

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



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ABSTRACT

Many fish that are exposed to a threat release disturbance cues, which are chemicals that alert conspecifics to the presence of the threat. The release of disturbance cues has been well demonstrated in various species of laboratory-reared fish. Migratory fish species often exhibit increased cortisol levels and are exposed to numerous stressors during their migrations, which could trigger the release of disturbance cues. We tested the responses of wild migrating sockeye salmon (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*) to the odours of disturbed and undisturbed conspecifics to determine whether these fish release disturbance cues following exposure to a simulated stressor. Furthermore, we tested the responses of sockeye salmon to water-borne cortisol, following evidence from past studies that this chemical is excreted through the gills of stressed fish, and speculation that endogenous cortisol correlates of stress might function as disturbance cues. We found that sockeye salmon avoid the odour of disturbed conspecifics, whereas pink salmon do not. Avoidance occurred in both female and male sockeye salmon, and was associated with an increase in plasma cortisol levels in females, but not in males. We also found no behavioural response to water-borne cortisol, which suggests this chemical does not act as an exogenous disturbance cue in sockeye salmon. Avoidance of disturbed conspecifics could limit exposure to risks during the sockeye salmon spawning migration, but could also delay the rate of migration and thereby accrue reproductive costs.

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1. 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; Ferrari et al., 2010), as opposed to the more commonly studied “damage-released cues” (Mathis et al., 1995; Wisenden, 2000; Brown, 2003) that are released following injuries. Disturbance cues appear to function as an early warning, eliciting behavioural and physiological responses in conspecifics (Wisenden, 2015), including avoidance (Jordão and Volpato, 2000), increased vigilance (Wisenden and Barbour, 2005), decreased foraging (Giaquinto and Hoffmann, 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 Nile tilapia and jundiá (*Oreochromis niloticus* and *Rhamdia quelen*, respectively; Barcellos et al., 2011), zebrafish (*Danio rerio*; Barcellos et al., 2014), salmonids (Salmonidae; e.g. Ferrari et al., 2008) and pacu (*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 our 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.

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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 may 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, 2007; Wong et al., 2008; Zuberi et al., 2011). Fish demonstrate behavioural differences after exposure to 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 possess 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 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 by Bett and Hinch, 2016).

In this study we examined whether wild adult sockeye and pink salmon exhibit avoidance responses to the odours of disturbed conspecifics. We subjected salmon to a disturbance consisting of a handling event, and then measured the effect of their odours on the behaviour of conspecifics. We predicted that sockeye and pink salmon would avoid the odour of disturbed conspecifics. Additionally, we tested the response of sockeye salmon to waterborne cortisol to determine whether this chemical, which could be excreted through the gills of disturbed salmon, might act as a disturbance cue.

2. Methods

2.1. 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)). We captured all sockeye and pink salmon 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 dur-

ing the peak of the respective populations' runs. Our experimental set-up was located approximately 100 m from the capture location.

2.2. Experimental set-up

We used submersible pumps 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 1136 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. 1). We used a Y-maze 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 × 1.22 m wide × 1.22 m high. A 2.44-m-long divider, made from fibreglassed plywood, divided the upstream end into two equally sized halves (or two "arms"). We 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. We used valves to maintain the amount of water entering each arm at 40 L min⁻¹. We also used plywood to cover the top of the Y-maze. We monitored behaviour through a video system, using an infrared camera (securitycamera2000.com, Hong Kong) connected to a monitor.

2.3. Experimental protocol

We conducted the experiments during daylight hours (0700–1700). At the beginning of each day, we captured 8–12 sockeye or pink salmon and immediately transferred them to an aerated 1000 L transport tank, which we then drove to the experimental set-up. We held the salmon in individual isolation chambers, constructed from PVC pipe (75 cm length × 15.3 cm diameter) with mesh ends, which we placed inside a 10,000 L holding tank receiving a continuous flow of water from Seton River. We transferred the salmon individually from the holding tank to the Y-maze, located directly adjacent, until each fish had been tested. The isolation chambers allowed us to remove individual fish from the holding tank without disturbing the others. Concurrently, we captured an additional 3 salmon from the same capture location at the beginning of each day, and we transferred them 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. Each batch of source fish contained at least one member of each sex (i.e. two males and one female, or two females and one male). 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 s during the transfer from the fishway to the transport tank using a dipnet, followed by 0.5–1.5 h in the aerated transport tank, then another 15 s of air exposure during transfer from the transport tank to the source tank. They remained in the source tank for up to 8 h. Physiological stress responses to handling events last up to 24 h 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. Manipulating wild fish is inherently stressful for them, so 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 h of confinement in net pens in the Seton River. Instead, we sacrificed the control fish immediately upon capture (<10 s) by cerebral percussion, before a physiological stress response could

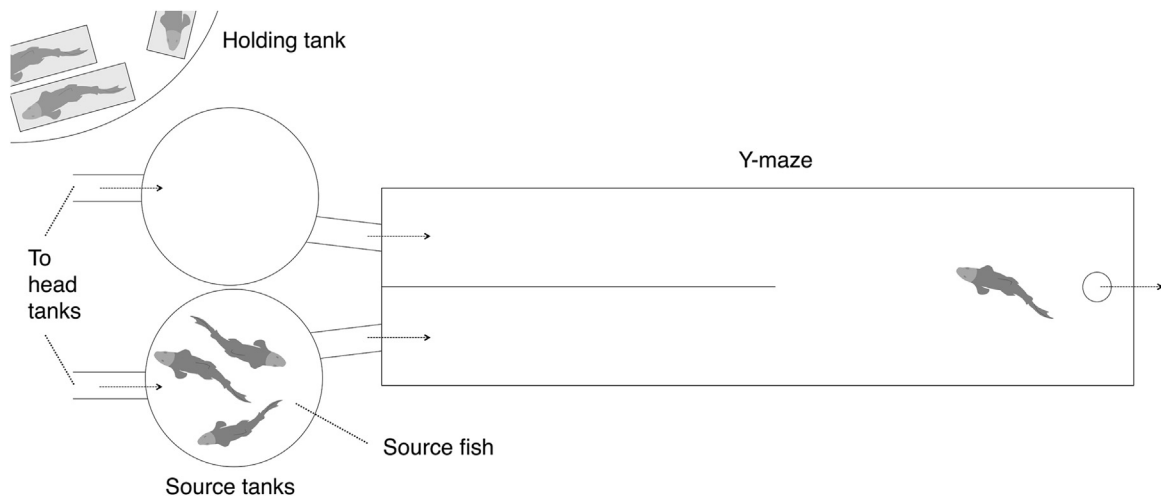


Fig. 1. Experimental set-up, with arrows indicating the flow of water. Source fish provided a disturbed or control conspecific odour into one arm of the Y-maze. Test subjects were transferred to the Y-maze from isolation chambers in the holding tank, and were tested independently. For the water-borne 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.

occur, and then placed them in a source tank. A previous study using Gates Creek sockeye salmon in Y-maze experiments (Bett and Hinch, 2015) 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 h 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 our study and all salmon were exposed to odours of lethally percussed conspecifics within 8 h post-death. The salmon tested in the Y-maze were captured and handled in a similar manner as the disturbed source fish, and were therefore also “disturbed” themselves. We 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 (Casselmann et al., 2013).

To test the potential role of cortisol as a disturbance cue released by threatened salmon, we 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. We added cortisol (Sigma Aldrich) 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. We used a peristaltic pump (Masterflex L/S model 7536-04, Cole-Parmer, QC, Canada) 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 min acclimation period, we removed the gate and recorded the behaviour of the fish for 20 min. We recorded the amount of time the fish spent in each arm, as well as the number of times it entered each arm. We 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 min trial, we removed the fish from the Y-maze and 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). We centrifuged each sample at 7000g 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 240 pc microplate reader (Molecular Devices, Sunnyvale, CA, USA) at the Fisheries and Oceans Canada Centre for Aquaculture and Environmental Research (West Vancouver, B.C.). We did not collect blood samples from the salmon prior to their introduction to the Y-maze in order to avoid causing additional handling stress. We 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 analysed following the same methods to provide an estimate of baseline plasma cortisol levels. Following collection of the blood sample, we measured fork length (Table 1), then returned the fish to the river. Source fish were held in their tank until the end of the experimental day (maximum 8 h), at which point they were released into the river. Before release, we clipped the adipose fin of each salmon to ensure no fish were recaptured and tested a second time.

2.4. 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). To test for normal distribution and equal variance, we used the Shapiro-Wilk test and Levene’s test, respectively, and applied square root transformations when the assumptions for parametric tests were not met. We used paired *t*-tests ($\alpha = 0.05$) to compare the amount of time and number of entrances in each arm of the Y-maze. 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), we used one-sample *t*-tests. We used linear regressions to test for relationships between the holding time prior to the trial and the proportion of time in the arm contain-

Table 1
Sample sizes, mean fork lengths, and dates for behavioural choice experiments.

Species	Treatment odour	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.

ing the conspecific odour. We 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). We ran all statistical analyses in R Studio V 0.98.501.

3. 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 ($t_{45} = -5.59$, $P < 0.001$; Fig. 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 control arm; $t_{45} = -3.32$, $P = 0.002$; Fig. 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. 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. 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 ($t_{44} = -1.17$, $P = 0.25$; Fig. 2a), enter it less frequently ($t_{44} = -0.92$, $P = 0.36$; Fig. 2b), spend a lower proportion of time in that arm than 0.5 ($t_{44} = -1.18$, $P = 0.25$; Fig. 2c), nor enter it a lower proportion of time than 0.5 ($t_{44} = -1.04$, $P = 0.30$; Fig. 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 (disturbed conspecific trials: $R^2 = 0.02$, $P = 0.35$; control trials: $R^2 = 0.02$, $P = 0.42$). 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 plasma cortisol levels between sexes of sockeye salmon, 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 (following square root transformation, $t_{28} = -4.67$, $P < 0.001$) and males ($t_{16} = -2.93$, $P = 0.001$). There were also fewer entrances in this arm by females (following square root transformation, $t_{28} = -2.58$, $P = 0.02$), and there was a similar trend in males ($t_{16} = -1.95$, $P = 0.07$), although the difference did not meet statistical significance. 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.

Sockeye salmon did not show any behavioural response to water containing 2×10^{-10} M cortisol. There was no difference in the amount of time spent in each arm ($t_{35} = -1.285$, $P = 0.21$; Fig. 4a), the number of entrances in each arm ($t_{35} = -0.713$, $P = 0.48$; Fig. 4a), nor the proportion of time ($t_{35} = -1.166$, $P = 0.25$; Fig. 4b) or entrances in the arm with water-borne cortisol compared to 0.5 ($t_{35} = -0.667$, $P = 0.51$; Fig. 4b).

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. 5a). 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. 5a). 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. 5b), 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. 5b). The proportion of time spent in the arm with the conspecific odour was not affected by the amount of time each pink salmon was held prior to their trial ($R^2 = 0.03$, $P = 0.12$), and as with sockeye salmon, sex did not affect the behavioural response ($P > 0.05$ for all variables). Due to the lack of responses to the odour of disturbed conspecifics, we deemed tests using control conspecific odours unnecessary and do not include cortisol data from these salmon. For this same reason, we also chose not to test the responses of pink salmon to water-borne cortisol.

4. Discussion

The responses of sockeye salmon to the odour of the handled conspecifics suggest disturbance cues were released following the

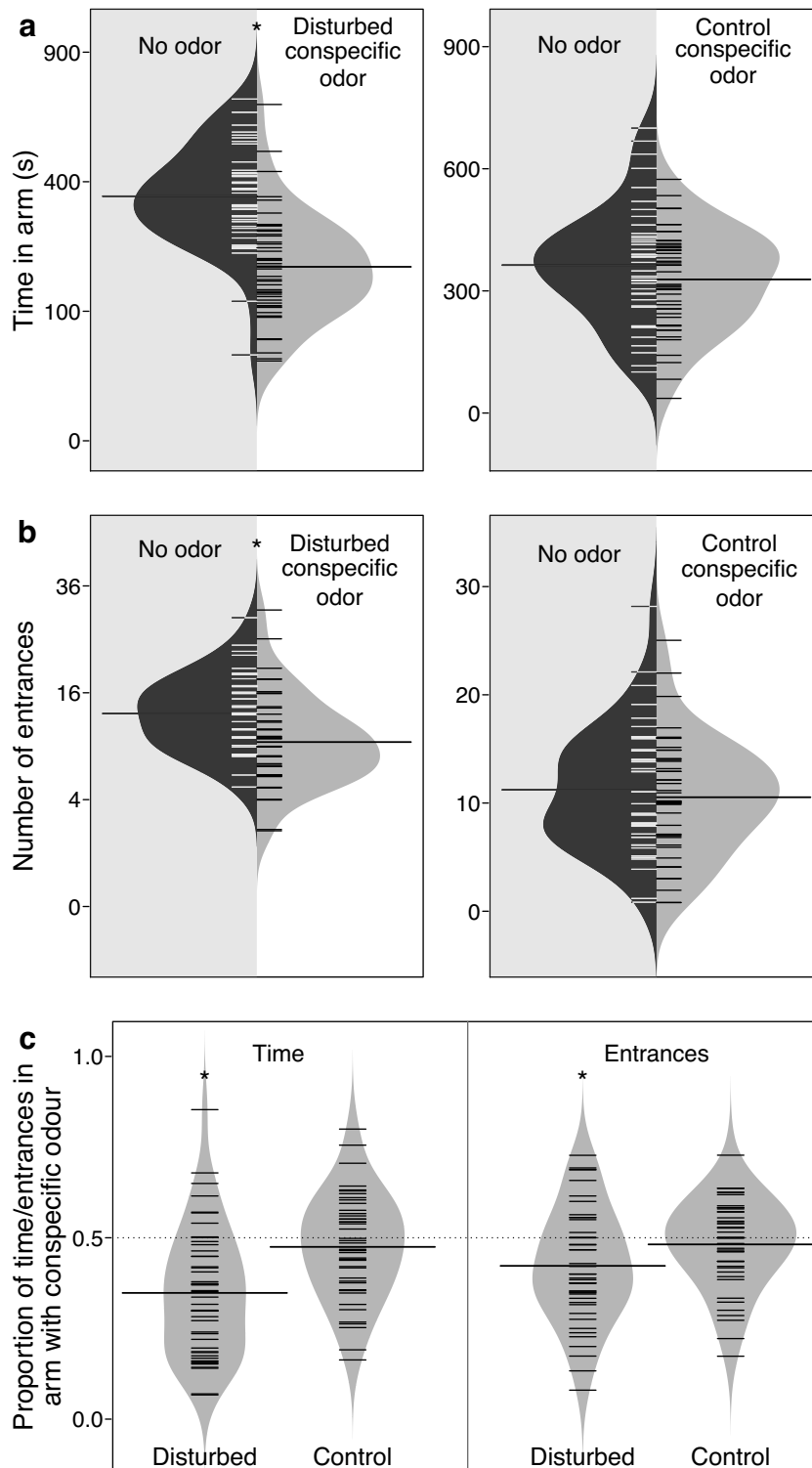


Fig. 2. Behavioural responses of sockeye salmon (*O. nerka*) in a Y-maze when the odours of disturbed and control conspecifics were introduced in one arm. The responses include (a) the amount of time the salmon spent in each arm, (b) the number of times they entered each arm, and (c) the proportion of 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.

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 (as

controls to the disturbed fish), test subjects did not display avoidance or attraction behaviours, further confirming that the handled fish did indeed emit disturbance cues. Our finding suggests that disturbance cues can be released by migrating adult sockeye salmon.

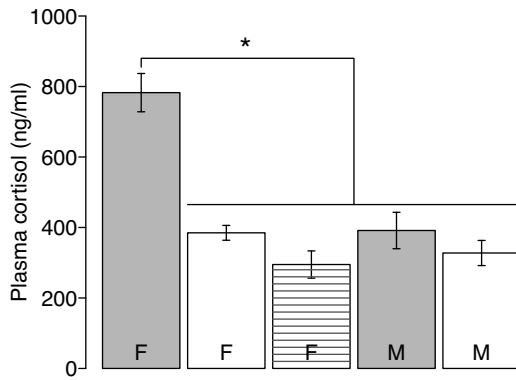


Fig. 3. Plasma cortisol concentrations in female (F) and male (M) sockeye salmon (*O. nerka*) exposed to the odours of disturbed conspecifics (grey bars; $n_F = 29$, $n_M = 17$) and control conspecifics (white bars; $n_F = 22$, $n_M = 23$), as well as baseline plasma cortisol in females sampled immediately upon capture (hatched bar; $n = 22$). Asterisks indicate a significant difference at $P < 0.05$.

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 (*O. mykiss*) exhibited a stress response when exposed to the odour of conspecifics that were

chased with nets (Toa et al., 2004), as did jundiá and Nile tilapia (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, at least at the concentration we tested. Barcellos et al. (2014) found a similar result in zebrafish. 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 we 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 – such as a barrier or a predator – 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 stan-

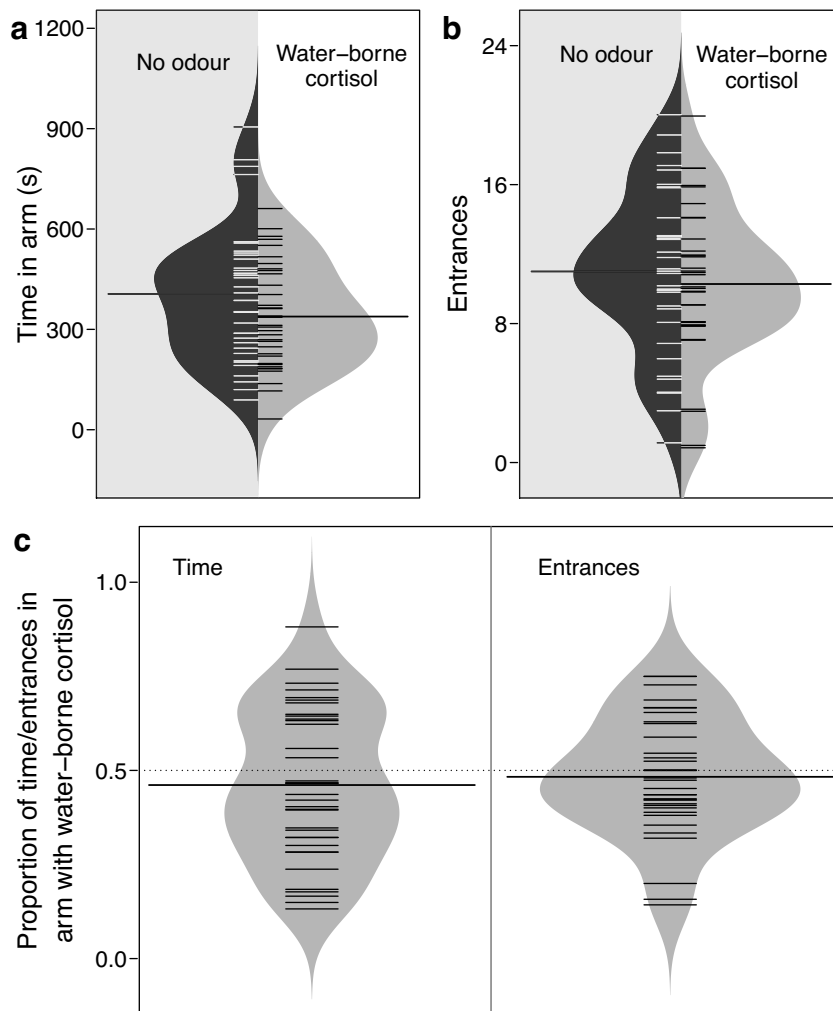


Fig. 4. Behavioural responses of sockeye salmon (*O. nerka*) in a Y-maze when water-borne cortisol was introduced into one arm to achieve a concentration of 2×10^{-10} M. The responses shown are (a) the amount of time the salmon spent in each arm, (b) the number of times the salmon entered each arm, (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.

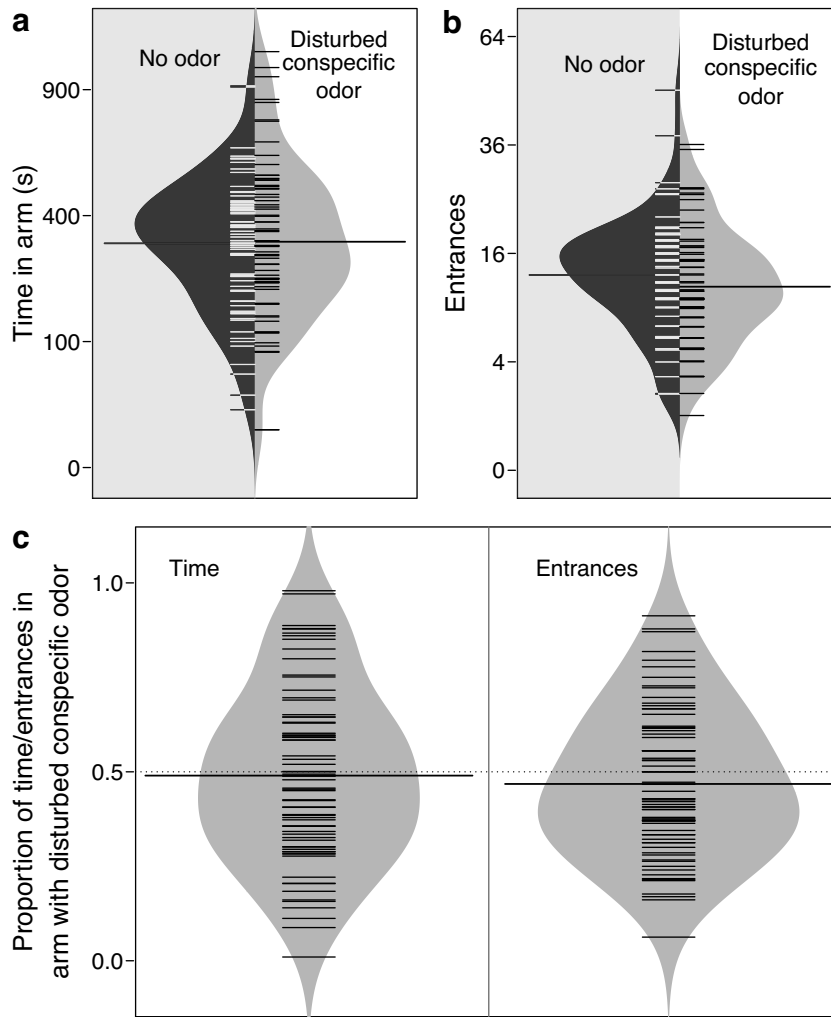


Fig. 5. Behavioural responses of pink salmon (*O. gorbuscha*) in a Y-maze when the odours of disturbed conspecifics were introduced in one arm. The responses include (a) the amount of time the salmon spent in each arm, (b) the number of times the salmon entered each arm, and (c) the proportion of time 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.

standardized 3 min 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 in general to chemical cues. Although direct evidence supporting this is lacking, as olfactory sensitivity has rarely been studied in pink salmon (Bett and Hinch, 2016), there is some 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 released, 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 our experimental protocol.

The sex of the fish did not have an effect on the behavioural response of sockeye or pink salmon in our study. However, there was a differential response in sockeye salmon 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). Our 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 we 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; Fisheries and Oceans Canada, 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., 2008; Martins et al., 2012). 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, our 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 potentially increasing migratory success.

Ethical note

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. Lethally percussed salmon were sacrificed immediately upon capture to minimize undue stress. All other salmon were held in flow-through tanks prior to their trials, and were released back into the river immediately following their trials.

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