

Prey naivety in the behavioural responses of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to an invasive predator

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SUMMARY

1. Non-native predators might inflict proportionally higher mortality on prey that have no previous experience of them, compared to species that have coexisted with the predator for some time.
2. We tested whether juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were less able to recognise a non-native than a native predator, by investigating behavioural responses to the chemical cues of the invasive smallmouth bass (*Micropterus dolomieu*) and the native northern pikeminnow (*Ptychocheilus oregonensis*) in both laboratory and field experiments.
3. Laboratory results demonstrated strong innate antipredator responses of individual juvenile Chinook salmon to northern pikeminnow; fish spent 70% of time motionless and exhibited 100% greater panic response than in controls. By contrast, antipredator responses to the chemical cues of smallmouth bass did not differ from controls.
4. These results were supported by similar differences in recognition of these predator odours by groups of juvenile Chinook salmon in fully natural conditions, though responses reflected a greater range of antipredator behaviours by individuals. In field trials, responses to northern pikeminnow odour resulted in increased flight or absence, reductions in swimming and foraging, and increased time spent near the substratum, compared to smallmouth bass odour.
5. Given that survival of juvenile fish is facilitated by predator recognition, our results support the hypothesis that naivety may be an important factor determining the effect of non-native predators on prey populations. Efforts to manage the effect of native and non-native predators may benefit by considering complex behavioural interactions, such as these at the individual and group levels.

Keywords: chemical cues, innate behaviour, invasive species, *Oncorhynchus tshawytscha*, prey naivety

Introduction

Human-assisted dispersal of non-native species has caused widespread changes in the global distribution of organisms (Olden *et al.*, 2004; Hobbs *et al.*, 2006). The ecological effects of non-native species on recipient ecosystems have ranged from positive to severely negative (Parker *et al.*, 1999; Mack *et al.*, 2000; Schlaepfer, Sax & Olden, 2011), spawning a suite of hypotheses to explain this variability. Important factors determining the effect of the invader include characteristics of its dispersal (Wilson

et al., 2009), its release from enemies or pathogens, and adaptations to or suitability in human environments (Sax & Brown, 2000). In the invaded range, factors associated with impact include biotic resistance (Fridley *et al.*, 2007), alteration of habitat (Sax & Brown, 2000) and inability of native species to cope with novel invaders (Sih *et al.*, 2010). Of these, reduced or ineffective responses by native species to novel predators because of a lack of prior experience (also known as 'prey naivety') have been identified as a possible contributor to the decline of the former (Schlaepfer *et al.*, 2005). Research on a range of

taxa including mammals (Banks, 1998; Barrio *et al.*, 2010; Fey *et al.*, 2010), invertebrates (Freeman, 2006; Edgell & Neufeld, 2008), amphibians (Pearl *et al.*, 2003; Gomez-Mestre & Diaz-Paniagua, 2011) and fish (McLean, Barbee & Swearer, 2007) has demonstrated reduced recognition, less effective defences or increased mortality in response to non-native than to native predators in laboratory experiments. Prey naivety has also been hypothesised to play a particularly important role in freshwater environments because of spatial habitat heterogeneity and barriers to dispersal, which promote adaptation to localised predation regimes and the potential for greater vulnerability to introduced predators (Cox & Lima, 2006).

Recent research demonstrates that naive prey do not respond consistently differently to, or suffer higher mortality from, non-native predators (Rehage, Dunlop & Loftus, 2009; Kovalenko *et al.*, 2010). Some studies have suggested that the ability to respond to novel predators is predominantly driven by factors such as level of predation risk in a system (Murray, Roth & Wirsing, 2004), similarity of non-native predator behaviour or morphology to that of native predators (Rehage *et al.*, 2009), or reliance of prey species on learned versus innate systems (Epp & Gabor, 2008). Ambiguity in past research findings may be partly due to experimental limitations on prey naivety, which ultimately demands testing of prey vulnerability to native and non-native predators in ecologically relevant settings. Laboratory settings typically suffer from a 'fence effect', where the prey are behaviourally constrained, while experiments using wild organisms cannot control for differences in experience and learning. Native and non-native predators used in comparative studies may also have subtly different hunting modes, making comparisons of prey behavioural response and subsequent mortality challenging (Rehage *et al.*, 2009). Prey populations in the wild inevitably have access to additional chemical, visual or social cues that may mediate the effects of naivety, but can be difficult to replicate adequately in laboratory settings. For these reasons, there are recognised gaps in research between laboratory and natural environments, and difficulties in assessing the relevance of prey naivety at a population level (Sih *et al.*, 2010). To compare responses in controlled and fully natural settings, we investigated the innate behavioural responses of juvenile Chinook salmon (*Oncorhynchus tshawytscha* Walbaum) to the chemical cues of native northern pikeminnow (*Ptychocheilus oregonensis* Richardson) and non-native smallmouth bass (*Micropterus dolomieu* Lacepède) predators in laboratory and field environments.

Our choice of prey and predator species reflects not only the significant management interest in the impacts of predation on endangered juvenile Pacific salmon, but the relatively comprehensive knowledge of the biology, evolutionary history and distribution of these species in the Pacific Northwest region of North America. The northern pikeminnow is a native cyprinid found in lentic and lotic systems from northern Oregon to British Columbia (Wydoski & Whitney, 1979; Li *et al.*, 1987); one of the largest members of the minnow family native to North America, it has been identified as a significant predator of emigrating salmon smolts in the Columbia River hydro-power systems and has been actively controlled for two decades (Tabor, Shively & Poe, 1993; Zimmerman, 1999). The smallmouth bass was chosen as a highly invasive predator that is of concern, because of its widespread distribution (Vander Zanden *et al.*, 2004; Sharma *et al.*, 2007) and well-known negative effects on the diversity and abundance of native fish (MacRae & Jackson, 2001; Jackson, 2002). The first known introductions of smallmouth bass from eastern North America to the Pacific Northwest were in the 1920s; subsequent decades saw continued stocking by state agencies and private citizens (Carey *et al.*, 2011). Smallmouth bass have become an increasingly important predator of juvenile salmon over the last two decades (Sanderson, Barnas & Rub, 2009). Although estimated predation rates vary across the region, analysis over several emigration periods (March–June) suggests that smallmouth bass predation could account for between 4 and 35% of wild autumn-run Chinook smolts in the lower Yakima River, depending on environmental conditions (Fritts & Pearsons, 2004). However, juvenile Chinook salmon are unlikely to have evolved chemosensory recognition of smallmouth bass. One study (involving frogs) has showed development of innate predator recognition after 20 generations under heavy selection pressure (Kiesecker & Blaustein, 1997), whereas others have not found this in a similar number of or more generations (Gomez-Mestre & Diaz-Paniagua, 2011; Paoletti, Olson & Blaustein, 2011).

We hypothesised that juvenile Chinook salmon would show an innate recognition and response to the chemical cues of northern pikeminnow, but not to smallmouth bass. We first tested responses of individual juvenile salmon in a laboratory, using two channel choice flumes. A similar chemical cue experiment was later conducted with groups of wild Chinook salmon under fully natural field conditions, to test the validity of the laboratory results and examine the ecological implications of any observed differences in behaviour.

Methods

Experiment 1: Chemosensory recognition of predators under laboratory conditions

Study organisms. Spring-run (Yakima River population) Chinook salmon eggs were acquired by the Cle Elum Supplementation Research Facility from 15 families of natural origin spawned during September 2009. The eggs were transferred to the Northwest Fisheries Science Centre (National Oceanic and Atmospheric Administration) 2 months later and incubated at 5 °C until they began feeding in mid-February 2010. In early April, the fish (approximate fork length 40 mm) were transported to the Western Fisheries Research Centre (WFRC; United States Geological Survey) and reared in sand-filtered and UV-treated fresh water from Lake Washington at ambient intake temperatures (mean \pm SD = 10.1 \pm 1.0 °C) in a 700-L circular tank. Juvenile Chinook salmon were held on a natural photoperiod regime and fed twice daily *ad libitum* (Bio Oregon bioVita) until the trials began in early June.

Smallmouth bass ($n = 3$; fork length: mean \pm SD = 390 \pm 10 mm; mass: mean \pm SD = 1018 \pm 85 g) and northern pikeminnow ($n = 5$; fork length: mean \pm SD = 398 \pm 27 mm; mass: mean \pm SD = 812 \pm 192 g) were captured from Lake Washington and housed in 700-L circular tanks at WFRC. Predators were closely matched in biomass, as juvenile salmon have a sophisticated ability to distinguish between predator concentration as well as low- and high-risk predators based on chemical cues (Vilhunen & Hirvonen, 2003; Hawkins, Magurran & Armstrong, 2007). Northern pikeminnow and smallmouth bass in our targeted size class (350–400 mm) are effective and efficient predators of juvenile Chinook salmon in the size range tested (Tabor *et al.*, 1993; Zimmerman, 1999); this was corroborated by our own separate predation trials (L. Kuehne, unpubl. data). Predator diets were restricted to earthworms for 7 days prior to experiments to control for juvenile salmon responses to diet cues in predator faeces.

Experimental protocol. Two channel choice flumes were used to test avoidance and preference of fish to chemical cues of different predator species. The flumes were constructed from opaque acrylic (20 cm $h \times$ 30 cm $w \times$ 90 cm l) with a clear acrylic wall (20 cm $h \times$ 60 cm l) dividing the channels. The inflow areas were separated by a white plastic screen, creating a 5 \times 15 cm mixing zone for incoming water, and a white mesh plastic sliding gate created a 20 \times 30 cm acclimation (pre-choice) area at the rear of the flume (Fig. 1). The outflow pipe in the rear was screened off, and water levels were set to a depth of 10 cm. Four flumes were arranged side-by-side, with two

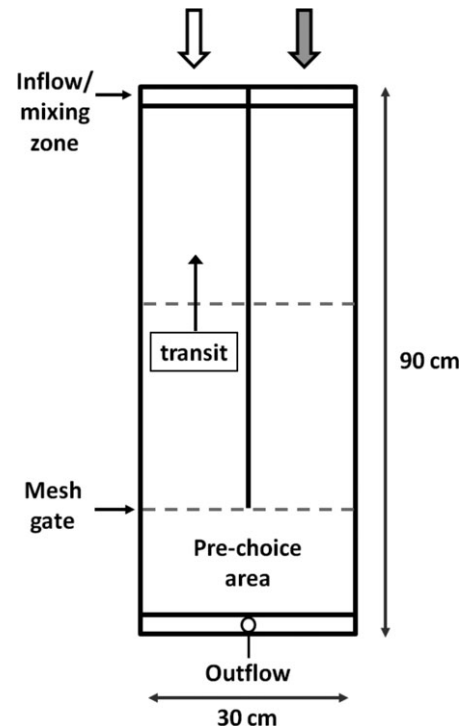


Fig. 1 Schematic of two channel choice flume and grid overlay for video analysis. A 'transit' constituted moving from one section (delineated by dashed lines) into another. Time spent in the pre-choice area did not count as time spent in either channel.

video cameras (Q-See Model QR208–811, 3.6 mm lens) positioned overhead to record trials, allowing the operator to remain out of sight. The tanks were set on rubber pads to reduce influence of vibration and surrounded by black plastic sheeting; incident light was kept low (mean \pm SD = 42 \pm 14 lux) to reduce shadowing.

Water was gravity-fed to individual channels from 75-L 'stimulus' tanks containing either two pikeminnow, or two smallmouth bass, or one pikeminnow and one smallmouth bass, or nothing (control). Each day, these predator pairs were moved to stimulus tanks 1 h prior to the start of the first trial and were removed at the end of the day. Water was delivered to the channels at 1 L min⁻¹, resulting in residence time of *c.* 40 min in stimulus tanks (which were aerated to compensate for changes in oxygen from predator respiration). Stimulus tanks always contained the same predator species or combination; tanks were drained at the end of each day, cleaned with Argentyne solution and refilled overnight.

To reduce variations in behaviour because of hunger, Chinook salmon were fed *ad libitum* by hand every 3 h on trial days. For each trial, we placed a single salmon in the pre-choice area of each flume and, after allowing 20-min acclimation time, water lines with treatment or control

water were connected to each channel. Pre-trial dye tests revealed that incoming water reached the pre-choice area after *c.* 2 min (mean \pm SD = 2.2 \pm 0.6). Accordingly, the gates were raised remotely, using a pulley system of fluorocarbon fishing line, 2 min after the water lines were connected, allowing the salmon to enter the channels. All trials were recorded by video from the time of placement into the pre-choice area to the end of the trial (10 or 15 min following the gate raise). Juvenile salmon averaged 65 \pm 5 mm (fork length: mean \pm SD), and individual fish were used only once.

We analysed the video recordings to quantify three behavioural metrics over discrete time intervals after the gate was raised: number of transits, activity and channel occupancy. 'Transits' were counted as movement across sections within the tank, as determined by a grid overlay (Fig. 1), summed every 20 s for comparison over time (i.e. 30 time points for 10 min trials). 'Activity' was characterised every 20 s using a modified movement index (Johnsson, Höjesjö & Fleming, 2001) which described: (i) movement of fins or body without displacement, (ii) swimming, (iii) no motion (hereafter 'freezing') and (iv) swimming burst or darting response (hereafter 'panic'). Of these behaviours, freezing and panic are routinely used as indicators of antipredator response in juvenile salmonids (Healey & Reinhardt, 1995; Vilhunen & Hirvonen, 2003; Scheurer *et al.*, 2007). 'Channel occupancy' was assessed as the channel where the fish was located at the end of each 20-s interval. Because responses of individual fish were very similar over short time periods (e.g. a fish that was frozen remained so for many minutes or a fish that was swimming comfortably continued that response over time), our use of discrete time intervals gave a good representation of the amount of time spent in each behaviour; therefore, for ease of reference, we hereafter report the per cent of intervals as 'per cent time'. The observer quantifying the behavioural metrics was blind to the treatment.

We conducted 24 replicates over 10-min trials of four odour combinations (treatments): (1) bass : control, (2) pikeminnow : control, (3) bass : pikeminnow, (4) bass + pikeminnow : control. In addition, we performed 36 control trials (control : control) and an additional 18 replicates of treatments (1) and (2) over 15 min. This allowed control trials to be later truncated for comparison with 10-min trials or used in full for comparison against the additional replicates of treatments (1) and (2) over the longer 15-min time period. Flumes were drained and cleaned between trials with Argentyne-treated brushes and control water. In addition, treatments were assigned to a different flume each day and the treatment channel

switched at midday to allow testing for the effect of flume position and treatment channel.

Statistical analysis

The behaviour of each juvenile Chinook salmon was summarised for each 10-min trial using three metrics: per cent of time frozen, per cent of time exhibiting panic behaviour and per cent of time in the stimulus channel (note: the stimulus channel was randomly assigned for the control : control treatments. As the bass : pikeminnow treatment contained a potential stimulus in both channels, we defined pikeminnow as the stimulus in that treatment). To meet assumptions of normality and heteroscedasticity, all variables were arcsine transformed prior to conducting separate ANOVAs. Although some slight departures from normality existed within some treatment groups following data transformation, ANOVA is considered robust to departures given sufficient sample sizes and equality of variance (Zar, 1999, p. 185). *Post-hoc* comparisons were made using one-tailed Dunnett's test against the control treatment and Tukey's HSD between other treatments. To examine responses over time, time series of mean and variance in number of transits were generated by treatment (15-min trials only). Smallmouth bass predators remained healthy throughout trials, but two northern pikeminnow had to be replaced; potential consequences of using different predators were therefore tested with no statistical difference in the effect on any behaviours (ANOVA, $P > 0.3$ for all variables). There being also no effect of flume position or treatment channel (ANOVA, $P > 0.4$), data from all of these groups were pooled in subsequent analysis.

Experiment 2: Chemosensory recognition of predators under natural conditions

Study organisms and sites. We tested for chemosensory recognition of predators by juvenile Chinook salmon in Swauk Creek (47.32 N, 120.68 W); a third-order tributary to the upper Yakima River, Kittitas County, Washington State (Fig. 2). Lower Swauk Creek is privately owned and minimally disturbed and contains a wild population of Chinook salmon. An initial survey prior to the experiment identified sites with at least 6 (mean \pm SD = 10 \pm 4) juvenile Chinook salmon visible to a snorkeler holding position in the stream, ensuring that video footage taken for subsequent analysis would allow two focal follows per site. Adult northern pikeminnow and smallmouth bass are present in the mainstem Yakima River, but have not been seen in Swauk Creek [Pearsons *et al.*, 1994, G.

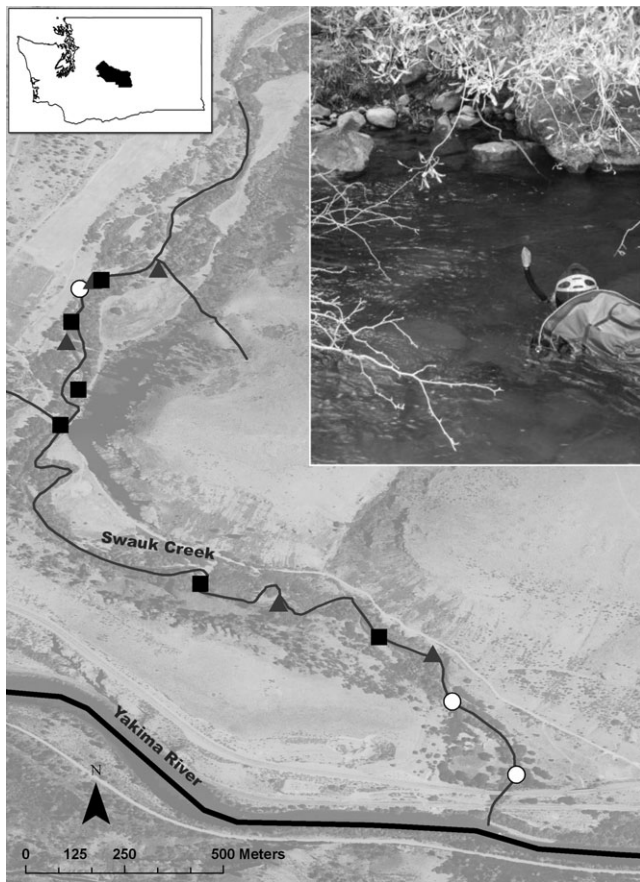


Fig. 2 Experimental sites along lower Swauk Creek (Kittitas County, Washington State, inset) and example of pool habitats surveyed before and after introduction of predator odour. Treatments are identified as pikeminnow (*Ptychocheilus oregonensis*, black square), smallmouth bass (*Micropterus dolomieu*, grey triangle) and control (well water, open circle).

Temple, Washington Dept. of Fish and Wildlife (WDFW) Ecological Interactions Team, pers. comm].

Predator stimuli. Four days before the experiment, two northern pikeminnow (fork length: 330 and 380 mm) and two smallmouth bass (fork length: 300 and 310 mm) were collected using electrofishing and angling from the mainstem Yakima River. Following capture, predator fish were held at the Yakama Nation Hatchery in Prosser, WA and deprived of food for 72 h to decrease the possibility of a reaction by Chinook salmon to diet-related cues. This is a shorter holding period than predators in the laboratory, but should still ensure gut evacuation (Rogers & Burley, 1991). To replicate laboratory conditions as closely as possible, predator odour was prepared by placing the two individuals of each species in 75-L plastic tubs of well water for 1 h. Predator water was collected in 4-L containers for transport on ice in insulated coolers, and

well water with no predator odour was collected for control treatments. There is little information on the length of time predator odour remains viable in storage, and therefore, the experiment was conducted as quickly as possible and the ice refreshed daily to maintain constant temperature. The last trial with predator-scented water was conducted within 48 h of water collection.

Experimental protocol. The experiment was carried out between 21 and 23 September 2010 by a two-person team consisting of a snorkeler and an observer. Predator odour treatments (northern pikeminnow, $n = 6$; smallmouth bass, $n = 6$; and control, $n = 3$) were determined by the observer so that treatments were alternated over time and space without bias by the snorkeler. Approaching from downstream, the snorkeler installed a clear 13-mm nylon hose c. 1 m upstream of the observed fish. Installation of the hose did not have a noticeable effect on the abundance of juvenile Chinook salmon in a pool or the area in which they were distributed; only one site was abandoned because fish were sufficiently disturbed that they vacated the pool.

After allowing 10 min for acclimation, the snorkeler re-entered the pool with a small hand-held camera (GoPro HD Surf HERO) and slowly moved into a position 45° downstream and to the side of the main school of fish. Once the snorkeler was in position, the observer started the experiment. Every minute the snorkeler was cued to count fish in the pool; after 10 min, the observer dispensed treatment water through the hose over a 20- to 40-s period. Counts were recorded for the 10 min following (min 11–20), at which point the experiment was terminated. Pool depth, length and water velocity were measured, and the dominant substratum and other species abundances recorded; dye tests conducted at three sites determined the approximate residence time of the 4-L volume of treatment water added to pools (mean \pm SD = 69 \pm 4 s). Analysis of environmental variables indicated no significant differences in flow, temperature or pool size between treatment groups (ANOVA, $P < 0.18$ for all variables).

We later used the video recordings to conduct focal follows on two randomly selected fish per site using standard media player software (Quicktime Pro). Juvenile salmon in Swauk Creek exhibited the territorial feeding behaviour common in stream-dwelling salmonids (Quinn, 2005), allowing relative ease in focal follows of individuals before and after the stimulus. Without knowledge of the treatment, an observer measured the following behavioural variables for 10 min before and after introduction of predator cue: (i) time absent from a feeding

territory, (ii) time on the substratum, (iii) number of feed strikes and (iv) time spent swimming. Focal follows could not be conducted for one pikeminnow site because of poor video quality. During video analysis, it also became clear that at one smallmouth bass site, the observer was visible to fish, so that site was removed from all analysis; this resulted in a final n of six, five and three snorkel observations of pikeminnow, smallmouth bass and control, respectively, and five, five and three for focal follow data.

Statistical analysis

We tested antipredator responses by subtracting mean pre-stimulus from post-stimulus scores for all variables, resulting in difference scores between the two periods; statistical tests were then conducted on difference scores with predator stimulus as a factor. The change in fish observed (based on snorkeler observation) was converted to a per cent increase or decrease to standardise for differences in abundance between sites; treatment effect was tested on this variable using one-way ANOVA.

As fish received the same stimulus at each site, scores for the two focal follow fish were averaged for a site score per variable. If a fish perceived a predator threat, we expected an increase in time absent, a shift in water column position towards the substratum, reduced feeding and reduced swimming activity (Berejikian, Tezak & LaRae, 2003; Leduc *et al.*, 2007). Given expected correlations among these responses, multivariate analysis of variance was used to test an overall effect of stimulus type, with subsequent analysis of variance tests on individual variables. The assumption of normality was not consistently met by all variables; therefore, we used permutational analysis of variance (PERMANOVA; Anderson, 2001) with 9999 permutations for all multivariate and univariate tests of global significance; PERMANOVA as implemented by the vegan package in R also has the advantage of allowing testing of unbalanced designs (Oksanen *et al.*, 2009). Significant pairwise differences were determined with Tukey–Kramer *post-hoc* tests.

Results

Innate recognition and response in the laboratory

Juvenile salmon spent significantly more time frozen and exhibiting panic responses in the presence of native northern pikeminnow odour compared to the control (Fig. 3a,b; $P < 0.03$ for both variables, ANOVA with Dunnett's *post-hoc* test), whereas no differences were observed

between non-native smallmouth bass and control treatments ($P > 0.38$ for both variables, ANOVA, Dunnett's). All treatments containing pikeminnow, whether alone (Dunnett's, freeze: $P < 0.001$, panic: $P = 0.01$), combined with bass (Dunnett's, freeze: $P < 0.001$, panic: $P = 0.03$) or as alternative to bass odour (Dunnett's, freeze: $P < 0.001$, panic: $P = 0.01$), differed significantly from controls, but not from each other (ANOVA, Tukey's, $P > 0.98$ for both variables). There was no statistical effect of treatment on the per cent of time spent in the stimulus channel (Fig. 3c; ANOVA $F_{4,127} = 1.68$, $P = 0.16$).

Behavioural responses of juvenile salmon varied over time across treatments, as shown by the time series of transits during the 15-min trials (Fig. 4). There was minor variation in response to the gate opening in control and

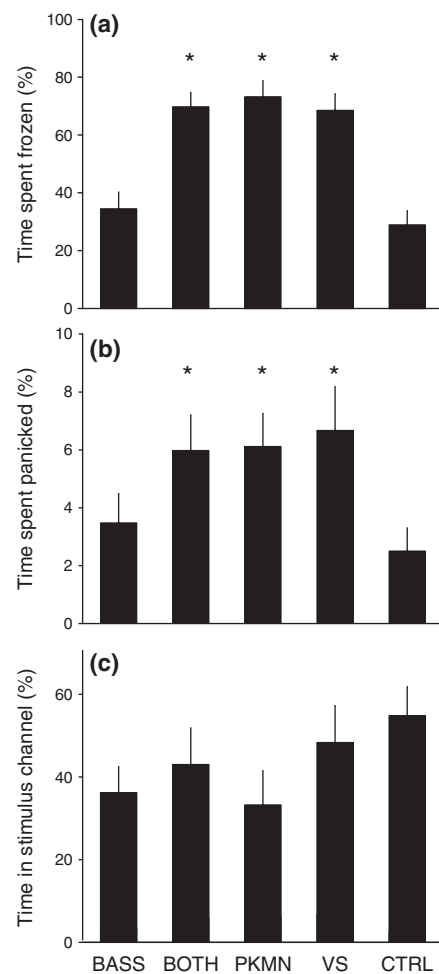


Fig. 3 Per cent of time (mean \pm SE) spent (a) frozen (b) panicked and (c) in stimulus channel by treatment, and significant *post-hoc* comparisons for 10-min trials. Treatments are bass : control (BASS), bass + pikeminnow : control (BOTH), pikeminnow : control (PKMN), bass : pikeminnow (VS) and control : control (CTRL). *Treatments that are significantly different from the control (Dunnett's test, $\alpha = 0.05$).

smallmouth bass treatments, but response to pikeminnow odour was marked by an initial period of very high transit rates followed by long periods of immobility; short bursts of panic and high transits were often repeated later in the trial (Fig. 4a). This difference in patterns of panic and immobility was further reflected in the time series of standard deviation in transits (Fig. 4b); smallmouth bass and control treatments exhibited similarly moderate variation in the first 3 min, which stabilised over time, whereas responses to northern pikeminnow showed much greater overall variation.

Recognition and response in a natural stream

In the Swauk Creek experiment, the percentage of fish visible to the snorkeler decreased in northern pikeminnow treatments and increased in smallmouth bass and control treatments following introduction of predator or control odour (Fig. 5; PERMANOVA, $F_{2,11} = 3.63$, $P = 0.06$). There was a significant treatment effect (PERMANOVA, $F_{2,10} = 3.21$, $P = 0.03$) on the overall behaviour of focal fish (combination of time absent, time on the substratum, feed strikes and time swimming). Subsequent PERMANOVA

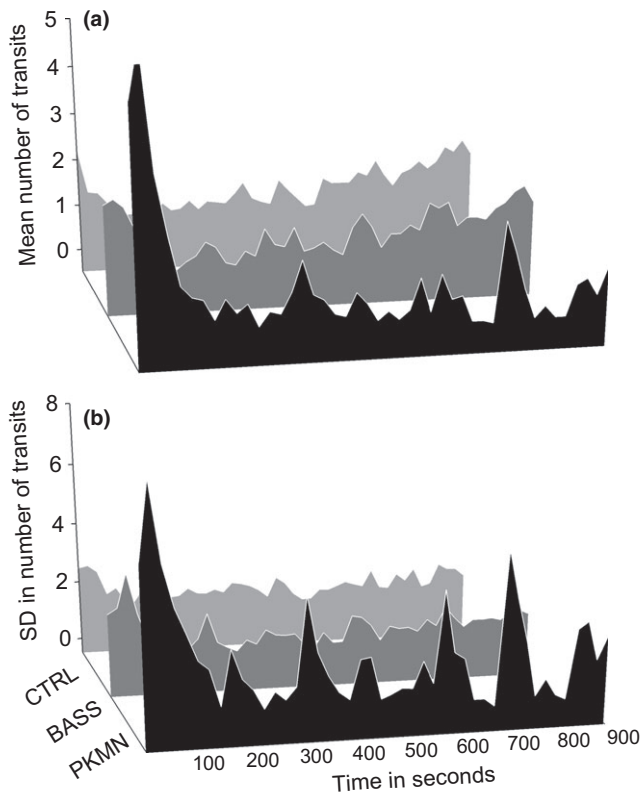


Fig. 4 (a) Mean number and (b) standard deviation of transits by treatment in 15-min trials following gate raise. Treatments are pikeminnow (PKMN), smallmouth bass (BASS) and control (CTRL).

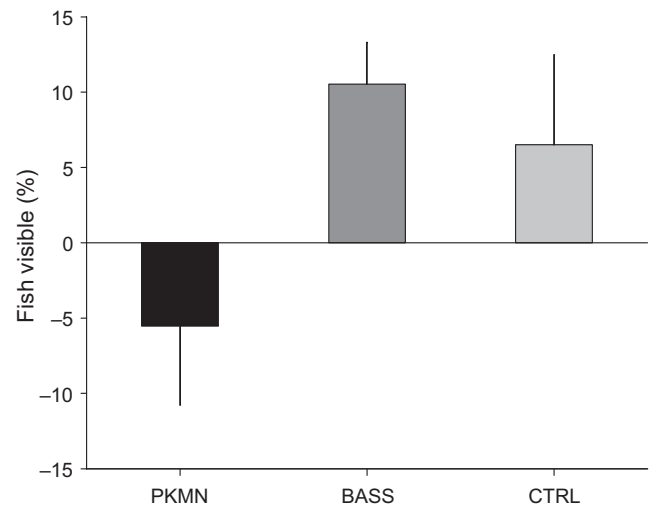


Fig. 5 Change (mean \pm SE) in per cent of fish visible to snorkeler by treatment for sites exposed to odour of pikeminnow ($n = 6$), smallmouth bass ($n = 5$) and control ($n = 3$). Treatments are pikeminnow (PKMN), smallmouth bass (BASS) and control (CTRL).

analysis on the individual variables showed a significant increase in time spent on the substratum (Fig. 6b; $F_{2,10} = 4.81$, $P = 0.03$) and reduction in time swimming (Fig. 6d; $P = 0.045$) in northern pikeminnow treatments. Though not statistically significant, visual inspection of time absent and feeding strikes showed increased flight or hiding and reduced foraging in response to northern pikeminnow odour (Fig. 6a,c; $P > 0.07$). Response to inputs of control and bass odour resulted in consistently opposite or neutral trends from northern pikeminnow odours, including a tendency towards increased visibility, swimming and foraging (Fig. 6a–d).

Discussion

Our study supported the prey naivety hypothesis by demonstrating a strong difference in innate responses of juvenile salmonids to a native versus non-native predator. Although similar results have been witnessed in other taxonomic groups (mammals: Banks, 1998; Barrio *et al.*, 2010; Fey *et al.*, 2010; invertebrates: Freeman, 2006; Edgell & Neufeld, 2008; amphibians: Pearl *et al.*, 2003; Gomez-Mestre & Diaz-Paniagua, 2011) and, to a lesser extent, other fish species (McLean *et al.*, 2007), our study is unusual in testing these responses in both laboratory and field settings to reflect more accurately the potential relevance of naivety under natural conditions. In aquatic systems, despite the existence of innate chemical recognition of native predators being reported for some species (reviewed in Ferrari, Wisenden & Chivers, 2010),

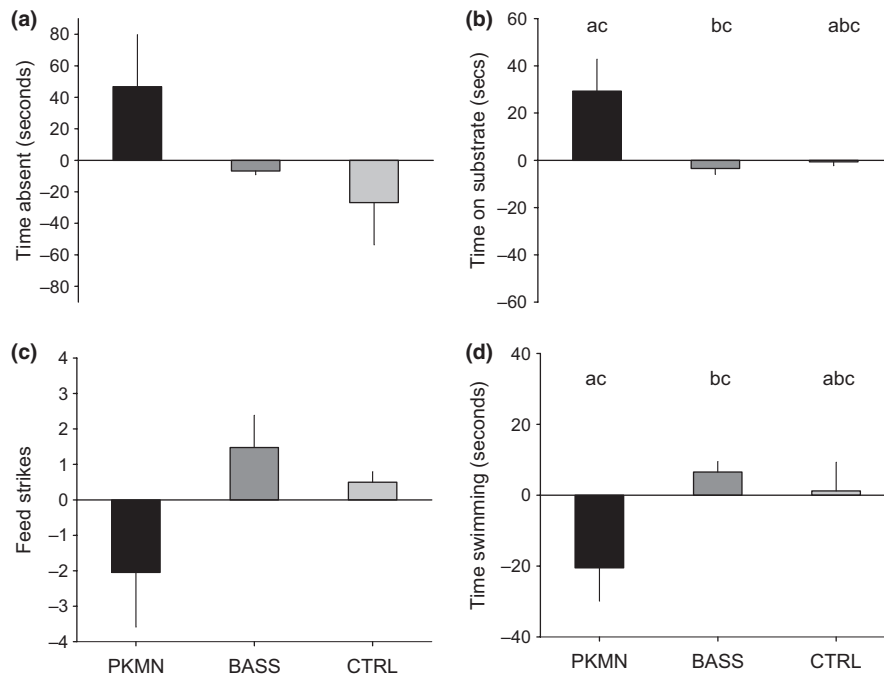


Fig. 6. Change (mean \pm SE) in focal follow variables: (a) time spent absent from feeding territories (s), (b) time spent on substratum (s), (c) feeding strikes and (d) time spent swimming (s) after introduction of predator odour. Sites were exposed to the odour of pikeminnow ($n = 5$), smallmouth bass ($n = 5$) and control ($n = 3$). Treatments are pikeminnow (PKMN), smallmouth bass (BASS) and control (CTRL). Significant treatment differences are indicated by letter groupings above bars (Tukey–Kramer test, $\alpha = 0.05$).

this is not considered a universal phenomenon. On the contrary, aquatic species are understood usually to require learning of predator odours (Ferrari *et al.*, 2010). Recent studies have shown that aquatic prey can acquire predator recognition in the egg stage (Ferrari & Chivers, 2009), raising questions as to whether prey from wild eggs are actually naive. In our study, wild parents spawned in a hatchery upstream of populations of northern pikeminnow or smallmouth bass. While tests of innate recognition must carefully consider prey origins, we believe our study considerably strengthens the case for the existence of innate responses in some species.

Predator-naive juvenile Chinook salmon exhibited a strong antipredator response to the chemical cues of northern pikeminnow but failed to respond to the odour of an introduced centrarchid predator (smallmouth bass). Previous research testing response of aquatic species to chemical cues of native and non-native predators has found differences in species of frogs (Pearl *et al.*, 2003; Bosch *et al.*, 2006; Gomez-Mestre & Diaz-Paniagua, 2011), salamanders (Murray *et al.*, 2004; Gall & Mathis, 2010), fish (McLean *et al.*, 2007) and crayfish (Shave, Townsend & Crowl, 1994). However, generalising these findings is difficult because yet other studies have failed to find definitive innate differences, including for frogs (Murray *et al.*, 2004; Smith *et al.*, 2008), fish (Kovalenko *et al.*, 2010)

and crayfish (Gherardi *et al.*, 2011). It is most informative in this context to compare our results with studies that also used fish prey, as processing and use of chemical cues are likely to be more similar among vertebrates that inhabit similar environments (Derby & Sorensen, 2008). Kovalenko *et al.* (2010) found no difference in the response of tetras (*Hemigrammus marginatus* Ellis) to recently introduced peacock bass (*Cichla kelberi* Kullander and Ferreira) and native wolfish (*Hoplias malabaricus* Bloch). However, prey were captured in the wild, and prior experience or learning could not be controlled. In addition, it is unclear how predator diets were controlled prior to experimental trials, which may have resulted in similar recognition. Our results are more consistent with those of McLean *et al.* (2007), who found a strong antipredator response of both newly recruited and juvenile galaxiids (*Galaxias maculatus* Jenyns) to odours of native eel predators (*Anguilla australis* Richardson), but only mild avoidance to the odour of introduced rainbow trout (*Oncorhynchus mykiss* Walbaum).

Our study further revealed that similar treatment effects were found even in the dynamic environment of a rearing stream. Both group and individual fish behaviour showed a coordinated trend in northern pikeminnow treatments towards absence from feeding territories, reduced swimming and feed strikes and shifting to a

benthic position. These results are consistent with those of Berejikian *et al.* (2003), who tested the response of juvenile Chinook salmon to northern pikeminnow in flow-through aquaria with a gravel substratum. Although assays were done with individual fish, they reported similar changes in reduced activity, feeding and slightly increased time near the substratum. By contrast, fish in our control and smallmouth bass trials generally increased activity and visibility; this is a reasonable result given that, unless a predator threat was perceived, one might expect increasing acclimation to the snorkeler over time. Leduc *et al.* (2007) witnessed a similar slight increase in activity and visibility by juvenile Atlantic salmon (*Salmo salar* Linnaeus) in control treatments of a natural stream experiment. It is also possible that a pulse of new water attracted fish as a potential source of food or by stirring up detritus.

Although our study demonstrated a strong difference in innate recognition and suggested its adaptive value (flight and increased caution), we were not able to test the survival implications explicitly. To our knowledge, only three studies so far have tested explicitly the relationship between innate recognition of chemical cues and increased survival in direct predator trials. Kovalenko *et al.* (2010) found no difference in recognition or behaviour by tetras in response to chemical cues of a native and non-native predator, but were unsuccessful in testing differential mortality, as predators did not actively pursue prey. By contrast, Kiesecker & Blaustein (1997) found reduced response in allotopic versus syntopic populations of red-legged frog (*Rana aurora* Baird and Girard) to chemical cues of introduced bullfrogs (*Rana catesbeiana* Shaw) resulting in increased rates of predation. Similarly, Gomez-Mestre & Diaz-Paniagua (2011) reported that Iberian green frogs (*Pelophylax perezi* Seoane) failed to exhibit induced antipredator defences in response to invasive crayfish (*Procambarus clarkii* Girard) and also suffered significantly higher mortality compared with a native dragonfly (*Anax imperator* Leach) predator. While not precisely parallel, chemical ecology and aquaculture research contain examples of survival studies of naive fish trained to recognise novel predator odours (Mirza & Chivers, 2000; Gazdewich & Chivers, 2002; Vilhunen, 2006). These and other studies, reviewed in Ferrari *et al.* (2010), consistently associate higher survival with advance warning and greater predator recognition. Although our study does not address differences in behaviour or mortality in the presence of actual predators, our research largely supports the hypothesis that prey naivety may be an important factor in population impacts of non-native predators.

To be useful in a management context, these results should be considered along with other factors thought to influence the outcome of novel predator invasions: these include degree of taxonomic similarity between native and invaded communities, competitive interactions and existing species diversity in the invaded range (Sih *et al.*, 2010). Smallmouth bass possess several advantages in being an opportunistic predator that is widely spread by humans (Carey *et al.*, 2011). Evidence also suggests that smallmouth bass can outcompete and displace many native predators (Fritts & Pearsons, 2004; Johnson *et al.*, 2008), a trend that may be augmented in the Pacific Northwest by historically low-diversity fish communities that contained no centrarchids. In this particular management scenario, prey naivety is likely to give smallmouth bass an additional competitive edge, to the detriment of native communities and salmon populations. Even more troubling is that (as elsewhere) smallmouth bass are only one of a suite of novel non-native predators found in the Pacific Northwest (Sanderson *et al.*, 2009). Further, our results suggest that management priorities for salmon conservation, which seek to reduce pikeminnow population density (i.e. state-funded bounty programme and targeted removals) while favouring smallmouth bass (i.e. catch limits), may be misguided.

Despite the need to improve understanding of how novel predators impact native fish communities, nevertheless, we recommend that these results be interpreted cautiously and used as a basis for future work. Logistical constraints in our field location resulted in small sample sizes, particularly in the number of control sites. In addition, our research does not take into account how behavioural responses of wild fish may shift with additional cues available to prey through predator diet, or chemical and social learning; however, we consider this work to be a foundation from which to pursue these questions.

Although laboratory and field results were similar, the difference in effect in the two environments is enlightening. Laboratory results suggested flight or escape as a uniformly prominent response to chemical cues of northern pikeminnow, but this response was only one of the several antipredator responses witnessed in the field. Our field study indicates that behavioural responses by individuals ranged from abandonment of feeding territories, reductions in movement (including foraging attempts) and increasing time near the substratum. The timing and extent of these responses further varied from less than a minute to the full 10 min following introduction of the predator cue. This variation is consistent with several factors that could not be controlled in the field, such as

hunger, habitat, cue concentration and group membership: all of these factors have been demonstrated to mediate the strength of antipredator responses (Ferrari *et al.*, 2010). However, we suggest our laboratory and field comparison also strongly reflect context-dependent differences in behavioural decision-making by individuals in confined laboratory spaces, by groups (field sites) and by individuals within groups. Unravelling the interactions between biotic, abiotic and community factors that mediate behavioural decision-making may be critical to scaling our understanding of prey naivety to the population or landscape level. In a recent presentation of a theoretical framework for naivety in ecosystems, Sih *et al.* (2010) identified the integration of behavioural, sensory and invasion ecology as a key area for research. From the field of chemical ecology, Ferrari *et al.* (2010) specifically call for field experiments that bring an 'ecological realism of natural populations' to tests of predator-prey hypotheses. We argue that by taking an integrated approach, our results contribute not only to the question of the existence and adaptive value of innate responses to chemical cues, but offer insight into mechanisms by which prey naivety operates at an ecological scale.

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