2 µg/L for Nile tilapia (*Oreochromis niloticus;* Stephenson 1982); 0.67 µg/L for freshwater catfish (*Heteropneustes fossilis*; Saha and Kaviraj 2003); and 111.4 and 30.8 µg/L for embryo and adult Japanese medaka (*Oryzias latipes*; Kim et al. 2008), respectively.

The migratory routes of pink salmon overlap with open net pen sites, however little information exists on the effects of anti-sea lice chemotherapeutants on proximal outmigrating pink salmon (Krkošek et al. 2005; Krkošek et al. 2007). The objective of this study was to determine the lethal and sublethal effects of these 5 anti sea lice chemotherapeutant active ingredients on pink salmon fry in order to determine risk, inform regulatory policy, and identify best practices to control sea lice outbreaks while minimizing non-target ecological receptor impacts.

Materials and methods

Chemicals

The following chemicals were obtained from Sigma-Aldrich (Oakville, ON): AZ, (> 99% pure), CAS: 35575-96-3; CP, (> 98% pure), CAS: 52315-07-8; DM, (> 99% pure), CAS: 52918-63-5; EB, (> 99% pure), CAS: 155569-91-8; HP, (30%), CAS: 7722-84-1; acetone, CAS: 67-64-1; methanol, CAS: 67-56-1; dichloromethane, CAS: 75-09-2; chloroform, CAS: 67-66-3; sodium chloride (NaCl), CAS 7647-14-5: and copper chloride dehydrate (CAS: 10125-13-0).

Fish

Fertilized pink salmon embryos were obtained from the Tenderfoot Creek hatchery (Brackendale, BC) and were raised under standard conditions for salmonids in heath trays supplied with dechlorinated municipal water at ambient temperature (average 10.2 °C) and in the dark until fish reached the swim-up fry stage (Lin et al. 2021). All phenotypically normal fry were transferred into 200-L fiberglass rearing tanks supplied with flow-through water for an additional 2 weeks. A gradual salinity acclimation regime was used to acclimate fry to seawater conditions by increasing tank salinity by 5‰ every 2 d until 28‰ was achieved. Seawater-acclimated fry were reared in 28‰ seawater at 11.9 °C under a 16 h light: 8 h dark photoperiod until fry were approximately 3 months old (post-hatch; mass 0.67 ± 0.01 g [mean ± SE]). Fish were fed twice daily at *ad libitum* with commercial salmonid fry feed (Skretting, Vancouver, BC). The care and experimental use of pink salmon were approved by the University Animal Care Committee according to Canadian Council on Animal Care guidelines.

Chemotherapeutant exposure

Sediments for experiments were collected from the upper 10 cm at an acceptable uncontaminated reference site (Boundary Bay Assessment and Monitoring Program [BBAMP; 2009–2015] [Hemmera 2017]) at Centennial beach (Tsawassen, BC). Sediment from this region has an organic carbon content ranging from 0.02–0.2% (Hemmera 2014). Sediment was sieved during collection using a 1 mm metal sieve to remove debris and was dried prior to use. Sieved sediment was weighed into batches of 2.5 kg and suspensions of chemicals prepared as above were used for sediment spiking. Sediments were wetted with chilled seawater and aliquots of the suspensions were added to each individual exposure aquarium (to achieve a depth of 2 cm) using glass serological pipettes to attain target chemical sediment concentrations. Sediments were mechanically mixed for 3 min using a stainless-steel spoon mounted to a drill. Sediments added were incubated in the dark for 24 h. Following this, filtered seawater was added to each tank, after which animals were introduced for exposure (Strachan and Kennedy, 2021).

For all exposures, seawater acclimated fry were randomly distributed into glass aquaria (40 L) containing seawater (28‰ and 12 °C) only (water exposures) or seawater and sediments (sediment exposures) ($n = 8$–12 fish per tank, 2-3 replicate tanks for each concentration treatment group, depending on the experiment). For avoidance assays, fish were not exposed to chemicals prior to the experiments. Chemical concentrations were measured in all water and sediment samples in tests as described below.

Chemical analysis

Representative water (5) and sediment (5) samples from spiked and representative exposure tanks (duplicates samples per concentration) were collected in amber vessels and analyzed as in Strachan and Kennedy (2021). HP samples were analyzed immediately using a Fluorometric Hydrogen Peroxide Assay Kit, read at $\lambda_{ex} = 540$/ $\lambda_{em} = 590$ nm. AZ samples were preserved with 2 g NaCl and 5 mL chloroform, shaken and then stored at 4 °C until analysis. For AZ, water samples were extracted using 2 g NaCl/100 mL water (Burridge et al. 1999), followed by DCM (Van Geest et al. 2014). Sediments were air-dried, and extracted with DCM. Extracts were analyzed by HPLC according to Strachan and Kennedy (2021). EB samples were collected and stored at −20 °C until analysis as described in (Park 2013). For EB, water samples were adjusted to pH 4 with orthophosphoric acid and along with sediments, extracted with DCM. Samples were analyzed by HPLC according to (Xie et al. 2011) and (Strachan and Kennedy 2021). DM and CP samples were preserved with dichloromethane (~ 5% v/v), shaken and then stored at 4 °C until analysis. For CP and DM, water and sediments were extracted with dichloromethane and analyzed by gas chromatography as in Strachan and Kennedy (2021).

Water and sediment lethality bioassays

Acute 48-h static toxicity tests (water exposures) were performed for all compounds according to standard methods outlined in ECCC (2017) with modifications following acclimation to laboratory conditions. Animals were randomly distributed into test tanks (40 L glass aquaria, $n = 11$ per tank) containing test solutions (HP: 1–1000 mg/L, AZ: 10–1000 µg/L, CP: 0.05–10 µg/L, DM: 100–2000 ng/L, EB: 100–2000 µg/L). Tests were run at 12 °C with 3 replicates for each test concentration and controls under a 16 h light: 8 h dark photoperiod with minimal aeration. Loading density for fish was <0.2 g/L. Water quality (temperature, pH, salinity, O_2 concentration) and mortality was assessed for each treatment. Tests were deemed acceptable if there was > 91% control survival (US EPA 2002). Mortality was confirmed by checking for movement following a gentle nudge with a glass rod. $CuCl_2$ was used as a reference toxicant for between test standardization.

Subchronic 10-d static toxicity tests (sediment exposures) with pink fry were performed for CM, DM and EB according to modified methods from ECCC (1992, 2001). Animals were randomly distributed into exposure tanks (40 L glass aquaria, $n = 11$ per tank) that contained 30 L of seawater and 2.5 kg of sediment (prepared as above). 10-d tests were run at 12 °C with 3 replicates for each test sediment concentration (CP: 10–500 µg/kg, DM: 200–2000 ng/kg, EB: 1–1000 µg/kg) and controls. Tests were deemed acceptable if there was >91% control survival (US EPA 2002).

Chemical avoidance

Avoidance assays were performed using a shuttle box automated system (Loligo® Systems, Tjele, Denmark) equipped with a shuttle box (total system l x w 45 ×22.5 cm) consisting of two cylindrical chambers (diameter 20 cm; depth 7 cm) connected by a trough (5.5 ×3.5 cm) that allows for the free movement of fish between the two chambers. The use of individual glass reservoirs generate separate circular and opposing flows in each chamber that prevents water mixing between them. A black curtain isolated the shuttle box system to minimize disturbances and black polyethylene was placed above the tanks to limit external light exposure. Fish were placed individually into the shuttle box (chamber side assigned randomly) and allowed to acclimate to the system for 30 min. Following the acclimation period, chemicals (AZ: 0.1 µg/L, CP: 0.1 µg/L, DM: 0.04 µg/L, EB: 0.1 µg/L, and HP: 50 mg/L) were added to glass reservoirs which supplied water the side of the shuttle box that the fish resided in at the start of the test. Video was analyzed for the time fish spent in each chamber of the shuttle box over a 10-min test duration. A uEye® USB camera (Imaging Digital Systems, MA, USA) captured fish spatial position and time in the shuttle box by the tracking software ShuttleSoft behaviour software v.2.6.4 (Loligo® Systems, Tjele, Den).

Olfaction

In order to test the olfactory ability of pink fry following exposure to chemotherapeutants, fry ($n = 10$ fish, 3 replicates per concentration) were exposed to varying sublethal concentrations (HP: 20–150 mg/L, AZ: 1–40 µg/L, CP: 0.01–3 µg/L, DM: 5–500 ng/L, EB: 10–750 µg/L) for 48 h as above and assessed using the shuttle box automated system described above. Fish were acclimated for 30 min the system, and food extract (0.1 ml of a ground TetraMin® [VA USA] tropical flake [40% protein, 12% lipid] solution in water filtered with a 20 µm filter) was added to the

chamber that the fish did not reside in through Tygon tubing that introduced the solution into the chamber without disturbance. If a fish moved to the chamber with the food extract within 30 sec and remained predominantly in that side (>2 min/3 min total test time) it was considered to have 'responded' to the olfactory stimulus. In a second set of experiments, fish were exposed to individual chemicals at a concentration at which no olfactory inhibition occurred in experiment 1 (HP: 20 mg/L, AZ: 10 µg/L, CP: 0.5 µg/L, DM: 100 ng/L, EB: 500 µg/L) for 96 h and assessed for olfactory ability as described. In a third set of experiments, pink salmon were exposed to sediments containing individual chemicals (CP: 0.5–50 µg/kg, DM: 10–500 ng/kg, EB: 10–1000 µg/kg) for 10-d and olfactory ability assessed as above.

Swim performance tests

The swimming performance of pink fry was examined following exposure to varying sublethal concentrations of chemotherapeutants (HP: 5–150 mg/L, AZ: 1–50 µg/L, CP: 0.05–3 µg/L, DM: 10–500 ng/L, EB: 50–750 µg/L) for 48 h as above using a mini swim tunnel system (Loligo® Systems). The apparatus consisted of a 1.5 L cylindrical glass chamber equipped with an electric propeller submerged inside a water reservoir. The temperature in the reservoir was regulated to 12 °C; DO was maintained at >95%. Water velocity was calibrated using slow-motion video and dye test following the manufacturer's protocol. Immediately following exposure, fish from each concentration group ($n = 7$) were transferred from exposure tanks to the swim tunnel chamber and allowed to acclimate for 15 min at a water velocity of approximately 5 cm/s (1.5 body lengths per second [BL/s]), after which they were then swum through a ramped critical (ramp-Ucrit) swimming protocol (Goulding et al. 2013). Briefly, after the acclimation period, water velocity within the test chamber was ramped to approximately 50% of the estimated Ucrit (based on non-exposed test fish) in 5 min. After the ramp period, water velocity was increased in a step-wise manner by approximately 0.3 BL/s every 20 min until the fish was exhausted (inactively resting on rear baffle for >2 s). Fish were removed, euthanized with MS 222 and wet weight (g) and fork length (cm) measured. Critical swimming speed (Ucrit) was calculated as the maximum speed attained by each fish normalized to fork length (BL/s [Osachoff et al. 2014]). The cross-sectional area of all swim-tested fish were less than 10% of swim tunnel cross-sectional area and fish density were under 0.2 g/L, therefore no correction for a solid blocking effect was needed (Webb 1971).

Statistics and calculations

Calculations and statistical analyses for toxicological parameters were performed using the Comprehensive Environmental Toxicity Information System CETIS (Version 1.8.7.16, Tidepool Scientific LLC). Point estimate techniques were used to calculate endpoints (LC50 [lethal concentration] and LOEC/NOEC [lowest and no-observered effect concentrations] values) using appropriate hypothesis testing techniques.

Avoidance or attraction was determined using the time spent in the contaminated chamber *v*. time spent in the uncontaminated side of the shuttle box. Rv values were defined as TT-TB (time in test side [contaminated] – time in blank side [uncontaminated] and calculated for individual fish. The percent of negative Rv values (more time on blank side) were calculated for each group of 10 fish tested for each concentration of a chemical. Average time spent on the TT side were also calculated to indicate the 'degree' of avoidance or attraction. For each chemical, the correlation between exposure concentration and the% -Rv values of fish ($n = 10$) were tested by simple linear regression analysis using JMP 16 (SAS Institute, Cary, NC). The regression coefficients, intercepts, and p values of regressions were individually calculated.

Logistic regression models using Proc Genmod or Proc Logistic were used to test for differences in mean olfactory responses between concentrations in the first experiment, and for a given concentration in water exposures between time points (0, 24, 96, 120 and 168 h) for each chemical separately in the second experiment. Post hoc tests using Dunnett's (comparing time 0 to each time period) or Tukey Kramer (all pairwise comparisons between time points) methods were used to determine which pairwise comparisons are statistically significantly different than each other. Time was considered to be a fixed effect categorical factor in the models. A total of three replicates of each time-chemical combination were used in the logistic regression models.

Ucrit values were calculated as the velocity of the last full step swum plus the temporal fraction of the step of fatigue (Brett 1964). The swim speed data were examined for normality, sample independence, and variance equality. Results of those three assumption tests fulfilled the requirements for a one-factor ANOVA analysis. For each chemical, the mean swim speed of fish in the control group was compared to the means of exposed groups using a one-factor ANOVA analysis followed by a Tukey-Kramer post-hoc test ($p < 0.05$). Analyses were performed using JMP 15 (SAS Institute, Cary, NC).

Results

Water chemistry

The recovery for HP (96% with a between-day variability of 4.1%) was determined by comparing spiked water samples (50 mL). The detection limit was 3.4 µg/L. AZ recovery was determined by comparing spiked water (1 L) was 94%, with between-day variability of 4.5%. The detection limit for AZ was 1.5 µg/L. EB recovery was determined by comparing spiked samples in water and sediment (in 5 g dry wt) and were 89 and 86%, respectively, with an in between-day variability of 6.7%. The detection limit for EB samples was 4.8 ng/L and 7.9 ng/kg in sediment. Recoveries for CP and DM from water and sediment using spiked water and sediment as above were 92 and 86%, respectively, with a between-day variability of 6.1%. The detection limit for DM and CP in water was 0.05 µg/L. The detection limit for DM and CP in sediment was 0.10 µg/kg. Chemical analysis of water samples for hydrogen peroxide, azamethiphos, emamectin benzoate, cypermethrin and deltamethrin on samples within the entire range for each compound (methods fully described in Strachan et al., [2021]) resulted in measured concentrations being 87–92% of target concentrations. Chemical analysis of sediment samples for emamectin benzoate, cypermethrin and deltamethrin on samples within the entire range for each compound (methods fully described in Strachan and Kennedy [2021]) resulted in measured concentrations being 76–88% of target concentrations. Due to the high correlation between nominal and measured concentrations, nominal concentrations were used in all calculations and statistical analyses.

Acute and subchronic lethal toxicity

The calculated toxicological parameters (LC50, [95% confidence intervals], NOEC, LOEC) for acute lethality tests in the 48-h water exposure tests for all 5 chemicals are as follows in order of least to most toxic to pink salmon fry: HP (227 [138–418], 10, 30 mg/L), EB (1090 [676–2006], 30, 100 µg/L), AZ (80 [52–161], 3, 10 µg/L), CP (5.1 [3.0–10.5], 0.3, 1 µg/L), and DM (980 [640–1800], 30, 100 ng/L). The calculated toxicological parameters (LC50, [95% confidence intervals], NOEC, LOEC) for subchronic lethality tests in the 10-d sediment exposure tests for 3 chemicals are as follows in order of least to most toxic: EB (2065 [1384–3720], 100, 300 µg/kg), CP (97 [58–190], 3, 10 µg/kg), and DM (1035 [640–2000], 100, 300 ng/kg).

Avoidance/attraction

Avoidance/attraction was examined for each of the 5 chemotherapeutants at various water concentrations by determining the proportion of fish that responded to and the time spent in the contaminated chamber *v*. time spent in the uncontaminated chamber of the test system. Behaviour varied between chemicals and with test concentration, although no chemical attracted pink salmon at any concentration (Fig. 1). Fish exposed to CP (0.5–4 µg/L) and DM (50–400 ng/L) were not attracted to and did not avoid the chemicals. Hydrogen peroxide initiated limited avoidance in fish at the higher concentrations used (50–80 mg/L range) ($p < 0.05$). Emamectin benzoate caused avoidance at lower concentrations (>300 µg/L, $p < 0.05$) than HP. Pink fry avoidance was most pronounced by AZ exposure compared to all other compounds, with avoidance occurring up to 80% of fish in the 150 µg/L treatment group. Avoidance behaviour to AZ occurred at water concentrations as low as 50 µg/L (Fig. 1) ($p < 0.05$).

Olfactory inhibition

The olfactory responsiveness of pink fry to a food extract following exposure to all chemicals individually for 48-h in water, or to sediments containing EB, CP or DM for 10-d at varying concentrations was examined. The concentrations used in the water and sediment exposures were all less than the LC50 values determined in the acute water and sublethal sediment toxicity assays and no mortality occurred in any exposure. In control fish, typical positive responses to the odorant were >95%. No significant decrease in responsiveness to food extract was seen in fry exposed to any water concentration of hydrogen peroxide or emamectin benzoate (Fig. 2) ($p > 0.05$). A concentration-dependent decrease in responsiveness was seen for AZ, CP and DM (Fig. 2). Significant decreased responsiveness was seen for both CP and DM at concentrations of 3 µg/L and 500 ng/L ($p < 0.05$), respectively (Fig. 2). At the highest concentration of both CP (3 µg/L) and DM (500 ng/L), the proportion of fish responding were 66 ± 11 and 66 ± 11% of controls, respectively. The proportion of fish responding (60 ± 8.2%) were significantly reduced with AZ exposures as low as 20 µg/L ($p < 0.05$). Maximum reductions occurred at 40 µg/L with 53 ± 4.7% responding ($p < 0.05$).

Concentrations that caused either no statistical olfactory inhibition following a 48-h exposure were used to determine if increased exposure duration (to 7 d) at these concentrations causes reductions in the proportion of responding fish. No effect on the proportion of responding fish occurred with either HP or EB at any concentration used (Fig. 3). At 10 µg/L, AZ exhibited a significant time-dependent decrease responding fish to a maximum of 42 ± 17% at 168 h (Fig. 3). ($p < 0.05$). Similar decreases in the proportion of fish that responded with time were seen for both CP (0.5 µg/L) and DM (100 ng/L) to minimum values of 53 ± 16 and 32 ± 13% responding by 7 d (Fig. 3) ($p < 0.05$).

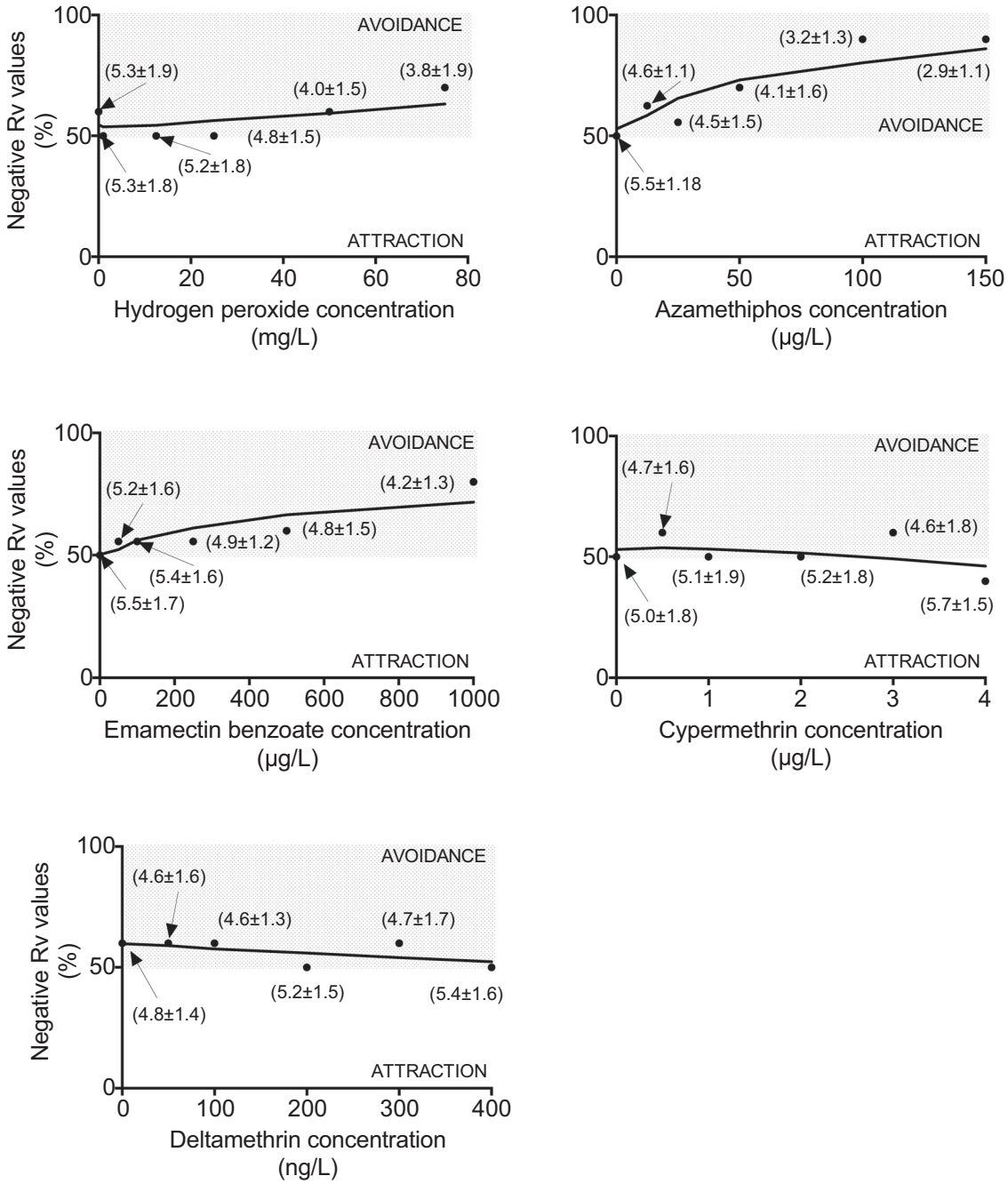


Fig. 1 Avoidance or attraction of pink salmon to various concentrations of chemotherapeutants based on the time spent in a contaminated chamber *v*. time spent in an uncontaminated chamber of the shuttle-box. Rv values are defined as TT-TB (time in test side [contaminated]—time in blank side [uncontaminated] and calculated for individual fish ($n = 10$–12 fish for each concentration). Negative Rv values indicate more time spend in the uncontaminated side. The percent of negative Rv values (more time on blank side) is the percent of fish tested for each concentration of a chemical. Control fish spent equal time in both sides of the chamber (-Rv values approximately 50% [slope = 0] indicating no preference or avoidance). Values in parentheses are mean value ± standard error of the mean (SEM) for the time spent in the contaminated side (10 min) to indicate the 'degree' of avoidance

Ten-d sediment exposures to EB, CP, or DM (at sub-lethal concentrations below the determined 10-d LC50 values) affected pink fry olfactory responses; the proportion of tested fish that responded to food extract can be seen for each chemical in Fig. 4. As in the previous experiments, EB had no effect on the olfactory responsiveness of fry. A concentration-dependent decrease in responsiveness was seen for CP but not DM (Fig. 4). Significant decreased responsiveness was seen for CP at a concentration of 50 µg/kg (Fig. 4) ($p < 0.05$). At the highest concentration of CP (50 µg/kg), maximum responding proportion of fish was 73 ± 11% of controls.

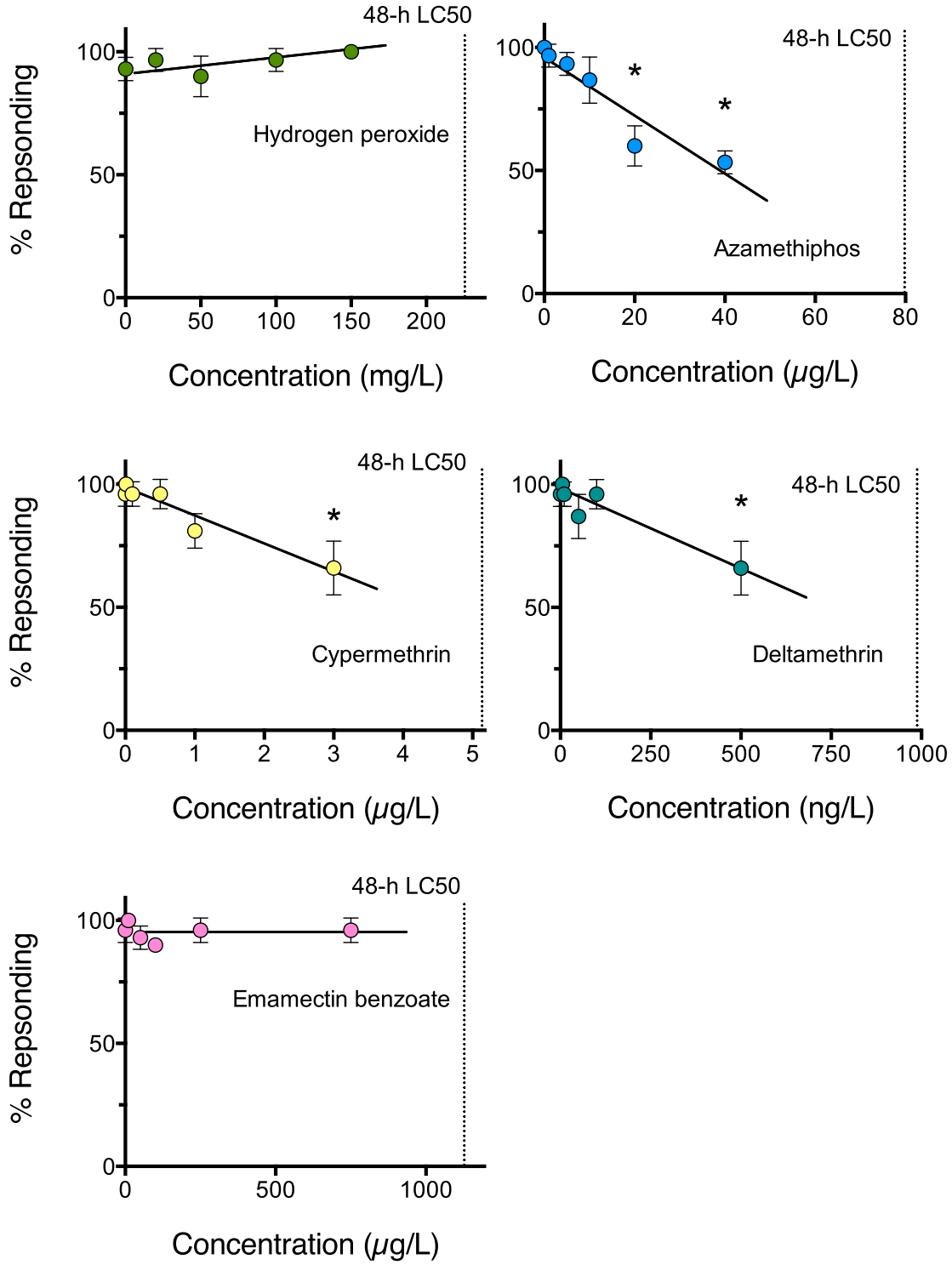


Fig. 2 Olfactory responses presented as the mean % ± SE of 10 fish in 3 replicates that responded to a food extract following a 48-h exposure to varying concentrations below the 48-h LC50 value (dashed line) of each chemotherapeutant. Significant differences in olfactory responsiveness in fish exposed to chemicals in water from controls were detected using post hoc tests using Dunnett's or Tukey Kramer methods and are denoted by asterisks ($*p < 0.05$)

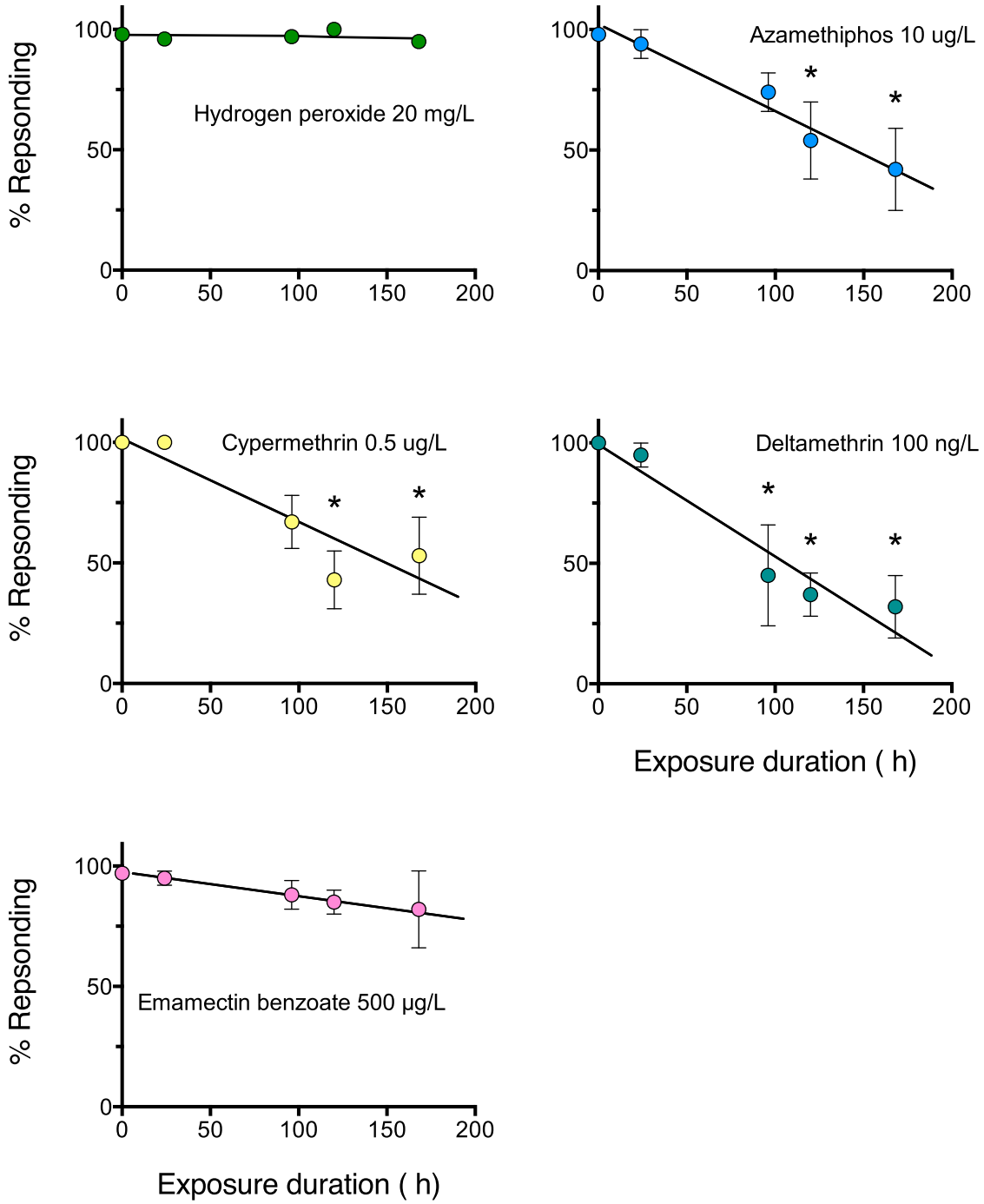
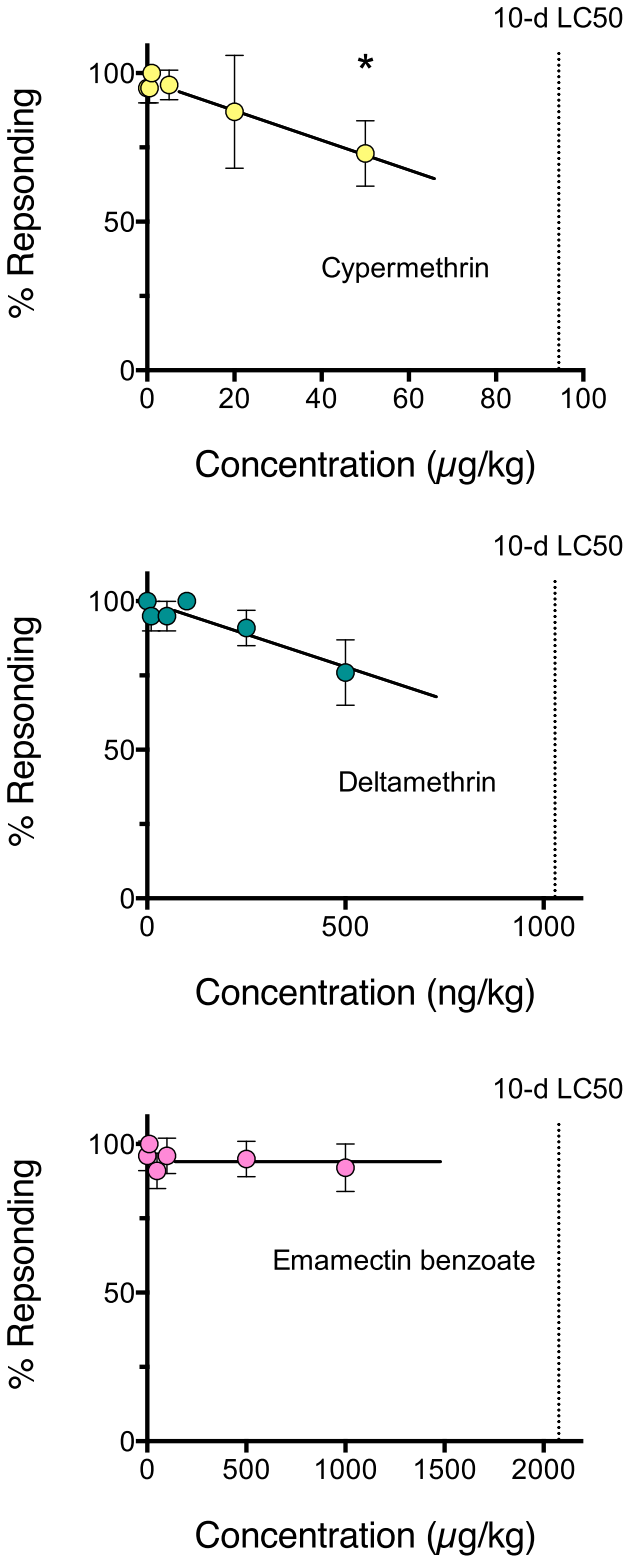


Fig. 3 Olfactory responses presented as the mean% ± SE of 10 fish in 3 replicates that responded to a food extract following an exposure to one concentration of each chemotherapeutant for various time periods (0–168 h). Significant differences in olfactory responsiveness in fish exposed to chemicals in water from the start of exposures (exposure duration 0 h) to all other time points were detected using post hoc tests using Dunnett's or Tukey Kramer methods and are denoted by asterisks (*$p < 0.05$)

Swim performance

Ucrit determinations were made for pink salmon fry exposed for 48-h to the 5 chemicals in water at varying concentrations less than the determined LC50 values in the acute water exposures; no mortality occurred at any concentration. In control fish, Ucrit values ranged from 5.0 to 6.3 BL/s. Concentration-dependent decreases in Ucrit was



◀**Fig. 4** Olfactory responses presented as the mean% ± SE of 10 fish in 3 replicates that responded to a food extract following a 10-d exposure to varying concentrations below the 10-d LC50 value (dashed line) of each chemotherapeutant. Significant differences in olfactory responsiveness in fish exposed to chemicals in sediments from controls were detected using post hoc tests using Dunnett's or Tukey Kramer methods and are denoted by asterisks (*$p < 0.05$)

seen for 4 of the chemicals (HP, AZ, CP and DM) tested (Fig. 5) ($p < 0.05$). The lowest concentrations (and % decrease compared to controls) that significantly decreased Ucrit for each chemical were: HP (100 mg/L; 55.4%), CP (2 µg/L; 78%), DM (200 ng/L; 63.8%) and AZ (10 µg/L; 80%) ($p < 0.05$). The concentration that resulted in the maximum significant decrease in Ucrit (and maximum reduction [% of controls]) for each chemical were: HP (100 mg/L; 55.4%), CP (3 µg/L; 62.8%), DM (500 ng/L; 46.6%) and AZ (50 µg/L; 66%).

Discussion

Organisms rely on constituent chemical defense mechanisms to avoid the potential toxic effects of foreign compound exposure (Tierney 2016). Ideally, behavioural avoidance acts to limit exposures to toxic substances by sensing the substance and moving into a cleaner environment. The avoidance/attraction responses to each of the 5 chemotherapeutants were chemical- and concentration-dependent. No attraction behaviour was exhibited for any of the chemicals, and pink salmon did not avoid either CP or DM at any concentration tested. Hydrogen peroxide initiated limited avoidance in fish at concentrations in the 50–80 mg/L range, emamectin benzoate resulted in more avoidance at concentrations >300 µg/L, but avoidance was most pronounced to AZ occurring at water concentrations as low as 50 µg/L.

There are many examples of responses to pesticides including avoidance (e.g. acrolein; [Folmar 1976]; aschlorpyrifos [Hansen et al. 1972]; 2,4-D [Tierney et al. 2011]), and attraction (e.g. bentazone; (Saglio et al. 2001). Avoidance behaviour cannot be extrapolated for all compounds within a class or for all fish species; for example, fenitrothion was avoided by goldfish (Scherer 1975) and medaka (Hidaka and Tatsukawa 1989) but sheepshead minnow (*Cyprinodon variegatus*) did not avoid malathion or carbaryl formulations (Hansen 1969). Avoidance has been shown for other organophosphates like AZ including malathion (in *G. affinis*; Hansen et al. 1972) and parathion (in *G. affinis*; Kynard 1974). There have been limited studies on the avoidance of these compounds in water or when associated with sediments; some evidence exists showing that AZ and EB provoke some level of avoidance behaviour in marine organisms. Under continuous exposure to AZ, juvenile American lobsters (*Homarus americanus*) exhibited an avoidance response (exiting shelters) with increasing water AZ concentrations, however, at concentrations used

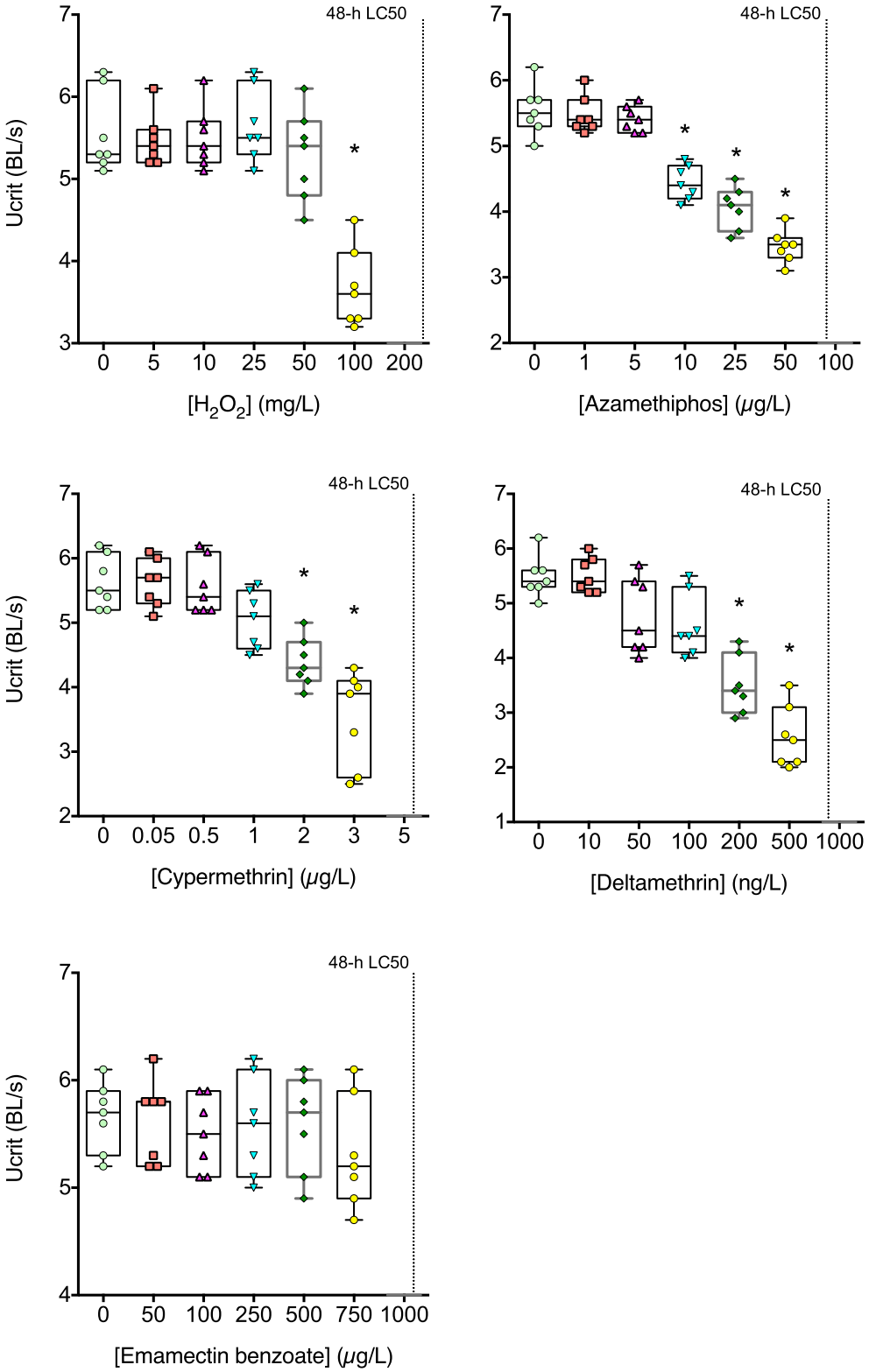
by aquaculture operations (100 µg/L and short exposure times), avoidance responses and effects in this species were not seen (Abgrall 1999). Naïve and chronically pre-exposed *E. estuarius* (marine amphipod) or *Neresis virens* (marine polychaete) placed into sediment containing 0.5 to 200 µg/kg of EB in avoidance assay chambers, showed no significant differences in the proportions found on the non-seeded/uncontaminated side of test chambers (Woof 2021).

The calculated toxicological parameters for acute lethality in the 48-h water and 10-d sediment exposure tests for pink salmon show that toxicity trends were similar regardless of the exposure media and that toxicity occurred within the range tested for each chemical. DM was consistently the most toxic to pinks (LC50 values 1 µg/L and 1 µg/kg in water and sediment, respectively) with values which are similar to those reported for fish (juvenile starry flounder [*Platichthys stellatus*], adult threespine stickleback [*Gasterosteus aculeatus*], and adult tidepool sculpin [*Oligocottus maculosus*]) (500–870 ng/L and 510 ng/kg [in Strachan and Kennedy 2021] and approximately 20-fold less sensitive than crustaceans (Burridge et al. 2014b; Fairchild et al. 2010). Few other comparable studies with DM exist; a study with Atlantic salmon supports this sensitivity range (Sievers et al., 1995). CP was the next most acutely lethal chemical (LC50 values of 5 µg/L and 97 µg/kg in water and sediment, respectively) with values in the range of those previously reported (summarized in Clark et al. 1989 and Haya 1989; Ernst et al. 2001; Strachan and Kennedy 2021). Comparatively, crustaceans are approximately 3–10 fold more sensitive to CP than fish species; LC50 values for crustaceans range from 0.005 µg/L (Clark et al. 1989) to 0.82 µg/L (Strachan and Kennedy 2021). AZ was the next most toxic chemotherapeutant to pink salmon in water (LC50 value 80 µg/L); this value is approximately 10-fold less toxic than reported for other marine fish species (Strachan and Kennedy 2021). This was within the range of toxicity values have been reported for a variety of crustaceans (1.03 µg/L to 191 µg/L) (Burridge et al. 1999; Burridge et al. 2014b). EB was the second least toxic compound to pink fry (LC50 values 1090 µg/L and 2100 µg/kg in water and sediment, respectively). Comparable values are 96 h LC50s in the range of 200–1300 µg/L for rainbow trout, bluegill sunfish (*Lepomis macrochirus*), and the Sheepshead minnow (*Cyprinodon variegatus*) (McHenery and Mackie 1999; Lumaret et al. 2012; Chukwudebe et al. 1996) and marine fish species (Strachan and Kennedy 2021). HP was the least toxic of all chemicals to both pink salmon fry (227 mg/L) with similar values to other marine species (Strachan and Kennedy 2021). Similar relative insensitivity of fish to HP has been widely reported (Burridge et al. 2014b; Kiemer and Black 1997).

The results for survival in the sediment exposure tests (with EB, CP and DM) for pink salmon fry exhibited similar toxicity trends with DM being the most toxic, followed by CP and then EB and were similar to those reported for adult tidepool sculpin [*Oligocottus maculosus*]) (Strachan and Kennedy 2021). 10-d LC50 values in the present study indicate that these 3 compounds, which due to low log Kow values, partition to sediments relative to the water phase, and appear to be bioavailable to pink fry which are considered a pelagic species. Pink salmon fry feed mainly on planktonic and epibenthic prey; in one study, between 38 and 51% of the diet comprised epibenthic prey (Godin 1981). Kaczynski et al. 1973 also reported the predominant occurrence of epibenthic prey in the diets of pink salmon fry in littoral areas of the marine environment; this feeding strategy suggests that there exists an exposure pathway and potential bioavailability of sediment-associated contaminants for pink salmon in their early marine life stages.

Chemical information from the environment is received by the olfactory and gustatory systems in fishes and the relayed information can be critical to many activities including food location, predator avoidance, mating, kin discrimination, and particular to salmonids, migration and homing behaviours. Although the underlying mechanisms may vary, xenobiotics can impair olfactory function by gross anatomical alteration or by inhibiting key specific molecules, resulting is aberrant or dysfunctional behaviours to naturally occurring chemical stimuli.

The olfactory responsiveness of pink fry to a food extract was examined following exposure to sublethal concentrations of chemotherapeutants in water or sediments for varying time periods. Control pink salmon showed a typical positive response to the food odorant used in the test system. Olfactory systems function as important screening systems for both the respiratory and the gastrointestinal systems, and the classification of odors into the food or non-food category is of eminent survival value (Boesveldt et al. 2010). Concentration-dependent decreases in olfactory responsiveness was seen after 48-h AZ, CP and DM exposures at values lower than those causing acute mortality (20 µg/L *v.* 80 µg/L, 3 µg/L *v.* 5.1 µg/L, and 500 ng/L *v.* 980 µg/L [LOEC for olfactory effects *v.* LC50 value). Interestingly, pinks avoided AZ without prior exposure that may be protective, however, following a 48-h exposure to AZ and olfactory inhibition, it is unclear whether this avoidance response would still occur. Exposure to chemotherapeutants in sediments only resulted in olfactory inhibition with CP. Longer exposures to low concentrations of chemotherapeutants (that did not result in olfactory inhibition at 48 h) increased the potential for olfactory dysfunction suggesting that the threshold for inhibition is likely much lower and a function of exposure duration. Longer exposure durations are not likely to occur with water exposures, as models predict rapid dilution of chemotherapeutants released following treatment (Ernst et al.



2014) even though half lives in seawater can be long (e.g. AZ 13 d, CP 20 d, DM 18 d [Strachan and Kennedy 2021]); however, sediment-bound chemicals can be available for uptake for long periods due to their long half-lives (e.g. CP 560 d, DM 45 d, EB 230 d [Strachan and Kennedy 2021]) and limited dilution under farms.

◀ **Fig. 5** Critical swimming speeds (Ucrit) in pink salmon exposed to each chemical for 48 h at concentrations below the 48-h LC50 value (dashed line). Swimming speeds are presented as body lengths per second (BL/s). Boxes denote upper and lower quartiles; line is median value; bars are minimum and maximum data points; symbols are individual swim speed of $n = 7$ fish. For each chemical, the mean swim speed of fish in the control group was compared to the means of exposed groups using a one-factor ANOVA analysis followed by a Tukey-Kramer post-hoc test and differences from controls are denoted by asterisks ($*p < 0.05$)

Several classes of pesticides affect fish olfactory responses including carbamates, organophosphates and triazine herbicides (Tierney et al. 2010). Other organophosphates like AZ that have shown olfactory inhibition through behavioural measures include diazinon (*O. tshawytscha*; Scholz et al., 2000), parathion (*C. auratus*; Rand et al. 1975) or olfactory sensory neuron impairment including diazinon (Moore and Waring 1998) and chlorpyrifos (O. kisutch; Sandahl et al. 2004). As in this study, alterations in olfaction were seen with CP exposure in *Salmo salar* (Moore and Waring 2001).

In most species of fish, swimming performance is a main determinant of survival and strongly influences the ability of a fish to obtain food, find mates, avoid unfavourable conditions, and migrate (Plaut 2001), and the Ucrit test has been used as an ecologically relevant definitive test for rover–predator teleosts with direct application to assessing their Darwinian fitness. In control fish here, Ucrit values ranged from 5.0 to 6.3 BL/s which are similar to the average swimming speeds determined in a study comparing critical swimming speed and maximal swimming speed in pink salmon fry (2.8 g), where mean swimming speeds ranged from 4.54 to 5.2 BL/s (Nendick et al. 2009).

Concentration-dependent decreases in Ucrit were seen following exposure to HP [threshold 100 mg/L], AZ [10 µg/L], CP [2 µg/L] and DM [200 ng/L]. It is well established that exposure to a variety of contaminants including metals (Waiwood and Beamish 1978; Beaumont et al. 1995; Taylor et al. 2000; Rajotte and Couture 2002), petroleum (Kennedy and Farrell 2006; Alderman et al. 2020), pesticides (Little et al. 1990; Mackinnon and Farrell 1992; Nikl and Farrell 1993), and other contaminants (Howard 1975; Wood et al. 1996) can alter swimming performance in teleosts. This includes effects on critical swimming speed following exposure to organophosphate (OP) pesticides ([*Cyprinodon variegatus*] Cripe et al. 1984, [*Salvelinus fontinalis*] Peterson 1974, [*O. kisutch*] Tierney et al. 2007; [*Oreochromis niloticus*] McKenzie et al. 2017)) such as AZ. The outcomes of OP exposure on locomotor activity appears to be two-fold; hyperactivity or decreases in swimming activity or ability (Tierney et al. 2007). For example, increased swimming activity was seen in eastern rainbow fish (*Melanotaenia duboulayi*) exposed to profenofos (Kumar and Chapman 1998) and in goldfish (*Carassius auratus*) following carbofuran exposure (Bretaud et al. 2001) In contrast, Coho salmon displayed a concentration-dependant decrease in swimming activity rather than hyperactivity following exposure to chlorpyrifos (Tierney et al. 2007). Little et al. 1990 reported an incremental decrease in swimming activity in rainbow trout exposed to methyl parathion. The OP pesticide trichlorfon caused prolonged impairments to swimming performance in *O. niloticus*, but individuals varied widely in their relative sensitivity to the pesticide (McKenzie et al. 2017).

Swim performance in the present study was affected by pyrethroid exposure; however, this class of chemical has shown contradictory results with respect to swim performance. For example, Goulding et al. (2013) showed no effects of permethrin exposure on the swimming performance in juvenile rainbow trout, but found reductions in Ucrit following deltamethrin exposure under the same conditions (Goulding et al. 2013). Effects on swimming performance have been explained by considering the sublethal toxicity generally described for pyrethroids that include muscle tremors, and rapid and erratic swimming (Glickman and Lech 1982; Haya 1989; Velíšek et al. 2007; Werner and Moran 2008). It is suggested that these manifestations of toxicity interrupted the constant gait required for prolonged swimming tests, forcing fish to transition to burst swimming earlier, which is unsustainable at the step durations used in Ucrit protocols (Farrell 2008).

Although not directly applicable to water exposures, an i.p dose of EB administration (3 d prior) caused dose-dependent decreases in swimming performance in juvenile rainbow trout at 5 mg/kg, an unlikely internal dose to be achieved in the present study. Three different swimming outcomes were examined and at these higher doses, Ucrit, burst swimming and schooling were affected (Kennedy et al. 2014). Although the contribution of glutamate-gated chloride ion channels and GABA-neurosynaptic transmission in the CNS to fish swimming performance is unknown, signs of avermectin toxicity (Katharios et al. 2001) suggests that increased accumulation of this neurotoxin in the brain of fish leads to altered parameters related to swimming. Avermectins inhibit signal transmission at GABA-gated and glutamate-gated chloride channels by binding GABA receptors, which leads to hyperpolarization of the neuronal cells (MSD, 1988). Data from the present study indicates that altering GABA transmission affects swimming performance and behaviour.

The postulated toxic modes of action of each chemotherapeutant provide clarity in the effects observed. EB, CP, DM and AZ are all neurotoxic agents and target specific biomolecules, however HPs mechanism of action is general with multiple targets.

Organophosphorous pesticides (OPs, e.g. AZ) inhibit acetylcholinesterase (AChE) which hydrolyzes acetylcholine (ACh) in cholinergic neuropathways leading to ACh accumulation and repeated post-synaptic action potentials and insensitivity to further signalling; this translates as convulsions, twitching, agitation, and eventual partial or complete paralysis (Fulton and Key 2001; Xuereb et al. 2009). AChE-impairing pesticides inhibit both olfaction (Sandahl et al. 2004) and muscle performance (Tierney et al. 2007).

In coho salmon, chlorpyrifos-induced declines in Ucrit performance are associated with reduced AChE activity in slow-twitch aerobic muscle and compromised neuromuscular coordination (Tierney et al. 2007). OPs are also reported to influence both the metabolism and cardiorespiratory physiology of fishes, reducing metabolic rate, heart rate, ventilatory activity and spontaneous swimming activity (da Silva et al. 1993, De Aguiar et al. 2004, Gehrke 1988, Tryfonos et al. 2009). For example, trichlorfon exposure decreased the ability of Nile tilapia (*Oreochromis niloticus)* to regulate aerobic metabolism due to an impaired capacity to hyperventilate (Thomaz et al. 2009). Reduced exercise performance following OP exposure in fishes may therefore reflect direct effects on swimming muscles but also on the ability of the cardiorespiratory system to meet the oxygen demands of activity (Mackenzie et al. 2017). Impaired neuromuscular coordination presumably due to AChE inhibition (Tierney et al. 2007) suggests that higher oxygen consumption is required to power swimming at any given speed. Guimarães et al. 2007 showed that trichlorfon exposure had no direct effect on respiratory metabolism, suggesting that the mechanism underlying Ucrit declines was a decline in swimming efficiency in *O. niloticus*

Pyrethroids such as cypermethrin and deltamethrin interact with voltage-gated Na^+ channels, and with other ion channels including voltage-gated Cl^- channels (Burr and Ray 2004) leading to repetitive neuronal firing (Vijverberg and Van Den Bercken 1979). Acute pyrethroid poisoning in fish manifests with symptoms that include muscle tremors, rapid and erratic swimming, loss of equilibrium, jaw spasms, gulping respiration, and lethargy (Werner and Moran 2008).

The effects of individual pyrethroid exposure on the swimming performance of fish depends on compound-specific interactions with Na^+ channels. For example, exposure of rainbow trout to 2 pyrethroids (permethrin and DM) resulted in reduced swim performance only with deltamethrin (Goulding et al. 2013). The divergent effects seen between these pyrethroids was attributed to their differing effects on peripheral motor neurons (Vijverberg et al. 1982), where permethrin causes repetitive action potential firing in response to stimulus (Vijverberg et al. 1982), while deltamethrin causes a frequency-dependent depression of action potentials due to the gradual depolarization of the cell membrane (Vijverberg and Van Den Bercken 1979). Gradual depolarization occurs more rapidly at higher action potential frequencies such as those needed for elevated tail beat frequencies at faster swimming speeds (Goulding et al. 2013).

Another contributing factor to pyrethroid-associated reductions in Ucrit may be due to an increase in resting metabolic rate and aerobic capacity through increased energy requirements associated with physiological stress, tissue repair, and detoxification (Kumaraguru and Beamish 1983 1986; Philip and Anuradha 1996; Velíšek et al. 2007). Histological studies have shown that acute DM exposure causes damage to gill, liver, and kidney in the common carp (*Cyprinus caprio*: Cengiz 2006), and gill, liver, and gut tissue of the mosquito fish (*Gambusia affinis*: Cengiz and Unlu 2006).

The precise mechanism(s) of action of EB is not fully understood. In invertebrates, avermectins are thought to interfere with GABA- and glutamate-gated Cl^- channel receptors in nerve and muscle cells by stimulating the influx of chloride ions (Burridge et al. 2010; Lumaret et al. 2012; Benchaoui and Mckellar 1996), leading to hyperpolarization of the neuronal cells and subsequent paralysis (Reddy 2012; Lumaret et al. 2012; Benchaoui and Mckellar 1996). Avermectins can affect fish swimming and perhaps other systems that rely on glutamate-gated chloride ion channels and GABA-neurosynaptic transmission in the CNS; signs of avermectin toxicity (Katharios et al. 2001; Kennedy et al. 2014).

The mechanism of toxicity of HP is non-specific and not fully understood. As with other reactive oxygen species (ROS), high concentrations have been attributed to cell damage (Cabiscol et al. 2000), cell death (Saito et al. 2006) and carcinogenesis (Liou and Storz 2010). In sea lice HP is believed to invoke mechanical paralysis through the formation of gas bubbles in the haemolymph (Burka et al. 1997; Bruno and Raynard 1994; Grant 2002).

Each of the chemotherapeutants examined have different environmental fates and resulting water and/or sediment concentrations, due to modes and concentrations of application, chemical characteristics, and environmental conditions, and therefore different levels of risk to non-target organisms. The highest exposure concentrations for pink salmon will occur in the water phase at the time of application and immediate release: Interox® Paramove 50 (AI HP), is applied in Canada as a bath treatment at 1500 mg. L^{-1} for 20–30 min (PMRA 2014), and elsewhere at 1200–1800 mg L^{-1} for 30 min (Burridge 2013; Burridge and Van Geest 2014; Grant 2002); Salmosan® (AI AZ) is applied as a bath treatment at 100 µg AZ L^{-1} for 30–60 min in well boats and tarps and at 150 µg AZ L^{-1} in skirt treatments (Burka et al. 1997; Van Geest et al. 2014; Burridge et al. 1999; Burridge et al. 2010; Grant 2002; Haya

2001; Haya et al. 2005); Excis® (AI CP) is applied as a bath treatment at 5 µg CP L^{-1} for 60 min (Burridge and Van Geest 2014); Alphamax® (AI DM) is applied as a bath treatment at 2–3 µg DM L^{-1} for 40 min (Burridge and Van Geest 2014). Following application, tides and currents strongly dictate the dilution and distribution of the chemical in the water column. For example, A field study in Atlantic Canada analyzed marine concentrations following the release of Salmosan®-treated baths using rhodamine dye as a tracer in an effort to characterise contaminant plume distribution (Ernst et al. 2014). Azamethiphos concentrations ranged from 1.1–11 µg/L and 0.2–1 µg/L approximately 1 and 1000 m from application release areas, respectively, 2–3 h after treatment. A dispersion study utilizing simulated bath treatments with the pyrethroid cypermethrin found that the pesticide remained detectable for up to 5.5 h, and at distances up to 3000 m from the site of release; however, concentrations quickly diluted to levels 10–1000 times lower than the treatment concentration (Ernst et al. 2001). EMB concentrations in the water column in the vicinity of a salmon farm undergoing treatment have been found between 0.006–0.635 ng/L in Canada (DFO 2012). Risk quotients (RQs) can be generated using the toxic effects thresholds determined here (e.g. LC50, NOEC values) and estimated environmental concentrations. Generated RQs can be used to compare chemotherapeutant risk as a group, or indiviudally by comparing RQ values to a jurisdiction and/or receptor-specific level of concern (US EPA 2021).

In areas where wild salmon migration routes co-exist with aquaculture, such as the coastal waters of Canada, juvenile wild salmon as small as 0.2 g (*O. gorbuscha*) may be exposed (Heard 1991). Exposure will be largely site-specific, influenced by local currents and tides. Each of the toxicity endpoints used here have different effects levels and can be compared to initial application or dilution rates (for HP, AZ, CP and DM) or highest measured sediment levels for EB (or CP and DM if available) to determine the risk for effects. For example, at initial chemotherapeutant application concentrations with bath applications, lethality of pink salmon would occur, however rapid dilution would reduce concentrations to non-lethal levels. The potential for sublethal effects on olfaction and swimming ability in pink salmon is a distinct possibility near farms. Dilution models in conjunction with toxicity effects levels should be used to make such risk determinations; this research highlights the importance of concentration-response data for regulators in this regard.

Data availability

Data is available upon request from the corresponding author.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval This project was approved by the Simon Fraser University University Animal Care Committee according to the guidelines outlined by the Canadian Council on Animal Care

Consent to participate All authors consent to participate.

Consent for publication All authors consent to publication.

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