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). In subchronic 10-d lethality sediment exposure tests, LC50 values (95% confidence intervals) in order of least to most toxic were: 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 168 h). 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.

1. 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 are the greatest source of mortality and economic loss at farms, estimated to be $500 million annually worldwide, representing 6% of product value (Mustafa et al., 2001; Costello, 2009). 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 coinciding 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).

Anti-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. However, the predominant management of outbreaks is with anti-sea lice chemotherapeutants (Burridge and Van Geest, 2014). The first anti-sea lice chemotherapeutants were issued in 1994 in Atlantic Canada in response to sea lice infestations in New Brunswick (Burridge and Van Geest, 2014). Salartect® (active ingredient (AI): hydrogen 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 to 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 Meyers, 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 near-shore waters housing open net pen operations (Godin; 1981; Quinn and Meyers, 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., 2002). Due to its hydrophobic nature (LogK_{OW} = 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 (Amdt 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; Velšek et al., 2007); 0.06 to 1.65 µg/L for the common carp (Cyprinus carpio; Svoboda et al., 2003; Çalış 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 value 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; Hedyati et al., 2012) 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 out-migrating 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.

2. Materials And Methods

2.1 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).

2.2 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. 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.

2.3 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.

### 2.4 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 $\lambda_{\text{ex}} = 540/\lambda_{\text{em}} = 590$ 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).

### 2.4 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).

### 2.5 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 x 22.5 cm) consisting of two cylindrical chambers (diameter 20 cm; depth 7 cm) connected by a trough (5.5 x 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).

### 2.6 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.

## 2.5 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 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).

## 2.6 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 and LOEC/NOEC 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).

3. Results

3.1 Water chemistry

The recovery for HP was determined by comparing spiked water samples (50 mL) and was 96% with a between-day variability of 4.1%. 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.

3.2.6 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).

3.2 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 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 more avoidance compared to HP, at concentrations > 300 µg/L (p < 0.05). 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).

3.3 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 34 ± 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).
Ten-d sediment exposures to EB, CP, or DM (at sublethal 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.

### 3.3 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 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%).

### 4. Discussion

This study provides important information on the effects of historically and presently used therapeutants, drugs, and pesticides on an economically and ecologically important salmonid species that can migrate past aquaculture sites during or following treatment of sea lice in Atlantic salmon in areas such as the Broughton Archipelago, BC, an area of active aquaculture (Krkošek et al., 2006; Jones and Hargreaves 2007) and may be at risk from exposure to chemotherapeutants.

All 5 chemotherapeutants were lethal to pink salmon fry in the concentration range tested; lethal concentrations varied by orders of magnitude. The chemicals in order of most to least toxic were DM > CP > AZ > EB > HP in both water and sediment exposures. Pink fry avoided HP, EB and AZ and were not attracted to any of the chemicals at the concentrations tested. AZ, CP and DM altered the olfactory ability that was both concentration and time-dependent. Swimming performance was also affected by all chemicals except EB, and again, concentrations resulting in effects thresholds varied by orders of magnitude.

Organisms rely on constituent chemical defense mechanisms to avoid the potential toxic effects of foreign compound exposure (Tierney et al., 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]; aschlopyrifos [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 et al., 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 et al., 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 and Kennedy 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 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; Chukwedebe 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 (Burrage et al., 2014; 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 in 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 (ref) 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.

Several classes of pesticides affect fish olfactory responses including carbamates, organophosphates and triazine herbicides (Tierny et al., 2010). Other organophosphates like AZ that have shown olfactory inhibition through behavioural measures include diazinon (O. tshawytscha; Scholtz 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, 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] Tierny 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 (Tierny 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 (Breutaud et al., 2001) In contrast, Coho salmon displayed a concentration-dependant decrease in swimming activity rather than hyperactivity following exposure to chlorpyrifos (Tierny 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).

Exposure to pyrethroids has shown contradictory results with respect to swim performance. Goulding et al. (2013) showed no effects of permethrin exposure on the swimming performance in juvenile rainbow trout, results contrary to another study showing Ucrit declines following permethrin exposure in the same species (Kumaraguru and Beamish 1983). The discrepancy was attributed to differences in the size of
fish used as it has been shown that permethrin toxicity in trout is inversely related to mass (Kumaraguru and Beamish 1981, 1983, 1986). Reductions in Ucrit were seen following deltamethrin exposure under the same conditions (Goulding et al., 2013). Effects on swimming performance were 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 was 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). In a study with zebrafish, no effects on swimming performance were seen following exposure to DM at 2 µg/L (Strungaru et al., 2019). DM caused behavioural effects including rapid swimming, loss of balance, aggressiveness and increases in the surface activity in brown trout (Salmo trutta) at concentrations as low as 1 µg/L (Karatas et al., 2019). Killifish (Jenynsia multidentata) exposed to cypermethrin at 4 µg/L showed a decrease in swimming activity and an increase in the time spent at the bottom of test tanks (Bonansea et al., 2016).

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 and Tierney, 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.

Organophosphates (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 (Couillard and Burridge, 2014; 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
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, 1990). 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; Balint et al., 1995; Philip and Anuradha, 1996; Velišek et al., 2007). Histological studies have shown that acute DM exposure causes damage to gill, liver, and kidney in the common carp (*Cyprinus carpio*: 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 (MSD, 1988) 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 and Tierney 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 sediment concentrations near farms due to modes of application, chemical characteristics, and environmental conditions. The highest exposure concentrations 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 et al., 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 m 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). The following half-lives of the AIs in formulation have been reported in the literature: Salmosan (AI: AZ), 9–50 d (Mayor et al., 2008; Strachan and Kennedy 2021); Excis® (AI:CP), 19.8–80 d (Mayor et al., 2008; Strachan and Kennedy 2021); Alphamax® (AI:DM), 17.9–285 d (Benskin et al., 2016; Strachan and Kennedy 2021); Paramove® 50 (AI: HP), 8–19 d (Lyons et al., 2014; Strachan and Kennedy 2021); and Slice® (AI: EB), 164–175 d (Mayor et al., 2008) and > 400 d (Benskin et al., 2016). The Slice® formulation (AI EB) has an optimal prescribed dose of 50 µg kg\(^{-1}\) d\(^{-1}\) in feed for 7 d (Stone et al., 1999). The highest EB accumulation in sediment is generally within 25–60 m of the net pens but can be detected greater than 300 m away (Weston 1990, Schendel et al., 2004) as a result of seawater hydrodynamics (Tefler et al., 2006; DFO 2012). 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). Some sampling events found concentrations as high as 140 and 366 µg/kg (McHenery and Mackie 1999, Boxall et al., 2002, Lalonde et al., 2012).

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 rates (for HP, AZ, CP and DM) or highest measured sediment levels for EB (or CP and DM if available) to determine the potential 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.

**Declarations**

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**Consent to participate:** all authors consent to participate

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**References**

1. Abgrall JF (1999) Short and medium term impact of aerial application of insecticide against the winter moth (Operophtera brumata L.). Revue Forestière Française 51(3):395–404. https://doi.org/10.4267/2042/5445
2. Alderman SL, Dilkumar CM, Avey SR, Farrell AP, Kennedy CJ, Gillis TE (2020) Effects of diluted bitumen exposure and recovery on the seawater acclimation response of Atlantic salmon smolts. Aquat Toxicol 221:105419. https://doi.org/10.1016/j.aquatox.2020.105419
3. Arndt RE, Wagner EJ (1997) The Toxicity of Hydrogen Peroxide to Rainbow Trout Oncorhynchus mykiss and Cutthroat Trout Oncorhynchus clarki Fry and Fingerlings. J World Aquaculture Soc
4. Beaumont MW, Butler PJ, Taylor EW (1995) Exposure of brown trout, Salmo trutta, to sub-lethal copper concentrations in soft acidic water and its effect upon sustained swimming performance. Aquat Toxicol 33(1):45–63. https://doi.org/10.1016/0166-445X(95)00007-Q

5. Benchaoui HA, Mckellar QA (1996) Interaction between fenbendazole and piperonyl butoxide: pharmacokinetic and pharmacodynamic implications. J Pharm Pharmacol 48(7):753–759. https://doi.org/10.1111/j.2042-7158.1996.tb03965.x

6. Benskin JP, Ikonomou MG, Surridge BD, Dubetz C, Klaassen E (2016) Biodegradation potential of aquaculture chemotherapeutants in marine sediments. Aquac Res 47(2):482–497. https://doi.org/10.1111/are.12509

7. Bloodworth JW, Baptie MC, Preedy KF, Best J (2019) Negative effects of the sea lice therapeutant emamectin benzoate at low concentrations on benthic communities around Scottish fish farms. Sci Total Environ 669:91–102. https://doi.org/10.1016/j.scitotenv.2019.02.430

8. Boesveldt S, Frasnelli J, Gordon AR, Lundström JN (2010) The fish is bad: Negative food odors elicit faster and more accurate reactions than other odors. Biol Psychol 84(2):313–317. https://doi.org/10.1016/j.biopsycho.2010.03.006

9. Bonansea RI, Wunderlin DA, Amé MV (2016) Behavioral swimming effects and acetylcholinesterase activity changes in Jenynsia multidentata exposed to chlorpyrifos and cypermethrin individually and in mixtures. Ecotoxicol Environ Saf 129:311–319. https://doi.org/10.1016/j.ecoenv.2016.03.043

10. Boxall AB, Fogg LA, Blackwell PA, Blackwell P, Kay P, Pemberton EJ (2002) Review of veterinary medicines in the environment. R&D Technical Report P6-012/8/TR. Bristol, UK

11. Bretaud S, Saglio P, Toutant J-P (2001) Effets du Carbofuran sur l’activité de l’acetylcholinesterase cérébrale et sur l’activité de Nage Chez Carassius Auratus (Cyprinidae) par. In Cybium (Vol. 25, Issue 1)

12. Brett JR (1964) The Respiratory Metabolism and Swimming Performance of Young Sockeye Salmon. J Fish Res Board Can 21(5):1183–1226. https://doi.org/10.1139/f64-103

13. Bruno DW, Raynard RS (1994) Studies on the use of hydrogen peroxide as a method for the control of sea lice on Atlantic salmon. In Aquaculture International (Vol. 2)

14. Burka JF, Hammell KL, Horsberg TE, Johnson GR, Rainnie DJ, Speare DJ (1997) Drugs in salmonid aquaculture – A review. J Vet Pharmacol Ther 20(5):333–349. https://doi.org/10.1046/j.1365-2885.1997.00094.x

15. Burr SA, Ray DE (2004) Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicol Sci 77(2):341–346. https://doi.org/10.1093/toxsci/kfh027

16. Burridge LE (2013) A review of potential environmental risks associated with the use of pesticides to treat Atlantic salmon against infestations of sea lice in southwest New Brunswick, Canada. DFO Can. Sci. Advis. Sec. Res. Doc, pages 2013/050. iv + 25 p
17. Burridge LE, Van Geest JL (2014) A review of potential environmental risks associated with the use of pesticides to treat Atlantic salmon against infestations of sea lice in Canada. *DFO Can. Sci. Advis. Sec. Res. Doc. 2014/002* vi + 36p

18. Burridge LE, Haya K, Zitko V, Waddy S (1999) The lethality of Salmosan (azamethiphos) to American lobster (Homerus americanus) larvae, postlarvae, and adults. *Ecotoxicol Environ Saf* 43(2):165–169. https://doi.org/10.1006/eesa.1999.1771

19. Burridge LE, Lyons MC, Wong DKH, MacKeigan K, VanGeest JL (2014) The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquaculture* 420–421:180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041

20. Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K (2010) Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. In *Aquaculture* (Vol. 306, Issues 1–4, pp. 7–23). Elsevier. https://doi.org/10.1016/j.aquaculture.2010.05.020

21. Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol* 3(1):3–8. https://doi.org/10.2436/im.v3i1.9235

22. Çalta M, Ural M (2004) Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, *Cyprinus Carpio*. *Fresenius Environ Bull* 13(11 A):1179–1183.

23. Cengiz EI (2006) Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. *Environ Toxicol Pharmacol* 22(2):200–204. https://doi.org/10.1016/j.etap.2006.03.006

24. Cengiz EI, Unlu E (2006) Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. *Environ Toxicol Pharmacol* 21(3):246–253. https://doi.org/10.1016/j.etap.2005.08.005

25. Chukwudebe AC, Andrew N, Drottar K, Swigert J, Wislocki PG (1996) Bioaccumulation Potential of 4′′-epi-(Methylamino)-4′′-deoxyavermectin B1a Benzoate (Emamectin Benzoate) in Bluegill Sunfish. *J Agric Food Chem* 44:2894–2899

26. Clark JR, Goodman LR, Borthwick PW, Patrick JM, Cripe GM, Moody PM, Moore JC, Lores EM (1989) Toxicity of pyrethroids to marine invertebrates and fish: A literature review and test results with sediment-sorbed chemicals. *Environ Toxicol Chem* 8(5):393–401. https://doi.org/10.1002/etc.5620080505

27. Costello MJ (2009) How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. In *Proceedings of the Royal Society B: Biological Sciences* (Vol. 276, Issue 1672, pp. 3385–3394). https://doi.org/10.1098/rspb.2009.0771

28. Cripe GM, Goodman LR, Hansen DJ (1984) Effect of chronic exposure to EPN and to Guthion on the critical swimming speed and brain acetylcholinesterase activity of *Cyprinodon variegatus*. *Aquat Toxicol* 5(3):255–266. https://doi.org/10.1016/0166-445X(84)90024-9

29. da Silva HC, Medina HSG, Fanta E, Bacila M (1993) Sub-lethal effects of the organophosphate folidol 600 (methyl parathion) on Callichthys callichthys (pisces:teleostei). *Comparative Biochemistry Physiology Part C Comparative* 105(2):197–201. https://doi.org/10.1016/0742-8413(93)90194-P
30. De Aguiar LH, Moraes G, Avilez IM, Altran AE, Corrêa CF (2004) Metabolic effects of Folidol 600 on the neotropical freshwater fish matrinxã, Brycon cephalus. Environ Res 95(2):224–230. https://doi.org/10.1016/S0013-9351(03)00119-1

31. DFO (Fisheries and Oceans Canada) (2012) Assessment of the fate of emamectin benzoate, the active ingredient in SLICE®, near aquaculture facilities in British Columbia and its effect on the Pacific spot prawn (Pandalus platyceros). Canadian Science Advisory Secretariat, Science Advisory Report 2011/082

32. Ernst W, Doe K, Cook A, Burridge L, Lalonde B, Jackman P, Aubé JG, Page F (2014) Dispersion and toxicity to non-target crustaceans of azamethiphos and deltamethrin after sea lice treatments on farmed salmon, Salmo salar. Aquaculture 424–425:104–112. https://doi.org/10.1016/j.aquaculture.2013.12.017

33. Ernst W, Jackman P, Doe K, Page F, Julien G, MacKay K, Sutherland T (2001) Dispersion and toxicity to non-target aquatic organisms of pesticides used to treat sea lice on almon in net pen enclosures. Mar Pollut Bull 42(6):432–443. https://doi.org/10.1016/S0025-326X(00)00177-6

34. Fairchild EA, Sulikowski JA, Rennels N, Howell WH, Tsang PCW (2010) Effects of moving acclimation cages before release of cultured fish: Alternate release strategies for a juvenile winter flounder Pseudopleuronectes americanus stock enhancement effort. Aquac Res 41(4):602–606. https://doi.org/10.1111/j.1365-2109.2009.02343.x

35. Farrell AP (2008) Comparisons of swimming performance in rainbow trout using constant acceleration and critical swimming speed tests. J Fish Biol 72(3):693–710. https://doi.org/10.1111/j.1095-8649.2007.01759.x

36. Folmar LC (1976) Overt avoidance reaction of rainbow trout fry to nine herbicides. Bull Environ Contam Toxicol 15(5):509–514. https://doi.org/10.1007/BF01685696

37. Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ Toxicol Chem 20(1):37–45. https://doi.org/10.1002/etc.5620200104

38. Garibaldi A, Turner N (2004) Cultural keystone species: Implications for ecological conservation and restoration. Ecol Soc, 9(3). https://doi.org/10.5751/ES-00669-090301

39. Gehrke PC (1988) Acute cardio-respiratory responses of spangled perch, leiopotherapon unicolor (Gunther 1859), to sublethal concentrations of zinc, temephos and 2, 4-d. Marine Freshwater Research 39(6):767–774. https://doi.org/10.1071/MF9880767

40. Glickman AH, Lech JJ (1982) Differential toxicity of trans-permethrin in rainbow trout and mice. II. Role of target organ sensitivity. Toxicol Appl Pharmacol 66(2):162–171. https://doi.org/10.1016/0041-008X(82)90281-2

41. Godin JGJ (1981) Daily patterns of feeding behavior, daily rations, and diets of juvenile pink salmon (oncorhynchus gorbuscha) in two marine bays of british columbia. Can J Fish Aquat Sci 38(1):10–15. https://doi.org/10.1139/f81-002
42. Golow AA, Godzi TA (1994) Acute toxicity of deltamethrin and dieldrin to Oreochromis niloticus (LIN). Bull Environ Contam Toxicol 52(3):351–354. https://doi.org/10.1007/BF00197820

43. Goulding AT, Shelley LK, Ross PS, Kennedy CJ (2013) Reduction in swimming performance in juvenile rainbow trout (Oncorhynchus mykiss) following sublethal exposure to pyrethroid insecticides. Comparative Biochemistry Physiology - C Toxicology Pharmacology 157(3):280–286. https://doi.org/10.1016/j.cbpc.2013.01.001

44. Grant AN (2002) Medicines for sea lice. Pest Manag Sci 58(6):521–527. https://doi.org/10.1002/ps.481

45. Gross M, Maycock P, Crane M (2008) Environmental assessment report for Alpha Max according to VICH Phase I and II guidance. Unpublished Report WCA Environment Ltd. Oxfordshire UK, 86 p

46. Guimarães ATB, Silva de Assis HC, Boeger W (2007) The effect of trichlorfon on acetylcholinesterase activity and histopathology of cultivated fish Oreochromis niloticus. Ecotoxicol Environ Saf 68(1):57–62. https://doi.org/10.1016/j.ecoenv.2006.08.005

47. Hansen DJ, Matthews E, Nall SL, Dumas DP (1972) Avoidance of pesticides by untrained mosquitofish, Gambusia affinis. Bull Environ Contam Toxicol 8(1):46–51. https://doi.org/10.1007/BF01684503

48. Hansen DJ (1969) Avoidance of Pesticides by Untrained Sheepshead Minnows. Trans Am Fish Soc 98(3):426–429. https://doi.org/10.1577/1548-8659(1969)98[426:aopbus]2.0.co;2

49. Haya K (1989) Toxicity of pyrethroid insecticides to fish. Environ Toxicol Chem 8(5):381–391. https://doi.org/10.1002/etc.5620080504

50. Haya K (2001) Environmental impact of chemical wastes produced by the salmon aquaculture industry. ICES J Mar Sci 58(2):492–496. https://doi.org/10.1006/jmsc.2000.1034

51. Haya K, Burridge LE, Davies IM, Ervik A (2005) A Review and Assessment of Environmental Risk of Chemicals Used for the Treatment of Sea Lice Infestations of Cultured Salmon. In: Environmental Effects of Marine Finfish Aquaculture (Vol, 5. Springer-Verlag, pp 305–340. https://doi.org/10.1007/b136016

52. Heuch PA, Bjørn PA, Finstad B, Holst JC, Asplin L, Nilsen F (2005) A review of the Norwegian “National Action Plan Against Salmon Lice on Salmonids”: The effect on wild salmonids. Aquaculture 246(1–4):79–92. https://doi.org/10.1016/j.aquaculture.2004.12.027

53. Hidaka H, Tatsukawa R (1989) Avoidance by olfaction in a fish, medaka (Oryzias latipes), to aquatic contaminants. Environ Pollut 56(4):299–309. https://doi.org/10.1016/0269-7491(89)90075-4

54. Howard TE (1975) Swimming Performance of Juvenile Coho Salmon (Oncorhynchus kisutch) Exposed to Bleached Kraft Pulpmill Effluent. J Fish Res Board Can 32(6):789–793. https://doi.org/10.1139/f75-103

55. Intorre L, Soldani G, Cognetti-Varriaile AM, Monni G, Meucci V, Pretti C (2004) Safety of azamethiphos in eel, seabass and trout. Pharmacol Res 49(2):171–176. https://doi.org/10.1016/j.phrs.2003.08.002

56. Jones SRM, Hargreaves NB (2007) The abundance and distribution of Lepeophtheirus salmonis (Copepoda: Caligidae) on pink (Oncorhynchus gorbuscha) and chum (O. keta) salmon in coastal
57. Kaczynski VW, Feller RJ, Clayton J, Gerke RJ (1973) Trophic analysis of juvenile pink and chum salmon (Ottoriyncitus gorbuscha and O. keta) in Puget Sound. J Fish Res Board Can 30:1003–1008

58. Karatas T, Yildirim S, Arslan H, Aggul AG (2019) The effects on brown trout (Salmo trutta fario) of different concentrations of deltamethrin. Comparative Biochemistry Physiology Part - C: Toxicology Pharmacology 226:108606. https://doi.org/10.1016/j.cbpc.2019.108606

59. Katharios P, Iliopoulos-Georgudaki I, Kapata-Zoumbos K, Spiropoulos S (2001) Toxicity of intraperitoneally injected ivermectin in sea bream, Sparus aurata. Fish Physiol Biochem 25(2):99–108. https://doi.org/10.1023/A:1020574810332

60. Kennedy CJ, Farrell AP (2006) Effects of Exposure to the Water-Soluble Fraction of Crude Oil on the Swimming Performance and the Metabolic and Ionic Recovery Postexcercise in Pacific Herring (Clupea Pallasi). In Environmental Toxicology and Chemistry (Vol. 25, Issue 10)

61. Kennedy CJ, Tierney KB, Mittelstadt M (2014) Inhibition of P-glycoprotein in the blood-brain barrier alters avermectin neurotoxicity and swimming performance in rainbow trout. Aquat Toxicol 146:176–185. https://doi.org/10.1016/j.aquatox.2013.10.035

62. Kiemer MCB, Black KD (1997) The effects of hydrogen peroxide on the gill tissues of Atlantic salmon, Salmo salar L. Aquaculture 153(3–4):181–189. https://doi.org/10.1016/S0044-8486(97)00037-9

63. Kim Y, Jung J, Oh S, Choi K (2008) Aquatic toxicity of cartap and cypermethrin to different life stages of Daphnia magna and Oryzias latipes. Journal of Environmental Science Health - Part B Pesticides Food Contaminants Agricultural Wastes 43(1):56–64. https://doi.org/10.1080/03601230701735029

64. Köprücü SS, Köprücü K, Ural MS (2006) Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Fingerling European Catfish, Silurus glanis L. Bull Environ Contam Toxicol 76:59–65. https://doi.org/10.1007/s00128-005-0889-3

65. Krkošek M, Ford JS, Morton A, Lele S, Myers RA, Lewis MA (2007) Declining wild salmon populations in relation to parasites from farm salmon. Science 318(5857):1772–1775. https://doi.org/10.1126/science.1148744

66. Krkošek M, Lewis MA, Volpe JP (2005) Transmission dynamics of parasitic sea lice from farm to wild salmon. Proceedings of the Royal Society B: Biological Sciences, 272(1564), 689–696. https://doi.org/10.1098/rspb.2004.3027

67. Kumar A, Chapman JC (1998) Profenofos Toxicity to the Eastern Rainbow Fish (Melanotaenia Duboulayi). In Environmental Toxicology and Chemistry (Vol. 17, Issue 9)

68. Kumaraguru AK, Beamish FWH (1981) Lethal toxicity of permethrin (NRDC-143) to rainbow trout, salmo gairdneri, in relation to body weight and water temperature. Water Res 15(4):503–505. https://doi.org/10.1016/0043-1354(81)90061-0

69. Kumaraguru AK, Beamish FWH (1983) Bioenergetics of acclimation to permethrin (NRDC-143) by rainbow trout. Comparative Biochemistry Physiology Part C Comparative 75(2):247–252. https://doi.org/10.1016/0742-8413(83)90188-3
70. Kumaraguru AK, Beamish FWH (1986) Effect of permethrin (NRDC-143) on the bioenergetics of rainbow trout, Salmo gairdneri. Aquat Toxicol 9(1):47–58. https://doi.org/10.1016/0166-445X(86)90005-6

71. Kynard, B. (1974). Avoidance Behavior of Insecticide Susceptible and Resistant Populations of Mosquitofish to Four Insecticides. *Transactions of the American Fisheries Society, 103*(3), 557–561. https://doi.org/10.1577/1548-8659(1974)103<557:ABOISA>2.0.CO;2

72. Lalonde BA, Ernst W, Greenwood L (2012) Measurement of oxytetracycline and emamectin benzoate in freshwater sediments downstream of land based aquaculture facilities in the Atlantic Region of Canada. Bull Environ Contam Toxicol 89(3):547–550. https://doi.org/10.1007/s00128-012-0724-6

73. Liou G-Y, Storz P (2010) Reactive oxygen species in cancer. Free Radical Res 44(5):479–496. https://doi.org/10.3109/10715761003667554

74. Little EE, Archeski RD, Flerov BA, Kozlovskaya VI (1990) Behavioral indicators of sublethal toxicity in rainbow trout. In *Arch. Environ. Contam. Toxicol* (Vol. 19)

75. Lumaret J-P, Errouissi F, Floate K, Rombke J, Wardhaugh K (2012) A Review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Curr Pharm Biotechnol 13(6):1004–1060. https://doi.org/10.2174/138920112800399257

76. Mackinnon DL, Farrell AP (1992) The effect of on juvenile Coho salmon (*Oncorhynchus kisutch*): sublethal toxicity testing 2-(thiocyanomethylthio) benzothiazole. In *Environmental Toxicology and Chemistry* (Vol. 11)

77. Mayor DJ, Solan M, Martinez I, Murray L, McMillan H, Paton GI, Killham K (2008) Acute toxicity of some treatments commonly used by the salmonid aquaculture industry to Corophium volutator and Hediste diversicolor: Whole sediment bioassay tests. Aquaculture 285(1–4):102–108. https://doi.org/10.1016/j.aquaculture.2008.08.008

78. McHenery JG, Mackie CM (1999) Revised expert report on the potential environmental impacts of emamectin benzoate, formulated as Slice® for salmonids. *Cordah Report No. SCH001R5.*

79. McKenzie DJ, Blasco FR, Belão TC, Killen SS, Martins ND, Taylor EW, Rantin FT (2017) Physiological determinants of individual variation in sensitivity to an organophosphate pesticide in Nile tilapia Oreochromis niloticus. Aquat Toxicol 189:108–114. https://doi.org/10.1016/j.aquatox.2017.06.001

80. Mcleese DW, Metcalfe CD, Zitko V (1980) Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp. Bull Environm Contam Toxicol 25:950–955

81. Moore A, Waring CP (1998) Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (Salmo salar L.) parr. Pestic Biochem Physiol 62(1):41–50. https://doi.org/10.1006/pest.1998.2366

82. Moore A, Waring CP (2001) The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (Salmo salar L.). Aquat Toxicol 52(1):1–12. https://doi.org/10.1016/S0166-445X(00)00133-8

83. Mustafa A, Rankaduwa W, Campbell P (2001) Estimating the cost of sea lice to salmon aquaculture in eastern Canada. Can Vet J 42(1):54–56
84. Nendick L, Grant A, Gardner M, Sackville M, Brauner CJ, Farrell AP (2009) Swimming performance and associated ionic disturbance of juvenile pink salmon *Oncorhynchus gorbuscha* determined using different acceleration profiles. J Fish Biol 75(7):1626–1638. https://doi.org/10.1111/j.1095-8649.2009.02388.x

85. Nikl DL, Farrell AP (1993) Reduced swimming performance and gill structural changes in juvenile salmonids exposed to 2-(thiocyanomethylthio)benzothiazole. Aquat Toxicol 27(3–4):245–263. https://doi.org/10.1016/0166-445X(93)90057-8

86. Osachoff HL, Osachoff KN, Wickramaratne AE, GunawardaneEK, Venturini FP, Kennedy CJ (2014) Altered burst swimming in rainbow trout *Oncorhynchus mykiss* exposed to natural and synthetic oestrogens. J Fish Biol 85:210–227. https://doi.org/10.1111/jfb.12403

87. Overton K, Samsing F, Oppedal F, Dalvin S, Stien LH, Dempster T (2018) The use and effects of hydrogen peroxide on salmon lice and post-smolt Atlantic salmon. Aquaculture 486:246–252. https://doi.org/10.1016/j.aquaculture.2017.12.041

88. Park A (2013) The biological effects of emamectin benzoate (Slice®) on spot prawn (*Pandalus platyceros*). University of Victoria

89. Peterson RH (1974) Influence of fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). J Fish Res Board Can 31(11):1757–1762. https://doi.org/10.1139/f74-223

90. Philip GH, Anuradha J (1996) Role of phosphatases during transport and energy metabolism in *Labeo rohita* after exposure to cypermethrin. Biomedical Environmental Sciences : BES 9(1):52–59. https://europepmc.org/article/med/8721628

91. Plaut I (2001) Critical swimming speed: Its ecological relevance. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 131(1), 41–50. https://doi.org/10.1016/S1095-6433(01)00462-7

92. PMRA (Pest Management Regulatory Agency) (2014) Proposed Registration Document PRD2014-11: Hydrogen Peroxide. Pest Management Regulatory Agency. Health Canada. Ottawa, ON, 40pp

93. PMRA (Pest Management Regulatory Agency) (2016) *Registration Decision RD2016-18, Hyrodgen Peroxide*. Retrieved 25, November 2018 from https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/registration-decision/2016/hydrogenperoxide-2016

94. PMRA (Pest Management Regulatory Agency) (2017) *Registration Decision RD2017-13, Azamethiphos*. Retrieved 25, November 2018 from https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/registration-decision/2017/azamethiphos-2017

95. Quinn TP, Myers KW (2004) Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. In *Reviews in Fish Biology and Fisheries* (Vol. 14, Issue 4, pp. 421–442). https://doi.org/10.1007/s11160-005-0802-5

96. Rajotte JW, Couture P (2002) Effects of environmental metal contamination on the condition, swimming performance, and tissue metabolic capacities of wild yellow perch (*Perca flavescens*).
97. Rand G, Kleerekoper H, Matis J (1975) Interaction of odour and flow perception and the effects of parathion in the locomotor orientation of the goldfish Carassius auratus L. J Fish Biol 7(4):497–504. https://doi.org/10.1111/j.1095-8649.1975.tb04624.x

98. Reddy VS (2012) Effect of general anesthetics on the developing brain. In Journal of Anaesthesiology Clinical Pharmacology (Vol. 28, Issue 1, pp. 6–10). Wolters Kluwer – Medknow Publications. https://doi.org/10.4103/0970-9185.92426

99. Roth M, Richards RH, Sommerville C (1993) Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. J Fish Dis 16(1):1–26. https://doi.org/10.1111/j.1365-2761.1993.tb00844.x

100. Roy WJ, Sutherland IH, Rodger HDM, Varma KJ (2000) Tolerance of Atlantic salmon, Salmo salar L., and rainbow trout, Oncorhynchus mykiss (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture 184(1–2):19–29. https://doi.org/10.1016/S0044-8486(99)00307-5

101. Saglio P, Olsén KH, Bretaud S (2001) Behavioral and olfactory responses to prochloraz, bentazone, and nicosulfuron-contaminated flows in goldfish. Arch Environ Contam Toxicol 41:192–200. https://doi.org/10.1007/s002440010237

102. Saha S, Kaviraj A (2003) Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. Bull Environ Contam Toxicol 80:49–52

103. Saha S, Kaviraj A (2008) Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. Bull Environ Contam Toxicol 80(1):49–52. https://doi.org/10.1007/s00128-007-9314-4

104. Saito Y, Nishio K, Ogawa Y, Kimata J, Kinumi T, Yoshida Y, Noguchi N, Niki E (2006) Turning point in apoptosis/necrosis induced by hydrogen peroxide. Free Radical Res 40(6):619–630. https://doi.org/10.1080/10715760600632552

105. Sandahl JF, Baldwin DH, Jenkins JJ, Scholz NL (2004) Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (Oncorhynchus kisutch) exposed to copper, chlorpyrifos, or esfenvalerate. Canadian Journal of Fisheries Aquatic Sciences 61(3):404–413. https://doi.org/10.1139/F04-011

106. Scherer E (1975) Avoidance of fenitrothion by goldfish (Carassius auratus). Bull Environ Contam Toxicol 13(4):492–496. https://doi.org/10.1007/BF01721858

107. Schindler D (2003) Pacific salmon and coastal ecology. Front Ecol Environ 1(1):31–37. https://doi.org/10.1890/1540-9295(2003)001[0031:PSATEO]2.0.CO;2

108. SEPA (Scottish Environmental Protection Agency) (1998) SEPA policy on the use of cypermethrin in marine fish farming risk assessment, EQS and recommendations. Policy No. 30

109. Skilbrei O, Espedal P, Nilsen F, Garcia E, Glover K (2015) Evaluation of emamectin benzoate and substance EX against salmon lice in sea-ranched Atlantic salmon smolts. Diseases of Aquatic Organisms 113(3):187–194. https://doi.org/10.3354/dao02832
110. Stephenson RR (1982) Aquatic toxicology of cypermethrin. I. Acute toxicity to some freshwater fish and invertebrates in laboratory tests. Aquat Toxicol 2(3):175–185. https://doi.org/10.1016/0166-445X(82)90014-5

111. Stone J, Sutherland IH, Sommerville CS, Richards RH, Varma KJ (1999) The efficacy of emamectin benzoate as an oral treatment of sea lice, Lepeophtheirus salmonis (Kroyer), infestations in Atlantic salmon, Salmo salar L. J Fish Dis 22(4):261–270. https://doi.org/10.1046/j.1365-2761.1999.00176.x

112. Stone J, Roy WJ, Sutherland IH, Ferguson HW, Sommerville C, Endris R (2002) Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon, Salmo salar L., smolts in freshwater, as a preventative treatment against infestations of sea lice, Lepeophtheirus salmonis (Krøyer). Aquaculture 210(1–4):21–34. https://doi.org/10.1016/S0044-8486(01)00822-5

113. Strungaru SA, Radojković P, Dumitru G, Nicoara M, Plavan GI, Todirascu-Ciornea E (2019) Oxidative stress and changes in swimming performances at zebrafish model (Danio rerio h. 1822) produced by acute exposure to deltamethrin. Journal of Survey in Fisheries Sciences 5(2):121–137. https://doi.org/10.18331/sfs2019.5.2.12

114. Svobodová Z, Lusková V, Drasticová J, Svoboda M, Îlábek V (2003) Effect of Deltamethrin on Haematological Indices of Common Carp (Cyprinus carpio L.). Acta Veterinaria Brno 72:79–85. http://www.vfu.cz/acta-vet/actavet.htm

115. Tarkhani R, Imanpoor MR (2012) Mortality response of Xiphophorus maculatus (Cyprinodontiformes Poeciliidae) to some agricultural pesticides Isolation and Identification of potentially probiotic bacteria from adult Caspian roach intestine and possible effects on roach fry View project Mortality Response of Xiphophorus maculatus (Cyprinodontiformes: Poeciliidae) to Some Agricultural Pesticides. World Journal of Fish Marine Sciences 4(5):512–516. https://doi.org/10.5829/idosi.wjfms.2012.04.05.64100

116. Taylor LN, Mcgeer JC, Wood CM, Gordon Mcdonald D (2000) Physiological Effects of Chronic Copper Exposure to Rainbow Trout (Oncorhynchus Mykiss) in Hard and Soft Water: Evaluation of Chronic Indicators. In Environmental Toxicology and Chemistry (Vol. 19, Issue 9)

117. Thomas CR, Hose GC, Warne MSJ, Lim RP (2008) Effects of river water and salinity on the toxicity of deltamethrin to freshwater shrimp, cladoceran, and fish. Arch Environ Contam Toxicol 55(4):610–618. https://doi.org/10.1007/s00244-008-9147-0

118. Thomaz JM, Martins ND, Monteiro DA, Rantin FT, Kalinin AL (2009) Cardio-respiratory function and oxidative stress biomarkers in Nile tilapia exposed to the organophosphate insecticide trichlorfon (NEGUVON®). Ecotoxicol Environ Saf 72(5):1413–1424. https://doi.org/10.1016/j.ecoenv.2008.11.003

119. Tierney K, Casselman M, Takeda S, Farrell T, Kennedy C (2007) The Relationship Between Cholinesterase Inhibition and Two Types of Swimming Performance in Chlorpyrifos-Exposed Coho Salmon (Oncorhynchus Kisutch). In Environmental Toxicology and Chemistry (Vol. 26, Issue 5). http://www.cerc.usgs.gov/pubs.center/pdfDocs/
120. Tierney KB, Sekela MA, Cobbler CE, Xhabija B, Gledhill M, Ananvoranich S, Zielinski BS (2011) Evidence for behavioral preference toward environmental concentrations of urban-use herbicides in a model adult fish. Environ Toxicol Chem 30(9):2046–2054. https://doi.org/10.1002/etc.588

121. Tierney KB (2016) Chemical avoidance responses of fishes. In Aquatic Toxicology (Vol. 174, pp. 228–241). Elsevier B.V. https://doi.org/10.1016/j.aquatox.2016.02.021

122. Tierney KB, Baldwin DH, Hara TJ, Ross PS, Scholz NL, Kennedy CJ (2010) Olfactory toxicity in fishes. In Aquatic Toxicology (Vol. 96, Issue 1, pp. 2–26). Elsevier. https://doi.org/10.1016/j.aquatox.2009.09.019

123. Tomlin C (ed) (1994) The pesticide manual: A world compendium.10th ed. Incorporating the Agrochemicals handbook. British Crop Protection Council and Royal Society of Chemistry, Thornton Heath, UK

124. Tomlin CDS (ed) (1997) The pesticide manual - A world compendium. British Crop Protection Council, Surrey

125. Tryfonos M, Papaefthimiou C, Antonopoulou E, Theophilidis G (2009) Comparing the inhibitory effects of five prototoxicant organophosphates (azinphos-methyl, parathion-methyl, chlorpyriphos-methyl, methamidophos and diazinon) on the spontaneously beating auricle of Sparus aurata: An in vitro study. Aquat Toxicol 94(3):211–218. https://doi.org/10.1016/j.aquatox.2009.07.003

126. U.S. Environmental Protection Agency (US EPA) (2002) Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Firth Edition. Office of Water. Washington, DC. EPA-821-R-02-012

127. Ural M, Sağlam N (2005) A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (Oncorhynchus mykiss Walbaum, 1792). Pestic Biochem Physiol 83(2–3):124–131. https://doi.org/10.1016/j.pestbp.2005.04.004

128. Van Geest JL, Burridge LE, Fife FJ, Kidd KA (2014) Feeding response in marine copepods as a measure of acute toxicity of four anti-sea lice pesticides. Marine Environmental Research 101(1):145–152. https://doi.org/10.1016/j.marenvres.2014.09.011

129. Velišek J, Jurčíková J, Dobšíková R, Svobodová Z, Piačková V, Máchová J, Novotný L (2007) Effects of deltamethrin on rainbow trout (Oncorhynchus mykiss). Environ Toxicol Pharmacol 23(3):297–301. https://doi.org/10.1016/j.etap.2006.11.006

130. Vera LM, Migaud H (2016) Hydrogen peroxide treatment in Atlantic salmon induces stress and detoxification response in a daily manner. Chronobiol Int 33(5):530–542. https://doi.org/10.3109/07420528.2015.1131164

131. Vijverberg HPM, Van Den Bercken J (1979) Frequency-dependent effects of the pyrethroid insecticide decamethrin in frog myelinated nerve fibres. Eur J Pharmacol 58(4):501–504. https://doi.org/10.1016/0014-2999(79)90325-X

132. Vijverberg HPM, Ruigt GF, van den Bercken J (1982) Structure-related effects of pyrethroid insecticides on the lateral-line sense organ and on peripheral nerves of the clawed frog, Xenopus laevis. Pestic Biochem Physiol 18(3):315–324. https://doi.org/10.1016/0048-3575(82)90072-4
133. Vijverberg HPM, Bercken V, J., & Van Den Bercken J (1990) Neurotoxicological Effects and the Mode of Action of Pyrethroid Insecticides. Crit Rev Toxicol 21(2):105–126. https://doi.org/10.3109/10408449009089875

134. Viran R, Erkoç F, Polat H, Koçak O (2003) Investigation of acute toxicity of deltamethrin on guppies (Poecilia reticulata). Ecotoxicol Environ Saf 55(1):82–85. https://doi.org/10.1016/S0147-6513(02)00096-9

135. Waiwood KG, Beamish FWH (1978) Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (Salmo gairdneri Richardson). Water Res 12(8):611–619. https://doi.org/10.1016/0043-1354(78)90141-0

136. Webb PW (1971) The Swimming Energetics of Trout II. Oxygen Consumption and Swimming Efficiency. In Exp. Biol (Vol. 55)

137. Werner I, Moran K (2008) Effects of pyrethroid insecticides on aquatic organisms. ACS Symposium Series, 991, 310–334. https://doi.org/10.1021/bk-2008-0991.ch014

138. Wood AW, Johnston BD, Farrell AP, Kennedy CJ (1996) Effects of didecyldimethylammonium chloride (DDAC) on the swimming performance, gill morphology, disease resistance, and biochemistry of rainbow trout (Oncorhynchus mykiss). In Can. J. Fish. Aquat. Sci (Vol. 53)

139. Woof L, Kennedy CJ (submitted) The lethal and sublethal effects of anti-sea lice chemotherapeutants in marine benthic invertebrates. Archives of Environmental Contamination and Toxicology

140. Xie X, Gong S, Wang X, Wu Y, Zhao L (2011) Simplified RP-HPLC method for multi-residue analysis of abamectin, emamectin benzoate and ivermectin in rice. Food Additives Contaminants 28(1):19–25. https://doi.org/10.1080/19440049.2010.527377

141. Xuereb B, Lefèvre E, Garric J, Geffard O (2009) Acetylcholinesterase activity in Gammarus fossarum (Crustacea Amphipoda): Linking AChE inhibition and behavioural alteration. Aquat Toxicol 94(2):114–122. https://doi.org/10.1016/j.aquatox.2009.06.010

142. Zitko V, Mcleese DW, Metcalfe CD, Carson WG (1979) Toxicity of Permethrin, Decamethrin, and Related Pyrethroids to Salmon and Lobster. In Bull. Environm. Contam. Toxicol (Vol. 21)

143. Zitko, V., Mcleese, D. W., Metcalfe, C. D., & Carson, W. G. (1979). Toxicity of Permethrin, Decamethrin, and Related Pyrethroids to Salmon and Lobster. In Bull. Environm. Contam. Toxicol (Vol. 21).

Figures
Figure 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 shuttlebox. 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 spent 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.

Figure 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).

Figure 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).

Figure 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).

Figure 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).