The effects of the anti-sea lice chemotherapeutants Salmosan® and Interox® Paramove® 30 on marine zooplankton

by

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Abstract

Sea lice infestations can be harmful to both wild and farmed salmon. The Atlantic salmon aquaculture industry relies on the use of chemotherapeutants to control sea lice outbreaks, which can have both economic and ecological impacts. With treatment, several chemotherapeutants are released directly into the water column, potentially exposing non-target organisms. The lethal and sublethal effects of two anti-sea lice chemotherapeutants, Interox® Paramove® 30 and Salmosan®, were examined in wild zooplankton assemblages, wild brachyuran and porcelain crab zoea, and cultured marine copepods (*Acartia tonsa*). The lowest LC50 values for Interox® Paramove® 30 and Salmosan® of 4 mg/L (CI 4 – 6.9 mg/L) and 54 µg/L (CI 32 – 90 µg/L), respectively) were found for wild zooplankton exposed for 3-h with a 48-h recovery period. The highest Interox® Paramove® 30 LC50 value was 55 mg/L (CI 30 – 95 mg/L) for brachyuran crab zoea using a 1-h exposure, and the highest LC50 value found for Salmosan® was 529 µg/L (CI 333 – 900 µg/L) using a 1-h exposure for wild zooplankton. In terms of sublethal affects, *Acartia tonsa* naupliar development was more sensitive to both chemicals compared to hatching and reproductive success. After exposure to Interox® Paramove® 30 or Salmosan®, the 3-h naupliar development EC50 values were 0.12 mg/L (CI 0.08 – 0.18 mg/L) and 30 µg/L (CI 20 – 41 mg/L), respectively. The least sensitive *Acartia tonsa* endpoint tested was immobility after hatching: eggs exposed for 1-h to Interox® Paramove® 30 had an immobility EC50 value of 7.3 mg/L (CI 3.2 – 72 mg/L). In contrast, Salmosan® had no observable effect after a 1-h exposure of *Acartia tonsa* eggs up to 7500 µg/L. Collectively, these results provide novel toxicity data for two chemotherapeutants to planktonic organisms which will support the safe and appropriate regulation of these aquaculture chemicals in Canada.

Keywords: Aquaculture; sea lice; chemotherapeutants; Salmosan®; zooplankton; Interox® Paramove® 30; zooplankton; toxicity

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List of Acronyms

ACh	Acetylcholine
AChE	Acetylcholinesterase
ANOVA	Analysis of Variance
BC	British Columbia
BHC	Benzene Hexachloride
DDT	Dichlorodiphenyltrichloroethane
EC50	Median Effective Concentration
EMB	Emamectin Benzoate
GluCls	Glutamate-gated Chloride channels
H_2O_2	Hydrogen Peroxide
ISO	International Organization for Standardization
LC50	Median Lethal Concentration
Log K_{ow}	Octanal-water partition coefficient
NOAEC	No Observed Adverse Effects Concentration
OECD	Organization for Economic Cooperation and Development
PMRA	Pest Management Regulatory Agency
SCP	Sodium Carbonate Peroxyhydrate
SW	Seawater
USEPA	United States Environmental Protection Agency

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Chapter 1. Introduction

1.1. Salmon farming in BC

Since the late 1970s, the salmon aquaculture industry has been rapidly growing to meet the global demand for seafood (Naylor et al., 2005; Ford and Meyers, 2008). Atlantic salmon farming produces nearly 60% of the world's harvested salmon, generating approximately $1.4B in revenue annually (Brauner et al., 2012). The largest producers of farmed salmon worldwide are Norway, Chile, and the United Kingdom, followed by Canada (Overton et al., 2018). In Canada an estimated 95% of employment and wages created from aquaculture benefit rural and coastal communities (DFO, 2018a). British Columbia (BC) holds the majority of aquaculture tenures in Canada (Brauner et al, 2012), where wild salmon outnumber farmed salmon by 1000:1 (Beamish et al., 2007).

Farmed salmon are held in open, submerged net pens permitting sea water (SW) to flow through. This allows salmon to remain within their tolerable ranges of abiotic factors such as temperature, pH, and salinity (Rust et al., 2014), and reduces costs related to water quality and waste management. Fish farms in BC culture Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch)*, Chinook salmon (*Oncorhynchus tshawytscha)*, sturgeon (*Acipenserida*e), rainbow trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis niloticus*), and sablefish (*Anoplopoma fimbria*) (DFO, 2017). Of these species, the most commonly farmed in net pens is Atlantic salmon, largely due to their efficient body conversion rate, low maintenance, and adaptability to the limited space in the pens (Statistics Canada, 2018; DFO, 2018).

Open net pen systems are associated with potential ecological risks due to the release of biological and chemical pollutants, as well as escapees. Released biological pollutants include bacteria, viruses, and parasites, as well as fecal waste and food remnants. Chemical pollutants include any chemotherapeutants or other additives in the fish food (antioxidants, dyes), and chemicals used in construction and maintenance of the farm (preservatives, antifoulants), and chemotherapeutants applied directly into net pens to treat the bacteria, viruses, and parasites (Burridge and Van Geest, 2014; Haya et al., 2001). With increased numbers of fish farms, the risk of escaped salmon increases considerably. This may potentially lead to interference with competition for mates, hybridization of Atlantic or Pacific wild salmon species, or increased transmittance of sea lice which has the potential to cause disease outbreaks and population declines (Ford and Meyers, 2008).

1.2. Sea lice

Sea lice are naturally occurring parasites found on fish including wild Atlantic salmon, sea trout, Arctic char (*Salvelinus alpinus*), and all species of pacific salmon (Burridge and Van Geest, 2014). As ectoparasites of fish, sea lice typically attach near the anal fin, or posterior to the dorsal fin resulting in skin erosion and sub-epidermal hemorrhaging (Beamish et al., 2005; Krkošek et al. 2009; Burridge and Van Geest, 2014). Sea lice infections that do not lead to direct mortality can instead lead to physiological and behavioral changes in their salmon hosts. Symptoms of sublethal parasitic infection may include reduced appetite, reduced swimming performance, osmoregulatory dysfunction, slower growth, and premature return to fresh water for anadromous salmonids (Costello, 1993; Wagner et al., 2014). These sublethal effects may eventually lead to indirect mortality as well through predation and disease onset.

In areas without fish farms, sea lice illicit no or few harmful effects on their host due to the extremely low number of parasites (Roth et al., 1993). Unfortunately, Atlantic salmon farming operations may facilitate transmission of sea lice between farmed and wild salmon, increasing onset of outbreaks in wild populations. Transfer of sea lice from farmed to wild salmon is understood to occur through two key exposure routes, specifically from infected escapees or from close swimming proximity of wild salmon to infected net pens (Krkošek et al. 2009). In Norway it has been estimated that escaped Atlantic salmon carry 10x more sea lice than wild Atlantic salmon (Heuch et al., 2005). This results in a need to treat cages repeatedly during the months of high natural transmission, typically March to June, coinciding with the outmigration of wild juvenile salmon (BCSFA, 2017). Sea lice have been found up to 30 km away from fish farms, suggesting that wild salmon swimming within this distance are at risk of becoming infected (Heuch et al., 2005; Krkosek et al., 2005).

There are 12 species of sea lice on the coast of BC, but the most commonly found in salmon farms are *Caligus clemensi* ('the herring louse') and *Lepeophtheirus salmonis* ('the salmon louse') (BCSFA, 2017; Brooks, 2005). *C. clemensi* is a generalist parasite that can be found on salmon but is more frequently found on other species of fish (Marty et al., 2010). *L. salmonis* is only found on salmon species and can be found in both the Pacific and the Atlantic (Marty et al., 2010).

The life cycle of a sea louse involves 10 separate stages, 3 of which are free swimming, followed by 4 parasitic life stages, and ending with 3 mobile stages (Burridge

and Van Geest, 2015; Brauner et al., 2012). After the female's eggs hatch directly into SW from egg strings, the larvae are positively phototactic and move passively as they drift in the water (Stien et al., 2018). The nauplii then molt into the infective copepodid stage (Burridge and Van Geest, 2015). At the length of approximately 0.7 mm and width of 0.3 mm, the sea louse develops the ability to attach to host tissue. When the louse has reached the adult stage, they can then transfer from host to host, and can survive up to 7 d without a host (Bravo et al., 2010). In their final life stage, males will reach approximately 7 mm in length while females can reach over 18 mm in length, with egg strings up to an additional 7 mm (Pike, 1990). Hatching and developing to an adult takes the sea louse 3 to 4 weeks and is a temperature dependant process. A female may produce between 6 and 11 broods in her lifetime, which is up to 210 d (Costello, 2006).

The first formal record of a sea lice infestation on wild salmon was in 1938 on the east coast of Nova Scotia (Torrissen et al., 2013). Atlantic salmon became infected with *L. salmonis* in early summer and the infestation peaked in mid-August where several fish had up to several hundred pre-adult and adult sea lice on them (Saksida et al., 2015). In the late 1980s, several of the first studies on sea lice and cultured salmon were performed. From this research 3 separate sea lice species were identified, including *Caligus curtus, Caligus elongatus* and *L. salmonis* (Burridge and Van Geest, 2014). *L. salmonis* infestations at aquaculture sites had not been common in BC and were not considered a serious health concern until 2003; sea lice were only reported if there were serious health risks to the fish (Roth et al., 1993). Routine monitoring and reporting of sea lice on Atlantic salmon farms is now a standard practice in Canada. Fish farms are expected to report sea lice and treatment details to government authorities on a monthly basis (Overton et al., 2018).

1.3. Sea lice management

1.3.1. Non-chemical methods

Attempts to control sea lice on fish farms have been in place since 1926. Previously implemented measures include adding turpentine and kerosene into the net pens or suspending bags of garlic or onions in the water (Roth et al., 1993). In recent years, preventative measures have been put into place to decrease the likelihood of sea lice infestations. These include consistent monitoring, site fallowing, and infrastructure modifications, and its further actions are required, chemical or non-chemical measures can be taken. Current non-chemical methods for sea lice control include mechanical and

thermal delousing, as well as the use of “cleaner fish” that eat the lice (Gonzalez et al., 2017). All three methods have limited published data on their efficacy but have shown potential in reducing the use of chemotherapeutants to control sea lice populations (Overton et al., 2018).

Currently, 3 mechanical delousing systems have been developed non-commercially as Flatsetsund Engineering AS, SkaMik AS, and the Hydrolicer® (Overton et al., 2018). All 3 machines require fish to be pumped into a treatment system where sea lice are mechanically removed from the fish. The Flatsetsund Engineering AS system pumps the fish through two low pressure washers that remove 81 to 100% of the mobile stages of sea lice, and 50 to 70% of attached sea lice from salmon (Overton et al., 2018). The SkaMik system uses a similar method, however it includes brushes that steer the salmon through the system. The SkaMik system has been reported to remove 85-95% of sea lice from salmon (Overton et al., 2018). The Hydrolicer® system pumps fish into a closed pipe filled with water, where inverse turbulence is used to ‘vacuum’ lice from fish. The Hydrolicer® system has been reported to remove 82-100% of mobile stages of sea lice (Overton et al., 2018). Negative impacts on fish observed with all 3 of these methods include the loss of scales, gill bleeding and wounds, as well as mortality (Hjeltnes et al. 2018).

Two systems of thermal delousing have also been developed and include the Thermolicer® and the Optilicer®. Both systems involve moving the salmon from the net pen into warm water (30 to 34° C) for 20 to 30 sec before returning the salmon back to the net pen (Overton et al., 2018). The differentiating factor between the two systems is that the Thermolicer® relies only on pumps to move the fish into the warmer water, whereas the Optilicer® also incorporates paddles which push the fish at a pre-set speed. The Thermolicer® removes 75-100% of mobile stages (Grøntvedt et al., 2015), while the Optilicer® can remove up to 99% of mobile stages (Roth, 2016). While effective against mobile stages, these methods are ineffective at removing attached sea-lice (Overton et al., 2018).

A third method of removing sea lice from fish involves the addition of “cleaner” fish to the net pens. Species used include wrasses (Labridae sp.), which are most commonly used at water temperatures above 10°C, and for cultivated fish at colder temperatures, lumpfish (*Cyclopterus lumpus*) are most commonly used; however, limited published literature exists that documents the details of their efficacy. The symbiotic cleaning behavior of wrasse was first identified by Geoff Potts in 1973, but “cleaner” fish weren’t

put into practical use to remove sea lice until goldsinny wrasse (*Ctenolabrus rupestris*) were used in salmon farms in Norway in 1988 (Bjordal, 1988). Just one goldsinny wrasse has proven to be effective in controlling the effects of sea lice for up to 150 salmon at fish farms in Norway (Blanco and de Boer, 2017). While found to be largely successful, the wrasse population can be difficult to maintain in a net pen due to escapees, salmonid predation, and high female mortality during sensitive spawning seasons (Blanco and de Boer, 2017).

1.3.2. Anti-sea lice chemotherapeutants

While non-chemical measures can be used to treat sea lice infestations to some success, the use of chemotherapeutants is the most commonly used treatment measure. Health Canada regulates chemotherapeutant use in the Canadian aquaculture industry; chemotherapeutants are considered either a drug (if administered in feed or by injection) or a pesticide (if administered topically or in a water bath). Net pens are usually treated repeatedly during the months of high infestation, which are typically March to June, coinciding with the outmigration of wild juvenile pacific salmon (BCSFA, 2017). Regulation of each chemotherapeutant is determined by assessing environmental fate and toxicity data which is submitted by registrants to determine if the level of risk to the environment or humans is acceptable (PRMA, 2016). Health Canada may bypass regulatory assessment if an emergency infestation occurs, and the approved chemicals are deemed unacceptable or ineffective due to sea lice resistance. For a summary of chemotherapeutant use in Canada including years in use and dosing regimens, refer to Tables 1 and 2.

Table 1. A history of the chemotherapeutants used in Canada.

Year(s) in use in Canada	Chemotherapeutant
1994-2000	Ivermectin®
1994-2000	Salartect®
1995-2002	Salmosan®
1999-2009	SLICE®*
2009-2010	Alphamax®
2009-current	Salmosan®, Interox® Paramove® 30 , SLICE®*

*SLICE® has been in use since 1999 however was discontinued on the east coast of Canada in 2009 (Burridge and Van Geest, 2014).

Table 2. A summary of chemotherapeutants and their treatment information (Roth et al., 2013; Burridge et al., 2014; Burridge and Geest, 2014).

Chemotherapeutant Formulation	Active Ingredient	Life stage impact	Concentration	Exposure Time
Ivermectin®	Ivermectin®	All	100 µg/kg bw	Fed 2x weekly
Salartect®	H_2O_2	Mobile	500 mg/L	40 min
Salmosan®	Azamethiphos	Mobile	100 µg/L	60 min
SLICE®*	EMB	All	50 µg/kg bw	Daily for 7 d
Alphamax®	Deltamethrin	All	2 µg/L	40 min
Excis®	Cypermethrin	All	5 µg/L	60 min
Paramove®30	H_2O_2	Mobile	1200 to 1800 mg/L	40 min

In use from 1994 to 2000, ivermectin and the pesticide Salartect® (active ingredient hydrogen peroxide) were the first chemotherapeutants used to treat sea lice infestations on fish farms in Canada (Burridge et al., 2010). Ivermectin is an in-feed treatment that binds to glutamate-gated chloride channels (GluCls) in the membranes of nerve and muscle cells (increasing the permeability to chloride). The influx of chloride ions causes hyperpolarization in nerves, which leads to the disruption of nerve impulses, and is followed by paralysis and death (Burridge and Van Geest, 2014) in invertebrates. Ivermectin was widely used but has a very low margin of safety in fish (Saksida et al, 2015). Hydrogen peroxide (H_2O_2) is the active ingredient of Salartect® (and Interox® Paramove® 30) and is known to temporarily paralyze sea lice, causing them to fall off the host tissue and float to the surface. The mechanism of action (MOA) of H_2O_2 is not entirely understood, however the majority of evidence indicates that H_2O_2 induces temporary mechanical paralysis in sea lice by creating bubbles in the gut and haemolymph (Burridge et al., 2014). Other suggested MOAs include lipid peroxidation of cellular organelle membranes, inactivation of enzymes and DNA replication, and mechanical paralysis (Cotran et al., 1989.). H_2O_2 is a strong oxidizing agent and is toxic to living cells. During the oxidation reaction, H_2O_2 attracts electrons from proteins, membrane lipids, DNA, and in the case of plants and bacteria, cell walls, thus impairing and potentially destroying them (Hassan et al., 1979). Salartect® was found to be far less effective at controlling increasing sea lice infestations compared to ivermectin, so was rarely used (Burridge and Van Geest, 2014).

Salmosan® (active ingredient azamethiphos) was in used from 1995 until 2002, and then reapproved in 2009. As an organophosphate, azamethiphos interferes with the nervous system *via* phosphorylation and subsequent acetylcholinesterase (AChE)

inhibition (Xuereb et al., 2009). AChE hydrolyses acetylcholine (ACh) into choline and acetic acid. Hydrolysis of acetylcholine allows neurons to return to their resting state after activation (Colovic et al., 2013). When AChE is inhibited, acetylcholine builds up at neural synapses, causing neurons to repeatedly fire and become insensitive to new signals (Burridge and Van Geest, 2014; ; Weinbroum, 2004). Symptoms of the affected organism include agitation, spasming, paralysis and direct or indirect death (Burridge and Van Geest, 2014; Xuereb et al., 2009). The direct cause of death is caused by hypoxia, due to the inability to circulate oxygen, followed by cardiac arrest and death (Dounia et al., 2016). An indirect cause of death is paralysis, which may lead to death by inhibiting the ability to forage or escape from predators (Xuereb et al., 2009). Crustaceans may be particularly sensitive to the effects of AChE inhibition because in many species ACh is their primary sensory neurotransmitter (Florey, 1973).

Following the introduction of Salmosan® in 1995, SLICE® (active ingredient emamectin benzoate [EMB]), began to be used in 1999 until 2009 on the east coast of Canada and is still the most commonly used treatment on the west coast of Canada (Burridge and Van Geest, 2014). SLICE® is an in-feed treatment that is absorbed in the gut of the fish, distributed throughout the skin, and finally ingested by the sea lice (Burridge et al., 2010). EMB modulates specific glutamate and gamma aminobutyric acid-gated anion channels and allows an influx of chloride ions. This results in hyperpolarisation, leading to the disruption of nerve impulses, and eventually paralysis and death.

In 2009, both AlphaMax® (AI: deltamethrin) and Interox® Paramove® 30 (AI: hydrogen peroxide; see above MOA for Salartect®) were introduced. Deltamethrin is a pyrethroid, which interacts with sodium channels, causing depolarization of nerve endings, and interfering with nerve membrane function (Miller and Adams, 1992). AlphaMax® was used under emergency registration in Canada from 2009 to 2010 but was discontinued as it showed potential to adversely impact non-target species below recommended treatment concentrations (Burridge and Van Geest, 2014).

The reliance on anti-sea lice chemotherapeutants over the last 20 years has led to some sea lice resistance in heavily treated areas in Canada (Lees et al. 2008; Aaen et al. 2015). When sea lice develop a resistance to chemotherapeutants the risk to the salmon increases immensely, not only because of the direct effects of the sea lice, but also because of the necessary increased frequency in treatments (Helgesen et al., 2014). Resistance to azamethiphos is not widespread but was identified in populations in Canada in 2002 (Aaen et al., 2015). At this time, Canada discontinued the use of Salmosan® and

relied solely on SLICE® until 2009, when Salmosan® was brought back into use (Table 1). SLICE® is still effective at treating sea lice on the west coast of Canada, however there is cause for concern due to resistance shown in Chile in 2005, and on the east coast of Canada in 2009 (Bravo, 2009, Burridge et al., 2010). While only used briefly in Canada, pyrethroids such as deltamethrin have also resulted in evidence of resistance in Norway in 2000 (Sevatadal and Horsberg, 2003). Relative to other anti-sea lice pesticides, Paramove® 30 has encountered considerably less sea lice resistance. The first incident of sea lice resistance to H_2O_2 was reported in Scotland in 2000 (Treasurer et al., 2000), and again in 2015 in Norway (Helgesen et al., 2015). It has been noted that in net pens that have been treated with Paramove® 30 multiple times, the pesticide has less efficacy, indicating that the development of resistance may occur over time (Treasurer et al., 2000).

1.3.3. Salmosan®

The anti-sea lice chemotherapeutant Salmosan® is an organophosphate formulation composed of 47.5% active ingredient (AI) azamethiphos and 52.5% proprietary ingredients (Burridge et al., 2010) Azamethiphos has a log-octanol-water partition coefficient (log K_{ow}) of 1.05, indicating that it is unlikely to bioaccumulate and adhere to organic matter. Additionally, due to its high solubility in water (1.1 g/L) (Tomlin, 1997), azamethiphos is likely to remain in the aqueous phase upon entering the environment, therefore the rate of dilution following application will likely determine its environmental concentration. In seawater, azamethiphos has a half-life of approximately 8.9 d, and decomposes by hydrolysis and photolysis (Burridge and Van Geest, 2014).

Salmosan® is applied either in a well-boat, or directly into the net pens. For the well-boat method, salmon are pumped into chambers aboard a specialized vessel before being treated. Salmon typically show a low bioaccumulation towards organophosphates as well as fast depuration times, resulting in little to no effect on the farmed salmon itself (Burridge et al., 2010). After fish are returned to the net pens, the pesticide exposed water can then be released at a different location. If applied directly to the net pen, one or more water-proof tarps or skirts is used to enclose the pen before application of the pesticide. In both methods, Salmosan® is directly released into the water column after use (Burridge et al., 2014).

The target concentration recommended by Salmosan Vet® for effective sea lice treatment is 100 µg/L azamethiphos for 30 to 60 min; the time of treatment is dependent on the temperature of the water (Burridge and Van Geest, 2014). Based on the target

concentration, Burridge et al. (2000) used a mathematical approach to estimate the range of concentrations in sea water within 0–10 m of net pens to be between 0.1 to 10 µg/L azamethiphos within the first 3 h of release (Burridge et al., 2000). Using the same target concentration of 100 µg/L, one dispersion study done by Ernst et al (2014), reported an azamethiphos concentration of 10 µg/L within 10 m of a fish farm following the release of Salmosan® at an undisclosed time after release.

While Salmosan® is effective in removing >85 % of adult and pre-adult lice, it is ineffective against larval stages or egg strings of sea-lice (Roth et al., 1993; Bravo et al., 2015).

1.3.4. Azamethiphos toxicity

The effects of azamethiphos have been examined in a wide variety of organisms, particularly crustaceans, which may have similar sensitivities to pesticides as sea lice due to their physiological similarities (Table 3). The brine shrimp (*Artemia salina*) is the most tolerant crustacean reported, with a 24-h LC50 value >10,000 µg/L (Ernst et al., 2001). The majority of known lethal thresholds for crustaceans, however, are much lower. In the mid-range of toxicity is the Southern rock crab (*Metacarcinus edwardsii*) with a 30-min LC50 of 2.85 µg/L (Gebauer et al., 2017). The lowest toxicity value available for a crustacean was the water flea *Daphnia magna* which has a 24-h LC50 value of 0.167 µg/L (Rose et al., 2016).

The majority of toxicity studies investigating the effects azamethiphos have been performed on the American lobster (*Homarus americanus*) due to its sensitivity and its economic importance on the east coast of Canada. Toxicity values indicate that adults are the most sensitive life stages, with the least sensitive being the larval stage one, and toxicity increasing with each life stages (Table 3) (Burridge et al., 1999; Burridge et al., 2014; Pahl and Opitz, 1999). For example, 24-h LC50 value for Salmosan® in *H. americanus* larval stage I is 8.9 µg/L, while the 48-h LC50 value is 3.57 µg/L (Burridge et al., 1999; Burridge et al., 2014). In comparison, the adult *H. americanus* 24-h LC50 value is 2.8 µg/L, while the 48-h LC50 value is 1.39 µg/L (Burridge et al., 2014).

The lethal toxicity values for non-crustaceans are limited but exist for various species of fish, as well as microscopic marine organisms. The highest reported toxicity values were for the bacterium *Vibrio fischeri*, with a 15-min LC50 of 11,000 µg/L, and the rotifer *Branchionus plicatilis*, which had a 24-h LC50 >10,000 µg/L (Ernst et al., 2001). In the mid-range of toxicity values were Atlantic salmon, with a 1-h LC100 value of 5000 µg/L

and a 1-h LC15 of 1000 µg/L. The lowest reported toxicity value specific to fish were for the rainbow trout, (*Onchorhynchus mykiss*), with a 2-h LC100 value of 100 µg/L (Intorre et al., 2004) (Table 3).

A more direct comparison of lethal thresholds can be made using similar exposure times, temperatures, and endpoints. The following are the 1-h LC50s generated from studies ranging from 8 to 14 °C in temperature, ranked from least to most sensitive. The general planktonic copepods have an LC50 of >500 µg/L, while the larval stage I American lobsters (*Homarus Americanus*) have an LC50 of >86.5 µg/L, and the shrimps *Crangon septemspinosa* and *Mysid sp.* share an LC50 of >85.5 µg/L, (Burridge et al., 2014; Van Geest et al., 2014). Lastly, the target species, pre-adult and adult sea lice (*L. salmonis*), are the most sensitive organisms to azamethiphos, with a 1-h LC50 ranging from 0.07 to 0.18 µg/L (Roth et al., 1996).

Sublethal effects associated with azamethiphos include feeding inhibition, a decrease in AChE activity (brain, hemolymph, abdominal), delayed development, decrease in spawning, effects on hepatosomatic and gonadosomatic indices, change in lipid and water content in hepatopancreas and decreased fertilization. Similar to lethal studies, the majority of sublethal toxicity studies for crustaceans are on *H. americanus*. Much of the research on this species utilized pulse exposure scenarios, which subject the organisms to repeated short duration exposures. Among the most sensitive endpoints for *H. americanus* was a decrease in abdominal AChE activity in adult males, with an EC50 of 0.5 µg/L after 5 sets of 1-h pulse exposures at 0, 4, 28, and 48 h (Dounia et al., 2016). One of the least sensitive *H. americanus* endpoints tested was the effect on spawning incidence, which resulted in an EC50 of 10 µg/L after 4 sets of 1-h pulse exposures to Salmosan® (Table 4).

The sublethal toxicity values for non-crustaceans exposed to azamethiphos is sparse, however multiple species have been investigated. The least sensitive endpoint tested was decreased fertilization for the sea urchin (*Lytechinus pictus*), which had a 20 min EC50 of 6.84 mg/L (Ernst et al., 2001). In the mid sensitivity range were endpoints for the blue mussel (*Mytilus edulis*), including reduced AChE activity and feeding inhibition for times ranging from 30 min to 24 h, and EC50s all >100 µg/L. The most sensitive endpoint found was for decreased brain AChE activity for rainbow trout (*O. mykiss*), European bass (*Dicentrarchus labrax)*, and European eel (*Anguilla Anguilla*), which all shared 2-h EC50s of 100 µg/L (Intorre et al., 2004).

Table 3. Summary of the lethal toxicity of azamethiphos to aquatic species.

Species	Life stage	Measured or Nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Azamethiphos µg/L (95%CI)	Reference
Crustaceans								
Artemia salina	-	-	F	24-h	15	LC50	> 10 000	(Ernst et al., 2001)
Copepod (various)	-	M	F	1-h	9	LC50	> 500	(Van Geest et al., 2014)
Crangon septemspinosa	-	M	F	24-h	8-14	LC50	191	(Burridge et al., 2014)
Crangon septemspinosa	-	M	F	1-h	8-14	LC50	> 85.5	(Burridge et al., 2014)
Crangon septemspinosa	-	M	F	24-h	13-15	LC50	4.8 – 33.6	(Ernst et al., 2014)
Daphnia magna	-	N	AI	24-h	19-21	LC50	0.167	(Rose et al., 2016)
Eohaustorius estuarius	-	-	F	48-h	14-16	LC50	> 20	(Ernst et al., 2001)
Eohaustorius estuarius	-	-	F	48-h	14-16	LC50	3.0	(Ernst et al., 2001)
Homarus americanus	Larval stage I	M	F	12-h	10-12	LC50	0.90 – 1.33	(Pahl and Opitz, 1999)
Homarus americanus	Larval stage I	M	F	6-h	10-12	LC50	3.50 – 5.40	(Pahl and Opitz, 1999)
Homarus americanus	Larval stage I	M	F	1-h	10-12	LC50	20.70 – 26.50	(Pahl and Opitz, 1999)
Homarus americanus	Larval stage I	M	F	30-min	10-12	LC50	27.01 – 37.70	(Pahl and Opitz, 1999)
Homarus americanus	Larval stage I	M	F	5-min	10-12	LC50	33.90 – 50.40	(Pahl and Opitz, 1999)
Homarus americanus	Larval stage I	M	F	48-h	14-16	LC50	3.57	(Burridge et al., 1999)
Homarus americanus	Larval stage I	M	F	24-h	8-14	LC50	8.9	(Burridge et al., 2014)
Homarus americanus	Larval stage I	M	F	1-h	8-14	LC50	> 86.5	(Burridge et al., 2014)
Homarus americanus	Larval stage II	M	F	48-h	14-16	LC50	1.03	(Burridge et al., 1999)
Homarus americanus	Larval stage III	M	F	48-h	14-16	LC50	2.29	(Burridge et al., 1999)
Homarus americanus	Larval stage IV	M	F	48-h	14-16	LC50	2.12	(Burridge et al., 1999)
Homarus americanus	Adult	M	F	48-h	11-14	LC50	0.61 – 3.24	(Burridge et al., 2005)
Homarus americanus	Adult	M	F	48-h	14-16	LC50	1.39	(Burridge et al., 1999)
Homarus americanus	Adult	M	F	24-h	8-14	LC50	2.8	(Burridge et al., 2014)
Homarus americanus	Adult	M	F	1-h	8-14	LC50	24.8	(Burridge et al., 2014)
Homarus americanus	Male adult	M	F	5x1-h at t = 0, 4, 24, 28, 48-h	10-12	93% mortality	5	(Dounia et al., 2016)
Gammarus spp	-	-	F	96-h	14-16	LC50	< 5	(Ernst et al., 2001)
Homarus americanus	Male adult	M	F	5 µg/L	10-12	LT50	26-h	(Dounia et al., 2016)

Species	Life stage	Measured or Nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Azamethiphos µg/L (95%CI)	Reference
Homarus americanus	Adult	M	F	3x1-h at t = 0, 4, 24 10-d + simulated stress	11-13	33% mortality	0.061	(Couillard and Burridge, 2014)
Lepeophtheirus salmonis	Adult and pre-adult	N	F	1-h	7.8-10	LC50	0.07 – 0.18	(Roth et al., 1996)
Metacarcinus edwardsii	Zoea I	N	F	30-min	15	LC50	2.85	(Gebauer et al., 2017)
Metacarcinus edwardsii	Zoea I	N	F	30-min	15	LC50	0.94	(Gebauer et al., 2017)
Mysid sp.	-	M	F	24-h	8-14	LC50	12.5	(Burridge et al., 2014)
Mysid sp.	-	M	F	1-h	8-14	LC50	> 85.5	(Burridge et al., 2014)
Mysis stenolepsis	-	M	F	24-h	13-15	LC50	4.8 – 23.5	(Ernst et al., 2014)
Tisbe battagliai	Copepodid	M	AI	48-h	18-22	LC10	3.6	(Macken et al., 2015)
Tisbe battagliai	Copepodid	M	AI	48-h	18-22	LC50	7.7	(Macken et al., 2015)
Tisbe battagliai	Nauplius	M	AI	48-h	18-22	LC10	3.4	(Macken et al., 2015)
Tisbe battagliai	Nauplius	M	AI	48-h	18-22	LC50	6.7	(Macken et al., 2015)
Fish	-	-						
Gasterosteus aculeatus			F	96-h	14-16	LC50	190	(Ernst et al., 2001)
Salmo salar	Adult	N	F	1-h	15	15% mortality	1000	(Sievers et al., 1995)
Salmo salar	Adult	N	F	1-h	15	100% mortality	5000	(Sievers et al., 1995)
Anguilla anguilla	Adult	N	AI	240-min	17-19	LC50	> 100	(Intorre et al., 2004)
Dicentrarchus labrax	Adult	N	AI	240-min	17-19	LC50	> 100	(Intorre et al., 2004)
Onchorhynchus mykiss	Adult	N	AI	120-min	11-13	LC50	> 100	(Intorre et al., 2004)
Onchorhynchus mykiss	Adult	N	AI	240-min	11-13	100% mortality	100	(Intorre et al., 2004)
Other								
Branchionus plicatilis	Juvenile	-	F	24-h	14-16	LC50	> 10 000	(Ernst et al., 2001)
Polydora cornuta			F	96-h	14-16	LC50	2310	(Ernst et al., 2001)
Strongylocentrotus droebachiensis	Adult	-	F	96-h	14-16	LC50	> 1000	(Ernst et al., 2001)
Vibrio fischeri	-	-	F	15-min	14-16	LC50	11000	(Ernst et al., 2001)

Notes: M=measured; N= nominal; F= formulation; AI= active ingredient; LC50= median lethal concentration; EC50= median effects concentration; CI= confidence interval; - = No value.

Table 4. Summary of the sublethal toxicity of azamethiphos to aquatic species.

Species	Life stage	Measured or nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Azamethiphos µg/L	Reference
Crustaceans								
Copepod (various)	various	M	F	1-h	9	EC50 Feeding inhibition	> 500	(Van Geest et al., 2014)
Crangon septemspinosa	-	M	F	24-h	8-14	LOEC	1.20	(Burridge et al., 2014)
Homarus americanus	Juvenile	N	AI	3x10-min 1-h intervals	9-11	EC50 Shelter exit	< 1000	(Abgrall et al., 2000)
Homarus americanus	Larval stage I	M	F	1-h	8-14	LOEC	11.1	(Burridge et al., 2014)
Homarus americanus	Larval stage IV	M	F	9x120-min over 3-d at 3-h intervals	14-16	NOEC	1.03	(Burridge et al., 2000b)
Homarus americanus	Male adult	M	F	5x1-h at t = 0, 4, 24, 28, 48-h	10-12	Decrease in abdominal AChE activity	0.5	(Dounia et al., 2016)
Homarus americanus	Male adult	M	F	5x1-h at t = 0, 4, 24, 28, 48-h	10-12	Increase in hemolymph [lactate]	5	(Dounia et al., 2016)
Homarus americanus	Male adult	M	F	5x1-h at t = 0, 4, 24, 28, 48-h	10-12	Increase in hemolymph [calcium]	5	(Dounia et al., 2016)
Homarus americanus	Female adult	N	F	4x1-h 2-wk intervals	12-14	48% decrease in spawning incidence	10	(Burridge et al., 2008)
Homarus americanus	Adult	M	F	10-d + Subjected to shipping stress	11-13	Effects on hepatosomatic and gonadosomatic indices; lipid and water content in hepatopancreas	0.061	(Couillard and Burridge, 2014)
Homarus americanus	Adult	M	F	1-h	8-14	LOEC	3.70	(Burridge et al., 2014)

Species	Life stage	Measured or nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Azamethiphos µg/L	Reference
Homarus americanus	Male adult	M	F	3x1-h at t = 0, 4, 24-h	10-12	NOEC	0.5	(Dounia et al., 2016)
Tisbe battagliai		M	AI	7-d	18-22	EC50 Development	> 3.6	(Macken et al., 2015)
Fish								
Anguilla anguilla	Adult	N	AI	240-min	17-19	Decreased brain AChE activity (with recovery)	100	(Intorre et al., 2004)
Dicentrarchus labrax	Adult	N	AI	240-min	17-19	Decreased brain AChE activity (with recovery)	100	(Intorre et al., 2004)
Onchorhynchus mykiss	Adult	N	AI	120-min	11-13	Decreased brain AChE activity (with recovery)	100	(Intorre et al., 2004)
Onchorhynchus mykiss	Adult	N	AI	240-min	11-13	Decreased brain AChE activity (no recovery)	100	(Intorre et al., 2004)
Other								
Lytechinus pictus	-	-	F	20-min	14-16	EC50 Fertilization	6840	(Ernst et al., 2001)
Lytechinus pictus	-	-	F	20-min	14-16	EC25	3340	(Ernst et al., 2001)
Mytilus edulis	-	N	AI	30-min *in vitro*	14-16	EC50 AChE activity (gill)	736	(Canty et al., 2007)
Mytilus edulis	-	N	AI	30-min *in vitro*	14-16	EC50 AChE activity (hemolymph)	1300	(Canty et al., 2007)
Mytilus edulis	-	N	AI	1-h	14-16	EC50 Feeding rate	> 100	(Canty et al., 2007)
Mytilus edulis	-	N	AI	24-h	14-16	EC50 Feeding rate	> 100	(Canty et al., 2007)
Mytilus edulis	-	N	AI	1-h	14-16	EC50 Phagocytic activity	> 100	(Canty et al., 2007)
Mytilus edulis	-	N	AI	24-h	14-16	91% inhibition of phagocytic activity	> 100	(Canty et al., 2007)

Notes: M=measured; N= nominal; F= formulation; AI= active ingredient; LC50= median lethal concentration; EC50= median effects concentration; CI= confidence interval; - = No value.

1.3.5. Interox® Paramove® 30

The chemotherapeutant Interox® Paramove® 30 (hereby called Paramove® 30) is a formulation composed of 30% of the active ingredient of hydrogen peroxide (H_2O_2). This formulation is also commercially available as Interox® Paramove® 50, composed of 50% H_2O_2. H_2O_2 is completely miscible in water and is likely to remain in the aqueous phase, therefore the rate of dilution will determine its environmental concentration (Burridge and Van Geest, 2014). In seawater, H_2O_2 has a half-life from 1 to 7 d (depending on formulation) before dissociating into water and oxygen (Kiemer and Black, 1997). H_2O_2 has a log K_{OW} of -1.5, indicating that it is unlikely to bioaccumulate (Health Canada, 2014).

Similar to the application of Salmosan®, Paramove® 30 is administered either in a well-boat, or directly into the net pens. If applied directly to the net pen, an additional step is required in which the paralyzed sea lice floating on the surface of the water must be removed (Burridge et al., 2014). In a bath treatment, the target concentration of Paramove® 30 is a range of 1200-1800 mg/L for 40 min, however the therapeutant may not be effective below 10˚C. This is because at lower temperatures the rate of H_2O_2 decomposition is lowered, resulting in decreased oxidation reactions and cell toxicity (Treasurer et al., 2000). Predicted concentrations in the water column surrounding the net pens immediately following release of Paramove® 30 from the net pens range from 14 to 270 mg/L (PMRA, 2014). To prevent resistance, a minimum of 7 d is required between applications, and no more than 50 applications per year is permitted (Solvay, 2015; PMRA).

The sensitivity of H_2O_2 varies depending on the life stage of the sea louse. While Paramove® 30 is typically effective in removing >85 % of adult and pre-adult lice, it is not effective against larval stages of sea-lice (Roth et al., 1993). Exposure to H_2O_2 results in reduced egg string viability however once hatched, H_2O_2 is ineffective towards larval stages of sea lice (Johnson et al., 1993; Mitchell and Collins, 1997; McAndrew et al., 1998). In the case of mobile sea lice, 85 to 100% of organisms may be effectively removed. If adult sea lice survive treatment a reduced ability to attach to the host has been observed (Bruno and Raynard, 1994).

H_2O_2 can also be used for biological purposes beyond sea lice management. In an effort to reduce fungal and bacterial infections, H_2O_2 is commonly used to disinfectant fish eggs in aquaculture and is registered in Canada by PMRA for this purpose (Rach et al., 1997). H_2O_2 is a superior disinfectant compared to competition such as formalin due to its

lack of odor and toxicity, and the dissociative ability of H_2O_2 to break into oxygen and water. H_2O_2 is also used as the active ingredient in sodium carbonate peroxyhydrate (SCP), a relatively new algaecide. H_2O_2 oxidizes algal cells causing intracellular and cell membrane damage that leads to cell death (Geer, 2016). Application of H_2O_2 has also been used as an oxygen source during the transport of fish (Taylor and Ross, 1988).

1.3.6. Hydrogen peroxide toxicity

The effects of H_2O_2 have been tested on a number of organisms, notably crustaceans and a variety of fish (Table 5). In the available literature, only two toxicity values for the American lobster can be found. Stage 1 *H. americanus* had an LC50 of 1.64 g/L, while the adult had a 1-h LC50 of >3.75 g/L (Burridge et al., 2014). The highest toxicity value for a crustacean was for the shrimp *Crangon septemspinosa* with a 1-h LC50 value of 3.18 g/L (Burridge et al., 2014). In the mid-range of toxicity values is the brine shrimp, with 24-h and 96-h LC50s (*Artemia salina*) of 800 mg/L and 168 mg/L, respectively (Matthews,1995; Smit et al., 2008). The lowest toxicity value available for a crustacean is for the water flea *Daphnia pulex* which has a 48-h LC50 of 2 mg/L (Shurtleff, 1989) (Table 5).

The majority of toxicity studies investigating the effects Paramove® 30 on non-crustaceans have been performed on Atlantic salmon, as there is evidence of a narrow margin between the prescribed treatment concentration and the concentration that can harm farmed fish. The highest toxicity value available is an EC50 for Atlantic salmon at approximately 2.38 g/L H_2O_2 (Mitchell and Collins, 1997). Mid-range toxicity values were for those of the early life stages of rainbow trout (*Onchorhynchus mykiss*) which were found to exhibit signs of stress and gill damage at >1 g/L H_2O_2, while larger and older fish were more sensitive (< 500 mg/L) (Rach et al., 1997). This is suggested to be because of the larger gill surface area in older fish. Of numerous fish tested, including *O. mykiss* and the brown trout, *Salmo trutta*, walleye (*Stizostedion vitreum*) were found to be the most sensitive, exhibiting stress, gill damage, and death at just 100 mg/L after a 45 min exposure to H_2O_2.

For a more direct comparison of lethal thresholds among species, the following 1-h LC50s were selected due to their similar range of habitat temperature, which is 8 to 14 °C. The least sensitive was the shrimp *C. septemspinosa* with 3182 mg/L, followed by the freshwater fish *Lepomis macrochirus* with 2560 mg/L (Rach et al., 1997; Burridge et al., 2014). In the middle range are the lobster *H. americanus* (larval stage 1) at 1637 mg/L

(Burridge et al., 2014), and the rainbow trout, *O. mykiss*, at 1.26 g/L (Burridge et al., 2014). The most sensitive 1-h LC50 was for the shrimp, *Mysid sp.* at 973 mg/L (Burridge et al., 2014).

Sublethal effects associated with H_2O_2 include a decrease in aerobic and metabolic rate and intracellular pH, development rate, immobility, reduced ability of sea lice to attach to the host, and gill damage. Sublethal H_2O_2 toxicity studies are currently unavailable for non-crustaceans, but those for crustaceans include the caridean shrimp *Crangon crangon* and two species of sea lice, *C. rogrecresseyi* and *L.* salmonis. The highest toxicity values were for adult *L. salmonis* with a 30 min EC50 of 890 mg/L, closely followed by this is the adult *L. salmonis* 20 min EC50 of 800 mg/L (Thomassen, 1993; Bruno and Raynard, 1994). In the mid-ranges of toxicity values were the *C. crangon* 5-h EC50 (decrease in aerobic rate and intracellular pH) of 680 mg/L (Abele-Oeschger et al., 1997), while the 20 min EC50 of *C. rogrecresseyi* eggs after exposure to H_2O_2 was 710 mg/L. The same exposure to adult *C. rogrecresseyi* was 700 mg/L (Marin et al., 2017). The lowest toxicity value recorded was for pre- adult *L. salmonis* with a 30 min EC50 of 503 mg/L (Table 6).

Table 5. Summary of the lethal toxicity of hydrogen peroxide to aquatic species in seawater and freshwater (**).

Species	Life stage	Measured or Nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Hydrogen peroxide g/L (95%CI)	Reference
Crustaceans								
Artemia salina	-	N	AI	24-h		LC50	0.8	(Matthews, 1995)
Artemia salina	Adult	-	AI	96-h	25	LC50	0.168	(Smit et al., 2008)
*Daphnia carinata***	Adult	-	AI	48-h	21	LC50	0.0057	(Reichwaldt et al., 2012)
Daphnia pulex	-	-	AI	48-h		LC50	0.0024	(Shurtleff, 1989)
C. septemspinosa	-	N	AI	1-h	8-14	LC50	3.182	(Burridge et al., 2014)
Corophium volutator	Adult	-	AI	96-h	15	LC50	0.046	(Smit et al., 2008)
*Eulimnogammarus verrucosus***	Adult	-	AI	24-h	6-8	LC50	1.1526	(Fedoseeva and Stom, 2013)
*Eulimnogammarus vittatus***	Adult	-	AI	24-h	6-8	LC50	0.238	(Fedoseeva and Stom, 2013)
*Gammarus lacustris***	-	-	AI	24-h	6-8	LC50	0.231	(Fedoseeva and Stom, 2013)
*Moina sp.***	Adult	-	AI	48-h	21	LC50	0.0019	(Reichwaldt et al., 2012)
Mysid sp.	-	N	AI	1-h	8-14	LC50	0.973	(Burridge et al., 2014)
H. americanus	Stage 1	N	F	1-h	8-14	LC50	1.637	(Burridge et al., 2014)
H. americanus	Adult	N	F	1-h	8-14	LC50	>3.75	(Burridge et al., 2014)
Pimephales promelas	-	-	AI	96-h	-	LC50	0.0164	(Shurtleff, 1989)

Species	Life stage	Measured or Nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Hydrogen peroxide g/L (95%CI)	Reference
Fish								
Ictalurus punctatus	-	M	AI	0.5-h	7, 12, 17, and 22	LC50	>5	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	0.5-h	7 and 12	LC50	>5	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	0.5-h	7	LC50	>5	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	24-h	7	LC50	0.369	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	3-h	12	LC50	1.52	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	24-h	12	LC50	0.0766	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	1-h	17	LC50	2.860	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	3-h	17	LC50	0.332	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	24-h	17	LC50	0.0574	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	1-h	22	LC50	0.002	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	3-h	22	LC50	0.210	(Rach et al., 1997)
Ictalurus punctatus	-	M	AI	24-h	22	LC50	0.0555	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	0.5-h	7	LC50	>5	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	1-h	7	LC50	3.19	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	3-h	7	LC50	1.62	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	24-h	7	LC50	0.29	(Rach et al., 1997)

Species	Life stage	Measured or Nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Hydrogen peroxide g/L (95%CI)	Reference
Lepomis macrochirus	-	M	AI	0.5-h	12	LC50	3.54	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	1-h	12	LC50	2.56	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	3-h	12	LC50	1.24	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	24-h	12	LC50	0.165	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	0.5-h	17	LC50	3.54	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	1-h	17	LC50	2.18	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	3-h	17	LC50	0.683	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	24-h	17	LC50	0.152	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	0.5-h	22	LC50	2.01	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	1-h	22	LC50	1.46	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	3-h	22	LC50	0.406	(Rach et al., 1997)
Lepomis macrochirus	-	M	AI	24-h	22	LC50	0.0715	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	1-h	7	LC50	2.38	(Mitchell and Collins, 1997)
Onchorhynchus mykiss	-	N	AI	1-h	22	LC50	0.218	(Mitchell and Collins, 1997)
Onchorhynchus mykiss	-	M	AI	0.5-h	7	LC50	>5	(Rach et al., 1997)

Species	Life stage	Measured or Nominal	Formulation or active ingredient	Exposure	Temperature (˚C)	Endpoint	Hydrogen peroxide g/L (95%CI)	Reference
Onchorhynchus mykiss	-	M	AI	1-h	7	LC50	2.38	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	3-h	7	LC50	0.506	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	24-h	7	LC50	0.0694	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	0.5-h	12	LC50	8.66	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	1-h	12	LC50	1.26	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	3-h	12	LC50	0.363	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	24-h	12	LC50	0.042	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	0.5-h	17	LC50	0.52	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	1-h	17	LC50	0.311	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	3-h	17	LC50	0.119	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	24-h	17	LC50	0.034	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	0.5-h	22	LC50	0.393	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	1-h	22	LC50	0.218	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	3-h	22	LC50	0.102	(Rach et al., 1997)
Onchorhynchus mykiss	-	M	AI	24-h	22	LC50	0.0313	(Rach et al., 1997)

Notes: M=measured; N= nominal; F= formulation; AI= active ingredient; LC50= median lethal concentration; EC50= median effects concentration; CI= confidence interval; - = No value.

Table 6. Summary of the sublethal toxicity of hydrogen peroxide to crustaceans.

Species	Life stage	Measured or nominal	Formulation or active ingredient	Exposure	Temperature (°C)	Endpoint	Hydrogen peroxide g/L	Reference
Crustaceans								
crangon crangon	-	N	AI	5-h		Decrease in aerobic metabolic rate and intracellular pH	0.68	(Abele-Oeschger et al., 1997)
Caligus rogrecresseyi	Egg	-	AI	20 min	11.5	Development to copepodid	0.71	(Marin et al., 2017)
Caligus rogrecresseyi	Adult	-	AI	20 min	11.5	Immobility	0.7	(Marin et al., 2017)
Lepeophtheirus salmonis	Adult	-	AI	20 min	6-9	Immobility	0.8	(Thomassen, 1993)
Lepeophtheirus salmonis	Pre-adult	-	AI	30 min	10	Immobility	0.5	(Bruno and Raynard, 1994)
Lepeophtheirus salmonis	Adult	-	AI	30 min	10	Immobility	0.89	(Bruno and Raynard, 1994)
Lepeophtheirus salmonis	Pre-adult	-	AI	30 min	12	Immobility	0.538 to 0.693	(Helgesen et al., 2015)
Lepeophtheirus salmonis	egg	-	AI	20 min	10	Egg hatching success and larval survivability	0.47 to 1.99	McAndrew et al., 1998

Notes: M=measured; N= nominal; F= formulation; AI= active ingredient; LC50= median lethal concentration; EC50= median effects concentration; CI= confidence interval; - = No value.

1.3.7. Zooplankton

The term “plankton” is derived from the Greek word “planktos” meaning “wanderer” or “drifter”. Due to the inability of these organisms to control their swimming, beyond vertical movement, plankton travel *via* ocean currents. To avoid predation, zooplankton communities utilize vertical migration, which is the habit of decreasing depth during sunset and increasing depth during sunrise, to limit their visibility to predators (O'Brien, 1979). Zooplankton play a key role in marine food web dynamics, biogeochemical cycling, and fish recruitment. As these organisms cover a range of species and sizes, zooplankton occupy both the primary and secondary trophic levels and are key components in the diets of many organisms. When zooplankton populations are altered, a cascade of consequences can occur at higher trophic levels of the food web (Rodriguez et al., 2018).

Zooplankton range in length from 2 µm (protozoans) to 8 ft (jellyfish) in size and can generally be divided into two groups; holoplankton and meroplankton. Holoplankton are permanent planktonic organisms, such as most copepods, salps, and chaetognaths (Lindeque et al., 2013). Meroplankton are planktonic during early life stages, typically as eggs and larvae, and then grow into a larger, more developed organism. Examples of meroplankton are benthic organisms, such as sea urchins, fish larvae, or crab zoea. Many species of fish are only planktivorous during their early life stages and commonly feed on benthic invertebrates or fish in their adult stages (Lurling and De Senerpont Domis, 2013).

Marine copepods have been used to assess environmental hazards in the marine environment since 1977, however their use did not enter common practice until the 1990s (Ward 1995; Kwok et al., 2015). Since then, scientists have been increasing the use of plankton studies relating to climate change analysis, investigating population changes, potential effects on marine life, including the consequences in response to changing environmental dynamics, and environmental toxicology. Typically, acute, chronic, and full life-cycle tests are performed on zooplankton species, which are among the most sensitive marine taxa to many chemicals (Wang et al., 2014). Despite their importance in marine environments, our knowledge of the toxicity effects to zooplankton from many chemicals, including aquaculture chemotherapeutants, is extremely limited.

1.3.8. Using *Acartia tonsa* in toxicology

Acartia tonsa are a species commonly used in toxicity tests for marine ecosystems (Rodriguez et al., 2018). Important considerations when selecting a reference species for toxicity testing include availability, relevance, sensitivity, and ease of handling (Kwok et

al., 2015). Regarding these criteria, *Acartia tonsa* are an ideal laboratory species. *Acartia tonsa* dominate most zooplankton communities and can be found in estuaries and coastal waters in subtropical, temperate, and polar regions, including both the Atlantic and Pacific Ocean surrounding salmon farming sites in Canada (Almeda et al., 2013; Kwok et al., 2015). *Acartia tonsa* are considered a fairly robust species due to their ability to survive in temperatures ranging from 0 to 30° C (Marcus and Wilcox, 2007). The only criteria that *Acartia tonsa* do not fulfill as a reference species is ease of handling. As with most copepods, *Acartia tonsa* are fragile, and cannot withstand rough treatment, vigorous aeration, or gravity outside of water (Marcus and Wilcox, 2007). While fragile, this species is still ideal for toxicology because they have continuous egg production regardless of fertilization, and lay eggs freely in the water column, opposed to keeping their eggs attached to their body via egg strings (Kwok et al., 2015). This allows toxicity tests to involve single egg exposures and the ability to track an individual egg's hatching success (Marcus and Wilcox, 2007). For these reasons, *Acartia tonsa* are one of the most commonly used marine zooplankton species and have been recommended by the International Organization for Standardization (ISO) to evaluate the acute effects of marine contaminants.

Acartia tonsa have rapid life cycles that begin with sexual reproduction. All 13 life stages (6 naupliar stages, 6 copepodid stages, and 1 adult stage) are distinct, providing well-defined endpoints and easy tracking of development (Kwok et al., 2015). They range from 0.5 to 1.5 mm in length and eggs are 70 to 80 µm in diameter (Mauchline, 1998). Fertilized eggs can take from 24 to 48 h to hatch at 20°C and under optimal food conditions takes approximately 10 to 14 d for the hatched nauplii to reach adulthood (Mauchline, 1998). The short development of *Acartia tonsa* means that toxicity tests can be used to assess various life stages and endpoints including hatching success, mobility of the nauplii, development, and the number of eggs hatched as endpoints (Barata et al., 2002).

1.4. Purpose of study

The effects of Paramove® 30 and Salmosan® on marine zooplankton have previously only been studied on a few species from Europe and the east coast of Canada. The goal of the current study was to expand this knowledge base by using natural zooplankton assemblages from the Pacific Ocean off the coast of BC to determine the acute lethality of Salmosan® and Paramove® 30 formulations. Additionally, because assessing sublethal testing on wild assemblages would be a difficult and lengthy process,

a laboratory species was used to investigate potentially sensitive and environmentally relevant endpoints. The effects on growth, development, and reproduction following exposure to Paramove® 30 and Salmosan® was investigated on the copepod species *Acartia tonsa*. Collectively, this study was performed to provide valuable information regarding the potential impacts to the marine environment following use and release of Salmosan® and Paramove® 30 formulations, and to contribute to regulatory decisions in sea lice management and the Canadian aquaculture industry. In order to determine the potential effects of these two chemicals on marine zooplankton, both lethal and sublethal effects were examined under environmentally relevant exposure scenarios.

Chapter 2. Materials and methods

2.1. Zooplankton collection, transport, and holding

The collection of wild marine zooplankton took place in Nanoose Bay, BC (see Figure 1) 48 h prior to the start of each experiment. Zooplankton assemblages were collected *via* horizontal plankton tow using a 150 µm mesh plankton net with a width of 1 ft (model E-411-426-A22). As recommended by Keen (2013), tow speed was maintained at 1 to 1.5 knots. The net was lowered to a depth of approximately 15 m, to correspond with the depths of most net-pens used in salmon farming which range from 15 m to 30 m (BC salmon farmers association, 2017). The net was towed for 7 min; collections were repeated 8 times per collection event. Further guidance on collection methods can be found in Appendix A.

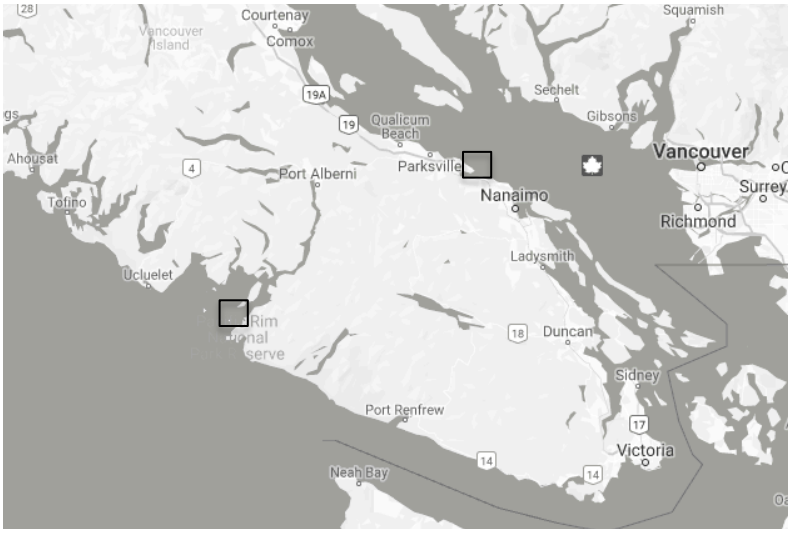


Figure 1. An aerial view of Vancouver Island (Google earth, version 2017). The two collection sites are identified by black boxes. Bamfield is located on the western side of the Island, south of Tofino. Nanoose Bay is located on the eastern side of the island, north of Nanaimo.

Water conductivity, pH, and temperature were measured on site at a depth of 10m. *In-situ* water to be used for experiments was collected using a basic water sampler and stored in Nalgene bottles (Thermo Fisher Scientific). Organisms were collected in the bottle attached to the bottom of the net and funneled into two 3.8 L Nalgene bottles (Thermo Fisher Scientific) and placed into a dark cooler. After transport to Simon Fraser University, plankton were placed in a temperature-controlled room at 12 °C; water was

aerated and zooplankton were allowed to acclimate overnight. All organisms were used in an experiment within 48 h of collection.

Zooplankton assemblages collected in Bamfield were performed using the same methods; all experiments for these zooplankton were performed at the Bamfield Marine Science Centre. For collection of brachyuran and porcelain crab zoea, zooplankton assemblages were collected as described above. Concentrations of these assemblages were then sorted under a stereomicroscope (Richter Optica s6T trinocular stereomicroscope) and the category of crab zoea (brachyuran or porcelain) to be used was removed with a Pasteur pipette and placed into a 500 mL mason jar (10/jar, 42 jars). After a sufficient number of crab zoea were identified, experiments were performed immediately (< 6h after collection).

2.2. Copepod culture

Acartia tonsa stocks of mixed life stages were purchased from *AlgaGen* (Florida, USA). Copepods were cultured based on the guidelines proposed by Marcus and Wilcox (2007). The copepods were housed in 9 L glass aquaria (<1 copepod/5 ml) and maintained at a temperature of 22 ± 2 ˚C with a 12:12 photoperiod. SW was renewed every 48 h. This was done using submerged stacked sieves (150 and 20 µm mesh) which allowed for a 100% water renewal. Immediately following each water renewal copepods were fed a diet of 25,000 cells/mL of both *Rhodomonas lens* and *Rhodomonas salina*. Salinity, temperature, pH, and dissolved oxygen content were monitored daily. The temperature for the cultured *Acartia tonsa* was maintained at 22 ± 2 °C. The pH remained at of 8.35 ± 0.45. The salinity remained at 36 ± 3 µS/cm^2. The dissolved oxygen remained at 7 ± 1 mg/L.

Male and female copepods were visually differentiated using 3 key characteristics; size, antennae, and swimmerets. Adult females are typically slightly larger (0.72 to 0.94 mm and 0.71 to 0.82 mm, respectively) than males, with longer and straighter antennae, and their swimmerets are thicker and more filamentous than those of the males (Figure 2) (Mauchline, 1998; Krupa et al., 2015).

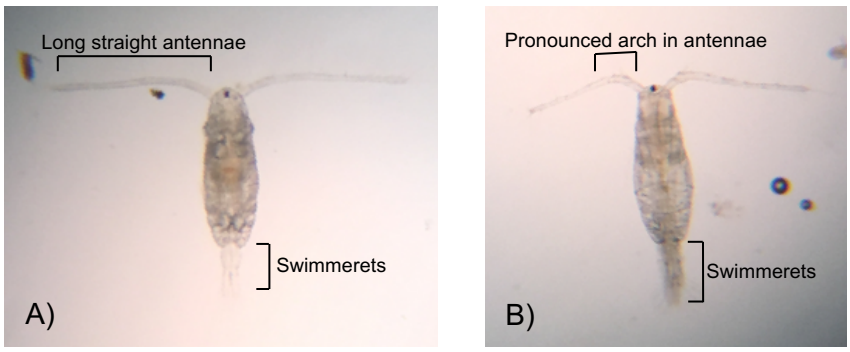


Figure 2. Examples of female and male *Acartia tonsa.* A) A female adult *Acartia tonsa* at 40x magnification, identified by long, straight antennae and thick swimmerets. B) A male adult *Acartia tonsa* at 40x magnification, identified by the pronounced arch in the antennae, and thin swimmerets.

2.3. Algal culture

A combination of two species of algae were used to feed the copepods; *R. lens* and *R. salina.* These were cultured based on the protocol set forth by Marcus and Wilcox (2007). Seed stocks of both species were acquired from the Canadian Centre for the Culture of Microorganisms (Vancouver, BC, Canada). Seed stocks (30 mLs) were stored in 50 mL glass vessels and were maintained at 23° C, under a 14:10 photoperiod. Seed stocks were agitated daily to maintain suspension. To create additional seed stocks, autoclaved Fernbach flasks were filled with 15 mL original seed stock solution, along with 1.5 L natural SW media. Solutions were held in a Fernbach flask for one month before transfer into a newly autoclaved Fernbach flask. To begin an algal culture, an autoclaved 1.5L Fernbach flask was inoculated with 2 mL of F/2 stock, which is a nutrient solution composed of trace metals and vitamins (see recipe in appendix D), Guilliard's Enrichment (GE) at 3.5 mL/L SW, 1.6 g sodium bicarbonate ($NaHCO_3$ at 3mM final concentration), and 500 mL algal solution from the 1.5 L incubator Fernbach vessels. Aeration was maintained at 10 mL/ sec of air. All flasks were agitated twice daily to maintain suspension Additional F/2 stocks were added to the algal culture every other day. Cell counts were done every 48-h using a hemocytometer to determine the cell count of algae required to feed the copepods. Enough stock to provide each 9L copepod tank with 135 x 10^6 cells from each species was removed and set aside to feed the copepods. Each culture was maintained until cell counts no longer increased, which typically occurred around 3.5 x 10^6 cells/ mL. The density in each culture was controlled using dilution and reducing the amount of GE added.

2.4. Chemicals

Salmosan® (Fish Vet Group®, Inverness, Scotland) is a wettable powder that consists of 49.5% azamethiphos, the active ingredient. Prior to experiments, Salmosan® and seawater were mixed for 1 h to create a stock solution. Interox® Paramove® 30 (Solvay, ON, Canada) is a liquid mixture composed of 30% hydrogen peroxide (H_2O_2). A stock solution was created by combining Interox® Paramove® 30 with seawater before a short mix period and immediate use. Neutral red (Abcam, Cambridge, United Kingdom) is a wettable powder used as a histology stain (also known as toluylene red, 3-Amino-7-dimethylamino-2-methylphenazine hydrochloride, and basic red 5). A stock solution was made by combining neutral red powder and SW for a final concentration of 0.01g/mL and stirring overnight in dim light (Elliott and Tang., 2009). All euthanizations were performed using 3.7 % unbuffered formaldehyde (Anachemia, Lachine, QC).

2.5. Acute lethal tests

Exposures for acute lethality tests were performed on wild zooplankton assemblages, wild brachyuran and porcelain crab zoea, and *Acartia tonsa*. Methods were based on Kusk and Wollenberger (1999) and the International Organization for Standardization (ISO, 1999). Wild zooplankton assemblages collected from Nanoose Bay were concentrated using a submerged 150 µm mesh sieve. From this concentrate, approximately 200 organisms were removed and euthanized in 3.7% unbuffered formaldehyde followed by storage at 4° C for later species identification. As well from this concentrate, approximately 30 organisms (in 10 mL of concentrate) were removed via a serological pipette and placed in each 250 mL jar, containing 190 mL of seawater (control) or one of 7 test concentrations (in triplicate) of Salmosan® or Paramove® 30 in seawater.

Prior to full experiments, range-finding trials were performed on the wild zooplankton and *Acartia tonsa*. The dosing concentrations used in the range-finding trials for Paramove® 30 exposures on the zooplankton harvested in Nanoose Bay were 0, 180, 900, and 1800 mg/L (AI). All exposures resulted in 100% mortality at 180 mg/L and higher, resulting in the final concentration series to be lowered. The concentrations used to treat both the Nanoose Bay zooplankton assemblages and *A. tonsa* were 0, 0.2, 1, 2, 10, 20, and 100 mg/L (AI). Due to the opportunistic nature and limited test species in the Bamfield zooplankton assemblages, including both species of crab zoea, no range-finding experiments were performed. For all Paramove® 30 exposures performed in Bamfield, general concentrations of 0, 0.1, 1, 10, 100, 500, 1000 mg/L (AI) were chosen. The

concentrations used in the range-finding trials for Salmosan® were 50, 100, and 1000 µg/L. Limited effects were seen in the 1 h exposure at all 3 concentrations, so another trial was performed at 1500 µg/L, which resulted in increased mortality, but not 100%. The final Salmosan® concentrations used to expose the wild assemblages collected from Nanoose Bay were 0, 7.5, 15, 75, 150, 750, 1500, 7500 µg/L (AI). The *Acartia tonsa* range-finding study resulted in 100% mortality at 1000 µg/L. This led to the final concentrations of 0, 1.5, 7.5, 15, 75, 150, 750 µg/L (AI).

There were 4 exposure periods used for the testing of each chemical. In the first, the zooplankton were incubated in the pesticide for 1 h, followed by immediate termination and analysis. In the second, zooplankton were incubated with test chemicals for 1 h and then transferred into clean SW for 48 h, followed by termination and analysis. The purpose of the 48-h period in clean seawater was to account for recovery or delayed effects. In the third exposure regime, zooplankton were incubated with pesticide for 3 h followed by immediate termination and analysis. In the fourth exposure regime, zooplankton were incubated with pesticide for 3 h before being transferred into clean seawater for 48 h, followed by termination and analysis.

During the last 30 min of each exposure scenario, neutral red dye vital stain was added to provide a quantifiable means of determining survival or death among zooplankton within a treatment group (Elliott and Tang, 2009). Living zooplankton absorb the dye and are stained bright red/pink, while dead organisms are left translucent and unstained (Elliott and Tang, 2009). Following the staining protocol outline by Elliott and Tang (2009), the stock was added to the exposure vessels at a volume of 1.5 mL/1L sample. After 30 min, the dye was then quickly filtered out using a 150 µm sieve, and the zooplankton were returned to the vessel. To euthanize and preserve the stained and unstained zooplankton, 1.5 mL 3.7 % unbuffered formaldehyde was then added, followed by storage at 4 °C. Prior to any exposures, collections of all live organisms as well as all dead organisms were exposed to stain and assessed for the stains ability to differentiate them (Figure 3).

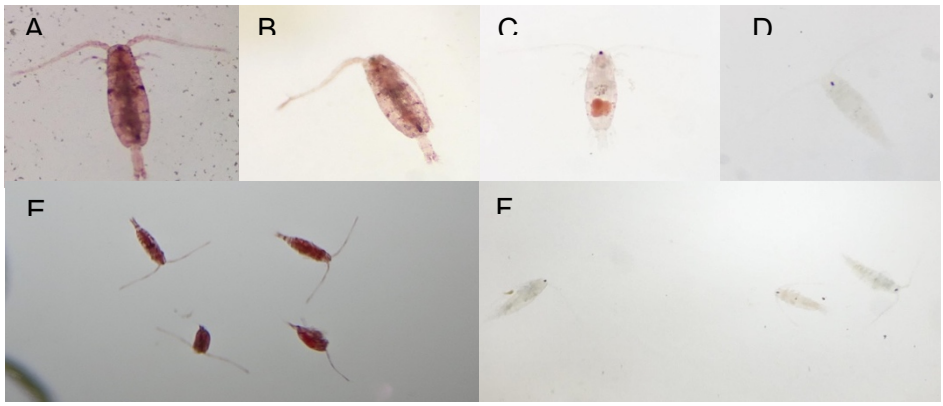


Figure 3. Appearance of neutral red-treated *Acartia tonsa* copepods under a stereomicroscope. A) darkly stained male *A. tonsa* at 40x magnification. Colour indicates that it is alive. B) A male *A. tonsa* lightly stained and classified as alive. C) A female presenting patchy staining, with majority translucent and classified as dead. D) A completely translucent female presenting no vital stain and classified as dead. Panel E) shows 100% survival of all *A. tonsa* at 10x magnification, and Panel F) shows 100% mortality.

Wild zooplankton assemblages as well as Brachyuran (*Brachyura*) and porcelain (*Porcellanidae*) crab zoea were used as test species in the experiments done in Bamfield. Exposure methods were similar to the Nanoose Bay methods except each test group was only exposed to the pesticide for 1 h followed by 4 h in clean SW before analysis. Due to a laboratory limitation, no neutral red was used to analyze organisms in Bamfield, so the more traditional approach of identifying movement or a heartbeat were used (USEPA, 1985). Each organism was classified as dead, mobile, or immobile. Each organism was determined to be immobile if it had a heartbeat and showed no response to probing or was visibly unable to control its movement.

The final step of each exposure was to identify the species present in the concentrate of approximately 200 organisms that was removed at the beginning of each experiment. Each organism was examined using a stereomicroscope and identified down to its order. It is not recommended for non-taxonomists to identify down to the family or genus due to increased difficulty and likelihood of inaccuracy due to the small size and similar morphology of marine zooplankton (Bron et al., 2011). To identify each organism, a reference guide was created by compiling images from online resources, predominantly “an image-based key to the zooplankton of north America” (University of New Hampshire) but also “Zooplankton” (Scripps Institution of Oceanography), of the most common marine zooplankton in the Pacific Ocean. If an organism could not be identified or was the only one of its kind it was placed in the category “other holoplankton.”

2.6. Sublethal toxicity assessments

Three separate sublethal experiments were performed for each pesticide: determination of effects on egg hatching success, naupliar development, and reproductive success. Based on Gorbi et al. (2011), approximately 48 h before the beginning of the first two experiments (egg hatching success and naupliar development), 35 - 40 adult females and 20 - 25 males were transferred using a plastic Pasteur pipette into a crystalizing dish containing 700 mL of seawater and 14 mL of both *R. lens* and *R. salina* with a stock solution density of 10^7 cells/mL. Organisms were maintained at a temperature of 22 ± 2 ˚C with a 12:12 photoperiod. Approximately 15 h before the beginning of the experiment both sexes were randomly placed into smaller crystallizing dishes (10-12 organisms total/ dish) containing 200 mL seawater and 25 mL of both *R. lens* and *R. salina* as described above. The eggs that were produced during the subsequent 15 h were collected via Pasteur pipette and used for the first two experiments, described below.

The egg hatching success experiment was based on an acute egg test described in Gorbi et al (2011). Individual *Acartia tonsa* eggs were transferred *via* micropipette into 150 mL glass jars (10 eggs/vessel in triplicate). The eggs were then exposed to100 mL of the test concentration for one of two exposure times (1 or 3 h). Concentrations used for the Paramove® 30 exposures were 0, 0.1, 0.5, 1, 5, and 10 mg/L .Concentrations used for the Salmosan® exposures were 0, 1.5, 7.5, 15, 75, 150, 1500, and 7500 µg/L No adverse effects resulted from the 2 lowest concentrations of Salmosan®, resulting in the addition of the 2 higher concentrations. After each exposure, the eggs were located *via* stereomicroscope, and transferred by micropipette into microplates with 300 µL clean seawater (1 egg/well, 10 eggs/microplate, 3 microplates/concentration). Immediately after the eggs were transferred into the microplates, 2 µL of both *R. lens* and *R. salina* with a stock solution density of 10^7 cells/mL were then added to each well to provide nutrients once the eggs hatched. At 24 and 48 h after exposure to the egg, nauplii survival, and mobility were observed. Nauplii were determined to be immobile if they displayed uncontrollable twitching of limbs or the inability to move vertically and horizontally. They were determined to be dead if there was no indication of movement after 30 sec of observation. No vital stain was required to determine if the organism was dead. This was because the shallow microplates allow for a higher magnification to be used than was used in the lethal experiments, as well as the ability to gently probe the organism in each microwell. Due to the larger vessel used in the lethal experiments, this was not possible.

The test was considered valid if control eggs had ≥ 80% hatch success and naupliar immobilization was ≤ 20% (Gorbi et al., 2011).

The naupliar development experiment followed the protocol for the chronic naupliar exposure test described by Andersen et al. (1999). Adult male and female A. tonsa were transferred from the culture into a crystallizing dish, and eggs were collected as described above. Collected eggs were placed into 96 well microplates (1 egg/well, 12 eggs/microplate, 3 microplates/ concentration, 6 concentrations plus a control). Each well was filled with 300 µL of seawater, and 2µL of both *R. lens* and *R. salina* stock solution with a density of 10^7 cells/mL. The eggs were then left for 24 h to hatch into nauplii at the temperature of 22 ± 2 ˚C with a 12:12 photoperiod. Once hatched, the organisms were exposed to the test concentration for 1 or 3 h. Concentrations used for the Paramove® 30 exposures were 0, 0.01, 0.05, 0.1, 0.5, and 1 mg/L (AI). Concentrations used for the Salmosan® exposures were 0, 15, 75, 150, 750, and1500 µg/L (AI). Each microwell was treated with the pesticide *via* a micropipette. At the end of the exposure period approximately 80% of the test solution was removed from each well using a 26G 3/8 single use needle, while viewed under a stereomicroscope. Fresh SW was then added, swirled, and 80% was once again removed using a 26G 3/8 single use needle. Fresh SW was then added once more, along with 2µL of both *R. lens* and *R. salina* as previously described. SW was renewed daily for 5 d, and nauplii monitored for death. After 5 d, one control was analyzed to determine if a minimum of 50% had reached the copepodite stage. If this was not the case the organisms would be allowed another day to develop, before another control was analyzed to check the development. In this case, after 5 d at least 50% had reached the copepodite stage when the exposure was terminated and all organisms were classified as a nauplii or copepodites (Andersen et al., 1999).

The third sublethal experiment (reproductive success) was based on the methods of Bellas and Thor (2007). This was done to determine the number of eggs laid, and their hatching success, by an ovigerous adult female after exposure. Eggs and nauplii were filtered out of the assemblages using submerged stacked sieves (150 and 20 µm mesh) and placed back into a clean 9 L glass aquarium. Adults and copepodites were examined using a stereomicroscope, through which female and male adults were identified. Five female and 1 male *Acartia tonsa* were transferred via Pasteur pipette into 250 mL glass jars containing 100 mL of SW and exposed to a test concentration for 1 or 3 h (5 females/ vessel, 4 vessels/ concentration, 3 concentrations and a control). Exposures occurred at the temperature of 22 ± 2 ˚C with a 12:12 photoperiod. Concentrations were chosen based

on range-finding studies in order to limit mortality while testing highest concentrations. Concentrations used for the Paramove® 30 exposures were 0, 0.1, 0.5, and 1 mg/L (AI). Concentrations used for the Salmosan® exposures were 0, 10, 50, and 100 mg/L (AI). Tests were run in quadruplicate at each concentration. The females were then carefully filtered through a 150 µm sieve and placed into a clean glass petri dish. The females were then located under the microscope and placed in 96 well microplates via a Pasteur pipette (1 female/well, 5 females/microplate 4 microplates/ concentration) with 300 µL SW. Males were removed and euthanized by placing into a glass jar of 3.7 % unbuffered formaldehyde. The number of eggs laid and successfully hatched were recorded, as well as survivorship of the female after 24 and 48 h in the clean microplates. Eggs and nauplii were viewed in each microwell by placing the microplates under a compound microscope at 40x objective. Eggs are negatively buoyant and were found at the bottom of each well. The nauplii were located by scanning upward through the water column using the course adjustment knob of the microscope.

2.7. Statistical analysis

Median lethal concentration (LC50, LC25, LC10), median effect concentration (EC50, EC25), and 95% confidence interval values were estimated using Probit analysis *via* JMP® Version 13.1.0 (SAS Institute Inc., 2016). The highest concentration resulting in no statistically significant adverse effects or mortality compared to the control was used as the no observed adverse effects concentration (NOAEC). Differences in the number of eggs laid, development, hatching success, mobility, and survival were tested for significance (p-value of < 0.05) by means of a one-factor analysis of variance (ANOVA). When significant differences were found among the groups, Tukey's multiple comparisons test was used to determine differences between treatment groups.

Concentration response curves for all lethality and sublethal experiments, except the total eggs laid in the adult reproduction experiment, were fit on on GraphPad Prism Version 8 (La Jolla California, 2017). They were generated by plotting the normalized response against the log of the concentration. For the total eggs laid endpoint, a multiple linear regression was performed.

Chapter 3. Results

3.1. Water quality

Temperature, pH, salinity, and dissolved oxygen content were measured every 48 h throughout each experiment. The temperature for the wild plankton assemblages from Nanoose was maintained at 12 ± 2 °C. The salinity remained at 45 ± 3 μS/cm^2. The dissolved oxygen remained at 8 ± 1 mg/L. The pH remained at 8.4± 0.6. The temperature for the cultured *Acartia tonsa* was maintained at 22 ± 2 °C. The pH remained at of 8.35 ± 0.45. The salinity remained at 36 ± 3 μS/cm^2. The dissolved oxygen remained at 7 ± 1 mg/L. The temperature for the organisms collected in Bamfield was maintained at 18 ± 0.5 °C. The pH remained at 7 ± 0.8. The salinity remained at 39 ± 3.5 μS/cm^2. The dissolved oxygen remained at 6.15 ± 0.15 mg/L.

3.2. Acute lethality

The median 1-h LC50 (95% confidence interval) value calculated for Paramove® 30 for the calanoid copepod *Acartia tonsa* was 23 mg/L (CI 17- 34 mg/L), while the value for 1-h LC50 with 48 h in clean SW was 13 mg/L (CI 9.3 - 20) (

Table 7). The 3-h LC50 calculated for the calanoid copepod *Acartia tonsa* was 10 mg/L (CI 6 - 19), while the 3-h LC50 with 48 h in clean SW was 9.1 mg/L (CI 5.4 - 17 mg/L). For brachyuran and porcelain crab zoea calculated EC50 (immobility + mortality) values were 55 mg/L (CI 30 - 95) and 46 mg/L (CI 27 - 76 mg/L), respectively. The 1-h LC50 value calculated for Paramove® 30 for the wild assemblages from Nanoose Bay was 8.2 mg/L (CI 6.2 - 11 mg/L), while the 1-h LC50 with 48 h in clean SW was 10 mg/L (CI 8.3 - 13 mg/L). The 3-h LC50 for the wild assemblages from Nanoose Bay was 5.5 mg/L (CI 4.4 - 6.9 mg/L), while the value for 3-h LC50 with 48 h in clean SW was 4.0 mg/L (CI 4.0 - 6.9 mg/L). The wild assemblages collected from Bamfield were found to have a 1-h LC50 with 4 h in clean SW of 7 mg/L (CI 4 - 12 mg/L).

Table 7. Estimated LC50 and EC50 values and 95% confidence intervals of different zooplankton groups subjected to lethal exposures to Interox® Paramove® 30.

Species	Length of Exposure	Endpoint	LC50 (mg/L)	95% CI (mg/L)
Acartia tonsa	1-h	LC50	23	(17 - 34)
Acartia tonsa	1-h + 48 h in clean water	LC50	13	(9.3 - 20)
Acartia tonsa	3-h	LC50	10	(6 - 19)
Acartia tonsa	3-h + 48 h in clean water	LC50	9.1	(5.4 - 17)
Brachyura zoea	1-h + 4 h in clean water	EC50	55	(30 - 95)
Porcellanidae zoea	1-h + 4 h in clean water	EC50	46	(27 - 76)
Wild assemblage*	1-h	LC50	8.2	(6.2 - 11)
Wild assemblage*	1-h + 48 h in clean water	LC50	10	(8.3 - 13)
Wild assemblage*	3-h	LC50	5.5	(4.4 - 6.9)
Wild assemblage*	3-h + 48 h in clean water	LC50	4.0	(4 - 6.9)
Wild assemblage**	1-h + 4 h in clean water	EC50	7.0	(4 -12)

Note: Wild assemblages with a single asterisk indicate that the organisms are from Nanoose Bay. Two asterisks indicate that the organisms were from Bamfield inlet.

The 1-h LC50 value calculated for Salmosan® for the calanoid copepod *Acartia tonsa* was 220 µg/L (CI 160 - 331 µg/L), while the value for 1-h LC50 with 48 h in clean SW was 166 µg/L (CI 115 - 262 µg/L) (Table 8). The 3-h LC50 calculated for the calanoid copepod *Acartia tonsa* was 98 µg/L (CI 60 - 177 µg/L), while the 3-h LC50 with 48 h in clean SW 127 µg/L (CI 78 - 231 µg/L). For brachyuran and porcelain crab zoea calculated EC50 (immobility + mortality) values were 203 µg/L (CI 128 - 320 µg/L) and 103 µg/L (CI 56 - 187 µg/L), respectively. The 1-h LC50 value calculated for Salmosan® for the wild assemblages from Nanoose Bay was 529 µg/L (CI 332 - 900 µg/L), while the 1-h LC50 with 48 h in clean SW was 360 µg/L (CI 223 - 623 µg/L). The 3-h LC50 for the wild assemblages from Nanoose Bay was 103 µg/L (CI 66 - 165 µg/L), while the value for 3-h LC50 with 48 h in clean SW was 54 µg/L (CI 320 - 90 µg/L). The wild assemblages collected from Bamfield were found to have a 1-h LC50 with 4 h in clean SW of 83 µg/L (CI 40 - 177 µg/L).

Table 8. Estimated LC50 and EC50 values and 95% confidence intervals of different zooplankton groups subjected to lethal exposures to Salmosan®.

Species	Length of Exposure	Endpoint	LC50 (µg/L)	95% CI (µg/L)
Acartia tonsa	1-h	LC50	220	(158 -331)
Acartia tonsa	1-h + 48-h in clean water	LC50	166	(115 - 262)
Acartia tonsa	3-h	LC50	98	(60 - 177)
Acartia tonsa	3-h + 48-h in clean water	LC50	127	(78 - 231)
Brachyura zoea	1-h	EC50	203	(128 - 320)
Porcellanidae zoea	1-h	EC50	103	(56 - 187)
Wild assemblage*	1-h	LC50	529	(332 - 900)
Wild assemblage*	1-h + 48-h in clean water	LC50	360	(223 - 623)
Wild assemblage*	3-h	LC50	103	(66 - 165)
Wild assemblage*	3-h + 48-h in clean water	LC50	54	(32 - 90)
Wild assemblage**	1-h	EC50	83	(40 - 177)

Note: Wild assemblages with a single asterisk indicate that the organisms are from Nanoose Bay. Two asterisks indicate that the organisms were from Bamfield inlet.

Zooplankton were collected from Nanoose Bay during various seasons, however there was little variation of species diversity in each tow (Table 9). The majority of the species present in the assemblages removed for identification from Nanoose Bay and Bamfield inlet fell under the order calanoida (Table 9 and 10) but differed in species diversity. In Nanoose Bay, calanoid copepods made up an average of 84% of the population, while the other 16% of species identified were in the orders cyclopoida, and harpacticoida. In Bamfield, the wild assemblages used for the Paramove® experiment consisted of 52% calanoid copepods, and the wild assemblages used for the Salmosan® experiment consisted of 49% calanoid copepods, while the remaining organisms from Nanoose Bay were among the orders cyclopoda, harpacticoida, and other holoplankton, and the remaining organisms in Bamfield were the same, with the addition of the orders diplostraca and decapoda.

Table 9. The average makeup of zooplankton assemblages collected from Nanoose Bay throughout 2018, as well as January and February 2019. Nauplii and any organisms that could not be identified or were the only one of their kind was placed in the “other holoplankton” category.

Order	Average	Range
Calanoida	84%	79 - 88%
Cyclopoida	8%	6 - 11%
Harpacticoida	7%	4 - 9%
Other holoplankton	1%	0 - 3%

Table 10. The makeup of zooplankton from Bamfield inlet for each chemotherapeutant experiment, collected in November 2018. Nauplii and any organisms that could not be identified or were the only one of their kind was placed in the "other holoplankton" category.

Order	Interox® Paramove® 30	Salmosan®
Calanoida	52%	49%
Cyclopoida	19%	13%
Harpacticoida	7%	17%
Diplostraca	8%	8%
Decapoda	12%	7%
Other holoplankton	5%	5%

3.3. Sublethal Toxicity

3.3.1. Egg hatching success

Egg hatching success was determined by exposing *Acartia tonsa* eggs to Paramove® 30 or Salmosan® for 1 or 3 h and analyzing them after 24 and 48 h to determine the number of eggs hatched, and the number of mobile, immobile, or dead hatched organisms. For both the 1-h and 3-h Paramove® 30 exposures, hatching success decreased as concentration increased (Figure 4A). For both the 1-h and 3-h Salmosan® exposures, there were no effects on hatching success, but immobility and death increased as concentration increased (Figure 4B). A summary of the effect concentration values found for this experiment including EC25, EC50, LC10 and NOAECs are presented in Table 18.

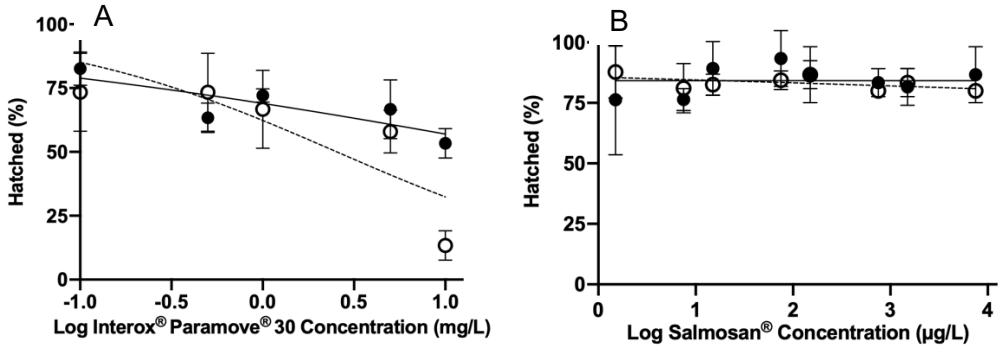


Figure 4. The effects of Paramove® 30 and Salmosan® on hatching success after exposure to the egg. The top two graphs represent the proportion hatching success of *Acartia tonsa* eggs after exposure to Paramove® 30 for 1 h (*closed circles*) and 3 h (*open circles*) (A), and Salmosan® for 1-h and 3-h (B).

In terms of hatching success, the Paramove® 30 1-h exposure resulted in a median effective concentration (EC50) of 1.21 mg/L (CI 0.41 - 4.3 mg/L) and the 3-h exposure resulted in an EC50 of 0.82 mg/L (CI 0.16 - 53 mg/L) (Table 11). For both Salmosan® experiments, no effect concentrations could be calculated, and instead no observed adverse effect concentrations (NOAEC) of 7500 µg/L were reported.

Table 11. Estimated EC50 and NOAEC (hatching success) values and 95% confidence intervals of *Acartia tonsa* eggs subjected to sublethal exposures of Interox® Paramove® 30 or Salmosan®. If unable to calculate EC50 the NOAEC was determined from the highest concentration tested that caused no effects. Error bars represent the standard deviation for each treatment (n= 3).

Chemotherapeutant	Length of exposure	Endpoint	Hatching effect concentration	95% CI
Interox® Paramove® 30	1-h	EC50	1.21 mg/L	(0.41 - 4.3 mg/L)
Interox® Paramove® 30	3-h	EC50	0.82 mg/L	(0.16 - 53 mg/L)
Salmosan®	1-h	NOAEC	7500 µg/L	
Salmosan®	3-h	NOAEC	7500 µg/L	

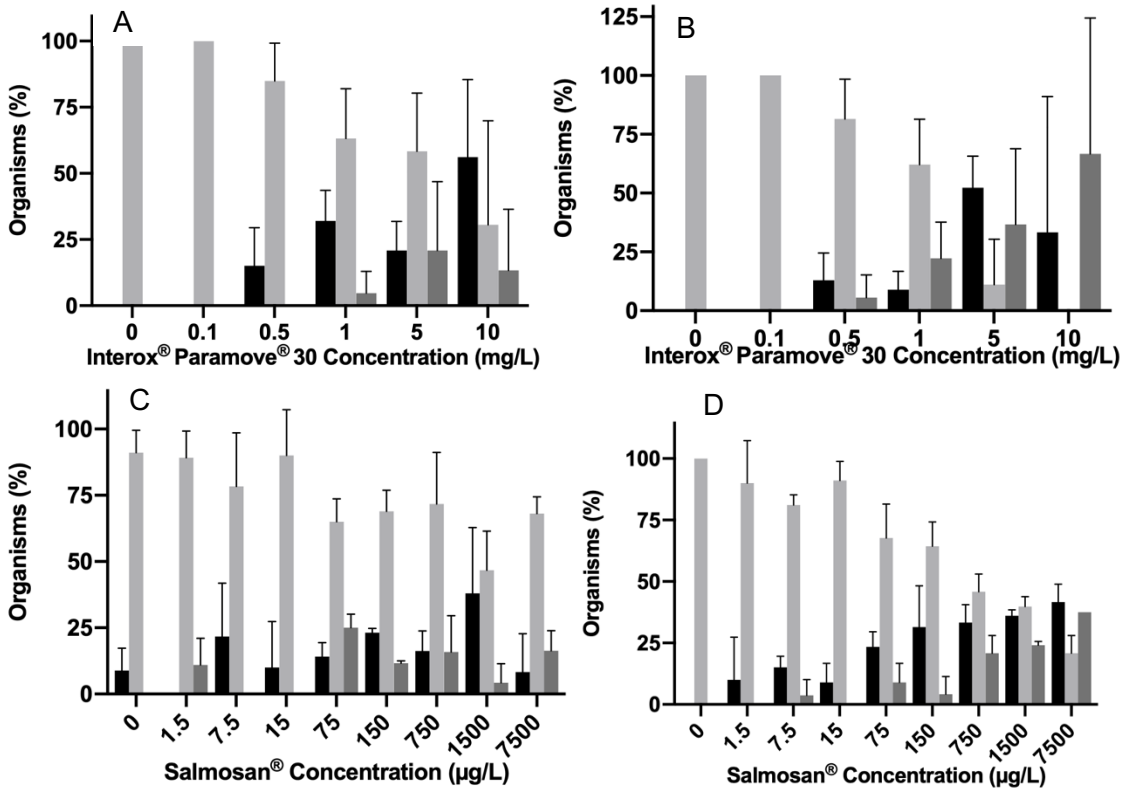


Figure 5. A summary of the effects on hatched *Acartia tonsa* after exposure to Paramove® 30 or Salmosan® as an egg. Effects on mobility (light grey), immobility (black), and death (dark grey) are presented after exposure to Paramove® 30 for 1-h (A), and 3-h (B), and Salmosan® for 1-h (C) and 3-h (D). Error bars represent the standard deviation for each treatment (n= 3).

After the eggs hatched, they were then classified as immobile, mobile, or dead (Figure 5). For both the 1-h and 3-h Paramove® 30 exposures, immobility and death increased as concentration increased, and mobility decreased (Figure 6). For the 1-h Salmosan® experiments there was no effect on immobility, mobility, or death (Figure 6 and 7). For the 3-h Salmosan® exposure, immobility and death increased as concentration increased (Figure 6 and 7).

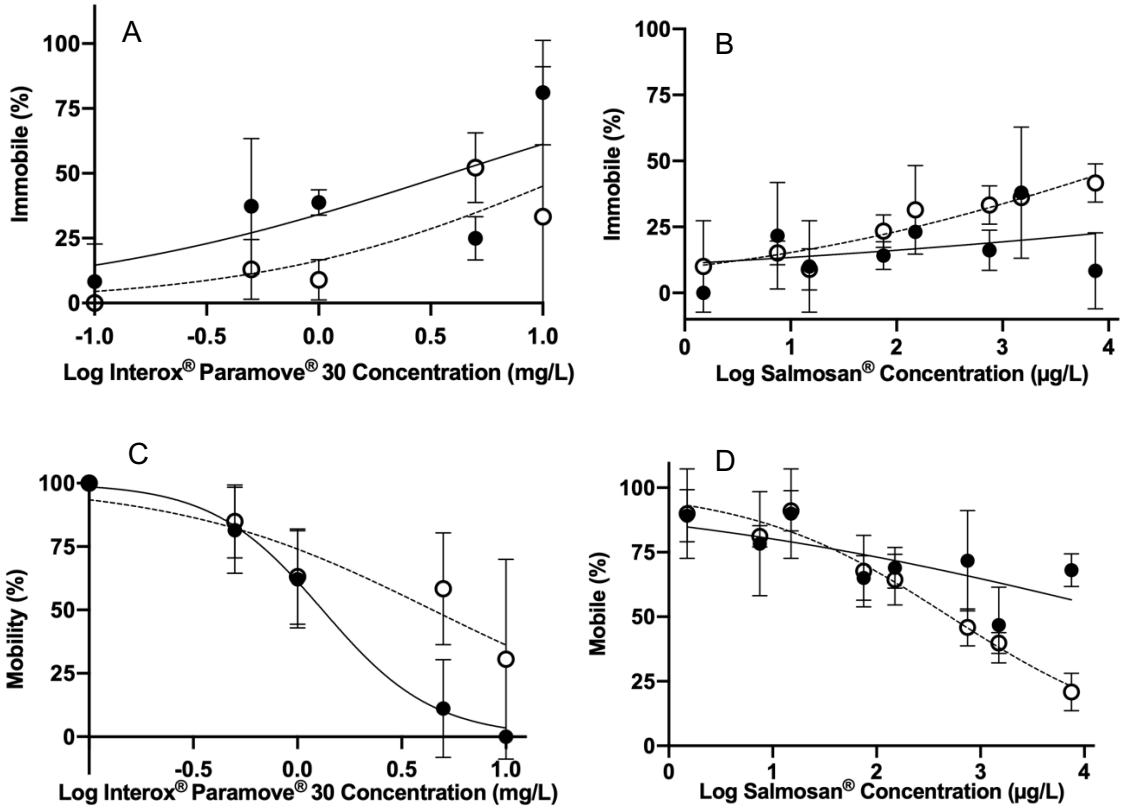


Figure 6. The effects of Paramove® 30 and Salmosan® on immobility after exposure to the egg. The top two graphs represent the immobility of hatched *Acartia tonsa* nauplii after exposure as an egg to Paramove® 30 for 1-h (*closed circles*) and 3-h (*open circles*) (A), and Salmosan® for 1-h and 3-h (B). The bottom two graphs represent the mobiltiy hatched *Acartia tonsa* nauplii after exposure as an egg exposed to Paramove® 30 for 1-h, and 3-h (C), Salmosan® for 1-h and 3-h (D). Error bars represent the standard deviation for each treatment (n= 3).

For immobility, Paramove® 30 1-h exposure resulted in an EC25 of 1.9 mg/L (CI 0.91 - 5.3 mg/L) and the 3-h exposure resulted in an EC25 of 0.57 mg/L (CI 0.037 - 1.4 mg/L) (Table 12). For this endpoint EC25s are reported instead of EC50s due to increased

error in the latter calculation. The 1-h Salmosan® experiment had no observed effect on mobility, resulting in a NOAEC of 7.5 mg/L, while the 3-h EC25 was calculated to be 131 µg/L (CI 16 - 750 µg/L).

Table 12. Estimated EC50 and NOAEC (immobility of hatched eggs) values and 95% confidence intervals of *Acartia tonsa* eggs subjected to sublethal exposures of Interox® Paramove® 30 or Salmosan®. If unable to calculate EC50 the NOAEC was determined from the highest concentration tested that caused no effects.

Chemotherapeutant	Length of exposure	Endpoint	Immobility concentration	95% CI
Interox® Paramove® 30	1-h	EC25	01.9 mg/L	(0.91 - 5.3 mg/L)
Interox® Paramove® 30	3-h	EC25	0.57	(0.036 - 1.4 mg/L)
Salmosan®	1-h	NOAEC	7500 µg/L	
Salmosan®	3-h	EC25	131 µg/L	(16 - 750 µg/L)

For mobility, Paramove® 30 1-h exposure resulted in an EC50 of 4.1 mg/L (CI 2.2 - 10 mg/L) and the 3-h exposure resulted in an EC50 of 1.5 mg/L (CI 1.0 - 2.5 mg/L) (Table 13). The 1-h Salmosan® exposure resulted in an EC50 of 7.5 mg/L (CI 2.4 – 5.7 µg/L), while the 3-h EC50 was 480 µg/L (CI 220 - 73 µg/L).

Table 13. Estimated EC50 and NOAEC (mobility) values and 95% confidence intervals of *Acartia tonsa* eggs subjected to sublethal exposures of Interox® Paramove® 30 or Salmosan®. If unable to calculate EC50 the NOAEC was determined from the highest concentration tested that caused no effects.

Chemotherapeutant	Length of exposure	Endpoint	Mobility	95% CI
Interox® Paramove® 30	1-h	EC50	4.1 mg/L	(2.2 - 10 mg/L)
Interox® Paramove® 30	3-h	EC50	1.5 mg/L	(1.0 - 2.5 mg/L)
Salmosan®	1-h	EC50	7500 µg/L	(2400 - 5700 µg/L)
Salmosan®	3-h	EC50	480 µg/L	(220 - 73 µg/L)

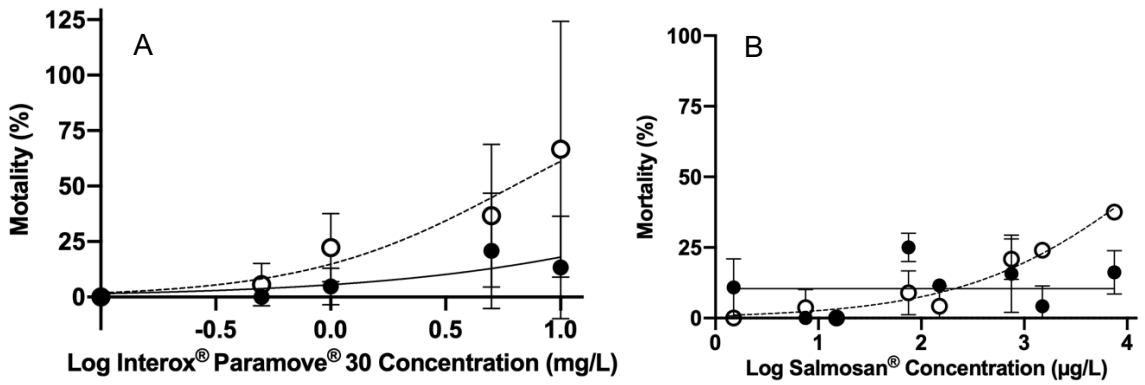


Figure 7. Mortality of hatched *Acartia tonsa* nauplii after exposure as an egg to Paramove® 30 or Salmosan®. A) Paramove® 30 1-h (*closed circles*) and 3-h (*open circles*) results and B) Salmosan® 1-h and 3-h results. Error bars represent the standard deviation for each treatment (n= 3).

For mortality, Paramove® 30 1-h exposure resulted in an LC10 of 3.7 mg/L (CI 0.12 - 12 mg/L) and the 3-h exposure resulted in an LC10 of 0.76 mg/L (CI 0.2 - 1.5 mg/L) (Table 14). LC10s were calculated instead of LC50s due to low mortality among treatment groups. The 1-h Salmosan® exposure resulted in a NOAEC of 7.5 mg/L, while the 3-h LC10 was 190 µg/L (CI 39 - 490 µg/L).

Table 14. Estimated LC10 (mortality) values and 95% confidence intervals of Acartia tonsa eggs subjected to sublethal exposures of Interox® Paramove® 30 or Salmosan®. If unable to calculate LC10 the NOAEC was determined from the highest concentration tested that caused no effects.

Chemotherapeutant	Length of exposure	Endpoint	Lethality concentration	95% CI
Interox® Paramove® 30	1-h	LC10	3.7 mg/L	(0.14 - 12 mg/L)
Interox® Paramove® 30	3-h	LC10	0.76 mg/L	(0.2 - 1.5 mg/L)
Salmosan®	1-h	NOAEC	7500 µg/L	
Salmosan®	3-h	LC10	190 µg/L	(39 - 490 µg/L)

3.3.2. Naupliar development

Naupliar *Acartia tonsa* (< 24 h old following hatching) were exposed to Paramove® 30 or Salmosan® for 1 or 3 h. They were fed and maintained in clean SW for 5 d, at which point the control groups had reached the copepodite stage. Each organism was then terminated and classified as a nauplii or copepodite. For both the 1-h and 3-h Paramove®

30 and Salmosan® experiments, the proportion of nauplii that developed into copepodites decreased as concentration increased (Figure 8). A summary of the effect concentration values found for this experiment including EC25, EC50, and NOAECs are presented in Table 18.

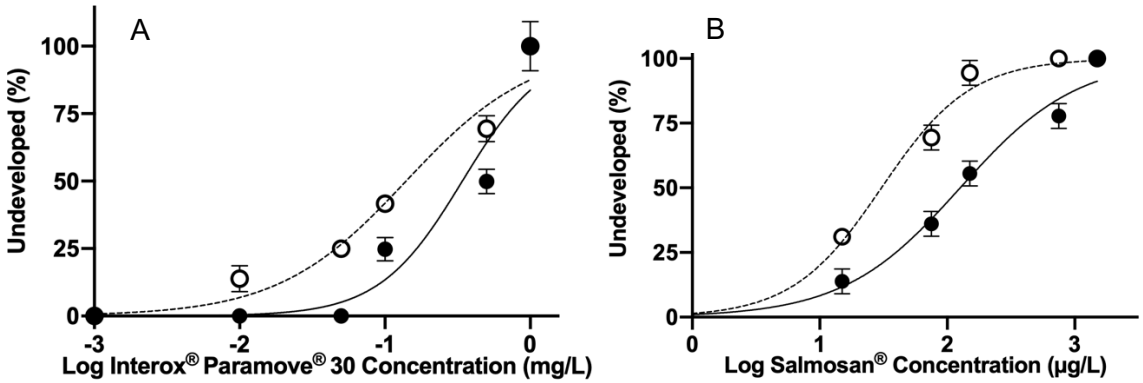


Figure 8. Development of *Acartia tonsa* nauplii to the copepodite stage after an exposure to Interox® Paramove® 30 or Salmosan®. The proportion undeveloped nauplii after exposure for 1-h (*closed circles*) and 3-h (*open circles*) to A) Interox® Paramove® 30 and B) Salmosan® are shown. Error bars represent the standard deviation for each treatment (n= 3).

In terms of naupliar development, Paramove® 30 had a 1-h and 3-h EC50 of 0.39 mg/L (CI 0.29 – 0.53 mg/L) and 0.12 mg/L (CI 0.08 – 0.18 mg/L) , respectively (Table 15). Salmosan® had a 1-h and 3-h EC50 of 120 µg/L (CI 80 – 170 µg/L) and 29.9 µg/L (CI 30.2 – 41 µg/L), respectively. An example of the observed endpoints can be seen in Figure 9.

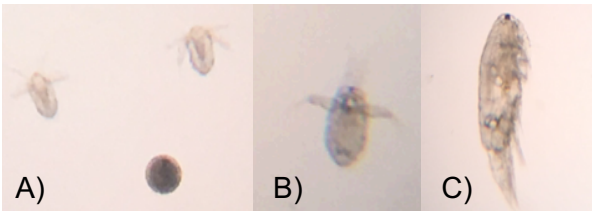


Figure 9. Life stages of *Acartia tonsa* at 40x magnification. A) One egg and two recently hatched nauplii B) A stage VI nauplii C) A stage II copepodid

Table 15. Estimated EC50 (naupliar development) values and 95% confidence intervals of Acartia tonsa nauplii subjected to sublethal exposures of Paramove® 30 or Salmosan®. Nauplii were exposed to the pesticide for the indicated period and analyzed after 5 d to determine if each organism had developed into a copepodite.

Chemotherapeutant	Length of exposure	EC50 (AI)	95% CI
Interox®Paramove®30	1-h	0.39 mg/L	(0.29 - 0.53 mg/L)
Interox®Paramove®30	3-h	0.12 mg/L	(0.08 - 0.18 mg/L)
Salmosan®	1-h	120 µg/L	(80.4 - 173 µg/L)
Salmosan®	3-h	29.9 µg/L	(20.2 - 40.9 µg/L)

3.3.3. Reproductive success

Fertilized female *Acartia tonsa* were exposed to Paramove® 30 or Salmosan® for 1 or 3 h. The number of eggs laid within 48 h were then recorded, as well as the hatching success of each egg. For both the 1-h and 3-h Paramove® 30 experiments, the number of eggs laid decreased as concentration increased (Figure 10A). The eggs laid from the highest concentration group also had a significantly lower egg hatching success proportion for both the 1-h and 3-h exposures (Figure 11A). For both the 1-h and 3-h Salmosan® experiments, the number of eggs laid decreased as concentration increased (Figure 10B). The 1-h Salmosan® experiment did not result in decreased hatching success as concentrations increased. The 3-h Salmosan® experiment did, however, result in decreased hatching success as concentrations increased (Figure 11B). A summary of the effect concentration values found for this experiment including EC25, EC50, and NOAECs are presented in Table 18.

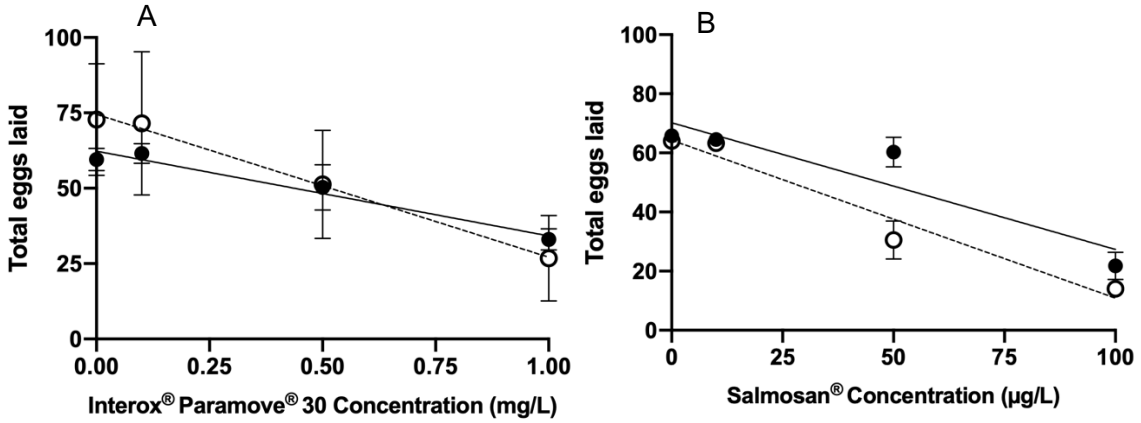


Figure 10. The total eggs laid by adult female *Acartia tonsa* after exposure to Interox® Paramove® 30 (active ingredient 30% H_2O_2) or (B) Salmosan. Females were exposed for

1-h (*closed circles*) or 3-h (*open circles*) to (A) Interox® Paramove® 30 or (B) Salmosan®. Error bars represent the standard deviation for each treatment (n= 3).

After a 1-h exposure of Paramove® 30 to adult female *Acartia tonsa*, an EC50 of 1.2 mg/L (CI 1.1 - 1.4 mg/L) was found for egg laying (Table 16). After a 3-h exposure, the calculated EC50 was 0.63 mg/L (CI 0.56 - 0.71 mg/L). For Salmosan®, the EC50 for a 1-h exposure was 84 µg/L (CI 76 - 94 mg/L), and the 3-h EC50 was 51 µg/L (CI 47 - 56 mg/L).

Table 16. Estimated EC50 (egg laying) values and 95% confidence intervals of Acartia tonsa adult females subjected to sublethal exposures of Paramove® 30 or Salmosan®. Adult females were exposed to the pesticide for the indicated period and analyzed at 24 and 48 h for the number of eggs laid.

Chemotherapeutant	Length of exposure	Endpoint	EC50	95% Confidence Intervals
Interox® Paramove® 30	1-h	EC50	1.2 mg/L	(1.1 - 1.4 mg/L)
Interox® Paramove® 30	3-h	EC50	0.63 mg/L	(0.56 - 0.71 mg/L)
Salmosan®	1-h	EC50	84 µg/L	(76 - 94 µg/L)
Salmosan®	3-h	EC50	51 µg/L	(47 - 56 µg/L)

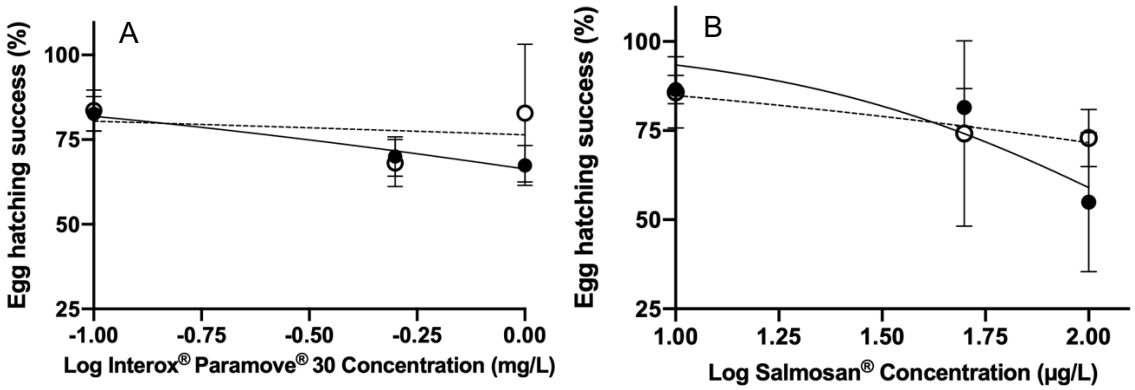


Figure 11. Hatching success of *Acartia tonsa* eggs from females exposed to Interox® Paramove® 30 (A) or Salmosan® (B). Exposures were for 1 h (*closed circles*) or 3 h (*open circles*). Error bars represent the standard deviation for each treatment (n= 4).

Hatching success of eggs after a 1-h exposure of Paramove® 30 to adult female *Acartia tonsa* resulted in an EC50 of 5.8 mg/L (CI 2.4 - 16 mg/L), while the EC50 after the 3-h exposure was 5.0 mg/L (CI 2.4 - 16 mg/L) (Table 17). The hatching success of *Acartia tonsa* eggs after the same exposure to Salmosan® of 1 and 3 h resulted in EC50s of 50

µg/L (CI 33 - 90 µg/L) and 29 µg/L (CI 16 - 66 µg/L), respectively. An example of what the microwells looked like after both 24 and 48 h can be seen in Figure 12.

Table 17. Estimated EC50 values and 95% confidence intervals for hatching success in Acartia tonsa adult females subjected to sublethal exposures of Paramove® 30 or Salmosan® . Adult females were exposed to the pesticide for the indicated period and analyzed at 24 and 48 h for the number of laid eggs that have hatched.

Chemotherapeutant	Length of exposure	Endpoint	EC50	95% Confidence Intervals
Interox® Paramove® 30	1-h	EC25	5.8 mg/L	(2.4 - 16 mg/L)
Interox® Paramove® 30	3-h	EC25	5.0 mg/L	(1.5 - 160 mg/L)
Salmosan®	1-h	EC25	50 µg/L	(33 - 90 µg/L)
Salmosan®	3-h	EC25	29 µg/L	(16 - 66 µg/L)

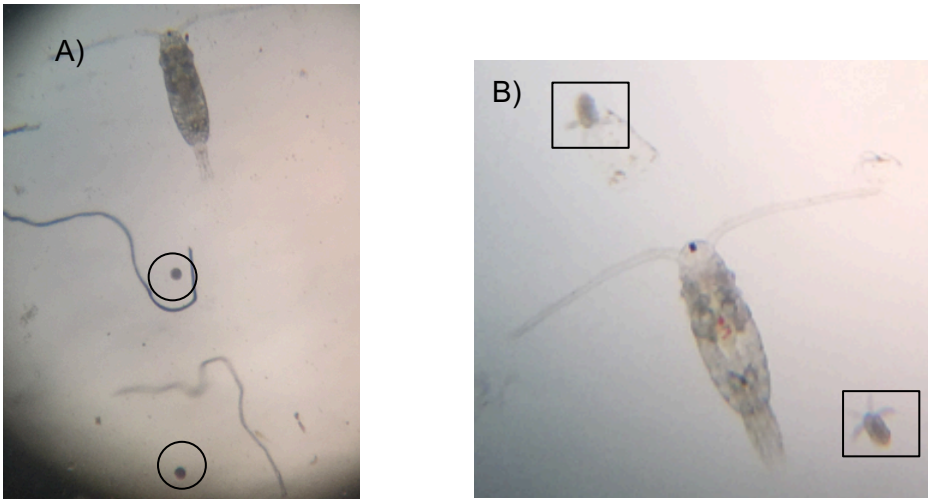


Figure 12. Examples of a female *Acartia tonsa* with hatched and unhatched eggs. A) A female *Acartia tonsa* in a 360µL microwell observed 24 h after exposure. Laid eggs can be seen in the black circles. B) A female *Acartia tonsa* in a 360µL microwell observed 48 h after exposure. Two hatched nauplii can be seen in the black squares. Both images are 40x magnification.

Table 18. Summary of calculated toxicological parameters for all sublethal experiments using *Acartia tonsa*. This includes EC25, EC50, and NOAECs, as well as LC10 and LC25 values for observed deaths in the egg-hatching experiment.

Endpoint	Time	Result	Interox® Paramove® 30 (CI)	Salmosan® (CI)
Hatching Success (post exposure to egg)	1-h	EC25	0.11 (0.0033- 0.33) mg/L	> 7.5 g/L
		EC50	1.21 (0.41- 4.3) mg/L	> 7.5 g/L
		NOAEC	0.1 mg/L	7.5 g/L
Hatching Success (post exposure to egg)	3-h	EC25	0.049 (0.00019- 0.22) mg/L	> 7.5 g/L
		EC50	0.82 (0.16- 53) mg/L	> 7.5 g/L
		NOAEC	0.1 mg/L	7.5 g/L
Immobility (post exposure to egg)	1-h	EC25	1.9 (0.91- 5.3) mg/L	> 7.5 g/L
		EC50	7.3 (3.2- 72) mg/L	> 7.5 g/L
		NOAEC	0.1 mg/L	7.5 g/L
Immobility (post exposure to egg)	3-h	EC25	0.57 (0.036- 1.4) mg/L	131 (16- 754) µg/L
		EC50	4.7 (2.1- 16) mg/L	> 7.5 g/L
		NOAEC	0.1 mg/L	7.5 g/L
Mobility (post exposure to egg)	1-h	EC25	16 (6.9- 92) mg/L	> 7.5 g/L
		EC50	4.1 (2.2- 10) mg/L	> 7.5 g/L
		NOAEC	0.1 mg/L	7.5 g/L
Mobility (post exposure to egg)	3-h	EC25	3.1 (2.0- 7.0) mg/L	7.5 (2.4- 57) g/L
		EC50	1.5 (1.0- 2.5) mg/L	480 (220- 73) µg/L
		NOAEC	0.1 mg/L	150 µg/L
Mortality (post exposure to egg)	1-h	LC10	3.7 (0.14- 12) mg/L	> 7.5 g/L
		LC25	12 (5.1- 1600) mg/L	> 7.5 g/L
		NOAEC	0.5 mg/L	7.5 g/L
Mortality (post exposure to egg)	3-h	LC10	0.76 (0.2- 1.5) mg/L	190 (39- 490) µg/L
		LC25	2.1 (1.1- 5.1) mg/L	> 7.5 g/L
		NOAEC	0.1 mg/L	7.5 g/L
Naupliar development (post nauplii exposure)	1-h	EC25	0.17 (0.11- 0.23) mg/L	38 (20- 59) µg/L
		EC50	0.39 (0.29- 0.53) mg/L	120 (80- 170) µg/L
		NOAEC	0.05 mg/L	< 15 µg/L
Naupliar development (post nauplii exposure)	3-h	EC25	0.037 (0.02- 0.06) mg/L	13 (7.0- 20) µg/L
		EC50	0.12 (0.08- 0.18) mg/L	30 (20.2- 41) µg/L
		NOAEC	< 0.01 mg/L	< 15 µg/L
Egg laying (post adult exposure)	1-h	EC25	2.1 (1.7- 3.1) mg/L	150 (130- 180) µg/L
		EC50	1.2 (1.1- 1.4) mg/L	84 (76- 94) µg/L
		NOAEC	0.5 mg/L	50 µg/L
Egg laying (post adult exposure)	3-h	EC25	1.3 (1.1- 1.6) mg/L	86 (79- 97) µg/L
		EC50	0.63 (0.56- 0.71) mg/L	51 (47- 56) µg/L
		NOAEC	0.1 mg/L	10 µg/L
Hatching success (post adult exposure)	1-h	EC25	5.8 (2.4- 16) mg/L	50 (33- 90) µg/L
		EC50	5.4 (1.3- 660000) mg/L	312 (150- 2100) µg/L
		NOAEC	0.1 mg/L	10 µg/L
Hatching success (post adult exposure)	3-h	EC25	5.0 (1.5- 160) mg/L	29 (16- 66) µg/L
		EC50	1.4 (0.13- 16) mg/L	260 (98- 8700) µg/L
		NOAEC	0.1 mg/L	10 µg/L

Chapter 4. Discussion

The target concentration of Paramove® 30 in Atlantic salmon aquaculture facilities ranges from 1200 to 1800 mg/L. Predicted concentrations in the water column immediately following release of Paramove® 30 from the net pens range from 14 to 270 mg/L. In all experiments using Interox® Paramove® 30, the LC50s and EC50 values generated fall significantly below the target concentration, as well as the predicted concentration in water following release from the net pen. This indicates that all wild assemblages and life stages of *Acartia tonsa* exposed to the full treatment concentrations of Paramove® 30 in the net pens during sea lice treatment or exposed to the chemotherapeutant immediately after release will likely experience adverse effects, including reduced hatching success, juvenile development, immobility, reproductive success, and overall survival, especially if entrained in an effluent plume for >1 h.

The prescribed treatment concentration of Salmosan® administered in aquaculture is 100 µg/L. Predicted concentrations in the water column immediately following release of Salmosan® from the net pens range from 0.1 to 10 µg/L (Burridge et al., 2000). In the Salmosan® experiments the 1-h wild assemblages, brachyuran crab zoea, and *Acartia tonsa* eggs all resulted in LC50s and EC50s significantly greater than the target concentration. The 3-h wild assemblages, porcelain crab zoea, and *Acartia tonsa* naupliar development and reproduction all had LC50s and EC50s below 100 µg/L, indicating that ≥ 50% organisms may be harmed if they are exposed to full treatment concentrations of Salmosan®. No LC50s or EC50s for any group tested were found at concentrations ranging from 0.1 to 10 µg/L, indicating that there is low risk, and it is unlikely that 50% of any population will be harmed if entrained in the diluted effluent plume for 1 or 3 h (Burridge et al., 2000).

Before a pesticide may be sold in Canada, the PMRA must assess the potential impacts to humans and the environment and approve that it is not expected to result in adverse impacts (PMRA, 2014). The goal is to cause no adverse effects in non-target species after the recommended exposure time. To accomplish this, the most sensitive values from this study would be used, which are those for the sublethal effects of naupliar development of *Acartia tonsa.* The lowest Interox® Paramove® 30 1-h NOAEC was 0.05 mg/L, and the lowest Salmosan® 1-h NOAEC was <15 mg/L. This, however, would not be a high enough treatment concentration to effect sea lice, and is therefore impractical.

4.1. Acute Lethality

After exposure to Paramove® 30, all LC50s from this study were far lower than those in the literature based on the exposure time. The lowest toxicity value in the literature is for *Daphnia carinata*, with an LC50 of 5.7 mg/L, however this is for an exposure time of 48-h (Reichwaldt et al., 2012). In terms of a similar exposure time, the lowest 1-h toxicity value in the literature is for the mysid shrimp at 973 mg/L (Burridge et al., 2014). Due to the limited number of studies involving H_2O_2 and marine organisms, there are fewer toxicity values in the literature to compare these results with than Salmosan®. The most sensitive test group was the Bamfield wild assemblage, followed by the wild assemblages from Nanoose Bay. *Acartia tonsa* threshold values for the Paramove® 30 results fell in the middle of all groups tested. The least sensitive test groups were the brachyuran and porcelain crab zoea. All LC50s fell lower than or within the predicted environmental concentrations of 14 to 270 mg/L after immediate release of Paramove® 30.

After exposure to Salmosan®, the LC50s from this study were in the mid/high ranges of those in the literature, based on the exposure time. The highest 1-h LC50 in the literature was that of Atlantic salmon, which was 5000 µg/L, and the highest LC50 calculated in this study was 529 µg/L (CI 332 – 900), for the wild assemblages from Nanoose (Burridge et al., 2014). The most sensitive test group was the Bamfield wild assemblage, followed by the brachyuran and porcelain crab zoea. *Acartia tonsa* threshold values for the Salmosan® results fell in the middle of all groups tested. The least sensitive test groups were the wild assemblages from Nanoose Bay. No LC50s fell lower than or within the predicted environmental concentrations of 0.1 to 10 µg/L after immediate release of Salmosan®.

4.1.1. Wild zooplankton assemblages

Lethal experiments involving wild assemblages collected in Nanoose Bay included 1-h, 3-h, 1-h with 48 h in clean SW, and 3-h with 48 h in clean SW for both Paramove® 30 and Salmosan®. Lethal experiments involving wild assemblages collected in Bamfield included 1-h with 4 h in clean SW for both pesticides.

Zooplankton are highly sensitive to the effects of H_2O_2. Paramove® 30 1-h LC50s for wild zooplankton assemblages ranged from 7 to 55 mg/L, and the 3-h LC50s ranged from 4 to 10 mg/L. All wild assemblage LC50s from the 1-h or 3-h exposure scenarios were not significantly different from their counterparts after 48 h in clean water. All LC50 values were within or lower than predicted environmental concentrations; no evidence of

a recovery trend was identifiable. In numerous studies, recovery has been highly evident at varying times after exposure of sea lice to H_2O_2. For example, Thomassen (1993) reported that 3 to 6 h after exposure to 1500 mg/L H_2O_2 for 20 min, 60 to 80% of sea lice had recovered. More recently, Helgesen et al. (2015) found that the sea-lice characterized as “immobile” immediately after treatment were re-evaluated as “alive” after 24 h. Marin et al (2017), reported that after exposure to H_2O_2, sea lice are less active for 2 h post exposure.

With Salmosan® exposure time and concentration increased, so did mortality. Zooplankton wild assemblage 1-h LC50s ranged from 83 to 529 µg/L and 3-h LC50s ranged from 54 to 127 µg/L. This indicates that when the zooplankton are incubated in the prescribed treatment concentration of Salmosan® (100 µg/L) for only 1 h there will be less than 50% mortality, and no effects are likely to occur when exposed to predicted environmental concentrations. At the treatment concentration for 3-h (3x the length of treatment in a fish farm), ≥ 50% of an assemblage would be killed. Similar to Paramove® 30, there was no evidence of recovery following exposure.

When analyzing the wild assemblages from Nanoose Bay, neutral red was used to stain live organisms. This removed the possible observational bias when determining if an organism is simply immobile, dead, or not moving at the moment of analysis. The use of neutral red, however, removed the ability to differentiate between mobile and immobile organisms. This limited the ability to identify if any recovery that may have occurred. When analyzing the Bamfield wild assemblages, lethality was determined without the use of the neutral red vital stain. Instead, the more traditional method of identifying the presence of a heartbeat, and response to a probe were used to determine if an organism was alive. This method allowed the added endpoint of immobility, as well as mortality to be measured.

Numerous factors contributed to the sensitivity of the wild assemblages, namely geographical, seasonal, and daily variation in the species and life stages present (Van Geest et al., 2014). In this experiment, the sensitivity to Paramove® 30 and Salmosan® was determined in wild assemblages from two locations, Nanoose Bay and Bamfield. The LC50s generated from both locations were not statistically different from each other. One notable observation was that the calanoid copepods found in Nanoose Bay were the last of the organisms to be impacted by either anti-sea lice pesticide, indicating higher resilience than other orders. No observation of one particularly resilient order was prominent in the Bamfield experiments.

Zooplankton species varied in each assemblage, as did life stages. When considering the presence of holoplankton, which is planktonic for its entire life cycle, and meroplankton, which is only planktonic during larval stages, the sensitivity of one organism may be vastly different from another. For example, adult stages of zooplankton such as copepods, krill, and amphipods, are the least sensitive stages in their life cycle, while meroplankton in their larval form are frequently considered to be one of the most sensitive life stages to chemicals and environmental change compared to their adult life stages (Conner, 1972; O'Brien et al., 1998). Throughout all experiments with wild organisms, the majority of holoplankton were present in their adult stage, but meroplankton were in their larval stage. This life stage makeup of the assemblages was due, in part, to the mesh size of the net used (150 µm). The mesh size also resulted in a small quantity of nauplii collected. Nauplii of varying species are generally quite similar in morphology and are therefore difficult to classify. Due to this, the few that were collected were placed in the "other holoplankton" category when identifying the species present in any tow. Had a larger quantity of nauplii been present, the wild assemblage LC50s for both chemotherapeutants may have been decreased, as the results from the naupliar development experiment indicate that it is the most sensitive life stage of the calanoid copepod *Acartia tonsa*.

4.1.2. Brachyuran and porcelain crab zoea

Brachyuran and porcelain crab zoea were opportunistically harvested from Bamfield inlet; due to limited numbers, only one experiment for each pesticide was performed. A 1-h exposure duration was chosen because it realistically reflects the duration of sea lice treatment. In the Paramove® 30 experiments, both Brachyuran and porcelain crab zoea generated EC50 values within predicted environmental concentrations and significantly below the treatment concentration of Paramove® 30 used in aquaculture. Brachyuran crab zoea had a 1-h EC50 (median effect concentration) (immobility and mortality) of 55 mg/L (CI 30 – 95 mg/L), while porcelain crab zoea had a 1-h EC50 of 46 mg/L (CI 27 – 76). After 4 h in clean water after exposure, the majority of effected crab zoea were paralyzed, but not dead. Recovery of sea lice (*L. salmonis*) has been reported just 2 h post exposure to 1500 mg/L H_2O_2 (Bruno and Raynard, 1994). It can be speculated that both species of crab of crab zoea may have required a longer period to fully recover than sea lice do, or that recovery was unlikely.

In the Salmosan® experiments, Brachyuran and porcelain crab zoea had 1-h EC50s below predicted environmental concentrations and similar to that of the treatment concentration of Salmosan® used in aquaculture. Brachyuran crab zoea had a 1-h EC50 value (immobility and mortality) of 203 µg/L (CI 128 – 320), while porcelain crab zoea had a 1-h EC50 value of 103 µg/L (CI 56 – 187). Similar to the effects of Paramove® 30, after 4 h in clean water after exposure, the majority of effected crab zoea were paralyzed but not dead. In a similar study by Gebauer et al (2017), a Chilean species of crab zoea (*Metacarcinus edwardsii*) showed increasing mortality at concentrations ranging from 0.0625 to 0.5 µg/L after repeated exposure of 1 h for 7 d. This suggests that either the Chilean crab zoea is more sensitive, or that multiple exposures of this pesticide could substantially reduce the EC50 values in the zoea.

4.1.3. Acartia tonsa

Lethal experiments involving *Acartia tonsa* included 1-h, 3-h, 1-h with 48 h in clean SW, and 3-h with 48 h in clean SW for both Paramove® 30 and Salmosan®. Paramove® 30 1-h LC50s for *Acartia tonsa* ranged between 13 and 23 mg/L, while 3-h LC50s were 9.1 and 10 mg/L. All Paramove® 30 toxicity values for *Acartia tonsa* were below or within predicted environmental concentrations and more than 120x dilution from the treatment concentration of 1200 to 1800 mg/L. Salmosan® 1-h LC50s were between 166 and 220 µg/L, while 3-h LC50s were between 54 and 127 µg/L. This indicates that after the recommended treatment concentration of 100 µg/L, some of the population would be affected by this chemotherapeutant. For both pesticides, all *Acartia tonsa* LC50s from the 1-h or 3-h exposure scenarios were not significantly different from their counterparts after 48 h in clean water.

The Paramove® 30 LC50 values for *Acartia tonsa* fell in the middle of the results for all test groups in this study, for both the 1-h and 3-h experiments. Both brachyuran and porcelain crab zoea were the least sensitive to Paramove® 30. Wild assemblages from Nanoose Bay and Bamfield were the most sensitive groups used in lethal testing.

4.2. Sublethal exposures

After exposure to Paramove® 30, all EC50s from this study were lower than those in the literature based on the exposure time. The lowest sublethal toxicity value in the literature is for immobility of *L. salmonis*, with an EC50 of 500 mg/L, however this is for a 30 min exposure (Bruno and Raynard, 1994). Due to the limited number of studies involving H_2O_2 and marine organisms, there are no 1-h sublethal toxicity values in the

literature to compare these results with. All LC50s fell lower than the predicted environmental concentrations of 14 to 270 mg/L after immediate release of Paramove® 30.

After exposure to Salmosan®, all EC50s from this study were far higher than those in the literature based on the exposure time. The highest sublethal toxicity value in the literature is for the fertilization success of *Lytechinus pictus* (painted urchin) of 6.84 mg/L, however this is for an exposure of 20 min (Ernst et al., 2001). The highest 1-h EC50 in the literature was that of wild assemblages of copepods, which was >500 µg/L. No EC50s fell lower than or within the predicted environmental concentrations of 0.1 to 10 µg/L after immediate release of Salmosan®.

4.2.1. Hatching success

Hatching success was determined by exposing *Acartia tonsa* eggs to Paramove® 30 or Salmosan® for 1 or 3 h and allowing them to hatch over a 48-h period. Exposure to Paramove® 30 resulted in decreased egg hatching success, and the nauplii that hatched experienced increased immobility and mortality below predicted environmental concentrations. The hatching success EC50s for the 1-h and 3-h exposures were 1.2 mg/L and 0.82 mg/L, respectively. Upon hatching the 1-h and 3-h naupliar immobility EC25s were 1.9 mg/L (CI 0.91 – 5.3 mg/L) and 0.57 mg/L (CI 0.036 – 1.4 mg/L), the mobility EC50s were 4.1 mg/L (CI 2.2 – 10 mg/L) and 1.5 mg/L (CI 1.0 – 2.5 mg/L), and the mortality LC25s were 12 (CI 5.1 – 1600 mg/L) and 2.1 mg/L (CI 1.1 – 5.1 mg/L). Similarly, decreased egg hatching success and larval survivability have also been reported for sea lice (*L. salmonis*) at similar time points and concentrations ranging from 470 to 1700 mg/L, indicating that *Acartia tonsa* is far more sensitive (Aaen et al, 2014; McAndrew et al., 1998). McAndrew et al. (1998) also noticed a significantly greater effect of H_2O_2 on immature egg strings, when compared to the mature egg strings of sea lice.

In this study, H_2O_2 exposure resulted in adverse effects upon *Acartia tonsa* eggs, results similar to those for sea lice eggs (Bravo et al., 2015). The specific MOA of this is unknown, but it can be speculated that they were affected by a mechanism similar to the that of most living cells. H_2O_2 is a reactive oxygen species (ROS) which can be partially reduced into a hydroxyl radical, or fully reduced into water (Wink et al., 1995). Hydroxyl radicals can result in peroxidation of lipids, protein, and DNA by attracting electrons, resulting in damage and potential destruction of each (Hensley et al., 2000). Inhibition of

any of these macromolecules would explain the decreased hatching success and increased mortality and immobility in *L. salmonis* and *Acartia tonsa*.

Exposure to Salmosan® did not affect egg hatching success at predicted environmental concentrations, and the nauplii that hatched did not experienced increased immobility and mortality after the 1-h exposure at concentrations tested but did after the 3-h exposure. Salmosan® exposure did not affect egg hatching success in *Acartia tonsa* eggs at concentrations as high as 7500 µg/L at either exposure duration. Of the eggs that hatched, the 1-h exposure had no effect on survivorship or mobility, however the 3-h exposure resulted in minor mortality (a calculated LC10 of 190 µg/L) and an EC25 for immobility of 131 µg/L (13 – 754 µg/L). Similar effects were seen in a study by Bravo et al (2015), who measured the effects of azamethiphos on sea lice (*Caligulus rogercresseyi*) egg strings at two concentrations (0.4 and 2 µg/L) for 24 h. Hatching success was not affected in the majority of exposed egg strings, however no larvae survived after hatching at either concentration. While not directly comparable due to the difference in the duration of the exposure, these results indicate that *C. rogercressyi* is potentially more sensitive due to the effects observed at far lower concentrations. An explanation for these results could be that the longer exposure time of azamethiphos was not enough to prevent the eggs from hatching, but enough to allow it to absorb into the egg and cause delayed effects in the organism.

4.2.2. Naupliar development

The naupliar development experiment was performed to test the toxicity to *Acartia tonsa* at their potentially most sensitive stage (Medina et al., 2002). Larval/juvenile testing is common in toxicity studies involving copepods due to the major metamorphosis between the last nauplius stage to the first copepodite stage. This requires a more extensive genetic to physiological change and can be considered the most sensitive developmental window (OECD, 2007). The easily detectable morphological distinction between these two life stages allow for a simple analysis of the endpoint.

Effects on naupliar development were determined by exposing nauplii to Paramove® 30 or Salmosan® for 1 or 3 h and allowing the organisms to develop over the next 5 d. Paramove® 30 and Salmosan® do not affect the larval stages of sea lice, so due to the similarities in physiology between sea lice and *Acartia tonsa,* one line of reasoning was that the larval stages of *Acartia tonsa* would not be affected by these chemotherapeutants. This was not the case for either chemotherapeutant, but Paramove®

30 showed far more sensitivity at concentrations below the treatment concentration, compared to Salmosan®.

The EC50s for naupliar development effects for the 1-h and 3-h Paramove® 30 experiments were 0.39 mg/L (0.29 – 0.53 mg/L) and 0.12 mg/L (0.08 – 0.18 mg/L). These were the lowest values determined of all life stages tested and below predicted environmental concentrations. This is in-line with previous copepod studies involving metals and organic pesticides that found nauplii to be 1.3 to 5x more sensitive than adults in 48-h and 96-h lethality tests (Verriopoulos and Moraittou-Apostolopoulou, 1982; O'Brien et al., 1988; Forget et al., 1998). In contrast to these results, Van Geest et al. (2014) reported a wild copepod assemblage that was dominated by nauplii to be less sensitive to Paramove® 30 than other assemblages that had more adult copepods present. This was thought to corroborate with previous studies which indicate that H_2O_2 is ineffective against larval stages of sea lice (Burridge et al., 1998; Torrissen et al., 2013).

The naupliar development EC50 values for the 1-h and 3-h Salmosan® experiments were 120 µg/L (CI 80 – 170 µg/L) and 30 µg/L (CI 20.2 – 41 µg/L), respectively. The 1-h EC50 is comparable to that of the associated Salmosan® treatment concentrations used in fish farms (100 µg/L). Unlike sea lice, larval stages of *Acartia tonsa* were affected by Salmosan®, but this is not dissimilar to the effect of this chemotherapeutant on the first larval stage of the crab *M. edwardsii*. In one study, mortality of *M. edwardsii* zoea increased after exposure to concentrations of Salmosan® ranging from 0.0625 to 0.5 µg/L after daily exposure of 1-h for 7 d. Development of the *M. edwardsii* zoea (larval crab life stage), however, was not adversely affected (Gebauer et al., 2017). This indicates that while *M. edwardsii* zoea was more sensitive to Salmosan® than *Acartia tonsa*, the results are in-line with previous studies.

4.2.3. Reproductive success

Reproductive success was determined by exposing adult female *Acartia tonsa* to Paramove® 30 or Salmosan® for 1 or 3 h and allowing each organism 48 h to reproduce. Reproductive success was defined by the number of eggs laid and hatching success. After exposure of Paramove® 30 to adult females, both egg laying and hatching success were affected at concentrations far below predicted environmental concentrations. For both the 1-h and 3-h experiments hatching success EC50 values (5.4 and 1.4 mg/L) were less sensitive than those of the EC50 values of the total eggs laid (1.2 and 0.63 mg/L). Very few similar reproductive studies have been done with Paramove® 30 and reproductive

success. Johnson et al. (1993) collected egg strings from wild female sea lice (*L. salmonis*) in fish farms 9 weeks after treatment with H_2O_2 and found that a proportion of them had a decreased hatching success and decreased development post-hatch compared to control eggs. It cannot be determined at this time if *Acartia tonsa* is more or less sensitive than *L. salmonis*, how it can be said that they both show sensitivity towards H_2O_2. All effects were observed at concentrations lower than the predicted environmental concentrations of 14 to 270 mg/L after immediate release of Paramove® 30.

One caveat for using the lethal and sublethal *Acartia tonsa* results from Paramove® 30 on cold water Pacific copepods is ambient temperature. As temperature increases, so does the toxicity of H_2O_2 (Rach et al., 1997). The *tonsa* were held in a temperature-controlled room at 22 ± 2°C which is within their optimal temperature range (Marcus and Wilcox, 2007), while BC fish farms range from 4 to 18 °C throughout the year. This may have caused the effects of Paramove® 30 to be exacerbated and result in lower LC50s and EC50s. The values reported in this study for the effect of these two chemotherapeutants on *Acartia tonsa* may not be indicative of the effects of seen at 4 to 18 °C temperatures.

After exposure of adult females to Salmosan®, both reproductive endpoints (egg laying and hatching success) were affected above below predicted environmental concentrations. For both the 1-h and 3-h experiments, hatching success EC50 values (312 and 260 µg/L), were less sensitive than those of the EC50 values for the total eggs laid, which were above the highest treatment group concentration, and estimated to be 84 and 51 µg/L (EC25s were 150 and 86 µg/L). In a previous study, egg production in the American lobster (*Homarus americanus*) was reported to decrease by 34% after a 1-h bi-weekly exposure to 0.1 µg/L azamethiphos (Urbina et al., 2019). This indicates that *H. americanus* egg-laying is more sensitive to the effects of azamethiphos than *Acartia tonsa*.

In most organisms, acetylcholine ACh is rapidly broken down by acetylcholinesterase (AChE) to prevent over stimulation of post-synaptic nerves, muscles, and exocrine glands. As the AI of azamethiphos is an organophosphate and subsequently an AChE inhibitor, exposure is likely to adversely affect physiological functioning in organisms. The MOA acting upon adult reproduction in this study is unknown, however similar results have been observed in the sea urchin *Paracentrotus lividus*. For fertilization of *P. lividus* to occur, ACh is required to depolarize the egg membrane (Angelini et al., 2004). This indicates that if the concentration was not high enough to impact the nervous system of the adult male and female, the presence of an AChE inhibitor is not likely to

affect fertilization. AChE would not be able to break down the ACh, which would not affect the ability of ACh to depolarize the cell membrane of the oocyte, enabling the sperm to fuse to the egg. It can be hypothesized that a similar mechanism of fertilization may take place in *Acartia tonsa*, resulting in the lack of effect of azamethiphos on hatching success. If there was a high enough concentration to impact the nervous system of the adult, the lack of effect on hatching success can perhaps be explained by exposure post fertilization.

A potential source of error in the hatching success endpoint of this experiment is the unknown time of fertilization. All females were removed from cultured stocks of *Acartia tonsa* and were therefore possibly carrying fertilized eggs at the time of each experiment. Unlike egg laying, which is not dependent on fertilization to occur, fertilization is vital to hatching success. A male was placed in each experiment vessel in an effort to ensure that each female was fertilized. If unfertilized, the adult female or male may have experienced symptoms such as seizing or paralysis, which may have inhibited fertilization from taking place. If the female was already carrying a fertilized egg, her exposure to the pesticide may have interrupted the development of the offspring. Lastly, the viability of the egg or egg sac may have simply been impaired.

A fewer number of concentrations were used in the reproductive success experiments compared to any other sublethal or lethal experiments in this study. Using a larger number of concentrations was not feasible due to the length of time required to sort the male and female *Acartia tonsa*, and the limited number of stereomicroscopes at our disposal. Future studies should consider including more concentrations.

4.3. Conclusions and future recommendations

This study was conducted to provide Fisheries and Oceans Canada National Contaminants Advisory Group information regarding the environmental fate and toxicity of Paramove® 30 and Salmosan® to marine zooplankton. When anti-sea lice pesticides are administered into an aquaculture site, zooplankton in and around the net-pens are likely to be exposed due to the method of release and dilution of treated water into the environment. The exposure time to non-target species in the water column varies due to potential plume formation or immediate dilution and hydrolysis but is likely only a few hours in length. The concentration of effluent plumes can also be affected by treatment of multiple net pens at a site in one day and the proximity of multiple aquaculture sites, which may cause prolonged or pulse exposure scenarios. In consideration of these factors, both 1-h and 3-h toxicity tests were performed.

Results from the current study indicate that Salmosan® presents less risk than Interox® Paramove® 30 does to both cultured *Acartia tonsa* and wild zooplankton from British Columbia. Studies such as this one are important to developing regulatory protocols and guidelines for the use of anti-sea lice pesticides in Canada. The results of this study add to our knowledge base and will hopefully assist in allowing decision makers to make informed regulatory decisions in the future.

A limitation of this study is that the majority of wild assemblages consisted of calanoid copepods, which may have skewed the results. Species composition was relatively consistent in Nanoose Bay, but in Bamfield the experiments were conducted only in November 2018, resulting in unknown species composition during the rest of the year. From these results is not recommended to do future toxicity testing with *Acartia tonsa* or marine zooplankton from Georgia strait for these two chemotherapeutants. To further investigate the effects of these chemotherapeutants on marine zooplankton in BC, it is suggested to repeat the Bamfield experiments during different seasons. The sensitivity of wild assemblages may fluctuate due to seasonal change in species composition, and the presence or absence of larval species such as brachyuran and porcelain crab zoea. As well, the use of other marine organisms in short-term toxicity tests is recommended to fill the data gaps in the literature. Further toxicity testing is currently ongoing that is studying the effect of these two pesticides on other non-target species that inhabit areas surrounding net-pens. Crustaceous species including the spot prawn (*Pandalus platyceros*), and amphipod (*Eohaustorius estuaris*) are being tested in the Kennedy lab at Simon Fraser University due to the potentially similar mechanism of action for azamethiphos. Testing, however, is not limited to organisms with similar physiology as the target sea-lice. Other species used are those that may be found in areas surrounding salmon farming net-pens, including two species of polychaetes (*Nereis virens* and *Neanthes Arenaceodentata*), sea urchins (*Strongylocentrotus purpuratus*), and starry flounder (*Platichthys stellatus)*.

While numerous toxicity tests are ongoing to determine the effects of these chemotherapeutants on non-target organisms, the best measure to prevent these effects would be to limit the use of chemotherapeutants. As mentioned above, there are alternative measures that could be performed to control sea louse populations on fish farms. Adding cleaner fish such as wrasse has proved successful in Norway, as well as using mechanical and thermal delousing techniques (Burridge et al., 2010). Until an

alternative method is used as the primary treatment option, non-target organisms including zooplankton are at risk.

References

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends Parasitol*. 31, 72-81.

Abdel-Lateif, H. M., Donker, M. H. and Van Straalen, N. M. (1998). Interaction between temperature and cadmium toxicity in the isopod *Porcellio scaber*. Funct. Ecol. 12, 521–527.

Abgrall P., Rangeley R.W., Burridge L.E., Lawton P. (2000). Sublethal effects of azamethiphos on shelter use by juvenile lobsters (*Homarus americanus*). Aquaculture 181, 1-10.

Andersen, H. R., Halling-Sørensen, B., & Kusk, K. O. (1999). A parameter for detecting estrogenic exposure in the copepod *Acartia tonsa*. *Ecotox and environ safety*. 44, 56-61.

Angelini, C., Baccetti, B., Piomboni, P., Trombino, S., Aluigi, M. G., Stringara, S. & Falugi, C. (2004). Acetylcholine synthesis and possible functions during sea urchin development. *European Journal of Histochem*. 235-244.

Barata, C., Medina, M., Telfer, T., Baird, D.J. (2002). Determining demographic effects of cypermethrin in the marine copepod *Acartia tonsa*: stage-specific short tests versus life-table tests. *Arch. Environ. Contam. Toxicol.* 43, 373-378.

Beek, B., Bohling, S., Bruckmann, U., Franke, C., Johncke, U., Studinger, G. (2000). The assessment of bioaccumulation. *The handbook of environmental chemistry (Part J): Bioaccum. New Aspects and Devel.* 2, 239–276.

Birkeland, K. (1997). Consequences of premature return by sea trout (*Salmo trutta*) infested with the salmon louse (*Lepeoptheirus salmonis* Kroyer): migration, growth and mortality. Can J. Fish. Aquat. Sci. 53, 2808–2813.

Bjordal Å. (1988). Cleaning symbiosis between wrasse (Labridae) and lice infested salmon (*Salmo salar*) in mariculture. ICES, *Mariculture Committee*. 188, 8.

Bateman, A.W., Peacock, S.J., Connors, B., Polk, Z., Berg, D., Krkošek, M. and Morton, A. (2016). Recent failure to control sea louse outbreaks on salmon in the Broughton Archipelago, British Columbia. *Canadian J. Fish and Aquat Sci*. 73, 1164-1172.

BC Farmers Association. (2018). http://bcsalmonfarmers.ca/ (accessed December 18, 2018)

Beamish, R.J., Neville, C.M., Sweeting, R.M. and Ambers, N. (2005). Sea lice on adult Pacific salmon in the coastal waters of Central British Columbia, Canada. *Fish Res.* 76,198-208.

Bellas, J., & Thor, P. (2007). Effects of selected PAHs on reproduction and survival of the calanoid copepod *Acartia tonsa*. *Ecotox.* 16, 465-474.

Brauner C.J., Sackville M., Gallagher Z., Tang S., Nendick L., Farrell A.P. (2012). Physiological consequences of the salmon louse (*Lepeophtheirus salmonis*) on juvenile pink salmon (*Oncorhynchus gorbuscha*): implications for wild salmon ecology and management, and for salmon aquaculture. *Philosophical Transactions of the Royal Society B: Biol Sci.* 367, 1770-9.

Bravo, S., Sevatdal, S., & Horsberg, T. E. (2010). Sensitivity assessment in the progeny of *Caligus rogercresseyi* to emamectin benzoate. *Bulletin of the European Association of Fish Pathologists*. 30, 99-105.

Bravo, S., Silva, M. T., Agusti, C., Sambra, K., & Horsberg, T. E. (2015). The effect of chemotherapeutic drugs used to control sea lice on the hatching viability of egg strings from *Caligus rogercresseyi*. *Aquac*. 443, 77-83.

Bron, J.E., Frisch, D., Goetze, E., Johnson, S.C., Lee, C.E. and Wyngaard, G.A. (2011). Observing copepods through a genomic lens. *Frontiers in zoology*. 8, 22.

Brooks, K.M. (2005). The Effects of Water Temperature, Salinity, and Currents on the Survival and Distribution of the Infective Copepodid Stage of Sea Lice (*Lepeophtheirus Salmonis*) Originating on Atlantic Salmon Farms in the Broughton Archipelago of British Columbia, Canada. *Rev in Fish Sci*. 3, 177-204

Burridge L.E., Haya K., Waddy S.L., Wade J. (2000). The lethality of anti-sea lice formulations Salmosan®(Azamethiphos) and Excis®(Cypermethrin) to stage IV and adult lobsters (*Homarus americanus*) during repeated short-term exposures. *Aquac.* 82, 27-35.

Burridge L.E., Haya K., Waddy S.L. (2005). Seasonal lethality of the organophosphate pesticide, azamethiphos to female American lobster (*Homarus americanus*). *Ecotox and environ safety*. 60, 277-81.

Burridge L.E., Haya K., Waddy S.L. (2008). The effect of repeated exposure to azamethiphos on survival and spawning in the American lobster (*Homarus americanus). Ecotoxicol and Environ Safety.* 69, 411-5.

Canty M.N., Hagger J.A., Moore R.T., Cooper L., Galloway T.S. (2007). Sublethal impact of short-term exposure to the organophosphate pesticide azamethiphos in the marine mollusk *Mytilus edulis*. *Mar Pollut*. 54, 396-402.

Carroll, Carol. (1999). Curating Curious Collections: An Interdisciplinary Perspective. *Predatory Pub Quarterly. 16,* 3-134.

Coburn, M., Schuel, H., & Troll, W. (1981). A hydrogen peroxide block to polyspermy in the sea urchin *Arbacia punctulata*. *Dev biol.* 84, 235-238.

Conner, P. M. (1972). Acute toxicity of heavy metals to some marine larvae. *Mar. Poll. Bull*. 3, 190-192.

Costello, M.J. (2009). The global economic cost of sea lice to the salmonid farming industry. *J. Fish Dis.* 32, 115-118.

Costelloe M., Costelloe J., Roche N. (1996). Planktonic dispersion of larval salmon-lice, *Lepeophtheirus salmonis*, associated with cultured salmon, Salmo salar, in western Ireland. *J. Mar Biol Assoc*. 76, 141−149.

Cotran, R.S., Kumar, V., and Robbins, S.L. (1989). Pathological Basis of Disease; 4 ed.; Saunders: Toronto.

Couillard C.M., Burridge L.E. (2015). Sublethal exposure to azamethiphos causes neurotoxicity, altered energy allocation and high mortality during simulated live transport in American lobster. *Ecotox and environ safety.*115, 291-9.

Cruz, J., Garrido, S., Pimentel, M.S., Rosa, R., Santos, A.M.P. and Ré, P). (2013). Reproduction and respiration of a climate change indicator species: effect of temperature and variable food in the copepod *Centropages chierchiae*. *J. plankton research.* 35, 1046-1058.

DFO (2018). Aquaculture statistics. Aquaculture statistics and reports. Retrieved 25 November, 2018 from www.dfo-mpo.gc.ca/aquaculture.

DFO (2013). National Peer-review Meeting on Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments in the Bay of Fundy, New Brunswick; November 2-3, 2011. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2012/050.

Dounia D., Andrea B., Lefort N., Van Geest J.L. (2016). Repeated sublethal exposures to the sea lice pesticide Salmosan®(azamethiphos) on adult male lobsters (*Homarus americanus*) causes neuromuscular dysfunction, hypoxia, metabolic disturbances and mortality. *Ecotox and environ safety.*134, 106-15

Ernst, W., Doe, K., Cook, A., Burridge, L., Lalonde, B., Jackman, P., Aubé, J. G. and Page, F. (2014). Dispersion and toxicity to non-target crustaceans of azamethiphos and deltamethrin after sea lice treatments on farmed salmon, Salmo salar. *Aquac.* 424–425, 104–112.

Florey, E. (1973). Acetylcholine as sensory transmitter in Crustacea. J. Comp. Physiol. 83, 1–16.

Forget, J., Beliaeff, B. and Bocquené, G. (2003). Acetylcholinesterase activity in copepods (Tigriopus brevicornis) from the Vilaine River estuary, France, as a biomarker of neurotoxic contaminants. *Aqua Tox*. 62,195-204.

Forget, J., Pavillon, J. F., Menasria, M. R., & Bocquene, G. (1998). Mortality and LC50Values for Several Stages of the Marine Copepod *Tigriopus brevicornis* (Müller) Exposed to the Metals Arsenic and Cadmium and the Pesticides Atrazine, Carbofuran, Dichlorvos, and Malathion. *Ecotox and envi safety*. 40, 239-244.

Gebauer P., Paschke K., Vera C., Toro J.E., Pardo M., Urbina M. (2017). Lethal and sub-lethal effects of commonly used anti-sea lice formulations on non-target crab *Metacarcinus edwardsii* larvae. *Chemosphere.* 185, 1019-29.

Geer, T. D. (2016). Responses of Aquatic Organisms to Exposures of Sodium Carbonate Peroxyhydrate.

Gonzalez, E.B. and de Boer, F. (2017). The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. *Fish. sci. 83*, 661-670.

Grøntvedt R.N., Nerbøvik I.K.G., Viljugrein H., Lillehaug A., Nilsen H., Gjevre A.G. (2015) Norwegian Veterinary Institute's Report Series: 13. Thermal de-licing of salmonid fish documentation of fish welfare and effect. Norwegian Veterinary Institute.

Gorbi, G., Invidia, M., Savorelli, F., Faraponova, O., Giacco, E., Cigar, M. & Sei, S. (2012). Standardized methods for acute and semichronic toxicity tests with the copepod *Acartia tonsa*. *Environ tox and chem.* 44, 2023-2028.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. pp 26-60. In Smith W.L. and Chanley M.H (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA.

Guillard, R.R.L. and Ryther, J.H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can. J. Microbiol. 8: 229-239.

Hassan, H. M., and Fridovich, I. (1979). Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. *Archives of biochemistry and biophysics*. 196, 385-395.

Helgesen, K.O., Bravo, S., Sevatdal, S., Mendoza, J., Horsberg, T.E. (2014). Deltamethrin resistance in the sea louse *Caligus rogercresseyi* (Boxhall and Bravo) in Chile: bioassay re- sults and usage data for antiparasitic agents with references to Norwegian conditions. *J. Fish Dis*. 37, 877–890.

Helgesen, K. O., Romstad, H., Aaen, S. M., & Horsberg, T. E. (2015). First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. *Aquac reports*. 1, 37-42.

Helgesen K.O., Bakke M.J., Kaur K., Horsberg T.E. (2017). Increased catalase activity—a possible resistance mechanism in H_2O_2 resistant salmon lice (*Lepeophtheirus salmonis*). *Aquaculture*. 468, 135-40.

Hjeltnes B., Bang-Jensen B., Bornø G., Haukaas A., Walde C.S. (2018) The Health Situation in Norwegian Aquaculture 2017. *Norwegian Veterinary Institute.* 108.

Hensley, K., Robinson, K. A., Gabbita, S. P., Salsman, S., & Floyd, R. A. (2000). Reactive oxygen species, cell signaling, and cell injury. *Free Radical Biol. and Med*. 28, 1456-1462.

Intorre L., Soldani G., Cognetti-Varriale A.M., Monni G., Meucci V., Pretti C. (2004). Safety of azamethiphos in eel, seabass and trout. *Pharmacol res*. 49, 171-6.

Johnson, S. C., Constible, J. M., & Richard, J. (1993). Laboratory investigations on the efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis* and its toxicological and histopathological effects on Atlantic salmon *Salmo salar* and chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms*. 17, 197-204.

Katija, K. and Dabiri, J.O. (2009). A viscosity-enhanced mechanism for biogenic ocean mixing. *Nature*. 460, 624-626.

Keen, E. (2013). A practical designer's guide to mesozooplankton nets. available at (last access: 15 October 2014): http://acsweb. ucsd.edu/~ekeen/resources/Choosing-a-Net. pdf.

Kennedy, C. J., Gill, K. A. and Walsh, P. J. (1989). Thermal modulation of benzo[a]pyrene uptake in the gulf toadfish, *Opsanus beta*. Environ. Toxicol. Chem. 8, 863–869.

Kiemer, M.C., Black, K.D. (1997). The effects of H_2O_2 on the gill tissues of Atlantic salmon, *Salmo solar* L. *Aquac.* 153, 181-9.

Kiørbe, T., Møhlenberg, F., Hamburger, K. (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar Ecol Prog Ser 26:35–97

Krkošek, M., Ford, J. S., Morton, A., Lele, S., Myers, R. A., & Lewis, M. A. (2007). Declining wild salmon populations in relation to parasites from farm salmon. *Science*. 318, 1772-1775.

Krkošek, M., Morton, A., Volpe, J.P. and Lewis, M.A. (2009). Sea lice and salmon population dynamics: effects of exposure time for migratory fish. *Proceedings of the Royal Society of London B: Bioll Sci*. 276, 2819-2828.

Krupa, E., Barinova, S., & Alyimov, M. (2015). Redescription of *Acartia* (Acanthacartia) *tonsa* Dana, 1849 (Copepoda: Calanoida) from the Caspian Sea. *Advanced Studies in Biol.*7, 393-402.

Kusk, K.O. and Wollenberger, L. (1999). Fully defined saltwater medium for cultivation of and toxicity testing with marine copepod *Acartia tonsa*. *Environ Tox and Chem: An International Journal*. 18, 1564-1567.

Kwok, K.W., Souissi, S., Dur, G., Won, E.J. and Lee, J.S. (2015). Copepods as references species in estuarine and marine waters. *Aquatic Ecotox*. 281-308. Academic Press.

Lewandowska, A.M., Boyce, D.G., Hofmann, M., Matthiessen, B., Sommer, U. and Worm, B. (2014). Effects of sea surface warming on marine plankton. *Ecol letters.* 17, 614-623.

Lindeque, P.K., Parry, H.E., Harmer, R.A., Somerfield, P.J. and Atkinson, A. (2013). Next generation sequencing reveals the hidden diversity of zooplankton assemblages. *PloS one*. 8, 81327.

Lurling, M. and De Senerpont Domis, L.M. (2013). Predictability of plankton communities in an unpredictable world. *Freshwater biol*. 58, 455-462.

Marcus, N. H., & Wilcox, J. A. (2007). Guide to the meso-scale production of the copepod *Acartia tonsa*.

Marín, S. L., Gonzalez, M. P., Madariaga, S. T., Mancilla, M., & Mancilla, J. (2018). Response of Caligus rogercresseyi (Boxshall & Bravo, 2000) to treatment with Hydrogen Peroxide: Recovery of parasites, fish infestation and egg viability under experimental conditions. *Journal of fish diseases*. 41, 861-873.

Mauchline, J. (1998). The Biology of Calanoid Copepods. *Advances in marine biology*, *33*.

Miller, T.A. and Adams, M.E. 1982. Mode of action of pyrethroids. In: Coats, J.R. (Ed.), Insecticide Mode of Action. *Academic Press*. 3–27.

Mitchell, A. J., & Collins, C. (1997). Review of the therapeutic uses of hydrogen peroxide in fish production. *Aquacult Mag*. 23, 74-79.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nat*. 405, 1017.

O'Brien, W.J. (1979). The predator-prey interaction of planktivorous fish and zooplankton: recent research with planktivorous fish and their zooplankton prey shows the evolutionary thrust and parry of the predator-prey relationship. *American Scientist*. 67, 572-581.

O'Brien, P., Feldman, H., Grill, E. V., & Lewis, A. G. (1988). Copper tolerance of the life history stages of the splashpool copepod *Tigriopus californicus* (Copepoda, Harpacticoida). *Mar. Ecol. Prog. Ser*. 44.

Ohs, C. L., Rhyne, A. L., & Stenn, E. (2009). Viability of subitaneous eggs of the copepod, *Acartia tonsa* (Dana), following exposure to various cryoprotectants and hypersaline water. *Aquacult*. 287. 114-119.

Overton K., Dempster T., Oppedal F., Kristiansen T.S., Gismervik K., Stien L.H. (2018). Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. *Reviews in Aquac*.

Page, F.H. and Burridge, L. (2014). Estimates of the effects of sea lice chemical therapeutants on non-target organisms associated with releases of therapeutants from tarped net-pens and well-boat bath treatments: a discussion. *Canadian Science Advisory Secretariat.*

Pahl B.C., Opitz H.M. (1999). The effects of cypermethrin (Excis) and azamethiphos (Salmosan) on lobster *Homarus americanus* H. Milne Edwards larvae in a laboratory study. *Aquac res.* 30, 655-65.

Potts G.W. (1973). Cleaning symbiosis among British fish with special reference to *Crenilabrus melops* (Labridae). J Mar Biol Assoc UK 53:1–10

Rach J.J., Schreier T.M., Howe G.E., Redman S.D. (1997). Effect of species, life stage, and water temperature on the toxicity of H_2O_2 to fish. *The Progressive Fish-Culturist.* 59, 41-6.

Ratnarajah, L., Bowie, A.R., Lannuzel, D., Meiners, K.M. and Nicol, S. (2014). The biogeochemical role of baleen whales and krill in Southern Ocean nutrient cycling. PloS one. 9, 114067.

Rodríguez, L.P., Caliani, I., Brugnano, C., Granata, A., Guglielmo, R., Guglielmo, L., Zagami, G. and Minutoli, R. (2018). Biomarkers employment in planktonic copepods for early management and conservation of aquatic ecosystems: the case of the 'Capo Peloro'lakes (southern Italy). *Regional Studies in Marine Science*. 18, 161-169.

Roth, B. (2016). Avlusing av laksefisk med Optilicer: Effekt p#a avlusing og fiskevelford. Rapport 59/2016. Nofima, Norway, 41 p (in Norwegian).

Roth, M., Richards, R.H., Dobson, D.P., Rae, G.H. (1996). Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). *Aquac*. 140, 217-39.

Saksida, S., Bricknell, I., Robinson, S., Jones, S. (2015). Population ecology and epidemiology of sea lice in Canadian waters. DFO Canadian Science Advisory Secretariat Research Document.

Sauterey, B., Ward, B., Rault, J., Bowler, C. and Claessen, D. (2017). The implications of eco-evolutionary processes for the emergence of marine plankton community biogeography. *The American Nat*. 190, 116-130.

Environment and Climate Change Canada (1990). Biological test method: Acute lethality test using *Daphnia spp*. *Report EPS. 1*, 57-60.

Sevatadal, S., Horsberg, T.E. (2003). Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis*, Krøyer) against the pyrethroid deltamethrin using bioassays and probit modeling. *Aquac.* 218, 21–31.

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J of Exp Biol.* 218, 1856-1866.

Shurin, J.B., Havel, J.E., Leibold, M.A. and Pinel-Alloul, B. (2000). Local and regional zooplankton species richness: a scale-independent test for saturation. *Ecol*. 81, 3062-3073.

Shurtleff L.E. (1989). Interox America Sodium Percarbonate and H_2O_2-Acute Toxicity to the Freshwater Invertebrate *Daphnia pulex*. *Burlington Res Inc., North Carolina*.

Shurtleff, L.E. (1989). Intertox America Sodium Percarbonate and H_2O_2- Acute Toxicity to the Freshwater Fish *Pimephales promelas*. Burlington Research, Inc.

Sievers G., Palacios P., Inostroza R., Dölz H. (1995). Evaluation of the toxicity of 8 insecticides in *Salmo salar* and the in vitro effects against the isopode parasite, *Ceratothoa gaudichaudii*. Aquac. 134, 9-16.

Stien L.H., Lind M.B., Oppedal F., Wright D.W., Seternes T. (2018). Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. *Aquac.* 490, 281-7.

Sverdrup, L. E., Fürst, C. S., Weideborg, M., Vik, E. A., & Stenersen, J. (2002). Relative sensitivity of one freshwater and two marine acute toxicity tests as determined by testing 30 offshore E & P chemicals. *Chemosphere*. 46, 311-318.

Taylor, N. I., & Ross, L. G. (1988). The use of hydrogen peroxide as a source of oxygen for the transportation of live fish. *Aquac.* 70, 183-192.

Thomassen, J. M. (1993). Hydrogen peroxide as a delousing agent for Atlantic salmon. *Pathogens of wild and farmed fish: sea lice*. 290-295.

Treasurer, J.W., Wadsworth, S., Grant, A. (2000). Resistance of sea lice, *Lepeophtheirus salmonis* (Krøyer), to H_2O_2 on farmed Atlantic salmon, Salmo salar L. *Aquac. Res*. 31, 855–860.

USEPA. (1985). Acute Toxicity Test for Freshwater Invertebrates. Standard Evaluation Procedure. United States Environmental Protection Agency, Hazard Evaluation Division, Washington, DC, Report, EPA. 540/9-85-006, 12.

Wagner, G. N., Mckinley, R. S., Bjorn, P. A. and Finstad, B. (2004). Short-term freshwater exposure benefits sea lice-infected Atlantic salmon. J. Fish Biol. 64, 1593–1604.

Wang, Z., Kwok, K.W., Lui, G.C., Zhou, G.J., Lee, J.S., Lam, M.H. and Leung, K.M. (2014). The difference between temperate and tropical saltwater species' acute sensitivity to chemicals is relatively small. *Chemosphere*. 105. 31-43.

Ward, P. and Shreeve, R.S. (1995). Egg production in three species of Antarctic calanoid copepods during an austral summer. *Deep Sea Research Part I: Oceanographic Res Papers*. 42, 721-735.

Watershed Watch Salmon Society. (2007). Salmon farms, sea lice and wild salmon: a Watershed Watch report on risk, responsibility and the public interest. *Watershed Watch Salmon Society: Coquitlam, BC.*

Wheeler, M., Park, R. and Bailer, A. (2006). Comparing median lethal concentration values using confidence interval overlap or ratio tests. *Environ. Toxicol. Chem.* 25, 1441–1444.

Wilcox, C., Van Sebille, E., & Hardesty, B. D. (2015). Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proceedings of the National Academy of Sciences*. 112, 11899-11904.

Wink, D. A., Cook, J. A., Pacelli, R., Liebmann, J., Krishna, M. C., & Mitchell, J. B. (1995). Nitric oxide (NO) protects against cellular damage by reactive oxygen species. *Toxicology letters*. 82, 221-226.

Verriopoulos, G., & Moraittou-Apostolopoulou, M. (1982). Differentiation of the sensitivity to copper and cadmium in different life stages of a copepod. *Marine Pollution Bulletin*. 13, 123-125.

Xuereb, B., Lefèvre, E., Garric, J. and Geffard, O. (2009). Acetylcholinesterase activity in *Gammarus fossarum* (Crustacea Amphipoda): Linking AChE inhibition and behavioural alteration. Aquat. Toxicol. 94, 114–122.

Appendix A.

Wild zooplankton collection problems and solutions

Horizontal net tows

Problem: Overconcentrated organisms

Solution: Short tows should first be performed (~5 min) and the number of organisms collected should be examined. During transport the concentration should be ~1 organism/mL seawater, to prevent aggression. Lengthen tow time as needed.

Problem: Net staying at the surface of the water

Solution: To begin a tow the boat she be brought to a stop and the collection bottles should be filled with seawater before attaching them to the cod end of the net. The collection bottle and net should then be completely submerged by holding them underwater until they begin to sink. The boat should then move at a speed of 1 to 1.5 knots.

Inadvertent jellyfish collection

Problem: Jellyfish predation in transport

Solution: Bring a sieve with a large mesh size (~600 µm) to the collection event. To prevent predation in the presence of jellyfish, the organisms can be sieved as the collection bottles are poured into the Nalgene bottles. Most jellyfish and any large detritus will remain in the sieve and can then be returned to the ocean.

Aeration in transport

Problem: Overaerating/damage to the organisms

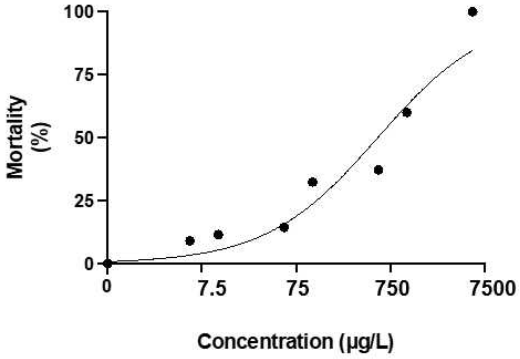
Solution: Do not aerate in transport. Once the organisms have been transported to the laboratory, they can be lightly aerated (~1 bubble/sec) and transferred into larger aquaria.

Temperature during transport

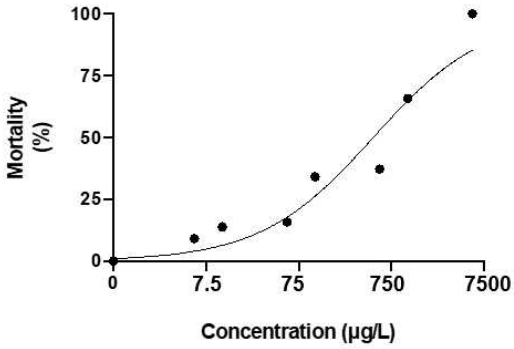
Problem: Maintaining a constant temperature during transport

Solution: Collect extra water in situ and place in Nalgene bottles to surround the organisms in a cooler. Do not open cooler again until arrival at the lab. Do not use ice.

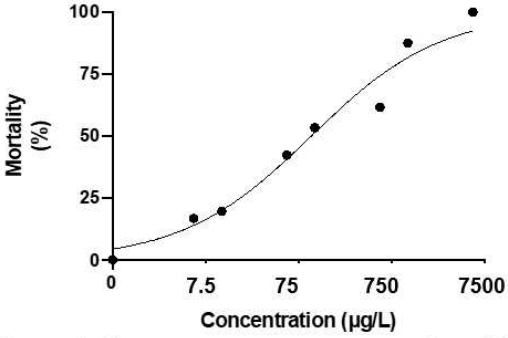
Appendix B.



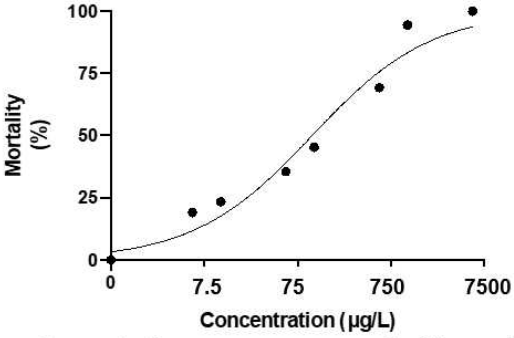
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Salmosan® for 1 h. The LC50 was determined to be 529 µg/L.



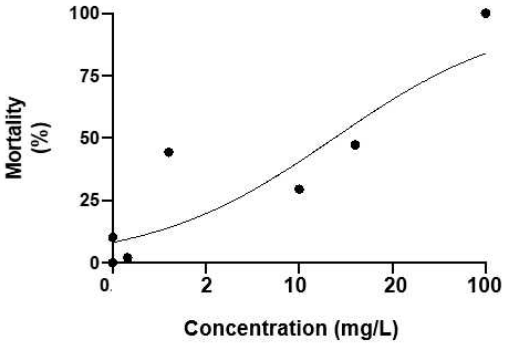
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Salmosan® for 1 h + 48 h in clean SW. The LC50 was determined to be 360 µg/L.



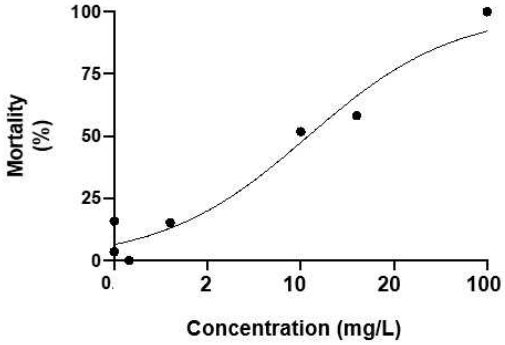
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Salmosan® for 3 h. The LC50 was determined to be 103 µg/L.



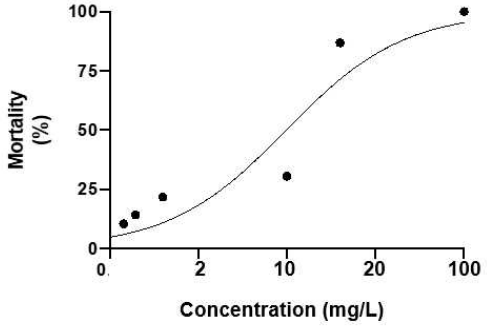
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Salmosan® for 3 h + 48 h in clean SW. The LC50 was determined to be 54 µg/L.



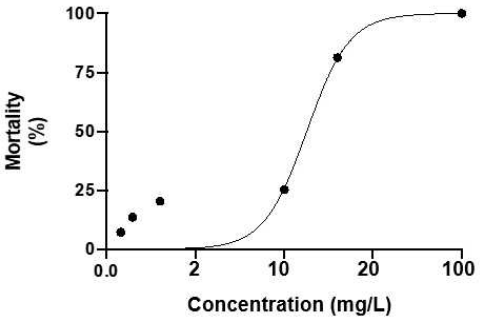
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Interox® Paramove® 30 for 1 h. The LC50 was determined to be 8.2 mg/L.



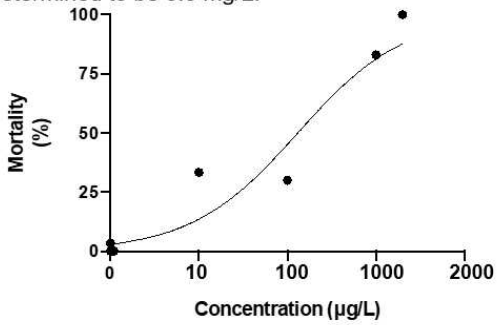
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Interox® Paramove® 30 for 1 h + 48 h in clean SW. The LC50 was determined to be 10 mg/L.



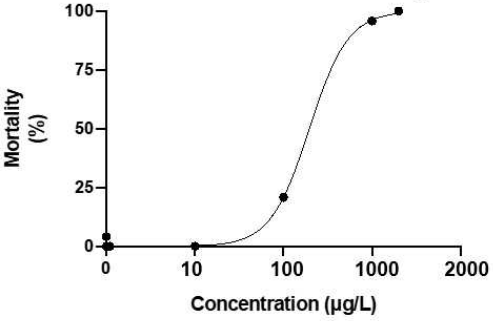
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Interox® Paramove® 30 for 3 h. The LC50 was determined to be 5.5 mg/L.



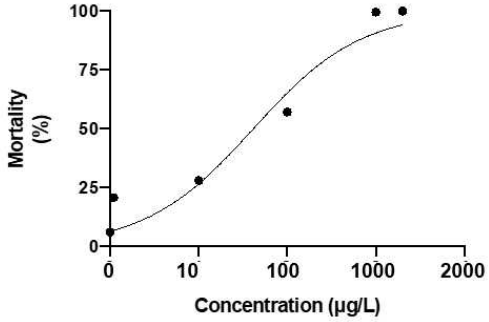
Concentration response curve of wild zooplankton assemblages from Nanoose Bay after exposure to Interox® Paramove® 30 for 3 h + 48 h in clean SW. The LC50 was determined to be 4 mg/L.



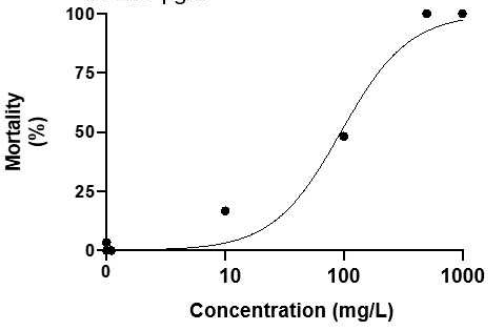
Concentration response curve of Porcelain crab zoea from Bamfield inlet after exposure to Salmosan® for 1 h. The LC50 was determined to be 103 µg/L.



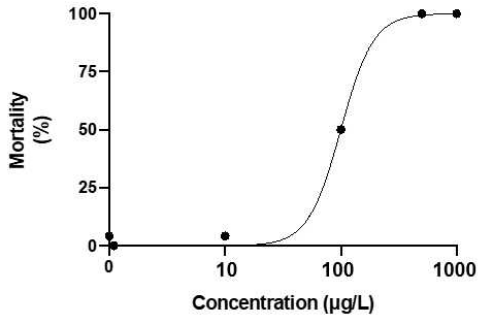
Concentration response curve of Brachyuran crab zoea from Bamfield inlet after exposure to Salmosan® for 1 h. The LC50 was determined to be 203 µg/L.



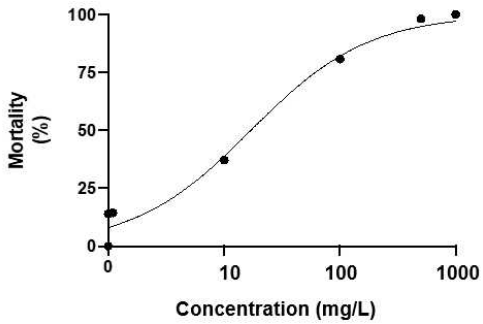
Concentration response curve of wild zooplankton assemblages from Bamfield inlet after exposure to Salmosan® for 1 h. The LC50 was determined to be 83 µg/L.



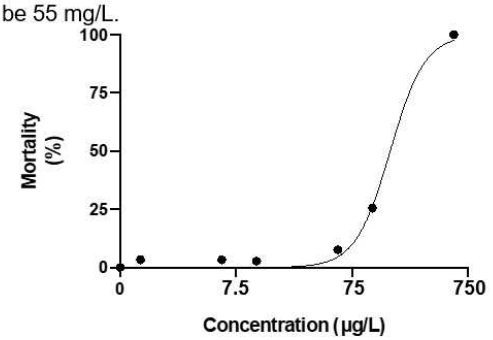
Concentration response curve of Porcelain crab zoea from Bamfield inlet after exposure to Interox® Paramove® 30 for 1 h. The LC50 was determined to be 46 mg/L.



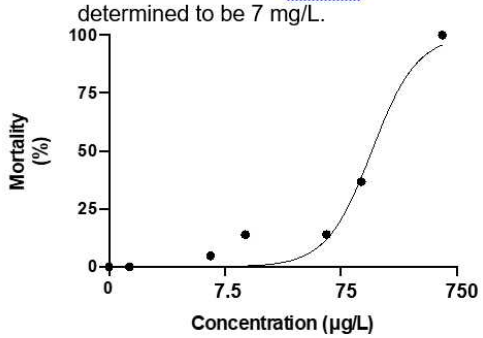
Concentration response curve of Brachyuran crab zoea from Bamfield inlet after exposure to Interox® Paramove® 30 for 1 h. The LC50 was determined to be 55 mg/L.



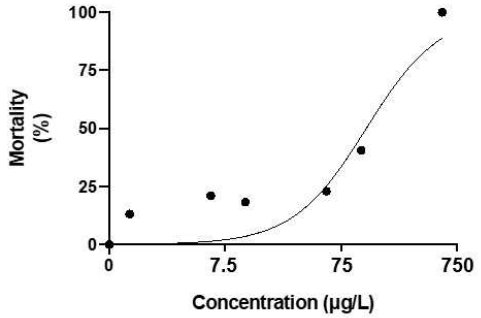
Concentration response curve of wild zooplankton assemblages from Bamfield inlet after exposure to Interox® Paramove® 30 for 1 h. The LC50 was determined to be 7 mg/L.



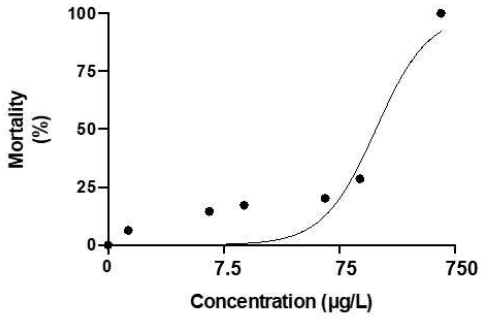
Concentration response curve of *Acartia tonsa* after exposure to Salmosan® for 1 h. The LC50 was determined to be 220 µg/L.



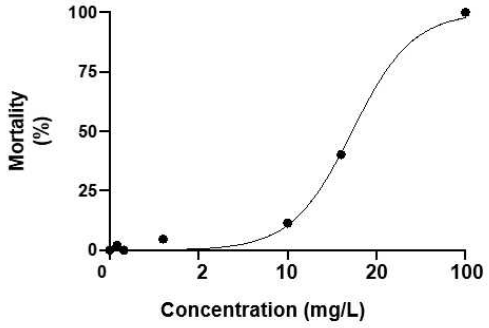
Concentration response curve of *Acartia tonsa* after exposure to Salmosan® for 1 h + 48 h in clean SW. The LC50 was determined to be 166 µg/L.



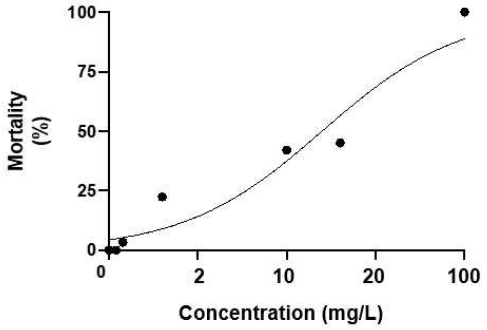
Concentration response curve of *Acartia tonsa* after exposure to Salmosan® for 3 h. The LC50 was determined to be 98 µg/L.



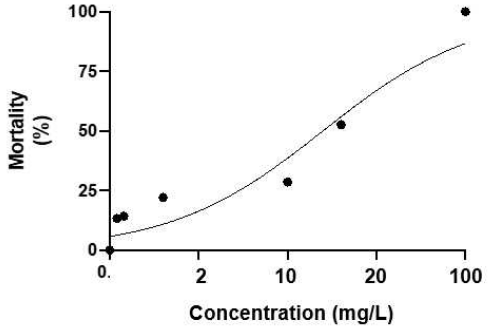
Concentration response curve of *Acartia tonsa* after exposure to Salmosan® for 3 h + 48 h in clean SW. The LC50 was determined to be 127 µg/L.



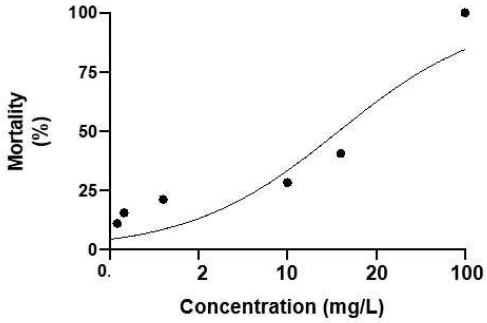
Concentration response curve of *Acartia tonsa* after exposure to Interox® Paramove® 30 for 1 h. The LC50 was determined to be 23 mg/L.



Concentration response curve of *Acartia tonsa* after exposure to Interox® Paramove® 30 for 1 h + 48 h in clean SW. The LC50 was determined to be 13 mg/L.

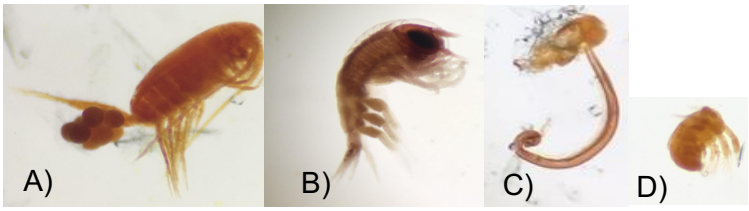


Concentration response curve of *Acartia tonsa* after exposure to Interox® Paramove® 30 for 3 h. The LC50 was determined to be 10 mg/L.



Concentration response curve of *Acartia tonsa* after exposure to Interox® Paramove® 30 for 3 h + 48 h in clean SW. The LC50 was determined to be 9.1 mg/L.

Appendix C.



Examples of wild zooplankton classified as “alive” after exposure to their specified chemotherapeutant and neutral red vital stain. A) A female calanoid copepod with a clutch of eggs still attached at 40X magnification, B) is a crustacean at 15X magnification, C) is an *Oikopleura dioica* at 40X magnification, and lastly D) is a nauplii of unknown species at 40X magnification.

Appendix D.

F/2 Medium recipe (Guillard and Ryther 1962, Guillard, 1975)

Component	Stock solution	Quantity	Molar concentration in final medium
$NaNO_3$	75 g/L	1 mL	8.82 x 10-4 M
NaH_2PO_4	5 g/L dH_2O	1 mL	3.62 x 10-5 M
Na_2SiO_3 9H2O	30 g/L dH_2O	1 mL	1.06 x 10-4 M
Trace metal solution	(see recipe below)	1 mL	-
Vitamin solution	(see recipe below)	0.5 mL	-

f/2 Trace metal solution recipe

Component	Primary stock solution	Quantity	Molar concentration in final medium
FeCl3 6H2O	-	3.15 g	1.17 x 10-5 M
Na2EDTA 2H2O	-	4.36 g	1.17 x 10-5 M
CuSO4 5H2O	9.8 g/L	1 mL	3.93 x 10-8 M
Na2MoO4 2H2O	6.3 g/L	1 mL	2.60 x 10-8 M
ZnSO4 7H2O	22.0 g/L	1 mL	7.65 x 10-8 M
CoCl2 6H2O	10.0 g/L	1 mL	4.20 x 10-8 M
MnCl2 4H2O	180.0 g/L	1 mL	9.10 x 10-7 M

f/2 Vitamin solution recipe

Component	Primary stock solution	Quantity	Molar concentration in final medium
thiamine HCl (vit. B1	-	200 mg	2.96 x 10-7 M
biotin (vit. H)	1.0 g/L dH2O	1 mL	2.05 x 10-9 M
cyanocobalamin (vit. B12)	1.0 g/L dH2O	1 mL	3.69 x 10-10 M