Salmon & Trout Conservation

Chemical Impacts:
The link between freshwater xenobiotics and reduced juvenile salmonid marine survival

Literature Review

I. Introduction

Many of the factors regulating salmonid populations remain poorly understood, although recent evidence suggests that environmental conditions experienced by juveniles in freshwater may have a significant effect on survival once the fish migrates into the marine environment (Fig. 1). Juvenile anadromous fish undergo a transition from parr to smolt during their downstream migrations. This transition, known as 'smoltification', is orchestrated by hormones and involves a plethora of physiological and behavioural changes that prepare juvenile salmonids for entry into seawater (Russell *et al*. 2012). The term xenobiotic denotes a substance, typically a synthetic chemical, which is foreign to an ecological system. The exposure of juvenile salmon to sublethal concentrations of xenobiotic compounds in freshwater can have adverse biological consequences on smolt quality and readiness for life at sea (Moore *et al*. 2003). The hypothesis is that sublethally stressed smolts will have reduced smolt-to-adult survival in the marine environment as a result of inhibited enzyme activities (important for maintaining physiological homeostasis in seawater), reduced growth, effects on migratory behaviour and impacts on predator avoidance (Finstad & Jonsson, 2001). This review explores the main categories of freshwater xenobiotics, examining their biological influences in juvenile salmonids and in turn how sublethal exposure can decrease marine survival probability.

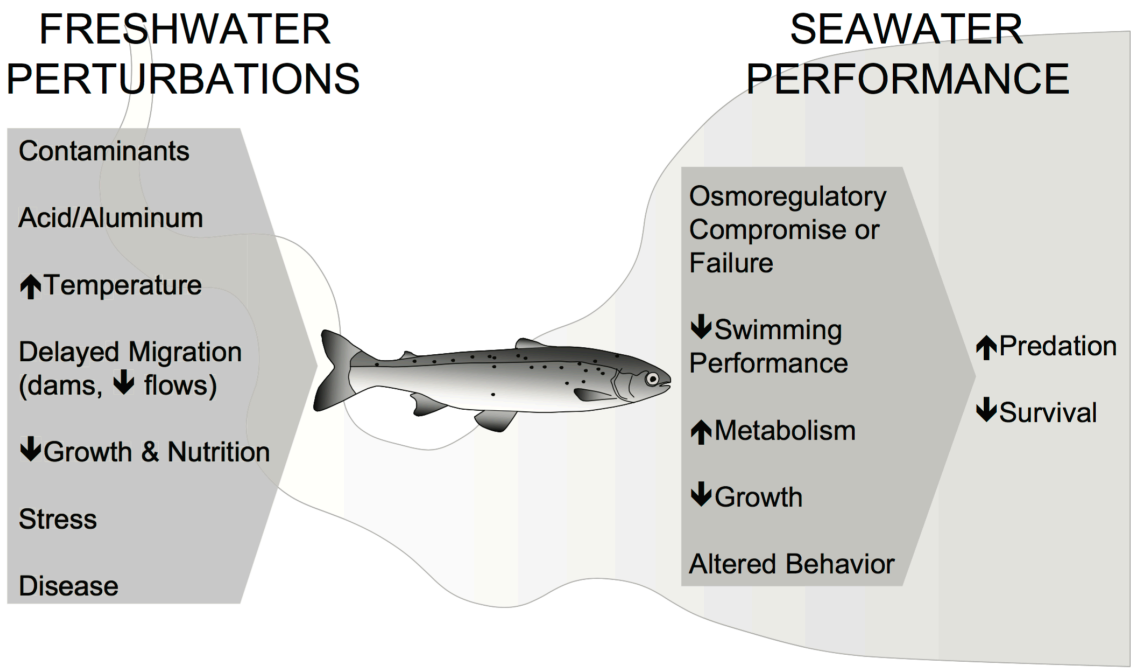

Figure 1: A summary of the outcomes of freshwater environmental impacts on salmonid seawater performance (McCormick *et al*. 2009)

II. Pesticides

Triazines

Atrazine is a common agricultural herbicide often applied in spring to control undesirable plants. It has high mobility through soil, so watercourses are particularly susceptible to contamination (Waring & Moore, 2004). Due to persistent groundwater contamination it was banned in the EU in 2004 (Sass & Colangelo, 2006).

However, its safety remains controversial with atrazine being amongst the most heavily used pesticides in the United States (Jablonowski *et al*. 2011).

Waring & Moore (1996) found Atlantic salmon smolt mortality of ~30% when smolts were exposed to sub-lethal concentrations of atrazine before transfer to seawater. This lead to speculation that atrazine inhibits the ability of juvenile salmon to adapt to the marine environment. A study exploring this further found that salmon smolts exposed to atrazine had significantly lower gill Na+/K+ ATPase activity than a control group with no exposure (Moore & Fewings, 2003). Gill Na+/K+ ATPase activity increase is one of the major physiological processes that allows salmon to adapt to saltwater environments. Activity of the enzyme increases two to five-fold during smoltification and signifies an increase in the ability of the fish to osmoregulate in saltwater. These results were corroborated by Waring & Moore (2004), where exposure of 2, 5 and 10 µg/L atrazine caused a significant reduction in Na+/K+ ATPase activity in Atlantic salmon smolts in freshwater. Moore *et al.* (2008) also found that exposure of Atlantic salmon smolts to a concentration of atrazine as little as 0.1 µg/L over a 72-h period significantly reduced gill Na+/K+ ATPase activity. Plus on transfer to 33% saltwater, 100% smolt mortality occurred. Contrastingly, Matsumoto *et al*. (2010) did not detect a significant effect on gill Na+/K+ ATPase activity in salmon smolts after short term exposure of up to 100 µg atrazine/L. As the concentrations of atrazine tested exceeded those likely to be experienced in the natural aquatic environment, it was concluded that atrazine poses no risk to Atlantic salmon during smoltification. However, it is important to take into account that in the wild fish are likely to be exposed to atrazine for long term periods. Atrazine is relatively persistent in freshwater with a half-life up to 350 days depending on environmental conditions (Nieves-Puigdoller *et al*. 2007). Interestingly, Strand *et al*. (2011) showed that early descending smolts with low gill Na+/K+ ATPase activity may delay their final sea entry and spend some time in the lower part of the river, thereby synchronising their final sea entry with later arriving smolts. This behaviour could give smolts the opportunity to recover from contaminant exposure, although it would be dependent on contaminant concentration and exposure time.

Slight decreases in growth and elevated plasma cortisol in response to atrazine exposure have also been observed. Atrazine at concentrations at and above 6.5 µg/L caused elevated plasma cortisol (32–37ng/ml) concentrations in salmon smolts compared to controls (18ng/ml) (Waring & Moore, 2004). Elevated plasma cortisol indicates that smolts are experiencing persistent physiological stress and prolonged elevation is considered harmful due to increased susceptibility to disease and suppression of reproductive processes (Carey & McCormick, 1998). Nieves-Puigdoller *et al*. (2007) found that prior exposure to atrazine in freshwater caused reduced growth of Atlantic salmon smolts reared in contaminant free seawater for 1 month. As early growth in seawater is widely recognised as critical to the overall marine survival of salmon, this impact of contaminant exposure in freshwater on seawater growth indicates a detrimental effect on marine survival (Beamish *et al*. 2004).

As well as physiological effects, atrazine exposure has also been shown to impact migratory behaviour of Atlantic salmon smolts. Moore *et al*. (2007) showed a significant effect on smolt active movement following atrazine exposure in freshwater. Control fish showed a higher degree of active movement compared to groups exposed to atrazine. The effect was also dose-responsive, with the higher atrazine dose group (5µg/L) showing significantly less migratory behaviour compared to the lower dose group (0.5µg/L). This has

particular significance to the survival of smolts in the marine environment as previous telemetry studies have indicated that smolts enter the open sea during a very brief window, so inhibition or delaying of migration means smolts may miss this (Moore *et al*. 1995).

Organophosphates and Carbamates

Organophosphate and carbamate insecticides are the most widely used group of insecticides in the world. Due to their acute toxicity, concern over their use in agriculture (particularly sheep-dips) and around the home in the UK has occurred. Although these compounds offer the advantage of rapid degradation in the environment, they generally lack target specificity and have high acute toxicity towards non-target species (Fulton & Key, 2001).

The primary function of organophosphates and carbamates is to inhibit acetylcholinesterase (AChE) activity, affecting normal cholinergic nerve transmission. The inhibition of AChE causes accumulation of acetylcholine in synapses and neuromuscular junctions, resulting in overstimulation of cholinergic receptors (Fukuto 1990). The key difference is that organophosphates bind the enzyme irreversibly while carbamates bind AChE reversibly. Therefore, recovery from exposure to organophosphates requires the synthesis of new cholinesterase enzyme, a process that can last up to several weeks whereas recovery from exposure to carbamates is brief and can occur on a timescale of a few hours (Baldwin *et al*. 2009). Monitoring of acetylcholinesterase (AChE) inhibition has been widely used in terrestrial and freshwater aquatic systems as an indicator of exposure and effects (Fulton & Key, 2001).

Research by Sandahl *et al*. (2005) found a close relationship between brain AChE inhibition and behavioural impairment in juvenile coho salmon exposed to the organophosphate chlorpyrifos. Brain AChE inhibition and reductions in feeding activity and spontaneous swimming were significantly correlated. Similarly, chlorpyrifos has been shown to significantly inhibit AChE in the nervous system and muscle of juvenile steelhead trout (Sandahl & Jenkins, 2002). The carbamate carbaryl has also been shown to cause decreased brain and muscle AChE activity in juvenile cutthroat trout after exposure to environmentally relevant concentrations (750 mg/L) for 6 hours (Labenia *et al*. 2007). Reduced swimming and feeding means reduction in somatic growth of juvenile salmon and as previously mentioned, juvenile growth is a critical determinant of freshwater and marine survival. Additionally, as the exposure in these studies typically replicated environmentally realistic concentrations, the effects were particularly concerning.

Synergistic AChE inhibition has also been shown in juvenile coho salmon when exposure to a mixture of organophosphates occurs (Laetz *et al*. 2013). Brain enzyme inhibition was concentration-dependent, with a 90% reduction and a significant decrease in spontaneous swimming speed at the highest binary mixture concentrations evaluated (diazinon-malathion at 2.6 and 1.1 µg/L, respectively; ethoprop-malathion at 2.8 and 1.2 µg/L, respectively). The authors highlighted an unusually steep concentration−response relationship across a mere 2-fold increase in mixture concentration. This emphasises the importance of evaluating pesticides as mixtures rather than on individual scales.

Pyrethroids

The increased use of pyrethroid pesticides has been largely driven by their high toxicity to target organisms and their relatively low toxicity to birds, mammals and amphibians (Köprücü *et al*. 2006). Because of their low mammalian toxicity, synthetic pyrethroid insecticides are extensively used in place of organochlorine, organophosphates and carbamates to control pests (Aydin *et al.* 2005). However, application of these compounds is highly toxic to freshwater fish even at very low concentrations (Suvetha *et al*. 2010). Pyrethroids interact with voltage-gated sodium channels in a similar manner to organochlorine pesticides, leading to repetitive neuronal firing (Clifford *et al*. 2005).

Pyrethroids mostly have a lethal effect on salmonids in freshwater so research on sublethal effects and their influence on juvenile marine survival is fairly scarce. Work by Sandahl *et al*. (2004) did indicate sublethal excitotoxicity (where nerve cells are damaged or killed by excessive stimulation by neurotransmitters) to juvenile coho salmon central networks after exposure to 0.2 µg/L of the pyrethroid esfenvalarate. Although a loss in sensory function in response to natural odorants did not occur, the natural odorant L-Serine triggered bursts of postsynaptic activity in the olfactory bulb. Interference with olfactory function has the potential to inhibit olfactory-mediated behaviours crucial for the survival and migration of salmonids. Goulding *et al*. (2013) also found swimming performance affected in juvenile rainbow trout after exposure to deltamethrin at 200 and 300 ng/L. The fish had significantly lower U_{crit} (the highest swimming speed that a fish can maintain for a period equal in magnitude to the time interval used in the test) values than the control fish. Neither U_{crit} nor U_{max} (maximum swimming speed) reduced after exposure to permethrin. Pyrethroids are divided into two classes based largely on their chemical structure and potency, with type II being the most potent. The fact deltamethrin is a type II pyrethroid may explain why it caused an effect but permethrin (type I) did not. Smolts with lower swimming speeds have reduced ability to avoid predators and more likely to become prey (Thorstad *et al*. 2007). Alternatively, they may miss the previously mentioned optimum window for entering the open sea. Both eventualities decrease their chances of marine survival.

III. Hormones

Xenoestrogens

Effluent discharges from wastewater treatment works can be major sources of oestrogenic compounds in freshwater environments (Bangsgaard *et al*. 2006). Xenoestrogens are synthetic and natural compounds that imitate oestrogen. The most potent form is the pharmaceutical oestrogen ethinyloestradiol, the active ingredient found in the contraceptive pill. This oestrogen is eliminated slowly from the body and degrades relatively slowly in the environment (Robinson *et al*. 2008). Alkylphenols varying in their side-chain lengths [ethyl-, propyl-, octyl-, and nonylphenol (EP, PP, OP, and NP, respectively)] and bisphenol A (BPA) represent a large group of structurally related xenoestrogens that have endocrine-disruptive effects. These compounds are widely used in detergents, paints, herbicides, pesticides, and plastic polymers and have the ability to accumulate in the environment (Kochukov *et al*. 2009).

Mechanistically, scientific evidence strongly suggests that oestrogen effects on somatic growth and osmoregulatory ability are mediated through the growth hormone (GH)/insulin-like growth factor (IGF-I)

system (Arsenault *et al*. 2004). Interference of environmental oestrogens in the GH/IGF-I regulation of smoltification was suspected to be a causal factor in the decline of salmon in Eastern Canada in the 1970s and 1980s. Mass forest application of the formulated pesticide Matacil 1.8D (which contains the xenoestrogen 4-nonylphenol (4NP)) coinciding with the period of smoltification of salmon was the suspected cause of the decline (Segner *et al*. 2013). Fairchild *et al*. (1999) tested this hypothesis and found significant negative relationships between pesticide spraying intensity and salmon returns. Studies by Madsen *et al*. (2004; 1997) found that injecting the natural oestrogen 17β-estradiol (E2) and 4NP into Atlantic salmon smolts both caused a significant decline in salinity tolerance, gill Na+/K+ ATPase activity and the number of gill chloride cells. These findings were corroborated by McCormick *et al*. (2005) who also found lowered salinity tolerance in injected smolts. Additionally, a decrease in plasma IGF-I levels was found, with the authors suggesting that substantial declines in smolt salinity tolerance after exposure to 4NP and E2 are caused by the suppressing effect these compounds have on plasma IGF-I levels.

Fairchild *et al.* (2000) looked at how water-borne concentrations of E2 and 4NP effect smoltification in Atlantic salmon. A portion of the exposed smolts experienced compromised growth and did not survive the transition from freshwater to seawater. Growth is an important factor when considering smolt survival at sea; poor growth measured close to the parr-smolt transformation has been linked to reduced survival and fewer returns of adult salmon to their native streams (Beckman *et al*. 1999). Bangsgaard *et al*. (2006) also investigated the effects of waterborne exposure to E2, but additionally looked at how the xenoestrogen 4-tert-octylphenol (OP) influenced Atlantic salmon smoltification and swimming behaviour. Two experiments were conducted, one with early exposure in November at the parr-stage and another with exposure during the spring (March) at the pre-smolt stage. Exposure to OP at 100µg/L and E2 at 500ng/L compromised smolting through reduced gill Na+/K+ ATPase activity and impairment in the ability of smolts to regulate muscle water content and plasma osmolality when subjected to 24h seawater challenge tests during the smolting period. The treated fish also migrated at a lower frequency than the control fish, indicating a reduction in migratory drive. As physiological and behavioural inhibitory effects occurred in pre-smolts and smolts, this study showed that xenoestrogen exposure has the capability to reduce marine survival even when parr are exposed significantly prior to the smolting period.

IV. Persistent Organic Pollutants (typically halogenated organic compounds)

Persistent Organic Pollutants (POPs) are highly toxic man-made organic compounds that enter aquatic systems largely from industrial and domestic origins. They exhibit high lipid solubility so are capable of bioaccumulation in fatty tissues of various organisms, increasing in concentration as they are processed and metabolised. Their tendency to degrade very slowly means they can travel long distances around the globe and persist in the environment for long periods of time (Hurwich & Chary, 2000). Well-documented examples of POPs include polychlorinated biphenyls (PCBs) and brominated flame-retardants (BFRs).

Polychlorinated biphenyls (PCBs)

Lerner *et al*. (2007) found that juvenile Atlantic salmon exposed to both 1 µg/L and 10 µg/L of a PCB mixture during smolting exhibited a dose-dependent reduction in preference for seawater. Fish treated with the

higher dose also exhibited a 50% decrease in gill Na+/K+ ATPase activity and a 10% decrease in plasma chloride levels in freshwater. Disruption of seawater preference by the PCB mixture appeared to be related to negative impacts on the endocrine parameters involved in smolt development, particularly the thyroid hormone triiodothyronine (T_3) and cortisol. Thyroid hormones have been proposed to play an integral part in controlling downstream migratory behaviour and seawater preference in juvenile salmonids (Iwata 1995). Olfactory imprinting is also facilitated by increases in levels of thyroid hormones during smoltification; disruption to this process by contaminants means returning adult salmon will struggle to find their native streams and could fail to complete their life cycle (Lema & Nevitt 2004). It was concluded from the study that freshwater exposure to PCBs has the potential to inhibit preparatory adaptations that occur during smolting, thereby reducing marine survival and sustainability of salmon populations. PCBs were also shown to impair smoltification and seawater performance in the anadromous Arctic charr (*Salvelinus alpinus*) by Jørgensen *et al.* (2004). Juvenile charr exposed to 100 mg/Kg of PCB had either a transient or a permanent reduction in plasma GH and IGF-I, alongside reduced titres of thyroxin and T_3 during the period of smoltification. These hormonal alterations corresponded with impaired hyposmoregulatory ability, plus reduced growth rate and survival after transference to seawater. Consequently, fewer juveniles matured compared to the control and lower concentration PCB treatment, indicating that if PCB levels are high enough impacts may occur at the level of the population by reducing the number of spawning adults.

Brominated flame-retardants

Brominated flame-retardants have similar properties to many classic organochlorine compounds and are structurally similar to PCBs. Within the general BFR category, there are three main divisions; tetrabromobisphenol-A (TBBPA), polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane (HBCD) (Palace *et al*. 2008). HBCD has a half-life of 2–25 days in water and studies around UK waters have shown evidence of bioaccumulation and biomagnification in the aquatic food chain (Law *et al*. 2006; Morris *et al*. 2004). Lower & Moore (2007) demonstrated that exposure to HBCD did not affect seawater adaptability in juvenile Atlantic salmon. However, disruption of plasma thyroid hormone levels and reduced olfactory function did occur. Over a 30-day period of HBCD exposure, olfactory response decreased significantly compared to the control smolts every week. A recently proposed alternative to PBDE's and HBCDs was the compound tetrabromoethylcyclohexane (TBECH), yet the toxicity and environmental impacts of this compound remain questionable. Work by Park *et al*. (2011) showed disruption of the thyroid axis in juvenile brown trout after TBECH exposure and highlighted the implications considering the importance of the thyroid axis in mediating the physiological adaptations required prior to migration from freshwater to saltwater.

V. Metals

Copper

Copper is a widespread source of water pollution in salmon habitat. The highest levels of copper pollution tend to be found in river systems that have been impacted by mining activities. However, copper also enters watercourses through a variety of nonpoint anthropogenic sources. Copper compounds are widely used in bio-fouling treatments on aquatic vessels and fungicides on agricultural crops where leaching can occur (Davis *et al*. 2001).

Salmon rely on chemical cues to form olfactory memories and return to their natal river system to spawn. Critically, the migratory patterns of adult salmon are disrupted when olfactory function is lost (Baldwin *et al*. 2003). Copper is broadly toxic to the salmon olfactory nervous system and short-term influxes of copper to surface waters have been shown to interfere with olfactory-mediated migratory behaviours (Mcintyre *et al*. 2008). Baldwin *et al*. (2003) exposed juvenile coho salmon to 1–20 µg/L of copper and a variety of natural odorants. After only 10 minutes of exposure the neurophysiological response to all odorants was impaired. Dissolved copper not only impairs sensory neurons in the salmonid nose, but also impairs the ability of juveniles to detect and respond to predation cues. A juvenile salmonid with disrupted predator avoidance behaviours stands a much greater risk of being eaten and therefore the likelihood of surviving to reproduce is reduced (Hecht *et al*. 2007). Sandahl *et al*. (2007) found that copper exposure up to 20 µg/L for 3 hours significantly impaired the sensory physiology and predator avoidance behaviours of juvenile coho salmon. They stated that as dissolved copper content in surface waters increases, the responsiveness of the peripheral olfactory system to predation cues will diminish until it falls below the threshold required to initiate an appropriate behavioural response. This means a likely outcome is that copper exposed fish will make behavioural decisions that are inappropriately risky for a particular ecological situation. McIntyre *et al*. (2012) also observed predator avoidance behaviour impairment in juvenile coho salmon exposed to 5–20 µg/L of dissolved copper for 3 hours. Unexposed fish exhibited a sharp reduction in swimming activity in response to two predation cues - conspecific skin extract and the upstream presence of a cutthroat trout predator previously fed juvenile coho – whereas no alarm response occurred in treated fish. The copper treatment also negatively influenced predator evasion once an attack was initiated. Therefore, it became increasingly likely that the juvenile coho would be captured on the first attack after exposure to higher copper exposure concentrations. It was hypothesised that the toxicity of copper to the lateral line mechanosensory system of the juvenile coho salmon may have contributed to the observed reduction in evasion success. Similarly to olfactory receptor neurons, copper is toxic to lateral line neurons that are directly exposed to contaminated waters.

Aluminium

Extensive research has been conducted on the effects of aluminium exposure on juvenile salmon life stages, particularly in combination with acidic conditions. Acid rain lowers the pH of river systems, causing aluminium leaching from soils to become more soluble and in turn increasing the presence of inorganic Al, the form most toxic to fish (McCormick *et al*. 2009). Labile (cationic/inorganic) forms of aluminium can accumulate in fish gills, where high concentrations result in mortality due to respiratory and ionoregulatory dysfunction. Work by Berntssen *et al*. (1997) demonstrated that Atlantic salmon smolts secrete excessive amounts of mucus in response to low pH/aluminium conditions and use this as a binding defence mechanism to protect from aluminium toxicity.

Population effects from sublethal aluminium/acid exposure can occur through the inhibition of gill Na+/K+ ATPase activity, reducing hyposmoregulatory capacity and in turn decreasing marine survival probability. Following exposure of Atlantic salmon smolts to sublethal acid/aluminium conditions, Kroglund *et al*. (2007) found gill aluminium concentrations exceeding 20µg/g and depression in gill Na+/K+ ATPase activity. Adult return rates were also impacted with smolt- to-adult survival declining by 20-50% relative to control fish.

These findings were similar to Kroglund & Finstad (2003), where exposure of Atlantic salmon smolts to very low levels of aluminium (6 mg/L) in acidic conditions reduced marine survival by 20–30%. The exposed fish also migrated downstream slightly later than the controls, increasing the probability of smolts missing the optimum migration window. Monette *et al*. (2010) explored further how acid/aluminium causes hypoosmoregulatory impairment in Atlantic salmon smolts, examining it on a cellular level. After exposure to acid and moderate aluminium concentration (42 µg/L) smolts exhibited a large reduction in the number of total gill mitochondria rich cells, accompanied by an increase in the surface expression of mitochondria rich cells in the filament epithelium. The loss of gill mitochondria rich cells and their individual ion uptake capacity explains impaired ion regulation in freshwater and after seawater exposure. It was hypothesised that the increased surface expression of mitochondria rich cells in the filament epithelium is an attempt to increase the number of active mitochondria rich cells in the gill to compensate for impaired ion regulation. Exposure to acid and low aluminium concentration (11 µg/L), impaired seawater tolerance occurred even though Na+/K+ ATPase activity and mitochondria rich cell numbers were not altered. However, exposure did alter the morphology of the mitochondria rich cells. Cells were smaller within the filament epithelium and less bright, and were more elongate within the lamellar epithelium. Seawater acclimated mitochondria rich cells are typically larger and stain brighter for Na+/K+ ATPase presence. Therefore, the results indicated that low acid/aluminium exposure causes a reversal from seawater to freshwater type mitochondria rich cells, therefore reducing the salt secretory capacity of the gill.

Thorstad *et al*. (2013) investigated the delayed impact of short-term aluminium exposure on Atlantic salmon smolts in moderately acidified freshwater during the first 37 km of their marine migration. Immediate mortality was 20% higher than the control group during the first 10km of the marine migration, but fish that made it past this point seemed to recover, maintaining a normal migration speed and survival. The authors speculated that the proximate cause for elevated mortality for smolts in the first 10km following exposure was predation by marine fishes, as exposed fish experienced impaired physiological function thus increasing their probability of being eaten. Decreased plasma concentrations of GH were also observed in exposed smolts but no affect on IGF-I was found. Interestingly work by Monette *et al*. (2008) found the opposite, with a decrease in plasma IGF-I but no affect on GH levels in exposed smolts. GH and IGF-I work both independently and in concert to increase seawater tolerance by stimulating gill Na+/K+ ATPase activity and chloride cell proliferation, so although the results found were different, decreased levels of either hormone indicates impaired hyposmoregulatory ability.

VI. Concluding Remarks

This review demonstrates how xenobiotic compounds in the freshwater environment are a significant driver in the decrease of returning marine salmonids, with lasting impacts on marine survival and population sustainability. Declining marine survival can no longer be attributed solely to changes in oceanic conditions and it is crucial that research into restoring salmon populations no longer considers the freshwater environment in isolation of the marine environment. Extensive evidence verifies impacts of individual contaminants on physiological development, migratory behaviour, early seawater survival, long-term seawater growth and natal stream homing in salmonids. However, cumulative effects of xenobiotic mixtures

and the time period between exposure in freshwater and entry into the sea may also be integral in terms of whether migration and survival in the marine environment is compromised. Therefore, future research efforts should be focussed on addressing these knowledge gaps.

For further information please contact Lauren Mattingley (Scientific Assistant for S&TC) at:
lauren@salmon-trout.org

VII. References

Arsenault, J.T.M. *et al*. (2004). Effects of water-borne 4-nonylphenol and 17beta-estradiol exposures during parr-smolt transformation on growth and plasma IGF-I of Atlantic salmon (*Salmo salar L.*). *Aquatic Toxicology*, 66(3), pp.255–265

Aydin, R. *et al*. (2005). Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio L .*) embryos and larvae. *Journal of Aquaculture International*, 13, pp.451–458

Baldwin, D.H. *et al*. (2009). A fish of many scales: Extrapolating sublethal pesticide exposures to the productivity of wild salmon populations. *Ecological Applications*, 19(8), pp.2004–2015

Baldwin, D.H. *et al*. (2003). Sublethal effects of copper on coho salmon: impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environmental toxicology and chemistry / SETAC*, 22(10), pp.2266–2274

Bangsgaard, K., Madsen, S.S. & Korsgaard, B. (2006). Effect of waterborne exposure to 4-tert-octylphenol and 17β-estradiol on smoltification and downstream migration in Atlantic salmon, *Salmo salar*. *Aquatic Toxicology*, 80(1), pp.23–32

Beamish, R.J., Mahnken, C. & Neville, C.M. (2004). Evidence That Reduced Early Marine Growth is Associated with Lower Marine Survival of Coho Salmon. *Transactions of the American Fisheries Society*, 133(1), pp.26–33

Beckman, B.R. *et al*. (1999). Growth, Smoltification, and Smolt-to-Adult Return of Spring Chinook Salmon from Hatcheries on the Deschutes River, Oregon. *Transactions of the American Fisheries Society*, 128(June 2016), pp.1125–1150

Berntssen, M.H.G. *et al*. (1997). Responses of skin mucous cells to aluminum exposure at low pH in Atlantic salmon (*Salmo salar*) smolts. *Can. J. Fish. Aquat. Sci*, 54, pp.1039–1045

Burnison, B.K., Sherry, J.P., Bennie, D.T., Brown, S.B. (2000). Effects of water-borne 4-nonylphenol and estrogen on the growth, survival and physiology of Atlantic salmon (*Salmo salar*) smolts. In: Proceedings of the 27th Annual Aquatic Toxicity Workshop, St. John's, Newfoundland, 1–4 October 2000, Can. Tech. Rep. Fish. Aquat. Sci. No. 2331, p. 58

Carey, J.B. & McCormick, S.D. (1998). Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. In *Aquaculture*. pp. 237–253

Clifford, M.A. *et al*. (2005). Synergistic effects of esfenvalerate and infectious hematopoietic necrosis virus on juvenile chinook salmon mortality. *Environmental Toxicology and Chemistry*, 24(7), pp.1766–1772

Davis, A.P., Shokouhian, M. & Ni, S. (2001). Loading estimates of lead, copper, cadmium, and zinc in urban runoff from specific sources. *Chemosphere*, 44(5), pp.997–1009

Fairchild, W.L. *et al*. (1999). Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption? *Environmental Health Perspectives*, 107(5), pp.349–357

Finstad, B. & Jonsson, N. (2001). Factors influencing the yield of smolt releases in Norway. *Nordic Journal of Freshwater Research*, (75), pp.37–55

Fukuto, T.R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. In *Environmental Health Perspectives*. National Institute of Environmental Health Science, pp. 245–254

Fulton, M.H. & Key, P.B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environmental toxicology and chemistry / SETAC*, 20(1), pp.37–45

Goulding, A.T. *et al*. (2013). Reduction in swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) following sublethal exposure to pyrethroid insecticides. *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP*, 157(3), pp.280–6

Hecht, S. A., Baldwin, D. H., Mebane, C. A., Hawkes, T., Gross, S. J., & Scholz, N. L. (2007). *An overview of sensory effects on juvenile salmonids exposed to dissolved copper: applying a benchmark concentration approach to evaluate sublethal neurobehavioral toxicity*. Technical White Paper

Hurwich, E. M., & Chary, L. K. (2000). Persistent Organic Pollutants (POPs) in Alaska: What Does Science Tell Us? *Circumpolar Conservation Union*

Iwata, M. (1995). Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: A review. *Aquaculture*, 135(1–3), pp.131–139

Jablonowski, N.D., Schäffer, A. & Burauel, P. (2011). Still present after all these years: Persistence plus potential toxicity raise questions about the use of atrazine. *Environmental Science and Pollution Research*, 18(2), pp.328–331

Jørgensen, E.H. *et al*. (2004). PCB impairs smoltification and seawater performance in anadromous Arctic charr (*Salvelinus alpinus*). *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, 138(2), pp.203–212

Kochukov, M.Y., Jeng, Y.J. & Watson, C.S. (2009). Alkylphenol xenoestrogens with varying carbon chain lengths differentially and potently activate signaling and functional responses in GH3/B6/F10 somatomammotropes. *Environmental Health Perspectives*, 117(5), pp.723–730

Köprücü, S.Ş. *et al*. (2006). Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis L.*). *Pesticide Biochemistry and Physiology*, 86(2), pp.99–105

Kroglund, F. *et al*. (2007). Exposure to moderate acid water and aluminum reduces Atlantic salmon post-smolt survival. *Aquaculture*, 273(2–3), pp.360–373

Kroglund, F. & Finstad, B. (2003). Low concentrations of inorganic monomeric aluminum impair physiological status and marine survival of Atlantic salmon. *Aquaculture*, 222(1–4), pp.119–133

Labenia, J.S. *et al*. (2007). Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl. *Marine Ecology Progress Series*, 329, pp.1–11

Laetz, C.A. *et al*. (2013). Interactive neurobehavioral toxicity of diazinon, malathion, and ethoprop to juvenile coho salmon. *Environmental Science and Technology*, 47(6), pp.2925–2931

Law, R.J. *et al*. (2006). Levels of the flame retardants hexabromocyclododecane and tetrabromobisphenol A in the blubber of harbor porpoises (*Phocoena phocoena*) stranded or bycaught in the U.K., with evidence for an increase in HBCD concentrations in recent years. *Environmental Science and Technology*, 40(7), pp.2177–2183

Lema, S.C. & Nevitt, G. A, (2004). Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. *The Journal of experimental biology*, 207(Pt 19), pp.3317–27

Lerner, D.T., Björnsson, B.T. & McCormick, S.D. (2007). Effects of aqueous exposure to polychlorinated biphenyls (Aroclor 1254) on physiology and behavior of smolt development of Atlantic salmon. *Aquatic Toxicology*, 81(3), pp.329–336

Lower, N. & Moore, A. (2007). The impact of a brominated flame retardant on smoltification and olfactory function in Atlantic salmon (*Salmo salar L.*) smolts. *Marine and Freshwater Behaviour and Physiology*, 40(4), pp.267–284

Madsen, S.S. *et al.* (2004). 17-β Estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon, *Salmo salar*. *Aquatic Toxicology*, 68(2), pp.109–120

Madsen, S.S., Mathiesen, A.B. & Korsgaard, B. (1997). Effects of 17ß-estradiol and 4-nonylphenol on smoltification and vitellogenesis in Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry*, 17, pp.303–312

Matsumoto, J., Hosmer, A.J. & Van Der Kraak, G. (2010). Survival and iono-regulatory performance in Atlantic salmon smolts is not affected by atrazine exposure. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, 152(3), pp.379–384

McCormick, S.D. *et al*. (2005). Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-

nonylphenol and 17beta-estradiol. *General and Comparative Endocrinology*, 142(3), pp.280–288
McCormick, S.D. *et al*. (2009). Taking it with you when you go: How perturbations to the freshwater environment, including temperature, dams, and contaminants, affect marine survival of salmon. *American Fisheries Society Symposium*, 69, pp.195–214
Mcintyre, J.K. *et al*. (2008). Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions. *Environmental Science and Technology*, 42(4), pp.1352–1358
McIntyre, J.K. *et al*. (2012). Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. *Ecological Applications*, 22(5), pp.1460–1471
Monette, M.Y. *et al*. (2010). Physiological, molecular, and cellular mechanisms of impaired seawater tolerance following exposure of Atlantic salmon, *Salmo salar*, smolts to acid and aluminum. *Aquatic Toxicology*, 99(1), pp.17–32
Monette, M.Y., Björnsson, B.T. & McCormick, S.D. (2008). Effects of short-term acid and aluminum exposure on the parr-smolt transformation in Atlantic salmon (*Salmo salar*): Disruption of seawater tolerance and endocrine status. *General and Comparative Endocrinology*, 158(1), pp.122–130
Moore, A. *et al*. (2003). The effects of 4-nonylphenol and atrazine on Atlantic salmon (*Salmo salar L*) smolts. *Aquaculture*, 222(1–4), pp.253–263
Moore, A. *et al*. (2007). The impact of a pesticide on migratory activity and olfactory function in Atlantic salmon (*Salmo salar L*.) smolts. *Aquaculture*, 273(2–3), pp.350–359
Moore, A. *et al*. (2008). The impact of a pesticide on the physiology and behaviour of hatchery-reared Atlantic salmon, *Salmo salar*, smolts during the transition from fresh water to the marine environment. In *Fisheries Management and Ecology*. Blackwell Publishing Ltd, pp. 385–392
Moore, A. *et al*. (1995). The migratory behaviour of wild Atlantic salmon (*Salmo salar*) smolts in the estuary of the River Conwy, North Wales. *Canadian Journal of Fisheries and Aquatic Sciences*, 52(9), pp.1923–1935
Moore, A. & Fewings, G. (2003). *The Effect of Atrazine Exposure on the Timing of Salmon (Salmo salar L.) Smolt Emigration.* In: R&D Technical Report, Environment Agency
Morris, S. *et al*. (2004). Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environmental Science and Technology*, 38(21), pp.5497–5504
Nieves-Puigdoller, K., Björnsson, B.T. & McCormick, S.D. (2007). Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. *Aquatic Toxicology*, 84(1), pp.27–37
Palace, V.P. *et al*. (2008). Biotransformation enzymes and thyroid axis disruption in juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to hexabromocyclododecane diastereoisomers. *Environmental Science and Technology*, 42(6), pp.1967–1972
Park, B.J. *et al*. (2011). Thyroid axis disruption in juvenile brown trout (*Salmo trutta*) exposed to the flame retardant B-tetrabromoethylcyclohexane (B-TBECH) via the diet. *Environmental Science and Technology*, 45(18), pp.7923–7927
Robinson, C.D. *et al*. (2008). Assessment of Oestrogenic Endocrine Disruption in Wild Scottish Salmon and Trout. *Fisheries Research Services Collaborative Report No 01/08*
Russell, I.C. *et al*. (2012). The influence of the freshwater environment and the biological characteristics of Atlantic salmon smolts on their subsequent marine survival. *ICES Journal of Marine Science*, 69(9), pp.1563–1573
Sandahl, J.F. *et al*. (2007). A sensory system at the interface between urban stormwater runoff and salmon survival. *Environmental Science and Technology*, 41(8), pp.2998–3004
Sandahl, J.F. *et al*. (2005). Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental toxicology and chemistry / SETAC*, 24(1), pp.136–45
Sandahl, J.F. *et al*. (2004). Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. *Canadian Journal of Fisheries and Aquatic Sciences*, 61(3), pp.404–413
Sandahl, J.F. & Jenkins, J.J. (2002). Pacific steelhead (*Oncorhynchus mykiss*) exposed to chlorpyrifos: Benchmark concentration estimates for acetylcholinesterase inhibition. *Environmental Toxicology and Chemistry*, 21(11), pp.2452–2458
Sass, J.B. & Colangelo, A. (2006). European Union bans atrazine, while the United States negotiates continued use. *International Journal of Occupational and Environmental Health*, 12(3), pp.260–267
Segner, H. *et al*. (2013). Impact of environmental estrogens on Yfish considering the diversity of estrogen signaling. *General and Comparative Endocrinology*, 191, pp.190–201
Strand, J. E. T., Davidsen, J. G., Jørgensen, E. H. & Rikardsen, A. H. (2011). Seaward migrating Atlantic salmon smolts with low levels of gill Na+, K+ -ATPase activity; is sea entry delayed? *Environmental Biology of Fishes,* 90, pp.317–321.

Suvetha, L., Ramesh, M. & Saravanan, M. (2010). Influence of cypermethrin toxicity on ionic regulation and gill Na+/K+-ATPase activity of a freshwater teleost fish *Cyprinus carpio*. *Environmental Toxicology and Pharmacology*, 29(1), pp.44–49
Thorstad, E.B. *et al*. (2007). Fjord migration and survival of wild and hatchery-reared Atlantic salmon and wild brown trout post-smolts. In *Hydrobiologia*. pp. 99–107
Thorstad, E.B. *et al*. (2013). Reduced marine survival of hatchery-reared Atlantic salmon post-smolts exposed to aluminium and moderate acidification in freshwater. *Estuarine, Coastal and Shelf Science*, 124, pp.34–43
Waring, C.P. & Moore, A. (2004). The effect of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh water and after sea water transfer. *Aquatic Toxicology*, 66(1), pp.93–104
Waring CP & Moore A (1996). *Environmental Atrazine: physiological effects on Atlantic salmon smolts in freshwater and after seawater exposure.* In: The Physiology of Migratory Fish (Eds.: S McCormick, M Sheridan, R Patino and D MacKinlay). International Symposium on the Biology of Fishes, (San Francisco) pp. 101-105