RESPONSES OF PACIFIC SALMON TO PHEROMONES, NATAL WATER, AND DISTURBANCE CUES DURING THE SPAWNING MIGRATION

by

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Abstract

Olfactory cues can provide many forms of information about an animal's environment. For some migratory species, these cues guide migrants towards foraging or reproductive grounds. Pacific salmon (*Oncorhynchus* spp.) use olfactory cues to locate their natal sites during the spawning migration. The objective of this thesis was to further our understanding of the olfactory-mediated movements of Pacific salmon as they return to their home streams to spawn.

Through a synthesis of past studies on olfactory navigation in anadromous fish, I identified critical knowledge gaps and future directions for research. Drawing from two long-standing hypotheses that seek to explain how salmonids navigate to their natal sites, I developed a new hypothesis that suggests salmonids use imprinted odours of their natal water as primary directional cues, pheromones as secondary cues, and non-olfactory environmental information as tertiary cues. One of the major implications of this hypothesis is that salmonids that have strayed from their natal migratory route might use pheromones to locate suitable spawning habitat. Using samples I collected from wild adult sockeye salmon (*O. nerka*), I found increased expression of potential pheromone receptors in strays. I also found that sockeye salmon are behaviourally attracted to the odour of conspecifics when imprinted natal cues are absent, as would be the case for a stray salmon, but not when the imprinted cues are present. Conspecific odours are not always attractive during the migration however, as I found sockeye salmon avoided the odour of conspecifics that were subjected to a handling event, suggesting these fish release chemical disturbance cues when stressed. Pink salmon (*O. gorbuscha*) did not avoid the odours of disturbed conspecifics, which might relate to species-level differences in life history. Finally, I analyzed the influence of altered flow composition resulting from hydroelectric developments on

olfactory navigation in sockeye and pink salmon. Alterations can disorient salmon as they swim upstream, and the results provide guidelines for managers to minimize such disorientation in this river system. The findings of my thesis contribute to our understanding of the olfactory process in salmonids, and point to future research directions in the field of salmonid homing.

Preface

All of the research in this thesis was conducted in accordance with animal use protocols approved by the University of British Columbia (A15-0205) and Fisheries and Oceans Canada, and with the guidelines set by the Canadian Council on Animal Care.

Chapter 2: A version of this chapter was published (Bett, N.N. and Hinch, S.G. 2015. Olfactory navigation during spawning migrations: a review and introduction of the hierarchical navigation hypothesis. Biological Reviews, doi: 10.1111/brv.12191). I conducted the literature review, synthesized the information, and wrote the manuscript under the supervision of Scott Hinch. Two anonymous reviewers provided manuscript edits.

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Chapter 1: Introduction

1.1 Animal navigation

Animals use navigation for many purposes. Whether locating a foraging patch, finding the way back to a nest or burrow, or one of many other routine tasks, animals navigate during much of their lives. The diversity in the physiology of these animals and the environments they inhabit has necessitated the use of different sensory systems. Bats, for example, use echolocation to track their prey, and more generally to "see" the world around them (Griffin 1958). Honey bees (*Apis* spp.) can use a combination of the sun, polarization, and the earth's magnetic field to locate food (Von Frisch 1971). Migratory birds use celestial cues to travel extraordinary distances between breeding and feeding grounds (Thorup and Holland 2009). Pacific salmon (*Oncorhynchus* spp.) use their sense of smell to return to their natal streams from the ocean, sometimes travelling thousands of kilometres upstream to do so (Groot and Margolis 1991). All of these methods of navigation are an essential part of the animals' lives, allowing them to accomplish basic necessities such as feeding or reproduction.

Many of the most complex forms of navigation are found in migratory animals, which often rely on input from multiple sensory systems, and can require highly developed sensory structures. In addition to celestial cues, for example, migratory birds also use an internal clock and the Earth's magnetic field (Emlen 1967; Wiltschko and Wiltschko 1996). In the aquatic environment, loggerhead sea turtles (*Caretta caretta*) use the unique magnetic signature of their natal coastline to return to their natal beaches and reproduce (Brothers and Lohmann 2015). Humpback whales (*Megaptera novaeangliae*) might use a combination of magnetic and solar cues to migrate between their winter calving and summer feeding grounds (Horton et al. 2011).

Due to the intricacies of receiving simultaneous input from multiple sensory systems, the specific directional cues that drive the movements of many migratory animals remain largely undefined.

For animals that migrate on land or in the ocean, some of the complexity of their navigation methods may be caused by their ability to move in any direction. Migratory fish in the open ocean, for example, appear to rely on several sensory cues, including the position of the sun, polarized light, currents and the geomagnetic field (Binder et al. 2011). In riverine habitats, on the other hand, migrations are restricted to two directions—upstream and downstream—and the relative simplicity of navigation in this environment seems to be reflected in the relative simplicity of the navigation methods that are used. There is overwhelming evidence that olfactory cues guide many upstream migrations (Northcote 1984). Salmonids, many of which are anadromous and migrate long distances from feeding to spawning grounds, have proved to be a model system for navigation research (Hasler and Scholz 1983). In particular, their attraction to olfactory cues while migrating upstream has been well documented.

1.2 Navigation during the spawning migration

1.2.1 *Homing in anadromous fish*

Anadromous fish are characterized by their unique life history that covers both freshwater and saltwater habitat. After hatching in rivers or lakes, these fish migrate to the ocean where they will feed and grow. When nearing maturity, they return to fresh water to spawn. Many species of anadromous fish return to the same tributaries in which they were born in a process known as homing. The first recorded observation of homing in these fish occurred in 1653, when anglers tagged Atlantic salmon (*Salmo salar*) smolts with ribbons, and watched the tagged fish return to the same site as adults (Hasler and Scholz 1983). Through the early 20th century, however, many biologists maintained that the fish did not leave coastal waters, and that returning adults simply

swam to the nearest river mouth. It was not until the 1930s that the precision of homing behaviour in anadromous fish was recognized (summarized by Scheer 1939), thanks largely to mark-and-recapture studies on salmonids. This work proved that fish could travel long distances through the world's largest oceans, yet still return to their natal tributaries to spawn. We now know that non-salmonids such as American shad (*Alosa sapidissima*) and Pacific lamprey (*Lampetra tridentata*) also home to varying degrees.

1.2.2 *Why do fish home?*

The adaptation of homing behaviours in anadromous fish has presumably been driven by fitness benefits afforded by philopatry. Hendry et al. (2004) proposed six theories that might explain why salmonids home. One theory is that the likelihood of finding suitable habitat and a mate is increased. Species that require specific spawning conditions could particularly benefit from this advantage. A second theory is that familiarity with local breeding conditions could be increased. For example, individuals could establish relationships at natal sites that subsequently influence mate choice and breeding success. Third, individuals could benefit from local adaptations. Environmental characteristics (e.g. temperature, flow, gravel size) can vary between potential reproductive sites, and philopatry allows for adaptations to the specific conditions of a given site. Other theories are that homing could be encouraged by spatial variation in habitat quality, that it could improve access to parental resources, and that it could allow migrants to avoid costs of excess movement associated with searching for habitat.

Homing may also play an ecological role. As juveniles migrate to the ocean, they take with them nutrients obtained while feeding. When adults return to spawn and die, however, they recycle nutrients back into the watershed (Krokhin 1975), which contributes to the survival of the offspring. Not only are nutrients from the natal watershed recycled, but also new, marine-

derived nutrients that were acquired while feeding in the ocean are brought back to the home stream (Naiman et al. 2002). This process is continuously repeated across generations.

1.2.3 *Olfactory navigation to spawning grounds*

Research on the migration of salmonids from the open ocean to coastal waters has been restricted by logistical difficulties associated with studying fish off-shore as well as the complexity of the sensory inputs they appear to use. In the ocean, homing salmonids may be directed by multiple environmental cues, including celestial cues, olfactory cues, visual cues, magnetic fields, currents, salinity, and temperature (McKeown 1984; Hinch et al. 2006). A recent study used a long-term data set on the migration patterns of sockeye salmon as they approached the Fraser River, and the results could improve our understanding of oceanic navigation (Putman et al. 2013). The authors identified magnetic fields and temperature as the most important factors influencing the movements of returning adults. The findings echo the discoveries made in sea turtles (Lohmann and Lohmann 1996), and could provide a basis for future research on geomagnetic navigation in salmonids.

During the freshwater phase of the migration, meanwhile, research has demonstrated that olfactory cues guide the fish as they move upstream. From the late 1950s through the 1980s, sensory impairment experiments were conducted to test whether olfaction and vision were necessary to homing. These experiments focused on a broad variety of salmonids, as well as some non-salmonids, and typically involved visual or olfactory ablation of adults as they swam upstream (for summaries, see Scholz and Hasler 1983 and Stabell 1992). Following impairment, the fish were released and allowed to resume their migration. In most cases, fish with plugged or cauterized nares had difficulty locating their home stream, whereas blinded fish did not, suggesting olfaction plays a necessary role in homing.

Building on the findings of the sensory impairment studies, electrophysiology research has explored the physiological capabilities of the fish's olfactory system. The electro-olfactogram (EOG) and electro-encephalogram (EEG) are instruments that monitor whether the olfactory system responds to a given chemical. EOG and EEG studies have identified which chemicals fish can detect, as well as the minimum thresholds for detection. The four known chemical compounds that the fish can detect are amino acids, steroids, prostaglandins, and bile acids (Hara 1992). Response thresholds to amino acids fall in the nanomolar to micromolar range, while thresholds to the other compounds are in the picomolar to nanomolar range (Zielinski and Hara 2007). These thresholds reflect the concentrations generally found in the aquatic environment (Hara 2005).

Electrophysiological responses indicate a fish's sensitivity to a chemical, but they do not inform its behavioural response. For example, increased sensitivity does not necessarily translate to a stronger attraction to (or repulsion from) a chemical. To analyze the behavioural response of migrating salmonids to different odours, behavioural choice experiments have been used. Fish are allowed to choose between water containing an odour of interest, and a control. Shoji et al. (2003) and Yamamoto et al. (2009) used such experiments to demonstrate that amino acids might act as critical olfactory cues to the Pacific salmon spawning migration. First, they analyzed the amino acid profiles of the salmons' natal rivers. Next, they created artificial "natal" water by adding the appropriate amounts of each amino acid to blank water. In subsequent two-choice experiments, the salmon preferentially selected the artificial natal water, suggesting they were attracted to the odour of the amino acids. Behavioural choice experiments have also been conducted in other salmon homing studies (e.g. Idler 1961; Brannon et al. 1984; Dittman et al. 1996).

Recently, researchers have also begun to examine the olfactory process on a molecular level. Dukes et al. (2004) found evidence that olfactory receptor genes are up-regulated during the parr-smolt transformation, when juveniles are potentially learning the unique odour of their natal water. Johnstone et al. (2011) found that expression of receptor genes differs between juveniles and adults in Atlantic salmon, which might relate to learning and recognition of odours. Olfactory activity on a molecular level in relation to homing is still largely unknown, however. The chemicals that bind to receptors during the migration, for example, remain unknown, and the relation between olfactory gene expression and homing behaviour is unclear.

1.2.4 *Olfactory imprinting*

The ability to orient towards an odour requires an olfactory system that is capable of detecting the odour's chemical constituents. Navigation towards the home stream, however, requires not just the ability to detect the natal odour but also a recognition of this odour as the desired destination. The most widely accepted theory that explains how salmonids locate and identify their natal site is the olfactory imprinting hypothesis (Hasler and Wisby 1951) which states that juveniles imprint on the unique odour of their natal tributary, and then migrate towards this odour as adults.

Scholz et al. (1976) conducted the first large-scale test of olfactory imprinting. In their experiment, juvenile coho salmon (*O. kisutch*) in Lake Michigan were imprinted on either morpholine or phenethyl alcohol. The fish were then released directly into Lake Michigan, thus minimizing the risk of imprinting on additional cues. When the fish reached maturity, the researchers released the two chemicals into two different rivers that flow into Lake Michigan. The majority of the salmon returned to the river containing the chemical, indicating they were

guided by the imprinted odour. Several similar studies were conducted by the same research group using other species of salmonids, and the results were similar (Hasler and Scholz 1983).

The time at which imprinting occurs in anadromous fish seems to vary across species, possibly reflecting differences in life history. Laboratory and field studies on coho salmon indicate that the imprinting period occurs during the parr-smolt transformation (PST), which is characterized by physiological and behavioural changes that prepare the fish for saltwater. For example, hatchery-raised coho salmon will imprint on chemicals if they are exposed as smolts, but not if exposed as parr or embryos (Dittman et al. 1996). Also, coho salmon that have been reared in one site and released from a second site will return as adults to the second site, so long as they were released prior to or during the PST (Donaldson and Allen 1957; Jensen and Duncan 1971). In contrast to this, there is evidence that sockeye salmon can imprint as alevins and emergent fry (Tilson and Scholz 1997), and potentially even as embryos (Quinn et al. 2006). Sockeye will often rear in a lake that is downstream from their incubation site, and do not return to the incubation site before migrating to the ocean. As adults, though, they migrate past the rearing lake to the incubation site. It should not be surprising, then, that they may be capable of imprinting during very early stages, before the PST. Similarly, Chinook salmon (*O. tshawytscha*) often migrate extensive distances downstream from their natal site before smolting (Beckmen et al. 2000), and there is evidence that they may imprint during earlier developmental stages as well (Dittman et al. 2010).

Thyroid hormones are associated with many of the changes that occur during the PST, and appear to be involved in the imprinting process. There is evidence in multiple salmonid species that olfactory sensitivity is enhanced during the PST, and that this period coincides with an increase in thyroid activity (Dittman and Quinn 1996). Artificially elevated thyroid hormones

have induced imprinting and the proliferation of olfactory receptor cells in coho salmon (Scholz 1980; Lema and Nevitt 2004). Also, the olfactory epithelium of masu salmon (*O. masou*) is enriched in thyroid hormone receptors during the PST (Kudo et al. 1994).

Some fish migrate distances that are presumably too great for them to navigate using imprinted home stream cues alone. Harden-Jones (1968) modified the olfactory imprinting hypothesis to account for this, suggesting that fish not only imprint on home stream cues, but also on chemical mixtures at other points along the migration route. He referred to the process as "sequential imprinting", and proposed that fish home towards these waypoints sequentially, until they are close enough to detect natal water. Transport experiments, in which juvenile salmon were transported and released in new locations before they migrated to sea, support this hypothesis. The returning adults tend to migrate towards the release site rather than the rearing site, although if the two locations are in close proximity, they often return to the rearing site (summarized in Dittman and Quinn 1996). These results suggest the release site acts as a waypoint, and that salmon will continue onwards to the rearing site only if it is detectable. Fish that are transported, however, also tend to stray more (Lister et al. 1981), indicating that homing is more complex than a series of simple imprinting events, and is likely influenced by a number of other factors.

1.2.5 *Pheromones as directional cues*

An alternative to olfactory imprinting is the pheromone hypothesis. This theory states that migrating fish are attracted to pheromones released by other members of their own population (Nordeng 1977). Juveniles are often present in natal tributaries as adults are migrating upstream, and they may release pheromones that attract the adults. Evidence of pheromone-based homing long pre-dates the hypothesis. White (1934), for example, described a river in Nova

Scotia that split into two branches, only one of which bore Atlantic salmon. It was only after the empty branch was stocked with parr that adult migrants entered and spawned. The migrants presumably detected olfactory cues released by the parr.

The best evidence of pheromones directing spawning migrations is found in lampreys, including sea lampreys (Li et al. 1995, Bjerselius et al. 2000), river lampreys (Gaudron and Lucas 2006), and Pacific lampreys (Yun et al. 2003, 2011). Lamprey larvae buried in the sediment of their home stream release a mixture of chemicals—including bile acids and sulfated steroids—that attract migrating adults (Sorensen et al. 2005). When larvae are removed from a stream, the pheromonal cues are lost and there is a significant decrease in the number of adults entering the stream (Moore and Schleen 1980). Unlike salmonids, however, lampreys do not home to their natal streams. Instead, they home to the streams with the largest numbers of conspecific larvae (Sorensen and Hoye 2007). The population of these larvae makes little difference, since different lamprey populations, and even different lamprey species, release the same pheromones (Fine et al. 2004). An imprecise form of homing results, as evidenced by a regional panmixia (Waldman et al. 2008).

The role of pheromones in salmonid migrations is not well understood. Given that salmonids exhibit precise natal homing, the use of pheromones as directional cues would necessitate the ability to distinguish pheromones produced by members of their own population. There is widespread evidence that population discrimination exists in several species of salmonids, including sockeye and coho salmon, rainbow trout (*O. mykiss*), brown trout (*Salmo trutta*), Arctic char (*Salvelinus alpinus*) and Atlantic salmon (Døving et al. 1974, Groot et al. 1986, Stabell 1992, Winberg and Olsén 1992, Griffiths 2003). Nordeng (1971) conducted the first major field study to test the role of pheromones in salmonid migrations, using Arctic char.

Despite being raised in a hatchery and therefore unable to imprint on home stream chemicals, most of the char swam to their natal river system, where juveniles of their population were present. More recently, Dittman et al. (2010) released juvenile hatchery-raised Chinook salmon at various locations, and found many adults in areas they had never experienced. Interestingly, most of these strays were recovered in spawning areas used extensively by wild Chinook salmon, suggesting that some fish may be guided by pheromones.

Despite these findings, however, the olfactory imprinting hypothesis is currently the favoured explanation for homing, at least in salmonids (Ueda 2011; Keefer and Caudill 2014). In addition to the large amount of experimental support behind olfactory imprinting, the use of pheromones as directional cues does not seem applicable to all salmonid species. Juvenile pink and chum salmon, for example, migrate to the ocean shortly after emerging from the gravel. Consequently, there are no juveniles present in the natal tributaries during the spawning migration, and therefore no pheromones present to guide adults. Nevertheless, salmonids appear capable of detecting pheromones and using them as directional cues. How these cues might be used alongside imprinted cues, however, remains unknown. One of the objectives of my thesis is to reconcile these two hypotheses.

1.2.6 *The influence of external stressors*

Pacific salmon face many external stressors during the spawning migration. For example, high flows can cause quick depletion of energy levels and lead to increased migration failure (Hinch et al. 2012). Barriers such as dams can confuse or delay migrants (Caudill et al. 2007), increasing physiological stress and the risk of en route mortality (Roscoe et al. 2011; Burnett et al. 2014). Other stressors have increased dramatically over recent years. The recreational fishery of sockeye in the Fraser River, for example, was nearly non-existent in 1990, but has grown to

such a degree that in 2010 approximately 100,000 sockeye were caught and released (Gale et al. 2011). Catch-and-release events elicit a strong stress response, and can lead to reduced survival and spawning success (Donaldson et al. 2012). Increasing temperatures in migratory rivers can also increase stress significantly. Pacific salmon have adapted to the specific thermal regimes of their natal tributaries (Hinch et al. 2012), but temperatures of many migratory routes are increasing considerably beyond historical norms. Peak summer temperatures in the Fraser River and Columbia River have increased by over 2^{o}C and 4^{o}C, respectively, in the past 60 years (Patterson et al. 2007; Crozier et al. 2008). Elevated water temperature acts as an acute and also a chronic stressor for migrating salmon, and may contribute to both en route and pre-spawn mortality (Hinch et al. 2012, Jeffries et al. 2012). The influence of such stressors on olfactory navigation during the spawning migration has not been explored. An objective of my thesis is to examine the influence of stressors—specifically handling events and confusing flow compositions in a regulated river—on olfactory navigation in migrating Pacific salmon.

1.3 Straying

1.3.1 *Straying behaviours in salmonids*

The majority of salmonids home successfully. Homing success in Pacific salmon, for example, is typically greater than 90% (Quinn 2005). The remaining individuals that do not return to their natal site are commonly referred to as "strays", and will attempt to spawn in a non-natal area. These fish may be unable to detect or recognize natal odours, or because they have stopped feeding in the marine environment and are utilizing energy reserves to complete migration and spawn, they may lack the energy stores required to reach their natal site. Straying has been a major field of study over the past several decades, largely due to interest in straying among hatchery fish (Keefer and Caudill 2014). In contrast, very few straying studies focus on

wild populations (Quinn 1993). Straying is a critical behaviour to wild populations, however, as it allows for colonization of new habitat and promotes gene flow between populations. Furthermore, diverse meta-populations that are created through straying buffer against the risk of localized disturbance ("portfolio effects") and ensure long-term survival (Schindler et al. 2010). Another objective of my thesis is to explore the straying behaviours of wild Pacific salmon, and to identify the olfactory cues they use to navigate.

1.3.2 *Evolutionary mechanisms that drive straying*

The low frequency of straying could be due, at least in part, to several negative consequences associated with this behaviour. Straying could be avoided for the same reasons that homing might be beneficial, as previously discussed. For example, straying could decrease the likelihood of locating suitable spawning habitat or finding mates. Site-specialist species, such as sockeye salmon, have very specific spawning requirements, and also have extremely low levels of straying (Quinn 2005). More generalist species such as pink salmon, on the other hand, have more lenient spawning requirements, and stray more frequently (Quinn 2005). Local adaptive advantages are also lost during straying. Adaptations can include characteristics such as optimized body shape or egg size (Crossin et al. 2004; Hendry et al. 2004). For example, sockeye salmon in Bristol Bay that breed in shallower streams experience a greater intensity of bear predation. Salmon in these populations have shallower bodies, an adaptation that reduces the risk of predation (Quinn et al. 2001). Fish that stray into non-natal sites can lose the fitness benefits associated with the local adaptive traits (Fraser et al. 2011), and experience reduced reproductive success.

While reduced reproductive success might keep straying rates low, there are several potential benefits to straying that could ensure straying continues to occur. Straying can reduce

resource competition if the strays locate a spawning ground utilized by fewer conspecifics. Furthermore, straying could counter inbreeding depression that might result from persistent philopatry. Straying can also buffer populations against temporal variation in habitat quality, such as flooding events that can cause mortality of eggs and alevins (Lapointe et al. 2000). Colonization is also made possible through straying, which can allow new populations to replace populations lost through localized extinction, and also to access new environments and expand their range. And in populations that have been previously isolated, straying can create a demographic rescue effect by providing a crucial influx of individuals as the population transitions towards self-sustainability, as found in Chinook and coho salmon upstream from a newly constructed fish ladder in Washington's Cedar River (Anderson et al. 2015). Consequently, straying continues to occur at low—but not negligible—levels in all salmonid species.

1.3.3 *Navigation in strays*

Although straying has been studied heavily by researchers and fisheries managers, very little is known about navigation in these fish. Whereas a homing salmon will migrate towards imprinted natal odours, stray salmon are not exposed to these cues. Instead, they must either select their migratory path at random, or orient themselves towards other cues. Past findings suggest the distribution of strays is not random. For example, stray salmon seem to prefer rivers that are occupied by conspecifics (Jonsson et al. 2003; Dittman et al. 2010) or have a higher discharge (Unwin and Quinn 1993). The behavioural responses of stray salmon to environmental cues has never been tested, however, and whether stray salmon are guided by olfactory or other cues is unknown. The identification of such cues could help predict the dispersal patterns of salmon, which can influence the health and stability of meta-populations. Furthermore, there is

concern that genetic introgression of strays into small or unstable recipient salmon populations might influence these populations' sustainability (Brenner et al. 2012; Johnson et al. 2012; Jasper et al. 2013; Hess and Matala 2014), and could lead to localized extinction or the loss of unique adaptations (Zhivotovsky et al. 2012).

1.4 Thesis objectives

The aim of my thesis was to explore the use of chemical cues by migrating adult Pacific salmon and to further our understanding of olfactory navigation during the spawning migration. In Chapter 2, I synthesized past research on olfactory navigation during spawning migrations. In this chapter, I also developed a new hypothesis that seeks to reconcile the olfactory imprinting and pheromone hypotheses. I then tested a fundamental component of this hypothesis—that stray salmon use pheromones as directional cues—using molecular (Chapter 3) and behavioural (Chapter 4) analyses. The molecular analysis is a novel approach to studying navigation during the Pacific salmon spawning migration, and has not been employed previously in the published literature. In this study, I focused on the expression of olfactory receptor genes potentially associated with pheromone detection in migrating stray and non-stray sockeye salmon. Chapter 4 builds on the findings of the molecular research, and is the first study to explore the behavioural responses of stray salmon to environmental cues. Here, I tested the responses of sockeye salmon to the odour of conspecifics when natal imprinted cues are absent (as in the case of strays) and when they are present (non-strays). Pacific salmon face many stressors during the spawning migration, so in Chapter 5 I explored the effects of a stressor on chemical conspecifc communication in sockeye and pink salmon. To do so, I replicated the experimental design used in Chapter 4, but this time using the odour of conspecifics that were stressed *via* a handling event. The results of this chapter demonstrate that conspecific cues can actually be repulsive,

rather than attractive, if the fish releasing the cues are stressed. In addition to handling during a fisheries capture, another stressor many salmon face during the spawning migration is migratory barriers such as dams. In Chapter 6, I studied the effects of an altered flow regime in a regulated river on the behaviour of migrating sockeye and pink salmon. The dam alters the release of natal chemical imprinted cues through the system such that returning salmon can become disoriented. The findings from this study can be used to inform management operations at the dam, and this work provides an example of how navigation research can contribute to fisheries management. Chapter 7 summarizes the findings from the previous chapters and provides future research directions within the field of salmon homing and navigation.

Chapter 2: Olfactory navigation during spawning migrations: a review and introduction of the Hierarchical Navigation Hypothesis

2.1 Synopsis

Migrations are characterized by periods of movement that typically rely on orientation towards directional cues. In this chapter, I synthesize research that explores the role of olfaction during the spawning migrations of anadromous fish. Research has largely focused on the family Salmonidae (salmonids), which contains the focal species of my research in subsequent chapters, as well as the family Petromyzontidae (lampreys). I draw attention to limitations in this research, and highlight potential areas of investigation that will help fill in current knowledge gaps. I also use the information assembled from my review to formulate a new hypothesis for natal homing in salmonids. My hypothesis posits that migrating adults rely on three types of cues in a hierarchical fashion: imprinted cues (primary), conspecific cues (secondary), and non-olfactory environmental cues (tertiary). I provide evidence from previous studies that support this hypothesis. I also discuss future directions of research that can test the hypothesis, which guided the research in chapters 3 and 4.

2.2 Introduction

Migrations are prevalent in a diverse array of species, including insects, birds, fish, amphibians, reptiles and mammals, and can provide insight into nearly all levels of life, from molecular to evolutionary (Dingle and Drake 2007). While the term ‘migration’ encompasses a broad variety of behaviours, all migrations involve some form of persistent, directed and predictable movement, which occur on broad spatial and temporal scales, and often span multiple types of habitat (Aidley 1981).

Many migratory species exhibit a specific form of migration known as natal homing, or movement to the natal location, which requires precise orientation capabilities. Oriented movements can be divided into three general categories (Ramenofsky and Wingfield 2007): (1) compass orientation, in which movement occurs in a fixed direction and relies on the detection of large-scale cues (such as geomagnetic or celestial cues); (2) true navigation, in which an animal's position is determined by a cognitive map (established either genetically or through experience); and (3) piloting, in which local cues such as landmarks or odours are used to provide direction. These categories of oriented movement are not mutually exclusive. For example, the combined use of compass orientation and cognitive maps has been well demonstrated in birds (Åkesson and Hedenström 2007).

Anadromous fish are well known for their distinctive life histories: after hatching in fresh water, they migrate to the ocean to feed, and then return to fresh water to spawn. As the fish progress through their different life stages, they utilize different types of oriented movement. In salmonids, the best-studied group of anadromous fish, the outward ocean migration appears to rely at least partially on compass orientation (Putman et al. 2014). The return or spawning migration, meanwhile, can be divided into two distinct phases. First, the fish must navigate the open ocean, possibly through compass orientation (Harden Jones 1968; Putman et al. 2013). Second, upon reaching fresh water they use olfactory cues to locate their upstream spawning grounds through piloting.

Herein, I provide an overview of how olfaction is used during spawning migrations, synthesize past research that explores this topic, and conduct a comprehensive assessment of our current understanding. I then discuss limitations in the research as a whole and provide directions for investigation that will help fill our existing knowledge gaps. Finally, I propose a new

hypothesis for natal homing that reconciles the two hypotheses that currently dominate this field of work.

2.3 Use of olfaction during the spawning migration

The freshwater phase of the spawning migration relies primarily on olfaction (Hasler and Scholz 1983; Dittman and Quinn 1996), and the olfactory system has been reviewed extensively (Hara 1994; Hamdani and Døving 2007; Zielinski and Hara 2007). The process begins at the peripheral olfactory organ(s), where odorants bind to receptors in the olfactory epithelia, and a signal is transmitted through the olfactory nerve to the olfactory bulb (Fig 2.1). These signals generate responses in different regions within the bulb, which vary with the chemical identity of the stimulant (Morin and Døving 1992; Laberge and Hara 2004). Chemical cues that signify the spawning destination are then interpreted in the brain, although the manner in which this information is processed has not yet been elucidated. Recent evidence suggests, however, that the NMDA receptor essential subunit NR1 plays a crucial role in olfactory memory formation and recall in the telencephalon (Ueda et al. 2016).

There are over 150 species of anadromous fish (Reide 2002), and the majority are philopatric, returning to their natal tributary to spawn. These 'natal homing' migrations have been most extensively studied in salmonids, and there are two primary hypotheses that seek to explain how natal homing occurs. The Olfactory Imprinting Hypothesis (Hasler and Wisby 1951; Hasler, Scholz and Horrall 1978) proposes that juveniles imprint on the unique odours of their natal tributary and then migrate towards these imprinted cues as adults. Harden Jones (1968) suggested that migrants not only imprint on natal water, but also on multiple 'waypoints' during the out-river migration. Returning adults then direct themselves towards these waypoints in the reverse sequence. A second hypothesis is the pheromone hypothesis (Nordeng 1971), which

proposes that adults migrate towards pheromones emitted from conspecific juveniles that are living in or migrating from the natal water.

Some other anadromous fish, like lamprey (Petromyzontidae), are not philopatric, and do not return to their natal water to spawn (Waldman, Grunwald and Wirgin 2008; Spice et al. 2012). Instead, adults migrate towards chemical cues released by larval conspecifics (Moser et al. 2015), with a preference for higher densities of larvae (Moore and Schleen 1980). Limited dispersal at sea, however, restricts their migration and spawning distribution to the same broad geographical area in which they were hatched (e.g. the west coast of North America; Spice et al. 2012). This type of migration strategy may be referred to as 'non-specific homing', which implies that migratory adults return to a general 'home' area, but not necessarily their natal tributary. A generalized attraction to pheromones is found in other migratory species of fish as well, such as in the family Galaxiidae (Baker and Hicks 2003).

These two groups of fish – salmonids and lamprey – comprise the overwhelming majority of research on olfactory navigation during the spawning migration, and are therefore the focus of this review. There are many aspects within this field of work, including molecular processes, the identification and interpretation of olfactory cues, and the effects of endogenous and exogenous factors, that remain largely unknown. The following review synthesizes the research that has been conducted to date, and highlights the gaps in our knowledge.

2.4 A review of olfaction and the spawning migration

I conducted a comprehensive search of the peer-reviewed literature in ISI *Web of Knowledge* and *Aquatic Sciences and Fisheries Abstracts* to identify studies that relate olfaction to migration in anadromous fish. I used the following topic search terms: "olfact* AND (homing OR migrat* OR selection OR spawn* OR navigat*)" in conjunction with the names of 171

known anadromous species (K. Reide, personal communication). A total of 248 relevant papers were collected. The search was supplemented by the inclusion of studies that did not appear in my search results but were cited by other authors. There is a broad range of research that falls within my focal topic, and to synthesize this work I grouped the studies into the following six categories, based on their specific aims: (1) detection of spawning grounds through sensory systems; (2) responses to natal water; (3) responses to conspecifics; (4) responses to specific chemical compounds; (5) olfactory imprinting; and (6) molecular ecology of olfaction and the spawning migration.

2.4.1 *Detection of spawning grounds through sensory systems*

Perhaps the most basic form of spawning migration research has focused on the relative importance of olfaction and other sensory systems in the detection of spawning grounds. These studies typically follow a similar methodology, in which the sensory systems of adult migrants are ablated, and their subsequent migratory behaviour is monitored. In most cases, impairment of the olfactory system significantly reduced homing success, whereas visual impairment had a more limited impact, such as increasing the time taken to reach natal water (Table 2.1). The earth's geomagnetic field may be a crucial component to the oceanic phase of the spawning migration (Putman et al. 2014), such as the detection of natal river mouths (Putman et al. 2013), although there is evidence that suggests otherwise (Yano et al. 1996; Ueda et al. 1998). The critical role of olfaction to the spawning migration in anadromous fish is now widely accepted, and recent studies rarely involve sensory impairment.

2.4.2 *Responses to natal water*

Many studies have focused on the responses, from cellular to behavioural levels, that are triggered by exposure to natal water (Table 2.2). Cellular responses to natal water have been

identified in the olfactory bulb of sockeye salmon (*Oncorhynchus nerka*; Bodznick 1978*a*), and magnetic resonance imaging has revealed that exposure to natal water induces an increase in blood perfusion to certain regions of the telencephalon (Bandoh, Kida and Ueda 2011). In addition, behavioural experiments and electrophysiology studies (which monitor electrical activity in the olfactory system) indicate that salmonids and alewifes (*Alosa pseudoharengus*) are attracted to natal water, or respond to it more strongly than to other water sources (Hara, Ueda and Gorbman 1965; Kaji et al. 1975; Sato, Shoji and Ueda 2000; Sutterlin and Gray 1973; Thunberg 1971; Ueda, Hara and Gorbman 1967; Ueda 1985). In coho salmon (*O. kisutch*) and Chinook salmon (*O. tshawytscha*), the natal water can be diluted down to 10% with little effect on the magnitude of the electrophysiological response (Hara et al. 1965; Ueda et al. 1967). The behavioural response is even more sensitive: Atlantic salmon (*Salmo salar*) are attracted to natal water that has been diluted to 0.1% (Sutterlin and Gray 1973), and sockeye salmon show a preference for pure natal water over natal water that has only been slightly diluted to 90% (Fretwell 1989). Interestingly, Sato et al. (2000) tested the electrophysiological response of lacustrine sockeye salmon to various waters collected from their natal pond and other regions of the same watershed, and found that the response to the pond water was the strongest, while the response to the water flowing into the pond was the weakest.

2.4.3 *Responses to conspecifics*

In addition to natal water, anadromous fish also respond to water that is conditioned by conspecifics (Table 2.3). An attraction to larval conspecifics has been demonstrated in several lamprey species, including sea lamprey (*Petromyzon marinus*; Fine, Vrieze and Sorensen 2004; Fine and Sorensen 2010; Li and Sorensen 1997; Sorensen et al. 2005; Teeter 1980; Vrieze and Sorensen 2001; Wagner, Twohey and Fine 2009), silver lamprey (*Ichthyomyzon unicuspis*; Fine

et al. 2004), river lamprey (*Lampetra fluviatilis*; Gaudron and Lucas 2006), and Pacific lamprey (*Entosphenus tridentatus*; Yun et al. 2011). Regional panmixia in sea lamprey (Waldman et al. 2008) and Pacific lamprey (Spice et al. 2012) suggests that they are not philopatric, and therefore do not appear to use population-specific odours as spawning migration cues. As a result, few studies have attempted to determine whether lamprey respond similarly to larvae from different locations. There is evidence, however, that they can be attracted to larvae of different lamprey species (Fine et al. 2004).

The effect of conspecific cues on migratory behaviour of salmonids is less clear. A variety of different study methods, including field experiments, electrophysiology, and behavioural trials, suggest they are able to detect and respond to conspecific odours that emanate from faeces, bile, intestinal content, urine, and skin mucus (Courtenay, Quinn and Dupuis 1997; Døving, Enger and Nordeng 1973; Hara and Maconald 1976; Selset and Døving 1980; Stabell, Selset and Sletten 1982). If these odours are used as directional cues during natal homing, however, adults must be able to differentiate their own population from other populations. There is evidence that salmonids are capable of population-level discrimination (Courtenay et al. 1997; Døving, Nordeng and Oakley 1974; Groot, Quinn and Hara 1986; McBride et al. 1964; Nordeng and Bratland 2006; Nordeng 1971, 2009; Quinn and Tolson 1986; Selset and Døving 1980), and even discrimination of siblings from non-siblings (Quinn and Busack 1985; Quinn and Hara 1986; Winberg and Olsén 1992). It should be noted, however, that the results in some of these studies are at least partially inconclusive. For example, Groot et al. (1986) found population discrimination in one population but not in another, and Courtenay et al. (1997) found that coho fry of certain populations appeared generally more attractive than fry of other populations. There

is also contrary evidence that discrimination does not occur (Keefe and Winn 1981; Fisknes and Døving 1982; Quinn, Brannon and Whitman 1983).

In a field test on the relative importance of pheromones and imprinted cues, Brannon and Quinn (1990) documented the homing behaviour of coho salmon. Adults returned to their juvenile release site rather than a nearby hatchery containing sibling conspecifics, suggesting that imprinted cues are more attractive than population- and family-specific odours. One of the groups in this study, however, returned to a hatchery they had never experienced. These fish had been moved downstream prior to their release as juveniles, which may have disrupted their ability to imprint on the outmigration route. Their return to the hatchery suggests that conspecific odours can be attractive. These results, taken together with the results from other studies, suggest that conspecific odours may act as a secondary directional cue if imprinting cues are undetectable.

2.4.4 *Responses to specific chemical compounds*

In addition to exploring the effects of exposure to natal water and conspecifics, many studies have tested the sensitivity of lampreys and salmonids to the component chemicals that contribute to these odours (Table 2.4). For migratory lamprey species, several bile acids have been identified as potent chemical cues that direct adults towards spawning grounds (Fine et al. 2004; Li and Sorensen 1997; Li, Sorensen and Gallaher 1995; Robinson et al. 2009; Siefkes and Li 2004; Sorensen et al. 2005; Vrieze and Sorensen 2001; Yun et al. 2011). These chemicals are released by larval lamprey, primarily through their faeces, at levels detectable by migrating adults (Fine and Sorensen 2010; Fine et al. 2004; Polkinghorne et al. 2001). When isolated, the chemicals can trigger an attractive response similar to that of larval water (Sorensen et al. 2005; Fine and Sorensen 2008). In salmonids, however, the directional cues have not yet been

identified. There are four classes of chemicals that are potent odours detected by the fish olfactory system. Amino acids (Belghaug and Døving 1977; Evans and Hara 1985; Fisknes and Døving 1982; Hara and Zhang 1998; Hara 1972, 1973; Hara et al. 1993; Laberge and Hara 2004; Quinn and Hara 1986; Rehnberg, Jonasson and Schreck 1985*b*; Satou and Ueda 1975; Shoji et al. 1996; Sutterlin and Sutterlin 1971; Sveinsson and Hara 1990*a*,*b*; Yamamoto, Ishizawa and Ueda 2008*a*; Zhang and Hara 2009) and some steroids (Essington and Sorensen 1996; Hara and Zhang 1998; Laberge and Hara 2003; Zhang and Hara 2009) are detected at the micromolar to nanomolar range, while bile acids and bile salts are detected at the nanomolar to picomolar range (Døving, Selset and Thommesen 1980; Giaquinto and Hara 2008; Hara and Zhang 1998; Zhang and Hara 2009; Zhang, Brown and Hara 2001), and prostaglandins are detected at the micromolar to picomolar range (Hara and Zhang 1998; Laberge and Hara 2003; Sveinsson and Hara 2000; Zhang and Hara 2009). Salmonids can also detect cations (such as calcium and magnesium) at millimolar to micromolar levels (Bodznick 1978*b*; Shoji *et al*. 1996). The detection thresholds for each of these classes of chemicals are within their range of concentrations in natural freshwater systems, and they are therefore all potential migratory cues (Zielinski and Hara 2007).

Salmonids and lampreys detect aquatic odorants through complex signal transduction mechanisms. Separate receptors exist for different classes of chemicals, such as amino acids, prostaglandins, bile acids and salts, and other steroids (Døving *et al*. 1980; Laberge and Hara 2004; Zhang and Hara 2009), allowing the fish to discriminate between the different classes. Furthermore, amino acids, bile acids, and prostaglandins can suppress responses to other chemical compounds within their class (Giaquinto and Hara 2008; Laberge and Hara 2003, 2004; Lo, Bradley and Rhoads 1991; Rehnberg and Schreck 1986), indicating that certain receptors are

able to bind multiple chemicals within a given class. There is also evidence, however, that different receptor types exist for certain chemical classes. There appear to be different types of receptors for amino acids (Sveinsonn and Hara 1990*b*) as well as for bile acids (Zhang and Hara 2009; Fine and Sorensen 2008; Siefkes and Li 2004), allowing for discrimination between, for example, free bile acids and amidated bile acids, or neutral and basic amino acids. Prostaglandins, meanwhile, may activate as few as a single receptor type (Laberge and Hara 2003). Reception of different chemical classes also triggers a response in different regions of the olfactory bulb (Døving *et al*. 1980; Fisknes and Døving 1982; Hara and Zhang 1998; Laberge and Hara 2004; Morin and Døving 1992). Taken together, this information suggests that the fish can discriminate among, and also within, different groups of chemical compounds (Fig 2.2).

Recently, amino acids have been identified as a primary candidate for migratory cues in salmonids. Shoji *et al*. (2000) created three types of artificial natal water by adding the appropriate concentrations of certain groups of chemicals to blank water, thus replicating the concentrations found in the natal water. The three separate artificial "natal waters" contained: (1) amino acids and inorganic salts; (2) bile acids and inorganic salts; or (3) inorganic salts alone. The electrophysiological response of mature masu salmon (*Oncorhynchus masou*) to the amino acid mixture was similar in strength to their response to the true natal water, whereas the response to the other artificial waters was substantially lower. Shoji *et al*. (2003) used behavioural choice tests to demonstrate that chum salmon (*O. keta*) are attracted to artificial natal water containing the appropriate concentrations of amino acids, taurine, urea, and ammonia, and Yamamoto *et al*. (2008*a*) produced similar results with sockeye salmon (*O. nerka*), masu salmon and pink salmon (*O. gorbuscha*). Furthermore, Yamamoto and Ueda (2009) determined that

chum salmon are attracted to artificial natal water containing only amino acids and salts, even when the amino acid that is most abundant in their natal stream is removed.

2.4.5 *Olfactory imprinting*

Olfactory imprinting, which is the most widely accepted explanation of natal homing in salmonids, has been tested extensively (Table 2.5). While the specific natural chemicals that are imprinted on have not yet been identified, artificial imprinting studies have convincingly demonstrated the imprinting abilities of salmonids. After being exposed as juveniles to a chemical that is absent from natural water systems, such as morpholine or phenethyl alcohol, adults become sensitive and attracted to that chemical. This has been demonstrated in various salmonid species, including coho salmon (*Oncorhynchus kisutch*; Cooper *et al*. 1976; Scholz *et al*. 1976; Rehnberg, Curtis and Schreck 1985*a*), Chinook salmon (*O. tshawytscha*; Hassler and Kutchins 1990), sockeye salmon (*O. nerka*; Tilson and Scholz 1997), steelhead (*O. mykiss*; Cooper and Scholz 1976), and brown trout (*Salmo trutta*; Scholz *et al*. 1978*a*).

Field studies indicate that coho salmon can successfully imprint in as few as two days (Jensen and Duncan 1971), while successful imprinting has been demonstrated in as few as 14 days in a laboratory study (Yamamoto, Hino and Ueda 2010). The precise moment at which imprinting occurs, however, has not been conclusively identified. Most studies on the timing of olfactory imprinting indicate that the parr–smolt transformation (PST) is a critical period. Thyroid hormones, which have been linked to memory development (Morin, Dodson and Doré 1989*a*,*b*; Nevitt *et al*. 1994), undergo a marked increase during this period of growth (Dickhoff, Folmar and Gorbman 1978), which may explain why the PST is a sensitive imprinting period. In some species of salmonids, however, juveniles migrate to a nursery lake or river before undergoing the PST. This implies that imprinting should occur prior to the PST, although

experimental evidence of pre-PST imprinting is limited (Tilson and Scholz 1997). Dittman and Quinn (1996) propose that the lack of evidence of imprinting before the PST may be due to a lack of environmental variability in imprinting studies. Juveniles in such studies are often reared in a hatchery environment (Hasler and Scholz 1983; Dittman, Quinn and Nevitt 1996; Dittman *et al*. 1997), where rearing conditions remain static (i.e. the fish are held in the same water throughout development). Static rearing conditions could limit imprinting to the developmentally controlled surge in thyroid hormones during the PST. In addition to developmentally controlled peaks of thyroid hormone levels, however, the thyroid axis is affected by exposure to novel water (Dickhoff *et al*. 1978; Hoffnagle and Fivizzani 1990) and other environmental cues (Dittman and Quinn 1996). Since hatchery fish remain in the same water, they do not experience an increase in thyroid levels that is associated with exposure to novel water. This may, at least in part, explain why experimental fish have historically been unable to imprint before the PST.

Large-scale field studies, in which the juvenile outmigration of salmonids is altered, also provide valuable insight into the imprinting process of juveniles. Juveniles that are reared in one location and released in another, typically during or close to the PST, tend to return to the site of release (Table 2.6). These results underline that the PST is a sensitive period for imprinting. Furthermore, juveniles that are released closer to their rearing site often return to the rearing site (e.g. Brannon and Quinn 1990; Nordeng and Bratland 2006), unlike those released further away (e.g. Donaldson and Allen 1958; Jensen and Duncan 1971; Johnson, Solazzi and Nickelson 1990), which may indicate that juveniles imprint to multiple waypoints, as proposed by Harden Jones (1968). In other words, as adults return, they may first locate the release site, then make their way onwards to the rearing site if it is within detection range.

2.4.6 *Molecular ecology of olfaction during the spawning migration*

Although the majority of studies to date have focused on the behaviour, physiology, and ecology of olfactory imprinting, recent genetic research has improved our understanding of the olfactory system in salmonids from a molecular standpoint. Various olfactory genes have been characterized in salmonids, particularly in Atlantic salmon (*Salmo salar*; Wickens, May and Rand-Weaver 2001; Dukes *et al*. 2006; Johnstone *et al*. 2009, 2012), but also in several species of Pacific salmon and trout (*Oncorhynchus* spp.; Onuma *et al*. 2005; Hino *et al*. 2007; Kudo *et al*. 2009; Johnson and Banks 2011), and most recently in sea lamprey (*Petromyzon marinus*; Chang 2013). Much of the research in these fish has focused on olfactory receptor genes, which encode for receptors in the olfactory epithelia. In teleosts, which lack a vomeronasal organ, these genes can be classified into four families: trace amine-associated receptors, vomeronasal family 1-like receptors, vomeronasal family 2-like receptors, and main olfactory receptors (Johnstone *et al*. 2012). These genes are highly conserved across various species (Morinishi *et al*. 2007; Johnson and Banks 2011), signifying their importance to the life history of salmonids. Also, the expression of olfactory genes is dynamic, changing over time and across life stages (Dukes *et al*. 2004; Johnstone *et al*. 2011), with an increase in expression, for example, during the PST (Dukes *et al*. 2004; Yamamoto *et al*. 2010).

2.5 Gaps in olfaction and spawning migration research

Based on my review of the research to date, I identified several research gaps that warrant further exploration.

2.5.1 *Species- and population-level differences*

Salmonids vary in their homing abilities across species, and this may be related to evolutionary differences. Ueda (2011), for example, suggests that species-level differences in homestream fidelity among Pacific salmon (*Oncorhynchus* spp.) may reflect their evolutionary history. Pink salmon (*O. gorbuscha*) exhibit the lowest level of homestream fidelity (Heard 1991), which may enable rapid colonization of new habitat (Pess *et al*. 2012). There is evidence that pink salmon are the most derived species of Pacific salmon (Murata *et al*. 1996), and Ueda (2011) suggests they may have evolved a less precise form of homing that has allowed them to become the most widely distributed species in their genus.

Ecological and life-history factors may also influence homestream fidelity. Extended freshwater experience might increase fidelity, as evidenced by a paired-release study by Westley, Quinn and Dittman (2013) that found greater homing success in stream-type Chinook salmon (*O. tshawytscha*) than ocean-type Chinook. Quinn (1984) hypothesized that fidelity is also higher in stable streams, because there is less risk of mortality due to a natural disaster. He also hypothesized that variation in age at maturity could reduce a population's susceptibility to a natural disaster, and therefore also increase homestream fidelity. This latter factor might suggest that, although both species migrate to the ocean within their first year, chum salmon (*O. keta*) have greater homestream fidelity than pink salmon, since chum salmon vary in their age at maturity, whereas pink salmon do not. Also, the proximity of suitable habitat in relation to the natal site may influence fidelity, as the frequency of straying decreases with increasing distance from the homestream (Keefer and Caudill 2014).

Currently, there are few data on homestream fidelity of salmonids, as noted by Keefer and Caudill (2014) in their review on this topic. As a result, estimates of homestream fidelity

among *Oncorhynchus* spp. are coarse, and the hypotheses proposed by Quinn (1984) remain largely untested. For example, the data currently available show similar homestream fidelity of pink and chum salmon (Keefer and Caudill 2014), contrary to what we might expect. There are several factors that restrict our ability to measure homestream fidelity (Keefer and Caudill 2014), such as the near-impossible task of monitoring all suitable habitat connected to the natal site, and quantitative estimates of fidelity may remain coarse for some time. Research that compares the responses (e.g. behavioural or physiological sensitivity) of different populations or species to migratory cues, however, could help determine the effects of the ecological factors on migratory behaviour, from which the relative degrees of homestream fidelity could be inferred.

2.5.2 *Effects of physiological and environmental factors on olfaction*

There are various physiological and environmental factors that may affect the olfactory ability of migrating adults, and therefore their ability to locate spawning grounds, but there is little information currently available. Cortisol, for example, increases dramatically with maturation level during the spawning migration (Schmidt and Idler 1962; Fagerlund 1967) although its effects on olfaction are unknown. Carruth, Jones and Norris (2002) suggest that increased cortisol levels might improve the fish's ability to recall imprinted odours, just as chronic stress has been linked to increased long-term memory retention in mammals.

Thermal elevation in migratory rivers could also affect olfaction. Recent studies on Atlantic salmon (*Salmo salar*) have found that rates of straying increase with warmer temperatures (Valiente, Beall and Garcia-Vazquez 2010; Horreo *et al*. 2011). Keefer *et al*. (2008*a*) also observed unusually indirect homing behaviours amongst Chinook salmon (*Oncorhynchus tshawytscha*) that were correlated with increased water temperatures. It is possible that migrants depart from their normal migratory route to limit the physiological costs

associated with warm migration temperatures (Martins *et al*. 2012). A potential effect of temperature on the olfactory system has not yet been distinguished from the possibility of thermoregulatory behaviours.

In addition to temperature and stress, CO_2 concentrations and pH levels may affect olfaction during the spawning migration. The olfactory response of salmonids is reduced at pH levels below 6.5 (Hara 1976; Moore 1994), and the behavioural response of juvenile Atlantic salmon (*Salmo salar*) to chemical alarm cues is temporarily reduced in slightly acidic streams (pH ~6) (Leduc *et al*. 2010). The effects of low pH do not appear to be permanent, although few studies have tested this. More subtle decreases in pH (or elevation in CO_2) – in the range of projected ocean acidification – impaired the ability of larval reef fish to select suitable settlement sites through olfactory cues (Munday *et al*. 2014). The lasting effects of modest reductions in pH (that reflect projected ocean acidification) on the behavioural response to migratory cues of salmonids, lamprey, or other anadromous fish have not been tested. Ongoing ocean acidification and increases in oceanic CO_2 could cause olfactory impairment that, even if not permanent, could continue into part of the freshwater phase of the migration. Future studies that focus on the behavioural response of migrating adults following exposure to acidic or elevated CO_2 conditions will help determine whether future climate change might reduce navigation abilities during the spawning migration.

2.5.3 *Effects of toxicity on the spawning migration*

Exposure to environmentally realistic concentrations of toxins interferes with the olfactory system in fish (Tierney *et al*. 2010). However, there have been few studies that explore the effects of toxicity on the spawning migration of salmonids (Saunders and Sprague 1967; Scholz *et al*. 2000; Sandahl et al. 2006), and there is a similar lack of information for lamprey

(Tierney *et al*. 2010). From a conservation perspective, pollution is perhaps the most critically understudied environmental factor affecting olfaction. Many major river systems that sustain large populations of anadromous fish contain large amounts of pollutants. In the Columbia River, for example, there are significant amounts of toxins such as polychlorinated biphenol, dichlorodiphenyltrichloroethane (DDT), and mercury, some of which are on the rise (Environmental Protection Agency 2009). In highly polluted systems, which are also often home to declining fish populations, research that focuses on the impact of toxins on the olfactory system and behaviour of migrating fish will help define the severity of the threat that pollution poses.

2.5.4 *Olfactory navigation in non-salmonids*

Of the 248 suitable papers that I found, the majority are on Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus* spp.) (Fig 2.3). The family Salmonidae constitutes 88% of the papers, with the genus *Oncorhynchus* contributing 57%. Most of the non-salmonids studied are lamprey species, particularly sea lamprey (*Petromyzon marinus*). Being semelparous, the ability of most *Oncorhynchus* spp. to locate spawning grounds is under particularly strong selection pressure. Their high level of homing success suggests that these fish have evolved fine-tuned homing mechanisms that could make *Oncorhynchus* a model genus for olfactory navigation research. This does not, however, make them representative of all anadromous fish. Additionally, some of the species currently being overlooked are critically endangered, such as the Atlantic sturgeon (*Acipenser oxyrinchus*). Given the direct influence of spawning site detection on reproductive success, conservation efforts for endangered species will benefit from a greater understanding of their navigation abilities.

There is also a strong bias towards hatchery populations for studies of salmon homing. Hatchery populations were used in 67% of the 224 relevant papers I found. Furthermore, of the studies focusing on wild populations of salmonids, only 44% used wild-born fish, while the rest used hatchery- or laboratory-reared offspring of wild populations. Hatchery-reared fish are suspected to differ from wild fish behaviourally and physiologically, and studies that use hatchery fish, such as imprinting experiments, have suggested that their unnatural rearing environment may affect the interpretation of their findings (Dittman and Quinn 1996). With laboratory imprinting studies, for example, the introduction of novel water or odorants during developmentally relevant periods would more accurately reflect natural conditions, and may produce informative results. Also, most imprinting studies to date have focused on coho salmon (*Oncorhynchus kisutch*), which reside in freshwater for one or more years before migrating to the ocean. Research on species with different juvenile life histories, such as pink salmon (*O. gorbuscha*) or chum salmon (*O. tshawytscha*) that migrate directly to the ocean, may produce results that differ from those found with coho.

2.5.5 *Identity and temporal consistency of candidate migratory cues*

While several bile acids used by lampreys have been chemically identified, the entire suite of directional cues has not yet been determined. Furthermore, the majority of this research has focused on land-locked sea lamprey (*Petromyzon marinus*), but the chemical compounds have not been tested on many of the other lamprey species. In salmonids, meanwhile, migratory cues have not yet been conclusively identified. Recent studies on amino acids provide the first steps in identifying the chemicals that act as migratory cues (Ueda 2011). One aspect in particular that has been missing from salmon homing research is consideration of changes in the components or concentrations of chemicals over time. In order for chemicals to act as migratory

cues, their concentrations or compositions during the outmigration and during the spawning migration, which can occur many years later, presumably must remain similar. For chemicals derived from conspecifics, this is likely true. Chemicals that are derived from other organisms or organic material present in the natal water, however, may not remain consistent over time. Yamamoto, Shibata and Ueda (2013) measured the concentration of amino acids in natal water at four-year intervals – first as juvenile chum salmon (*Oncorhynchus keta*) were migrating to the ocean, and then during their return as adults. The composition of amino acids varied between these four-year intervals, with approximately half the amino acids changing significantly in their relative concentrations. Mature adults, however, were equally attracted to both types of artificial natal water (one containing the concentrations found during the outmigration and the other containing the concentrations found during the return migration). The attraction of mature adults to waters with differing compositions and/or concentrations of amino acids is not yet understood and should be explored further. Additional studies that take into account natural changes in relative concentrations of amino acids and other chemical groups over time will be critical to the identification of migratory chemical cues.

2.6 Hierarchical Navigation Hypothesis: A new explanation of natal homing in salmonids

Taken together, research to date on salmonids suggests there is a need for further studies of the relative importance of imprinted cues and conspecific cues. Natal homing in salmonids appears to involve an imprinting component (the Olfactory Imprinting Hypothesis), but there is also substantial evidence that suggests that migrating adults are attracted to conspecific cues (the Pheromone Hypothesis). While not mutually exclusive, the two hypotheses are often discussed as competing explanations of natal homing, with imprinting favoured over pheromones due to

(1) the absence of juveniles in natal water during the spawning migration for some species, and (2) the abundance of evidence of imprinting (Ueda 2011; Keefer and Caudill 2014).

Based on the research synthesized herein, the following points outline my current state of knowledge on natal homing in salmonids: (*i*) they are capable of imprinting to chemical compounds; (*ii*) these chemicals are presumably consistent in their concentration and/or composition over time, at least to the extent that adults can recognize water they imprinted on as juveniles; (*iii*) it is exceedingly unlikely that the homestream odours are detectable from the estuarine habitat, particularly for long-distance migrants; (*iv*) fish are capable of detecting conspecific cues, and there is evidence of population-level discrimination; and (*v*) other members of the same population (e.g. juveniles) are not always present in the homestream during the adult migration.

With these points in mind, I have developed a hypothesis, referred to here as the Hierarchical Navigation Hypothesis, which seeks to explain how salmonids locate spawning grounds. This hypothesis unifies the widely acknowledged olfactory imprinting and pheromone hypotheses, and incorporates suggestions first made by Quinn *et al*. (1983), Brannon and Quinn (1990) and Dittman *et al*. (2010). I propose that migrating adults rely on three tiers of directional cues in a hierarchical fashion (Fig 2.4). Migrants first search for imprinted chemicals, which act as the primary directional cue. In the absence of imprinted cues, returning adults search for conspecific cues (or pheromones), which act as a secondary directional cue. If conspecific cues are also absent, they rely on other environmental indicators (such as flow, temperature, and substrate) that influence migratory conditions or spawning habitat. Such information would lead stray migrants to suitable non-natal spawning sites.

The imprinted cues likely include a wide spectrum of odorants present in the natal tributary, including conspecific cues, which combine to form a unique chemical mixture. I hypothesize that components of this imprinted mixture do not elicit an attractive response when disassociated from the other chemicals, with the exception of conspecific cues. Conspecific cues may therefore be incorporated in the primary tier of imprinted chemicals, but could also act as a secondary directional cue, in which they elicit an attractive response independently (e.g. when migrants have strayed away from the imprinted mixture), and without the necessity of imprinting. It is possible that within this secondary tier exists two levels, the first being cues from their own population, which would lead migrants to natal water, and the second being cues from other populations, which would lead them to non-natal habitat.

This hypothesis may be applied to previous research. First, there is evidence that adult migrants will select imprinted cues over pheromones when exposed to both. For example, Brannon and Quinn (1990) demonstrated that adult coho salmon (*Oncorhynchus kisutch*) will migrate directly past conspecifics and continue upstream to water they have imprinted on. Similarly, when adult Arctic char (*Salvelinus alpinus*) were taken out of their natal river and held in a nearby tributary, none of the other migrants were attracted into the nearby tributary, despite migrating directly past its ouflow (Black and Dempson 1986). Furthermore, when given the choice between natal water and foreign water that contains juveniles of their own population, adult coho salmon show a significant preference for the natal water (Brannon, Whitman and Quinn 1984). Although the number of studies that have directly tested the relative preferences of imprinted and conspecific cues is limited, they all suggest that imprinted cues are more attractive, and there is currently no evidence that indicates a preference in the opposite direction.

In the absence of any imprinted cues, such as when migrants have strayed from their normal route, salmonids migrate towards their conspecifics. Nordeng (1971, 2009), who first proposed the Pheromone Hypothesis, demonstrated this in Arctic char (*Salvelinus alpinus*). Juveniles were reared at a satellite location, with no opportunity to imprint on natural streams, and adults subsequently returned to the waters containing juveniles from their own population. In another study, Quinn, Brannon and Dittman (1989) released two groups of coho salmon (*Oncorhynchus kisutch*) smolts roughly 40 km and 18 km downstream from their rearing sites. The majority of coho returned to a hatchery a few kilometres upstream from the release site, despite (*a*) being transported well after the PST (and therefore presumably after imprinting occurred), and (*b*) never having experienced the water at that hatchery. As the authors suggest, the imprinted cues of the rearing sites were potentially out of detection range from the downstream location (both rearing sites are heavily diluted in Lake Washington before reaching the release site), and transportation prevented any opportunity for sequential imprinting. As a result, the migrants were instead attracted to conspecifics at the hatchery. Furthermore, the migrants did not return to an equally proximate hatchery that contained very few adult coho salmon (less than 1% of the number at the other hatchery), which suggests the migrants were attracted to the strong concentration of conspecific cues, and not another hatchery-related cue. Dittman *et al*. (2010) conducted a study in which Chinook salmon (*O. tshawytscha*) smolts were transported and released before their outmigration. The majority of returning adults that strayed from their release site entered rivers that were home to wild populations of conspecifics, especially when they had strayed a large distance (>25 km). A similar result, in which the majority of strays enter rivers with populations of conspecifics, has been found in Atlantic salmon (*Salmo salar*; Jonsson, Jonsson and Hansen 2003). There is also evidence that stocking a

fishless river with Atlantic salmon attracts stray adults from other populations (White 1934, Solomon 1973).

Johannesson (1987) observed spawning migration behaviour of Atlantic salmon that further supports the notion that migrants will return primarily to imprinted cues and secondarily to pheromones. Smolts were released in a harbour, and they returned as adults to the release point. The adults remained in this area until a new group of smolts were placed in pens in an experimental pool that empties into the harbour, after which point the adults swam into the experimental pool. The experiment was repeated multiple times, and the same result – adults remaining in the harbour until smolts were added into the pool – was achieved each time. While the author noted that the findings appear to simultaneously support and contradict the pheromone hypothesis, they are more comprehensible when viewed in the context of hierarchical navigation: the adults first returned to imprinted cues (the harbour), then towards conspecifics (the experimental pool).

In addition to published research, there is anecdotal evidence that salmonids migrate towards conspecifics in the absence of imprinted cues. In the summer of 2012, as part of an unrelated experiment, I held 20 sockeye salmon (*Oncorhynchus nerka*), in Cayoosh Creek (British Columbia, Canada), a tributary of the Seton River, which itself is a tributary of the Fraser River (Fig 2.5). Cayoosh Creek does not contain any salmon populations and sockeye salmon are not transiently found there. The Seton River is part of the migratory route for Gates Creek sockeye, but not for other populations. Within 12 h of placing the sockeye salmon in Cayoosh Creek, more than 50 free-swimming sockeye were observed alongside and directly downstream from those being held. DNA samples were collected from four of the free-swimming fish, and they were all identified as stray sockeye, originating from two non-Gates-

Creek populations. Since sockeye salmon do not occur in Cayoosh Creek under normal circumstances, these stray salmon may have been attracted to the odours of co-migrating adults that had been placed there for experimental purposes. That each of the salmon were identified as strays further suggests that salmon might migrate towards conspecifics specifically in the absence of imprinted cues.

If neither conspecifics nor imprinted cues are present, other environmental characteristics may become directional cues indicating suitable non-natal spawning habitat. There are a variety of non-olfactory environmental properties that might act as directional cues to stray migrants, such as discharge, temperature, and oxygen levels (Keefer and Caudill 2014). Higher flows, for example, can be attractive: in a study on Chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand, there was a positive relationship between the number of strays in a given river and its discharge (Unwin and Quinn 1993). Migrating adult salmon utilize thermal refugia to conserve energy as they swim upstream (Mathes *et al*. 2010), and stray migrants may be attracted to waters closer to their optimal temperature. There are other environmental factors, such as substrate, cover, stream width, and gradient, that can affect spawning site selection and the spatial distribution of spawners (Cram *et al*. 2013). There is very little information on the attractiveness of these and other environmental characteristics to stray migrants, however, and their potential role as tertiary directional cues has not been tested. Research in this area is therefore needed before this portion of the Hierarchical Navigation Hypothesis can be supported or challenged.

Non-olfactory environmental characteristics might also be used as a supplement to primary or secondary cues. For example, adult migrants entering a main stem river may not always need to rely on a complex system of sequentially imprinted waypoints, but might rather

simply remain in the high discharge until they are within close enough proximity to detect their natal tributary.

From an evolutionary standpoint, this method of homing could be adaptive. For adults that have strayed from their normal migratory route, movement towards conspecifics would increase the probability of locating suitable non-natal spawning habitat, as well as potential mates, and therefore increase fitness. Such an attraction can also encourage genetic mixing between populations, which can help reduce inbreeding depression (Hendry *et al*. 2004). When neither imprinted nor conspecific cues are present, an attraction to environmental characteristics that are indicative of high-quality spawning habitat would maximize reproductive success and offspring survival in unoccupied waters. Similarly, when imprinted and/or conspecific cues are present at peak concentrations, such as on spawning grounds, an attraction to favourable environmental characteristics will optimize reproductive success and offspring survival within that area. Furthermore, an attraction to uninhabited but environmentally suitable spawning grounds increases the probability of successful colonization, which could in part explain instances of rapid colonization such as in pink salmon (*O. gorbuscha*; Pess *et al*. 2012).

Future research can help test the Hierarchical Navigation Hypothesis further. As seen in Table 2.4, numerous studies have documented the olfactory sensitivity of salmonids to specific chemical compounds, any of which may guide migrating adults. In order to provide evidence for or against this or other natal homing hypotheses, studies will need to simultaneously incorporate various chemicals to determine their relative importance as migratory cues. Furthermore, while the commonly used electrophysiological approaches provide an estimate of basic sensitivity to individual chemicals, they do not demonstrate a behavioural response. To determine whether a chemical is acting as a migratory cue, the behaviour of the fish in response to the chemical must

therefore be analysed. As such, research that integrates different chemicals and assesses direct behavioural responses to these chemicals will test the homing hypotheses most effectively.

2.7 Conclusions

(1) Several decades of research have provided strong evidence that many anadromous fish rely primarily on olfaction to locate their spawning grounds during the freshwater phase of the spawning migration. In this review I focused on two types of spawning migrations, 'natal homing' (migration to natal water to spawn) and 'non-specific homing' (migration to the general geographic area of birth, but not necessarily natal water), and olfaction is important for both. Salmonids and lamprey have been the most extensively studied groups of species for each of these two migration types, respectively, and have provided the basis for our understanding of how olfaction directs adult migrants.

(2) The behavioural and physiological responses of lamprey and salmonids to various odours have been studied extensively. In general, these fish respond to amino acids, bile acids, salts, metals, prostaglandins, and other hormones, and the responses occur at concentrations that are relevant to natural freshwater conditions. Additionally, the ability of natal homing species to imprint on odours as juveniles has been conclusively demonstrated. Juveniles that are exposed to a unique odorant, particularly during the parr–smolt transformation stage, display an attractive response to the odorant as migrating adults. The recent introduction of molecular and genetic analyses in olfactory research has provided new directions for exploration and our first glimpse of the molecular processes that drive the olfactory system in anadromous fish.

(3) My synthesis also highlights limitations in past research and I have identified knowledge gaps that warrant investigation. These gaps include the potential effects of various environmental factors on the olfactory response of migrating adults. The effects of some factors, such as toxins

and stressors (e.g. high temperatures, acidity), may have significant conservation and management implications, as the ability to locate spawning grounds successfully is critical to reproductive success. In addition, variation in the olfactory responses of different species or populations of salmonids has rarely been studied directly. Combining such information with variation in life histories, such as the juvenile rearing stages, will help elucidate the role of olfaction during the spawning migration, and could also have implications with respect to the evolutionary history of olfactory sensitivity to spawning grounds. My review also highlights biases in focal species within this field of research. The majority of research has focused on salmonids, particularly those from hatchery populations, while little attention has been given to other species of anadromous fish, some of which are threatened or endangered.

(4) There are two hypotheses that currently dominate the field of natal homing in salmonids: the Olfactory Imprinting Hypothesis (or its modified form – the Sequential Imprinting Hypothesis) and the Pheromone Hypothesis. While there has been evidence to support each of these, they are often introduced as competing explanations for natal homing (e.g. Ueda 2011; Keefer and Caudill 2014). I have proposed an alternative explanation, the Hierarchical Navigation Hypothesis, which unifies and expands upon the two predominant hypotheses. The hypothesis posits that migrating adults respond to different cues in a hierarchical fashion. Imprinted odours, which are unique in composition and may be derived from many different components of the natal tributary, provide the primary directional cue. Conspecific odours provide a secondary directional cue, and may be particularly attractive to strays that are not able to detect imprinted cues. Non-olfactory environmental indicators, such as substrate or discharge, may act as tertiary cues and assist strays in finding suitable non-natal spawning habitat.

(5) While past studies have incidentally lent evidence that supports the Hierarchical Navigation Hypothesis, I have provided examples of future directions for research that can further test this hypothesis. This includes a movement away from the odorant-specific experiments that have been favoured in previous research, towards an integrated approach that assesses relative responses to different odours. Furthermore, while many studies have examined the electrophysiological responses of salmon, and more recently the molecular or genetic responses, these approaches only highlight responsiveness or sensitivity to chemical cues. In order to determine behavioural responses, some form of behavioural assay is necessary.

Fig 2.1 Dorsal view of the olfactory system in a salmonid. The peripheral olfactory organs are covered with the olfactory epithelia and are directly exposed to the surrounding water (Hara, 1992). Odorants bind to G protein-coupled receptors in the olfactory epithelium, which leads to the formation of a secondary messenger and the opening and closing of various ion channels. As ions pass through the channels, there is a change in the membrane potential that triggers an action potential. The action potential travels along the axons of the olfactory sensory neurons, which form synaptic contacts with mitral cells in the olfactory bulb. The mitral cells project to the brain, where the information is processed.

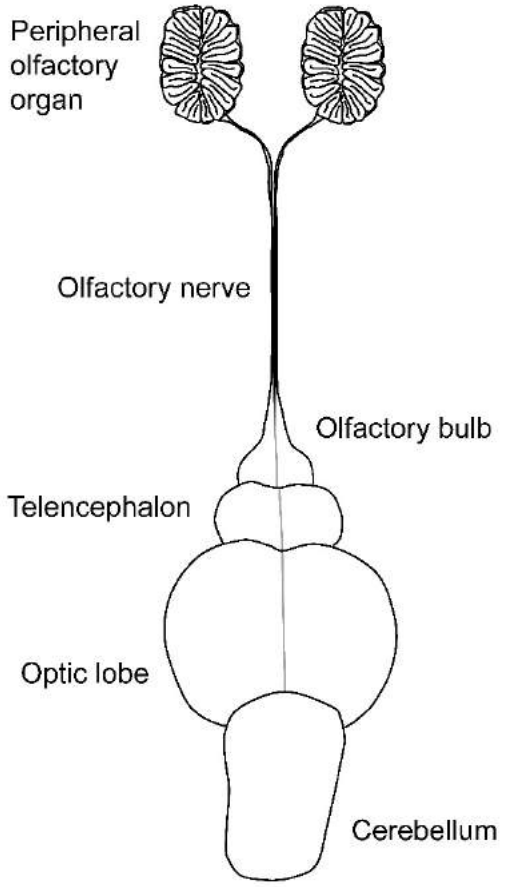


Fig 2.2 Interactions between different classes of chemicals in the olfactory system of anadromous fish. Solid lines indicate suppression, and therefore shared transduction mechanisms and/or olfactory receptors (signifying the ability to discriminate). *ECG, etiocholam-3α-ol-17-one glucuronide; DHP, 17α,20β-dihydroxy-4-pregnene-3-one. 1, Bodznick (1978*b*); 2, Brown and Hara (1981); 3, Cagan and Ziegler (1978); 4, Doving et al. (1980); 5, Fine and Sorensen (2008); 6, Giaquinto and Hara (2008); 7, Hara and Zhang (1998); 8, Laberge and Hara (2003); 9, Laberge and Hara (2004); 10, Li and Sorensen (1997); 11, Lo et al. (1991); 12, Lo et al. (1994); 13, MacLeod (1976); 14, Rehnberg and Schreck (1986); 15, Shoji et al. (1996); 16, Siefkes and Li (2004); 17, Sveinsson and Hara (1990*b*); 18, Zhang and Hara (2009).

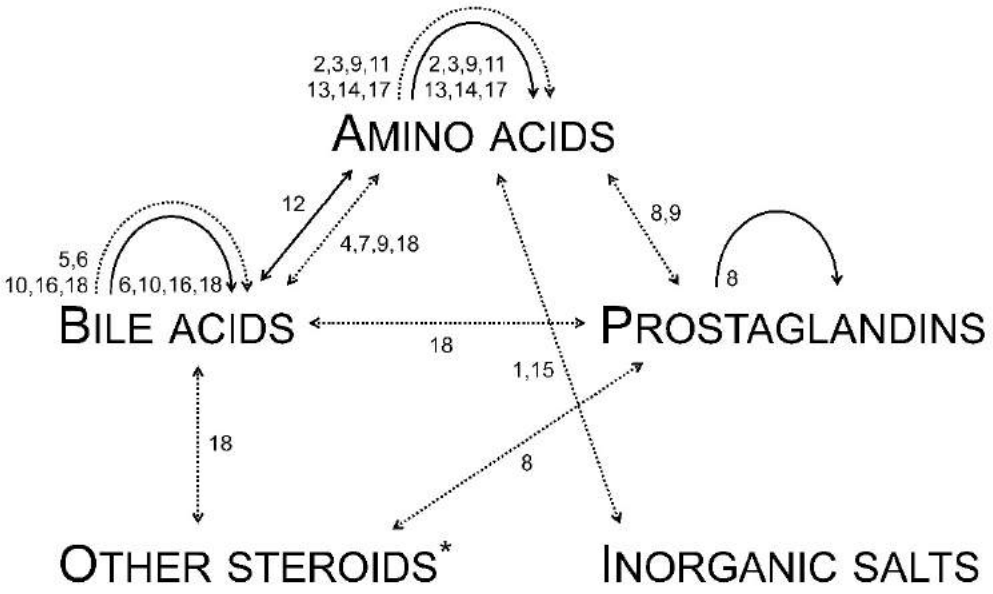


Fig 2.3 Distribution of the number of peer-reviewed publications relating olfaction to the spawning migration in different species of anadromous fish.

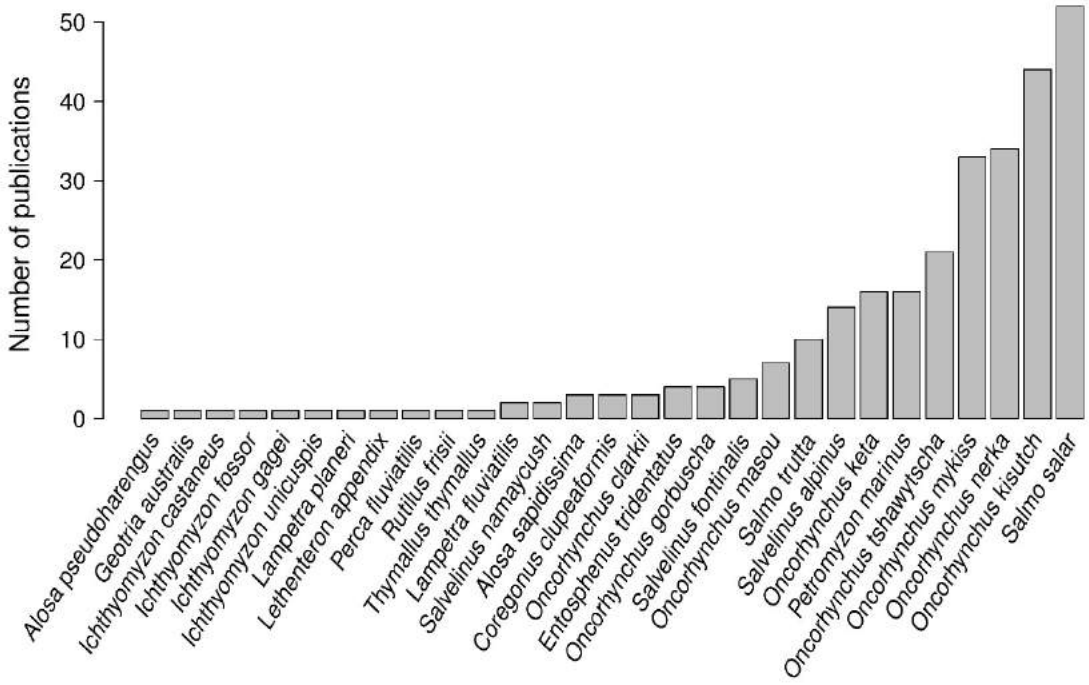


Fig 2.4 The Hierarchical Navigation Hypothesis: a new explanation of the navigation process in salmonids migrating upstream. Migrants primarily rely on imprinted olfactory cues (likely to include a wide spectrum of odorants present in the natal tributary, which combine to form a unique chemical mixture), and secondarily on conspecific cues. In the absence of these, they move towards other environmental indicators, such as optimal temperature or flow.

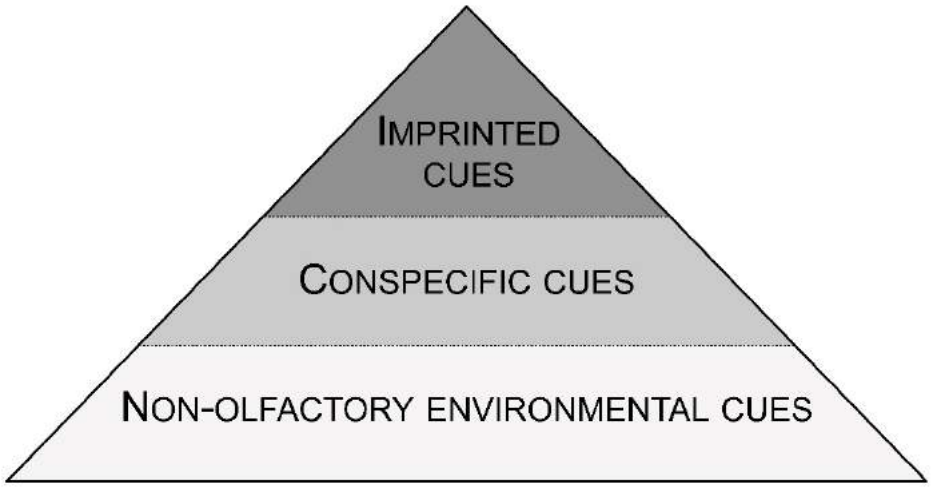


Fig 2.5 Map of the area in the Fraser River system of British Columbia, Canada (inset) where anecdotal evidence suggests that migrating salmon are attracted to conspecifics in the absence of imprinted cues. Sockeye salmon were captured in the Seton River (square symbol) during their upstream migration to Gates Creek, and held overnight in Cayoosh Creek (triangle symbol), a non-salmon-bearing stream.

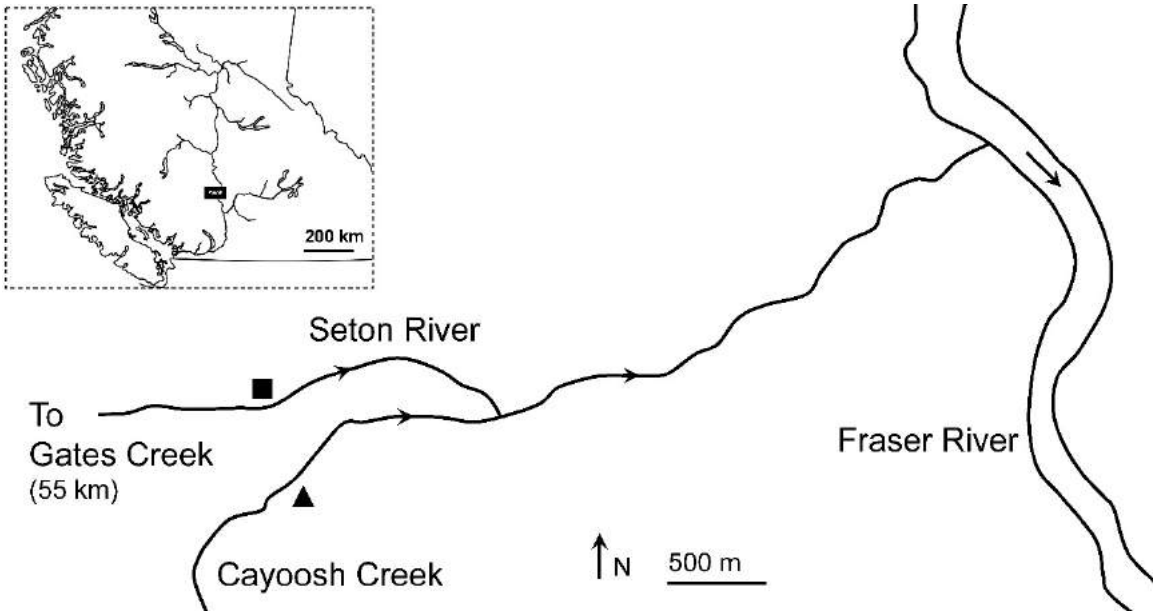


Table 2.1 Importance of sensory systems to successful migration to spawning grounds.

Reference	Species	Olfaction important?	Vision important?	Notes
Bertmar and Toft (1969)	*S. salar*	Y		
Brett and Groot (1963)	*O. clarkii*		N	Blind fish took longer to locate spawning grounds
Dodson and Legget (1974)	*A. sapidissima*	Y	Inconclusive	Site of release affected blind fishes ability to locate spawning grounds
Groves et al. (1968)	*O. tshawytscha*	Y	N	
Hansen et al. (1987)	*S. salar*	Inconclusive		Control fish did not successfully home either
Hiyama et al. (1966)	*O. keta*	Y	N	Sample sizes too small to determine statistical significance
Jahn (1969)	*O clarkii*	Y	Inconclusive	
Lorz and Northcote (1965)	*O. nerka*	Inconclusive	Inconclusive	No control for effect of sensory impairment
McCleave and Horrall (1970)	*O clarkii*	N	N	Blind fish took longer to locate spawning grounds
McCleave (1967)	*O clarkii*	N	N	
Rehnberg et al. (1985*b*)	*O. kisutch*	Y		Tested avoidance response to 10^{-6} M L-serine, did not avoid when anosmic
Ueda et al. (1998)	*O. nerka*		Inconclusive	Effect not significant, but blind fish took longer to locate spawning grounds. Impaired geomagnetic sensing had no effect
Vrieze et al. (2010, 2011)	*P. marinus*	Y		
Wisby and Hasler (1954)	*O. kisutch*	Y		

Table 2.2 Responses to natal water in anadromous fish.

Reference	Species	Life-history stage	Reference water/chemical	Method	Response (relative to reference)	Additional results/notes
Bandoh et al. (2011)	*O. nerka*	Maturing adult	L-serine	MRI	Positive	Strongest responses (blood perfusion) in lateral area of dorsal telencephalon
Bodznick (1975)	*O. nerka*	Migrating and spawning adult	Other lakes and spawning grounds, ground water	E	Neutral	Did not respond more strongly to natal water than other spawning grounds. En-route migrants responded more strongly to natal water and other spawning grounds than completed migrants
Bodznick (1978*b*)	*O. nerka*	Parr	Other rivers	CR	Neutral	Responses to lake water similar to responses to $CaCl_2$
Fagerlund et al. (1963)	*O. nerka*	Migrating adult	Connected rivers, other spawning grounds	BA	Positive	Response to lake outlet stronger than response to inlets. Decreased response to volatile fraction, indicating volatile and non-volatile fractions both important to recognition
Fretwell (1989)	*O. nerka*	Migrating adult	Diluted natal water	BC	Positive	Preference for pure natal water over natal water diluted to 90%
Hara et al. (1965)	*O. kisutch*, *O. tshawytscha*	Spawning adult	Other lakes, hatchery	E	Positive	Response to natal water maintained when diluted to as low as 10%
Idler et al. (1961)	*O. nerka*	Migrating adult	Other lakes and rivers	BA	Positive	Increased response to inlets of natal lake that have spawning populations, but not to inlets without spawning populations or to other spawning grounds. Response to volatile fraction, but not to non-volatile
Kaji et al. (1975)	*O. keta*	Migrating adult	Other rivers	E	Positive	Different (but not necessarily "stronger") response to natal water than other waters

Reference	Species	Life-history stage	Reference water/chemical	Method	Response (relative to reference)	Additional results/notes
Keefe and Winn (1991)	*S. fontinalis*	Migrating adult, parr	Other rivers	BC	Neutral	No preference for natal water over other rivers
			Ground water		Positive	Plugged nares of juveniles and made same choices when re-tested, suggesting gustation may be involved
McBride et al. (1964)	*O. nerka*	Smolt	Other nursery lake	BA	Positive	Smolts conditioned to respond more strongly to either own nursery lake or other lake
Oshima et al. (1969*b*)	*O. tshawytscha*	Spawning adult	Other rivers	E	Positive/ neutral	Response greater than two other waters, but similar to a third water
	O. kisutch	Spawning adult	Other rivers	E	Positive/ Neutral	Increased response to natal water for one population tested, but not for other population
	O. tshawytscha	Smolt	Other rivers	E	Positive	Were held in artificial salt water for two weeks prior to tests
	O. tshawytscha	Smolt	Other river	E	Neutral	Held in aquaria, and after three days developed similar electrophysiological response to holding water as natal water
Oshima et al. (1969*a*)	*O. tshawytscha*	Spawning adult (jack)	Other hatcheries	E	Inhibited	Injected with memory-blocking agents (antimetabolites), which temporarily inhibited natal water discrimination (4–7 h)
Sato et al. (2000)	*O. nerka*	Spawning adult	Other lakes and rivers	E	Positive/ neutral	Stronger response to natal water than all other waters except for water that fish had been held in for one week prior to tests
	O. masou	Spawning adult	Connected rivers and lake, other hatchery	E	Positive	
Sutterlin and Gray (1973)	*S. salar*	Spawning adult	Downstream river	BC	Positive	Natal water diluted to 0.1%, still attracted to it. Adding copper to natal water generated an avoidance response

Reference	Species	Life-history stage	Reference water/chemical	Method	Response (relative to reference)	Additional results/notes
Thunberg (1971)	*A. pseudo-harengus*	Spawning adult	Other lakes	BC	Positive	
Ueda et al. (1967)	*O. kisutch*, *O. tshawytscha*	Spawning adult	Other spawning grounds and rivers	E	Positive	Response to natal water maintained when diluted as low as 10%
Ueda (1985)	*O. keta*	Migrating adult	Other rivers	E	Positive	Lower frequency responses only when exposed to natal water. Response to nonvolatile fraction, but not to volatile

Table 2.3. Response to conspecific cues (C.C.) and ability to discriminate populations in anadromous fish

Reference	Species	Life-history stage	Attractants	Method	Response/ attraction to C.C.?*	Pop. Discr?	Notes
Bjerselius et al. (2000)	*P. marinus*	Migrating adult	Larva	BC	Y		
			Bile acids (larva)		Y		
		Spawning adult	Larva		N		
Black and Dempson (1986)	*S. alpinus*	Migrating adult	Adult and parr	F	N		Returned to home stream rather than tributary where fish from same population held
Brannon and Quinn (1990)	*O. kisutch*	Migrating adult	Adult and parr	F	N		Returned to home stream rather than hatchery where fish from same population held
Courtenay et al. (1997)	*O. kisutch*	Fry	Fry	BC	Y	Y/N	Prefer own population, but prefer other population if concentration higher
			Faeces (fry)		Y	Y/N	Some showed preference for own population, others did
Courtenay et al. (2001)	*O. kisutch*	Fry	Fry	BC	Y	Y	Preferred siblings over non-siblings
Dizon et al. (1973)	*O. kisutch*	Migrating adult	Adult	E	Y		Stronger response to home stream water with conspecific cues than without
Døving et al. (1973)	*S. alpinus*	Adult^	Adult	E	Y	N	
			Urine, skin mucus (adult)		Y		Strongest response to skin mucus
Døving et al. (1974)	*S. alpinus*	Migrating adult	Fry	CR	Y	Y	Response to different populations different
			Mucus (fry)		Y		
Fine and Sorensen (2010)	*P. marinus*	Migrating adult	Larva	BC	Y		
Fine et al.	*P. marinus*	Migrating	Larva	BC	Y		

Reference	Species	Life-history stage	Attractants	Method	Response/ attraction to C.C.?*	Pop. Discr?	Notes
(2004)		adult	Larva (*L. appendix*, *I. fossor*)		Y		
	I. unicuspis	Migrating adult	Larva (*P. marinus*)		Y		
Fisknes and Døving (1982)	*S. salar*	Subadult/ adult^	Urine, intestine, mucus	E	Y	N	Strongest response to intestinal content
Gaudron and Lucas (2006)	*L. fluviatilis*	Migrating adult	Larva	BC	Y		
Groot et al. (1986)	*O. nerka*	Migrating adult	Adult	BC	Y/N	Y/N	One population attracted to conspecifics and preferred its own population over another, other population showed no attraction or discrimination
			Adult and Smolt	E	Y	Y/N	
Hara and Macdonald (1976)	*O. mykiss*	Subadult/ adult^	Mucus	E	Y		
			Mucus (*C. clupeaformis*, *C. auratus*)		Y		
Keefe and Winn (1991)	*S. fontinalis*	Fry, migrating adult	Fry	BC	Y	N	Preferences persisted in anosmic fish, suggesting non-olfactory source
McBride et al. (1964)	*O. nerka*	Smolt	Smolt	BA	Y	Y	
Nordeng and Bratland (2006)	*S. alpinus, S. trutta*	Migrating adult	Fry	F	Y	Y	Returned to home stream after transport as juveniles
Nordeng (1971)	*S. alpinus*	Migrating adult	Fry	F	Y	Y	Returned to home stream after transport as juveniles
Nordeng (2009)	*S. alpinus*	Migrating adult	Fry	F	Y	Y	Returned to home stream after transport as juveniles
Quinn and Busack (1985)	*O. kisutch*	Fry	Fry	BC	Y	Y	Prefer siblings over unfamiliar non-siblings
Quinn and Hara (1986)	*O. kisutch*	Fry	Fry	BC	Y	Y	Preference for siblings, but discrimination appears to be learned, not innate

Reference	Species	Life-history stage	Attractants	Method	Response/ attraction to C.C.?*	Pop. Discr?	Notes
				E	Y		
Quinn and Tolson (1986)	*O. kisutch*	Fry	Fry	BC	Y	Y	
		Migrating jacks			Y	Y	
		Spawning adult				N	
Quinn et al. (1983)	*O. kisutch*	Migrating adult	Fry	BC	Y	N	
Selset and Døving (1980)	*S. alpinus*	Adult^	Smolt	BC	Y		
			Intestine, mucus		Y	Y	Response to intestinal content and bile, but not skin mucus
Siefkes and Li (2004)	*P. marinus*	Migrating/spawning adult female	Larva and spawning adult male	E	Y		
Sorensen et al. (2005)	*P. marinus*	Migrating adult	Bile acids (larvae)	BC	Y		
Stabell et al. (1982)	*S. salar*		Intestine mucus, (parr)	M		Y	Chemical composition of skin mucus and intestinal content different between populations
Sveinsson and Hara (2000)	*S. alpinus*	Adult		E	Y		
Teeter (1980)	*P. marinus*	Migrating adult	Larva	BC	Y		
Vrieze and Sorensen (2001)	*P. marinus*	Migrating adult	Larva	BC	Y		
		Subadult			N		
Wagner et al. (2009)	*P. marinus*	Migrating adult	Larva	F	Y		
Winberg and Olsén (1992)	*S. alpinus*	Parr	Fry	BC	Y	Y	Prefer siblings over unfamiliar non-siblings
Yun et al.	*E.*	Migrating	Larva	BC	Y		

Reference	Species	Life-history stage	Attractants	Method	Response/ attraction to C.C.?*	Pop. Discr?	Notes
(2011)	*tridentatus*	adult	Bile acids (larva)	E	Y		
Zhang et al. (2001)	*S. namaycush*	Subadult	Bile acids (subadult)	E	Y		

Species – *Carassius auratus*, goldfish; *Coregonus clupeaformis*, lake whitefish; *Entosphenus tridentatus*, Pacific lamprey; *Ichthyomyzon fossor*, northern brook lamprey; *I. unicuspis*, silver lamprey; *Lampetra appendix*, American brook lamprey; *L. fluviatilis*, river lamprey; *Oncorhynchus kisutch*, coho salmon; *O. mykiss*, rainbow trout; *O. nerka*, sockeye salmon; *Petromyzon marinus*, sea lamprey; *Salmo salar*, Atlantic salmon; *S. trutta*, brown trout; *Salvelinus alpinus*, Arctic char; *S. fontinalis*, brook trout; *S. namaycush*, lake trout.

Methods – BA, behavioural assays; BC, behavioural choice tests; CR, cellular response in olfactory epithelia/bulb; E, electrophysiology; F, field experiments; M, molecular analysis.

^ Specific life stage not known.

* Can only determine response, and not attraction, when using methods CR, E and M.

Y, yes; N, no.

Table 2.4 Sensitivity and attraction to odorants in anadromous fish

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
Belghaug and Døving (1977)	*S. alpinus*	Subadult/adult^	L-asparagine		2.5×10^{-8}	E	
			L-methionine		6.8×10^{-8}		
			L-glutamine		6.8×10^{-8}		
			L-alanine		3.2×10^{-7}		
			L-homoserine		6.3×10^{-7}		
			L-serine		9.6×10^{-7}		
			L-glutamic acid		1.0×10^{-6}		
			L-leucine		2.10^{-6}		
			L-histidine		6.8×10^{-6}		
			L-lysine		6.8×10^{-6}		
			4-hydroxy-proline		$> 10^{-4}$		
			Proline		$> 10^{-4}$		
			β-alanine		$> 10^{-4}$		
			Carboxylic acids		$> 10^{-4}$		
			Sugars		$> 10^{-4}$		
			Taurine		$> 10^{-4}$		
			Anserine		6.3×10^{-6}		
			Carnosine		$> 10^{-5}$		
			Glutathione		1.5×10^{-7}		
			Phenethyl alcohol		$> 10^{-4}$		
			Indole		$> 10^{-4}$		
Bjerselius et al. (2000)	*P. marinus*	Migrating adult	Petromyzonal sulfate and cholic acid	5×10^{-6}		BA	Positive
			Petromyzonal sulfate and allocholic acid	5×10^{-10}		BA	Positive
				5×10^{-10}		BC	Neutral
Bodznick (1978*b*)	*O. nerka*	Parr	Calcium		5×10^{-6}	E	
			Magnesium		10^{-4}		
			Sodium		$> 10^{-3}$		
			Potassium		$> 10^{-2}$		
			Calcium		10^{-5} to 10^{-6}	CR	
			Sodium		$> 10^{-4}$		

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
		Fry	Calcium	Additional 3.3×10^{-4}		BC	Neutral
			Calcium	Equivalent to rearing water			Neutral
Døving et al. (1980)	*S. alpinus, T. thymallus* (results not distinguished between species)	Subadult/adult^	Cholic acid		8.0×10^{-8}	E	
			Taurocholic acid		2.0×10^{-8}		
			Taurodeoxycholic acid		6.3×10^{-8}		
			Taurochenodeoxy-		4.0×10^{-8}		
			Taurolithocholic acid		6.3×10^{-9}		
			Sulfotaurolithocholic		1.0×10^{-8}		
			Methionine		1.3×10^{-6}		
			Taurine		1.0×10^{-5}		
Essington and Sorensen (1996)	*S. fontinalis*	Subadult	Testosterone	10^{-7}		E	Positive
			Prostaglandin $F_{2\alpha}$	10^{-7}			Positive
			15-keto-$PGF_{2\alpha}$	10^{-7}			Positive
			$PGF_{1\alpha}$	10^{-7}			Positive
			13,14 dihydro-15-keto $PGF_{2\alpha}$	10^{-7}			Positive
	S. trutta	Subadult	$PGF_{2\alpha}$	10^{-7}			Positive
			15-keto-$PGF_{2\alpha}$	10^{-7}			Positive
			$PGF_{1\alpha}$	10^{-7}			Positive
			ECG	10^{-7}			Positive
	S. fontinalis	Subadult	ECG	10^{-7}			Neutral
	S. trutta	Subadult	13,14 dihydro-15-keto $PGF_{2\alpha}$	10^{-7}			Neutral
	S. fontinalis, S. trutta	Subadult	Testosterone	10^{-7}			Neutral
			Testosterone-sulphate	10^{-7}			Neutral
			Oestradiol	10^{-7}			Neutral
			DHP	10^{-7}			Neutral
			17,20β-P sulphate	10^{-7}			Neutral
			17,20β-P-glucuronide	10^{-7}			Neutral
			Prostaglandin E_2	10^{-7}			Neutral

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
Evans and Hara (1985)	*O. mykiss*	Subadult/adult^	L-serine		10^{-9} to 10^{-10}	PS	
			L-leucine		10^{-7} to 10^{-8}		
Fine and Sorensen (2008)	*P. marinus*	Migrating adult	Petromyzonamine disulfate		10^{-13}	E	
				10^{-13}, 10^{-14}	10^{-13}	BC	Positive
			Petromyzosterol disulfate		10^{-13}	E	
				10^{-11}, 10^{-12}	10^{-11}	BC	Positive
			Petromyzonol sulfate		10^{-12}	E	
				10^{-11}, 10^{-12}	10^{-11}	BC	Positive
Fine and Sorensen (2010)	*P. marinus*	Larvae	Petromyzonamine			MS	Release 16 ng larva^{-1} h^{-1}; half-life 3 days
			Petromyzosterol				Release 24 ng larva^{-1} h^{-1}; half-life 3 days
			Petromyzonol sulfate				Release 10 ng larva^{-1} h^{-1}; half-life 3 days
Fine et al. (2004)	*P. marinus*	Larvae	Petromyzonol sulfate				Release 1.8 ng (g larva)$^{-1}$ h^{-1}
			Allocholic acid				Release 0.7 ng (g larva)$^{-1}$ h^{-1}
	L. appendix	Larvae	Petromyzonol sulfate				Release 2.3 ng (g larva)$^{-1}$ h^{-1}
			Allocholic acid				Release 0.5 ng (g larva)$^{-1}$ h^{-1}
	I. fossor	Larvae	Petromyzonol sulfate				Release 1.9 ng (g larva)$^{-1}$ h^{-1}
			Allocholic acid				Release 0.8 ng (g larva)$^{-1}$ h^{-1}
Fisknes and Døving (1982)	*S. salar*	Subadult/adult^	L-serine		4×10^{-5}	E	
			L-glutamine		8×10^{-6}		
Giaquinto and Hara (2008)	*O. mykiss*	Adult^^	Deoxycholic acid		10^{-10}	E	
			Chenodeoxycholic acid		10^{-11}		
			Cholic acid		10^{-10}		
			Taurochenodeoxy-cholic acid		10^{-11}		
			Taurocholic acid		10^{-10}		
			Taurolithocholic acid 3-sulphate		10^{-11}		
Hara (1972)	*O. kisutch*	Subadult	L-serine		10^{-6} to 10^{-7}	E	
			L-methionine		10^{-6} to 10^{-7}		
			L-alanine		10^{-6} to 10^{-7}		
	O. nerka	Subadult	L-serine		$> 10^{-6}$		
			L-methionine		$> 10^{-6}$		
			L-alanine		$> 10^{-6}$		

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
Hara (1973)	*O. mykiss*	Subadult/adult^	L-serine		10^{-7} to 10^{-8}	E	
Hara (1977)	*O. mykiss*	Subadult/adult^	L-alanine, L-serine, L-cysteine, L-glutamine	10^{-4} to 10^{-5}		E	Decreased response when amino group acetylated
			Glycine, L-alanine, L-serine, L-cysteine, L-methionine, L-leucine, L-isoleucine, L-histidine, L-glutamic acid	10^{-4} to 10^{-5}			Decreased response when alpha-carboxyl group esterified
			L-alanine, L-serine, L-leucine	10^{-4} to 10^{-5}			Decreased response when alpha hydrogen replaced by a methyl group
			Glycine, L-alanine, L-aminobutyric acid, L-norvaline, L-norleucine	10^{-4} to 10^{-5}			Greatest response in amino acids with three carbon atoms in the chain
Hara and Zhang (1998)	*O. mykiss, S. salar, S. trutta, S. alpinus, S. namaycush, C. clupeaformis*	Adult^^	L-cysteine		10^{-9} to 10^{-10}	E	
			Taurocholic acid		10^{-9} to 10^{-10}		
	S. salar, S. trutta, S. alpinus, S. namaycush, C. clupeaformis		Prostaglandin $F_{2\alpha}$		10^{-11}		
			15-keto-$PGF_{2\alpha}$		10^{-9}		
	O. mykiss, S. trutta, S. salar		ECG		10^{-10}		
Hara et al. (1993)	*S. fontinalis*	Adult^^	L-cysteine		10^{-8}	E	
			L-serine		10^{-8}		
			L-arginine		10^{-8}		

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
			L-glutamate		10^{-7}		
	S. alpinus	Adult^^	L-cysteine		10^{-8}		
			L-serine		10^{-8}		
			L-arginine		10^{-8}		
	S. namaycush	Adult^^	L-cysteine		10^{-9}		
			L-serine		10^{-8}		
			L-arginine		10^{-8}		
Laberge and Hara (2003)	*S. trutta*	Adult^^	Prostaglandin $F_{2\alpha}$	10^{-8}		BA	Positive
			15-keto-$PGF_{2\alpha}$	10^{-8}			Neutral
			13,14-dihydro-$PGF_{2\alpha}$	10^{-8}			Positive
	C. clupeaformis	Adult^^	Prostaglandin $F_{2\alpha}$	10^{-8}			Positive
			15-keto-$PGF_{2\alpha}$	10^{-8}			Positive
			13,14-dihydro-$PGF_{2\alpha}$	10^{-8}			Neutral
	O. mykiss	Adult^^	Prostaglandin $F_{2\alpha}$	10^{-8}			Neutral
			15-keto-$PGF_{2\alpha}$	10^{-8}			Neutral
			13,14-dihydro-$PGF_{2\alpha}$	10^{-8}			Neutral
	S. trutta	Adult^^	Prostaglandin $F_{2\alpha}$		10^{-11}	E	
			13,14-dihydro-$PGF_{2\alpha}$		10^{-10}		
			Prostaglandin $F_{1\alpha}$		10^{-9}		
			15-keto-$PGF_{2\alpha}$		10^{-8}		
			15-keto-13,14-dihydro-		10^{-6}		
			15-keto-$PGF_{2\alpha}$		10^{-6}		
			ECG		10^{-8}		
	C. clupeaformis	Adult^^	Prostaglandin $F_{2\alpha}$		10^{-8}		
			13,14-dihydro-$PGF_{2\alpha}$		10^{-9}		
			Prostaglandin $F_{1\alpha}$		10^{-8}		
			15-keto-$PGF_{2\alpha}$		10^{-10}		
			15-keto-13,14-dihydro-		10^{-8}		
			15-keto-$PGF_{2\alpha}$		$> 10^{-7}$		
			ECG		10^{-8}		
	O. mykiss	Adult^^	Prostaglandin $F_{2\alpha}$		10^{-6}		
			ECG		10^{-7}		
			Other PGFs		$> 10^{-5}$		
Laberge and	*O. mykiss*	Subadult/adult^	L-cysteine		10^{-9}	E	
			L-serine		10^{-7}		

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
Hara (2004)	*S. trutta*	Subadult/adult^	L-arginine L-glutamic acid L-cysteine L-serine L-arginine L-glutamic acid L-cysteine, L-serine, L-arginine, taurocholic acid, $PGF_{2\alpha}$		10^{-7} 10^{-7} 10^{-8} 10^{-6} 10^{-6} 10^{-6}	E	Response to bile acids in mid-dorsal region of bulb; to amino acids in latero-posterior region
Li and Sorensen (1997)	*P. marinus*	Migrating adult	Allocholic acid Cholic acid Deoxycholic acid Petromyzonol Petromyzonol sulfate Lithocholic acid 3-sulfate Glycolithocholic acid Taurolithocholic acid Taurocholic acid Taurodeoxycholic acid		10^{-12} 10^{-9} 10^{-8} 10^{-8} 10^{-12} 10^{-12} 10^{-11} 10^{-12} 10^{-8} 10^{-8}	E	
Li, Sorensen and Gallaher (1995)		Migrating adult	Allocholic acid Petromyzonol sulfate Petromyzonol Taurolithocholic acid 3-sulphate		10^{-12} to 10^{-13} 10^{-12} to 10^{-13} 10^{-8} 10^{-12}	E	
Lo, Bradley and Rhoades (1991)	*S. salar*	Subadult/adult^	L-[^{3}H]alanine L-[^{3}H]serine	10^{-7} 10^{-7}		B	
Lo et al. (1994)	*S. salar*	Subadult/adult^	Taurocholic acid	10^{-6} to 10^{-9}		B	
Morin and Døving (1992)	*S. salar*	Parr	L-alanine and taurocholate	10^{-4}		E	Even spatial distribution of response to the two chemicals

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
		Parr-smolt transformation	L-alanine and taurocholate	10^{-4}			Stronger response to amino acid in lateral region of bulb than in medial
Polkinghorne et al. (2001)	*P. marinus*	Larvae, adult	Petromyzonol sulfate, allocholic acid, petromyzonol			HPLC	Chemicals produced in liver and gallbladder of larvae, but not in adults
		Larvae	Petromyzonol sulfate				Released 16 ng larva^{-1} h^{-1}
			Allocholic acid				Released 5 ng larva^{-1} h^{-1}
			Petromyzonol sulfate, allocholic acid				Released primrily in faeces, half-life of one day
Quinn and Hara (1986)	*O. kisutch*	Parr	L-arginine		10^{-8} to 10^{-9}	E	
			L-cysteine		10^{-8} to 10^{-9}		
			L-serine		10^{-8} to 10^{-9}		
			Taurocholic acid		10^{-8} to 10^{-9}		
Rehnberg and Schreck (1986)	*O. kisutch*	Adult^	L-alanine		10^{-4}	B	
			L-threonine		10^{-4}		
			L-cysteine		10^{-4}		
			Glycine		10^{-4}		
			L-histidine		10^{-4}		
			β-alanine		10^{-4}		
			L-glutamic acid		10^{-4}		
			L-aspartic acid		10^{-4}		
			L-lysine		10^{-4}		
		Parr	L-threonine	10^{-7}		BC	Avoidance
			L-serine	10^{-7}			Avoidance
			L-alanine	10^{-7}			Avoidance
			L-histidine	10^{-7}			Avoidance
			L-cysteine	10^{-7}			Neutral
			β-alanine	10^{-7}			Neutral
			L-aspartic acid	10^{-7}			Neutral
			Glycine	10^{-7}			Neutral
			L-lysine	10^{-7}			Neutral
		Fry	L-alanine	35-100 × 10^{-7}		BC	Avoidance of L-serine supressed

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
			Glycine	35-100 × 10^{-7}			Avoidance of L-serine supressed
			L-threonine	35-100 × 10^{-7}			Avoidance of L-serine not supressed
			L-aspartic acid	35-100 × 10^{-7}			Avoidance of L-serine not supressed
			L-histidine	35-100 × 10^{-7}			Avoidance of L-serine not supressed
Rehnberg et al. (1985*b*)	*O. kisutch*	Age 0 parr	L-serine	10^{-6} to 10^{-9}	10^{-8}	BC	Avoidance at ≥ 10^{-8} M
		Age 1 parr	L-serine	10^{-6} to 10^{-8}	10^{-7}		Avoidance at ≥ 10^{-7} M
		Smolt (April)	L-serine	10^{-4} to 10^{-6}	10^{-5}		Avoidance at ≥ 10^{-5} M
		Smolt (June)	L-serine	10^{-6} to 10^{-7}	10^{-6}		Avoidance at ≥ 10^{-6} M
		Age 1 parr	L-alanine	10^{-5} to 10^{-8}	10^{-7}		Avoidance at ≥ 10^{-7} M
		Smolt (April)	L-alanine	10^{-5} to 10^{-7}	10^{-6}		Avoidance at ≥ 10^{-6} M
		Smolt (June)	L-alanine	10^{-5} to 10^{-6}	10^{-5}		Avoidance at ≥ 10^{-5} M
Robinson et al. (2009)	*E. tridentatus*	Migrating adult	Petromyzonol sulfate		10^{-8} to 10^{-9}	E	
			3-keto petromyzonal		10^{-8} to 10^{-9}		
			Allocholic acid		> 10^{-6}		
			Taurolithocholic acid		> 10^{-6}		
		Spawning adult	Petromyzonol sulfate	10^{-6}			Significantly less than migrating adults
			3-keto petromyzonal sulfate	10^{-6}			Significantly less than migrating adults
Sato and Suzuki (2001)	*O. mykiss*	Subadult/adult	L-alanine, L-arginine, L-glutamic acid, L-norvaline (combined)	10^{-3}		CR	Response in some ciliated olfactory receptor neurons (ORNs) and some microvillous ORNs
			L-threonine	10^{-3}			
			L-methionine	10^{-3}			
			Glycine	10^{-3}			
			ECG	10^{-4}			Response in some cORNs
			Taurine	10^{-3}			No response in cORNs or mORNS
			DHP	10^{-4}			
			DHP 20-acetate	10^{-4}			
			DHP 20-sulphate	10^{-4}			
			F-prostaglandin	10^{-4}			
			15-keto-PGF2α	10^{-4}			
			dPGF	10^{-4}			

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
			U-46619	10^{-4}			
Satou and Ueda (1975)	*O. mykiss*	Subadult/adult^	L-α-alanine		10^{-6} to 10^{-7}	E	
			L-α-alanine		10^{-6} to 10^{-7}		
			L-serine		10^{-6} to 10^{-7}		
			D-serine		10^{-6} to 10^{-7}		
			Amino acids	10^{-3}			Spectral patterns of responses vary among amino acids
Shoji et al. (1996)	*O. mykiss*	Subadult/adult^	NaCl		10^{-2} to 10^{-3}	E	
			$CaCl_2$		10^{-6} to 10^{-7}		
			$MgCl_2$		10^{-2} to 10^{-3}		
			L-glutamine		10^{-8}		
			L-serine		10^{-7}		
			L-glutamic acid		10^{-7}		
			L-methionine		10^{-8}		
			L-alanine		10^{-8}		
			L-arginine		10^{-8}		
Shoji et al. (2000)	*O. masou*	Subadult/adult^	Amino acids and salts	HS		E	Positive
			Inorganic salts				Neutral
			Bile acids and salts				Neutral
Shoji et al. (2003)	*O. keta*	Spawning adult	Amino acids, taurine, urea and ammonia	HS		BC	Positive
Shparkovskiy et al. (1981)	*O. gorbuscha*	Spawning adult	Amino acids (mixture)	10^{-5}		BA	Positive
			D,L-valine	10^{-5}			Neutral
			L-cysteine	10^{-5}			Neutral
			L-glutamine	10^{-5}			Positive
			D,L-asparagine	10^{-5}			Positive
			D,L-serine	10^{-5}			Positive
			D,L-leucine	10^{-5}			Positive
			L-arginine	10^{-5}			Neutral
			D,L-alanine	10^{-5}			Negative
			L-cystine	10^{-5}			Neutral
			L-lysine	10^{-5}			Neutral
			L-arabinose	10^{-4}			Neutral
			Sucrose	10^{-4}			Neutral

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
			D-lactose	10^{-4}			Neutral
			D-mannitol	10^{-4}			Neutral
			D-glucose	10^{-4}			Neutral
			D-maltose	10^{-4}			Neutral
	S. salar	Spawning adult	Amino acids (mixture)	10^{-5}		BA	Positive
			D,L-valine	10^{-5}			Negative
			L-cysteine	10^{-5}			Positive
			L-glutamine	10^{-5}			Positive
			D,L-asparagine	10^{-5}			Positive
			D,L-serine	10^{-5}			Neutral
			D,L-leucine	10^{-5}			Positive
			L-arginine	10^{-5}			Neutral
			D,L-alanine	10^{-5}			Negative
			L-cystine	10^{-5}			Neutral
			L-lysine	10^{-5}			Neutral
			L-arabinose	10^{-4}			Neutral
			Sucrose	10^{-4}			Neutral
			D-lactose	10^{-4}			Neutral
			D-mannitol	10^{-4}			Neutral
			D-glucose	10^{-4}			Neutral
			L-maltose	10^{-4}			Neutral
Shparkovskiy et al. (1983)	*S. salar*	Zero age parr, One year parr, Immature adult	Amino acids (mixture)	10^{-4}		BA	Positive
			L-alanine	10^{-4}			Neutral
			D,L-arginine	10^{-4}			Negative
			L-aspartic acid	10^{-4}			Positive
			D,L-valine	10^{-4}			Positive
			Histidine	10^{-4}			Negative
			L-glutamic acid	10^{-4}			Positive
			L-leucine	10^{-4}			Positive
			L-lysine	10^{-4}			Neutral
			D,L-methionine	10^{-4}			Neutral
			D,L-serine	10^{-4}			Neutral
			Threonine	10^{-4}			Neutral
			D,L-tryptophan	10^{-4}			Neutral
			L-cysteine	10^{-4}			Neutral
			L-cystine	10^{-4}			Neutral

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
Siefkes and Li (2004)	*P. marinus*	Migrating adult	3-keto petromyzonal 3-keto allocholic acid Petromyzonol sulfate Allocholic acid		10^{-12} 10^{-10} 10^{-10} 10^{-10}	E	
Sorensen et al. (2005)	*P. marinus*	Migrating adult	Petromyzonamine disulfate	10^{-12} to 10^{-14}	10^{-13} 10^{-13}	E BC	Positive at $\geq 10^{-13}$ M
			Petromyzosterol disulfate	10^{-11} to 10^{-12}	10^{-13} 10^{-11}	E BC	Positive at 10^{-11} M
			Petromyzonol sulfate	10^{-11} to 10^{-12}	10^{-12} 10^{-11}	E BC	Positive at 10^{-11} M
Sutterlin and Sutterlin (1971)	*S. salar*	Smolt	L-alanine L-threonine L-proline		3.2×10^{-9} 2.5×10^{-6} 3.2×10^{-5}	E	
			Various carboxylic acids, sugars, alcohols, amines				No response
Sveinsson and Hara (1990*a*)	*S. alpinus*	Not given	L-cysteine		$<10^{-9}$	E	
Sveinsson and Hara (1990*b*)	*S. alpinus*	Not given	L-arginine L-histidine L-alanine L-cysteine		$<10^{-8}$ $<10^{-7}$ $<10^{-7}$ $<10^{-9}$	E	
Sveinsson and Hara (2000)	*S. alpinus*	Subadult or early-stage mature adult	Prostaglandin $F_{2\alpha}$ 5-*trans*-$PGF_{2\alpha}$ 16-phenyl-tetranor-11β-$PGF_{2\alpha}$ 16,16-dimethyl-$PGF_{2\alpha}$ U-46619 15-keto-$PGF_{2\alpha}$ 15(R)-$PGF_{2\alpha}$ I-BOP Prostaglandin $F_{1\alpha}$ 13,14-dihydro-$PGF_{1\alpha}$ $PGF_{3\alpha}$		10^{-10} to 10^{-12} 10^{-9} to 10^{-10} 10^{-10} 10^{-8} to 10^{-9} 10^{-10} to 10^{-12} 10^{-10} to 10^{-12} $>10^{-8}$ $>10^{-8}$ 10^{-9} 10^{-9} to 10^{-10} 10^{-9} to 10^{-10} 10^{-9} to 10^{-10}	E	

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
			$PGF_{2\beta}$		10^{-9} to 10^{-10}		
Vrieze and Sorensen (2001)	*P. marinus*	Migrating adult	Petromyzonal sulfate Allocholic acid	10^{-10} 0.15×10^{-10}		BC	Neutral Neutral
Yamamoto and Ueda (2009)	*O. keta*	Spawning adult	Amino acids and Amino acids excluding L-glutamic acid	HS		BC	Positive Positive
			Amino acids and Amino acids excluding L-glutamic acid			E	Positive Positive
Yamamoto et al. (2008*a*)	*O. gorbuscha*	Spawning adult	Amino acids	HS		BC	Positive/neutral (no statistical tests)
	O. keta	Spawning adult	Amino acids			BC	Positive (no statistical tests)
	O. masou	Spawning adult	Amino acids			BC	Positive (no statistical tests)
	O. nerka	Spawning adult	Amino acids			BC	Positive (no statistical tests)
Yamamoto et al. (2008*b*)	*O. mykiss*	Parr	L-alanine		10^{-7}	E	
Yamamoto et al. (2013)	*O. keta*	Spawning adult	Amino acids	HS (during outmigration)		BC E	Positive Positive
			Amino acids	HS (spawning migration)		BC E	Positive Positive
Yun et al. (2011)	*L. tridentata*	Migrating adult	Petromyzonamine Petromyzosterol Petromyzonal sulfate 3-keto petromyzonal Allocholic acid 3-keto allocholic acid		10^{-14} 10^{-13} 10^{-14} 10^{-14} 10^{-9} 10^{-10}	E	
Yun et al. (2003)	*L. tridentata*	Larvae	Petromoyzonal sulfate			HPLC	Release 1.48 ng (g larva)$^{-1}$ h^{-1}
	L. richardsonii	Larvae	Petromoyzonal sulfate				Released 30.68 ng (g larva)$^{-1}$ h^{-1}

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
	P. marinus	Larvae	Petromoyzonal sulfate				Released 36.77 ng (g larva)$^{-1}$ h^{-1}
Zhang et al. (2001)	*S. namaycush*	Subadult	Taurocholic acid			HPLC	Release 268.5 ± 5.1 nmol µl^{-1} in bile, 23.4 ± 0.7 nmol mg^{-1} in feces, 2.6 ± 2.4 nmol h^{-1} in urine, 2.8 ± 1.1 nmol min^{-1} kg^{-1} in water
			Taurochenodeoxy-cholic acid				Release 15.0 ± 0/8 nmol µl-1 in bile, 3.1 ± 0.3 nmol mg^{-1} in faeces, 1.4 ± 1.4 nmol h^{-1} in urine, 0.9 ± 0.2 nmol min^{-1} kg^{-1} in water
			Taurolithocholic acid				Release 0.1 ± 0.1 nmol µl-1, trace in faeces, none in urine or water
			Taurooxocholanic acid				Release 20.5 ± 0.0 nmol µl^{-1} in bile, 3.5 ± 0.7 nmol mg^{-1} in faeces, none in water
			Taurooxodeoxycholic acid 3α-sulphate				Release 7.8 ± 0.2 nmol µl^{-1} in bile, 1.3 ± 0.1 nmol mg^{-1} in feces, 0.2 ± 0.2 nmol h^{-1} in urine, trace in water
			Taurocholic acid		10^{-9} to 10^{-10}		
			Taurochenodeoxy-cholic acid		10^{-9} to 10^{-10}		
			Sulphated fractions		10^{-9}		
Zhang and Hara (2009)	*S. namaycush*	Subadult/adult^	CD		2×10^{-11}	E	
			DA		2×10^{-9}		
			3-dehydrocholic acid		2×10^{-11}		
			Cholic acid		10^{-10}		
			Hyocholic acid		2×10^{-10}		
			7-Oxo-DA		10^{-9}		
			12-Oxo-CD		2×10^{-9}		
			3-Deoxycholic acid		2×10^{-11}		
			Isodeoxycholic acid		5×10^{-9}		
			Allocholic acid		10^{-8}		
			Lithocholic acid		10^{-8}		

Reference	Species	Life-history stage	Odorant	Concentration tested (M)	Detection threshold (M)	Method	Response
			Petromyzonol		2×10^{-8}		
			5β-petromyzonol		2×10^{-8}		
			Dehydrocholic acid		2×10^{-7}		
			Nordeoxycholic acid		2×10^{-7}		
			Cholanic acid		2×10^{-7}		
			5β-epicholestanol		2×10^{-6}		
			Taurochenocholic acid		5×10^{-10}		
			Taurocholic acid		5×10^{-10}		
			Tauro-DA		2×10^{-9}		
			Glycocholic acid		10^{-8}		
			Glycocheno-DA		2×10^{-8}		
			Taurolithocholic acid		2×10^{-8}		
			Glyco-DA		5×10^{-8}		
			Taurohyo-DA		10^{-7}		
			Taurourso-DA		10^{-7}		
			Glycolithocholic acid		5×10^{-7}		
			Taurodehydrocholic acid		5×10^{-7}		
			Taurocholanic acid		10^{-6}		
			CD diacetate methyl ester		10^{-6}		
			Taurolithocholic acid		2×10^{-11}		
			Taurocholic acid		10^{-10}		
			Petromyzonol sulphate		10^{-9}		
			Lithocholic acid 3α-S		2×10^{-9}		
			Glycolithocholic acid		5×10^{-9}		
			Deoxycholic acid		5×10^{-8}		
			Ursodeoxycholic acid		10^{-7}		
			Cholic acid 3α,7α,12α-		2×10^{-7}		
			Prostaglandin F2α		10^{-12}		

Species – *Coregonus clupeaformis*, lake whitefish; *Entosphenus tridentatus*, Pacific lamprey; *Ichthyomyzon fossor*, northern brook lamprey; *Lampetra richardsonii*, western brook lamprey; *Lethenteron appendix*, American brook lamprey; *Oncorhynchus gorbuscha*, pink salmon; *O. keta*, chum salmon; *O. kisutch*, coho salmon; *O. masou*, masu salmon; *O. mykiss*, steelhead/rainbow trout; *O. nerka*, sockeye salmon; *Petromyzon marinus*, sea lamprey; *Salmo salar*, Atlantic salmon; *S. trutta*, brown trout; *Salvelinus alpinus*, Arctic char; *S. fontinalis*, brook trout; *S. namaycush*, lake trout; *Thymallus thymallus*, grayling.

Odorants: CD, chenodeoxycholic acid; DA, deoxycholic acid; DHP, 17α,20β-dihydroxy-4-pregnene-3-one; ECG, etiocholam-3α-ol-17-one glucuronide; I-BOP, 7-[3-[3-hydroxy-4-(4-iodophenoxy)-butenyl]-7-oxabicycloheptyl]-5-heptanoic acid; PG, prostaglandin; U-46619, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$.

Concentration tested – HS, chemicals tested at the concentration present in the home stream.

Methods – B, binding of olfactory receptors; BA, behavioural assays; BC, behavioural choice tests; CR, cellular response in olfactory epithelia/bulb; E, electrophysiology; HPLC, high performance liquid chromatography; MS, mass spectrometry; PS, phospholipid staining.

^Specific life-history stage not given.

^^Have reached maturity, but reared in captivity (and therefore not classified as either ‘migrating adult’ or ‘spawning adult’).

Table 2.5 Studies exploring the timing of imprinting in salmonids

Reference	Species	Life stages imprinted	Imprinting chemical	Concentration	Duration of exposure	Method	Life stage of successful imprinting	Notes
Cooper and Scholz (1976)	*O. mykiss*	PST	Morpholine	10^{-10}	1 month	F	PST	
Cooper and Hasler (1974)	*O. kisutch*	PST	Morpholine	10^{-10}	5 weeks	E	PST	
Cooper et al. (1976)	*O. kisutch*	PST	Morpholine	10^{-7} to 10^{-10}	5–7 weeks	F	PST	Also tested reduced exposure duration (2 days) and monitored returns to decoy stream one year early. Increased number of jacks returning
Dittman et al. (1996)	*O. kisutch*	Alevin, parr, smolt	PEA	10^{-7}	1.5–3 weeks	BC	Smolt	
		Embryo-fry, parr, smolt	Hatchery water		1.5–9 weeks	F	Inconclusive	
Dittman et al. (1997)	*O. kisutch*	PST	PEA	10^{-7}	10 days	BC	PST	Olfactory cilia guanylyl cyclase sensitized to imprinted odorant in mature adults
Dukes et al. (2004)	*S. salar*					G	PST	Increased expression during PST
Hara and Brown (1979)	*O. mykiss*	Parr, PST, smolt	Morpholine	5.7×10^{-10}	5 months	E	None	No increase in response up to 12 months after exposure (did not test mature adults)
Hassler and Kutchins (1990)	*O. tshawytscha*	PST	Morpholine	10^{-10}	17–40 days	F	None	

Reference	Species	Life stages imprinted	Imprinting chemical	Concentration	Duration of exposure	Method	Life stage of successful imprinting	Notes
Johnsen and Hasler (1980)	*O. kisutch*	PST	Morpholine	10^{-10}	1 month	F	PST	
Lema and Nevitt (2004)	*O. kisutch*					CP	PST	Positive relationship between thyroxine and cellular proliferation during smoltification
Morin and Døving (1992)	*S. salar*	Parr, PST, smolt	Taurocholate or L-alanine	10^{-7}	0–10 weeks	E		Peak response during PST
Morin et al. (1989*a*,*b*)	*S. salar*	PST	L-cysteine	3.8×10^{-4}		CC		Strongest response during mid-point of smoltification (21–28 days after onset), suggesting this is key imprinting period
Morin et al. (1995)	*S. salar*		L-alanine	10^{-5} to 10^{-9}		E		Artificially increasing plasma L-thyroxine (to mimic the natural increase during smoltification) reduced olfactory sensitivity
Morin et al. (1997)	*S. salar*		L-alanine	10^{-5} to 10^{-9}		E		Plasma L-thyroxine surged during middle of PST, but no change in electrophysiological response
Nevitt et al. (1994)	*O. kisutch*	PST	PEA	10^{-7}	10 days	BC, CR	PST	Results suggest imprinting memory stored in the peripheral nervous system
Rehnberg et al. (1985*a*)	*O. kisutch*	Smolt	Morpholine	10^{-10}	15 days	F	Inconclusive	Released morpholine in their natal water. Control (unexposed) salmon returned in equal numbers to exposed salmon
Rehnberg et al. (1985*b*)	*O. nerka*		L-serine, D,L-alanine			BC		Threshold concentration for avoidance response higher during PST and post-PST than in parr, suggesting potentially reduced sensitivity

Reference	Species	Life stages imprinted	Imprinting chemical	Concentration	Duration of exposure	Method	Life stage of successful imprinting	Notes
Sahafi (2013)	*R. frisii*	Yolk sack, active fry, fingerling	Morpholine	10^{-7} to 10^{-12}	Not given	F	Active fry	Very small sample size (two control adults returned, 4–14 adults returned in treatment groups)
Scholz et al. (1976)	*O. kisutch*	PST	MorpholinePEA	10^{-10} (M), 8×10^{-9} (PEA)	1.5 months	F	PST	
Scholz et al. (1978*a*)	*S. trutta*	PST	Morpholine	10^{-10}	34 days	F	PST	
Scholz et al. (1978*b*)	*O. mykiss*	PST	Morpholine	10^{-10}	73 days	F	PST	
Shimizu et al. (1995)	*O. masou*							Various unknown proteins in the olfactory system appeared or disappeared during smoltification
Yamamoto et al. (2010)	*O. nerka*	Parr, PST, smolt	L-proline	10^{-6}	1, 6 h; 1, 7, 14 days	E	Parr, PST	Successful imprinting following 14 days of exposure, but not other exposure durations
		Parr, PST, smolt	L-proline	10^{-6}	1, 6 h; 1, 7, 14 days	BC	Parr, PST	

Species – *Oncorhynchus kisutch*, coho salmon; *O. masou*, masu salmon; *O. mykiss*, steelhead/rainbow trout; *O. nerka*, sockeye salmon; *O. tshawytscha*, Chinook salmon; *Salmo salar*, Atlantic salmon; *S. trutta*, brown trout; *Rutilus frisii*, kutum.

Method – BC, behavioural choice; CC, cardiac conditioning; CR, cellular proliferation; CR, cellular response; E, electrophysiology; F, field experiment (imprinting chemical released into decoy stream and adult returns monitored); G, olfactory receptor gene expression.

PEA, phenethyl alcohol.

PST, parr–smolt transformation.

Table 2.6 Studies in which juvenile salmonids were transplanted before or during their outmigration, and adult returns were monitored.

Reference	Species	Age at trans-port	Transport location	Distance (km)	Number released	Number returns	% to release site	% to rearing site	% to other sites	Imprinted site	Notes
Brannon and Quinn (1990)	*O. kisutch*	Fry	Within lake	10	19,637	82	100	0		Release	
	O. kisutch	Smolt	Within lake	10	10,020	38	84	16		Release	
Donaldson and Allen (1958)	*O. kisutch*	PST	Adjacent river	75	34,405	124	100	0		Release	
		PST	Adjacent river	150	36,833	70	100	0		Release	
Ebel et al. (1973)	*O. tshawytscha*	Smolt	Downstream	200 or 300	131,958						Increased returns to rearing site when transported downstream (relative to controls)
	O. mykiss				72,647						
Foster and Schom (1989)	*S. salar*	Kelt	Different river system	150	7	5	0	100		Rearing	Delayed release of nine other kelts, but none recovered
Hansen and Jonsson (1994)	*S. salar*	Smolt	Different river system	Not given	847	27	85	≥15	≥15	Release	Returns to rearing site and other rivers not distinguished
Jensen and Duncan (1971)	*O. kisutch*	Smolt	Downstream	260	650,000	1712	100	0		Release	
Johnson et al. (1990)	*O. kisutch*	Smolt	Different river system	177	398,265	2307*	99.9	0.1		Release	*Based on recovery estimates
			Downstream	11	208,100			121*			* Not possible to estimate percentages based on data collected
			Upstream	23	208,048			720*			
Keefer et al.	*O.*	Smolt	Downstream	>350		245		82.5	17.6		Smolts barged

Reference	Species	Age at trans-port	Transport location	Distance (km)	Number released	Number returns	% to release site	% to rearing site	% to other sites	Imprinted site	Notes
(2008*b*)	*tshawytscha*	None	NA	NA		161		92.6	7.4		downstream, but water from river circulated continuously
	O. mykiss	Smolt	Downstream	>350		409		75.6	24.4		
		None	NA	NA		238		88.7	11.3		
McIsaac and Quinn (1988)	*O. tshawytscha*	Eggs	Downstream	370		894		58	42		Reared from hatching until release at same site
Nordeng (1971)	*S. alpinus*	Smolt	Estuary	~1500	174	31	32		68		Reared in different river system, released 10 km from estuary of ancestral river. "Other sites" = ancestral river
			Estuary	~1500	143	27	96		4		Reared in different river system, released at estuary of ancestral river. "Release site" = ancestral river
Nordeng (2009)	*S. alpinus*	Smolt	Estuary	~1500	174	35	34		66		Reared in different river system, released at estuary 10 km from estuary of ancestral river. "Other sites" = ancestral river
			Coastal ocean	~1500	291	63		65	35		Released 15 km from estuary of ancestral river, therefore no freshwater "Release site". "Rearing site" = ancestral river
Nordeng and Bratland	*S. alpinus*	Smolt	Different river system	8	188	66	9	88	3	Rearing	

Reference	Species	Age at trans-port	Transport location	Distance (km)	Number released	Number returns	% to release site	% to rearing site	% to other sites	Imprinted site	Notes
(2006)			Coastal ocean	8, then 10	43	17	6	88	6	Rearing	Held 3 days in different river then released in ocean
	S. trutta	Smolt	Different river system	8	109	14	0	100	0	Rearing	
			Coastal ocean	8, then 4	80	24	4	96	0	Rearing	Held 3 days in different river then released in ocean
Quinn et al. (1989)	*O. kisutch*	Smolt	Downstream	18	10,020	51	86	14		Release	Held at rearing site until fully completed PST
		Smolt	Downstream	4	8491	34		100*	0	Rearing	* Hatchery site
		Smolt	Downstream	40	10,000	17	88	12		Release	Held at rearing site until fully completed PST
Savitz et al. (1993)	*O. kisutch*	Parr	Lake Michigan	Un-known	50,436	8	63		38		"Other sites" are other harbours in Lake Michigan
		Smolt			50,427	19	84		16		
	O. tshawytscha	Parr			148,937	52	52		48		
		Smolt			130,154	38	63		37		
Slaney et al. (1993)	*O. mykiss*	Smolts	Downstream	32 or 28	95,258	Unknown (return figures based on downstream fisheries)				Release	Reach of release correlated to reach where adults were caught
				22 or 18	97,051	Unknown (all other return figures based on downstream fisheries)				Release	
				12 or 1	92,282	Unknown (all other return figures based on downstream fisheries)				Release	
Solazzi et al.	*O. kisutch*	Smolt	No transport		750,798	1475		99.9	0.1		Smolts were transported

Reference	Species	Age at trans-port	Transport location	Distance (km)	Number released	Number returns	% to release site	% to rearing site	% to other sites	Imprinted site	Notes
(1991)			Downstream	205	(total)	29		96.6	3.4		downstream to lower Columbia River, estuary, or ocean. "Rearing site" = Columbia River, "Other Sites" = other river systems
			Downstream	232		49		95.9	4.1		
			Estuary	253		66		93.9	6.1		
			Ocean	253		100		79	21		
			Ocean	272		48		62.5	37.5		
Vreeland et al. (1975)	*O. kisutch*	Smolt	Downstream	223	100,914	347	97	3		Release	
Wagner (1969)	*O. mykiss*	Smolt	Downstream	50	81,000	161	71	29		Release	

Species – *Oncorhynchus kisutch*, coho salmon; *O. myskiss*, steelhead/rainbow trout; *O. tshawytscha*, Chinook salmon; *Salmo salar*, Atlantic salmon; *S. trutta*, brown trout; *Salvelinus alpinus*, Arctic char.

PST, parr–smolt transformation.

Chapter 3: Olfactory gene expression in migrating adult sockeye salmon (*Oncorhynchus nerka*)

3.1 Synopsis

In Chapter 2 I hypothesized that stray salmon rely on pheromones as directional cues, as opposed to salmon that have not strayed, which migrate towards imprinted cues emanating from their natal stream. The hypothesis is incidentally supported by past research on Pacific salmon homing, in addition to personal observations I described in Chapter 2. My objective in this chapter was to compare the expression of olfactory genes in stray and non-stray sockeye salmon. My analysis focused on vomeronasal type 2 receptor-like genes that are associated with the detection of pheromones, in addition to several other olfactory-related genes. I collected samples from stray and non-stray adult sockeye salmon in two different rivers, and measured the relative expression of these genes in the olfactory epithelia. Several of the vomeronasal type 2 receptor-like genes were differentially expressed in the stray and non-stray groups, while the other olfactory genes were not. Differences included upregulation in Stellako River sockeye salmon that strayed into the Seton River in comparison to individuals from the same population captured in their home stream. Similarly, expression of several of these genes was higher in Chilko and Stellako River sockeye salmon that strayed into the Seton River in comparison to Gates Creek sockeye salmon, which are native to the area. My findings indicate that stray and non-stray sockeye salmon exhibit differential expression of olfactory receptor genes, and, furthermore, that stray sockeye salmon may respond more strongly to pheromones.

3.2 Introduction

The homing migration of Pacific salmon (*Oncorhynchus* spp.) is guided by olfactory cues. These cues are learned by juvenile salmon prior to their downstream migration, through a process known as olfactory imprinting (Hasler and Scholz 1983). Upon their return as adults, the salmon are attracted to the familiar imprinted cues. The imprinting process enables these fish to migrate long distances upstream to spawning grounds while maintaining a high level of home stream fidelity. While the importance of olfaction to the spawning migration has been well established, however, the molecular basis is not currently understood.

In the salmonid olfactory system, odorants in the water bind to G protein-coupled receptors in the olfactory epithelia, which covers specialized peripheral olfactory organs (commonly known as "rosettes") (Hara 1992). Binding of the odorants ultimately leads to the production of a signal that is sent along the olfactory nerve to the olfactory bulb, which contains cells that project to various regions of the brain, where the information is processed. Olfactory receptors therefore direct the first step in the olfactory process, and their presence in the epithelia is necessary for the detection of olfactory cues.

Despite the importance of olfactory receptor genes—and other olfactory genes—to Pacific salmon homing, research on this topic is very limited, and the specific odorants involved remain largely unknown. Johnstone et al. (2012) found differential expression of olfactory receptor genes in adult and juvenile Atlantic salmon, and suggested the genes are involved in imprinting and homing. Yamamoto et al. (2009) found evidence that Pacific salmon imprint on amino acids in their natal rivers, and subsequent research identified an olfactory gene (SOIG) that is up-regulated during the parr-smolt transformation (Yamamoto et al. 2010), a critical stage

of imprinting (Dittman and Quinn 1996). An increased expression of olfactory receptor genes has also been associated with the parr-smolt transformation (Dukes et al. 2004).

Amino acids may not be the only directional cues used by migrating salmon. When salmon stray into unfamiliar rivers, for example, there is evidence to suggest they might instead be guided by pheromones from conspecifics, as I hypothesized in Chapter 2 (although it is possible that pheromones may comprise amino acids, Yambe et al. 2006). Straying behaviours influence the geographic distribution and genetic diversity of Pacific salmon meta-populations, which I expand on in Chapter 4, and they also provide a contrast to non-straying salmon that are migrating towards imprinted cues. If straying salmon are attracted to different odorants than salmon in their natal waters, these differences could be reflected in differential expression of olfactory receptor genes. Recent evidence suggests expression of olfactory receptor genes could be plastic, and could change in response to the presence of familiar odours (Claudianos et al. 2014). To test whether olfactory receptor genes are expressed differentially in stray and non-stray salmon, I collected samples from sockeye salmon in or near their home stream, as well as sockeye salmon that had strayed into a non-natal river. I analyzed the expression of various olfactory genes, the majority of which were vomeronasal type 2 receptor-like (V2R-like) genes. These receptors are similar to those found in the vomeronasal organ of reptiles and mammals, a specialized structure associated with the detection of pheromones (Firestein 2001). I predicted that olfactory gene expression in stray and non-stray salmon would differ, which could indicate differential responses to odorants. Furthermore, following the hypothesis developed in chapter 2, I predicted that expression of V2R-like genes would be greater in stray salmon, as these fish may be more sensitive to conspecific chemical cues.

3.3 Methods

3.3.1 *Sample collections and procedure*

Samples were collected from wild adult sockeye salmon in British Columbia's Fraser River system. The salmon were captured *via* dipnet out of the top pool of the Seton Dam fishway, on the Seton River, on August 24, 2012, as well as from beach seines in the Stellako River on September 12-14, 2012. Sockeye salmon captured in the Seton River originated from three populations: Gates Creek (n = 17), Stellako River (n = 3), and Chilko River (n = 6) (Fig 3.1). Gates Creek sockeye salmon migrate through the Seton River *en route* to their spawning ground, which is located 55 km upstream from the Seton River capture site. The other two populations spawn in Fraser River tributaries located much further upstream from the Seton River - the individuals captured from these populations had strayed into the Seton River. The salmon captured in the Stellako River all belonged to the Stellako River population (n = 20). The population identities of each salmon was determined by genetic analyses (Beacham et al. 2005) at the Pacific Biological Station, Nanaimo, BC.

Immediately upon capture, the salmon were sacrificed by cerebral percussion. Rosettes were then excised (< 30 sec following percussion) to quantify olfactory gene expression using qPCR. The rosettes were preserved in RNA*later* (Life Technologies, Grand Island, NY) at 4°C for 24 h and then placed in liquid nitrogen until they were transported to the laboratory and stored at −80°C, where they remained until analysis. Adipose fin clips were collected and preserved in ethanol for population identification.

3.3.2 *RNA extraction, amplification and quantification*

Total RNA from the rosettes was extracted using Magmax-96 for Microarray Kits (Ambion Inc., Austin, TX) and a Biomek FXP automated liquid-handler (Beckman-Coulter, Mississauga, ON). Both rosettes from each fish were homogenized with stainless steel beads in 600 µL of TRI-reagent (Ambion Inc.) on a MM301 mixer mill (Retsch Inc., Newtown, PA). Aliquots of 75 µL of the homogenates were pipetted into 96-well plates and extractions were carried out using the "no-spin procedure" according to the manufacturer's instructions. RNA was eluted and the RNA yield was determined measuring the A260 with a DTX 880 Multimode Detector (Molecular Devices, Sunnyvale, CA). Purity was assessed by measuring the A260/A280 ratio, and RNA was normalized to a concentration of 62.5 ng mL^{-1}. RNA was reverse transcribed to cDNA using a PTC-100 Thermal Cycler (Bio-Rad Laboratories Inc., Foster City, CA) following the manufacturer's instructions. Specific target amplification (STA) was performed with a PTC-100 Thermal Cycler. 1.25 µL of cDNA was added to 2.5 µL of 2X TaqMan PreAmp Master Mix (Applied Biosystems, Carlsbad, CA) and 1.25 µL of an STA primer mix. The primer mix contained primers for 16 olfactory genes and 6 housekeeping genes (Table 3.1). Samples were treated with ExoSAP-IT (USB Corp., Cleveland, OH) following the manufacturer's instructions. Samples were then diluted 5-fold with DNA Suspension Buffer (Teknova Inc., Hollister, CA). An assay mix was made on a 48x48 plate by adding 3 µL of 2X Assay Loading Reagent (Fluidigm Corp., San Francisco, CA), 2.4 µL of DNA Suspension Buffer, and 0.6 µL of forward and reverse primers to each well. A sample mix was made by adding 3 µL of SsoFast EvaGreen Supermix with Low ROX (Bio-Rad Laboratores, Hercules, CA), 0.3 µL of 20X DNA Binding Dye Sample Loading Reagent (Fluidigm Corp., San Francisco, CA), and 2.7 µL of sample to each well of a 48x48 plate. A Fluidigm Biomark HD

(Fluidigm Corp., San Francisco, CA) was used for qRT-PCR, following the manufacturer's instructions. Samples were run in duplicate and with non-template controls included. Relative expression of the target genes was determined using the comparative Ct method (Schmittgen and Livak 2008). Target gene expression was normalized to the two housekeeping genes with highest primer efficiencies (CoilP84 and 78d16.1).

3.3.3 *Statistical analysis*

Statistical differences between the four groups were analyzed by analysis of variance (ANOVA). When differences were found, post hoc pairwise comparisons using t tests with the Bonferonni adjustment were done. When assumptions of homogeneity of variances, as assessed by Bartlett's tests, or normality, as assessed by Shapiro-Wilk tests, were not met following transformation, statistical differences between groups were analyzed by Kruskal Wallis rank sum tests, followed by multiple pairwise comparisons using the pgirmess R package (Giraudoux 2016). All statistical tests were performed using R Studio V 0.98.501.

3.4 Results

The mean normalized expressions of six genes were different among the four groups (Fig 3.2): *Olf*C 2.1 ($F_{3,42} = 4.67$, $P = 0.0066$), *Olf*C 3.1 ($\chi^2_3 = 10.88$, $P = 0.013$), *Olf*C 13.1 ($F_{3,42} = 4.90$, $P = 0.0052$), OlfC 17.1 ($\chi^2_3 = 9.73$, $P = 0.021$), *Olf*C 17.2 ($\chi^2_3 = 10.73$, $P = 0.013$), and *Olf*C 17.p3 ($F_{3,42} = 5.86$, $P = 0.0019$). Post hoc tests determined significant differences between the Gates Creek and Stellako River populations captured in the Seton River (*Olf*C 2.1; *Olf*C 3.1; *Olf*C 13.1; *Olf*C 17.1; *Olf*C 17.2), as well as differences between Gates Creek and Chilko River populations captured in the Seton River (*Olf*C 3.1; *Olf*C 17.1). There were also significant differences between Stellako River sockeye salmon captured in the two different rivers (*Olf*C

2.1; *Olf*C 17.p3). The mean normalized expression of one gene also differed between the Stellako River sockeye salmon captured in their home stream and the Chilko River sockeye salmon captured in the Seton River (*Olf*C 17.p3). The direction of the differences in mean normalized expressions was consistent across these genes, with higher levels in the stray salmon relative to the non-strays.

There were no significant differences between the groups in the normalized expression of CYP1A ($\chi^2_3 = 5.99$, $P = 0.11$), CYP2K1 ($F_{3,42} = 2.73$, $P = 0.056$), CYP2M ($\chi^2_3 = 5.83$, $P = 0.12$), CYP3A ($\chi^2_3 = 3.65$, $P = 0.30$), *Olf*C 4.5 ($F_{3,42} = 0.27$, $P = 0.85$), *Olf*C 4.10 ($\chi^2_3 = 4.99$, $P = 0.17$), *Olf*C 16.1 ($\chi^2_3 = 6.09$, $P = 0.11$), *Olf*C 16.2 ($\chi^2_3 = 3.56$, $P = 0.31$), OR ($\chi^2_3 = 4.74$, $P = 0.19$), or SOIG ($\chi^2_3 = 6.95$, $P = 0.074$).

3.5 Discussion

I found differential expression of six olfactory receptor genes. Of these six genes, mean normalized expression in the Seton River was consistently higher in stray sockeye salmon from Stellako and Chilko River populations captured in the Seton River than native salmon from the Gates Creek population captured in the Seton River, and these differences were significant in two and five of the genes, respectively. Also, stray sockeye salmon from the Stellako River population captured in the Seton River had higher mean normalized expression levels than Stellako River sockeye salmon captured in their home stream, and the difference was significant in two of the genes.

Relative down-regulation of olfactory receptor genes as salmon near spawning grounds could indicate that high sensitivity to the imprinted cues is unnecessary. Stray salmon, on the other hand, might benefit from relatively higher expression levels if it increases their sensitivity

to the imprinted cues, which would enable them to detect these cues further from their home stream. Studies on olfactory gene expression plasticity are limited, but a recent finding in honeybees (*Apis mellifera*) suggested that down-regulation of olfactory genes may occur during exposure to familiar odorants. Claudianos et al. (2014) triggered the formation of a long-term memory of an odorant in honeybees through reward-based associative learning, then measured gene expression of a relevant olfactory receptor. They replicated the experiment with a second type of odorant, and in both cases found down-regulation of the receptor gene following exposure to the odorant. As salmon near their home stream, and the strength of the imprinted odour increases, they may be able to detect this odour even if the relevant receptors are down-regulated. Claudianos et al. (2014) suggested down-regulation of these receptors could allow for increased olfactory awareness of other important cues, and the same could be said here—salmon also use olfactory cues to detect predators and mates, and reducing the expression of receptors that bind imprinted chemicals could improve their ability to detect these other odours.

The olfactory genes I analyzed, however, may not be associated with various environmental chemicals that presumably make up the imprinted odour. Instead, they might be associated with the detection of pheromones. There are two main classes of olfactory receptors in mammals: main olfactory receptors, which are expressed in ciliated olfactory sensory neurons, and vomeronasal receptors, which are expressed in microvillar vomeronasal sensory neurons (Bargmann 1997). The latter are found in the vomeronasal organ, an auxiliary olfactory organ that is present in mammals and some other types of animals, but not in fish. The *Olf*C genes in salmon are classified as vomeronasal type 2 receptor-like genes (Johnstone et al. 2009), and are analogous to receptor genes found in the vomeronasal organ. Vomeronasal receptors are associated with the perception of pheromones, whereas the main olfactory receptors primarily

detect other environmental odours (Firestein 2001; Dukes et al. 2004). It is therefore possible that the increased expression of these *Olf*C genes in the stray salmon reflects a response to pheromones released by other sockeye salmon. Unlike natal imprinted cues, which are absent for strays and present for non-strays, pheromones from co-migrating conspecifics were present for all of the salmon I analyzed, and therefore presence alone would not result in a differential response. A difference in expression might arise, however, from a differential behavioural response to pheromones. Stray salmon may use pheromones as directional cues (Chapter 2), unlike non-stray salmon, and an attraction to these cues may be reflected by increased expression of the *Olf*C genes. There is evidence to suggest that olfactory genes may indeed be up-regulated when an attractive odorant is detected. In juvenile zebrafish (*Danio rerio*), exposure to an imprinted chemical increases expression of an olfactory-related transcription factor in the olfactory epithelia (Harden et al. 2006), and similar odorant-induced increases in expression have been found in rats (Moon et al. 1999) and mice (Norlin et al. 2005). The potential attraction of stray salmon to pheromones is something I test directly in Chapter 4.

There is currently no published research on olfactory gene expression in Pacific salmon, and although the data presented here is novel, it is also limited. Fish have an estimated 100 olfactory receptors (Firestein 2001), and my study was restricted to the analysis of only 11 receptor genes, in addition to 6 other olfactory-related genes (none of which were differentially expressed). The primers I used to analyze these genes were mostly taken from research on Atlantic salmon (*Salmo salar*; Johnstone et al. 2012). Primers for the other genes analyzed in the Atlantic salmon research had poor primer efficiencies on sockeye salmon samples. The findings in this chapter therefore only present a small component of olfactory receptor expression in homing salmon, and an analysis of a broader range of olfactory receptor genes could clarify the

extent to which expression in stray and non-stray salmon differs. We also do not know what chemicals the receptors bind, nor do we know what specific chemicals act as directional cues, which makes interpretation of expression data difficult aside from broad generalizations. Identification of the chemicals that bind to different receptors would allow researchers to use gene expression profiles of homing salmon to determine what environmental chemicals guide salmon as they search for their natal habitat.

As with the identification of the imprinted chemicals that guide salmon to natal habitat, olfactory expression profiles might also be used to determine the chemicals that guide stray salmon. In addition to uncertainty surrounding the affinities of olfactory receptors for different chemicals, however, studies on stray salmon are also limited by the difficulty of obtaining paired collections of stray and non-stray salmon from the same population. I was fortunate to collect samples from a small number of Stellako River sockeye salmon that strayed into the Seton River, which complemented my collections from the same population in their home stream. Fewer than 10% of salmon stray (Keefer and Caudill 2014), and the likelihood of locating and capturing stray salmon is small. This difficulty is compounded by the fact that different populations are often morphologically indistinguishable, and identification is reliant on subsequent genetic analyses (as in the data presented in this chapter). Further exploration of the influences of straying behaviours on olfactory gene expression, or vice versa, might therefore rely on salmon held in an artificial environment, in which the water may be manipulated. Identification of the roles of individual olfactory receptors will also be necessary to understand which chemical cues trigger any changes in expression. Nevertheless, my results are the first to suggest differential olfactory activity in stray and non-stray adult salmon on a molecular level. This variation likely

reflects differences in the chemical cues these salmon are responding to, some or many of which provide directional information.

Fig 3.1 Map of the Fraser River watershed, in British Columbia, Canada. Olfactory gene expression was analyzed in returning adult sockeye salmon from three populations (Stellako River [■], Chilko River [▲], and Gates Creek [●]), captured at two sites (×).

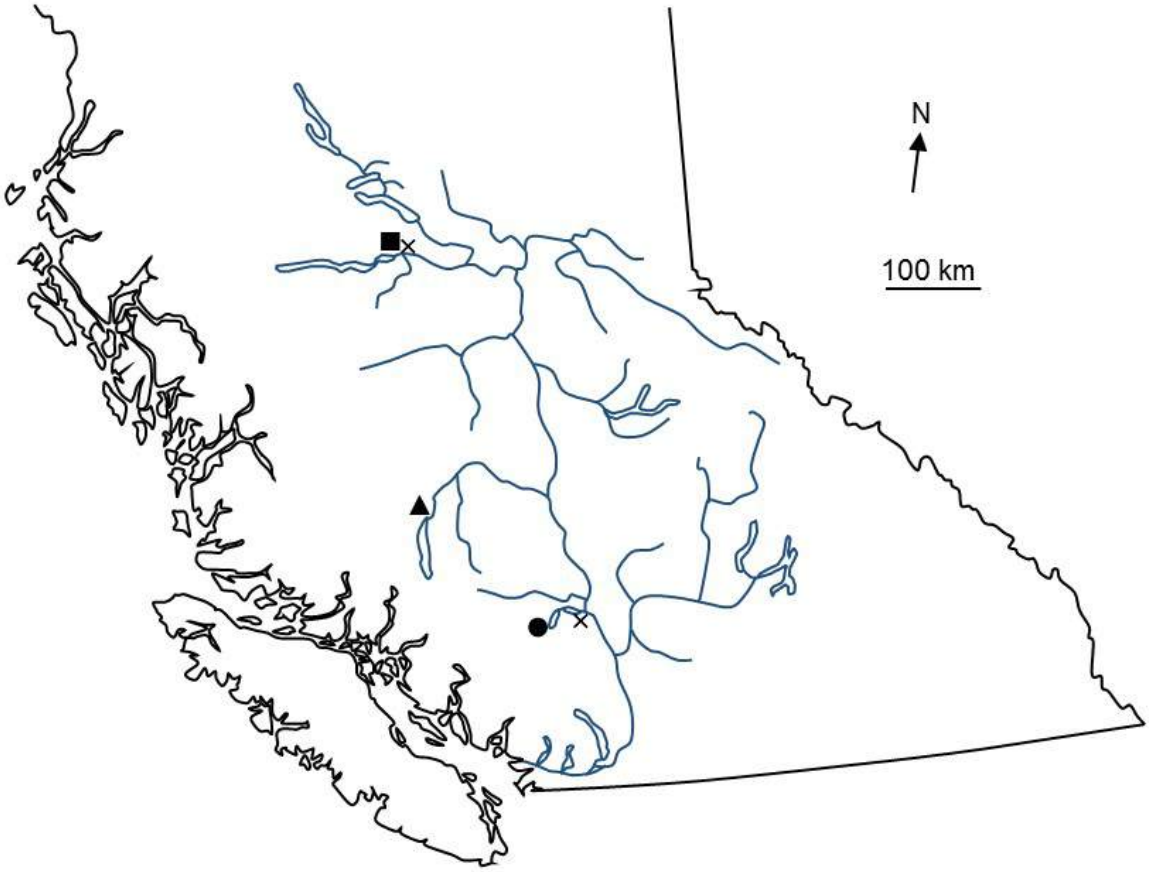


Fig 3.2 Normalized expression of olfactory receptor genes from three populations of sockeye salmon (Gates Creek, Chilko River and Stellako River) captured in the Seton River, which is downstream from the spawning grounds of the Gates Creek sockeye salmon, and the Stellako River. Solid horizontal lines indicate means and different letters indicate significant differences.

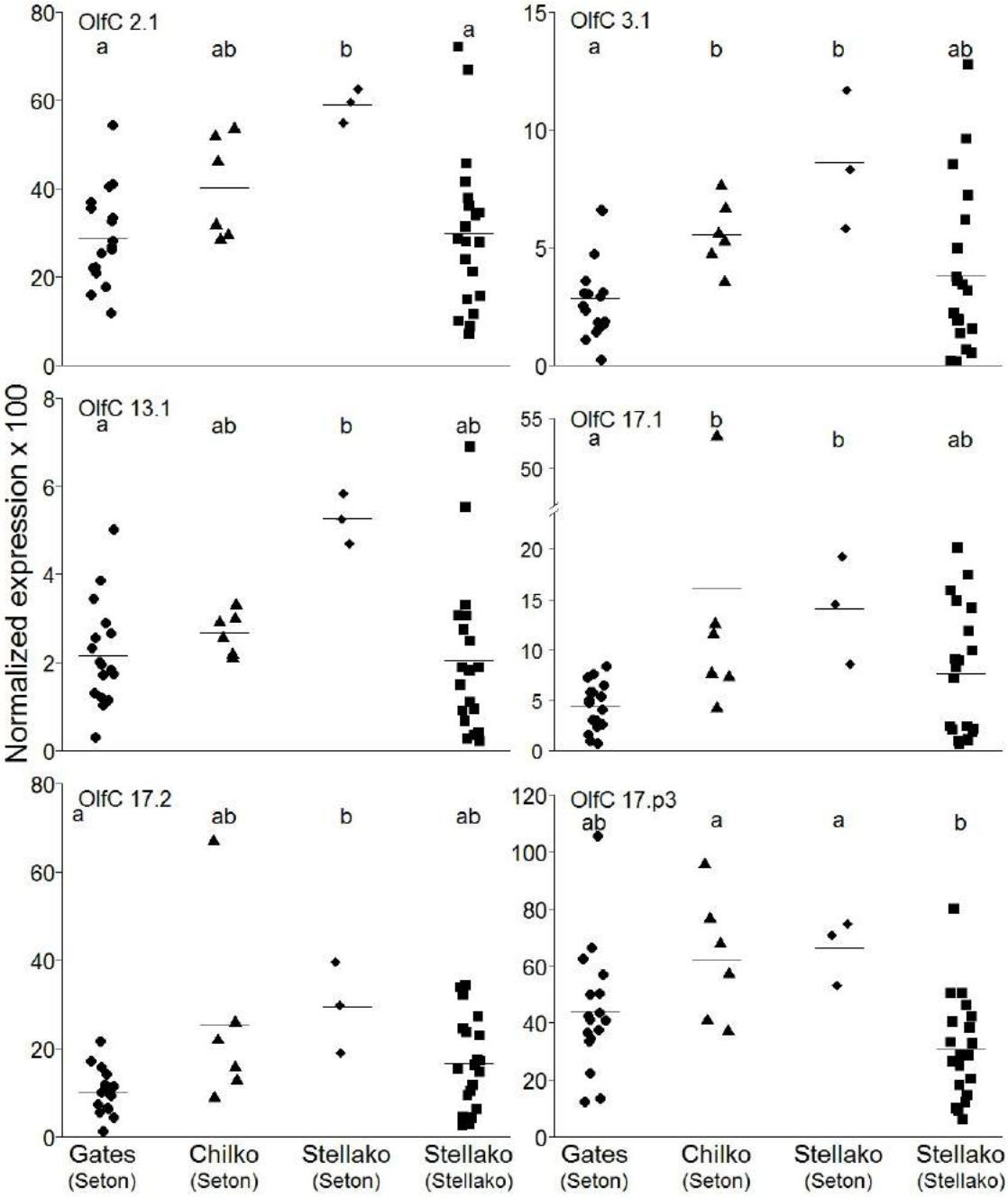


Table 3.1 Primer sequences for genes analyzed by quantitative real-time PCR analysis.

	Gene	Primer Direction	Sequence (5' to 3')	Reference
Olfactory genes	OlfC 2.1	Forward	TCC GGT TCT GCT CAG TCT ATT GTC G	Johnstone et al. 2011
		Reverse	TCA CCG AGG CAC GCG C	
	OlfC 3.1	Forward	ACA AGA GGA CAG CAG TGC CTC TTT T	Johnstone et al. 2011
		Reverse	CAT GGG GCA GTG GGC TCG AT	
	OlfC 4.5	Forward	TCA GAG GCT TGA CAT CGA GAG T	Johnstone et al. 2011
		Reverse	TCT CAC TGC ACA CTG ACA CAG G	
	OlfC 4.10	Forward	TAG AGT GTG ATG TGG GTT CAA	Johnstone et al. 2011
		Reverse	TGA AGT TAT CAG GCA GCT TCC G	
	OlfC 13.1	Forward	TGT CTG CTG CTT CGA CTG C	Johnstone et al. 2011
		Reverse	TGG AAC ACA GTG GTC TCT G	
	OlfC 16.1	Forward	TCA GAA CCA TCC CCA GTG ACG C	Johnstone et al. 2011
		Reverse	GAC CGG GCA GCG TAA ACT CCA TA	
	OlfC 16.2	Forward	CGA CTG CAT CTC CTG TGC TG	Johnstone et al. 2011
		Reverse	AGT CCT CTG GAC ATC TCA AGC	
	OlfC 17.1	Forward	CAG CTG TAT ATG CCA TTG CAC AT	Johnstone et al. 2011
		Reverse	TCA CCT CCT TCA GGT ACT GC	
	OlfC 17.2	Forward	AGA ATG ACA CAG ACA GCG GT	Johnstone et al. 2011
		Reverse	CCA GTT CAC TAG GTC GTA GC	
	OlfC 17.P3	Forward	GGC ATT TGA GCA GAC AGG TCC G	Johnstone et al. 2011
		Reverse	GGG CGC TGT GTC CCT CGA	

	Gene	Primer Direction	Sequence (5' to 3')	Reference
Olfactory genes	ORS	Forward	GCC TGG TTC TGC TTC TAA TGT TGG CAT GAG AAT GAT AGG G	Johnstone et al. 2011
		Reverse	CTT TCC CCT CCC GTT CTC T	
	SOIG	Forward	ACA CTC AAG TCC ATT GTG GG	Hino et al. 2007
		Reverse	GGA CGA CCA TTT TTG TCA GTC	
	CYP1A	Forward	AGT GCT GAT GGC ACA GAA CTC AA	Matsuo et al. 2008
		Reverse	AGC TGA CAG CGC TTG TGC TT	
	CYP2K1	Forward	CTC ACA CCA CCA GCC GAG AT	Matsuo et al. 2008
		Reverse	CTT GAC AAA TCC TCC CTG CTC AT	
	CYP2M1	Forward	GCT GTA TAT CAC ACT CAC CTG CTT TG	Matsuo et al. 2008
		Reverse	CCC CTA AGT GCT TTG CAT GTA TAG AT	
	CYP3A27	Forward	TCT GCT GAT GCC CAA ACG A	Matsuo et al. 2008
		Reverse	CGT TGT TGG ACT CTT CAG AGT GGT A	
Housekeeping genes	Beta-actin	Forward	GAC CCA CAC AGT GCC CAT CT	Matsuo et al. 2008
		Reverse	GTG CCC ATC TCC TGC TCA AA	
	78d16.1	Forward	GTC AAG ACT GGA GGC TCA GAG	
		Reverse	GAT CAA GCC CCA GAA GTG TTT G	
	MrpL40	Forward	CCC AGT ATG AGG CAC CTG AAG G	
		Reverse	GTT AAT GCT GCC ACC CTC TCA C	
	CoilP84	Forward	GCT CAT TTG AGG AGA AGG AGG ATG	
		Reverse	CTG GCG ATG CTG TTC CTG AG	

Chapter 4: Attraction of migrating adult sockeye salmon to conspecifics in the absence of natal chemical cues

4.1 Synopsis

The results of the gene expression analyses indicate there are differences in the olfactory responses of stray and non-stray salmon as they migrate upstream. Changes in olfactory gene expression, however, do not necessarily indicate changes in the salmon's behaviour. Following the finding that olfactory receptor genes that potentially bind to pheromones are up-regulated in stray sockeye salmon, I directly tested the behavioural responses of homing sockeye salmon to conspecific cues. As theorized in the Hierarchical Navigation Hypothesis (Chapter 2), I predicted that salmon are attracted to conspecific cues when imprinted natal cues are absent, but not when they are present.

4.2 Introduction

Natal philopatry, defined as an individual's return to its birthplace prior to reproduction, occurs in a variety of species (Greenwood 1980). Animals that exhibit such behaviour can benefit from local adaptation to their natal area or from the assurance of suitable habitat and potential mates upon arrival at breeding grounds (Hendry et al. 2004). They also avoid certain costs to leaving the natal area, such as 'risk costs' (e.g. movement into unsuitable habitat) and 'opportunity costs' (e.g. loss of familiarity-related advantages) (Bonte et al. 2012). There are consequences to natal philopatry, however, including increased competition and the risk of inbreeding depression (defined as a reduction in fitness of offspring resulting from inbreeding) (Lambin 1994). In contrast to natal philopatry is dispersal, which involves movement away from the natal area prior to reproduction (Howard 1960). Increased rates of dispersal have been

documented when population density (Matthysen 2005) and chance of inbreeding (Bollinger et al. 1993; Lambin 1994; Daniels and Walters 2000) are greater, suggesting that dispersal might help counter some of these drawbacks to natal philopatry.

Large-scale movement in animals, such as towards a natal site as in the case of philopatry, or away from it as in dispersal, is often directed by navigation using one or more sensory systems (Able 1991). Many species of anadromous fish, for example, are philopatric, and the use of sensory information as they navigate to spawning grounds, as well as the evolutionary implications of homing and dispersal, have been well documented (Hasler and Scholz 1983; Hendry et al. 2004). While navigation in the ocean requires complex coordination of multiple sensory systems (Harden Jones 1968; Dittman and Quinn 1996; Putman et al. 2014), anadromous fish rely primarily on their olfactory systems to navigate after entering freshwater (Stabell 1984; Ueda 2011). The use of olfactory navigation has been most thoroughly studied in the family Salmonidae (Chapter 2).

Research on anadromous salmonids has demonstrated "olfactory imprinting", a process that enables adults to identify their spawning grounds based on chemical cues (Hasler and Scholz 1983). The process begins when juveniles imprint on the chemical composition of their natal rearing site. Most evidence to date indicates that imprinting occurs primarily during the parr-smolt transformation (PST), a period when juveniles undergo substantial physiological change in preparation for the outward migration and entrance into saltwater (Groot and Margolis 1991). Upon return into freshwater during the spawning migration, adult migrants recall the imprinted chemical cues and swim towards them. In this manner, anadromous salmonids, and perhaps other

species of anadromous fish (e.g. Dodson and Leggett 1974; Sahafi 2013), can locate their natal sites with high precision (Quinn 2005).

While imprinting is known to occur in various different species of salmonids, an alternative hypothesis has also garnered attention. Nordeng (1971) proposed that these fish use the smell of conspecifics as directional cues. Juveniles often rear in freshwater before migrating to the ocean, and Nordeng suggested that adults might be attracted to the juveniles as they swim upstream, similar to the manner in which northern lamprey (Petrmoyzontidae) use larval pheromones as directional cues during the spawning migration (Moser et al. 2015). Known as the "pheromone hypothesis", the use of pheromones as directional cues could provide a simpler method of navigation when contrasted with olfactory imprinting (Nordeng 1977). As other researchers have noted (Selset and Døving 1980; Keefer and Caudill 2014), however, these two theories are not mutually exclusive: it is possible that conspecific cues comprise part of the "olfactory bouquet" that juveniles imprint on. Limiting the role of pheromones strictly to this interpretation (one component of the imprinted odor) would suggest that pheromones are only attractive when combined with the other components of the bouquet. There is evidence, however, that adult migrants are attracted to pheromones in the absence of other natal cues (Stabell 1984; Chapter 2). This implies that pheromones may act as a migratory directional cue even in isolation.

While few studies have attempted to address the relative importance of imprinted cues compared to conspecific cues, there is evidence that the attraction towards imprinted cues supersedes that of pheromones (Brannon et al. 1984; Black and Dempson 1986; Brannon and Quinn 1990). Pheromones, however, may act as a "secondary" cue, providing directional

information in the absence of imprinted, or "primary", cues. This method of olfactory navigation has been proposed as the hierarchical navigation hypothesis (Chapter 2). Studies have found that adult salmonids that stray from their natal migratory route enter rivers containing conspecifics more frequently than rivers without (Jonsson et al. 2003; Dittman et al. 2010), and that coho salmon (*Oncorhynchus kisutch*) are attracted to juvenile conspecifics when tested in city water (Quinn et al. 1983), suggesting that pheromones may indeed attract migrants when natal imprinted cues are absent.

These findings suggest a more refined olfactory navigation process than is typically discussed in salmonid research, with an adaptation that may be influenced by its effect on dispersal success. Since migrants that stray from their natal route are likely to attempt to spawn in new areas, an attraction to pheromones would increase the likelihood of successful dispersal by ensuring suitable habitat and the presence of potential mates. In addition to increasing the fitness of these stray migrants, the attraction to conspecifics could promote gene flow between populations, thereby reducing inbreeding depression. To date, however, there have been few direct tests on the potential "secondary" role of pheromones as directional cues.

The objective of this study is to determine whether adult sockeye salmon (*Oncorhynchus nerka*) that have strayed from their natal water will use conspecific odors as directional cues. To test this, I conducted a pair of behavioural choice experiments in which conspecific odors were introduced in one of the two following types of water: 1) natal water, which contains imprinted chemical cues, or 2) non-natal water, lacking the imprinted chemical cues. Following the hierarchical navigation hypothesis, I predicted that adult migrating sockeye salmon would be attracted to conspecific odors when placed in non-natal water, but not when in natal water.

4.3 Methods

4.3.1 *Study location and animals*

Experiments took place on the north bank of the Seton River, a tributary of the Fraser River, in the interior of southwestern British Columbia, Canada (Fig 4.1). They were conducted from August 14 to August 28 2014, during daylight hours (0700 – 1700). All sockeye salmon (*n* = 85) were captured by dipnet from the top pool of the Seton Dam fishway, located on the Seton River, and immediately transferred to an aerated 1,000 L transport tank on the back of a truck. The fish were driven approximately 100 m and unloaded them in a 10,000 L holding tank adjacent to the dam, with continuous flow of water from Seton River. Each fish was held in an individual isolation chamber, constructed from PVC pipe (75 cm length x 15.3 cm diameter) with mesh ends.

I tested Gates Creek sockeye salmon, which spawn approximately 55 km upstream from the capture location in the Seton River. These fish were therefore tested during the migratory phase of their life history, one to two weeks prior to arrival at spawning grounds and sexual maturation. Straying, or dispersal from the natal tributary, naturally occurs during the spawning migration of Pacific salmon (*Oncorhynchus* spp.), and sockeye salmon from disparate populations sometimes enter the Seton River system. To identify stray salmon captured for my experiments, a microwave energy metre (FM 692 Fish Fatmeter, Distell, Scotland, UK) was used to estimate gross somatic energy. Gates Creek sockeye salmon have gross somatic levels that are significantly lower than those of other populations bound for more northern locations but which can stray into the Seton River system (Casselman et al. 2012). DNA analysis of 64 stray sockeye salmon captured in the Seton River in 2012 (Casselman et al. 2012) and 2013 (unpublished)

determined that all originated from more northern populations up the Fraser River. The differences in gross somatic energy are likely attributed to the differences in distance to spawning grounds, as Fraser sockeye salmon with longer and steeper migration routes begin their migration with more somatic energy and expend less per unit of migration distance (Crossin et al. 2004). Furthermore, longer distance migrants in the Fraser system have a more fusiform body shape (Crossin et al. 2004), and are more silver upon arrival at Seton River when compared to Gates Creek sockeye salmon. Following the trials, DNA samples from the adipose fins of all fish that were abnormal in any of the three variables (GSE, body shape, or color) were analyzed at the Fisheries and Oceans Canada Pacific Biological Station (Nanaimo, BC) to determine their population identity (Beacham et al. 2005).

4.3.2 *Experimental set-up*

Water was stored in 11,365 L polyethylene "header" tanks (Premier Plastics Inc., Delta, BC), and the water was gravity fed through 2" diameter water suction hoses (Greenline, Delta, BC) to two 1,136 L polyethylene "mixing" tanks (Premier Plastics Inc.) (Fig 4.2). Water was gravity fed from each tank through 4" diameter water suction hoses into a Y-maze. A Y-maze was constructed from plywood and 2x4 supports, and sealed the interior with fiberglass and a fish-safe gelcoat (Rebel Fiberglass, Kamloops, BC). The Y-maze was rectangular in shape, 4.88 m long x 1.22 m wide x 1.22 m high. A 2.44 m divider, made from fiberglassed plywood, divided the upstream end into two equally sized halves (or two "arms"). I conducted a dye test to ensure no mixing occurred between water in each of the two arms. Water exited the Y-maze through a standpipe, and the water level was maintained at 17 cm. Valves regulated the amount

of water entering each arm of the Y-maze to 40 L/min. Plywood was placed on top of the Y-maze to block out light, and to reduce stress in the fish.

Water for the "natal water" experiment was obtained from the Seton River, using a submersible pump. Water for the "non-natal water" experiment was obtained from nearby Cayoosh Creek. Cayoosh Creek does not contain any spawning populations of Pacific salmon, due to an impassable barrier approximately 1 km upstream from the collection site, and I did not see any transient salmon in the creek during the time of the experiment. Water was transported from Cayoosh Creek to the header tanks using a gas-powered pump with 2" water suction hose and a 1,500 L transport tank on the back of a truck. Temperatures throughout the entire experimental period ranged between 16.9-20.4°C for Seton River water and 16.1-19.4°C for Cayoosh Creek, and the two arms were always within ± 0.2°C during any given trial. The optimal migratory temperature window for adult Gates Creek sockeye salmon is 12.9-20.7°C (Eliason Parsons 2011).

4.3.3 *Experimental protocol*

Sockeye salmon were captured in the morning of each experimental day, and 7-10 fish were transported to the holding tank, to be later tested in the Y-maze. Concurrently, three additional sockeye salmon (two females and one male, or one female and two males) were captured and immediately sacrificed by cerebral percussion. These sockeye salmon were then transferred to one of the two mixing tanks, where they acted as the source of conspecific odors during the trials. Experiments using live sockeye salmon in the mixing tanks demonstrated that these fish emit a stress-related chemical signal that triggers an avoidance response from conspecifics (Chapter 5), therefore live Gates sockeye salmon were unsuitable for this aspect of

the study. All of the sockeye salmon selected to provide the conspecific odor source were free of any visible tissue damage, to further minimize the possibility of alarm signals. Water containing the conspecifics was paired with water lacking the conspecifics, such that each water type entered a different arm of the Y-maze. The arm containing water with conspecifics was switched each day to mitigate any potential behavioural bias for one of the arms, although control tests using the same set-up demonstrated no bias (Casselman et al. 2013).

At the start of each trial, a single sockeye salmon was transferred directly from the holding tank to the downstream end of the Y-maze. In this manner, sockeye salmon were tested individually. A mesh gate prevented fish from entering either of the upstream arms. After a 10 minute acclimation period, the gate was removed and the behaviour of the fish was recorded for 20 minutes. Behaviour was monitored through a video system, using an Infrared camera (securitycamera2000.com, Hong Kong) connected to a monitor. I recorded the following behavioural variables: the amount of time spent in each arm, the number of entrances into each arm, and the number of times the sockeye salmon surfaced (breached the surface of the water with its head) at the upstream end of each arm, where water flowed in. The latter behaviour, which has been used in past behavioural choice experiments (e.g. Groot et al. 1986) was selected following observations made during previous experiments. Surfacing into the water entering the Y-maze was often immediately followed by attempts to jump into the intake hose, and appears to indicate a desire to swim further in this direction. I also calculated the proportion of time spent in the arm containing the conspecific odor using the following equation:

$$\frac{t\ arm\ wih\ odor}{t\ arm\ with\ odor + t\ arm\ without\ odor}.$$

At the end of the 30 minute trial, the fish were removed from the Y-maze and blood and DNA samples were collected, then the fish was returned to the river. The Y-maze was flushed before the introduction of the next test fish.

The research conformed to protocols approved by the University of British Columbia Committee on Animal Care (A12-0250-006) and met the Canadian Council for Animal Care Guidelines.

4.3.4 *Data analysis*

A Shapiro-Wilk normality test was used for each of the variables collected. The amount of time spent in each arm and the number of entrances in each arm were compared using paired t-tests ($\alpha = 0.05$). The proportion of time spent in the arm with the conspecific odor was evaluated using a one sample t-test ($\mu = 0.5$, $\alpha = 0.05$). The number of surfaces in the upstream end of each arm, which had a non-normal distribution, were compared using a Wilcoxon signed-rank test ($\alpha = 0.05$). All fish that did not enter each arm at least once during the trial were removed from the analysis as these fish did not experience a full concentration of each of the waters, and therefore could not exhibit choice or preference behaviours. I ran all statistical analyses in R Studio V 0.98.501.

4.4 Results

Three sockeye salmon in the natal water experiments and two sockeye salmon in the non-natal water experiments were identified as strays, and were removed from analyses. Eight sockeye salmon (four from each of the natal and non-natal water test) did not enter both arms and were removed from analyses. Of these eight fish, two did not enter either arm at all, and

three entered only very briefly (< 30 s). The remaining three entered one arm but then remained motionless.

When tested in non-natal water, sockeye salmon spent significantly more time (t_{28} = 2.45, P = 0.021; Fig 4.3a) in the arm with the conspecific odor. The trend remained similar in terms of the proportion of time in the arm with the conspecific odor (t_{28} = 1.85, P = 0.076; Fig 4.4) when tested in non-natal water. They did not, however, spend more time (t_{42} = -1.06, P = 0.295; Fig 4.3b), nor did they spend a greater proportion of time (t_{42} = -1.06, P = 0.293; Fig 4.4) in either arm when tested in their natal water.

There were also significantly more surfaces at the upstream end of the arm with conspecific odor in non-natal water tests (V = 30.5, n = 29, P = 0.017; Fig 4.5a), but not in natal water tests (V = 314.0, n = 43 P = 0.555; Fig 4.5b). The sockeye salmon did not enter either arm more frequently in non-natal water (t_{28} = 0.85, P = 0.401), nor in natal water (t_{42} = -0.95, P = 0.348).

Since natal imprinted cues were absent in the non-natal water experiments for all sockeye salmon, regardless of whether they are Gates sockeye salmon or strays, I also analyzed the results from this test with the two stray sockeye salmon included. In this analysis, the results were similar, although the preference for conspecific odors was more exaggerated: the sockeye salmon spent significantly more time (t_{30} = 2.86, P < 0.01) and a significantly greater proportion of time (t_{30} = 2.31, P = 0.028) in the arm with the conspecific odor when tested in non-natal water.

When sexes are analyzed separately, the trends remained similar for both males and females. In non-natal water, females spent significantly more time in the arm containing the

conspecific odor (t_{16} = 2.604, P = 0.019), as well as a greater proportion of time (t_{16} = 2.45, P = 0.026) in that arm. Males also appeared to spend more time (t_{13} = 1.298, P = 0.217) and a greater proportion of time in the arm containing the conspecific odor (t_{13} = 0.75, P = 0.470), although these differences were not significant at α = 0.05, which might be attributed to the small sample size. Both sexes surfaced at the upstream end of the conspecific arm more frequently, although the difference was not significant at α = 0.05 (females: V = 47.5, n = 16, P = 0.916; males: V = 8, n = 14, P = 0.672). In natal water, there were no significant differences nor any apparent trends in any of the behavioural variables in females (time in each arm: t_{21} = -0.18, P = 0.859; proportion of time in arm with conspecific odor: t_{21} = -0.28, P = 0.784; number of surfaces in the upstream end of each arm: V = 28.5, n = 22, P = 0.959). In males there were similarly no significant differences in any of the behavioural variables, although there was a slight trend towards an avoidance of the arm containing the conspecific odor (time in each arm: t_{20} = 1.51, P = 0.148; proportion of time in arm with conspecific odor: t_{20} = -1.39, P = 0.179; number of surfaces in the upstream end of each arm: V = 77, n = 21, P = 1).

4.5 Discussion

In terms of the amount of time spent, proportion of time spent, and number of surfaces in each arm, sockeye salmon showed an attraction to their conspecifics when they were tested in non-natal water, but not when they were tested in natal water. The attraction may be stronger in females, although further tests with a larger sample size would be needed to conclusively determine whether there is a sex-specific response. The results suggest that sockeye salmon do not use conspecific odors as a directional cue when imprinted chemicals are present, but do use conspecific odors as a directional cue when the imprinted chemicals are absent. This finding

supports the hierarchical navigation hypothesis, which proposes that philopatric, anadromous fish primarily seek imprinted cues, and secondarily seek conspecific cues during the spawning migration.

Direct comparison of the behaviours in the two water treatments highlights differences that may further reflect the relative importance of imprinted and conspecific cues. The sockeye salmon spent on average more time in the rear section of the Y-maze in non-natal water (mean ± standard error: 645 ± 37 s) than they did in natal water (505 ± 28 s). In addition, the sockeye salmon surfaced more frequently overall in the natal water than in the non-natal water (Fig 4.5). Together, these discrepancies suggest sockeye salmon tested in natal water were more strongly attracted to water entering the Y-maze than were sockeye salmon tested in the non-natal water, despite the addition of conspecific odor. From a behavioural perspective, it seems reasonable that sockeye salmon might be more motivated to swim towards imprinted chemicals, which indicate natal spawning grounds, than towards conspecific cues in unfamiliar waters. It is possible that conspecific cues act not only as secondary directional cues for strays, but also that they do not elicit as strong an attractive response, possibly because the stray fish are less confident in their navigational movements.

The two stray sockeye salmon tested in non-natal water showed a particularly strong preference for the conspecific odor: one spent 349 s in the arm with the conspecific odor and 33 s in the arm without (91% in the conspecific odor), and the other spent 512 s in the arm with the conspecific odor and 191 s in the arm without (73% in the conspecific odor). The seemingly strong preference behaviour in these fish may be attributed to the fact that they are ‘true’ strays and have never experienced the ‘non-natal’ Cayoosh Creek water, whereas Gates Creek sockeye

salmon were exposed to it briefly during their outmigration. In an unrelated research project, conducted in the same system in 2012, I found further evidence that stray salmon can be attracted to conspecifics in non-natal water. I placed 20 sockeye salmon in individual isolation chambers in Cayoosh Creek, at the same location where the "non-natal water" was collected in 2014 (Fig 4.1). No conspecifics were seen in the creek, which is not salmon-bearing, during the two weeks prior to this event. The salmon were held overnight, and the following morning I counted more than 50 freely-swimming sockeye salmon adjacent to, and downstream from, the isolation chambers. Four of these freely-swimming sockeye salmon were captured and biopsied, and DNA analyses (Beacham et al. 2005) determined all were strays (i.e. not from the Gates Creek population). Further behavioural tests, using completely unfamiliar waters, are needed to determine whether strays are as strongly attracted to conspecifics as these findings suggest.

In order to best interpret these results, as well as results from past studies, it is important to distinguish between an attraction to pheromones, and an attraction to a combination of imprinted cues that includes pheromones. It is believed that adult salmonids migrate towards an imprinted mixture of chemicals present in their natal water (Ueda 2011), and that this mixture could include pheromones (Keefer and Caudill 2014). In a laboratory setting, juvenile salmon can be imprinted on an individual chemical, such as an amino acid (Yamamoto et al. 2010) or morpholine (Hasler and Scholz 1983), and are attracted to this chemical as adults. There is currently no evidence, however, that salmon can imprint to a mixture of chemicals and subsequently be attracted to only one, except for pheromones, which can elicit an attractive response even when isolated from the rest of the imprinted mixture (summarized in Stabell 1984). In my natal water experiment, I tested the response of sockeye salmon to natal water, presumably containing its regular background concentration of conspecific cues, to natal water

containing a higher level of conspecific cues. It is not feasible to remove background cues from large volumes of natal water, nor would the effects of this removal accurately represent the choice a fish might make in a natural system. I therefore assumed that an attraction to pheromones in natal water, beyond that which comprises one component of the imprinted mixture, would be evidenced by an attraction to the arm containing the increased conspecific odor.

Past studies indicate stray migrants are attracted to conspecifics, in support of my findings. Quinn et al. (1983) and Quinn and Tolson (1986) found that adult and juvenile coho are attracted to juvenile conspecifics in non-natal water, and Groot et al. (1986) found similar results with sockeye salmon. Dittman et al. (2010) conducted a broad scale analysis of spawning distribution of Chinook salmon (*Oncorhynchus tshawytscha*) in the Yakima River, a tributary of the Columbia River, and found that strays tend to enter rivers occupied by conspecifics. Jonsson et al. (2003) recorded a similar result—a tendency of strays to enter rivers containing conspecifics—with Atlantic salmon (*Salmo salar*) in Norway. White (1934) documented adult Atlantic salmon migrating towards conspecifics of a different population, and suggested this attraction to conspecifics occurred after confusing flow patterns disoriented the fish and prevented them from detecting their natal water. In the study that introduced the pheromone hypothesis (Nordeng 1971; see Introduction), Arctic char migrated towards conspecifics from their own population after being raised in a distant hatchery, with no "natal water" to imprint on. The char in that study may, in a sense, be considered strays, as they were deprived of the opportunity to imprint on their ancestral home stream. Together, these studies support my finding in suggesting salmonids may use pheromones as a secondary directional cue during the spawning migration.

Straying occurs regularly in all species of Pacific salmon (Keefer and Caudill 2014)—as well as in other anadromous salmonids (Jahn 1969; Hendricks and Hoopes 2002; Jonsson et al. 2003)—and a secondary attraction to pheromones may occur in many of these fish, particularly if they are attracted to co-migrating adults, as demonstrated in my experiment. One source of confusion regarding Nordeng's (1971) pheromone hypothesis is that juvenile chum salmon (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) (as well as some populations of other species) swim to the ocean before the spawning migration occurs. These fish presumably do not encounter juvenile pheromones during the spawning migration, yet most behavioural choice studies have focused on the attraction of salmon to juvenile conspecifics (e.g. Quinn et al. 1983; Brannon et al. 1984, Groot et al. 1986, Quinn and Tolson 1986). By demonstrating an attraction towards conspecific adults, my findings suggest that a secondary attraction to conspecific cues may occur in any species of Pacific salmon, including pink and chum salmon.

There is a major evolutionary benefit to using conspecific cues as a secondary directional cue that might explain how this adaptation arose: it would increase the probability that stray migrants find suitable spawning habitat and successfully spawn. Animals that are philopatric may adapt to the environmental conditions of their natal habitat, and dispersal from this area reduces fitness (Weatherhead and Forbes 1994; Hendry et al. 2004). Salmon require highly specific environmental conditions to spawn successfully, such as adequate flow, temperature and substrate, and these requirements vary across species (Groot and Margolis 1991). Entering tributaries that are occupied by conspecifics not only ensures the presence of potential mates, but also that species-specific habitat requirements are met. Additionally, the use of conspecific cues could decrease the amount of time spent searching for suitable spawning habitat, and therefore reduce search-related movement costs (Stamps et al. 2005b). Adult salmon migrate upstream on

a fixed energy budget because they are no longer eating, and longer migratory times result in high energetic costs (Hinch and Rand 1998). Stray fish would therefore benefit from an attraction to conspecifics because it would increase their probability of reproductive success by leading them to suitable spawning habitat and reducing search-related movement costs.

The adaptation of such migratory behaviour, which improves the reproductive success of stray migrants, could also promote an increase in genetic diversity. Philopatric animals can be susceptible to inbreeding depression due to the lack of genetic mixing between populations (Lambin 1994; Gandon 1999; Hendry et al. 2004), but inbreeding depression can be partially avoided through dispersal (Greenwood 1980; Bollinger et al. 1993; Pusey and Wolf 1996; Szulkin and Sheldon 2008). The increased genetic diversity afforded by higher reproductive success of stray migrants could help reduce the threat of inbreeding depression.

Although rarely discussed in salmonid research, many studies have explored the use of conspecific information in the selection of breeding habitat, usually under the title of "social attraction" or "conspecific attraction" (see reviews by Stamps 1988; Reed and Dobson 1993). Much of this research has focused on birds, including both colonial and territorial species (e.g. Muller et al. 1997; Serrano et al. 2004; Ward and Schlossberg 2004). Conspecific attraction has been studied in other animals as well, such as mammals (Garrett and Franklin 1988; Smith and Peacock 1990; Weddell 1991), reptiles (Kiester 1979; Stamps 1991) and invertebrates (Gotceitas and Clifford 1983; Stamps et al. 2005a). In fish, there is evidence that larval coral reef fish are attracted to reefs containing conspecifics during the settlement stage (Öhman et al. 1998; Lecchini et al. 2007).

Unlike most of these animals that demonstrate conspecific attraction, salmon typically return to their natal habitat and therefore have little need to assess habitat quality when searching for spawning grounds. As a consequence, research on salmon pheromones as navigational cues has focused solely on their role as indicators of natal water. My findings, however, suggest stray salmon may use conspecific information to select suitable non-natal reproductive sites, similar to what has been documented in other animals. Future studies that assess the response of strays to conspecific cues will further our understanding of the navigational abilities of migrating salmon outside their natal migratory routes.

Fig 4.1 Study area, located in the Fraser River system in British Columbia, Canada (inset). Sockeye salmon were captured in the Seton River at location "a", approximately 55 km downstream from their spawning grounds in Gates Creek. This was also the site of the experiments, and the site of water collection for "natal water" experiments. Water in the "non-natal water" experiments was collected from Cayoosh Creek, at location "b".

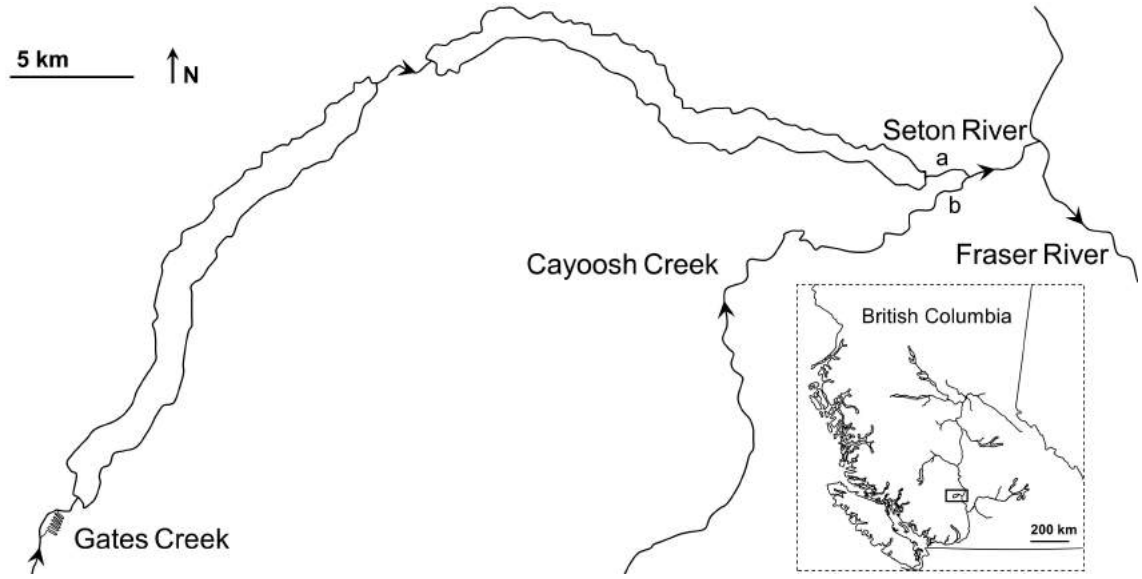


Fig 4.2 Experimental set-up for behavioural choice tests. Gravity-fed water passed from large header tanks (A) to smaller mixing tanks (B), where sockeye salmon that provided the source of conspecific odor were held. Water then continued into each arm of the Y-maze (C) at 40 L/min.

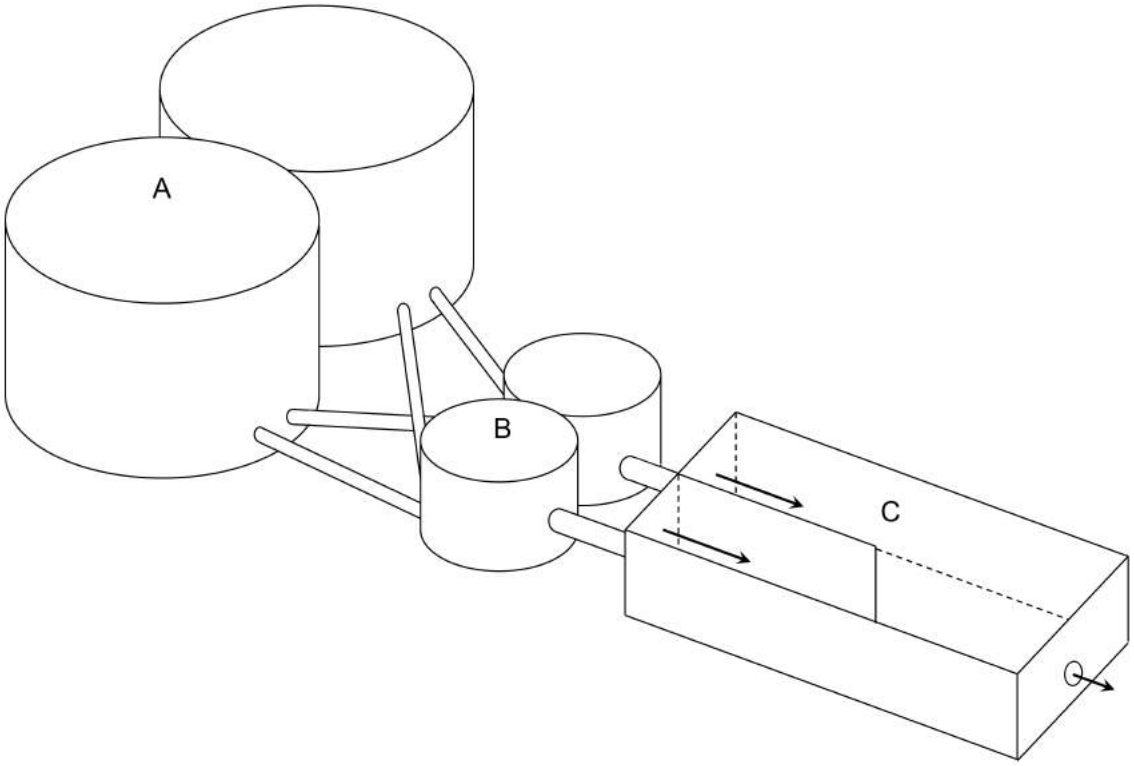


Fig 4.3 Amount of time spent by sockeye salmon in each of the two arms when tested in a) non-natal water, which does not contain imprinted chemical cues from spawning grounds (n = 29), and b) natal water, which does contain imprinted chemical cues from spawning grounds (n = 43). In each test, one arm contained the conspecific odor, while the other did not. * $P > 0.05$.

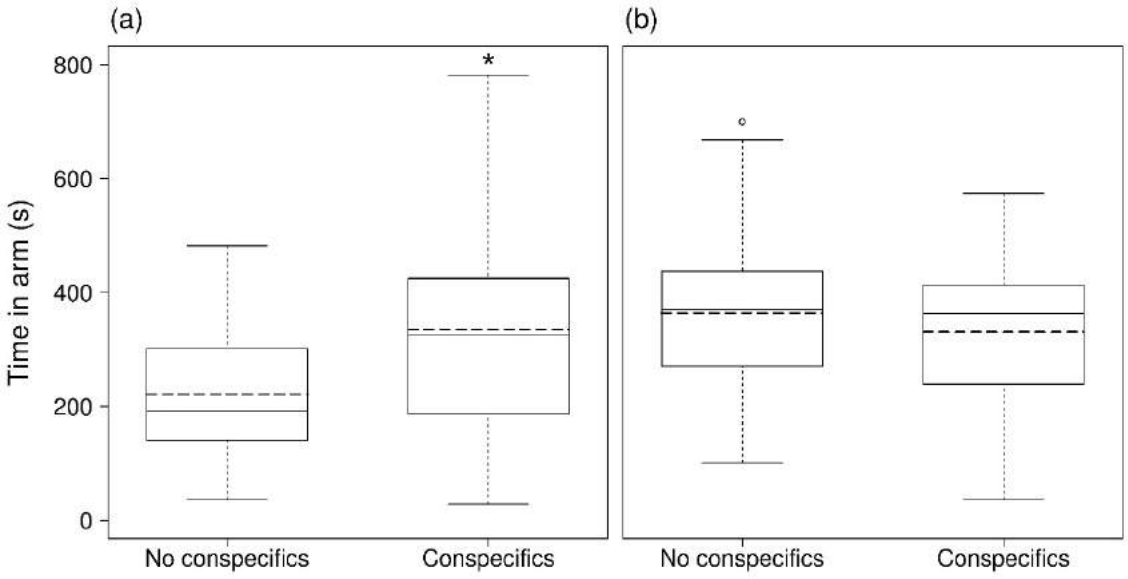


Fig 4.4 Proportion of time spent by sockeye salmon in the arm containing the conspecific odor when tested in non-natal water, which does not contain imprinted chemical cues from spawning grounds (n = 29), and when tested in natal water, which does (n = 43). The solid line designates 0.5, and '+' indicates a significant difference at P = 0.076).

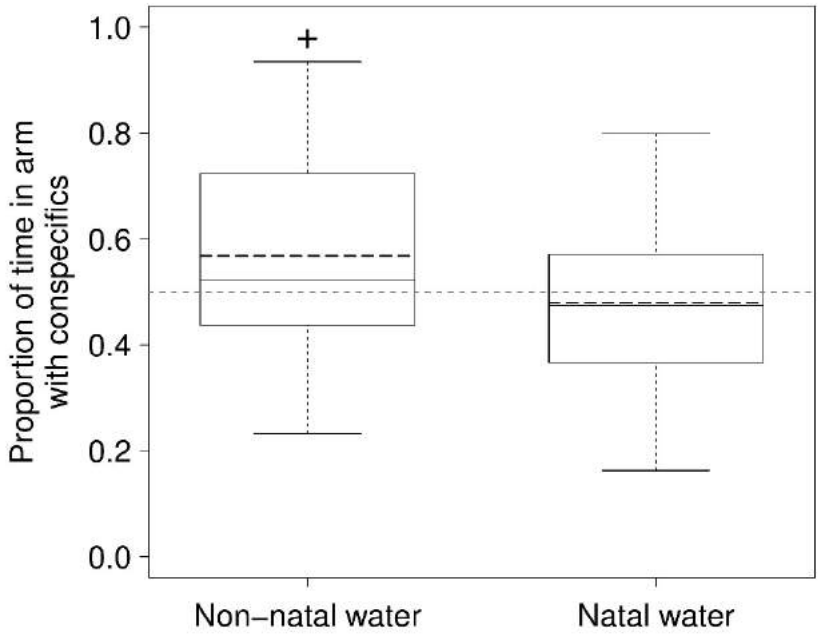
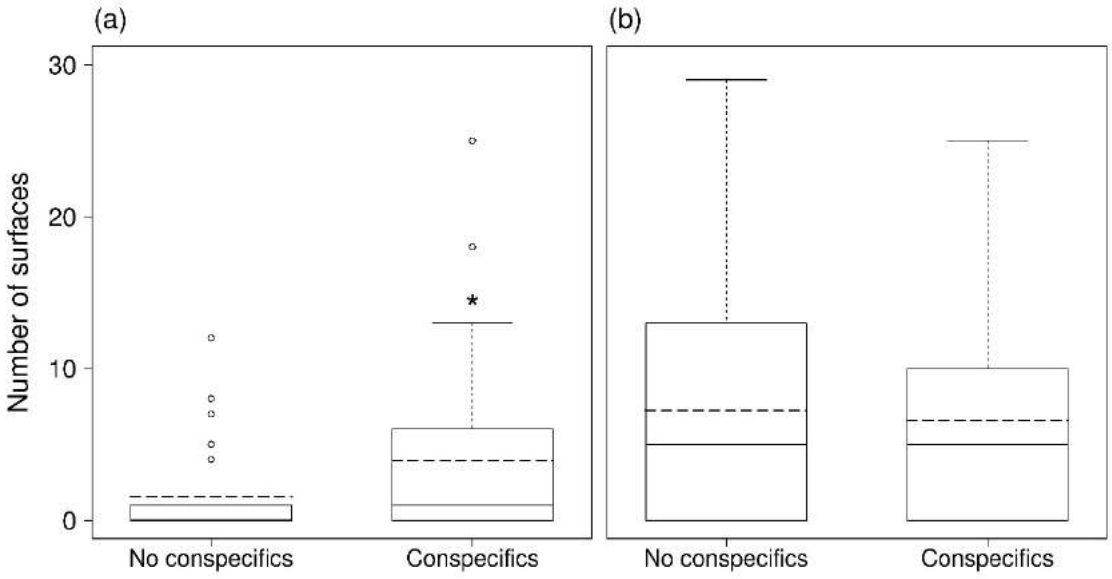


Fig 4.5 Number of surfaces per sockeye salmon at the upstream end of each arm when tested in a) non-natal water, which does not contain imprinted chemical cues from spawning grounds (n = 29), and b) natal water, which does (n = 43). In each test, one arm contained the conspecific odor, while the other did not. * $P < 0.05$.



Chapter 5: Behavioural responses of Pacific salmon to chemical disturbance cues during the spawning migration

5.1 Synopsis

Chapters 3 and 4 provide evidence that pheromones may act as directional cues for salmon that have strayed from their natal migratory route. The chemical environment provides much more information to a migrating salmon than directional cues, however, including the chemical cues released by conspecifics. For the first time, I test for the release of disturbance cues by adult salmon, which face many stressors during their spawning migration that could elicit the release of these cues. The results indicate that disturbed sockeye salmon release disturbance cues that trigger an avoidance response in conspecifics. Pink salmon, however, do not, which could reflect variation in the ecology and behaviour of the two species.

5.2 Introduction

In the aquatic environment, many animals use chemical cues as indicators of danger (Liley 1982; Smith 1992). One category of these chemical cues is "disturbance cues", which are released when danger is detected but no physical damage is incurred (Wisenden 2000; Ferarri et al. 2010), as opposed to the more commonly studied "damage-released cues" (Mathis et al. 1995; Wisenden 2000; Brown 2003) that result from predator-induced injuries. Disturbance cues appear to function as an early warning, eliciting moderate levels of behavioural and physiological responses in conspecifics (Wisenden 2015), including avoidance (Jordão and Volpato 2000), increased vigilance (Wisenden and Barbour 2005), decreased foraging (Giaquinto and Hoffman 2012), and increased plasma cortisol levels (Barcellos et al. 2011, 2014). They can also act as a primer for anti-predator behaviours in response to subsequent cues

(Ferrari et al. 2008; Vavrek et al. 2008). These cues have been documented in various fish species, including tilapia and catfish (*Oreochromis niloticus* and *Rhamdia quelen*, respectively; Barcellos et al. 2011), zebrafish (*Danio rerio*; Barcellos et al. 2014), and pacus (*Piaractus mesopotamicus*; Jordão and Volpato 2000).

Damage-released chemical cues have been well demonstrated in Pacific salmon and trout (*Oncorhynchus* spp.; e.g. Brown and Smith 1997; Berejikian et al. 1999; Scholz et al. 2000; Mirza and Chivers 2003; Scott et al. 2003; Tierney et al. 2006; McIntyre et al. 2012), as have disturbance cues (Toa et al. 2004; Ferrari et al. 2008; Vavrek et al. 2008; Brown et al. 2012). These studies, however, were conducted on juvenile salmonids reared in a hatchery or laboratory. To my knowledge, none have tested wild adult salmon during their spawning migration, despite the abundance of threats or stressors that migrating adult salmonids face. Stressors include predation risk, migration barriers (e.g. dams, rapids), and capture-and-release from commercial, recreational or subsistence fisheries. Avoidance of disturbance cues could limit exposure to such stressors, increasing migratory—and therefore spawning—success.

In addition to an absence of information on wild adult salmonids, the chemical identities of disturbance cues remain largely unknown (Wisenden 2015). There is evidence that they can be metabolites (Lebedeva et al. 1993) and that they may in part comprise ammonia (Kiesecker et al. 1999, but also see Vavrek et al. 2008) or urea (Brown et al. 2012). Plasma cortisol levels increase in response to danger, such as following a simulated stressor (e.g. chasing fish with a net or similar apparatus to simulate predation risk; Toa et al. 2004; Ellis et al. 2007; Barcellos et al. 2011; Donaldson et al. 2014), and excess cortisol is released into the water through the gills (Ruane and Komen 2003; Ellis et al. 2004; Ellis et al. 2007; Wong et al. 2008; Zuberi et al.

2011). Fish can detect released corticosteroids (Stacey 2015), and it is possible that cortisol or other endogenous correlates of stress may not only influence the release of disturbance cues, but could also act as cues themselves (Wisenden 2015).

Within the *Oncorhynchus* genus, species appear to differ in their sensitivity to olfactory cues. For example, sockeye salmon (*O. nerka*) have very high natal site fidelity (Keefer and Caudill 2014), whereas the closely related pink salmon (*O. gorbuscha*), whose distribution overlaps with sockeye salmon, stray more frequently to non-natal areas. One explanation for this difference may be that pink salmon spend considerably less time imprinting on freshwater cues prior to smolting and migrating to the ocean compared to sockeye salmon, although there is some evidence that sockeye salmon may additionally have greater olfactory sensitivity. Yamamoto et al. (2008) tested the responses of both species to mixtures of amino acids, believed to be a major component of the olfactory cues that signify natal water (Ueda 2011). The authors exposed the fish to amino acid mixtures that mimicked the amino acid profile of their natal water, and found a much stronger attraction response in sockeye salmon relative to pink salmon. A stronger olfactory sensitivity to migratory cues could be associated with a stronger sensitivity to other chemicals, such as disturbance cues, though this has not been investigated (reviewed, Chapter 2).

In this study I examined whether wild adult sockeye and pink salmon exhibit avoidance responses to the odours of disturbed conspecifics. I subjected salmon to a disturbance consisting of a handling event, and then measured the effect of their odours on the behaviour of conspecifics. I predicted that sockeye and pink salmon would avoid the odour of disturbed conspecifics. Additionally, we tested the response of sockeye salmon to water-borne cortisol to

determine whether this chemical, which could be excreted through the gills of disturbed salmon, might act as a disturbance cue.

5.3 Methods

5.3.1 *Study location and animals*

The experiments took place on August 18-23 and September 17-23 of 2013, and August 16-25 and October 7-9 of 2014. They were conducted on the north bank of the Seton River, a tributary of the Fraser River, in the interior of southwestern British Columbia, Canada. Sockeye salmon in this river system spawn in Portage Creek and Gates Creek, located 25 km and 55 km upstream from the capture site, respectively. Pink salmon spawn throughout the watershed, including the area surrounding the capture location. Both species must migrate approximately 300 km up the Fraser River from the ocean to reach the Seton River (the study site has been further described by Roscoe et al. [2011] and Burnett et al. [2014]). All sockeye and pink salmon were captured by dipnet from the top pool of the Seton Dam fishway, located on the Seton River 5 km from the Seton-Fraser confluence. Captures all took place during the peak of the respective populations' runs. The experimental set-up was located approximately 100 m from the capture location.

5.3.2 *Experimental set-up*

Submersible pumps were used to move water from the Seton River into two 11,365 L polyethylene head tanks (Premier Plastics Inc., Delta, BC), from which the water was gravity fed through 2" diameter water suction hoses (Greenline, Delta, BC) to two 1,136 L polyethylene source tanks (Premier Plastics Inc.). Water was gravity fed from each source tank through 4" diameter water suction hoses into a Y-maze (Fig 5.1). The Y-maze was constructed from

plywood and wood supports, and sealed the interior with fiberglass and a fish-safe gelcoat (Rebel Fiberglass, Kamloops, BC). The Y-maze was rectangular in shape, 4.88 m long x 1.22 m wide x 1.22 m high. A 2.44-m-long divider, made from fiberglassed plywood, divided the upstream end into two equally sized halves (or two "arms"). I conducted a dye test to ensure no mixing occurred between the two arms. Water exited the Y-maze through a standpipe, and the water depth was 17 cm. Valves were used to maintain the amount of water entering each arm at 40 L min^{-1}. Plywood was used to cover the top of the Y-maze. Behaviour was monitored through a video system, using an infrared camera (securitycamera2000.com, Hong Kong) connected to a monitor.

5.3.3 *Experimental protocol*

The experiments were conducted during daylight hours (0700 – 1700). At the beginning of each day, 8-12 sockeye or pink salmon were captured and immediately transferred to an aerated 1,000 L transport tank, which was driven to the experimental set-up. The salmon were held in individual isolation chambers, constructed from PVC pipe (75 cm length × 15.3 cm diameter) with mesh ends, which were placed inside a 10,000 L holding tank receiving a continuous flow of water from Seton River. The salmon were transferred individually from the holding tank to the Y-maze, located directly adjacent, until each fish had been tested. The isolation chambers allowed me to remove individual fish from the holding tank without disturbing the others. Concurrently, an additional 3 salmon were captured from the same capture location at the beginning of each day, and were transferred to one of the two source tanks. The "source fish" remained in the source tank for the duration of the day and provided an odour to one arm of the Y-maze. In the sockeye salmon experiment, the source fish were divided into two

treatment groups: “disturbed” and “control”. The disturbed source fish underwent the following handling procedure: first, air exposure for approximately 15 seconds during the transfer from the fishway to the transport tank using a dipnet, followed by 0.5-1.5 hours in the aerated transport tank, then another 15 seconds of air exposure during transfer from the transport tank to the source tank. They remained in the source tank for up to 8 hours. Physiological stress responses to handling events last up to 24 hours in Pacific salmon (Donaldson et al. 2014), and this, in addition to confinement stress, ensured the source fish remained in a disturbed state for the duration of the tests. As a consequence of testing wild adult salmon, it was not possible to capture, transport, and confine source fish in the control group in a truly undisturbed state. Roscoe et al. (2011), for example, found an increase in plasma glucose, a commonly used indicator of stress in salmonids (Barton 2002), in Gates Creek sockeye salmon following 5 hours of confinement in net pens in the Seton River. Instead, I sacrificed the control fish immediately upon capture (< 10 s) by cerebral percussion, before a physiological stress response could occur, and then placed them in a source tank. A previous study using Gates Creek sockeye salmon in Y-maze experiments (Bett and Hinch 2015b) found that individuals were neither attracted to nor deterred by the odour of lethally percussed conspecifics when tested in their natal waters. It is possible however that dead fish might eventually begin to release odours that deter conspecifics—indeed, odours emitted from zebrafish 10 hours post-death induced defensive behaviours in conspecifics (Oliveira et al. 2014). Exposure to conspecifics 8 or fewer hours post-death did not induce defensive behaviours or a physiological stress response. These sorts of behaviours were not observed in my study and all salmon were exposed to odours of lethally percussed conspecifics within 8 hours post-death. I alternated the arm containing the conspecific odour each day to account for potential bias of the salmon towards either arm of the Y-maze.

Furthermore, double-negative tests (i.e. no conspecific odour in either arm) previously conducted on Gates Creek sockeye salmon and pink salmon in the same experimental set-up indicated no bias for either arm (Casselman et al. 2013).

To test the potential role of cortisol as a disturbance cue released by threatened salmon, I conducted another experiment on sockeye salmon (in these tests on the Portage Creek population), using water-borne cortisol in place of conspecific odours. Similar experiments were not conducted on pink salmon—an explanation is provided in the results section. Cortisol (Sigma Aldrich) was dissolved in 99% ethanol to Seton River water in an acid-washed 20 L Nalgene carboy to create a 2×10^{-7} M stock solution. A peristaltic pump (Masterflex L/S model 7536-04, Cole-Parmer, QC, Canada) was used to introduce the cortisol stock solution into the upstream end of one arm of the Y-maze at 40 mL min^{-1}, to achieve a final cortisol concentration of 2×10^{-10} M. This concentration is similar to the concentration of cortisol released in water by disturbed Atlantic salmon (*Salmo salar*) following a handling stress comprising 90 s of air-exposure (Ellis et al. 2007).

At the start of each trial, a single salmon was transferred directly from the holding tank to the downstream end of the Y-maze. A mesh gate prevented the fish from entering either of the arms. After a 10 minute acclimation period, the gate was removed and I recorded the behaviour of the fish for 20 minutes. I recorded the amount of time the fish spent in each arm, as well as the number of times it entered each arm. I also calculated the proportion of time and entrances in the arm containing the conspecific odour relative to the total time or entrances in the two arms.

Following the 30 minute trial, the fish was removed from the Y-maze and I immediately (< 15 s) collected 2.5 mL blood samples by caudal puncture using a sterile 3.8 cm, 21-gauge

needle and a heparinized vacutainer (lithium heparin, 3 mL, Becton-Dickson, NJ, USA). Each sample was centrifuged at 7000 g for 5 min, then extracted the plasma and stored it in liquid nitrogen, followed by storage at −80°C until analysis. The plasma was analysed in duplicate for cortisol, using a commercial ELISA kit (ELISA no. 402710, Neogen Inc., Lansing, MI, USA) and a Spectramax 240pc microplater reader (Molecular Devices, Sunnyvale, CA, USA) at the Fisheries and Oceans Canada Centre for Aquaculture and Environmental Research (West Vancouver, B.C.). I did not collect blood samples from the salmon prior to their introduction to the Y-maze in order to avoid causing additional handling stress. I did, however, collect blood samples from a separate group of female sockeye salmon (n = 22) immediately upon capture from the fishway (< 15 s) on August 24, 2012, which were analyzed following the same methods to provide an estimate of baseline plasma cortisol levels. Following collection of the blood sample, fork length was measured (Table 5.1), then the fish was returned to the river. Source fish were held in their tank until the end of the experimental day (maximum 8 hours), at which point they were released into the river. Before release, I clipped the adipose fin of each salmon to ensure no fish were recaptured and tested a second time.

5.3.4 *Statistical analyses*

All fish that did not enter each arm at least once during the trial were not fully exposed to both waters, and were removed from analyses (n = 5; 2 sockeye salmon tested with the odour of disturbed conspecifics, and 3 sockeye salmon tested with the odour of control conspecifics). I tested for normal distribution and equal variance using the Shapiro-Wilk test and Levene's test, respectively, and applied square root transformations when the assumptions for parametric tests were not met. I used paired t-tests ($\alpha = 0.05$) to compare the amount of time and number of

entrances in each arm of the Y-maze. I used one-sample t-tests to compare the proportion of time or entrances in the arm containing the conspecific odour to a value of 0.5 (i.e. equal time in each arm). I used linear regressions to test for relationships between the holding time prior to the trial and the proportion of time in the arm containing the conspecific odour. I also compared the concentrations of plasma cortisol in sockeye salmon exposed to the odours of disturbed and control conspecifics using a two-way ANOVA on rank-transformed values, followed by Tukey's post hoc test to determine differences among groups. Sex was treated as a second explanatory variable as plasma hormones differ between sexes in Pacific salmon (Fagerlund 1967). I ran all statistical analyses in R Studio V 0.98.501.

5.4 Results

Sockeye salmon exhibited avoidance behaviours when exposed to water containing disturbed conspecifics. They spent 197 ± 18 s (mean ± standard error) in the arm containing the odour of disturbed conspecifics, compared to 371 ± 21 s in the arm without any conspecific odour. These times were significantly different (following square root transformation to meet parametric test assumptions, $t_{45} = -5.59$, $P < 0.001$; Fig 5.2a). They also entered the arm with the disturbed conspecific odour significantly less frequently (10.2 ± 0.8 entrances, compared to 13.5 ± 0.8 in the other arm; following square root transformation, $t_{45} = -3.32$, $P = 0.002$; Fig 5.2b). Furthermore, the proportion of time they spent in this arm (on a scale from 0 to 1.0) was 0.35 ± 0.03, which was significantly lower than 0.5 ($t_{45} = -5.88$, $P < 0.001$; Fig 5.2c), and the proportion of entrances was 0.42 ± 0.02, also significantly lower than 0.5 ($t_{45} = -3.42$, $P = 0.001$; Fig 5.2c). Sockeye salmon did not exhibit any avoidance (nor attractive) response when exposed to water containing the control conspecific odour. They did not spend significantly less time in the arm

containing the odour (328 ± 18 s in the arm with the odour and 363 ± 21 s in the arm without; t_{44} = -1.17, P = 0.25; Fig 5.2a), enter it less frequently (10.5 ± 0.8 in the arm with the odour, 11.2 ± 0.8 in the arm without; t_{44} = -0.92, P = 0.36; Fig 5.3b), spend a lower proportion of time in that arm than 0.5 (0.48 ± 0.02; t_{44} = -1.18, P = 0.25; Fig 5.2c), nor enter it a lower proportion of time than 0.5 (0.48 ± 0.02; t_{44} = -1.04, P = 0.30; Fig 5.2c).

There were significant main effects of sex ($F_{1,108}$ = 13.5, P < 0.001) and treatment group (exposed to the odours of disturbed conspecifics, exposed to the odours of control conspecifics, and baseline; $F_{2,108}$ = 26.0, P < 0.001) on plasma cortisol levels of sockeye salmon, as well as a significant interaction of sex and treatment group ($F_{1,108}$ = 6.8, P = 0.01) (Fig 3). A Tukey's pairwise comparison revealed significant differences between females exposed to disturbed conspecific odours (n = 29; 782 ± 54 ng mL^{-1}) and both females exposed to control conspecific odours (n = 22; 385 ± 21 ng mL^{-1}; P < 0.01) as well as baseline female values (n = 22; 295 ± 39 ng mL^{-1}; P < 0.01). Plasma cortisol concentrations were also significantly different between females exposed to disturbed conspecific odours and both males exposed to disturbed conspecific odours (n = 17; 391 ± 52 ng mL^{-1}; P < 0.01) as well as males exposed to control conspecific odours (n = 23; 328 ± 36 ng mL^{-1}; P < 0.01). There were no significant differences in plasma cortisol concentrations amongst females exposed to the control conspecific odour, baseline females, and males exposed to disturbed or control conspecific odours (P > 0.05 for each comparison).

The amount of time each test subject was held prior to their trial did not significantly predict the proportion of time they spent in the arm with the conspecific odour (sockeye salmon disturbed conspecific trials: R^2 = 0.02, P = 0.35; sockeye salmon control trials: R^2 = 0.02, P =

0.42; pink salmon disturbed conspecific trials: $R^2 = 0.03$, $P = 0.12$). For the trials using the odours of disturbed conspecifics, the amount of time each test subject was held was equivalent to the amount of time that elapsed between lethal percussion of the source fish and the start of the trial.

Despite differences in sockeye salmon plasma cortisol levels between sexes, separate analyses of their behavioural responses to the conspecific odours indicates no difference between sexes. There was less time spent in the arm containing disturbed conspecific odours in both females ($t_{28} = -4.67$, $P < 0.001$) and males (following square root transformation, $t_{16} = -2.93$, $P = 0.001$). There were also fewer entrances in this arm by females (following square root transformation, $t_{28} = -2.58$, $P = 0.02$) and males ($t_{16} = -1.95$, $P = 0.07$), although the difference in males is not significant at $\alpha = 0.05$. Similarly, the proportion of time spent in the arm containing the disturbed conspecific odour is lower than 0.5 in both females ($t_{28} = -4.73$, $P < 0.001$) and males ($t_{16} = -3.39$, $P = 0.004$), as is the proportion of entrances (females: $t_{28} = -2.61$, $P = 0.01$; males: $t_{16} = -2.17$, $P = 0.04$). In the control conspecific odour tests, none of these variables were significantly different ($P > 0.05$) for either sex.

Pink salmon did not show any behavioural response to water containing disturbed conspecifics. They spent 359 ± 27 s in the arm containing the disturbed conspecific odour, compared to 343 ± 21 s in the arm without the odour, which was not significantly different (following square root transformation, $t_{72} = 0.1077$, $P = 0.91$; Fig 5.4a). There was also no significant difference in the number of entrances in each arm (12.6 ± 0.9 in the arm with the conspecific odour and 14.0 ± 0.9 in the arm without; $t_{72} = -1.22$, $P = 0.23$; Fig 5.4a). The proportion of time that the pink salmon spent in the arm containing the conspecific odour was

0.49 ± 0.03, which was not significantly different from 0.5 (t_{72} = -0.374, P = 0.71; Fig 5.4b), and the proportion of entrances in that arm was 0.47 ± 0.02, also not significantly different from 0.5 (t_{72} = -1.424, P = 0.16; Fig 5.4b). Due to the lack of responses to the odour of disturbed conspecifics, I deemed tests using control conspecific odours unnecessary, and do not include cortisol data from these salmon for the same reason. For this same reason, I also chose not to test the responses of pink salmon to water-borne cortisol.

Sockeye salmon did not show any behavioural response to water containing 2×10^{-10} M cortisol. There was no significant difference in the amount of time spent in each arm (t_{35} = -1.285, P = 0.21; Fig 5.5a), the number of entrances in each arm (t_{35} = -0.713, P = 0.48; Fig 5.5a), nor the proportion of time (t_{35} = -1.166, P = 0.25; Fig 5.5b) or entrances in the arm with water-borne cortisol compared to 0.5 (t_{35} = -0.667, P = 0.51; Fig 5.5b).

5.5 Discussion

The responses of sockeye salmon to the odour of the handled conspecifics suggest disturbance cues were released following the handling event. Sockeye salmon in the Y-maze avoided the odour of disturbed conspecifics, spending less total time and a lower proportion of time in that arm, and entering it less frequently. When lethally percussed sockeye salmon were used as a source odour (i.e. controls in the experiment), test subjects did not display avoidance (or attraction) behaviours, further confirming that the handled fish did indeed emit disturbance cues. My finding suggests that disturbance cues can be released by migrating adult sockeye salmon. Similar responses to the odour of handled conspecifics have been found in other species of fish that were not undergoing migrations. For example, juvenile rainbow trout (*Oncorhynchus mykiss*) exhibited a stress response when exposed to the odour of conspecifics that were chased

with nets (Toa et al. 2004), as did jundiá (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) (Barcellos et al. 2011).

The lack of response in sockeye salmon to water-borne cortisol indicates that this compound is likely not a disturbance cue in this species. Barcellos et al. (2014) found a similar result in zebrafish (*Danio rerio*). For immediate threats such as predation, the latent time before plasma cortisol increases to levels sufficient for release through the gills may restrict its usefulness as a disturbance cue. It may still function, though, as an indicator of less acute stressors such as migratory barriers, which was one reason I examined it with a migratory species. It is possible that other hormones or chemicals associated with the stress response may act as disturbance cues. Furthermore, there is evidence that fish can distinguish chemical cues released by conspecifics exposed to different stressors (Giaquinto and Hoffmann 2012). Such information could allow fish to not only detect a stressor, but also assess its relative threat.

Whereas sockeye salmon demonstrated an avoidance response to disturbed conspecifics, pink salmon did not. One explanation could be that disturbance cues were not released by the handled pink salmon, perhaps due to a relatively weak physiological stress response to the handling event. However, our research group has extensively examined stress responses of Pacific salmon using standardized 3 minute chase-to-exhaustion and air exposure protocols and have found that pink and sockeye salmon have similar magnitudes of responses in terms of plasma metrics such as lactate and osmolality (Raby et al. 2013; Gale et al. 2014), suggesting that both species are capable of mounting similar physiological responses to handling. It is possible that migratory state influenced the behavioural differences, as the pink salmon were captured closer to spawning grounds than the sockeye salmon, and may have lost sensitivity to

disturbance cues as they prepared for spawning. Alternatively, pink salmon may be less responsive to chemical cues. Although direct evidence supporting this is lacking, as olfactory sensitivity has rarely been studied in pink salmon (Bett and Hinch 2015a), there is circumstantial evidence. The behavioural physiology of migrating adult sockeye and pink salmon is quite different. Based on EMG telemetry tracking in the Fraser River, adult migrating sockeye salmon exhibit much more variable and erratic swim speeds, commonly eliciting burst swimming behaviours during their upstream migration, whereas pink salmon rarely exhibit burst swimming though migrating through identical reaches as sockeye salmon (Hinch et al. 2002). Pink salmon are much more energetically efficient swimmers than sockeye salmon (Crossin et al.2003), and as they rarely burst swim, they are not producing large levels of plasma lactate or glucose (or other stress metabolites associated with fast and erratic swimming; Eliason et al. 2013) so it is possible that they generally do not excrete the same level of disturbance cues as sockeye during migration. If such cues are rarely elicited, pink salmon may not have developed advanced receptor systems for recognizing these cues. Pink salmon also exhibit a weaker behavioural response to olfactory directional cues during the migration (Yamamoto et al. 2008). Another possible explanation for their lack of response could be that their smaller size was insufficient to produce a detectable concentration of cues under my experimental protocol.

The sex of the fish did not have an effect on the behavioural response of sockeye salmon in my study. However, there was a differential response in plasma cortisol levels between sexes. Plasma cortisol was elevated in females exposed to the odour of disturbed conspecifics, but not in males, despite both sexes exhibiting the same avoidance response. Similarly, Donaldson et al. (2014) studied the physiological response of sockeye salmon to a simulated fisheries capture, and found a significant increase in the plasma cortisol concentrations of females but not males. There

is also a sex-specific response in the plasma cortisol levels of sockeye salmon as they navigate difficult hydraulic barriers. For example, plasma cortisol levels increased much more in female than in male sockeye salmon during passage through Hell's Gate, a major hydraulic barrier in the Fraser River (Hinch et al. 2006). The plasma cortisol levels of males and females were similar at other points in their migration, before and after Hell's Gate. Similarly, plasma cortisol levels of sockeye salmon captured at the top of the Seton Dam fishway were higher in females than in males (Pon et al. 2009; Roscoe et al. 2011). My findings that cortisol increased after exposure to potential disturbance cues in females but not males could indicate that female sockeye salmon have a more sensitive physiological stress response to conspecific disturbance cues, as they appear to have with hydraulic barriers. The behavioural response to disturbance cues, however, may not be accompanied by an elevation in plasma cortisol, given that the males I tested also exhibited an avoidance response.

During the Pacific salmon spawning migration, avoidance of stressors could reduce immediate risk of mortality or injury, improving the likelihood of successful spawning. Avoidance could also, however, slow or delay the spawning migration. Adult salmon migrate towards their spawning grounds on a fixed energy budget, and longer migrations are correlated with increased energy expenditure (Hinch and Rand 1998), leaving less energy available for gonad development and spawning (Crossin et al. 2004). Migrating sockeye salmon may balance the potential risk associated with disturbance cues and the potential reproductive costs of delaying their migration. Pink salmon, on the other hand, frequently migrate much shorter distances to spawning grounds (Quinn 2005), and consequently are less likely to encounter stressors. A lack of response to disturbance cues may therefore have limited impact on their migratory success.

Changes in the prevalence of disturbance could cause further divergence in the migratory behaviours of these two species. In the Fraser River, for example, recent changes in federal fisheries policy and management strategies are increasing the relative levels of harvest that take place during latter portions of their return spawning migration (e.g. in fresh water) (DFO 2015). If fisheries pressure, or stressors associated with hydraulic challenges such as with dam or other hydrologic developments, increases along freshwater migratory routes of sockeye salmon, the increased release of disturbance cues by migrants in response to such stressors could slow or impede the migration of conspecifics. This issue could be exacerbated by warming river temperatures, which are already negatively affecting sockeye salmon spawning migrations in the Fraser River and elsewhere (Keefer et al. 2008c; Martins et al. 2011). Pink salmon, on the other hand, have a higher thermal optimum (Clark et al. 2011) and are projected to broaden their distribution as river temperatures increase (Gordeeva et al. 2005; Irvine and Fukuwaka 2011). They may be less affected by thermal and fisheries-related stressors, and could continue to colonize new areas at a rapid rate (Pess et al. 2012).

In conclusion, my results provide the first evidence of the release of chemical disturbance cues in sockeye salmon during their spawning migration, and are contrasted by pink salmon, which appear to either not release or not respond to disturbance cues. In sockeye salmon, these cues trigger an avoidance response in conspecifics, and may be adaptive in decreasing exposure to risks, thereby increasing migratory success.

Fig 5.1 Experimental set-up, with arrows indicating the flow of water. Source fish provided a disturbed or control conspecific odour into one arm of the Y-maze. Test subjects were transferred to the Y-maze from isolation chambers in the holding tank, and were tested independently. For the water-borne cortisol test, there were no source fish providing a conspecific odour; instead, a peristaltic pump was used to introduce cortisol into one arm of the Y-maze.

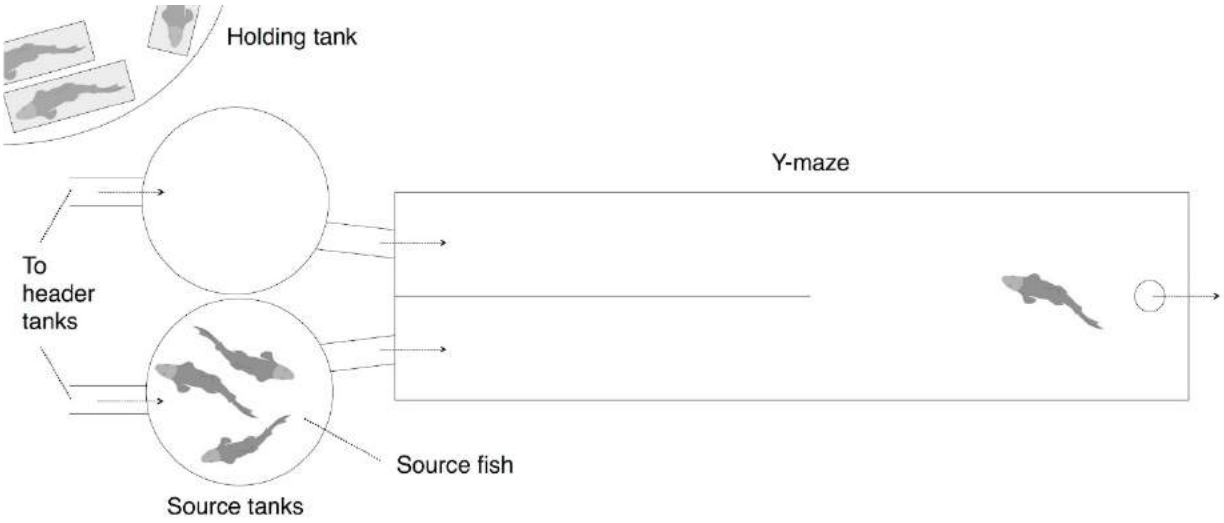


Fig 5.2 Behavioural responses of sockeye salmon in a Y-maze when the odours of disturbed and control conspecifics were introduced in one arm. The responses include (a) the amount of time the salmon spent in each arm, (b) the number of times they entered each arm, and (c) the proportion of time or entrances in the arm containing the conspecific odour. Short horizontal lines indicate the values of individual fish, long horizontal lines indicate mean values, and shaded areas are the estimated density of the distribution. The dotted horizontal lines in (c) indicate a proportion of 0.5. Asterisks denote statistical significance at $P < 0.05$ between the arm containing the odour and the arm without the odour (a,b), and between the arm containing the odour and a fixed value of 0.5 (c). Data was transformed for some statistical analyses, and the scales on the y-axes of the corresponding figures have been adjusted to reflect this.

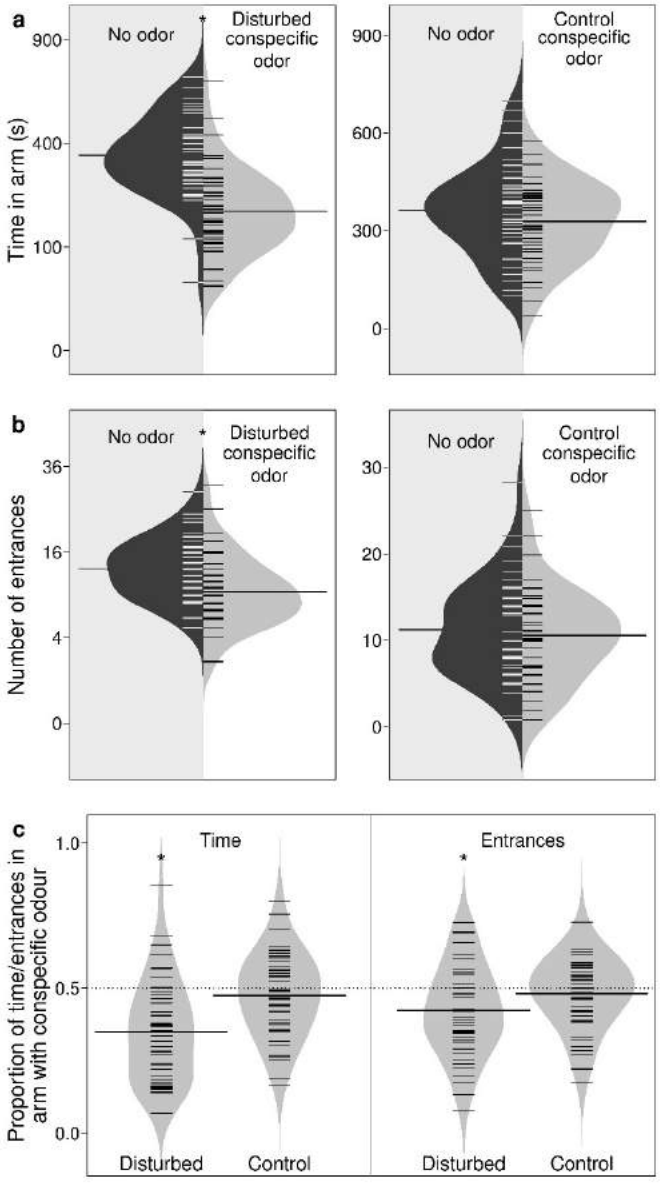


Fig 5.3 Plasma cortisol concentrations in female (F) and male (M) sockeye salmon (*O. nerka*) exposed to the odours of disturbed conspecifics (grey bars; $n_F = 29$, $n_M = 17$) and control conspecifics (white bars; $n_F = 22$, $n_M = 23$), as well as baseline plasma cortisol in females sampled immediately upon capture (hatched bar; $n = 22$). Asterisks indicate a significant difference at $P < 0.05$.

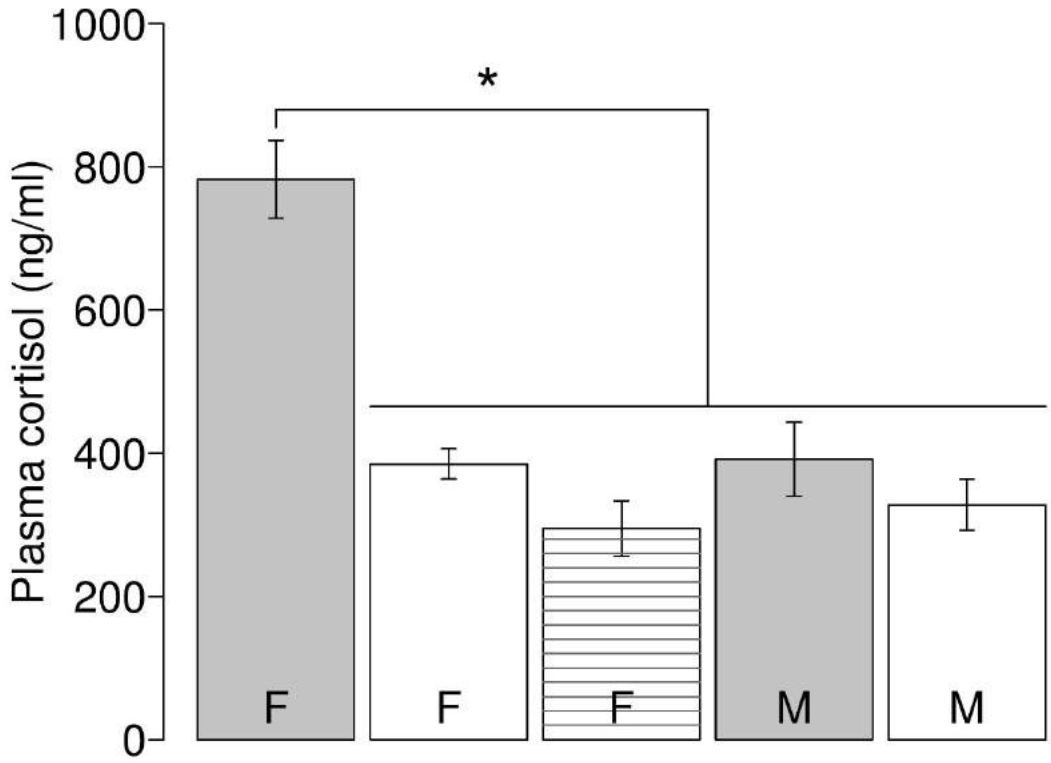


Fig 5.4 Behavioural responses of pink salmon (*O. gorbuscha*) in a Y-maze when the odours of disturbed conspecifics were introduced in one arm. The responses include (a) the amount of time the salmon spent in each arm, (b) the number of times the salmon entered each arm, and (c) the proportion of time or entrances in the arm containing the conspecific odour. Short horizontal lines indicate the values of individual fish, long horizontal lines indicate mean values, and shaded areas are the estimated density of the distribution. The dotted horizontal lines in (c) indicate a proportion of 0.5.

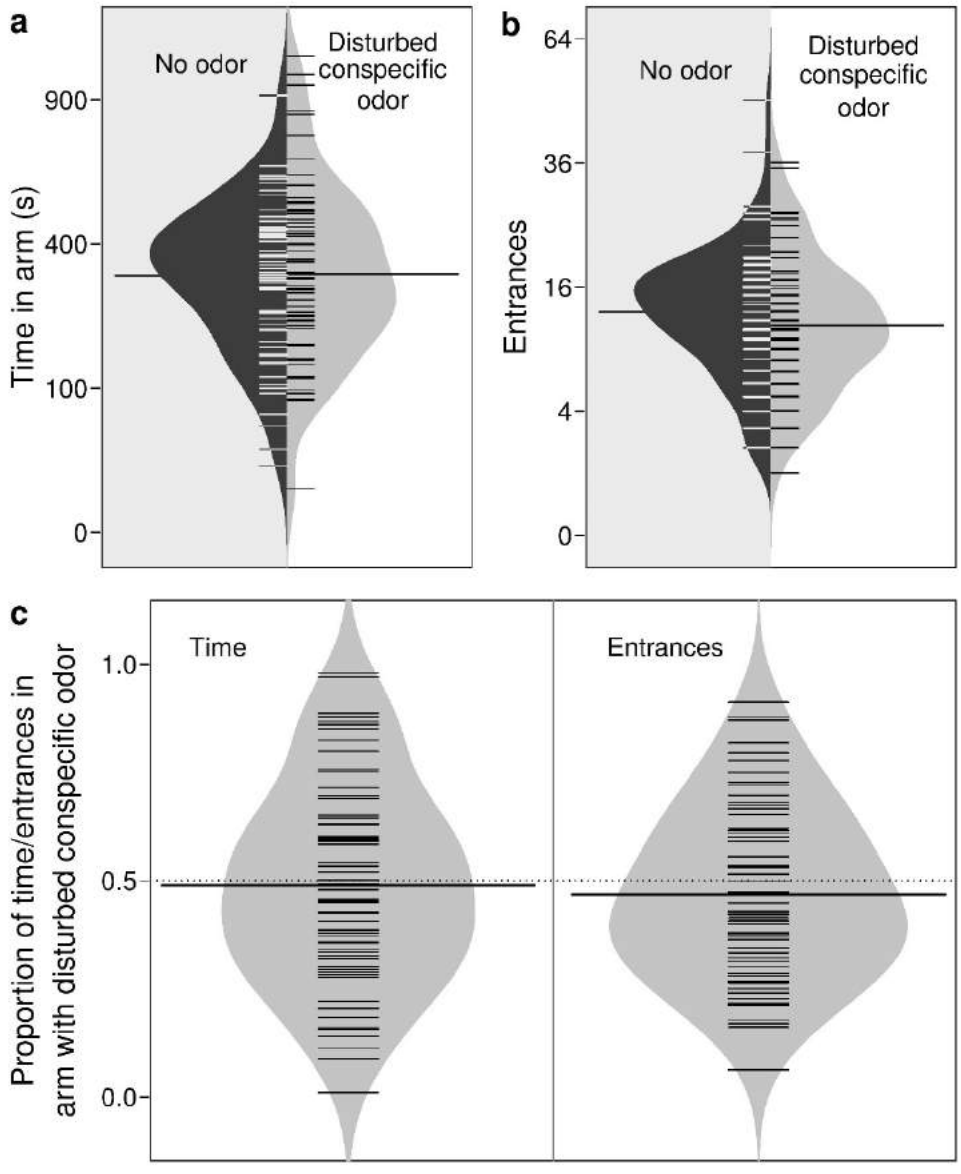


Fig 5.5 Behavioural responses of sockeye salmon (*O. nerka*) in a Y-maze when water-borne cortisol was introduced into one arm to achieve a concentration of 2×10^{-10} M. The responses shown are (a) the amount of time the salmon spent in each arm, (b) the number of times the salmon entered each arm, (c) the proportion of time the salmon spent in the arm containing cortisol, and the proportion of entrances the salmon made into the arm containing cortisol. Short horizontal lines indicate the values of individual fish, long horizontal lines indicate mean values, and shaded areas are the estimated density of the distribution. The dotted horizontal lines in (c) indicate a proportion of 0.5.

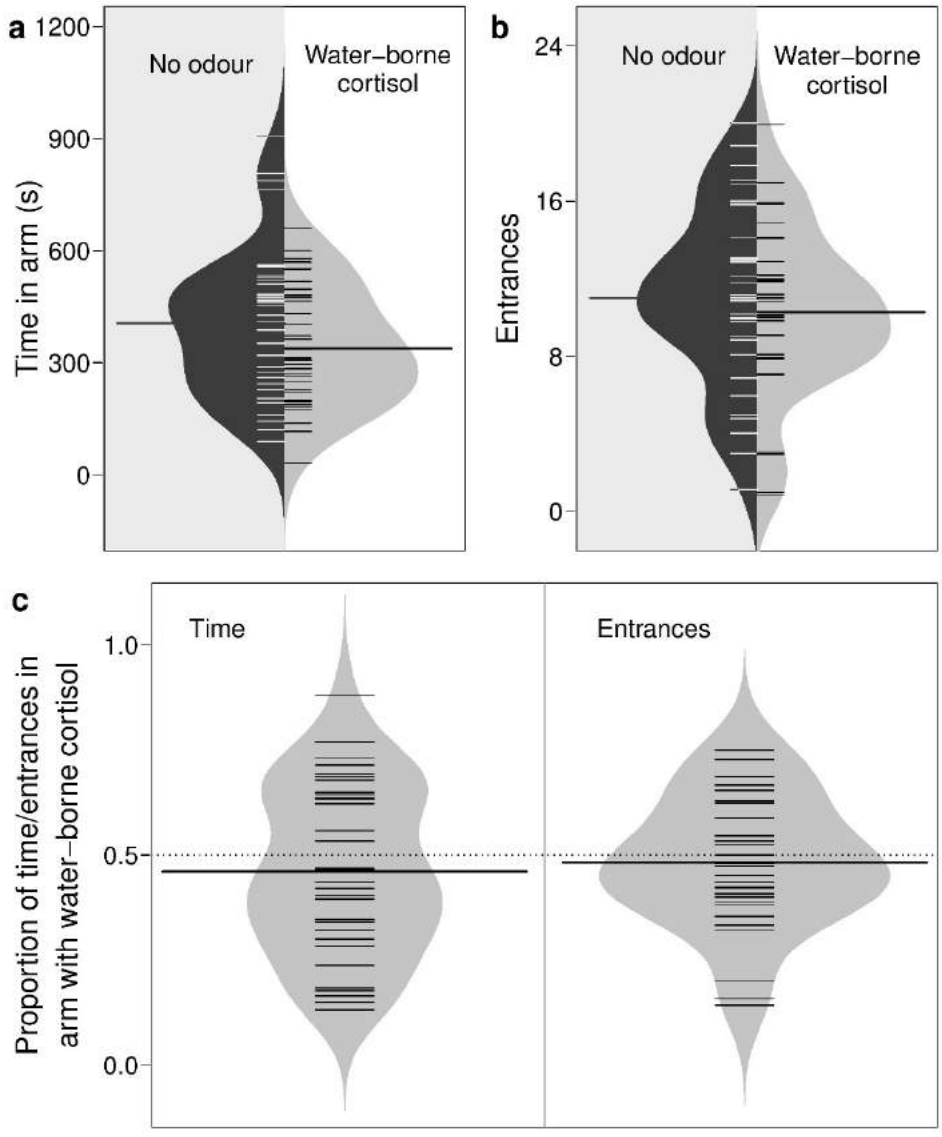


Table 5.1 Sample sizes, mean fork lengths, and dates for behavioral choice experiments.

Species	Treatment odor	*n*	Fork length (mean ± SE)	Dates
Sockeye salmon (Gates Creek)	Disturbed conspecifics	48[a] (30 F, 18 M)	Females 57.4 ± 0.5; Males 60.1 ± 0.6	Aug 18-23, 2013
Sockeye salmon (Gates Creek)	Control conspecifics	48[a] (24 F, 24 M)	Females 57.6 ± 0.5; Males 61.4 ± 1.0	Aug 16-25, 2014[b]
Sockeye salmon (Portage Creek)	Water-borne cortisol	36 (10 F, 26 M)	Females 57.6 ± 0.7; Males 62.3 ± 0.4	Oct 7-9, 2014
Pink salmon	Disturbed conspecifics	73 (25 F, 48 M)	Females 51.4 ± 0.4; Males 52.9 ± 0.4	Sep 17-23, 2013

a Values include salmon that were removed from analyses due to immobility during behavioural tests (two in the "disturbed conspecifics" group, three in the "control conspecifics" group)

b In 2013, concerns by management agencies over low spawning ground recruitment prevented us from sacrificing sockeye salmon during the study period. Therefore tests using the "control conspecifics" treatment odour were conducted in 2014.

Chapter 6: Effects of natal water dilution in a regulated river on the Pacific salmon migration

6.1 Synopsis

The previous chapters have focused on olfactory-mediated processes during unspecified periods of migration, which are broadly applicable to salmonid spawning migrations. In this chapter, I focus on the olfactory-mediated behaviours of sockeye and pink salmon within the context of their environment in a regulated river system in southern British Columbia. Alterations to flow regimes can have a negative effect on the navigation of salmon towards their home stream if they create confusing release patterns of natal water. I studied the effects of natal water dilution resulting from hydroelectric operations on upstream navigation, and the efficacy of current dilution targets set in place by local managers. I found that both local populations of sockeye salmon exhibit preferences for pure natal water when paired with diluted natal water, whereas pink salmon, which spawn throughout the system and exhibit lower levels of natal site fidelity, appear unaffected. My findings confirm that the current management operations are appropriate in minimizing the negative effects of natal water dilution on the sockeye salmon migration.

6.2 Introduction

Construction of dams continues to occur at a rapid pace in many river systems around the world (Northcote 1998; Rivinoja et al. 2001), which creates many issues for migratory fish. For example, migrating fish must locate the fishway entrance—assuming passage facilities are present—which can be problematic, as some of these structures are positioned in an area that is difficult for fish to locate (Bunt 2001). Additionally, passage facility discharge may not be high enough to sufficiently attract fish (Laine et al. 2002). After locating the entrance, fish often have

difficulty negotiating through the facility (Naughton et al. 2007), which can be energetically costly and stressful (Fenkes et al. 2016). The energetic costs incurred during passage not only influence migration success but can also induce a negative carryover effect on reproductive success (Burnett et al. 2014).

For Pacific salmon (*Oncorhynchus* spp.), which use a complex navigation system to locate their spawning grounds, the influence of a dam can extend beyond fishway passage. Pacific salmon use the unique chemical signature of their natal sites as directional cues during the spawning migration (Hasler and Scholz 1983). These cues are carried downstream, and are used by returning adult salmon to orient themselves towards their spawning grounds. Confusing release patterns can disorient the salmon as they attempt to locate their natal cues. Disorienting flow regimes can occur naturally (White 1934), but can also result from alterations to water release patterns caused by hydroelectric development (Fretwell 1989). The potential effects of altered flow composition (such as alterations to the concentration of natal water in the system) on the navigation of migrating fish has received little attention, perhaps because the flow composition and migratory routes are site-specific and dependent on the area's topography.

The aim of this chapter is to examine the influence of natal water dilution on the navigation of returning adult sockeye (*O. nerka*) and pink (*O. gorbuscha*) salmon in Seton River, a tributary of British Columbia's Fraser River. In this system, natal water flows into the mainstem (the Fraser River) from two locations—an impassable power canal, and the Seton River, which salmon must migrate through to reach spawning grounds. Salmon are capable of discriminating between different concentrations of natal water, and will preferentially select higher concentrations (Fretwell 1989). This creates a potential problem in the system, as the

power canal contains a higher concentration of natal water relative to the Seton River, which receives water from an uninhabited tributary that lacks natal chemical cues. A diversion dam on this tributary can be used to divert this water out of the tributary, thereby increasing the concentration of natal water in the Seton River. Research conducted by the International Pacific Salmon Fisheries Commission identified minimum natal water concentration targets that will encourage salmon to enter the Seton River (Fretwell 1989). These targets were adopted by the local water use plan, and have been adhered to ever since (BC Hydro 2011).

My objective is to examine the behavioural responses of sockeye and pink salmon in the Seton River system to diluted natal water, and to assess the current management guidelines. To do so, I conducted behavioural choice experiments that tested the salmons' responses to the current natal water concentration targets, as well as concentrations above and below the targets. The diluted natal water, which represents what they might encounter in the Seton River, was paired with undiluted natal water that was analogous to the water released from the power canal. I predicted that sockeye salmon would exhibit a preference for undiluted natal water when paired with natal water diluted to concentrations below the current targets, but not when undiluted natal water was paired with natal water concentrations above the targets. I also predicted that pink salmon, which spawn freely throughout the system, would be less sensitive to changes in concentrations, and not exhibit any behavioural preferences. The results of these tests confirm whether the current management targets are appropriate or need to be modified.

6.3 Methods

6.3.1 *Study site*

The study took place at the Seton River, a tributary of the Fraser River in southern British Columbia, Canada (Fig 6.1). Several species of Pacific salmon migrate through this river, including pink salmon as well as two populations of sockeye salmon. The sockeye salmon spawn further upstream in Gates and Portage Creeks, while the pink salmon spawn throughout the system. The average run sizes of the Gates and Portage Creek sockeye salmon populations are 54,000 and 41,000, respectively (DFO 2014). Pink salmon in this area have not been enumerated.

The Seton Dam is situated on the upstream end of the Seton River, at the outflow of Seton Lake (Fig 6.2). Some of the water from Seton Lake is released through the dam into the Seton River, while the rest is diverted down a power canal towards a generating station. The two sockeye salmon populations rear in Seton Lake, and this water contains the imprinted natal odour that will attract adults as they return to spawn. As adult salmon migrating up the Fraser River approach the area, they first encounter their natal water at the outflow of the power canal. The Seton River flows into the Fraser River approximately one kilometre further upstream. While the water in the power canal comes solely from Seton Lake, the water in Seton River originates from Seton Lake as well as a tributary, Cayoosh Creek. Cayoosh Creek is not inhabited by any spawning populations of sockeye salmon, and does not contain natal chemical cues. In the Seton River, the natal water from Seton Lake is diluted by water from Cayoosh Creek. This diluted natal water is less attractive to the migrating salmon than the undiluted natal water released from the power canal, which discourage salmon from entering the Seton River and attract them to the

power canal. Metal grates have been installed at the generating station that prevent salmon from attempting to enter the power canal.

The amount of Cayoosh Creek water entering the Seton River can be controlled via the Walden North diversion dam on Cayoosh Creek. This dam diverts water through a tunnel to Seton Lake. As more water is diverted from Cayoosh Creek to the lake, less water continues into the Seton River, and the natal water concentration in the Seton River increases. Operation of Walden North therefore determines natal water concentration in the Seton River. The current target for minimum natal water concentration during the Gates Creek sockeye salmon migration is 80% (i.e. the composition of Seton River is 80% water from Seton Lake and 20% water from Cayoosh Creek). Natal water concentrations below this target are believed to be detrimental to the migration, as the salmon may choose to return to the power canal outflow rather than enter the Seton River. The minimum natal water concentration target for the Portage Creek sockeye salmon migration is 90%. There is no minimum target set for pink salmon.

6.3.2 *Experimental design*

Behavioural choice tests were conducted from Aug 3-14 and Sep 9-15 2013, and Aug 1-7 and Sep 29 – Oct 6 2014, to determine the range of Seton River water dilutions at which sockeye and pink salmon might prefer undiluted water released through the power canal. Experiments took place on the north bank of the Seton River, adjacent to the Seton Dam, during daylight hours (0700 – 1700). All sockeye salmon (Gates Creek n = 112; Portage Creek n = 71) and pink salmon (n = 43) were captured by dipnet from the top pool of the Seton Dam fishway, located on the Seton River, and immediately transferred to an aerated 1,000 L transport tank on the back of a truck. The fish were driven approximately 100 m and unloaded into a 10,000 L holding tank

adjacent to the dam, with continuous flow of water from Seton River. Each fish was held in an individual isolation chamber, constructed from PVC pipe (75 cm length x 15.3 cm diameter) with mesh ends.

Water was stored in two 11,365 L polyethylene head tanks (Premier Plastics Inc., Delta, BC), and the water was gravity fed through 2" diameter water suction hoses (Greenline, Delta, BC) to two 1,136 L polyethylene tanks (Premier Plastics Inc.) (Fig 4.2). Water was gravity fed from each of these tanks through a 4" diameter water suction hose into a Y-maze. A Y-maze was constructed from plywood and 2x4 supports, and the interior was sealed with fiberglass and a fish-safe gelcoat (Rebel Fiberglass, Kamloops, BC). The Y-maze was rectangular in shape, 4.88 m long x 1.22 m wide x 1.22 m high. A 2.44 m divider, made from fiberglassed plywood, divided the upstream end into two equally sized halves (or two "arms"). A dye test confirmed no mixing occurred between water in each of the two arms. Water exited the Y-maze through a standpipe, and the water level was maintained at 17 cm. Valves regulated the amount of water entering each arm of the Y-maze to 40 L/min. Plywood was also placed on top of the Y-maze to block out light, and to reduce stress in the fish.

One head tank, which fed into one arm of the Y-maze, was filled with water from Seton Lake, to replicate the water exiting the power canal. The other head tank, which fed into the other arm of the Y-maze, was filled with a mixture of water from Seton Lake and from Cayoosh Creek, to replicate the water exiting the Seton River. Water was obtained from the outflow of Seton Lake using a submersible pump with a hose leading to the head tank. Water was obtained from Cayoosh Creek using a gas-powered pump with 2" water suction hose connected to a 1,500

L transport tank on the back of a truck. The truck was then driven to the experimental location and the water released into the head tank.

My selection of mixture concentrations was guided by the current natal water dilution targets. For Gates Creek sockeye salmon, I tested their response to the target of 80% natal water, as well as concentrations above and below this value (50%, 70% and 95%). I tested the response of Portage Creek sockeye salmon to the current target of 90% natal water, as well as 80%. I tested the response of pink salmon to a concentration of 50%. The conductivity of the Seton River and Cayoosh Creek was also measured over the period of the salmon migrations using a conductivity metre (YSI Pro 30, YSI Incorporated, Yellow Springs, OH, USA). These measurements provided a basic estimate of differences in the water chemistry of the two rivers.

6.3.3 *Behavioural trials*

Eight to twelve salmon were captured in the morning of each experimental day and transferred to the holding tank, to be individually tested in the Y-maze. At the start of each trial, a single salmon was transferred directly from the holding tank to the downstream end of the Y-maze. A mesh gate prevented the fish from entering either of the arms. After a 10 minute acclimation period, the gate was removed and the behavior of the fish was recorded for 20 minutes. Behaviour was monitored through a video system, using an Infrared camera (securitycamera2000.com, Hong Kong) connected to a monitor. I recorded the amount of time spent in each arm and the number of entrances into each arm. I also calculated the proportion of time spent in the arm containing the mixture of Seton Lake and Cayoosh Creek water relative to the time spent in the arm with pure Seton Lake water.

At the end of the 30 minute trial, the fish was removed from the Y-maze and blood and DNA samples were collected. The fish was then returned to the river, and the Y-maze was flushed before the introduction of the next test fish. The arm containing the Seton Lake and Cayoosh Creek mixture was switched each day to mitigate any potential behavioral bias for one of the arms. A control test was also run on the Gates Creek sockeye salmon using pure Seton Lake water in both arms to characterize any potential bias for one arm.

6.3.4 *Data analysis*

A Shapiro-Wilk normality test was used for each of the variables collected. The amount of time spent in each arm and the number of entrances in each arm were compared using paired t-tests ($\alpha = 0.05$). In instances where the distribution of time spent in each arm was not normal, even following transformation, a Wilcoxon signed-rank test ($\alpha = 0.05$) was used. The proportion of time spent in the arm with the test mixture was analyzed using a one sample t-test after performing an arcsine transformation ($\mu = 0.785$ [0.785 is a value of 0.5 after transformation, which would indicate the fish spent an equal proportion of time in each arm], $\alpha = 0.05$). All fish that did not enter each arm at least once during the trial were removed from the analysis as these fish did not experience a full concentration of each of the waters, and therefore could not exhibit choice or preference behaviors. Two Gates Creek sockeye salmon were removed for this reason (one in the 5% test, the other in the 50% test), as were two pink salmon (50% test). All statistical analyses were run in R Studio V 0.98.501.

6.4 Results

Based on the time spent in each arm, Gates Creek sockeye salmon did not exhibit any preferential bias for either arm during the control tests (time spent in arm: $V = 66$, $n = 19$, $P =$

0.26; proportion of time in arm: $V = 121$, $n = 19$, $P = 0.31$). Gates Creek sockeye salmon spent more time in the arm containing pure Seton Lake water when paired with the 50% and 70% mixtures (50%; $t_{25} = 4.325$, $P < 0.001$; 70%: $t_{29} = 5.639$, $P < 0.001$), but not when paired with the 80% or 95% mixtures (80%: $t_{25} = -0.584$, $P = 0.57$; 95%: $t_8 = 1.919$, $P = 0.091$) (Fig 6.3a). They also spent a greater proportion of time in the arm containing the pure Seton Lake water when paired with the 50% and 70% mixtures (50%: $t_{25} = 4.291$, $P < 0.001$; 70%: $t_{29} = 6.119$, $P < 0.001$), but not when paired with the 80% and 95% mixtures (80%: $t_{25} = -0.554$, $P = 0.59$; 95%: $t_8 = 2.096$, $P = 0.069$) (Fig 6.3b).

Portage Creek sockeye salmon spent more time in the arm containing pure Seton Lake water when paired with the 80% mixture ($t_{35} = 3.797$, $P < 0.001$) and a greater proportion of time in that arm ($t_{35} = 3.484$, $P = 0.0014$), but not when paired with the 90% mixture (amount of time in arm: $V = 337$, $n = 35$, $P = 0.73$; proportion of time in arm: $t_{34} = 0.754$, $P = 0.46$) (Fig 6.4).

Pink salmon did not spend more time in either arm when tested with pure Seton Lake water paired with a 50% mixture ($V = 160.5$, $n = 41$, $P = 0.64$), and they spent a greater proportion of time in the arm with the 50% mixture ($t_{40} = -4.261$, $P < 0.001$) (Fig 6.5).

Conductivity was consistently higher in Cayoosh Creek than in Seton River (Fig 6.6). Furthermore, over the course of the Gates Creek sockeye salmon migration in August and the Portage Creek sockeye salmon migration in September and October, there was an upwards trend in the conductivity of Cayoosh Creek and a downwards trend in Seton River, such that the differences between the two rivers became more pronounced.

6.5 Discussion

The behavioural responses of the Gates Creek and Portage Creek sockeye salmon indicate a preference for water from Seton Lake when paired with a test mixture of Seton Lake water diluted with Cayoosh Creek water. Gates Creek sockeye salmon exhibited a preference when the mixed water was diluted to 70% or below, but no preference when the mixture was 80% or above. This result suggests the salmon might enter the Seton River, rather than return downstream to the power canal, when the concentration of Seton Lake water in the river is at or above 80%. The Portage Creek sockeye salmon, meanwhile, exhibited a preference when the mixture was 80%, but not when it was 90%, suggesting they are more sensitive to the dilution of their natal water. Pink salmon did not show any preference for Seton Lake water even when the paired test mixture was diluted to 50%, which suggests that they may not be influenced by the dilution of water in the Seton River.

The results for Gates Creek and Portage Creek sockeye salmon are similar to those found by Fretwell (1989), despite the fact that he examined fish that were likely in different physiological condition, had migrated through different environmental conditions, and were tested using different experimental procedures. In the research conducted by Fretwell (1989), salmon were captured by braile net, a method that can cause injury to the fish, whereas I used the relatively benign capture method of dip-netting. Also, the somatic energy of Pacific salmon is influenced by open ocean productivity (Crossin et al. 2004), which varies across years and likely differed between my experimental years and those of Fretwell (1989). Further, temperatures in the Fraser River have steadily increased over the past several decades (Patterson et al. 2007), which could affect the physiological state and potentially the behaviour of the salmon. Finally,

salmon were tested in groups by Fretwell (1989), and the temperature of the Cayoosh Creek water used in these tests was increased to match the temperature of Seton Lake (this was done to eliminate any potential effect of temperature on the salmons' preference behaviours). In my experiment, I tested the salmon individually, and did not alter water temperatures in order to better emulate the conditions of the natural system.

In my experiment, both Gates Creek and Portage Creek sockeye salmon were slightly less sensitive to natal water dilution than in the study by Fretwell (1989). This difference might be explained by my decision to test salmon individually, rather than in groups. Berdahl et al. (2015) theorized that salmon might navigate more accurately as a collective group, rather than individually, and testing salmon in groups might increase their sensitivity to the dilution of their natal water. By treating each salmon as an individual data point, however, Fretwell (1989) made the assumption that each fish in the group acted independently. This is an assumption that is rarely met when measuring animal behaviour (Martin and Bateson 1993), especially for a schooling fish such as salmon.

The increased sensitivity of Portage Creek sockeye salmon relative to Gates Creek sockeye salmon could reflect population-level genetic variation (e.g. variation in olfactory abilities), or could be influenced by temporal variation in the chemical composition of the waters. Portage Creek sockeye salmon migrate later in the season than those from Gates Creek. Differences in the conductivity of water from Seton River and Cayoosh Creek increases over the course of the migration period for the two populations, such that the conductivity of Cayoosh Creek is much higher (indicating a higher concentration of ions) than Seton River during the Portage Creek migration. The specific chemicals that Pacific salmon use as directional cues are

unknown (Chapter 2), but it is possible that changes in the conductivity might reflect changes in the concentrations of directional cues. There is recent evidence that amino acids, which are believed to act as directional cues during salmon homing (Ueda 2011), fluctuate seasonally (Yamamoto et al. 2013), and it is possible that many other chemical concentrations change as well. Without knowing the chemicals responsible for directing the salmon towards spawning grounds, however, it is not possible to determine whether Cayoosh Creek and Seton River do in fact become less similar over the course of the season, from the perspective of the migrating salmon.

Pink salmon did not show any preference for the Seton Lake water, despite being tested with water that was strongly diluted to 50%, and they actually spent a greater proportion of time in the heavily diluted mixture. The lack of preference for Seton Lake water likely reflects that these salmon spawn throughout the system, unlike the sockeye salmon. Indeed, the pink salmon tested in this study were captured at the top pool of the fishway and were therefore unlikely to ever return to Cayoosh Creek, but nevertheless did not prefer the Seton Lake water. It is possible that pink salmon spawn freely in the area without reference to their specific natal site, and appears unlikely that the dilution of water from Seton Lake has an influence on their upstream navigation. These salmon might in fact rely on cues other than the odour of their natal water when navigating towards spawning habitat.

The issue of dilution in the Seton River can be addressed by the use of a tunnel that diverts water from Cayoosh Creek to Seton Lake. This reduces the relative concentration of Cayoosh Creek water in the Seton River and decreases the difference in concentrations between the river and the power canal. My findings suggest that the tendency for sockeye salmon to

return to the power canal after exploring the mouth of the Fraser River can be minimized by maintaining a minimum concentration of Seton Lake water in the Seton River. During the Gates Creek sockeye salmon migration, the minimum concentration is 80%, and during the Portage Creek sockeye salmon migration it is 90%. These values are the current management targets, and can be maintained by diverting water from Cayoosh Creek through the tunnel. Pink salmon do not appear to be affected by the dilution of water exiting Seton Lake, which suggests changes to operational conditions will not likely have a significant effect on their behaviour. These findings align with the current guidelines that were based on Fretwell's (1989) report. Interestingly, the report noted a preference for undiluted natal water when paired with 80% and 90% natal water for the Gates and Portage Creek populations respectively. It might be expected, then, that if operations allow concentrations to drop to these target levels, the tendency of the sockeye salmon to locate and ascend the Seton River may be reduced. My findings suggest a preference occurs when the amount of natal water drops below these concentrations, but not at these values. This would suggest that the salmon are unlikely to preferentially select the water exiting the power canal, even when natal water in the Seton River is diluted to 80% or 90%.

The design of the diversion dam on the Seton River provides two lessons that might be applied to future hydroelectric development in rivers with migratory fish. First, the diversion of water downstream from the river itself, which was necessary due to the topography of the region, should be avoided when possible. Fish moving upstream will detect the diverted water before they have the opportunity to detect the river. Furthermore, anadromous fish such as Pacific salmon, which imprint on the odour of their home stream, will be attracted to this diverted water, and may spend an extended period of time attempting to enter the diversion. This delay could incur energetic costs, and some fish could ultimately choose to return downstream and seek

alternate spawning habitat. Second, when water is diverted, the amount released through a dam and into the river decreases. If a tributary connects with this river, directional cues will be diluted, and the magnitude of dilution will increase as more water is diverted. Diverting water out of this tributary, as has been done in Cayoosh Creek, can minimize the level of dilution. My findings reconfirm that current management dilution guidelines in the Seton River system (BC Hydro 2011) will minimize the effects of natal water dilution on the upstream migration.

Fig 6.1 Map of study area within British Columbia's Fraser River system (inset). Sockeye salmon spawn in Gates Creek and Portage Creek, while pink salmon spawn throughout the area.

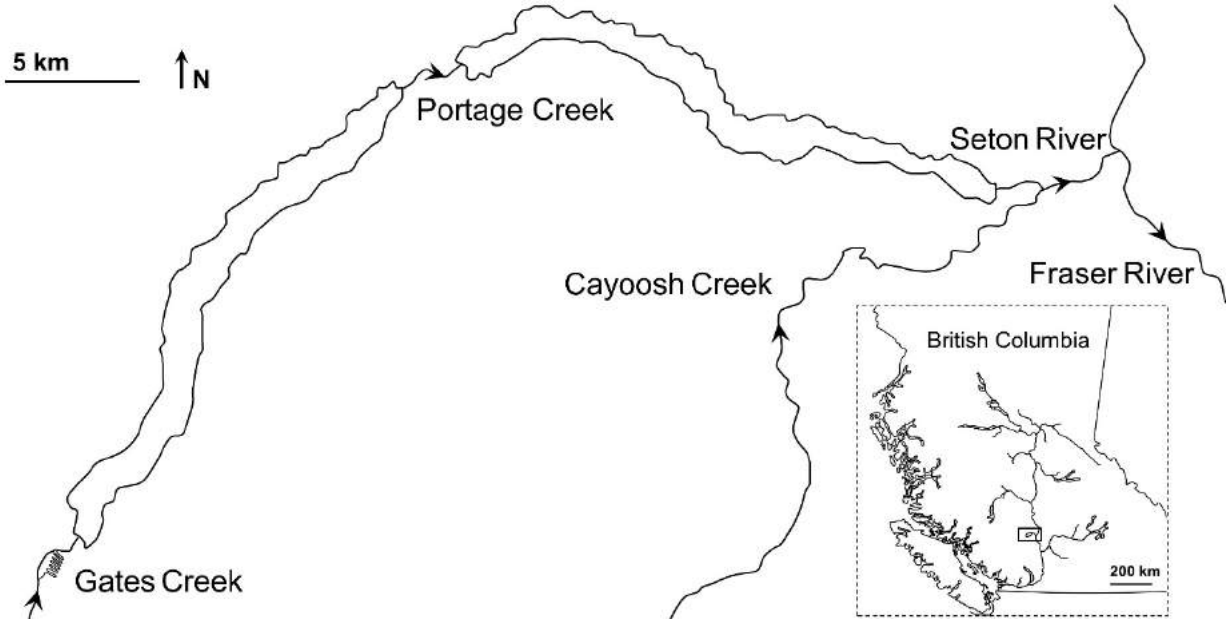


Fig 6.2 Diversion of water from the outlet of Seton Lake down a power canal. Water in the power canal exits through a generating station into the Fraser River, providing an attractive source of water to adult salmon migrating upstream.

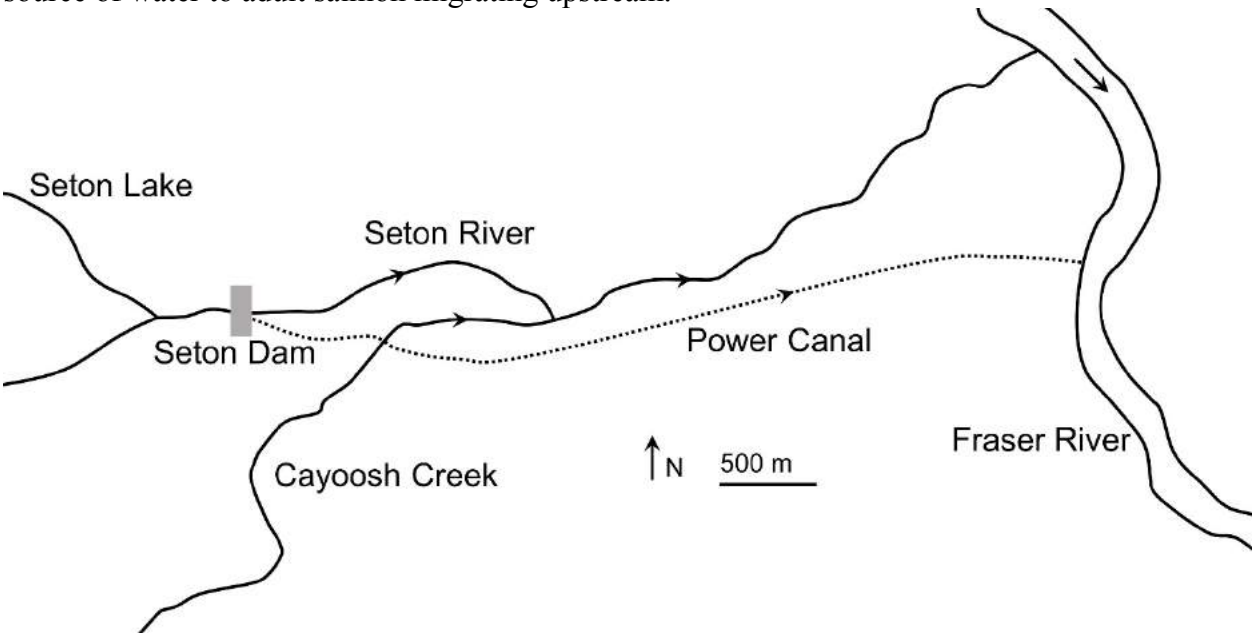


Fig 6.3 Time spent in each arm (a) and proportion of time in the Seton Lake arm (b) by Gates Creek sockeye salmon. Water from Seton Lake was paired with a mixture of Seton Lake and Cayoosh Creek water in a Y-maze, and the behavioural responses of the salmon was recorded. Mixtures are denoted in percentage of Seton Lake water. The axis in (b) has been altered to reflect an arcsine transformation, and the horizontal line indicates the value at which equal time is spent in the two arms of the Y-maze. Asterisks indicate a significant difference at $\alpha = 0.05$.

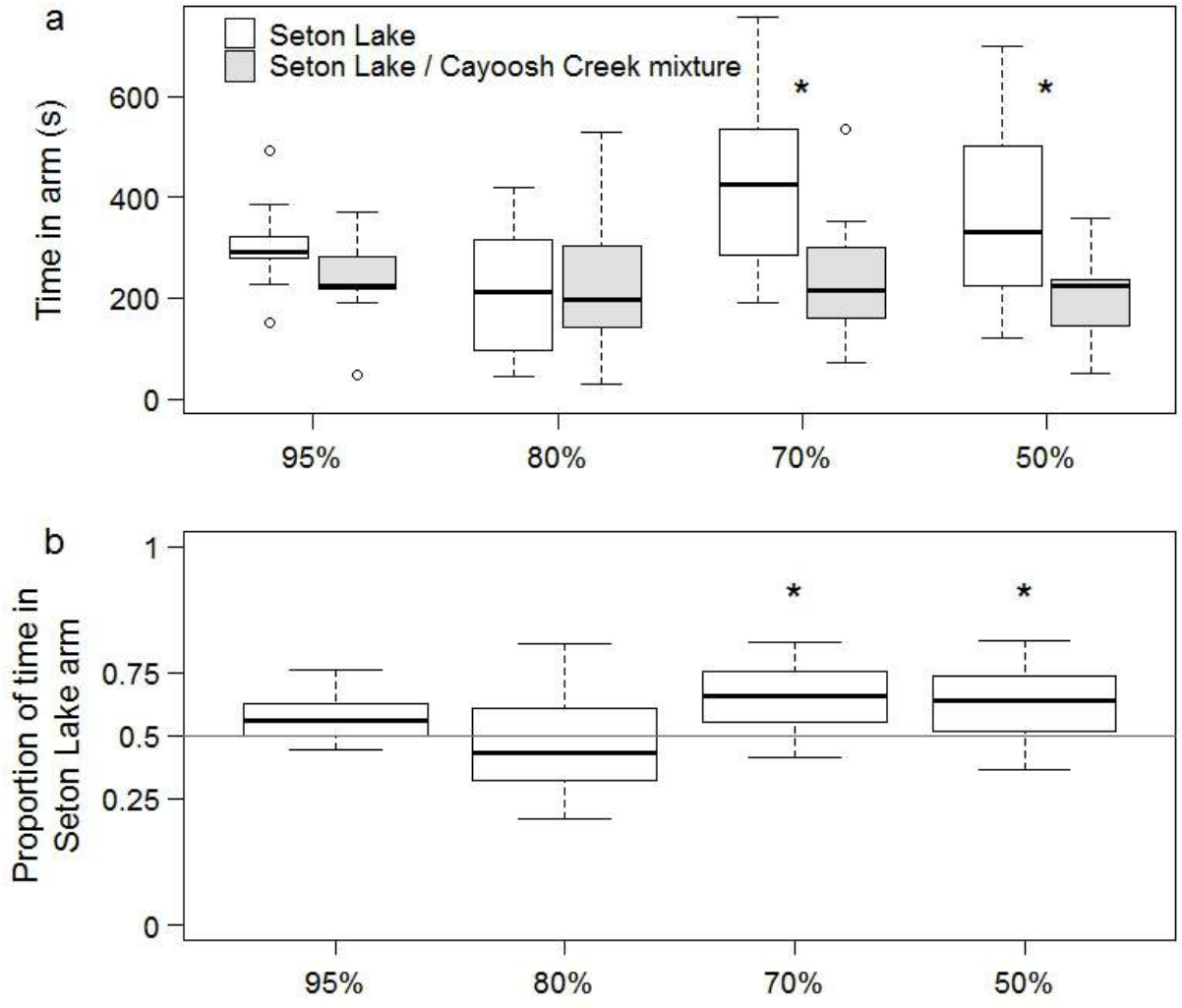


Fig 6.4 Time spent in each arm (a) and proportion of time in the Seton Lake arm (b) by Portage Creek sockeye salmon. Water from Seton Lake was paired with a mixture of Seton Lake and Cayoosh Creek water in a Y-maze, and the behavioural responses of the salmon was recorded. Mixtures are denoted in percentage of Seton Lake water. The axis in (b) has been altered to reflect an arcsine transformation, and the horizontal line indicates the value at which equal time is spent in the two arms of the Y-maze. Asterisks indicate a significant difference at $\alpha = 0.05$.

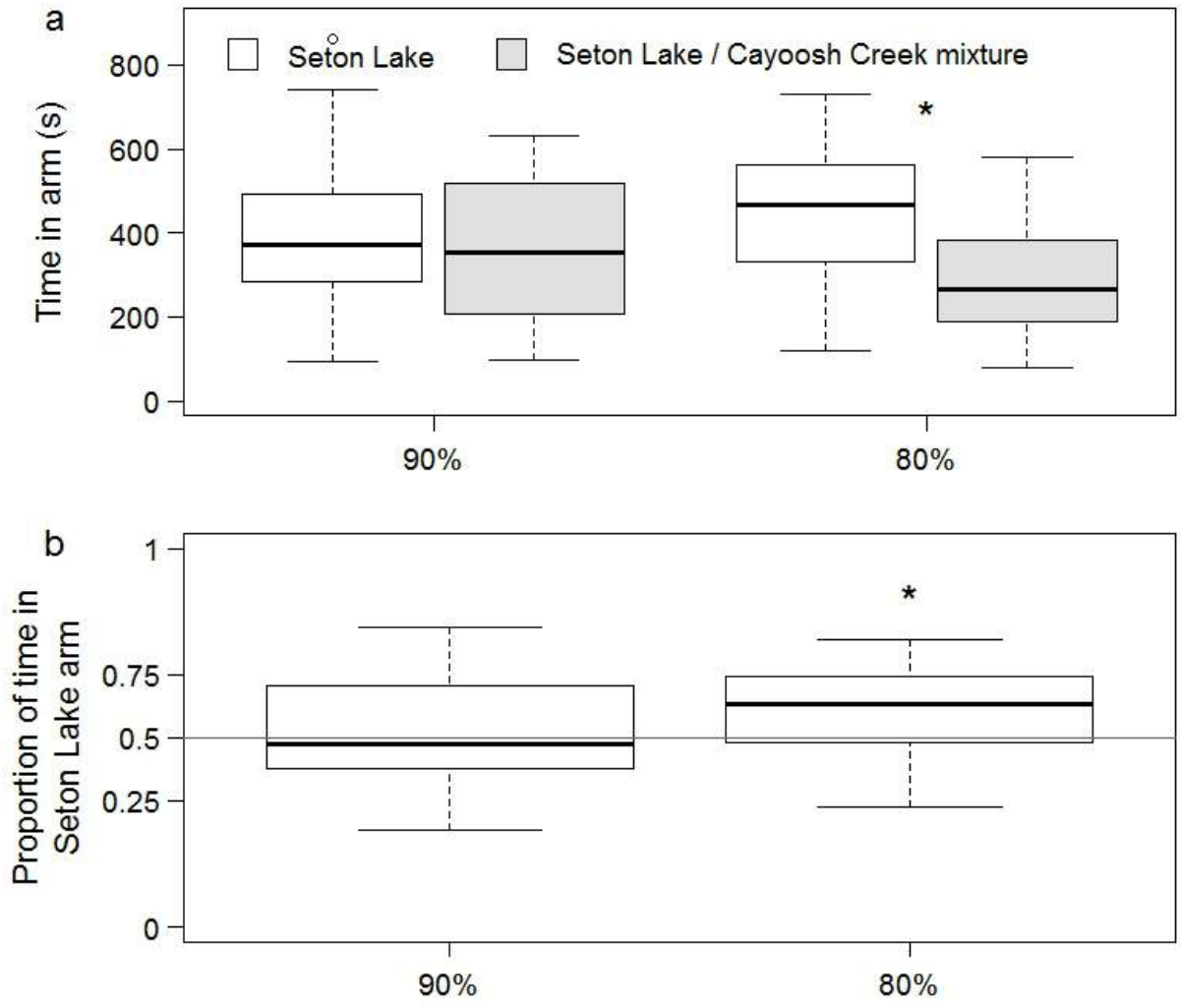


Fig 6.5 Time spent in each arm (a) and proportion of time in the Seton Lake arm (b) by pink salmon. Water from Seton Lake was paired with a mixture of equal parts Seton Lake and Cayoosh Creek water (i.e. 50% each) in a Y-maze, and the behavioural responses of the salmon was recorded. The axis in (b) has been altered to reflect an arcsine transformation, and the horizontal line indicates the value at which equal time is spent in the two arms of the Y-maze. Asterisks indicate a significant difference at $\alpha = 0.05$.

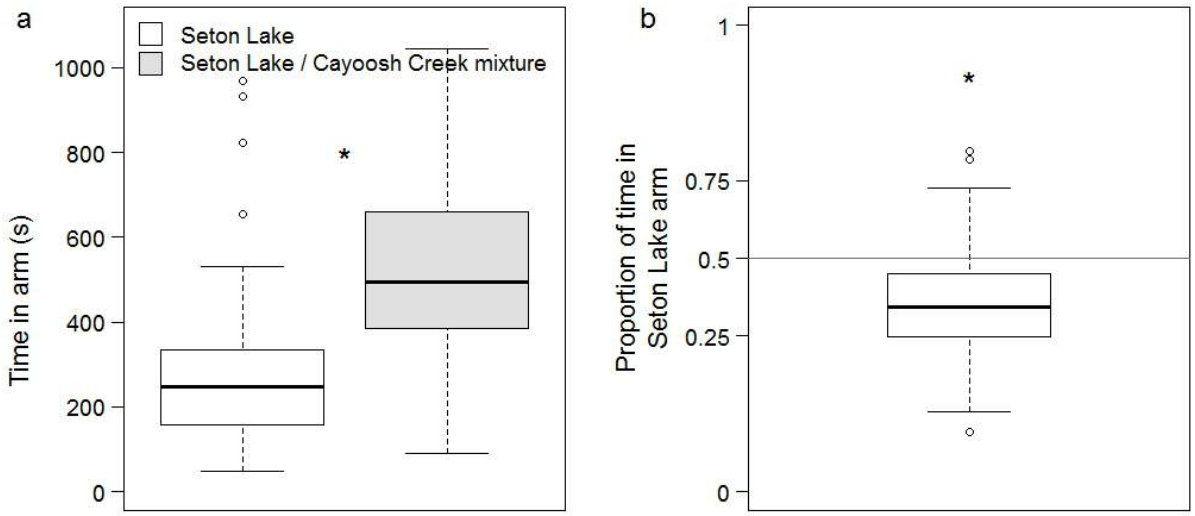
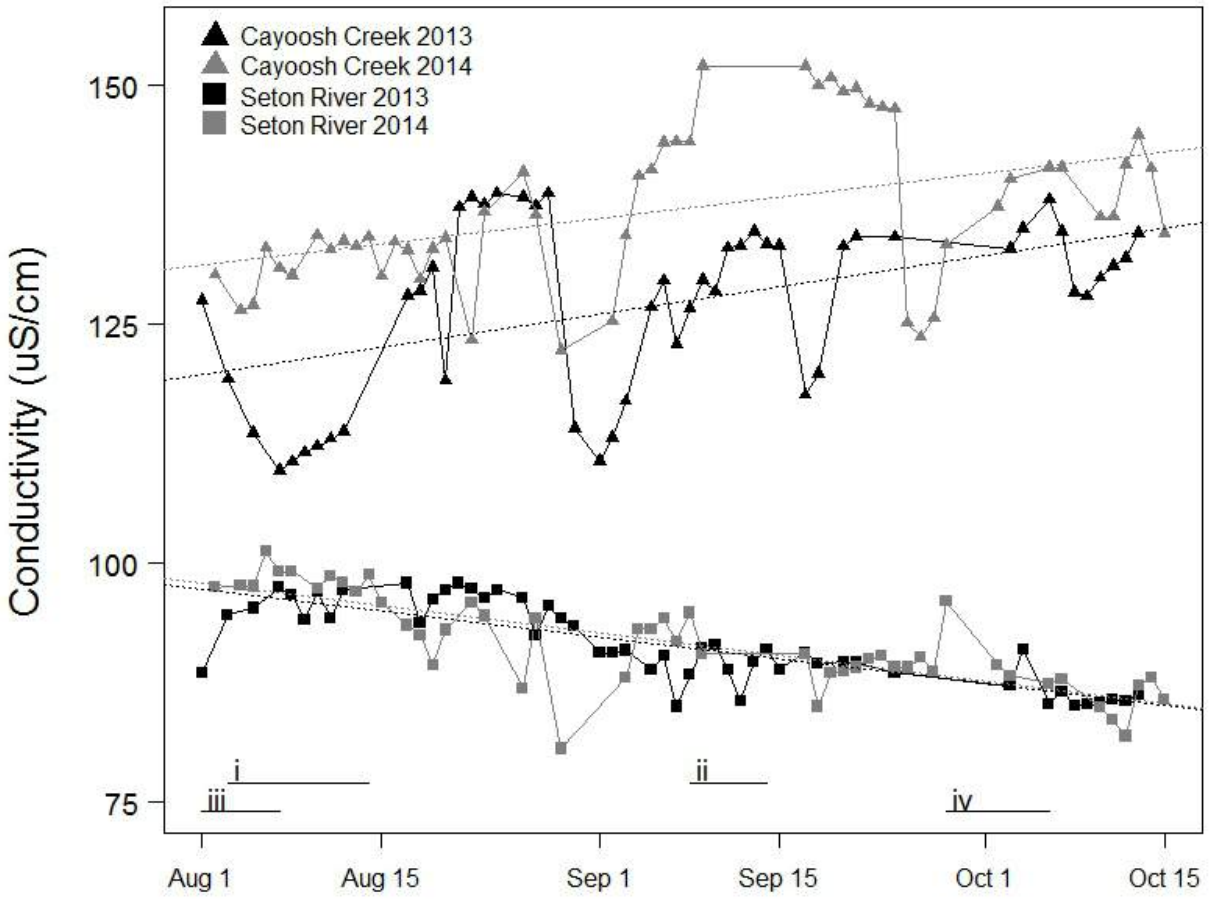


Fig 6.6 Conductivity of Seton River and Cayoosh Creek during the sockeye and pink salmon runs. Behavioural tests were conducted during the peak of the run for each group of salmon (i: Gates Creek sockeye salmon 2013; ii: Pink salmon 2013; iii: Gates Creek sockeye salmon 2014; iv: Portage creek sockeye salmon 2014). Regression lines are represented with dotted lines.



Chapter 7: Conclusions and future directions in salmonid homing research

My thesis explored the use of olfactory cues by Pacific salmon as they navigate to spawning grounds. There were two primary goals: 1) to develop a hypothesis that explains how salmon navigate to their natal waters, and 2) to examine the effects of external stressors on olfactory navigation during the spawning migration. Chapter 2 synthesized the large history of research on olfactory navigation to spawning grounds, and developed the hierarchical navigation hypothesis for salmon homing. A fundamental component of the hypothesis—that salmon use pheromones as secondary directional cues—was tested in chapters 3 and 4. In chapter 3, olfactory receptor genes were differentially expressed among stray and non-stray sockeye salmon. The analysis focused on vomeronasal receptor family 2-like genes, which are analogous to receptor genes expressed in the vomeronasal system in mammals and associated with the detection of pheromones. These genes were expressed at higher levels in the stray salmon, potentially indicating a sensitivity to pheromones. In chapter 4, olfactory navigation in stray salmon was tested in a behavioural choice experiment. The salmon exhibited an attraction to the odour of conspecifics, building on the findings of chapter 3 and providing further support for the hypothesis developed in chapter 2. Chapters 5 and 6 examined the effects of external stressors encountered during the spawning migration on olfactory navigation. Chapter 5 showed that in addition to releasing cues that are attractive to conspecifics (as demonstrated in chapter 4), sockeye salmon can also release cues that are repulsive. Such disturbance cues were released following a handling event, and could deter conspecifics from potential threats. Chapter 6 examined the effects of river regulation and the release of natal chemical cues on the choices made by salmon as they navigate upstream. Together, these findings contribute to our

understanding of olfaction in salmon and its role during the spawning migration, and have important management considerations.

7.1 Hierarchical navigation and conspecific attraction

The two prevailing hypotheses that seek to explain how salmon navigate to their spawning grounds are the olfactory imprinting hypothesis and the pheromone hypothesis. The hierarchical navigation hypothesis proposed in chapter 2 incorporates both imprinted cues and pheromones, and also provides a mechanism through which stray salmon increase their likelihood of locating mates and spawning habitat, thereby increasing reproductive success. Salmon have the unique characteristic of natal philopatry, which encourages the adaptation of an advanced navigation process such as olfactory imprinting. Animals that are not philopatric and seek habitat in unfamiliar areas, however, must rely on other environmental cues, such as conspecific cues (which can be olfactory, visual or auditory). Various species of larval reef fish, for example, are attracted to the odour of conspecifics when searching for a reef to inhabit (Öhman et al. 1998; Lecchini et al. 2007). Attraction to sites containing conspecifics can be found in nearly every other major group of animals as well, including birds (Muller et al. 1997; Serrano et al. 2004; Ward and Schlossberg 2004), mammals (Garret and Franklin 1988; Smith and Peacock 1990; Weddell 1991), reptiles (Kiester 1979; Stamps 1991) and invertebrates (Stamps et al. 2005). If a salmon has lost track of the familiar imprinted odours, it can either return downstream and search for those odours, or travel a new route in search of suitable habitat. In the latter scenario, the use of conspecific cues could provide similar benefits as those theorized for other animals. Stamps (1988) provided four potential benefits to conspecific attraction that might drive this behaviour. These include reproductive benefits (*e.g.* access to

mates), information about the habitat, protection from predators and defence against competitors. The first two of these benefits that might be most applicable to stray salmon. During the spawning migration, salmon are seeking habitat for the purpose of reproduction, and the reproductive benefits in the form of potential mates is therefore clear. Furthermore, however, salmon also require specific environmental conditions for spawning. The presence of conspecifics indicates that these conditions are met. In this manner, a migrating salmon could assess the quality of habitat before arriving at the site, based on the presence of conspecifics. This approach would limit the amount of energy and time required to locate suitable habitat.

Conspecific attraction has several potential conservation applications, including the re-establishment of breeding habitats (Reed and Dobson 1993). The use of recorded vocalizations has proven effective in attracting endangered songbirds to experimental sites, with as little as one year of playbacks potentially sufficient to establish a population (*e.g.* Ward and Schlossberg 2004; Ahlering and Faaborg 2006). Placement of decoy conspecifics has also been effective in songbirds as well as seabirds (Kress and Nettleship 1988; Ahlering et al 2010). There are numerous threatened populations of Pacific salmon throughout their range (Rand 2011), particularly in the Pacific Northwest and California, where widespread extirpations have occurred (Gustafson et al. 2007). The use of decoy salmon could encourage re-establishment of such populations without the need for translocations. In situations where a lag in natural recolonization is expected, such as in Washington's Elwha River where a major dam was recently removed (Pess et al. 2008), conspecific attraction might accelerate the recolonization process. The effectiveness of such a strategy has, in fact, already been demonstrated in salmon. In the 1930s, Atlantic salmon juveniles were held in a previously uninhabited river, and adults ascended the river the next fall (White 1934). In the 1950s, Atlantic salmon were stocked in an

unoccupied river, and again this appeared to attract conspecifics to the river (Solomon 1973). While conducting field work in August 2012, I used isolation tubes to hold sockeye salmon in the uninhabited Cayoosh Creek, a tributary of the Seton River. Overnight, a school of free-swimming sockeye salmon appeared adjacent to and downstream from the salmon I was holding. Interestingly, there did not appear to be any salmon present upstream, further suggesting they were attracted to those being held. If the habitat is suitable for spawning and rearing, this method of conspecific attraction could encourage the establishment of self-sustaining populations.

7.2 Straying into recipient populations

Several components of my thesis incorporate the movement behaviours and navigation of stray salmon, and I have discussed the importance of straying to colonizing new habitat and maintaining gene flow between populations. Recently, however, researchers have documented genetic introgression of stray Pacific salmon into small or threatened populations (Keefer and Caudill 2014). Historically, straying has been studied from the perspective of the donor population, to which the stray salmon belong. Studying straying from the perspective of the recipient populations, however, reveals potential conservation issues that these populations face.

Recently, researchers have found evidence of increasing genetic introgression as salmon stray into small recipient populations of conspecifics (Brenner et al. 2012; Johnson et al. 2012; Zhivotovsky et al. 2012; Jasper et al. 2013). Even low levels of straying, if sustained over multiple generations, can substantially alter the genetic composition of the recipient population (Hess and Matal 2014). Introgression from donor populations could cause the loss of local adaptive advantages, and reduce recipient population fitness (Grant 2012). The potential for fitness costs is greatest when straying occurs over large distances, as differences in fitness

increase with distance between populations (Fraser et al. 2011). The local adaptive advantages could be lost even when strays come from nearby donor populations, however, because adaptive advantages occur in Pacific salmon at fine spatial scales (Reisenbichler 1988; Fraser et al. 2011). Some of the recipient populations most threatened by straying are uniquely adapted ones, as the fitness costs might be greatest and unique behaviours could be lost. Zhivotovsky et al. (2012) provided evidence that a wild beach-spawning population of chum salmon (*O. keta*) in Kurilskiy Bay, Russia, are becoming swamped by a hatchery stock released in the same system. The potential for the hatchery salmon to spawn in beach habitats is unknown, and there is a risk that this behaviour could disappear if the hatchery stock continues to immigrate into the wild population. Straying of hatchery salmon into wild populations, as in this case, could incur particularly high fitness costs (Grant 2012; Rand 2012), especially if the hatchery stocks are domesticated and have reduced genetic variability. Spawning of hatchery salmon in the wild has been a long-standing concern in fisheries management (Grant 1997; Bisson et al. 2002), but continues to occur in many systems. In California's Sacramento River system, for example, Johnson et al. (2012) used isotopic analyses to estimate that over 90% of adults returning to the wild habitat of a small population of Chinook salmon were of hatchery origin. There is also evidence of hatchery chum salmon straying into wild populations in Prince William Sound, Alaska (Jasper et al. 2013).

In addition to the loss of local adaptations, straying could increase competition for breeding sites, which reduces spawning success (Essington et al. 2000). Straying could also expose the recipient population to foreign pathogens, although this has not yet been explored. The spread of pathogens among salmonids has been well documented (Naylor et al. 2005), such as infectious hematopoietic necrosis (IHN) virus, which in one instance was transferred from

hatchery steelhead to wild populations in the Columbia River (Kurath et al. 2003). In addition to the risk of direct pathogen transfer between stray and native fish, genetic introgression of stray salmon could reduce a recipient population's resistivity, given that populations of Pacific salmon can evolve resistance to endemic microparasites (Miller et al. 2015). Straying could also further increase extirpation risk through the reduction of "portfolio effects" (temporal stabilization resulting from the presence of numerous discrete populations within a meta-population; Schindler et al. 2010).

While these issues associated with genetic introgression warrant concern, however, there is also the potential for genetic and demographic rescue (Carlson et al. 2014; Whiteley et al. 2015) of small populations through straying. In recipient populations that have been previously isolated, for example, straying can create a demographic rescue effect by providing a crucial influx of individuals as the population transitions towards self-sustainability, as found in Chinook and coho salmon upstream from a newly constructed fish ladder in Washington's Cedar River (Anderson et al. 2015). Asymmetric gene flow into small recipient populations may also maintain genetic diversity, which might otherwise be lost through genetic drift (Consuegra et al. 2005).

Conspecific interactions could promote straying into recipient populations, as opposed to straying into uninhabited rivers. There is an apparent tendency of strays to enter rivers occupied by conspecifics (Jonsson et al. 2003; Dittman et al. 2010), and this might be explained by the evidence in chapters 3 and 4 that strays are attracted to conspecific olfactory cues. Large recipient populations may provide a stronger concentration of conspecific cues, and could thus attract more strays. Berdahl et al. (2014) also suggested that salmon may benefit from "collective

navigation" during the spawning migration, and that individuals in larger groups may be better able to sense and respond to navigation cues. Donor straying appears to decrease with increased abundance of the population (Sholes and Hallock 1979; Quinn and Fresh 1984; Hard and Heard 1999; Westley et al. 2015), supporting this hypothesis. Overlap in run timing of different populations could also affect straying. Brenner et al. (2012) noted that hatchery pink salmon stocks with higher stray rates typically had later run times, and that their migration overlapped with the run times of many wild pink salmon populations. Similarly, Ford et al. (2015) documented higher stray rates in populations originating from less abundant spawning areas. The authors suggest strays may have been attracted to the spawning grounds of larger populations, which have larger concentrations of conspecifics. Sizes of donor and recipient populations could therefore inversely affect straying: large donor populations could exhibit less straying due to a strengthened collective navigation, while large recipient populations could receive more strays by providing a stronger concentration of conspecific cues. Interestingly, each of these effects could potentially reduce the occurrence of recipient straying into small populations.

7.3 Olfactory navigation is complicated

The use of chemicals as directional cues during the spawning migration is complex. As seen in chapters 4 and 5, for example, the odour of conspecifics can be either attractive or repulsive, depending on the physiological state of the fish and the environmental conditions. Furthermore, different chemicals or odours might vary in their attractiveness, such that a fish may select some attractive odours over others when exposed to both. The hierarchical navigation hypothesis simplifies this variation in attractiveness, and while it provides a general framework for olfactory navigation in salmonids it does not cover all the intricacies involved. For example,

while pheromones might act as secondary cues, they may still be used by migrating salmon in conjunction with imprinted odours.

Identifying the chemicals used by migrating adults as directional cues will resolve a major question in the field of salmon homing research, but this could prove difficult based on the potential complexity of the cues involved. While experimental evidence suggests salmonids are able to imprint on just a single chemical cue in an artificial setting (Hasler and Scholz 1983; Yamamoto et al. 2010), this is likely not the case in their natural environment. Yamamoto et al. (2009) found that chum salmon were attracted to the amino acid profile of their natal water, even when the most abundant amino acid was removed. Furthermore, Sandoval (1980) demonstrated that coho salmon are able to distinguish mixtures of chemicals from the same chemicals in isolation. It appears that salmon are attracted to a combination of chemicals, or the olfactory "bouquet" (as suggested by Hasler and Scholz [1983]) of their natal site. If, for example, the amino acid profile is sufficient on its own to guide salmon to their home stream, what portion of this amino acid profile must remain intact for the water to be recognizable? Removing one amino acid does not appear to affect the salmons' response (Yamamoto et al. 2009), but what about the removal of multiple amino acids? A related unresolved question is how similarity in the odours of neighbouring streams affects the ability of migrating salmon to identify their natal site. If streams are similar in their chemical composition, as might be expected if they share a water source, the entirety of the olfactory bouquet might be necessary for recognition. If streams are dissimilar, however, salmon may need only recognize a fragment of their home stream's odour.

It is possible that molecular analyses of olfactory receptors might elucidate the chemicals salmon respond to. This approach would require several steps. First, the chemicals that bind to

the various receptors would need to be identified. Second, the expression of the receptor genes would need to be analyzed following exposure to the relevant chemicals. If the genes are either up- or down-regulated following exposure, it would then be possible to identify the directional cues guiding the salmon over the course of their migration by tracking changes in receptor gene expression. After identifying key chemicals that guide the fish, the findings could be verified by measuring their concentrations in the natal water. Simulated natal water could be created using these concentrations, and behavioural responses to this water could be tested, akin to the research conducted by Shoji et al. (2003) and Yamamoto et al. (2009).

7.4 Current assumptions in olfactory navigation

There are several assumptions that are currently made in the field of olfactory navigation during salmonid spawning migrations. These assumptions are grounded in logic, but lack direct empirical evidence. Future experiments could be useful in confirming these assumptions, and allow this field of work to continue in new directions.

7.4.1 *Assumption 1: Salmon use imprinted chemicals from natal water, and not pheromones, as directional cues*

As described in chapter 2, there is a large amount of evidence to support the theory that salmon imprint on the odour of their natal water, and they are attracted to this odour when they return as adults. As also described in chapter 2, however, salmon are able to detect pheromones as well, and can distinguish the pheromones of their own population from that of other populations. Currently the olfactory imprinting hypothesis is favoured (Keefer and Caudill 2014). A typical justification for acceptance of that hypothesis and rejection of the pheromone hypothesis is that juvenile pink and chum salmon migrate to the ocean prior to the adult

spawning migration, and therefore juvenile pheromones are not present to guide adults home (Ueda 2011). Experiments that test the two hypotheses against one another are currently limited, however. Brannon and Quinn (1984) provided adult coho salmon with a choice between their natal water and non-natal water containing juveniles from their population, and the salmon preferred the natal water. Black and Dempsen (1986) conducted a field test on Arctic char (*Salvelinus alpinus*), holding some char in an uninhabited tributary that fed into their normal migration route. None of the returning adults entered this tributary. I collected DNA samples from four of the salmon that entered Cayoosh Creek following my introduction of conspecific decoys, and microsatellite and major histocompatibility complex variation (methods detailed in Beacham et al. 2005) confirmed they were all strays that do not normally migrate through the Seton River. These findings, along with research by Quinn et al. (1983) and the results in chapter 4, suggest that salmon might be attracted to pheromones when natal water cues are absent—*i.e.* when the salmon have strayed. Revisiting the study in which the pheromone hypothesis was first proposed (Nordeng 1971), the char seemed to be guided by pheromones after being reared in a remote facility. These fish were therefore unable to imprint on any water in the system where they were released, and in this sense could be considered strays. Taken together, there is strong evidence to support the assumption that salmonids are guided primarily by imprinted cues, but with the corollary that they might be guided secondarily by conspecific cues.

7.4.2 *Assumption 2: The chemical composition of the imprinted natal odour is relatively static over time*

If a juvenile fish imprints on the odour of its natal water, the chemicals that create that odour are presumably similar in concentration or composition when the fish returns to spawn, at

least to the degree that the natal odour is recognizable. Based on their olfactory abilities, the chemicals that the fish imprint on are believed to be prostaglandins, bile acids, amino acids, or steroids, or a combination of these. The consistency of these chemicals' concentrations across seasons and years, however, has not been confirmed. The recent evidence that amino acids might be a critical component of imprinting (Shoji et al. 2003; Yamamoto et al. 2009) was followed with a study on the concentrations of amino acids in a natal river over time (Yamamoto et al. 2013). Of the 19 amino acids analyzed, the concentrations of 5-7 remained stable between the time at imprinting and the time of the spawning migration. It is possible that only a portion of the natal chemical bouquet must remain similar to retain its familiarity, but this has not been tested. Without knowing the temporal stability of a chemical in the natal river, it is difficult to assess its candidacy as a component of the imprinted odour.

7.4.3 *Assumption 3: Salmonids imprint to waypoints along the migration route*

Regardless of what chemicals contribute to the natal water, this odour will decrease in concentration as the distance from the natal site increases. It does not seem possible that a salmon entering fresh water hundreds or thousands of kilometres downstream from its natal site could detect the natal water from such a distance. This assumption led Harden Jones (1968) to suggest that juvenile salmon imprint on several waypoints as the migrate downstream, and recognize the sequence of these waypoints as adults. There is indirect evidence that salmon might imprint on multiple odours through transport studies, as detailed in chapter 2, but no experiments have been conducted that directly test sequential imprinting. To do so, one could sequentially expose juveniles to several different waters at relevant periods based on their natural outmigration. As adults, these fish could be exposed to the different waters and their behavioural

responses monitored. The waters could be paired sequentially such that each pair comprises water from two adjacent points in the theoretical river system. Based on the sequential imprinting hypothesis, we would expect to the fish to consistently prefer the water that is theoretically further upstream.

7.4.4 *Assumption 4: Imprinting occurs prior to the parr-smolt transformation*

If a fish imprints on the odour of its natal site, this imprinting presumably occurs while the fish is still present at that site. There is strong evidence that salmon imprint during the PST, but attempts to imprint prior to this period have been mostly unsuccessful (Hasler and Scholz 1983; Dittman et al. 1996). Based on the life histories of some species, however, imprinting should occur at earlier stages. Pink salmon, for example, do not transition through distinct parr and smolt stages because they migrate to the ocean shortly after emergence from the gravel (Heard 1991). They develop salinity tolerance during the brief window of emergence in a process that could be classified as smoltification (Gallagher et al. 2013), but whether they could imprint on their natal water during this period is unclear. An imprinting study on sockeye salmon indicated that 7 days of exposure to a chemical is insufficient for successful imprinting, although imprinting did occur after 14 days of exposure (Yamamoto et al. 2010). I led an experiment concurrent to the research presented in this thesis in which pink salmon successfully imprinted as alevins, prior to reaching the emergence stage. To my knowledge, the only other evidence of imprinting at such an early stage was found in a report on lacustrine sockeye salmon in Lake Roosevelt, Washington (Tilson and Scholz 1997). Following emergence, sockeye salmon migrate directly from their natal stream to a nearby lake, where they typically rear for one year before undergoing the PST and migrating to the ocean. Coho and Chinook salmon undergo a

similar process but migrate to another river, rather than a lake, sometimes travelling extensive distances in the process (Shrimpton et al. 2014). If any of these species use olfactory cues to recognize their natal stream, they presumably imprint on its odour before leaving that site following emergence. It is curious, then, that previous attempts to imprint coho salmon prior to the PST have been unsuccessful (Dittman et al. 1996). Dittman and Quinn (1996) theorized that rearing salmon in an artificial environment, without the introduction of novel odours to mimic movement away from the natal site, could interfere with pre-PST imprinting. Future imprinting studies that incorporate changes in water chemistry to reflect natural movement patterns could reveal imprinting processes that better align with the assumption that imprinting occurs prior to the PST.

These assumptions highlight several areas of potential research that could improve our knowledge of olfactory responses during spawning migrations, but as seen in my thesis there are other aspects of olfactory research that could influence salmonid conservation. The development of hydroelectric structures on salmon migratory routes could disorient salmon as they attempt to navigate towards spawning grounds, and the presence of stressors such as freshwater fisheries could delay migrations through alarm signaling. Hatcheries that supplement declining escapement numbers could cause genetic introgression of hatchery fish into wild populations through straying and an attraction to conspecifics. An understanding of olfactory-mediated behaviours during the spawning migration will help assess the likelihood of such threats, and could contribute to the conservation of salmonids.

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