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Distinct navigation behaviors in six species of disease vector mosquito larvae

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Abstract

Mosquitoes spread deadly diseases that impact millions of people every year. Understanding mosquito physiology and behavior is vital for public health and disease prevention. However, many important questions remain unanswered in the field of mosquito neuroethology, particularly in our understanding of the larval stage. In this study, we investigate the innate exploration behavior of six different species of disease vector mosquito larvae. We show that these species exhibit strikingly different movement paths, corresponding to a wide range of exploration behaviors. We also investigate the response of each species to an appetitive food cue, aversive cue or neutral control. By contrast to the large differences in exploration behavior, all species appeared to gather near preferred cues through random aggregation rather than directed navigation. Our results identify key behavioral differences among important disease vector species, and suggests that navigation and exploration among even closely related mosquito species may be much more distinct than previously thought.

Introduction

Mosquitoes are global disease vectors that transmit diseases such as malaria, chikungunya, and Dengue fever. To limit the spread of these disease vector mosquitoes, researchers have identified larval mosquito control as a highly effective public health tool (Weeks et al. 2019). In particular, naturally occurring larvicides such as methionine and *Bacillus* sp. bacteria have recently increased in popularity as an environmentally safe alternative to synthetic insecticides like DDT (Regis et al. 2000, Weeks et al. 2019). These larvicides must be ingested by larvae to be effective, and many factors affect larval feeding rate, including foraging strategy, chemosensory preference, and competition with conspecifics or individuals from other species (Ramoska and Hopkins 1981, Merritt et al. 1992, Hartman 2016). In addition, larval behaviors and development rate also play an important role in adult population levels. For instance, direct competition for limited food resources at the larval stage is thought to be a

major factor in the presence of certain disease vectors (Alto et al. 2005, Bevins 2008). Qualitative studies of mosquito larvae have shown different patterns of feeding and swimming behaviors (Ramoska and Hopkins 1981, Merritt et al. 1992, Yee et al. 2004) although these inter-species differences appear to be flexible and dependent upon the environment (Yee et al. 2004). For example, larvae employ different behaviors such as swimming under plant material or staying near walls to avoid predators. But despite growing interest (Skiff and Yee 2014, Zabouli et al. 2017, Reiskind and Janairo 2018), the strategies larvae use to locate sources of food, or respond to environmental stimuli, including sources of nutrients or toxic chemicals, remain poorly understood across many disease vector species. A better understanding of larval navigation and foraging behavior across mosquito species may help inform vector control techniques by suggesting where, when, and how much ingestible larvicide to apply to maximize

mosquito control while minimizing cost and environmental impact.

Comparative approaches can provide important insights into the sensory- and evolutionary-bases of behavior. Despite the limited data on chemosensory behaviors by mosquito larvae, previous work has demonstrated species-species differences in locomotory and responses to food-related stimuli (Skiff and Yee 2014), with these differences attributed to the adaptive use of nutrient resources and impacts of competition. For instance, multiple species of *Culex* or *Aedes* sp. larvae may inhabit spatially-restricted containers where individuals experience interspecific competition for limited resources (Bevins 2008, Yee et al. 2004, Julianno 2009, Workman and Walton 2003). Although these species may exhibit differences in locomotory and feeding responses, like suspension feeding or browsing on surfaces, they can exhibit similar behaviors when experiencing similar environmental resources, like browsing on the surface of a leaf (Yee et al. 2004). Larval resource use and competition has important effects on adult body size, and in the case of *Aedes albopictus*, can increase arboviral infection. Characterizing larval behaviors under different chemosensory conditions may allow determining how different species respond to different microhabitats or competitive environments.

Mosquito larvae may also provide important insight into the algorithms associated with search behaviors by aquatic insects. A previous study has shown that *Ae. aegypti* larvae find food randomly, rather than demonstrating directed motion toward preferred cues. Once a food-rich area is located, larvae decrease their swimming speed to remain in the favorable environment (Lutz et al. 2019). Previous studies in *Anopheles albimanus* (Aly and Mulla 1986) and *Aedes vexans* (Aly 1985) larvae showed that these species also discover food at random, although the methods used in these studies prevented deeper analysis into the mechanism of navigation. This is an unusually simple foraging strategy rarely found in other insects, or even in adult mosquitoes (Takken and Knols 2010). Do other disease vector mosquito species also forage by randomly encountering food cues?

In this study we investigated the foraging and navigation behavior of six species of mosquito

larvae, drawn from the three major disease vector genera *Aedes*, *Anopheles*, and *Culex* (Ruzzante et al. 2019) (Fig 1A). These species were selected for their importance to public health and for their diversity in ecological specialization and habitat choice. In the *Aedes* genus we investigated *Aedes aegypti* and *Aedes albopictus* - the most important vectors of Dengue fever and yellow fever. Although these two species are closely related, *Ae. aegypti* preferentially breeds in manmade containers (Christophers 1960) while *Ae. albopictus* is a generalist that may also inhabit rural and forested areas (Mousson et al. 2005). In the *Anopheles* genus we examined the malaria vectors *Anopheles arabiensis* and *Anopheles coluzzii*. Interestingly, although *An. arabiensis* and *An. coluzzii* inhabit similar human-associated larval habitats (Minakawa et al. 1999, Etand et al. 2016). *An. arabiensis* drastically outcompetes *An. coluzzii* in mixed-species larval competition (Hartman 2016). This suggests that these closely related species may rely on different foraging strategies, and that larval habitat specialization may not solely predict foraging behavior. Finally, we investigated *Culex quinquefasciatus*, a container-breeding mosquito, and *Culex tarsalis*, which breed in large vegetative areas such as rice fields. These two *Culex* species exhibit oviposition behavior that correspond to predation risk in their natural larval habitat (high risk and high predator avoidance for *Cx. tarsalis*, low risk and low predator avoidance for container-dwelling *Cx. quinquefasciatus*) (Van Dam and Walton 2008). Our exploratory study reveals striking differences in exploration behavior between all six species.

Methods

Insects

Six species of wild-type mosquitoes were obtained from BEI Resources (NIAID, NIH): *Ae. aegypti* (Strain COSTA RICA, MRA-726, contributed by William G. Brogdon), *Ae. albopictus* (Strain ATM-NJ95, Centers for Disease Control and Prevention for distribution by BEI, NR-48979), *An. arabiensis* (Strain DONGOLA, MRA-856, contributed by Mark Q. Benedict), *An. coluzzii* (Strain Ngousso, MRA-1279, contributed by Frédéric Simard), *Culex quinquefasciatus* (Strain JHB, NR-43025), and *Culex tarsalis* (Strain YOLO, NR-43026). All species were reared in milliQ water in a shallow

tray (26x35x4cm) and fed with fish food (Petco; Hikari Tropic First Bites). Larvae were reared using the circadian cycle recommended by species-specific BEI rearing guidelines (16:8 light:dark for *Cx. tarsalis*; 12:12 for all other species). One day before the experiment, L3-stage larvae were isolated in Falcon™ 50mL conical centrifuge tubes (Thermo Fischer Scientific, Waltham, MA, USA) containing ~15mL milliQ water. Starved larvae were denied food for at least 24 hours before the experiment. Animals that died before eclosion or pupated during the experiment were omitted, and all animals were tested during the light phase of their circadian cycle.

Preparation of Odor Stimuli

Stimuli were used that elicited robust behavioral responses across species. Two stimuli were used: an attractive food solution and quinine, a compound that elicits aversion in *Ae. aegypti* larvae (Lutz et al. 2019). The food extract solution was made fresh daily by dissolving 0.5% food (Petco; Hikari Tropic First Bites) in milliQ water for one hour, then passing the mixture through a 0.2µm filter (VWR International #28145-477) to remove solid particulates. Quinine hydrochloride was prepared at 10mM in milliQ water (Aldrich #Q1125). For all species, we saw no difference in mortality between the three treatments ($p=1$ for all species, Fig S2), suggesting that exposure to quinine or food extract did not significantly harm larvae physiologically.

Behavioral Arena and Imaging

We computed the trajectories of individual larvae in a custom behavior arena as previously described (Lutz et al. 2019, Bui et al. 2019). Briefly, individual larvae were introduced to a 8x3cm rectangular behavior arena containing 20mL of distilled milliQ water. Larvae were allowed to acclimate within the dark arena for 15 minutes, while being recorded by a Basler Scout Machine Vision GigE camera under infrared light. Subsequently, 100µL of one stimulus was pipetted gently into the upper left corner of the arena (Fig 1C), and larval behavior was recorded for an additional 15 minutes. In a separate experiment without larvae, we pipetted 100µL of fluorescein dye into an identically shaped arena, in order to map stimulus concentration within the arena throughout the 15 minute experiment (Lutz et

al. 2019) (Fig 1C). Trajectory paths were extracted from each video using Multitracker software by Floris van Breugel (van Breguel et al. 2018) and additional code developed previously (Lutz et al. 2019). We visually inspected each trajectory path and manually corrected errors and omissions introduced by the tracking software.

Trajectory Quantification

During foraging and swimming behaviors, mosquito larva can exhibit species specific differences in their swimming kinematics and behaviors, including changing the duration of activity (Sih 1986), increasing or decreasing their swim speeds, or exhibiting complex changes in locomotion 5. We thus quantified ten aspects of larval navigation in clean water to represent many of these ecologically relevant behaviors. Time spent moving was quantified as a proportion (0-100%), with movement defined as $>1\text{mm} \cdot \text{s}^{-1}$. Total distance traveled was measured in meters. To normalize for any size-specific differences across individuals or species (Fig S2), we converted larval speed measurements into body lengths per second. Experimenters were blind to larval species or sex when measuring body lengths. Thus, body lengths $\cdot \text{s}^{-1}$ were used for quantifying maximum speed, mean speed when moving, mean speed in first minute, and the difference in mean speed between first and last minutes. Spirals were defined as a distinct time period in which larvae engaged in >4 seconds of continuous spiraling movement. Sharp turns were defined as turns of $>45^\circ$ conducted at a speed of $>4\text{mm} \cdot \text{s}^{-1}$. Continuous paths were defined as sustained movement at the same Δ angle, not including spirals. Rests were defined as periods of time >10 seconds of no movement.

Statistical Analyses

Statistical analyses were performed in R (2015) and in Python 2015. We used a non-parametric Kruskal-Wallis test with Bonferroni correction to compare navigation characteristics across species for each of the ten aspects of larval navigation (Fig 2), because we found that not all variables followed a normal distribution (Shapiro-Wilk test, $p>0.05$). To create the Euclidean distance matrix for larval similarity analysis (Fig S1), we first standardized all variables to zero mean and unit variance. To compare larval trajectories across species, both

as a group of six and in species-species pairs, we used a perMANOVA and test of multivariate dispersion ANOVA with a Bonferroni correction (Table 1). We used a Monte Carlo permutation test to select significant eigenvectors for visualization in our PCA ordination (Fig 3). We used a pairwise t-test to compare larval preference for different stimuli for each species (Fig 4A). Preference was defined as the median concentration preferred by the larvae during the 15 minute experiment, normalized to the areas chosen by the same larva during the preceding 15 minute acclimation phase. This normalization was necessary to control for innate larval preference for corners or walls reported in some species (Lutz et al. 2019). Discovery time across different stimuli were compared for each species using a non-parametric Kruskal-Wallis test (Fig 4B). A non-parametric test was used because we found that discovery time data did not follow a normal distribution (Shapiro-Wilk test, $p > 0.05$). Discovery time was defined as the time taken (in seconds) to first encounter a section of the behavioral arena $\geq 50\%$ concentration, normalized to the time taken to first encounter the same area during the clean water acclimation period. We used a Fisher's Exact Test with Bonferroni correction to assess mortality differences among larval species, as well as among experimental treatments in larvae of the same species (Fig S2). We used a non-parametric Kruskal-Wallis test to compare body length between different species (Fig S2), because we found that body length data did not follow a normal distribution for all species (Shapiro-Wilk test, $p > 0.05$).

Results

Larval exploration behavior in clean water

To study the navigation behavior of each mosquito species, we used a semi-automated video analysis method previously reported in 14 (Fig 1B). We first investigated behavior in clean water during the 15 minute acclimation period (Ae. aegypti $n=67$; Ae. albopictus $n=70$; An. arabiensis $n=93$; An. coluzzii $n=108$; Cx. quinquefasciatus $n=110$; Cx. tarsalis $n=53$). We observed striking behavioral differences across mosquito species in many aspects of exploration behavior (Fig 2A). For example, Cx. tarsalis explored the environment slowly using distinctive sweeping circles (mean number of spirals = 13.1), while the two Anopheles species

interspersed long rests with fast, straight sprints (An. arabiensis mean number of spirals = 0.2; An. coluzzii 0.4). The two Aedes species, as well as Cx. tarsalis, spent the majority of the time moving (Ae. aegypti 56%; Ae. albopictus 52%; Cx. tarsalis 46%), albeit at a much lower mean speed (Ae. aegypti 0.8 body lengths s⁻¹; Ae. albopictus 0.7 BLs⁻¹; Cx. tarsalis 0.7 BLs⁻¹) compared to other species (mean speed 1.3~1.9 BLs⁻¹; time spent moving 9~35%). To further investigate these observations, we quantified ten different aspects of each larval trajectory, based on metrics we believed to be relevant to foraging and exploration behavior (Fig 2B-K). We found significant differences across species in all quantified measures (Fig 2B-K; $p < 0.001$, Kruskal-Wallis test with Holm-Bonferroni correction).

For example, some metrics measure the frequency of exploration behavior in starved larvae, such as time spent moving and total distance traveled. Other metrics quantify known search behavior patterns in insects, such as the looping spirals observed in local-search behavior (Bell 1990), frequency of sharp turns, and the number of continuous straight-line paths. Some metrics were added to assess larval response to disturbance. For example, introducing animals to the arena during the acclimation phase is likely to elicit disturbance response to mechanical movement. Thus, we measured the mean speed of animals during the initial minute following introduction to the arena, as well as throughout the entire 15 minute acclimation period. We also calculated a metric subtracting the speed during the initial minute from the last (15th) minute, to quantify the change in larval behavior post-disturbance. Because we found significant differences in larval size across species (Fig S2), we normalized all speed measurements to each individual's body length (body lengths $\cdot$ s⁻¹). Finally, some metrics were intended to measure the physiological capacity of the starved larvae, such as the longest rest period and maximum observed speed.

Exploration differences among species

We next investigated whether or not these observed differences were consistent with known phylogenetic relationships. In particular, do different species exhibit different trajectory patterns when all navigation variables are considered? To answer this question, we created

a Euclidean distance matrix of larval trajectories by incorporating all ten navigation variables - time spent moving, total distance traveled, mean speed, maximum speed, initial speed, speed modification, sharp turns, longest rest period, number of spirals, and number of continuous paths (Fig S1). We found significant differences among the six mosquito species (Fig 3, perMANOVA $p < 0.001$, pseudo $F = 70.7$). To visualize these results, we reduced the dimensionality of this data using Principal Component Analysis (PCA) based on the same Euclidean distance matrix (Fig 3A). PC1 and PC2 explained a significant proportion of variation in the data, but not subsequent PC axes (Monte Carlo permutation test, PC1 $p < 0.001$; PC2 $p < 0.001$; PC3-10: $p > 0.05$). In brief, we found that larvae appeared to cluster in ordination space into four distinct categories: very fast animals (upper left); animals that traveled a long distance and conducted many sharp turns (upper right); animals that rested for long periods of time (lower left); and animals that traveled in spiral patterns and changed their behavior drastically during the 15 minute period (lower right).

We next asked if sister mosquito species display exploratory behavior that is more similar to each other than to other species. A post-hoc pairwise perMANOVA for each species-species pair showed that all species differed significantly in navigation from each other, including sister species (Table 1). Although we observed that both *Aedes* species were more similar to each other than to any other species (comparison of pseudo-F statistics across species-species pairs), and both *Anopheles* species were also closest to each other than to non-sister species. However, *Cx. quinquefasciatus* was most similar to *Ae. aegypti*, while *Cx. tarsalis* was most similar to *Ae. albopictus*. It is interesting to note that *Cx. quinquefasciatus* and *Ae. aegypti* both inhabit man-made containers, while *Cx. tarsalis* and *Ae. albopictus* can inhabit large vegetated areas such as rice fields and lakes. Although our study only compares six species and is not intended to draw phylogenetic conclusions, our limited panel of results suggest that both evolutionary history and ecological specialization may correlate with similar navigation behaviors in different species.

Larval response to attractive and aversive cues

Next, we examined the change in larval behavior after introduction of 0.5% food extract, 10mM quinine, or distilled water. These stimuli were chosen to investigate larval cue-finding behavior, because previous studies have shown that these cues elicit robust preferences in *Ae. aegypti* mosquito larvae (Lutz et al. 2019, Bui et al. 2019). Corroborating a previous study (Lutz et al. 2019), we found that *Ae. aegypti* significantly preferred 0.5% food extract. To quantify preference, we normalized the median concentration preferred by each larvae during the experiment phase, to the corresponding larval behavior during the acclimation phase. *Ae. aegypti* preferred a median food concentration of 20% more than would be expected from their pre-experiment behavior ($p = 0.0002$, pairwise t-test). We observed similar attraction for all other species (Fig 4A): *Ae. albopictus* (+32%, $p < 0.001$), *An. arabiensis* (+13%, $p = 0.005$), *An. coluzzii* (+7%, $p = 0.04$), *Cx. quinquefasciatus* (+14%, $p = 0.006$), and *Cx. tarsalis* (+16%, $p = 0.001$). Further, we investigated changes in larval behavior after introduction of 10mM quinine, an aversive tastant. Similar to our previous study (Lutz et al. 2019), *Ae. aegypti* significantly avoided quinine, preferring a median concentration of 9% less than would be expected from their pre-experiment behavior ($p < 0.001$). We observed similar aversion in *Ae. albopictus* (-11%, $p < 0.001$), *An. arabiensis* (-7%, $p = 0.002$), and *An. coluzzii* (-7%, $p = 0.001$) (Fig 4A). Interestingly, neither *Cx. quinquefasciatus* nor *Cx. tarsalis* exhibited aversion to quinine (*Cx. quinquefasciatus* $p = 0.42$; *Cx. tarsalis* $p = 0.60$). In response to the addition of distilled water - a negative control for mechanical disturbance - all species exhibited no change in preference ($p > 0.05$).

We next explored the mechanism of larval navigation to food sources. In our previous work, we found that *Ae. aegypti* explore their environment using a non-directional search strategy that results in random discovery of preferred cues (Lutz et al. 2019). In all species in this study, we found that larval preference also appeared to be consistent with random cue discovery. Discovery time did not significantly differ between water, food, and quinine for any species ($p > 0.05$, Kruskal-Wallis test, Fig 4B), suggesting that larvae encounter environmental cues by random chance. In addition, we did not observe strong differences in orientation (Fig

4C) or turn frequency (Fig 4D) between different stimuli for any of the six species. Although surprising, these results are consistent with earlier literature using both video-tracking methods 14 and researcher observations (Aly 1985, Aly and Mulla 1986).

In our previous study, we were able to conduct deep analyses and simulations into the mechanism of *Ae. aegypti* navigation, using a dataset of over 500 individual animals observed independently, with approximately 2 million total data points. In this current study, we did not have the necessary data to conduct the simulations or 3,000 experiments necessary for similar analyses across species. Nevertheless, we visualized some of the same behavioral changes as a reference for future experiments (Fig S3, Fig S4, Fig S5). We found several interesting patterns in these datasets. For example, in the vast majority of cases, animals did not appear to change their behavior - such

as the number of looped searches or sharp turns - after addition of the stimulus. In cases where animals did change their behavior - such as *An. coluzzii*, which decreased initial speed in the post-stimulus period - animals seemed to exhibit the same behavioral changes for all experiments, independently of the stimulus added. Corroborating our previous results, we found that in our current work, *Ae. aegypti* also appear to aggregate near preferred cues by decreasing their movement speed near preferred areas (Fig S4). Interestingly, we observed the same pattern for *Ae. albopictus* but not for any other species (Fig S4). Although further experiments are necessary to understand these results, it is likely that the *Anopheles* and *Culex* species in our study use different kinematic changes to navigate with respect to chemosensory cues, such as adjusting their turning frequency.

Figure 1. Summary of experimental design and methods.

A: A simplified phylogeny of the six mosquito species investigated in this manuscript, adapted from 18 (branch lengths are not representative of phylogenetic distance). B: Diagram of the experimental rig, adapted from 14, including a Basler Scout Machine Vision GigE camera (1), infrared lighting (2) and a behavior arena filled with water (3). C: A map of stimulus distribution within the arena at 15 minutes post-stimulus addition, adapted from Lutz et al. 2019..

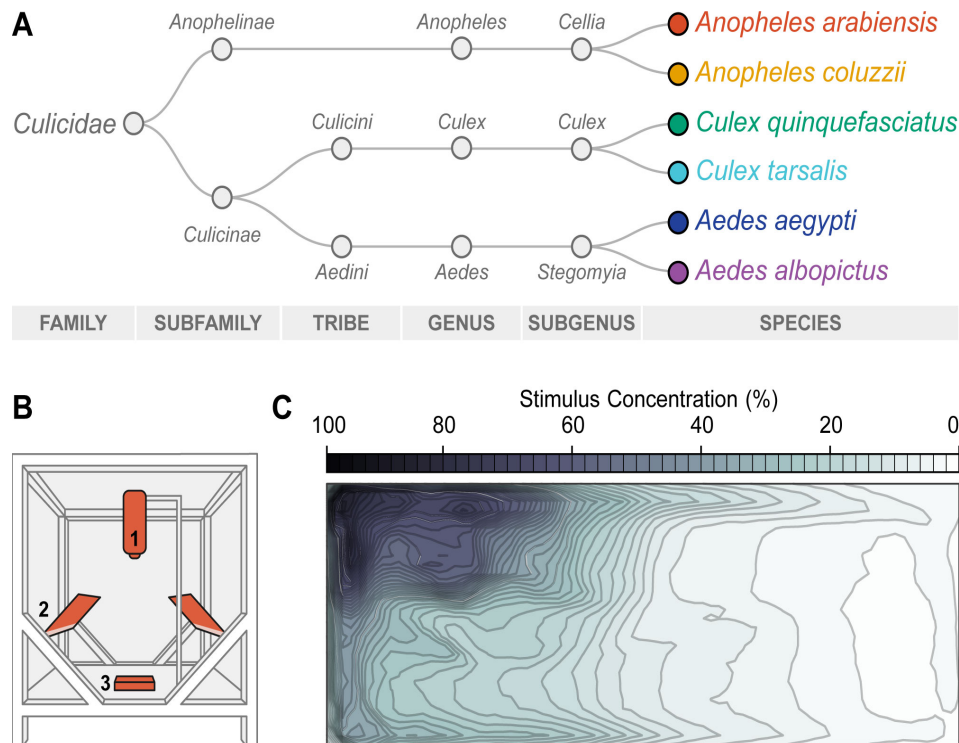


Figure 2. Larvae exhibit species-specific behavioral differences in the absence of chemosensory stimuli.

A: Example trajectories visualizing one individual from each species, navigating in clean water. **B-K:** Species-specific distributions for each of ten navigation variables quantified from larval behavior in clean water. For each variable a violin plot visualizes the distribution for each species, with scatter points showing values for individual animals. A black bar marks the mean value for each species. Asterisks above each plot represents the significance of differences across species (Kruskal-Wallis test; ***: $p < 0.001$). For all plots, a sample trajectory from a *Cx. quinquefasciatus* individual is shown on the right in gray, with red and black lines highlighting sections of the trajectory included in that variable. From left to right in all graphs: *Ae. aegypti* (navy); *Ae. albopictus* (purple); *An. arabiensis* (red); *An. coluzzii* (yellow); *Cx. quinquefasciatus* (green); *Cx. tarsalis* (aqua). **B:** Time spent moving (%) **C:** Total distance traveled (meters) **D:** Mean speed when moving (body lengths $\cdot s^{-1}$ [BL/s]) **E:** Maximum speed (body lengths $\cdot s^{-1}$ [BL/s]) **F:** Initial speed, or mean speed in first minute (body lengths $\cdot s^{-1}$ [BL/s]) **G:** Speed modification, or difference in mean speed between first and last minutes (body lengths $\cdot s^{-1}$ [BL/s]) **H:** Sharp turns (% total time spent turning $> 45^\circ$) **I:** Longest continuous rest period **J:** Number of spirals **K:** Number of continuous paths that are not spirals.

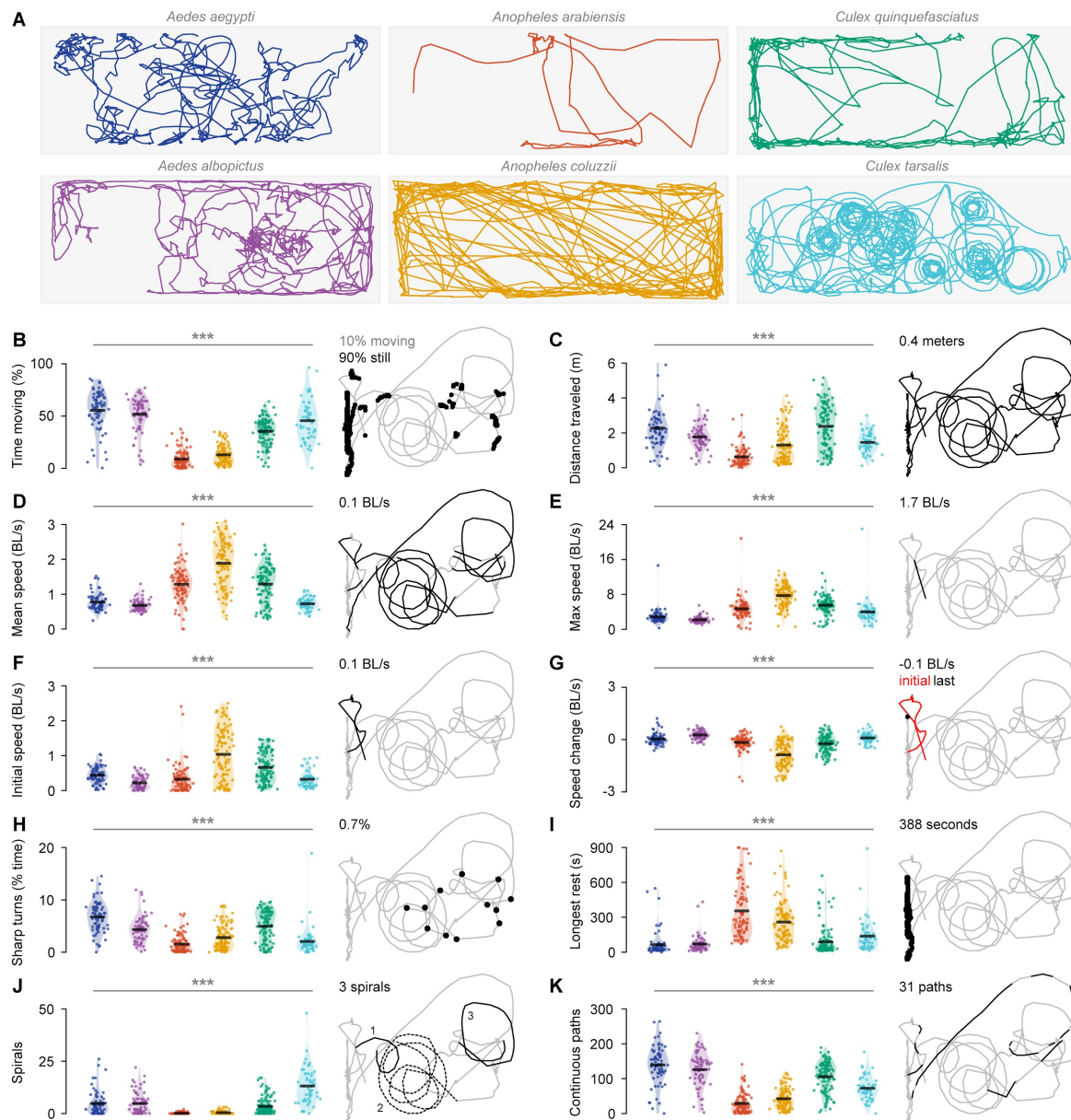


Figure 3. Larvae exhibit species-specific behavioral differences in the absence of chemosensory stimuli.

A: PCA biplot of ten individual variables describing larval trajectories based on the Euclidean distance matrix visualized in Fig S1. PC1 and PC2 explain a significant proportion of variance in the original data (Monte Carlo test, PC1: 41.5%, $p < 0.001$; PC2: 33.7%, $p < 0.001$), while all other PC axes did not ($p > 0.05$). Scatter points depict individual larvae in the ordination space, colored by species: *Ae. aegypti* (navy circles, $n=67$), *Ae. albopictus* (purple x markers, $n=70$), *An. arabiensis* (red squares, $n=93$), *An. coluzzii* (yellow diamonds, $n=108$), *Cx. quinquefasciatus* (green triangles, $n=110$), and *Cx. tarsalis* (aqua + markers, $n=53$). **B:** Vector arrows (1-10) indicate the direction of variable gradients in the ordination space. Ordered from highest to lowest contribution to PC1 and PC2, these variables include: **1:** Time spent moving (variable contribution to PC1: 21.4%; PC2: 0.8%); **2:** Distance traveled (PC1: 17.9%; PC2: 4.5%); **3:** Continuous runs (PC1: 21.5%; PC2: 0%); **4:** Mean speed (PC1: 0.4%; PC2: 24.5%); **5:** Initial speed in first minute (PC1: 0.1%; PC2: 24.6%); **6:** Sharp turns (PC1: 15.8%; PC2: 3.2%); **7:** Speed modification, or the difference in mean speed between first and last minutes (PC1: 2.2%; PC2: 19.4%); **8:** Maximum speed (PC1: 0.2%; PC2: 20.1%); **9:** Longest rest period (PC1: 16%; PC2: 0.1%); and **10:** Spirals (PC1: 4.6%; PC2: 2.8%)

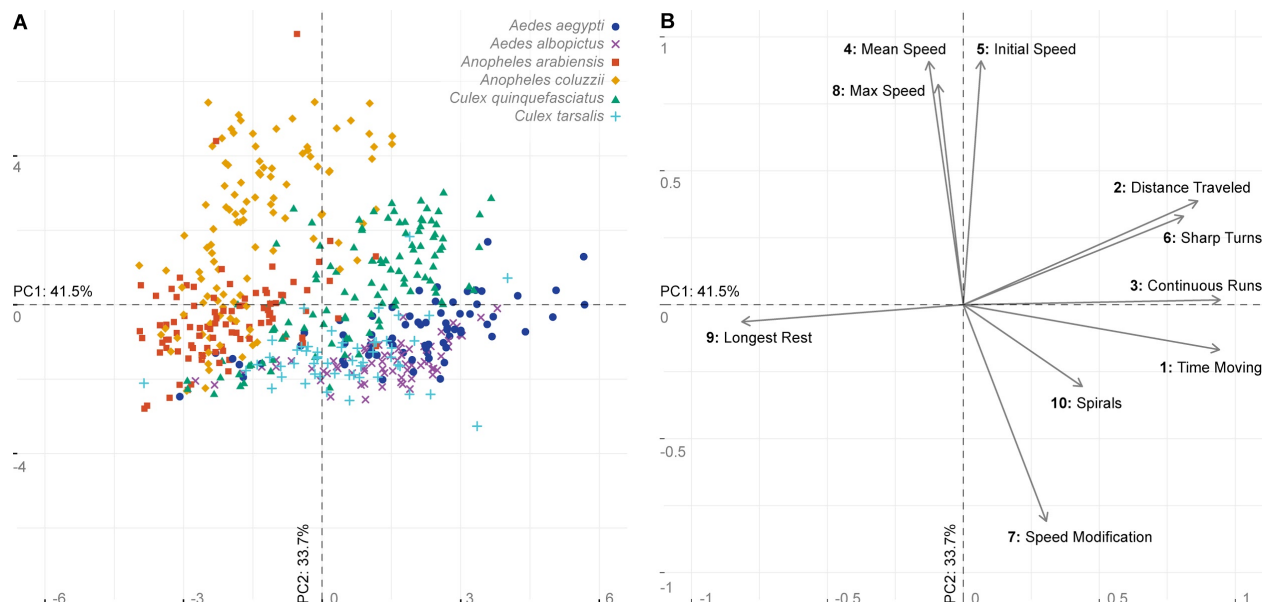


Figure 4. Larval gathering near preferred cues is more consistent with passive exploration than active navigation.

A: All larval species were significantly attracted to food extract. With the exception of the two *Culex* species, larvae were also significantly repelled by the aversive tastant quinine. **B:** However, none of the six larval species were able to change their navigation behavior to find food faster, or delay occupying high-concentration areas of quinine: *Ae. aegypti* ($p=0.60$, Kruskal-Wallis test); *Ae. albopictus* ($p=0.71$); *An. arabiensis* ($p=0.52$); *An. coluzzii* ($p=0.22$); *Cx. quinquefasciatus* ($p=0.64$); *Cx. tarsalis* ($p=0.93$). **C:** For all species, animals did not appear to change their behavior to move towards or away from any stimuli. Polar plots showing orientation for all animals throughout the 15 minute experiment for water (grey), food (pink) or quinine (light blue). The label “S” marks the approximate direction of each stimulus. Each bar in the polar plot represents the proportion of time that animals moved in that specific direction. For visualization purposes, all polar plots are normalized to the maximum frequency for each stimulus. **D:** Turning frequency for each species did not appear to change depending on the added stimulus. Frequency histograms for exploratory larval turning frequency in response to the addition of water (grey), food (pink) or quinine (light blue). Y axis ranges are identical for all six plots. **C-D:** To reduce noise from passively drifting animals, plots only include data where larvae were moving at $\geq 1\text{mm} \cdot \text{s}^{-1}$. Although our experiments did not have sufficient power to statistically inspect these differences, animals do not appear to significantly change orientation across stimuli.

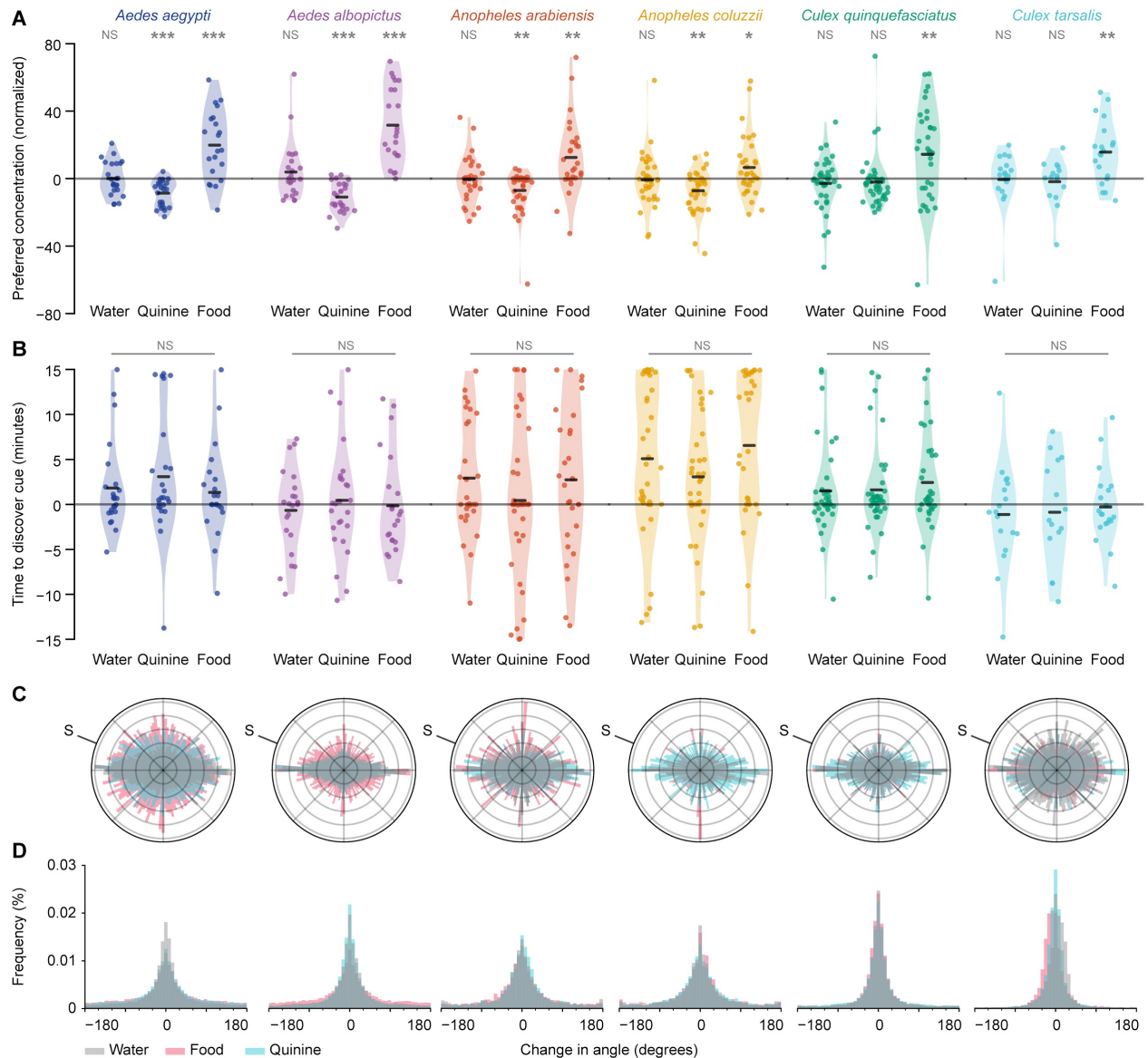


Table 1: Comparisons of swimming patterns among species.

Values in upper right half of the matrix represent pseudo-F statistics from pairwise perMANOVA tests. Asterisks after each value indicate the significance of the corresponding p-value after Bonferroni correction: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; no asterisk: not statistically significant. In the upper right, significant values with a high pseudo-F statistic represent species-species pairs that exhibit statistically significant differences in overall navigation behavior. Values in the lower left half of the matrix represent F statistics for a pairwise test of multivariate dispersion (ANOVA with Bonferroni correction). Significant values with a high F-statistic in the bottom half of the matrix represent species-species pairs with statistically significant differences in the intra-species variability of navigation behavior. These results suggest that some, but not all, of the observed differences between species-species pairs may be due to variance among individuals, rather than to differences in raw behavioral metrics.

	<i>Aedes aegypti</i>	<i>Aedes albopictus</i>	<i>Anopheles arabiensis</i>	<i>Anopheles coluzzii</i>	<i>Culex quinquefasciatus</i>	<i>Culex tarsalis</i>
<i>Aedes aegypti</i>		11.12 ***	89.92 ***	115.66 ***	25.66 ***	20.13 ***
<i>Aedes albopictus</i>	5.54		99.66 ***	118.58 ***	38.73 ***	16.96 ***
<i>Anopheles arabiensis</i>	0.76	0.03		33.10 ***	71.41 ***	48.50 ***
<i>Anopheles coluzzii</i>	1.44	6.79	18.19 ***		69.04 ***	63.56 ***
<i>Culex quinquefasciatus</i>	4.90	18.77 ***	21.33 ***	0.06		29.69 ***
<i>Culex tarsalis</i>	0.02	4.84	0.22	3.15	3.21	

Discussion

Our results raise several interesting questions for future research. In our experiments investigating larval cue-finding, we predicted that larvae may exhibit navigation strategies adapted to their environment, with species living in small containers displaying different strategies than those that breed in larger lakes or streams. However, our results showed that the six different species of mosquito larvae were strikingly homogeneous in their chemosensory responses to food: none of the species were able to change their behavior to find food cues faster in our experimental paradigm. Are there intrinsic physical properties to chemical diffusion in small, stagnant aquatic environments that makes more directed navigation particularly difficult? Although many of the species examined are naturally adapted to habitats of similar size to the experimental arena (Skiff and Yee 2014, Christophers 1960), it is possible that larvae may exhibit different navigation strategies in

larger environments where the chemical gradients may be shallower or influenced by turbulent kinetic motion. Additionally, are there physiological limitations to larval chemosensation, such as the sensitivity of receptors or complexity of neural processing circuits, that prevent larvae from utilizing more complex navigation processes?

Second, is there an evolutionary benefit to different navigation behaviors exhibited by each species in clean water? Interspecific larval competition significantly affects distribution of mosquito species in the wild and in laboratory experiments (Harman 2016, Juliano 1998, Braks et al. 2004). It is possible that this competitive environment drives larvae to exploit different foraging niches through different navigation strategies. Alternatively, it is possible that the different environmental conditions preferred by each species, such as lakes, streams, and containers, result in different navigation strategies consistent across habitats. Although our study did not examine enough species to quantitatively answer this question, it is

interesting to note that *Aedes* and *Anopheles* mosquito larvae exhibited greater similarity to sister species even when the sister species inhabited vastly different natural larval habitats. By contrast, the two *Culex* species exhibited the greatest similarity toward non-sister species that inhabited similar ecological environments.

It is important to note that our investigative study may not address important characteristics of mosquitoes found in the wild. For example, although all larvae analyzed in this study successfully completed development under the same laboratory rearing conditions, it is likely that environmental variables including temperature, humidity, and water depth were more optimal for some species than others. Indeed, species exhibited significantly different mortality rates post-experiment, suggesting that the 24-hour starvation period may have been more stressful for some species (Fig S2). In addition, our experimental trials only observed larvae for a total of 15 minutes after stimulus addition, and it is possible that larvae may exhibit different behaviors in longer time scales.

Nevertheless, we believe that this study reveals an important area of future research. To our knowledge, this is the first study to quantitatively compare exploration behavior among mosquito larvae using machine vision rather than researcher observations. Even among the small subset of species examined in this study, we saw immediate and clear differences in exploration, stimulus preference, and chemosensory navigation. Future studies incorporating additional mosquito species - especially outgroups that are not disease vectors - would add fascinating comparisons that may help clarify the evolutionary basis of exploration behavior in mosquitoes. Because laboratory arena sizes and rearing conditions are already within the natural range of many mosquito species, mosquito larvae may be a promising field for phylogenetic behavior questions from a practical perspective. Finally, our results underscore the importance of understanding disease vector behavior at all life history stages. We suggest that species-specific vector control research may be particularly important to improving disease prevention methods.

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Supplementary Figures



Fig S1: Euclidean distance matrix of individual differences between larvae.

Euclidean distance matrix incorporating all ten navigation behavior variables in clean water. Each row or column visualizes one individual larvae, arranged in species groups and then in order of experiment date. White lines mark boundaries between different species. Higher Euclidean distances (arbitrary units) represent lower similarity between individuals. The diagonal of black cells (upper left corner to the lower right corner) indicate self-self comparisons (distance = 0).

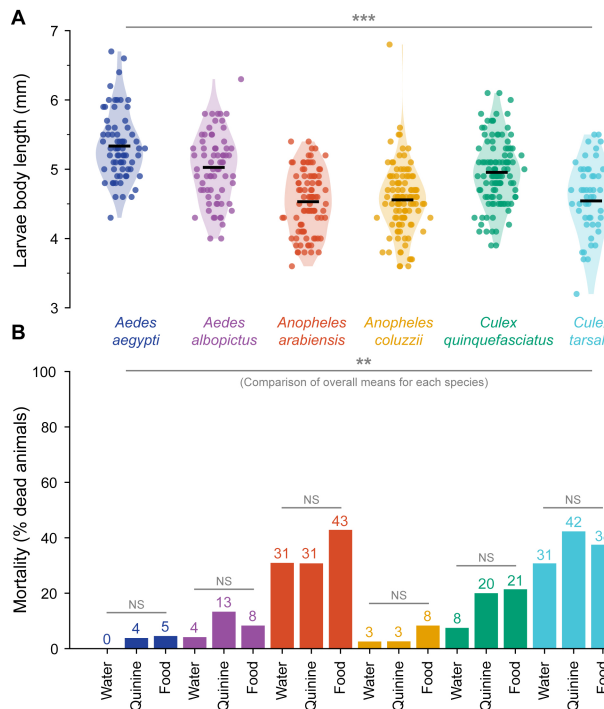


Fig S2: Larval size and mortality are significantly different across species.

A: Size of starved L3 larvae differed significantly across species ($p < 0.001$, Kruskal-Wallis test with Bonferroni correction). Scatter points depict individual larvae, colored by species from left to right: *Ae. aegypti* (navy, $n=67$, mean=5.33mm), *Ae. albopictus* (purple, $n=70$, mean=5.03mm), *An. arabiensis* (red, $n=93$, mean=4.53mm), *An. coluzzii* (yellow, $n=108$, mean=4.56mm), *Cx. quinquefasciatus* (green, $n=110$, mean=4.95mm), and *Cx. tarsalis* (aqua, $n=53$, mean=4.54mm). Horizontal black bars visualize the mean of each group. Due to these size differences across species, speed measurements were standardized to larval body size for each individual. **B:** Post-experiment mortality for each species and treatment. Numbers shown above each bar mark the percentage value for each treatment. Differences were statistically significant across species ($p=0.003$, Fisher's Exact Test with Holm-Bonferroni correction), but not across the three treatments for the same species ($p=1$ for all species). Asterisks indicate the significance of the corresponding p -value: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; NS: not statistically significant.

Fig S3: Changes in kinematic swimming responses following stimulus addition.

A-J: Changes in each of the ten navigational behavior measurements following the addition of water (W), quinine (Q), or food extract (F). From left to right in all plots: *Ae. aegypti* (navy); *Ae. albopictus* (purple); *An. arabiensis* (red); *An. coluzzii* (yellow); *Cx. quinquefasciatus* (green); and *Cx. tarsalis* (aqua). Each scatter point represents one individual, and the y axis shows the change in the navigational variable (experiment period - acclimation period). Thus, a point lying at 0 represents no change in behavior following addition of the stimulus. **A:** Time spent moving (%) **B:** Total distance traveled (meters) **C:** Mean speed when moving (body lengths·s⁻¹ [BL/s]) **D:** Maximum speed (body lengths·s⁻¹ [BL/s]) **E:** Initial speed, or mean speed in first minute (body lengths·s⁻¹ [BL/s]) **F:** Speed modification, or difference in mean speed between first and last minutes (body lengths·s⁻¹ [BL/s]) **G:** Sharp turns (% total time spent turning >45°) **H:** Longest continuous rest period **I:** Number of spirals **J:** Number of continuous paths that are not spirals. **K-N:** Representation of the same ten variables as in **A-J**, in PCA space. **K:** Reproduction of acclimation data for all animals visualized in Fig 3A. **L:** Experiment data from all animals responding to quinine, reprojected into the same PC space as in **K**. **M:** Experiment data from all animals responding to water, reprojected into the same PC space as in **K**. **N:** Experiment data from all animals responding to food, reprojected into the same PC space as in **K**.

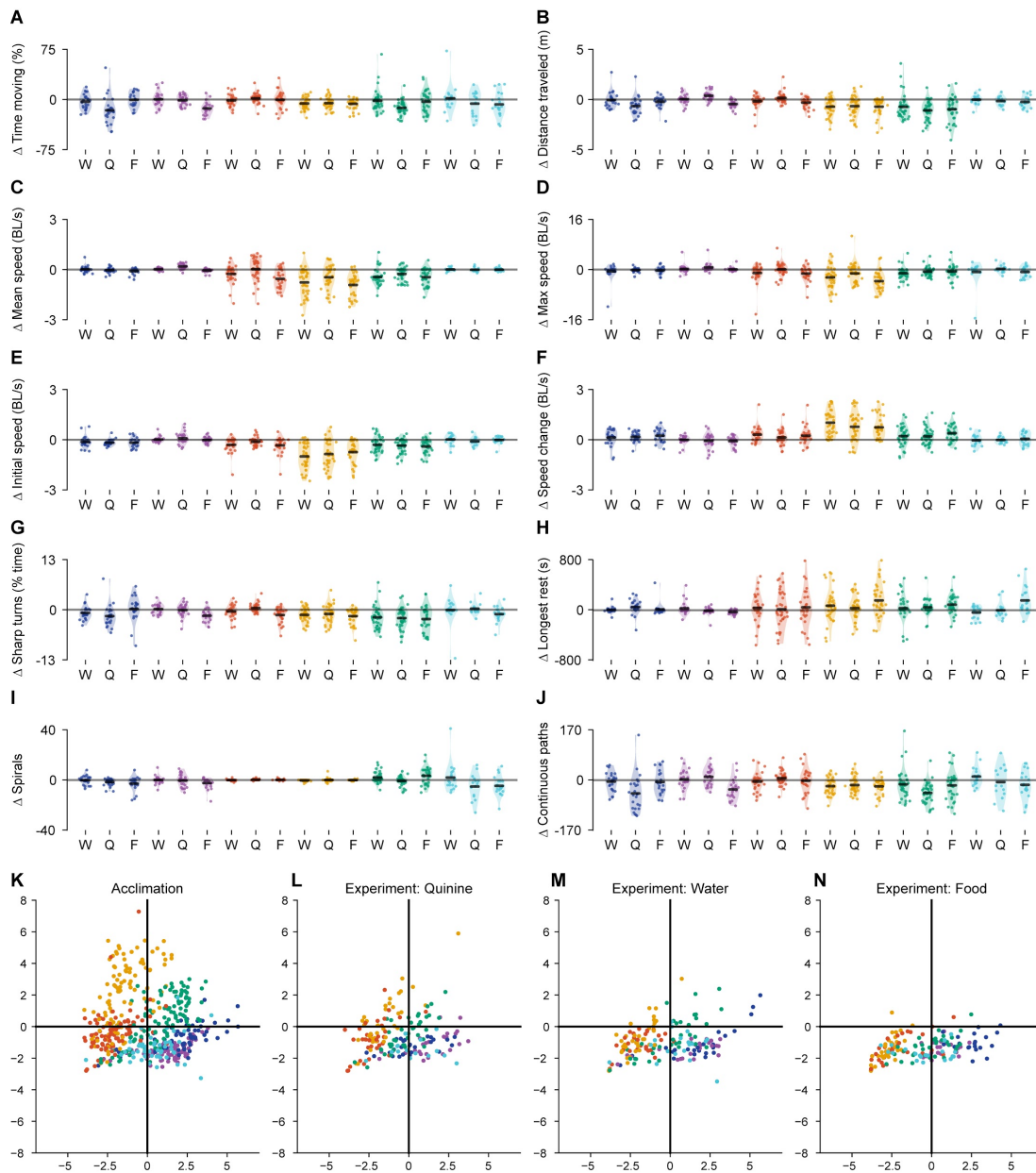


Fig S4: Species exhibit potential differences in navigation strategy toward preferred areas.

In a previous study, we conducted deep analyses into the mechanism of *Ae. aegypti* navigation, using a dataset of over 500 individual animals observed independently. This study revealed that *Ae. aegypti* locate preferred cues by decreasing their movement speed near preferred areas. In this current study, we did not have the reagent resources or manpower to conduct the 3,000 experiments necessary for an equally robust analysis. Nevertheless, we visualized the speed near and far from cues for each species and each stimulus. In this graph, each scatter point represents one individual, and the y axis shows the change in speed near the experimental cue (speed in areas >50% concentration - speed in areas <50% concentration), normalized to larval behavior in corresponding sections of the arena in the 15 minute acclimation period.

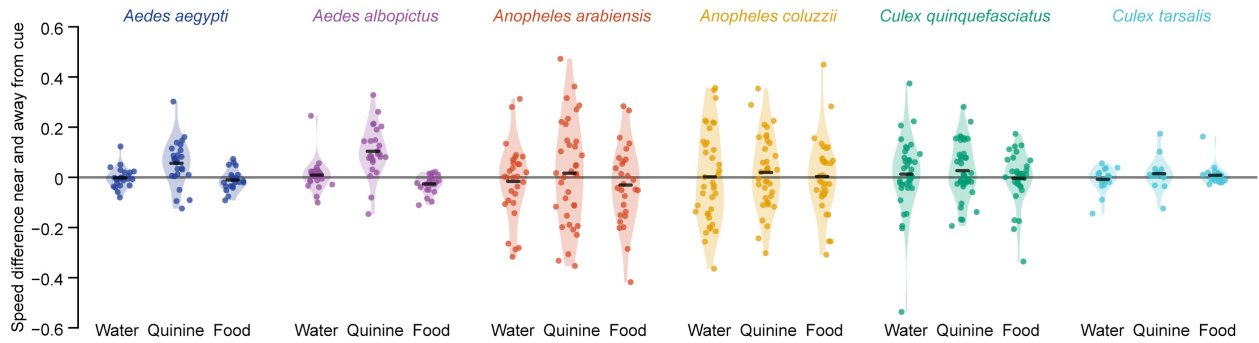
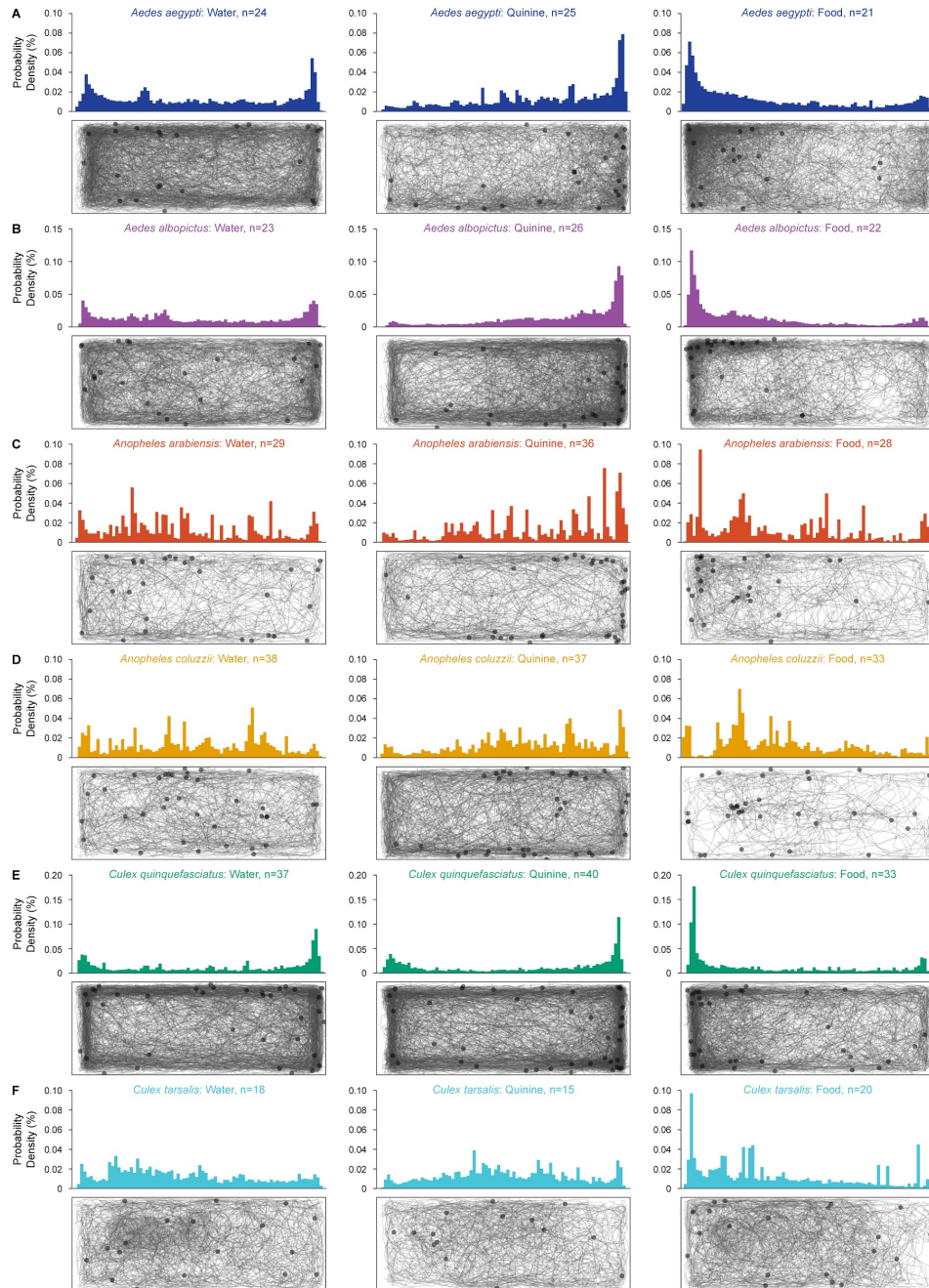


Fig S5 (following page): Larval distribution and trajectory maps for all species and experimental conditions. A-F: Distribution histograms across the x axis (above) and trajectory maps (below) of all starved animals during the experiment phase for water (left column), quinine (center column), and food (right column); *Ae. aegypti* (A), *Ae. albopictus* (B), *An. arabiensis* (C), *An. coluzzii* (D), *Cx. quinquefasciatus* (E), and *Cx. tarsalis* (F). Although trajectories are shown aggregated into one image for each panel, all animals were tested individually. Scatter points show the position of each animal at the end of the experiment. It is important to note that these histograms show the aggregated position data from all animals throughout the entire 15 minute experiment. Thus, a single animal exhibiting strong attraction or aversion may disproportionately influence this data visualization. For statistical tests reported in this paper, a single preference value was calculated for each animal (Fig 4A) to avoid such effects. Note that the distribution histograms for *An. arabiensis* and *An. coluzzii* appear particularly sparse, because these two *Anopheles* species spent the majority of the experiment at rest.



Evaluation of residential developer patch creation behavior

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Abstract

Newly constructed homes and yards are new urban patch mosaics, resulting from specific decisions by heterogeneous residential developers and their agents. I reconstructed the patch creation decisions of developers on 60 parcels, from a stratified random sample of 1,258 newly build and sold single-family residential parcels in the Seattle Metropolitan Statistical Area. By closely examining new residential land covers and plants, I provide empirical evidence of the outcomes of residential land use plan implementation across multiple jurisdictions. Exclusive of roads, each newly developed parcel generates about 276 m² of impervious surfaces and 115 m² of lawn. These land cover types prioritize human uses, providing little space for other species. The underlying land values and target buyer appear to drive many developer decisions. In response to higher land values at more northerly latitudes, developers allocated a larger proportion of parcel area to buildings, other pavement, and to mixed and single species perennial beds. By reconstructing plant material spending, I found that developers of more expensive homes spent more on non-Lawn vegetation patches. My work suggests that developers are responsible for the initial creation of the luxury effect, where wealthier homeowners have more abundant and diverse vegetation

Introduction

Residential development drives a large proportion of land use and land cover change (Irwin and Bockstael 2007; Pejchar et al. 2015). Despite the efforts of planners to mitigate negative environmental and social effects of residential development by encouraging density, most new residential development occurs at the urban fringe (Grant, Manuel, and Joudrey 1996; Pejchar et al. 2015). Given their spatial extent and large footprint compared to other urban land uses (Irwin and Bockstael 2007), understanding the creation of residential landscapes is therefore critical for urban planners, developers and ecologists.

Because newly constructed homes and yards are new urban patch mosaics, I view residential developers as urban ecosystem engineers (Jones, Lawton, and Shachak 1994), who create new habitat for other people and other species. Residential developers make specific decisions that shape the urban fabric: which lands to purchase; what previous structures, infrastructure, and landscaping to retain; and what to build, install, and plant (Coiacetto 2007; Kohlhepp and Kohlhepp 2018). Exploring the factors underlying developer decision-making should lead to an improved understanding of urban patch creation. Residential developers respond to underlying urban land values (Anas 1978; Coiacetto 2007), focus on controlling project costs to meet profit targets (Mohamed

2006), and target specific types of buyers (Coiacetto 2007; Kohlhepp and Kohlhepp 2018). The main goal of this paper is to explore how these developer behaviors shape the urban environment.

Urban land rent gradient theory links land values to competition for urban land and urban form (Harvey and Jowsey 2003). Different land users compete with each other for the most desirable locations. The most expensive lands are closest to the city center, because transportation and transactions costs decrease. Each urban sector (commercial office, retail, residential) and user have their own bid rent curve (how much they are willing to pay based on distance and other location desirability factors). Although this monocentric view of the city is an oversimplification of complex urban structure (Zhang, Sun, and Li 2017), it predicts that residential parcels exhibit a density gradient in response to the underlying land rent. As land value increases, parcel and yard sizes should decrease. Therefore, I predict proportion of the parcel allocated to buildings will increase as land value and density increase.

Many urban ecologists have found that money buys green, the so-called luxury effect (Hope et al. 2003). Wealthier households and neighborhoods have greater woody species richness and cover (Clarke, Jenerette, and Davila 2013; Schwarz et al. 2015). If landscaping is a cost to control, then only builders of more expensive homes would be willing to spend more on plant materials as a signal to the wealthier households they are targeting. Thus, plant material spending represents an opportunity to the test for the luxury effect in developer landscaping decisions in new yards. Therefore, I predict that spending will be positively correlated with appraised improvement value and median assessed value.

Methods

Study Area & Sample

The lower Green-Duwamish watershed (Figure 1) lies within the Seattle-Tacoma- Bellevue-Metropolitan Statistical Area (Seattle MSA), in the central part of the Puget Sound Region. Of the 3.87 million people who live in the MSA, the majority (2.2 million) live in King County (<https://www.census.gov/quickfacts/>). The sample of 60 single-family residential parcels is a stratified random sample that I drew

from a sampling frame of 1,258 newly built and sold homes and yards. To be included in the sampling frame, the parcel had to meet all of the following criteria: 1) be located within the study area; 2) have a single family home built in 2014 or 2015; 3) be sold by a developer to a household by July 1, 2016. I screened all potential parcels to confirm that both buyers and sellers were eligible agents. I excluded buyers whose names were those of firms, non-governmental organizations, or governmental agencies. I also excluded buyers who bought more than one parcel during that time period. After screening buyers, I then screened the sellers. I included sellers with obvious firm names, and excluded sellers who were governments or nongovernmental organizations.

After constructing the sampling frame, I assigned each parcel to one of our strata, depending on how many homes each unique developer had within the frame. Strata constructed to capture developer variation, because developers specialize in location, project types (infill vs new subdivisions), and building types (Coiacetto 2007). The four strata were quartiles of property sale counts for each unique developer (Figure 1). Representing infill development and small development firms, strata 1 and 2 are unique developers who have built and sold 1 or 2 parcels, respectively, in the sampling frame. Strata 3 and 4 represent small subdivisions developers (3-8 parcels) and large subdivision developers (9 or more parcels). Using mail and in-person visits to the new homeowners, I obtained permission to sample 15 parcels per strata.

The University of Washington Human Subjects Division determined that this human subjects research project is qualified for exempt status, based on its interview procedures, lack of risk to participants, and subject matter. All data except for identifying information (e.g. parcel numbers, homeowner names, and coordinates) may be requested from the author.

Yard Patch Types & Area

After obtaining permission from the new homeowners, my field crew and I surveyed all 60 parcels during the growing season of 2016. I noted all homeowner created patches and noted what the original developer patch type was. For example, several homeowners had converted some of the original backyard lawn into

perennial beds or garden plots, installed sheds or greenhouses, or established chicken coops.

Using a 50 m tape, we measured the dimensions of all patch types, noted their shapes, and used standard geometry equations to calculate area for circles, ovals, rectangles, triangles, and trapezoids. For irregularly shaped planting patches, we used the offset method (Christians and Agnew 2008) to estimate area. We placed the length line along the longest axis of the patch, and measured shape length along lines placed perpendicularly to the length line. To maximize accuracy of estimations for irregular patches, we divided the length line into 1 m segments. For each yard patch, I assigned one of nine patch categories, based on the dominant vegetation or surface type (Table 1, Figure 2,3).

After fieldwork, I measured the building, paved, and developed area, using the King County parcel viewer (<https://gismaps.kingcounty.gov/parcelviewer2/>). The viewer allowed me to overlay high-resolution aerial photos of the new construction with property boundaries. Developed area included sampled yard patches plus all parcel-associated impervious surfaces (building footprint plus driveways, sidewalks, other walkways, and patios). Buildings were the footprint areas of all houses, garages, and developer constructed buildings. Other impervious surfaces were sidewalks, walkways, and uncovered patios. Yard area was the difference of developed area and the area of all impervious surfaces.

For some parcels, developed area was not the same as lot size as in the assessor dataset. First, two parcels had non-buildable areas. One had a steep slope covered with mature trees that the developer was required to avoid per the issued building permit. The other had a large riverine wetland that covered more than 40% of the parcel area. Second, curb strips were sometimes within property boundaries and sometimes not. Third, I included sidewalks, because developers were required to build them in certain jurisdictions. I also wanted to include all parcel-associated impervious surfaces except streets. Fourth, developers did not always place fence lines precisely along property boundaries. Finally, two homeowners told me that they managed specific patches shared between themselves and their adjacent neighbor. Instead of dividing the patch by where I estimated the

property boundary to be, I included this shared area as part of the developed area.

Because I did not use survey grade approaches to measure patch area, I corrected patch area to account for measurement error. I summed the area of all yard patches to calculate field yard area and calculated the proportion of field yard area occupied by each patch. I multiplied this proportion by aerial photo estimate of yard area to obtain corrected patch area.

Perennial Plant Prices

In each patch, I counted and identified all perennial plants. Then, I assigned each plant to one of four origin categories: remnant, developer planted, household planted, or spontaneously establishing after initial landscaping. After creating a list of developer planted taxa, I obtained at least four different prices for each taxon and calculated median retail price. Prices are from visits to plant retailers, reviews of online catalogs, and from values printed on tags that were still on many of the plants when I sampled. All stores and nurseries that I visited were within the Seattle Metropolitan Statistical Area; some, but not all, were inside the study area boundaries. Turf price, the median price per m², does not include transport costs, which some sod companies included in their quotes. Cost estimates per vegetated patch only included plant material costs, not site preparation costs. I rounded all prices to the nearest dollar.

Independent Variables

All independent variables (Table 2) except yard area, patch area, and elevation are calculated from 2016 data from King County (<https://www.kingcounty.gov/services/gis/GISData.aspx>). Neighborhood variables were calculated at the Census Block Group extent.

Data Management & Analysis

I conducted all data manipulation and analysis in R. After calculating sample mean area, total area, mean plant spending, and total plant spending for all patch types, I used the survey package (Lumley 2019) to estimate the same metrics for the sampling frame, specifying a stratified random design.

Built patch matrix (60 x 11) consisted of the proportion of the developed area occupied by buildings (Buildings), other impervious surfaces

(Paved) and nine yard patch types (Table 1). Plant patch spending matrix (58 x 4) consisted of the total spending on the four vegetated yard types (Lawn, Mix, Mono, Tree). In the spending analysis, I omitted two extremes: one where the developer planted no vegetation and the other where the homeowner was related to the developer. I log transformed area related predictor variables and then center scaled them. For all other predictor variables, I center scaled them and divided by their standard deviation. I chose Euclidean distance as the distance metric, because joint absences should be included in comparing parcel similarity in patch areas and amount spent per plant patch type.

To test the relationships between predictor variables and patch matrices, I ran redundancy analyses (RDA) in the vegan package (Oksanen 2018). A constrained ordination similar to multivariate linear regression, RDA maximizes the predictability of multivariate response variables, given a suite of multivariate predictors (Legendre and Legendre 2012). RDA was appropriate for my data, given that the distance measure was Euclidean and that the patch matrices are linear (proportion area, amount spent).

Finally, I evaluated per patch spending, using simple and mixed level negative binomial regressions in the MASS (Ripley 2018) and lme4 (Bates et al. 2019) packages. I evaluated model fit with Akaike Information Criterion (AIC) values, Bayesian Information Criterion (BIC) values and likelihood tests compared to simpler models (Long 1997). The final model had the smallest AIC and BIC values and was significantly from simpler models.

Results

Comparing Sample to Population

Buildings, Lawn, and Paved cover the most area (Table 3). The sample has a larger yard mean than the sample frame. Lawn, Mix, and Tree area means are larger for the sample; all other means are similar. Impervious surfaces cover about 44% of the developed area in the sample and about 51% of the developed area in the sampling frame. Lawn, Mix, Tree, Mulch, and Gravel/Cobble cover the most area of the yard. Short stature, single species patches (Mono), Gravel/Mulch, Rock, and Bare are minor types.

Table 2. Mean area and total area, by sample and sample frame. Area values are estimates of area covered by that patch type. Error is standard error. Types: Building = houses and other buildings. Other Paved = sidewalks, walkways, driveways, steps, and patios. Yard = Area of Managed Parcel Area not covered by buildings or other paved surfaces. Italicized words are yard patch names used in the text.

All mean spending estimates for the sample are larger than for the population (Table 4). A typical planting for a new yard cost \$1,170 for the sample and \$1,027 for the population. Developers spent nearly \$70,000 for the vegetated patches in the sample and nearly \$1.3 M in the population. Of this, developers spent the most money on Mix and then Lawn patches. The maximum developer spent an order of magnitude more on plant materials than the minimum developer.

Multivariate Approaches to Understanding Drivers of Developer Decisions

The constraining site and neighborhood variables explained 29% of the variance in the patch area RDA and 24% of the variance in the patch spending RDA. Value and density variables influenced space allocation and plant patch spending decisions (Figure 4). In both RDAs, land value, latitude, and median assessed value were negatively loaded on axis 1, and median house foot print and median yard size were positively loaded. Greater building and pavement area are positively associated with each other, higher land and higher latitudes (closer to Seattle to the north) and negatively with tree and lawn area. Greater paved area (Paved) and mixed perennial area (Mix) were positively associated with each other, slightly with Mono and gravel/cobble and negatively with Lawn area.

In terms of plant spending, greater lawn and tree spending was positively associated with larger yard sizes and negatively with land values and latitude. Mix and Mono spending, median assessed value, appraised improvement value, and residential density were positively associated with each other and negatively with longitude, elevation, and median yard area. Less was spent on plant patches further from Seattle (negative loadings of latitude, longitude, and elevation).

Univariate Approaches to Understanding Drivers of Developer Spending

Patch area and patch type of developer on the parcel influenced per plant patch spending (Table 5; Figure 5). Lawn patches are less expensive and less variable per unit area than Mix, Mono, or Tree patches. For a typical patch

(mean patch area of all vegetated patches; 33 m²), a typical developer would spend about \$60 on a Lawn patch, \$221 on a Mix patch, \$120 on a Mono patch, or \$251 on a Tree patch. Increasing patch area by one standard deviation to 93 m² increases patch spending much more for all patch types except Lawn.

Table 1. The nine vegetated yard patch types and descriptions.

Vegetated Patch	Description
Lawn	Traditional turf grass lawn. Isolated trees in lawn patches not assigned to <i>Tree</i> . Kept within <i>Lawn</i>
Tree	Dominant cover woody perennials with mature height >2m. Includes patches of large, native trees as well as tall hedge species
Mix	Mostly mix of shrubs and herbs, but may have a few accent trees. Mixed, short stature borders and rain gardens classified here.
Mono	Single species planting of short stature perennial.
Unvegetated Patch	Description
Mulch	Wood chips, bark, or sawdust
Gravel/Mulch	Linear strip of mulch and gravel, often used as borders around lawns or in side yards.
Rock	Accent boulders and rock walls
Gravel/Cobble	Small particle size rocks, usually linear or curvilinear. Gravel often used for paths. Cobble is often used for drainage, alone or in combination with gravel.
Bare	Unplanted, bare mineral soil
Mono	Single species planting of short stature perennial.

Table 2. Summary of independent variables evaluated. All data directly from King County, unless specified otherwise. Area metrics only evaluated in univariate analyses.

Site Extent	Mean ± SD (for values >0)	Min	Max
Latitude (degrees)	Not shown to protect homeowner privacy		
Longitude (degrees)			
Elevation (m) ¹	104.7 ± 41.0	21.1	179.4
Land Value (\$/m ²) ²	265.3 ± 173.6	40.1	1039.1
Assessed Improvement Value (\$1000)	426.2 ± 152.7	172.0	933.0
Yard Area (m ²) ³	363.0 ± 319.4	42.7	1897.1
Total Area by Yard Patch Type (m ²) ³	75.5 ± 130.2	0.0	1179
Yard Patch Area (m ²) ³		0.0	
Census Block Group (CBG) Extent			
Residential Density (# residences/km ²) ²	624.1 ± 381.5	15.5	1760.0
Median Age (years) ²	52.8 ± 22.0	8	90
Median Assessed Value (\$1000) ²	325.9 ± 98.9	185.0	664.5
Median House Footprint (m ²) ²	125.4 ± 34.5	64.1	196.0
Median Yard Size (m ²) ²			

¹From Google maps (<https://google.maps.com/>)

²Calculated in R from 2016 King County parcel data

³Measured in yards in 2016 and corrected via aerial photo interpretation of sampled area

Table 3. Mean area and total area, by sample and sample frame. Area values are estimates of area covered by that patch type. Error is standard error. Types: Building = houses and other buildings. Other Paved = sidewalks, walkways, driveways, steps, and patios. Yard = Area of Managed Parcel Area not covered by buildings or other paved surfaces. *Italicized words are yard patch names used in the text.*

Patch Type	Sample (n=60)		Sample Frame (n=1,258)	
	Mean Total Area/Parcel (m ²)	Total Area (m ²)	Mean Total Area/Parcel (m ²)	Total Area (m ²)
Built Patches				
<i>Buildings</i>	185.79 ± 7.5	11,147.39	183.43 ± 8.76	230,759 ± 11,016
<i>Paved</i>	100.65 ± 9.5	6,038.86	92.93 ± 10.15	116,911 ± 12,768
<i>Yard</i>	363.00 ± 41.2	21,779.84	268.83 ± 25.84	338,191 ± 32,508
Impervious (%)	44.1	44.1	50.7	50.7
Yard Patches				
<i>Lawn</i>	189.29 ± 37.7	9,720.01	114.95 ± 17.9	144,603 ± 22,507
Mixed Perennials (<i>Mix</i>)	60.76 ± 8.8	3,645.35	38.92 ± 5.3	48,958 ± 6,624
<i>Tree</i>	51.69 ± 23.5	3,101.48	28.35 ± 8.5	35,658 ± 10,730
Single Species (<i>Mono</i>)	12.07 ± 4.1	724.28	10.15 ± 3.9	12,771 ± 4,889
<i>Mulch</i>	33.46 ± 7.5	2,007.53	29.78 ± 8.8	37,468 ± 11,104
<i>Gravel/Cobble</i>	23.59 ± 4.1	1,415.66	32.72 ± 6.9	41,164 ± 8,658
<i>Gravel/Mulch</i>	5.90 ± 1.6	354.17	4.75 ± 2.0	5,979 ± 2,463
<i>Rock</i>	1.70 ± 0.8	102.14	0.56 ± 0.3	709 ± 351
<i>Bare</i>	9.58 ± 4.7	574.55	7.51 ± 3.9	9,445 ± 4,929

Table 4. Estimated spending on vegetated yard patch types for the sample and sampling frame. Error is standard error.

Vegetated Patch Type	Sample (n=58)			Sampling Frame (N=1,258)	
	Mean	Range	Total	Mean	Total
<i>Lawn</i>	\$439 ± 63	\$0-\$2,906	\$25,450	\$316 ± 47	\$397,690 ± 58,837
<i>Mix</i>	\$588 ± 76	\$0 - \$3,119	\$34,077	\$571 ± 98	\$717,714 ± 123,268
<i>Tree</i>	\$110 ± 26	\$0 - \$998	\$6,431	\$83 ± 19	\$104,754 ± 23,702
<i>Mono</i>	\$62 ± 13	\$0 - \$473	\$3,601	\$57 ± 21	\$71,967 ± 25,661
All	\$1,170 ± 103	\$331 - \$3,803	\$69,559	\$1027 ± 116	\$1,292,124 ± 146,414

Table 5. Vegetated yard patch cost model selection overview and final model selected for interpretation (italicized). All models negative binomial to account for right skew. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion. *P* = model probability from likelihood tests. ICC = interclass correlation coefficient. Marginal and conditional R^2 calculated via delta method.

Cost of All Vegetated Patch Types (y=\$) 402 patches on 58 sites				
Model	AI C	BI C	P	
M0: Intercept Only Model	492 6.1	493 4.1	n/a	
M1: Patch Type	488 8.4	490 8.3	<0.0001***	
M2: Log 10 Patch Area	483 6.8	484 8.8	<0.0001***	
M3: Patch Type + Log 10 Patch Area	469 0.8	471 4.8	<0.0001***	
M4: Site Random Effect	491 9.0	493 1.0	<0.0001***	
M5: Patch Type+Log10 Patch Area + Site Random Effect	468 0.1	470 8.1	<0.0001***	
Vegetated Yard Patch Spending (p<0.0001***) Marginal Pseudo R ² = 0.524; Conditional Pseudo R ² = 0.5801				
Fixed Effects	Estimate	Std. Error	z value	P
Intercept (Lawn)	4.093	0.0813	50.31	<0.0001***
Mixed Perennials (Mix)	1.304	0.1025	12.72	<0.0001***
Single Species, Short Perennial (Mono)	0.704	0.1544	4.561	<0.0001***
Tree	1.436	0.1684	8.53	<0.0001***
Log10 Patch Area	0.7607	0.0479	15.90	<0.0001***
Random Effects	Variance	Std. Dev		ICC
Developer on Site	0.07294	0.2701		0.144

Figure 1. Map of study area (lower Green-Duwamish Watershed Resource Inventory Area, outlined in gray) and parcels within Washington State (upper left) and King County (center). Strata based number of parcels each unique developer has in population: 1= 1 parcel; 2 = 2 parcels; 3= 3-8 parcels; 4 = 9 or more parcels in population. Unsamped parcels are light grey.

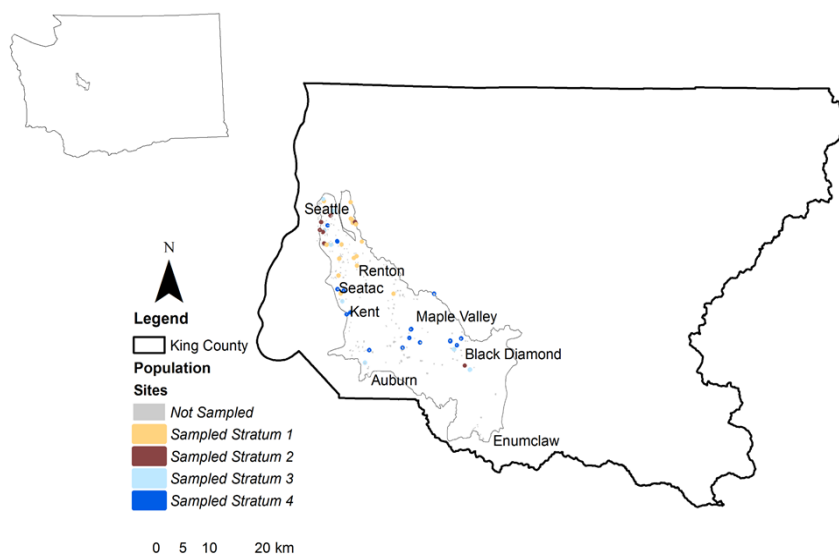


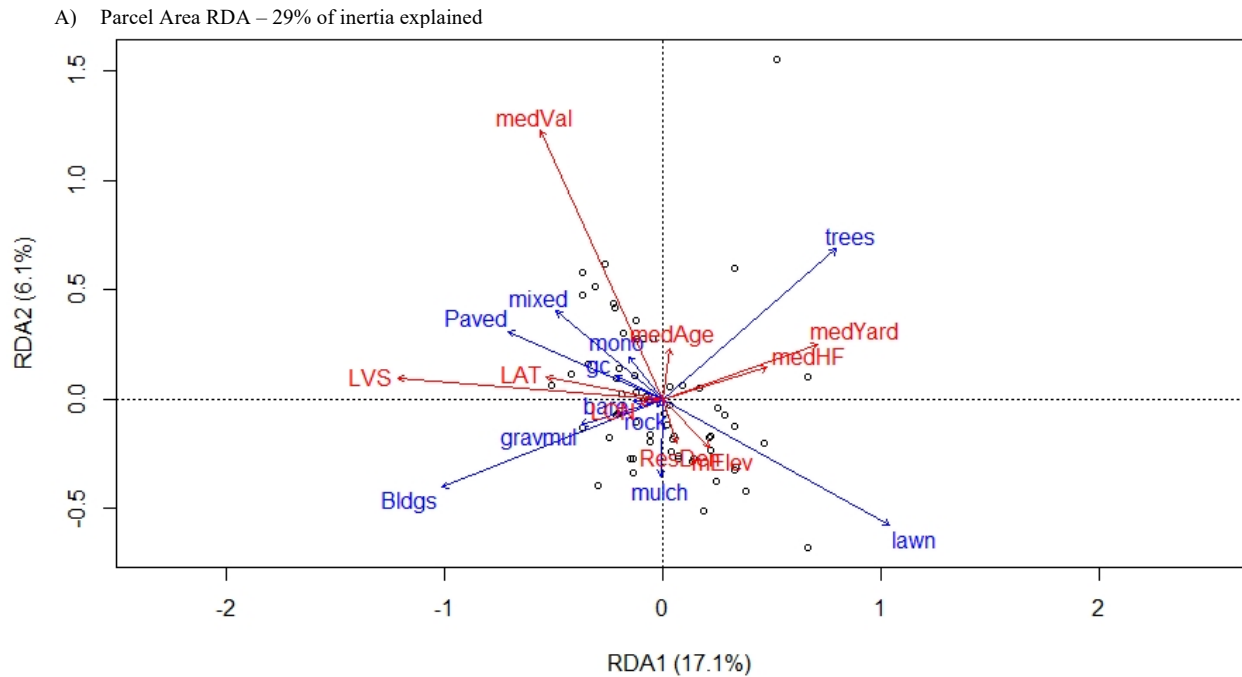
Figure 2. Examples of the four vegetated patch types found in sampled yards: A) Lawn; B) Tree – back yard patch of remnant *Pseudotsuga menziesii* (Douglas-fir); C) Mix – front yard patch with shrubs, herbs, and single accent *Acer palmatum* (Japanese maple); D) Mono – row of *Juniperus squamata* 'Blue Star' (blue star juniper); E) Tree – newly planted *Thuja occidentalis* (arborvitae); F) Mix – developer installed rain garden with several graminoid and shrub species.



Figure 3. Examples of the five non-vegetated patch types found in sampled yards: A) Mulch; B) Gravel/Mulch; C) Rock; D) Gravel/Cobble; E) Gravel/Cobble; F) Bare.



Figure 4. Redundancy analysis (RDA) triplots for A) patch area and B) plant patch spending. Sample sites represented by black circles, patch types by blue vectors, and predictor variables by red vectors. Variables: LVS=land value/m²; LAT = latitude; LON= longitude; mElev = elevation (m); medVal = median assessed value; medAge = median Age; medYard = median yard area; medHF= median house footprint; ResDen = residential density. Two additional variables included in spending RDA: Yard = yard area and ApprImpsVal = appraised improvement value.



B) Plant Patch Spending RDA - 24% of inertia

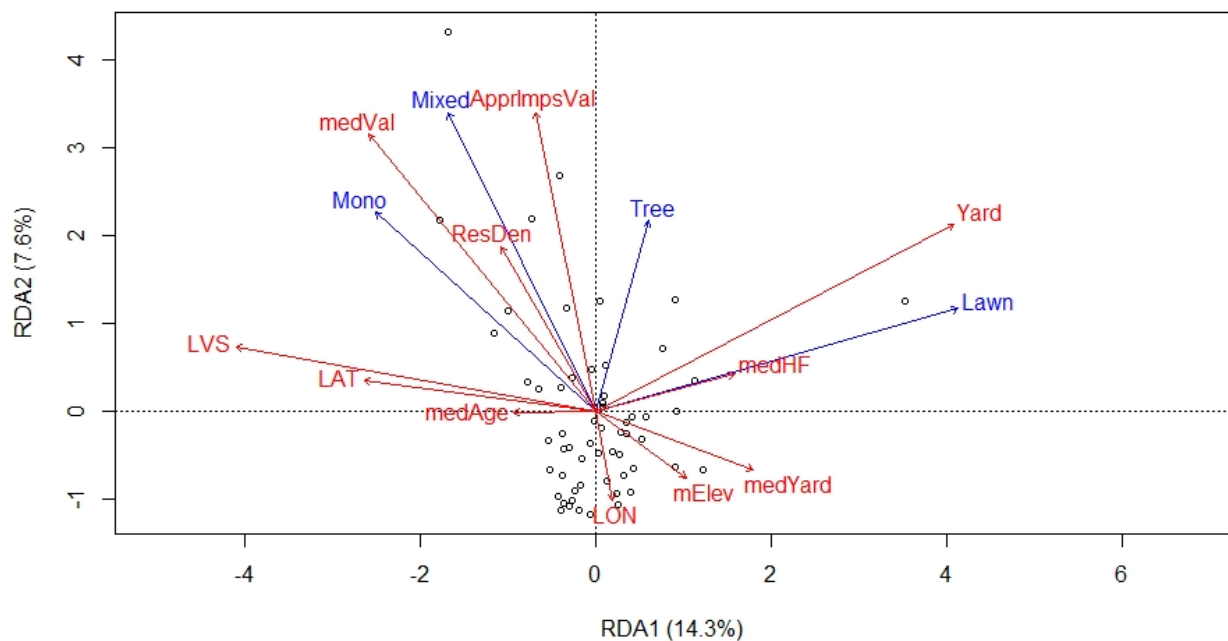
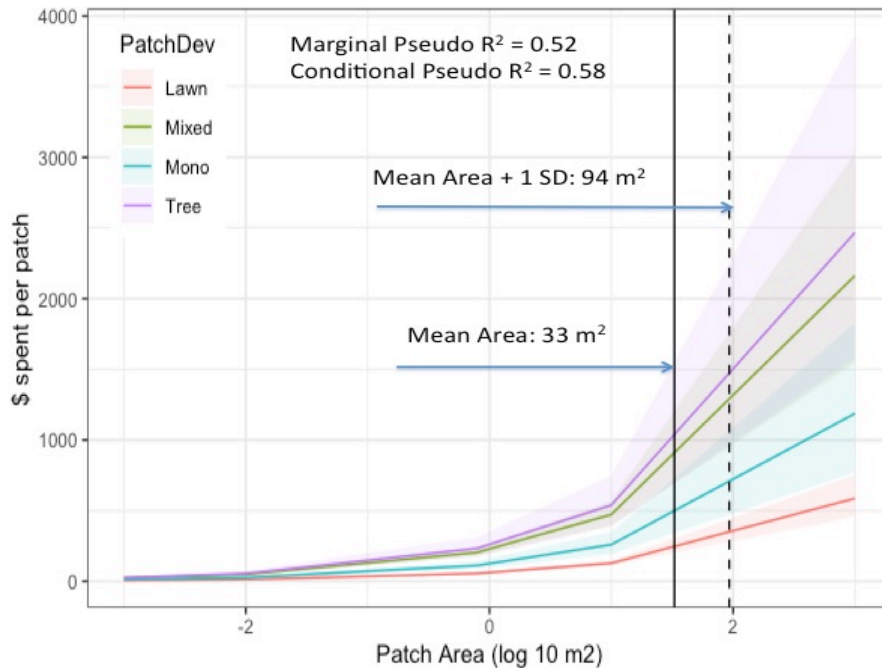


Figure 5. Fixed effects of patch area and patch type on per patch spending from mixed level model. Random effect is site. Lines represent the estimated spending per type for the typical patch (solid line; mean area 33m²) and a larger patch (dashed line; mean plus standard deviation; 94 m²).



Discussion

I focused on a single cohort of new residential landscapes, viewing construction of new residential landscapes as an act of urban patch creation. With the goal of documenting typical real estate developer patch creation activities and possible drivers, I examined two different decisions: 1) how developers allocated the developable area to different patch types, and 2) how much they spent on plant materials by yard and by patch type. I found broad support for my hypotheses that many of their decisions are linked to economic drivers.

Patch Area

In my reconstruction of developer patch creation decisions, I included both impervious and pervious patch types. Exclusive of roads, each newly developed parcel in the sampling frame generates about 276 m² of impervious surfaces (buildings and other paved surfaces; 44% of sample) and 115 m² of lawn. These land

cover types prioritize human uses, providing little habitat for other species. For yard patch types, Lawn, Mix, Tree, Mulch, and Gravel/Cobble are the predominant yard patch types, with Lawn covering the most area. My patch type areas correspond well with those of Nielsen and Jensen (2015), who found that grass was the most abundant vegetation type in Danish neighborhoods (170-243 m²/parcel), followed by tree cover (23-121 m²), bushes, and planting beds. Loose surface (2.2-6.1 m²) and loose ground cover (0.3-1.6 m²) correspond roughly to mulch and gravel types of my study. Similarly, cultivated borders, mown grass, path, trees, and uncultivated were some of the most frequently occurring types (more than 100 of 267 sites) in five UK cities (Loram et al. 2008).

Field mapping of urban land cover allows for finer resolution of more diverse cover types, but is time consuming, requires land owner permission for access, and is thus impractical for broader spatial scale studies of neighborhoods and cities (Nielsen and Jensen 2015). However, most remote sensing studies

were unable to distinguish many pervious patch types due to sensor limitations, overlapping cover types, and minimum mapping sizes. As a result, the most commonly used pervious patch types are tree/shrub, grass, and soil/rock/bare (Akbari, Rose, and Taha 2003; Li et al. 2014; Nielsen and Jensen 2015).

At the neighborhood and parcel extents, land rent gradients are well known to influence urban form (Anas 1978; Harvey and Jowsey 2003; Barr and Cohen 2014). As land values increase, building footprints tend to decrease and heights tend to increase, allocating building floor area upwards rather than outwards. I found building footprint and paved area to be positively associated with greater land values, consistent with land rent gradient theories. The positive association of non-Lawn plant patch types with land value metrics probably represents both a land rent gradient effect as well as a luxury effect, where wealthier homeowners and neighborhoods have more abundant vegetation (Clarke, Jenerette, and Davila 2013; Schwarz et al. 2015; Endsley, Brown, and Bruch 2018). Gravel/Cobble could also be a reflection of the underlying land rent gradient and a luxury effect. Because it was positively associated with Mix, Mono, and economic variables, developers may substitute Gravel/Cobble for Mulch or for Gravel/Mulch as land values increase.

Developer Plant Spending Behavior

In my review of the residential vegetation literature, I found no other estimates of developer spending or landscape valuation. Instead, most of the landscape valuation literature relies on hedonic modeling to disentangle the estimated effects of vegetation from the other structural, site, and neighborhood factors contributing to sale price of single-family residential homes. Most hedonic models find a positive effect on vegetation on sale price (3-10% increase) from the presence of trees or having higher quality landscaping (Anderson and Cordell 1988; Kadish and Netusil 2012).

Other researchers ask informed consumers how much they are willing to pay. By presenting different landscapes designs for a base model home, the conjoint analyses estimated how much more attendees of home and garden shows were willing to pay for better front yard landscaping (Behe et al. 2005). Compared to the base design of home, lawn, and straight driveway and walkway, consumers

in both studies preferred larger plant materials and more sophisticated designs (curved planting beds and hardscapes, mixes of deciduous and evergreen species, multiple beds and islands, and colored annual plants). These informed consumers were willing to pay 10-15% more for the most desirable landscapes.

I evaluated the overall perennial yard spending by yard and per patch as an exploration of residential developers' decisions to control costs and target specific buyers. For a typical patch area to vegetate, I found Lawn to be the least expensive patch type to install. The other three types cost at least twice as much per unit area. I also found extreme differences in how much each developer spent per patch type. The maximum developers spent at least twice as much per patch as much on Mix, Mono, and Tree patches compared to the minimum developers. Given the amount of yard area to vegetate, different developers appear to have made very different plant spending decisions, possibly because of their different target buyers (Boddy 2007; Tewari and Beynon 2017). Builders of more expensive homes spent more on non-Lawn plant patches. In contrast, developers spent more on lawn patches when yards were larger and land values lower. Combining my results with the other valuation approaches suggests that developers already consider this vegetation premium in their landscaping decisions. Wealthier buyers may expect new yards to have more than the base design of home, pavement, and lawn. Behe et al. (2006) noted that "design sophistication, plant size, and plant material" were the elements of landscape design that most contributed to perceived value. In fact, minimalist yards (small plants, little sophistication) decreased perceived value.

Strengths & Limitations

The sample size of 60 parcels limits generalizability. However, I drew the sample from a larger probability sample of newly built homes and yards. This cohort-based approach allowed investigation of the different choices that different residential developers make within the same real estate market. I chose to deliberately oversample parcels developed by small firms, in order to evaluate a variety of decisions of development firms who choose different locations and development types across the urban gradient. Homeowners who are

more interested in gardening may have been more likely to give permission for sampling. Yards in the sample may not be an accurate representation of all the different yard types built by developers in the sampling frame.

For the price evaluations I excluded two possible outliers: Site 2, where the developer installed no vegetated patches in the yard, and Site 41, where the homeowner turned out to be related to the developer. These factors may be common in the development process and should be incorporated in space allocation decisions. However, I felt they could be too influential in the spending analysis. Moreover, for Site 41, I was not confident that I could accurately separate patch-spending decisions of the developer and homeowner.

Spending estimates represent only plant material prices. The calculated retail costs of the planted perennials may be overestimates. Developers are probably more likely to get bulk discounts. I may not have been able to detect some of the plants that homeowners removed. I originally planned to include mulch, gravel, cobble, or rock in my estimates of patch prices. Quoted prices from landscaping firms and rock sources were too variable to reliably estimate price. Factors influencing quoted prices included parent material for rock walls and boulders, finer size and shape criteria than I had recorded for gravel and cobble, minimum quantity purchase requirements, and transport costs. Estimated prices may be higher than when the landscapes were installed in 2014 or 2015.

Conclusions

New residential construction is an act of product and patch creation, with developers as key agents of ecosystem change. The newly constructed homes and yards are new urban patch mosaics, resulting from specific decisions by specific residential developers. Using ecology field techniques, homeowner interviews, and archival photo analysis, I was able to reconstruct the initial conditions created by the developer. My research expands our knowledge of urban ecology by considering how developers shape residential landscape, by establishing the initial patches and plant communities. I found considerable heterogeneity in the established land covers

within a single land use of similar age, particularly for yard patches.

As predicted by land rent and luxury effect theories, some of the developer behavior appeared to be driven by underlying economic incentives. As land value increased, developers allocated more area to buildings and pavement and to vegetation patches other than lawn. Similarly, I found that developers who built more expensive homes spent more on non-Lawn plant materials.

A large body of urban ecology research links the luxury effect to neighborhood characteristics or homeowner preferences. My study is the first to show that residential developers create the luxury effect in the initial plantings. I detected the effect in both vegetated area and spending on non-Lawn patches. I conclude that residential developers directly create the luxury effect to attract wealthier homebuyers who would be more willing to pay more for better landscaping, just as they would be willing to pay for more expensive features within their new homes. Further research is needed in other urban ecosystems to replicate these results.

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Stream drift and fish diet response to disturbance from large-scale dam removal on the Elwha River, WA

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Abstract

Dams are being removed at increasing rates across the world, yet empirical data surrounding ecosystem response to removal actions are few and far between. Large-scale dam removals pose special risk to ecosystems due to the large quantity of stored sediment that often needs to be released. The Elwha River dam removals provided an opportunity to study food web response to large scale dam removal, as it was the largest managed sediment release in history. This study applied a suite of multivariate techniques on previously collected stream drift and fish diet data spanning five years (two pre-removal, two during, one after) and three sections of the Elwha River to investigate how stream drift and fish diet composition changes through space and time. The largest impacts of dam removal to drift and diet composition were observed in the lower section of the river, and many of these changes reversed in the year following dam removal. Trends of diet-drift mismatch were observed post dam removal, suggesting that fish may have responded to changes in drift composition and availability by shifting to a benthic prey base. Results from this study imply that ecosystems may resilient to short-term disturbances introduced by large-scale dam removal.

Introduction

Many dams in the United States are aging and in need of repair and dam removal is increasingly used as an alternative to repair due to economic and environmental benefits. Over 1400 dams have been removed in the US since the 1940s, with the rate of removal increasing every decade (O’Conner et al. 2015, American Rivers 2018). Despite these high rates of removal, empirical monitoring information has only been published for 181 dam removals in the United States and abroad (Duda et al. 2018). Large dam removals (i.e. greater than 8 m tall) are not common, accounting for less than 20% of total dam removals (DRIP 2018), although there have been several high-profile, large-scale dam removals over the last decade (Bellmore et al. 2016). Due to the lack of empirical data on dam removals, especially in terms of temporal scope and ecosystem recovery, ecological consequences of these large-scale dam removals are poorly understood.

Large dam removals are often associated with high volumes of impounded sediment and thus require a complex sediment management component (Grant & Lewis 2015). As sediment is released downstream during removal, short-term negative ecological consequences can be

expected (Tullos et al. 2016). The impact on benthic invertebrates has been widely studied (reviewed by Carlson et al. 2018), as have the effects of increased turbidity and bed disturbance on salmonid foraging in predominantly lab-based studies (Barrett et al. 1992; Gregory et al. 1993; Harvey & White 2008). Despite these studies, data seems to be lacking on the consequences of sediment release to food web dynamics, particularly prey availability and prey consumption.

The Elwha River dam removals in Washington State provided an opportunity to study food web response to large scale dam removal, as it was the largest managed sediment release in history (Warrick et al. 2015, Ritchie et al. 2018). Two dams on the Elwha River blocked anadromous migration as well as downstream transport of sediment and wood for nearly a century. A combined 30 metric tonnes (Mt) of sediment had accumulated behind the two dams by the time they were removed (Randle et al. 2015). These blockages resulted in a coarsened river bed and eroded coastal beaches (Warrick et al. 2009, Draut et al. 2011) - physical changes which further affected fish populations (Brenkman et al. 2008, Pess et al. 2008), alongside the associated changes to benthic food webs (Morely et al. 2008).

Removal of both dams began in September 2011 with the final blast in August 2014, using a recommended three-year staged removal strategy to balance the severity and duration of sediment release impacts (Randle et al. 2015).

The objectives of this study were to use multivariate statistics to explore how prey availability and diet composition for juvenile rainbow trout (*Oncorhynchus mykiss*) changed during three years of dam removal, across multiple Elwha River habitats. Specifically, my objectives were to 1) quantify how drift composition changed spatially and temporally (seasonally and relative to timing of removal activities/sediment releases), 2) quantify how prey composition in fish diets changed spatially and temporally, and 3) examine how changes in prey availability related to fish diets. I hypothesized that prey composition would change during dam removal, especially in times of high impact sediment release, and that changes in fish diets would mirror changes in prey availability.

Methods

Study Area

Largely protected in the coniferous forests of the Olympic National Park designated wilderness, the Elwha River is a short and steep river that originates in the Olympic Mountains and flows northward for 72 km into the Strait of Juan de Fuca 9 km west of Port Angeles, Washington (Fig 1). About 14 km of the Elwha flows through second-growth forests on private, state, and tribal lands downstream of the national park boundary. The Elwha River receives heavy precipitation (annual average of 143 cm), and seasonal discharge peaks during fall and early winter rainy and spring snowmelt (Duda et al. 2011). Mean annual flow at USGS gage 12045500 is 43 m³/s, and the system is also characterized by a relatively high annual sediment load of 0.34 ± 0.08 Mt (Magirl et al. 2015). The lower 7.9 km of the river which is free flowing contains five anadromous salmon (Chinook (*O. tshawytscha*), chum (*O. keta*), coho (*O. kisutch*), pink (*O. gorbuscha*), sockeye (*O. nerka*)), as well as anadromous steelhead (*O. mykiss*), cutthroat (*O. clarkii*), bull trout (*Salvelinus confluentus*), Pacific lamprey (*Entosphenus tridentatus*), and eulachon (*Thaleichthys pacificus*) (Pess et al. 2008). Resident fish species found in the river basin include sculpin (*Cottus* spp.), threespine stickleback (*Gasterosteus aculeatus*), reddsideshiner (*Richardsonius balteatus*), surf smelt (*Hypomesus pretiosus*), and non-native brook trout (*S. fontinalis*) (Brenkman et al. 2008).

Two dams on the Elwha river were built in early 1900s in order to power the logging town of Port Angeles. The oldest and smallest of the two, Elwha Dam was completed in 1913 with a height of 32-m. Elwha dam sat 7.9 rkm upstream of the mouth of the river and forming Lake Aldwell with a 10 million m³ capacity. Glines Canyon Dam was built not long after in 1927, to a staggering 64-m in height. Glines Canyon Dam was located just upstream of the national park at rkm 26.1 and impounded Lake Mills with a 50 million m³ capacity. At the time of dam removal, Lake Aldwell was estimated to have 7 Mt sediment and Lake Mills with an additional 23 Mt (Randle et al. 2015). Neither of these dams had fish passage options, despite the WA state requirement at the time of construction.

Data

I used previously collected data from a long-term monitoring program on the Elwha River dam removal project (Morley et al. in prep). Data collection spanned multiple years through the dam removal process including impacted and control reaches throughout the system. In the following sections I describe the experimental design and field sampling methods for this dataset before detailing the methods for my statistical analyses.

Experimental Design

Samples were collected from four sections of the Elwha River: 1) lower, 2) middle, 3) Aldwell, and 4) tributaries (Fig 1). The lower section stretched from the estuary to the former Elwha Dam (7 rkm), the former Aldwell Reservoir continued from the Elwha Dam to the Highway 101 bridge (4 rkm), and the middle section began upstream of the 101 bridge and ended at the former Glines Canyon Dam (12 km). These sections were all sediment impacted during the removal. Additionally, two different habitat types were sampled in the lower and middle sections: mainstem (MS) and side channel (SC). Four tributaries (TR) that flowed into the middle Elwha, between Glines Canyon and Elwha dams (Griff Creek, Madison Creek, Indian River, and Little River; elevations 68-115 m) served as a control reach since they were free from sediment impacts associated with dam removal.

This analysis included 162 unique sampling events across 37 sites. The distribution of sites was non-random, but dictated by access and overlap with long-term monitoring stations (Morley et al. 2008; Shaffer et al. 2009; Duda et al. 2011). The total number of sites sampled in a given year and season changed over time

due to variation in year-to-year river discharge and a rapidly shifting habitat mosaic during dam removal. In the river, high flows precluded sampling mainstem sites in spring and winter. All spring and winter sampling were limited to tributary sites and floodplain sites in the lower and middle sections, with no sampling in Aldwell, and only occurred in 2011, 2012, and 2013.

Sampling methods

Both aquatic and terrestrial invertebrates were collected via drift sampling (Baxter et al. 2017). Two 250- μ m-mesh, 0.14 m²-frame drift nets were placed side-by-side at the upstream end of each sample reach, directed perpendicular to flow, and secured above the stream bottom with rebar. Nets were left in place over a timed 30- to 60-minute interval and measured adjacent water velocity and depth to calculate the total water volume sampled. Invertebrates captured in the nets were rinsed through a 200- μ m-mesh soil sieve and combined into one sample preserved in ethanol.

Because there were no anadromous fish present upstream of the Elwha Dam prior to dam removal, our river sampling was limited to resident trout (*O. mykiss*, except for *O. clarkii* at one tributary site). In shallow (< 1.5 m) fast-flowing riverine habitats, a Smith-Root backpack electrofisher was used to collect salmonids within a 500-m sample reach. Multiple passes were made until either 10 individuals of each target species were captured, or a period of 90 minutes had elapsed. Captured fish were transferred to buckets containing aerated ambient water and kept cool until handling. Fish were anesthetized in a diluted solution of tricaine methanesulfonate (MS-222) to count, identify, and measure FL to the nearest mm and weight to the nearest mg. After recovery from anesthesia, all fish were released at the point of capture.

Stomach contents from a sub-sample of target salmonid species were extracted via non-lethal gastric lavage and preserved in ethanol. When possible, stomach contents from ten individuals of each species between 55-199 mm fork length (FL) were collected at each site during each sampling event. Due to incidental electrofishing mortality (0.03%), diets were also collected from six smaller fish (42-54 mm FL). Fish with no regurgitated prey were recorded as empty stomachs.

Fish diet and invertebrate samples were sent to a professional taxonomist for processing and identification to the lowest practical taxonomic level. Taxa were classified either as aquatic (aquatic in every life stage), of aquatic-origin

(terrestrial adults of aquatic larvae), or terrestrial. Aquatic and aquatic-origin taxa were typically identified to the genus or species level and terrestrial taxa to family or genus. Some less common taxa or partially-digested prey items were identified to Order or Class.

Body parameter measurements were recorded for all invertebrate taxa to the nearest mm, either as head-capsule width or total length. From these measurements, dry weight (g) and energy content (joules) of each invertebrate was calculated based on published regression equations or equations developed from individuals collected during this study (Morley et al. in prep). Dry weights were also converted into wet weights using percent dry mass (PDM) values from the literature. These calculations were life-stage specific and made at the family-level or next available taxonomic level reported in the literature.

Statistical analyses

I performed all data analysis in R version 3.5.3 (R Development Core Team 2019) using the packages Biostats (McGarigal 2009) and Vegan (Oksanen et al. 2011).

a. How did drift composition change through time and space?

A total of 162 unique sampling events were analyzed for drift composition. Due to the large number of taxa and the variability in taxon level, all taxa were grouped by family. Since I was ultimately interested in how fish prey availability changed over time, I only selected families that were present in fish diets. To further reduce the dataset and minimize effects of rare items, I dropped families with < 5% frequency of occurrence. The data matrix was standardized to relative proportions of wet weight, and transformed into a Bray-Curtis distance matrix for further analyses.

To identify dominant gradients of variation in the drift composition in relation to time and location, I conducted a principal coordinate analysis (PCoA) on the distance matrix. Significance of each axis was based on a broken stick model. Analysis only proceeded on an axis if its eigenvalues (measure of variance explained by that axis) exceeded the null broken stick model. I then fit variable loadings (taxa significance) onto the ordination by linear correlation analysis of each original variable (i.e. taxa) on the scored derived from the PCoA. Loadings were calculated using the *envfit* function in the Vegan package, which permutes 1000 iterations to generate a p-value estimating significance of the relationship, and an *r*² value representing the strength of the relationship to

that axis. On each axis, species loadings were included only if significantly correlated to the axis ($p < 0.01$ and $r^2 > 0.1$).

To test for differences in drift composition among different locations and years, I used permutational multiple analysis of variance (perMANOVA), which acts analogously to a traditional ANOVA by partitioning the within- and among- group sums of squares of a distance matrix and uses permutations to test for significance (Anderson 2001). Our experimental design had several potential grouping levels to nest: spatially, habitat types (MS and SC) were nested within sections and temporally, seasons were nested within years. Additionally, the temporal factors could be nested within the spatial factors. I ran a series of nested perMANOVA models to assess differences in time and space at different levels of groupings. First, I tested for differences between years within each of the sections. Next, I determined that the different habitat types showed different responses to time through dam removal. Finally, I was interested to see if there were different annual responses within the different seasons. Due to sample size limitations, I was not able to fully nest seasons within year, habitat, and section. Instead I chose to evaluate a model with season nested within year and section. While this model does not account for habitat differences, it still allows an opportunity to evaluate if seasonal differences exist in each year. PerMANOVA tests can be sensitive to differences in multivariate dispersion, so I also conducted permutational analyses of dispersion (permDISP) to assess differences in dispersion among different grouping levels (Anderson et al. 2006).

b. How did diet composition change through space and time?

Fish were only selected for analysis if they were collected at a sampling event during which drift was sampled. Gut contents were generally identified to higher taxa levels than drift samples and many items could not be identified to lower levels due to digestion level. Therefore, diet taxa were grouped at the family level and rare taxa were dropped ($< 0.5\%$ frequency occurrence). The data matrix was standardized to relative proportions of wet weight, and transformed into a Bray-Curtis distance matrix for further analyses. PerMANOVAs and PCoAs were conducted on diet samples following the same methods described above for drift samples. Because of the level of variation present in the fish diets, I also conducted multivariate dispersion tests to

explore changes in diet composition variability at individual sampling events through time.

c. How did changes in diet relate to changes in prey composition?

I used a Procrustes analysis to check for correlation between drift and diet multivariate structure. Procrustes analysis is a method of comparing two distance matrices containing the same objects by scaling each matrix and rotating one until the best fit superimposition of the two matrices is found, and then provides a measure of fit (m^2). Because fish were not collected at all sites where drift was collected, I removed all sample events at which fish were not collected from the drift data before comparing the Bray-Curtis distance matrices. To create the drift matrix for this analysis, I assigned each fish the corresponding drift variables from the sampling event at which they were collected. That is, all fish collected at the same site and date were given the same drift variables. In order to test for significance of the Procrustes analysis, I used a permutational approach to determine the probability that the observed m^2 is smaller than expected due to chance. I then plotted the residuals of the Procrustes analysis to determine which fish diets exhibited a good or poor fit with the corresponding drift matrix, and explore any trends in this fit through time and space.

d. How did sediment impact sites through space and time?

I visualized sediment pulses through time and space using two different datasets. I used suspended sediment concentration (SSC, mg/L) data from the US Geological Survey (USGS) to explore trends in sediment release through time. This data was derived from regression relations with turbidity at the USGS gaging station Elwha River Diversion (no. 12046260), collected at 15-min intervals (Ritchie et al. 2018). I averaged this data on a daily time step to visualize how sediment pulses through the river changed in the course of this food web study (Fig 2).

In order to visualize impacts of sediment release and deposition at individual sampling events, I analyzed pebble count data that was collected alongside a subset of drift and diet sampling events. Pebble counts were conducted at 21 sampling events from 2011 through 2014, across all four sections in order to classify stream bed composition (Wolman 1954). In the reach where stream drift and fish were sampled, 100 pebbles were selected at random, evenly disbursed throughout reach length and width. The length of intermediate axis was measured

for each pebble sampled, and anything less than 2-mm in length was categorically described as “fines.” For this analysis, I focused on the percent fines at each sampling event (Fig 3).

Results

How did drift composition change through time and space?

A total of 201 unique taxa were observed in drift samples grouped at the family level. After removing rare taxa and taxa not represented in the diet samples, this number was reduced to 60 unique taxa. A mantel test conducted between the full and reduced distance matrices on Pearson’s product-moment correlation indicated significant correlation ($p=0.001$, $r=0.922$), justifying analysis on the reduced dataset.

Significant axes on the PCoA explained 18% of the variation in the drift community data (PCO1: 12%, PCO2: 6%). Significant taxa positively correlated on PCO1 include two mayfly families Heptageniidae ($p=0.001$, $r = 0.1$) and Baetidae ($p=0.001$, $r = 0.1$) and the caddis fly Rhyacophilidae ($p=0.001$, $r=0.1$), while the Dipteran family Chironomidae ($p=0.001$, $r = 0.9$) was significantly negatively correlated on this axis. Baetidae ($p=0.001$, $r = 0.4$) and Heptageniidae ($p=0.001$, $r=0.2$) in addition to another mayfly Ephemerellidae ($p=0.001$, $r = 0.1$) were positively correlated with PCO2, while the terrestrial Bibionidae ($p=0.001$, $r = 0.4$) and Formicidae ($p=0.001$, $r = 0.1$) were negatively correlated on this axis.

Differences between habitats is visualized along PCO1 of the PCoA, which explained 12% of the total variation in the drift composition (Fig 4). Tributary sites are concentrated on the positive side of PCO1, with a lower relative abundance of Chironomidae. The centroids of most years of tributary data are concentrated in the top right of the plot, indicating a general pattern of high relative abundance Baetidae, Ephemerellidae, and Heptageniidae. Mainstem and side channel sites appear to have much more variation across both PCO1 compared to tributary sites. Mainstem sites don’t overlap much with tributary sites and have a higher tendency towards the negative end of PCO1, associated with higher abundances of Chironomidae, while side channel sites are spread throughout the range of mainstem and tributary sites.

While large amounts of site variation across PCO1 and PCO2 make it difficult to visualize trends in location and dispersion at multiple factor level groupings, boxplots of each PCO highlight seasonal, habitat, and section differences through the course of the dam

removal timeline. Boxplots allow visualization of the mean axis location of sites within each grouping, while the position and spread of the interquartile range (representing 50% of the sites) shows how representative those axis positions are among all sites within the group. Impact sites saw differences in position and distribution along both PCO1 and PCO2 in response to dam removal in 2012 and 2013, and these changes varied by section, habitat, and season (Fig 5).

Coinciding with release of sediment from Glines Canyon dam removal in 2013, Aldwell sites shifted negatively on PCO2 towards Formicidae and Bibionidae (Fig 5b). In 2014, Aldwell sites shifted back towards the origin on PCO2 and move negative on PCO1, indicating increasing dominance of Chironomidae in drift samples and decreased occurrence of Formicidae and Bibionidae. In the lower river, mainstem sites saw a shift away from Chironomidae on PCO1 in 2013, but there was a lot of variability in this response among sites (Fig 5a). These sites also shifted towards Formicidae and Bibionidae in both 2012 and 2013, before reversing this trend in 2014. Side channels in the lower river did not vary as much across years on PCO1, although there is a slight positive shift away from Chironomidae in 2012. Interestingly, response during dam removal in 2012 was opposite in the mainstem and side channel habitats, with mainstem sites continuing to be dominated by Chironomidae while composition in side channel sites shifted in the opposite direction. Side channel sites had a similar response as mainstem sites on PCO2 in 2012 and 2013, shifting negative before returning back near the origin in 2014, indicating species composition shifts towards Formicidae and Bibionidae during dam removal. Middle river sites did not elicit strong response to dam removal across PCO1. In the mainstem, drift compositions shifted towards Chironomidae dominance in 2012, but most sites remained on this side of PCO1 through 2013 and 2014, although this was more variable in 2013 and 2014 compared to 2012. On PCO2, mainstem sites did show a negative response in 2013, which reversed in 2014 and returned to positions similar to pre-removal. Side channel sites were more correlated with Heptageniidae, Baetidae, and Rhyacophilidae similar to the tributaries, and this did not change much through time. Side channels responded to dam removal in 2013 negatively along PCO2 showing increased dominance of Formicidae and Bibionidae.

Seasonal changes through time were also apparent along PCO1 and PCO2 (Fig 5c,d).

During spring in the lower river, there was a drastic shift away from Chironomidae dominance in 2012. This distinction was not observed in any other seasons (Fig 5c). Additionally, Spring samples were more associated with Formicidae and Bibionidae in 2012 compared to 2011, a response also seen in the summer months (Fig 5d). Winter compositions responded with decreasing association with Baetidae, Heptageniidae, and Ephemerellidae in 2012 and 2013 and 2013 (Fig 5d). Chironomidae dominance appeared to drive changes in composition from 2012 to 2013 (Fig 5c). In the middle river, spring and winter sample composition was driven by Heptageniidae, Baetidae, and Rhyacophilidae throughout dam removal. The shift on PCO1 towards Formicidae and Bibionidae was much more dramatic in winter and spring compared to summer. The only seasonal response observed in the tributaries was heavy loading on PCO2 during the winter, indicating higher abundances of Baetidae, Heptageniidae, and Ephemerellidae compared to spring and summer (Fig 5d).

Both section and habitat type contributed to significant differences in drift community composition, as did year and season when nested within those factors (Table 1). The highest amount of variation was explained by the season within year within section model ($R^2=0.195$, Table 1), followed by the year within habitat within section model ($R^2=0.163$, Table 1). Significant differences in multivariate dispersion within the factor groupings were present in all models, indicating that differences in drift community between factor groupings could be a result of differences in multivariate space and dispersion (Table 1).

In fact, multivariate dispersion varied significantly within sections and habitats through time (Fig 6, Table 2). Generally, dispersion was higher in side channel habitats compared to the mainstem habitats. Low sample size in Aldwell 2012 ($n=2$) makes it difficult to infer effects of dam removal, however multivariate dispersion (mean distance to the centroid) is significantly higher in 2013 compared to 2012 and 2014 (Fig 6, Table 2). In the lower mainstem, dispersion increased from 2011 to 2012, coinciding with removal of the Elwha dam and during removal of the upper Glines Canyon dam in 2013, dispersion was significantly higher than before removal in 2011 and after removal in 2014. A similar pattern was seen in the lower side channels where 2012 had significantly higher dispersion than 2010 and 2011. Dispersion decreased significantly from 2012 and 2013 after removal had finished in 2014, although this should be

interpreted conservatively due to the low sample size in 2014. These patterns did not hold in the middle section. Dispersion decreased significantly in the mainstem from 2011 to 2012, increased slightly in 2013, and then significantly decreased in 2014. In the side channel habitats, dispersion was significantly higher in 2013 compared to pre-removal in 2012, but the magnitudes for these changes are smaller than observed in the lower river. Elevated dispersion in 2013 across both side channel and mainstem habitats is consistent with the timing of the upper Glines Canyon Dam removal in 2013. Tributary sites did not experience a change in dispersion in response to dam removals. However, dispersion in 2010 is significantly lower than in any of the other years, potentially signaling natural variation in dispersion through time due to other factors. Patterns of multivariate dispersion may have been strongly influenced by Chironomidae frequency and abundance across samples. Mean dispersion of each habitat, section, and sample year grouping was negatively correlated with the mean proportion of Chironomidae among drift samples at each site ($R^2 = 0.79$, $p < 0.001$, Fig S2). This relationship was not influenced by taxa diversity however, as the regression of mean group dispersion on the mean number of taxa present in drift samples was not significant.

How did diet composition change through space and time?

Diets were analyzed from a total of 907 fish collected across 132 sampling events. A total of 172 unique taxa at the family level was observed across all diets. Diets were dominated by Chironomidae and Baetidae, occurring in 60% and 45% of all fish diets, respectively. The Ephemeropteran families Ephemerellidae and Heptageniidae were also relatively frequent, occurring in 25% and 23% of diets, respectively. There was a lot of variability present in diet samples, with vast majority of diet items were considered “rare” - 146 out of the 172 taxa occurred in less than 5% of diets and 112 taxa occurred in less than 1% of diets. Due to this diversity in taxa, I only dropped taxa if they occurred in fewer than 0.5% of diets. There were 112 variables in this reduced dataset. A mantel test based on Pearson’s product-moment correlation conducted between the full and reduced dataset showed significant correlation ($p=0.001$, $r=0.983$), justifying continued analysis on the reduced dataset.

Ordination of diets highlighted minimal trends in diet composition across time and space and explained minimal variation in the

prey composition. I explored different levels of taxa grouping to reduce the variable size, but none of those ordinations improved variation explained or the significance. Additionally, I ran linear correlations between the variables and each orthogonal PCO axis from the ordination to select the most significant taxa on each axis. I attempted another ordination using only those variables which resulted in a marginal increase in variation explained by the first two axes, but none of the axes were significant. Only PCO1 was significant, which explained a mere 6% of the total variation in the data. Significant arching was present in the PCoA, which was caused by diet samples dominated by Baetidae and Chironomidae (Fig S1, supplementary). Significant spread in the data and overlap between factors was also apparent in the ordination, and clear distinction between factor groups was not apparent. Because of this, boxplots were used to visualize changes across PCO1 just as in the drift samples.

Variation across PCO1 was mainly influenced in the negative direction by Chironomidae ($p = 0.001$, $r = 0.92$) and in the positive direction by Ephemerellidae ($p = 0.001$, $r = 0.15$). Among tributary fish, mean values on this axis all clearly fell on the positive side, associated with presence of Ephemerellidae (Fig 7a). Though there is still a large amount of variation in this data, the majority of fish in each year fell on the positive side of this axis, distinctly different from the other river sections. A common trend among sections and habitats was a negative shift on PCO1 towards Chironomidae in 2014, observed in Aldwell, lower mainstem, and middle side channel habitats. Until 2014, the majority of fish in middle side channel habitats were positioned on the positive side of PCO1 associated with higher reliance on Ephemerellidae, similar to the tributaries. Diets did not change much on PCO1 in relation to dam removal timing in the middle mainstem habitats, although there was a slight trend towards more fish relying on Chironomidae prey in 2012 and 2014. In the lower and middle river, most diets in spring and winter were positively associated with Ephemerellidae and negatively associated with Chironomidae, and this relationship did not exhibit trends through dam removal (Fig 7b). The trend towards Chironomidae during dam removal and especially in 2014 was apparent in summer months in the lower and middle sections. Tributary diets were similar through seasons and years with the exception of winter 2013 where diets shifted a little more negative.

Although all factors were significant ($p < 0.001$), perMANOVA analysis on fish diets did not indicate meaningful differences between composition within any of the factor groupings ($R^2 < 0.05$), probably owing to very large sample size. Analysis of multivariate dispersions at all factor grouping levels were also all significant ($p < 0.001$). An exploration of the mean multivariate dispersion in diets within sections and habitats indicated some shifts in diet variability through time (Fig 8, Table 3). The main trend was a significant decrease in dispersion (distance to centroid) in 2014 observed in Aldwell, lower mainstem, lower side channel, and middle side channels (Fig 8, Table 3). Interestingly, level of dispersion does not seem to be impacted during dam removal in 2012 and 2013. Dispersion in the middle mainstem habitats was not significantly different among years. Although there are significant differences in dispersion across tributary sites, the magnitudes of change are not as extreme and do not follow the same trend indicating that changes in impacted sites are likely in response to dam removal activities.

How did changes in diet relate to changes in prey composition?

Variable loadings on the first PCO of diet and drift ordination showed similar trends. Chironomidae and Ephemerellidae were both driving dominant trends in variation within drift and diet samples. Trends on PCO1 loosely matched between drift and diet samples, with noticeable shifts towards Chironomidae in 2014 at Aldwell, the lower mainstem, and middle side channel sites (Fig 5a, Fig 7a). Positive shifts away from Chironomidae towards dominant Ephemeroptera (Heptageniidae, Baetidae, and Ephemerellidae) and Trichoptera (Rhyacophilidae) families were also consistent between drift and diet ordinations in 2013 at Aldwell and lower mainstem sites.

Procrustes analysis between the drift and diet distance matrices indicated a significant relationship between the two matrices ($p = 0.001$, m^2 : 0.89, Correlation in symmetric Procrustes rotation: 0.323). Residuals of the Procrustes analysis showed trends in goodness of fit between the two matrices across space and time (Fig 9). Residuals were higher in 2014 compared to previous years in Aldwell, lower mainstem, lower side channel, and middle side channel, indicating diet compositions did not match drift compositions as well as previous years. Diet composition matched drift availability the best during years impacted by dam removal in the lower mainstem and side channels (2012, 2013) and in

the middle side channels (2013). Residual values appear to be fairly consistent across years in tributary habitats and in the middle mainstem.

How did sediment impact sites through space and time?

Sediment impacts of dam removal were concentrated in 2012 and 2013 (Fig 2, Ritchie et al. 2018). The first spike in suspended sediment loads occurred in the spring of 2012. Elevated suspended sediment spiked again to similar levels through the fall and winter of 2012, coinciding with fall and winter rain events, and these levels remained through June 2013. The highest sediment loads occurred

between September 2013 and March 2014, but Spring of 2014 returned to low sediment levels for the duration of spring and summer. Sediment deposition at sample sites, as measured by percent fines from pebble counts, was most extreme during 2013 and 2014 at side channel habitats in the middle section (Fig 3). At mainstem sites in the middle section, sediment deposition peaked in 2012 and by 2014 returned to levels seen in 2011. In the lower river, the highest sediment deposition in mainstem sites was observed in 2012, while in side channel sites, it increased from 2012 to 2013. Fine sediments remained at consistent low levels in Aldwell and tributary sites through time.

Table 1. Results from permutational multivariate analysis of variance (perMANOVA) and permutational analysis of multivariate dispersion tests on drift composition, testing differences between years nested in sections, years nested in habitats nested in sections, and seasons nested in years nested in sections.

Model	Effect	perMANOVA				dispersion		
		df	F-ratio	R ²	p	df	F	p
Section (Year)	Section	3	5.139	0.103	<0.001	3	5.190	0.002
	Section:Year	14	1.429	0.134	0.003	17	5.377	<0.001
Habitat (Year)	Habitat	2	8.265	0.110	<0.001	2	20.44	<0.001
	Habitat:Year	11	1.454	0.106	0.004	13	7.580	<0.001
Section (Habitat (Year))	Section	3	5.259	0.103	<0.001	3	5.190	0.002
	Section:Habitat	2	2.597	0.034	<0.001	5	9.465	<0.001
	Section:Habitat:Year	19	1.311	0.163	0.006	24	7.596	<0.001
Section (Year (Season))	Section:Year:Season	17	1.959	0.195	<0.001	34	3.817	<0.001

Table 2. Pairwise comparisons of multivariate dispersion in drift composition between sample years, significant at $p < 0.05$. All p -values presented are from a pairwise permutational test of significance. Habitats include mainstem (MS) and side channels (SC).

Section	Habitat	Contast	p
Aldwell		2012 v 2013	0.001
		2013 vs 2014	0.015
Lower	MS	2011 vs 2013	0.014
		2013 vs 2014	0.007
		2010 vs 2012	0.009
	SC	2011 vs 2012	0.049
		2012 vs 2014	0.005
		2013 vs 2014	0.037
Middle	MS	2011 vs 2012	0.003
		2011 vs 2014	0.002
		2013 vs 2014	0.028
	SC	2011 vs 2013	0.005
Tributaries		2010 vs 2012	0.02
		2010 vs 2013	0.015
		2010 vs 2014	0.011

Table 3. Pairwise comparisons of multivariate dispersion in diet composition between sample years, significant at $p < 0.05$. All p -values presented are from a pairwise permutational test of significance. Habitats include mainstem (MS) and side channels (SC).

Section	Habitat	Contrast	p
Aldwell		2013 vs 2014	0.009
Lower	MS	2012 vs 2014	0.024
		2010 vs 2011	0.038
		2010 vs 2012	0.002
		2010 vs 2014	0.039
		2011 vs 2014	0.001
		2012 vs 2013	0.015
		2012 vs 2014	0.001
Middle	SC	2010 vs 2011	0.042
		2010 vs 2013	0.001
		2010 vs 2014	0.009
		2011 vs 2014	0.001
		2012 vs 2014	0.001
		2013 vs 2014	0.001
Trib		2010 vs 2012	0.002
		2010 vs 2013	0.001
		2010 vs 2014	0.001
		2011 vs 2013	0.009
		2012 vs 2013	0.023

Fig 1. Map of Elwha River, study sites, and former dam locations. Credit: Randall McCoy, Lower Elwha Klallam Tribe.

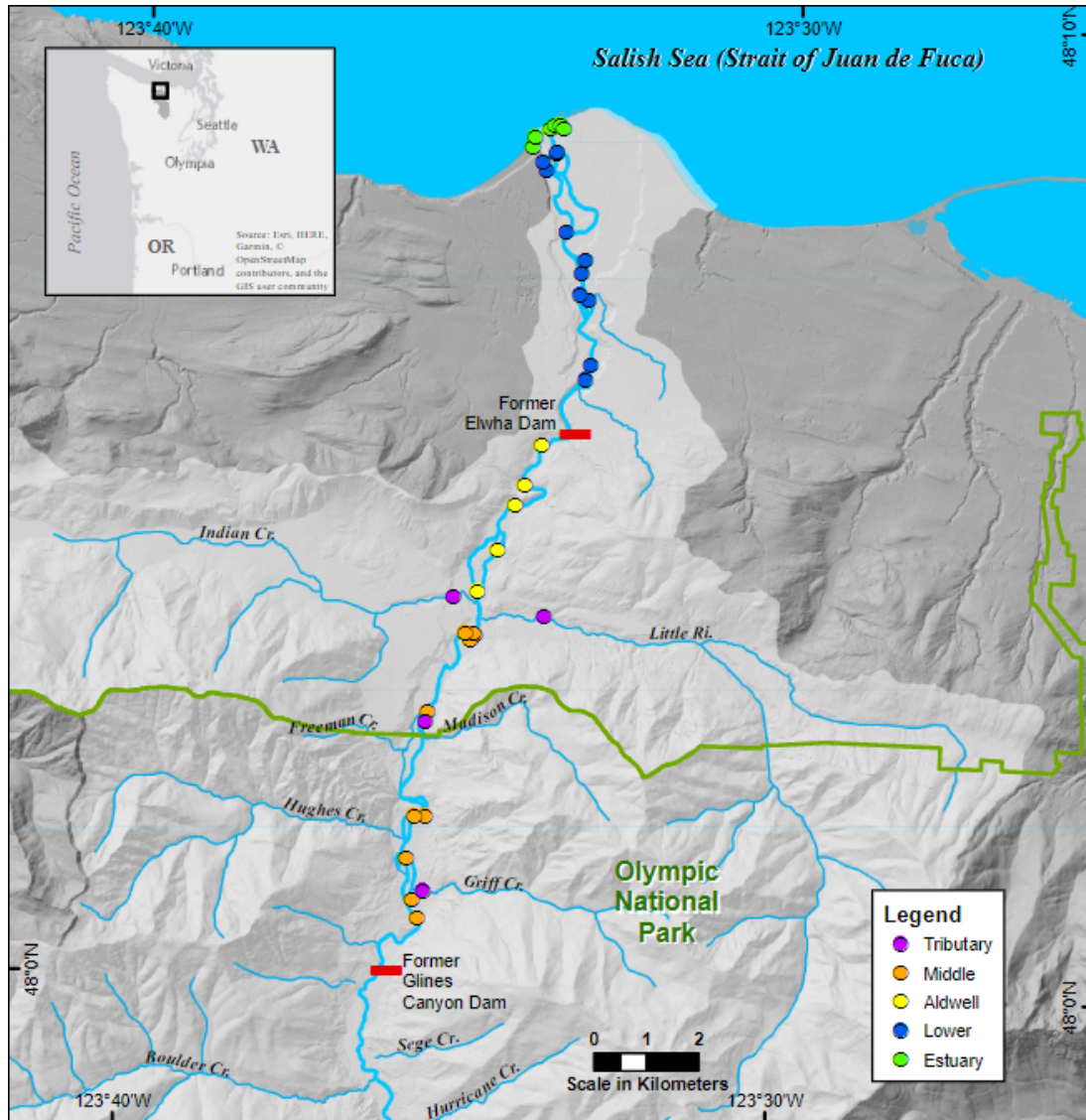


Fig 2. Daily averages of suspended sediment concentration (mg/L) in the lower Elwha River at USGS gage 12046260. Data downloaded from <https://doi.org/10.5066/F7PG1QWC> on 12/1/2019 (Ritchie et al. 2018).

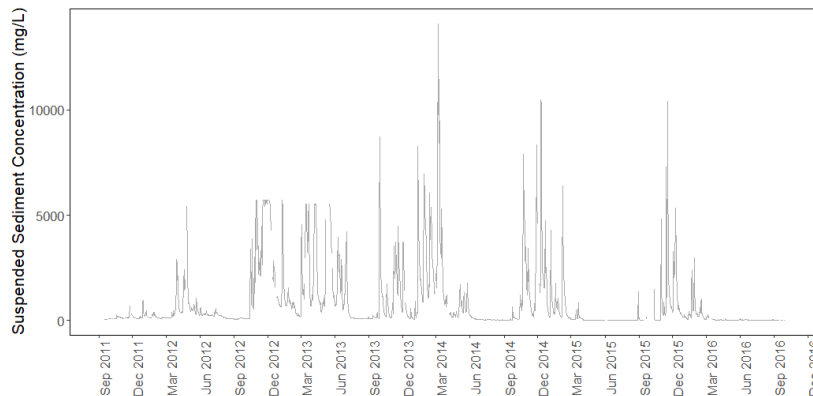


Fig 3. Percent fines calculated from pebble count data grouped by section, habitat, and year. Habitats include mainstem (MS), side channel (SC), and tributary (TR). Colors differentiate years of data collection with 2010 and 2011 being classified as pre-removal, 2012 and 2013 are during removal, and 2014 is after removal.

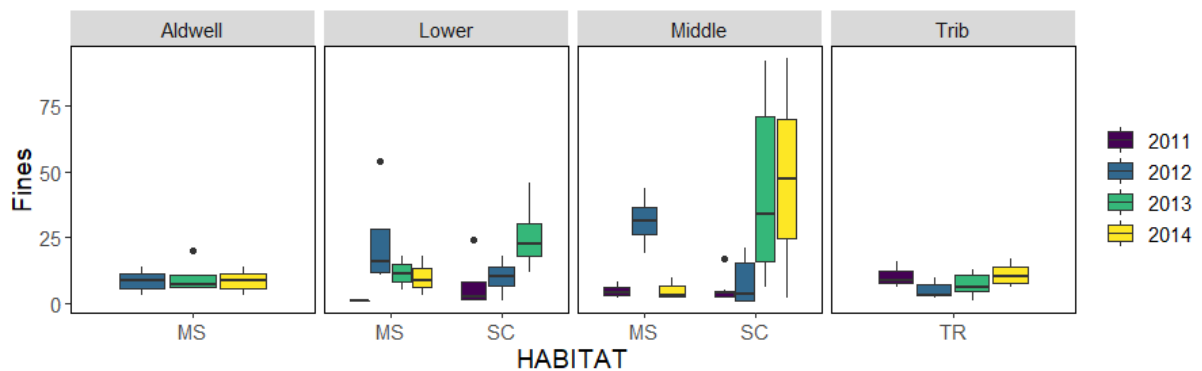


Fig 4. Principal coordinate analysis (PCoA) biplot of drift species composition and significant species loadings. Shapes of points correspond to year and removal stage (Pre: 2010, 2011; Mid: 2012, 2013; Post: 2014). Color of points correspond to habitat type (MS: mainstem, SC: side channel, TR: tributary). Small transparent points represent individual sampling events while year text represents the centroid of each Year + Habitat grouping.

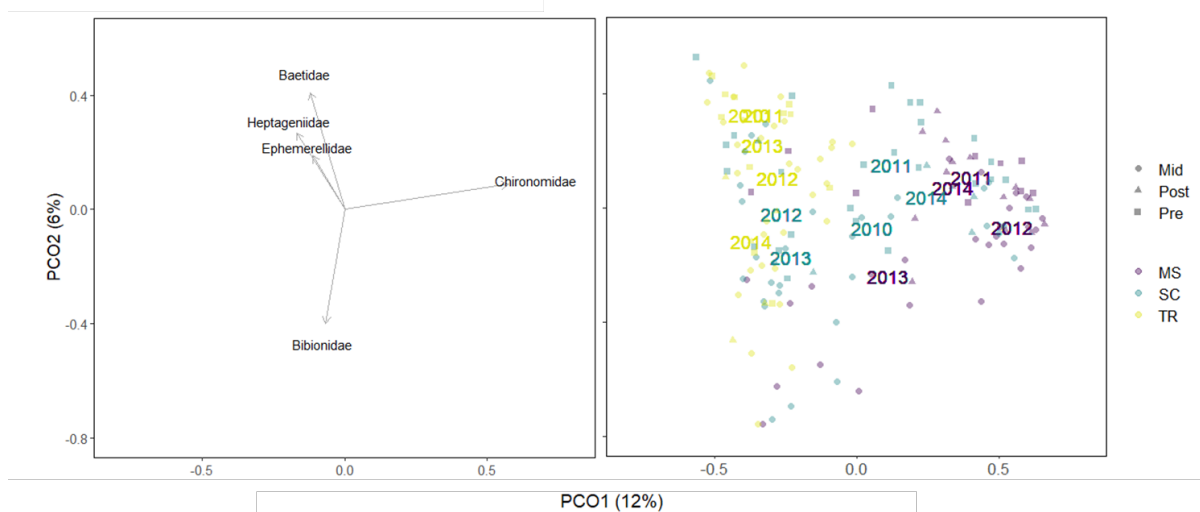


Fig 5. Principal coordinate (PCO) scores from drift composition PCoA on the first two axes. Colors differentiate years of data collection with 2010 and 2011 being classified as pre-removal, 2012 and 2013 are during removal, and 2014 is after removal. The y-axis represents from the corresponding PCO. Taxa listed in red along the y-axis of each row correspond to taxa that were significantly correlated on that axis ($p < 0.001$). Positions of taxa on the axis correspond to the direction of the correlation and are scaled based on the strength of their correlation. Panel A shows changes on PCO1 through time by river section and habitat (MS: mainstem, SC: side channel), panel B shows changes on PCO2 by section and habitat, panel C shows changes through time on PCO1 by river section and season, and panel D shows changes on PCO2 by river section and season.

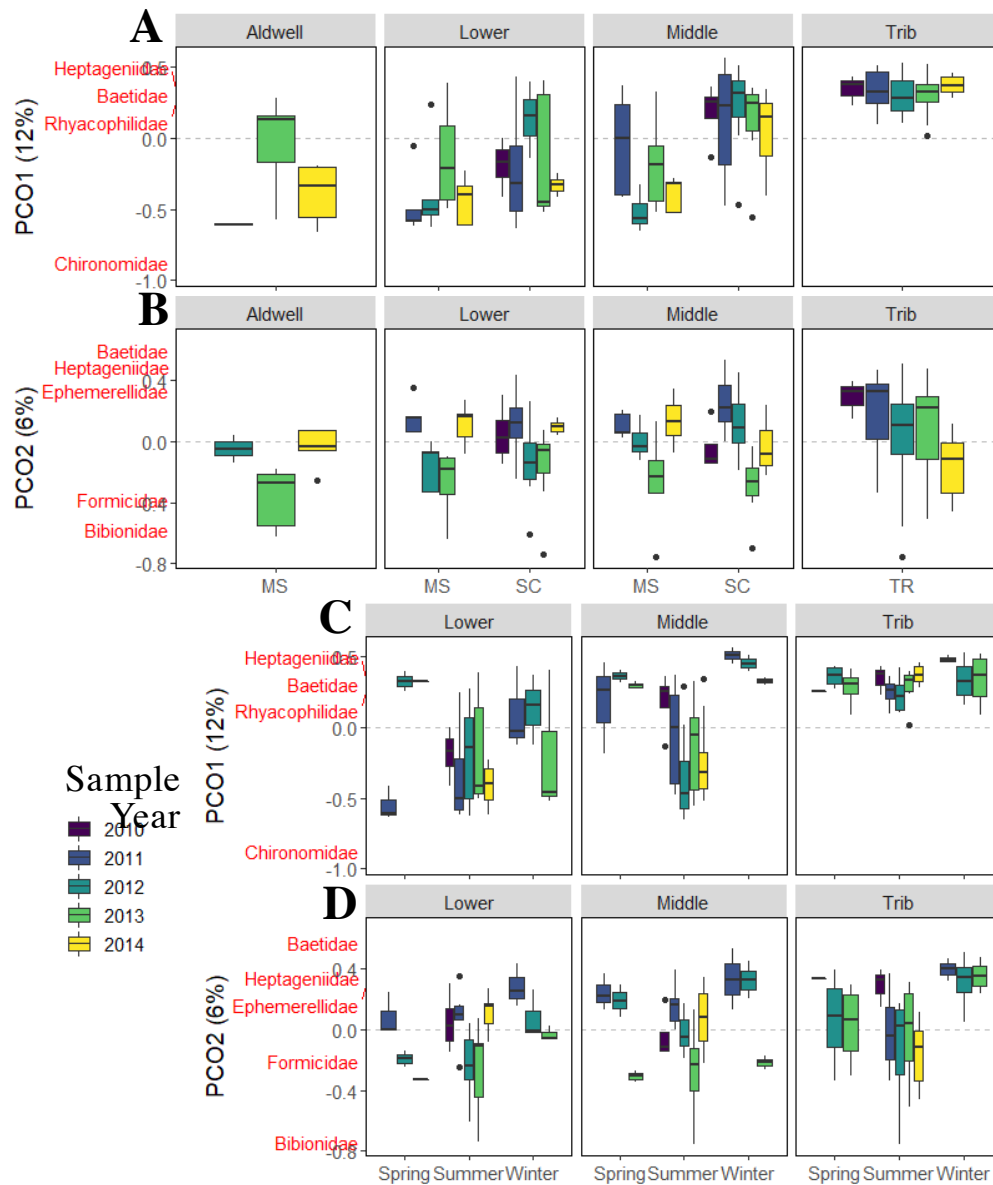


Fig 6. Multivariate dispersion of drift sample composition. Drift sample events were grouped by section, habitat, and year and distance to the multivariate group centroid was plotted for each sample. Colors differentiate years of data collection with 2010 and 2011 being classified as pre-removal, 2012 and 2013 are during removal, and 2014 is after removal. Habitats include mainstem (MS), side channel (SC) and tributary (TR).

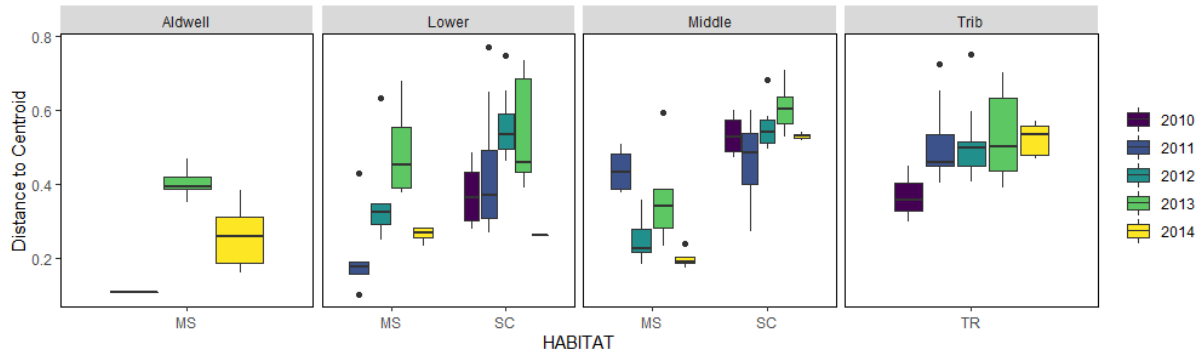


Fig 7. Principal coordinate (PCO) scores from diet composition PCoA on the first axis. Colors differentiate years of data collection with 2010 and 2011 being classified as pre-removal, 2012 and 2013 are during removal, and 2014 is after removal. The y-axis represents from the corresponding PCO. Taxa listed in red along the y-axis of each row correspond to taxa that were significantly correlated on that axis ($p < 0.001$). Positions of taxa on the axis correspond to the direction of the correlation and are scaled based on the strength of their correlation. Panel A shows changes on PCO1 through time by river section and habitat (MS: mainstem, SC: side channel and panel B shows changes on PCO1 by river section and season.

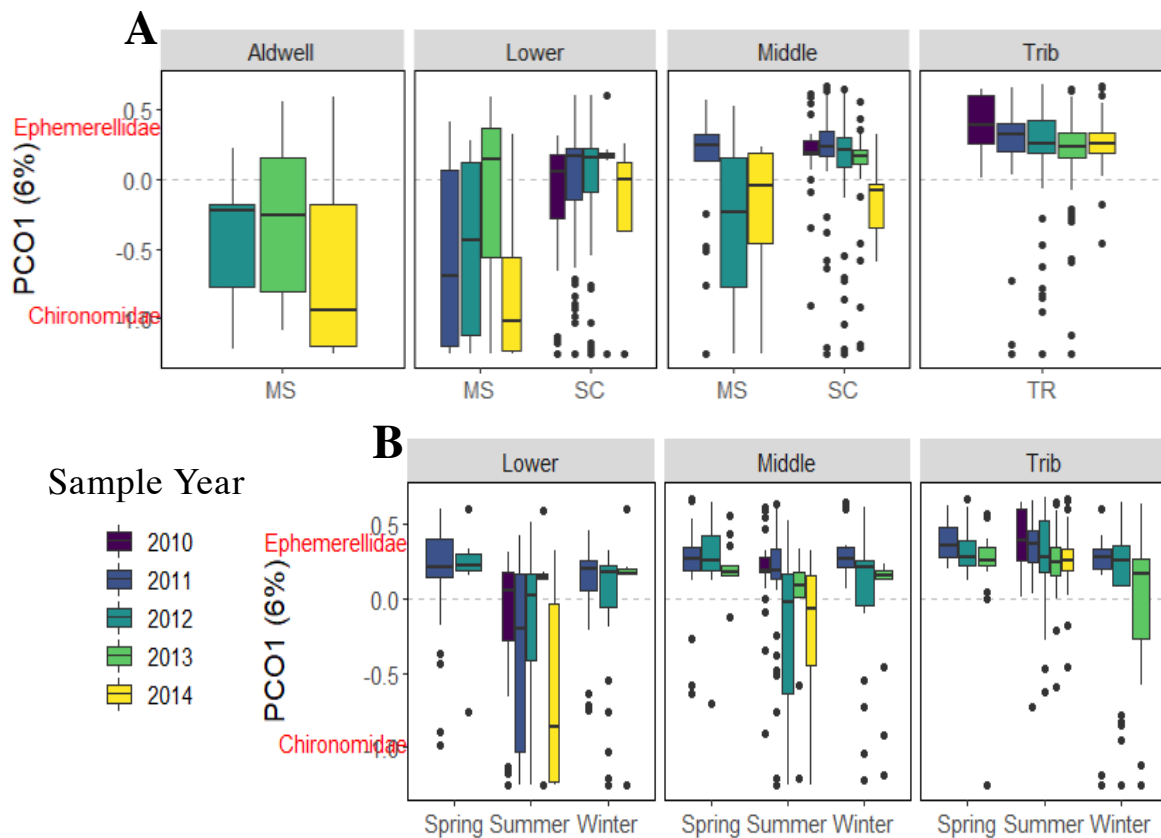


Fig 8. Multivariate dispersion of fish diet composition. Diet sample events were grouped by section, habitat, and year and distance to the multivariate group centroid was plotted for each sample. Colors differentiate years of data collection with 2010 and 2011 being classified as pre-removal, 2012 and 2013 are during removal, and 2014 is after removal. Habitats include mainstem (MS), side channel (SC) and tributary (TR).

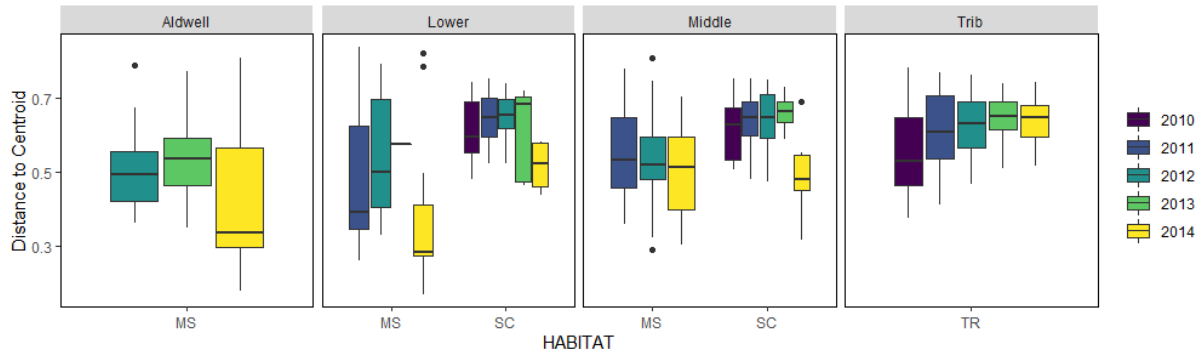
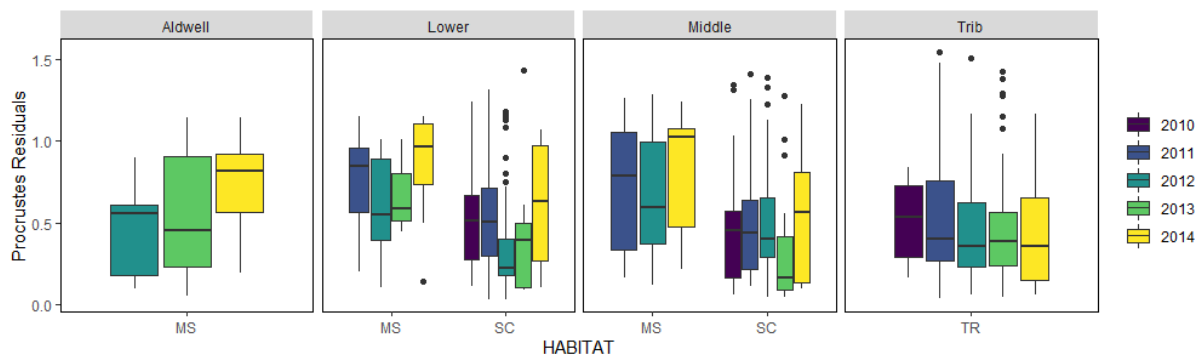


Fig 9. Procrustes analysis residuals grouped by section, habitat and year. Residuals are plotted on the y-axis signifying the amount of discrepancy between each object in the diet matrix and each object in the drift matrix. Habitats include mainstem (MS), side channel (SC), and tributaries (TR). Colors differentiate years of data collection with 2010 and 2011 being classified as pre-removal, 2012 and 2013 are during removal, and 2014 is after removal.



Discussion

Drift composition changed through space and time in response to dam removal activities. The lower river was impacted most by dam removal, by exposure to sediment pulses during removal of the Elwha Dam in 2012, as well as in 2013 during removal of the Glines Canyon Dam (Fig 2). In contrast, the middle river was only impacted during 2013. It's no surprise then that the most significant shifts in drift composition occurred in the lower river in 2012 and 2013. These changes included a shift towards more terrestrial influence in the drift, with increased abundances of the terrestrial ant family Formicidae and the fly family Bibionidae driving the change which was most pronounced during the spring and winter, but still prevalent in the summer. This change also coincided with a decreased number of sites dominated by Chironomidae, a family of aquatic flies. The middle river saw a similar response during

removal in 2013 and the tributaries experienced no obvious change, implying that sediment deposition negatively impacted aquatic species during dam removal. Though no other studies to my knowledge have looked at dam removal impacts to fish prey availability and acquisition, one study found a similar shift from aquatic to terrestrial energy subsidies in the diet of swallows during dam removal (Sullivan et al. 2018).

Despite impacts of dam removal to Chironomidae, this species occurred in drift samples at many sites. Throughout the study period this family had a high frequency of occurrence in drift and diet samples and appeared in high relative proportions in many samples as well. Chironomidae were significant taxa across drift samples in Aldwell, lower mainstem, lower side channel, and middle mainstem sites (Fig 5a). In fact, by 2014 (one year following removal) Chironomidae in the

lower river and estuary comprised > 90% of the drift samples (Morley et al. in prep). In the Elwha estuary high deposition of fine sediment (favored burrowing invertebrates, including subfamilies of Chironomidae, e.g. Tanypodinae (Foley et al. 2017; Morley et al. in prep). This could also be the case in the lower mainstem and tributary sites which saw increases in fine sediment deposition. Increase in Chironomid abundance has been positively associated with sediment pulse duration as well as deposition of fine sediments in other studies of stream disturbance (Culp et al. 1986; Vuori & Joensuu 1996; Shaw & Richardson 2001), while sedimentation has been associated with decreases in the larger, and longer living Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (Chiu et al. 2013). However, this pattern does not hold for middle river side channel sites which saw the highest fine sediment loads after removal of the Glines Canyon dam in 2013 and 2014, where drift compositions maintained a positive association with the Heptageniidae, Baetidae, and Rhyacophilidae and a negative association with Chironomidae. This could be due to the intense modification of side channel habitats as the river reconnected with its floodplain after the first winter with a natural flow regime. Old side channels became filled with sediment and new channels were carved out, which may have provided new habitats that were better suited to these aquatic Ephemeroptera and Trichoptera taxa. The middle river side channel habitats were also much closer to major tributaries in the system, which could have acted as a dispersal source of those aquatic invertebrates that were not able to thrive in the middle mainstem or lower river.

While I have discussed some dominant trends in drift composition across time and space during dam removal, it is important to consider that the drift ordinations only explained 18% of total variation in multivariate space. Thus, there may be other important shifts in species occurring that were not apparent in the ordinations of this analysis. For example, the high occurrence of Chironomidae in the data could have masked other trends in taxa composition among less dominant taxa as a result of dam removal impacts.

Changes in multivariate dispersion may provide more valuable insight into how diversity in prey communities were affected by dam removal. High dispersion in drift samples within the same section and habitat occurred in the lower river mainstem and side channel habitats during dam removal in 2012 and 2013, suggesting more variability in habitat and

environmental conditions during dam removal. This increase in dispersion related to dam removal appeared to be a consequence of the drastic decline in the abundance of drift invertebrates, including a decline in relative abundance of Chironomidae, during dam removal. Before dam removal and in the year following, Chironomidae were dominant in the drift similarly across all sites. During dam removal however, relative abundances of Chironomidae decreased and sites became more variable in taxa composition. This suggests that macroinvertebrate changes responding to removal activities varied on a site-specific basis, as opposed to a regional basis, a response found during dam removals in other systems as well (Pollard & Reed 2004; Poulos et al. 2019).

In the diet samples, similar trends in drift were observed through space and time, although there was significant variation among fish that dampened these trends. This variation could be an effect of diet sampling, which only offers a snapshot of prey consumption through time. Rainbow trout are minimally selective in their foraging, with prey size being the dominant factor in their prey selectivity (Bisson 1978; Nakano et al. 1999), so it's likely that the significant variation among diets within sites, habitats, and sections is mainly due to heterogeneity in drift invertebrate dispersal. However, dispersion of fish diets did decrease in 2014, the first year following removal activities.

Shifts in foraging patterns and diet subsidies have been an observed response to habitat disturbance in multiple species of salmonids (Perry et al. 2003, Kraus et al. 2016). In our system, association between diet samples and the drift prey composition at each site was significant, with a reasonable correlation (0.323), but evaluation of the residuals of the Procrustes analysis showed evidence of a temporal change in drift prey-diet mismatch associated with dam removal in some reaches. The larger mismatch in drift and diet composition in 2014 implies that fish might be responding to decreased aquatic drift availability by relying more on benthic resources associated with the new sedimentation. This result has support from previous laboratory studies which have observed successful benthic foraging by salmonids in turbid flowing waters (Harvey & White 2008).

Many trends in species composition observed during dam removal had reversed by the following year. The largest rebound was observed in Diptera taxa such as Chironomidae, characterized by smaller size and shorter

lifespans, which have previously been shown to be more resilient to dam removal in the short term (Chiu et al. 2013). In addition, mismatch in fish diets and drift samples in the most impacted areas suggest that fish are responding to these environmental changes by shifting their prey base. In fact, Morley et al. in prep found that fish condition factor in the lower Elwha was highest in 2014, despite major changes to the drift prey base. Considering the acute impacts of large-scale dam removal, this analysis suggests ecosystem resiliency in the face of disturbance. However, long-term monitoring of the ecosystem will be required to assess long-term recovery and stability. As the natural connectivity is restored to this system through sediment and debris transport, in addition to recolonization of anadromous salmon, the evolution of more long-term ecological responses is expected to occur (Bellmore et al. 2019).

Acknowledgements

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Supplementary Figures

Fig S1. Principal coordinate analysis (PCoA) biplot of drift species composition and significant species loadings. Shapes of points correspond to year and removal stage (Pre: 2010, 2011; Mid: 2012, 2013; Post: 2014). Color of points correspond to habitat type (MS: mainstem, SC: side channel, TR: tributary). Small transparent points represent individual sampling events while year text represents the centroid of each Year + Habitat grouping. Note: only PCO1 explains a significant amount of variation, and PCO2 was included in this biplot for illustrative purposes only.

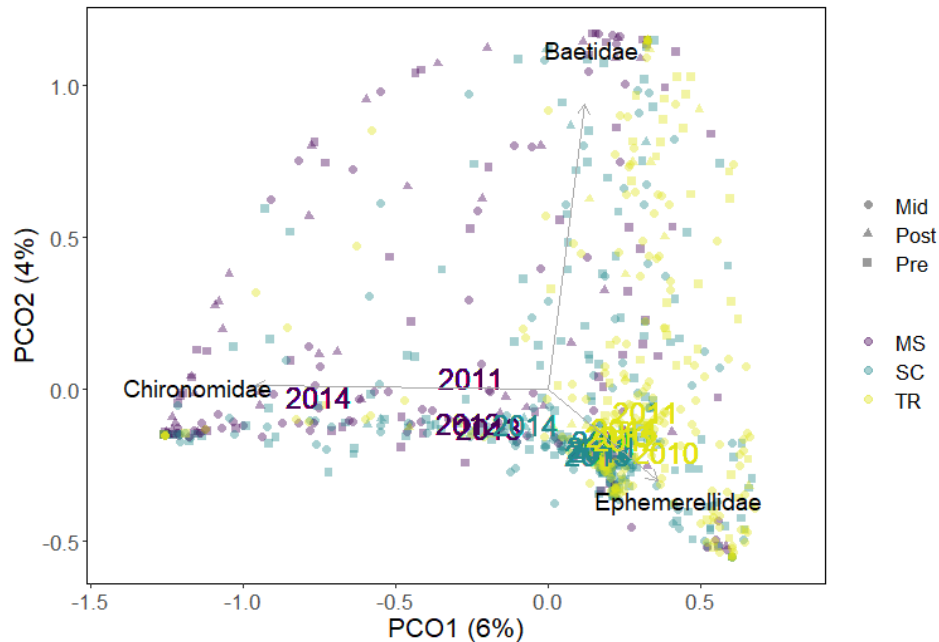
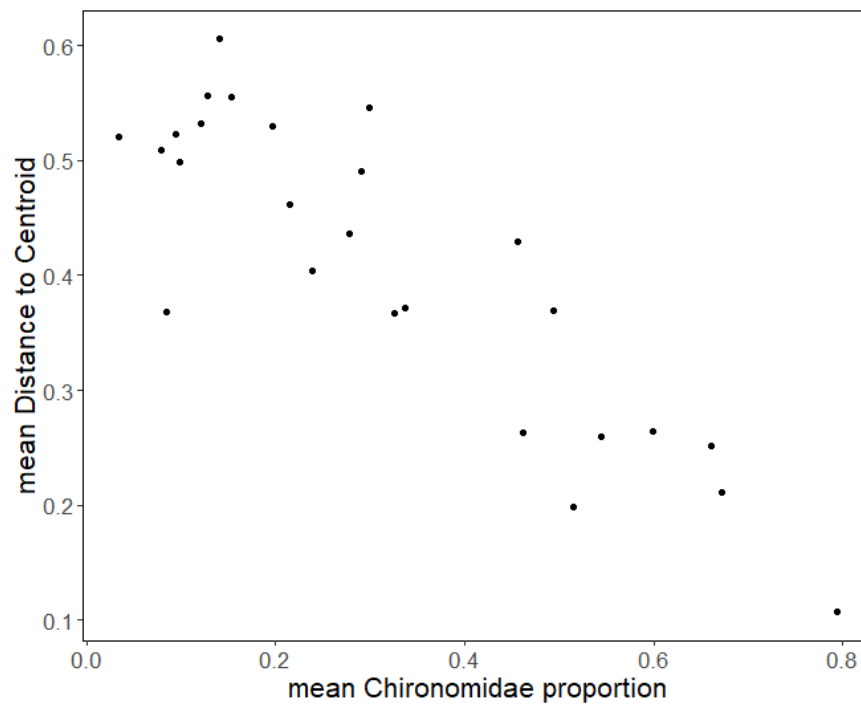


Fig S2. Mean group dispersion versus mean proportion of Chironomidae in drift samples.



Environmental drivers of pelagic fish distribution in the U.S. Continental Shelf Region of the Chukchi Sea

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Abstract

The Pacific Arctic is an area of high seasonal productivity driven by the input of Pacific water across the shallow Chukchi Sea shelf. These dynamics support a large ecosystem, though efforts to characterize the pelagic fish community have been limited. In 2012 and 2013, acoustic-trawl surveys were conducted in the northern Bering and Chukchi Seas to describe the pelagic fish community and determine the potential environmental drivers that influence their spatial distribution, and identified Arctic cod, saffron cod, capelin, and Pacific herring as the dominant pelagic fishes. The environmental gradients observed across the survey were investigated using principal component analysis, which indicated that abiotic factors explain the greatest amount of variability across sites. Canonical correspondence analyses (CCA) were performed to investigate the relationship between water column properties and abundances. The CCAs showed that a) the abundances and spatial separation of the major pelagic fishes correspond to variability in their local environment and b) the size-frequency distribution of Arctic cod across sites is at least partially explained by these same environmental gradients. These species- and size-specific preferences may lead to changes to the ecosystem structure as further warming leads to competition for habitat and resources among these major species.

Introduction

The shallow continental shelf of the Chukchi and northern Bering Seas comprise the Pacific Arctic Region (PAR), functioning as a transition zone between the Pacific and Arctic Oceans. The PAR serves as the Pacific gateway to the Arctic Basin, concentrating the flow of heat, freshwater, and nutrients through the Bering Strait and across the Chukchi Sea shelf as currents move north, driven by winds and the pressure head between the two basins (Woodgate et al., 2005a; Danielson et al., 2014). The continental shelf has high seasonal variability, with cool water and sea ice cover for much of the year, and rapid warming in summer as sea ice retreats. The seasonal flushing of Pacific water across the shallow Chukchi Sea drives strong seasonal dynamics, triggering ice melt and sparking seasonal productivity that supports a large Pacific Arctic ecosystem (Grebmeier et al., 2006).

In the Chukchi Sea, the typical environmental conditions are characteristic of the Bering and Chukchi Sea summer water masses (Danielson et al., 2017). In the summer, two distinct water masses enter the Chukchi Sea through the Bering Strait: 1) the Alaskan Coastal Water, a relatively fresh and warm water mass heavily influenced by river discharge onto the Bering shelf, and 2) Bering Sea Water, a colder, more saline mixture of Bering Shelf Water and Gulf of Anadyr Water (Coachman et al., 1975). North of the strait, the water flows towards the Arctic Basin in three main branches (generally constrained by topography): east along the Alaska coast towards Barrow Canyon, west and towards Herald Valley and Long Strait, and between these two, a third branch through the Central Channel (Weingartner et al., 2005). Changes in hydrography across the shelf are found to drive variations of the phytoplankton, zooplankton, and ichthyoplankton communities (Danielson et

al., 2017; Pinchuk and Eisner, 2017). These plankton communities across the shelf have been shown to be structured based on advantageous growth in specific water conditions (Neely et al, 2018), though the role these distinct water masses play in the distribution of fishes is unclear.

There are no large-scale commercial fisheries in the Chukchi Sea and, until recent decades, efforts to characterize the ecosystem have been limited. Interest in the Arctic, initially driven by oil exploration and development, has led to increased research and efforts to evaluate the ecological impact of further disturbance and changes in climate (Grebmeier et al., 2015; Loggerwell et al., 2015). The region is considered a benthic-dominated system where high benthic biomass supports a benthic-based food web (Grebmeier et al., 2006; Moore and Stabeno 2015). As such, much of the sampling effort has been focused on the benthic fauna and demersal fish community, where Arctic cod (*Boreogadus saida*) and saffron cod (*Eleginus gracilis*) have been identified as major components of the ecosystem (Norcross et al.; 2013; Loggerwell et al., 2015).

The Arctic has been undergoing rapid changes associated with surface warming and loss of sea ice, and the PAR is one of the seasonally ice-covered areas where this decrease is clearly observed (Frey et al., 2014). With sea ice in the Chukchi Sea melting earlier by 2.3 days decade⁻¹ (Stroeve et al., 2014), more extensive phytoplankton blooms in open water may shift the balance of the benthic-pelagic coupling towards a pelagic-dominated ecosystem (Moore and Stabeno, 2015). The pelagic fish community is likely to be impacted by such environmental change, but we lack the data to quantify this since sampling of pelagic species in the Chukchi Sea has been sparse. Similar to the demersal community, the pelagic fish community on the shelf is known to be dominated by a few species, the most abundant of which is Arctic cod, and previous sampling conducted in 1970 using midwater trawls found an abundance of age-0 Arctic cod in the northeast Chukchi Sea (Quast, 1974). More recently, however, sampling of the pelagic fish community has been limited in the northern Bering and Chukchi Seas.

Arctic cod are a circum-Arctic distributed gadid, found throughout the shallow shelves of the Arctic marginal seas as well as in the Central Arctic Basin (Mecklenburg et al., 2018). Arctic cod are a key pelagic secondary consumer and serve as a central trophic link between plankton and higher trophic levels

(Bradstreet et al., 1986). Arctic cod spawn in fall and winter under sea ice on the shallow shelves of the Arctic marginal seas (Ponomarenko, 2000). The fertilized eggs are buoyant, and they develop under ice cover at the ice-water interface (Graham and Hopp, 1995; Ponomarenko, 2000). Development time is dependent on the temperature of the water column, with number of days to reach 50% hatching ranging from 75 days at -1 °C to 32 days at 3.5 °C. Hatching across the Arctic has been observed from December through August with a peak during May/June (Bouchard and Fortier, 2011). As larvae, their swim bladder begins to fill at 6-7mm length which corresponds with improved motility, leading to active feeding and reduced susceptibility to predation (Aronovich et al., 1975). As they age, they exhibit an ontogenetic migration, residing deeper in the water column as they age. Age-1+ fish assemblages have been observed at depth along the continental slope into the Arctic Basin (Parker-Stetter et al., 2011). Adults become sexually mature at 2-3 years and have a maximum life span of 6-7 years (Hop and Gjøsæter, 2013).

While their biology has been well studied, few studies have investigated the ecology of Arctic cod in order to assess their distribution and movement, particularly in the Pacific Arctic. Continued loss of sea ice and warming has the potential to negatively impact Arctic cod growth and survival in this region, and further investigation of how the environment influences their abundance and distribution is necessary to better predict ecosystem changes with further climate change. Thus, the objective of this work is two-fold, first to characterize the composition and distribution of the major pelagic fish species in the PAR, and in particular Arctic cod. Second, we aim to identify the environmental conditions that influence the pelagic fish community structure to better understand the role of the strong advective regime on the Chukchi shelf in shaping the distribution of fishes and what that may look like under future environmental conditions.

Methods

Acoustic-trawl surveys

Acoustic-trawl (AT) surveys were conducted in 2012 (7 August to 24 September) and 2013 (7 August – 25 September) as part of the Arctic Ecosystem Integrated Survey (Arctic Eis) program. These surveys were conducted alongside multidisciplinary sampling efforts in the northern Bering and Chukchi Seas on the

F/V Bristol Explorer along a 1° longitude by 0.5° latitude station grid. Station operations included conductivity temperature depth (CTD) casts, surface trawls, and zooplankton sampling, and transit between stations occurred at a speed of approximately 4.5 m s^{-1} . In 2012, a large Cantrawl rope trawl was used for both surface (fitted with headrope floats) and midwater trawls. In 2013, a smaller Marinovich midwater trawl was used for midwater sampling, and the Cantrawl was used only for surface trawling. The smaller Marinovich trawl was found to be less selective and better at retaining the small fishes within the study area. The average vertical opening of the Cantrawl was $18.7 \pm 3.3 \text{ m}$ during surface trawls and $13.5 \pm 2.9 \text{ m}$ during midwater trawls. The Marinovich averaged $5.7 \pm 0.7 \text{ m}$ vertical opening in 2013. Catches were weighed, and organisms were identified to species and enumerated. Measurements of fork length were recorded for a subsample of up to 50 fish of each species to the nearest 1.0mm.

Acoustic observations were collected along transect between stations during daylight hours. In addition to trawl sampling at stations, midwater trawls were conducted along transect to target fish aggregations observed in the acoustics. More effort was allocated in the Chukchi Sea for midwater sampling as this was the primary area of interest, and due to light limitations and other survey objectives, some portion of the acoustic transect in the northern Bering had to be completed at night, with fewer midwater trawls completed. The northern extent of the survey region was limited by sea ice extent which resulted in the relocation or dropping of grid stations which varied between years.

Acoustic backscatter was collected at 38 and 120 kHz using a split-beam Simrad ES60 echosounder, operating hull-mounted ES38-10 and ES120-7C transducers at a depth of 3.7m. Bottom depths during the survey averaged 38 m, and 95% of the survey area ranged between 18 and 55 m. In these shallow conditions, acoustic data were collected at 2.5 Hz ping rate using a 0.512 ms pulse length. Calibrations were conducted before and after the survey in 2012, and after the survey in 2013 (for further details, see De Robertis et al., 2017a). Data were analyzed using Echoview (v. 6.1, Echoview Pty Ltd.). Acoustic backscatter from fishes were determined based on the frequency response of 5 m moving average smoothed sampled where samples with a $\Delta S_{v120-38\text{kHz}}$ in the range of -16 to 8 dB were used to determine fish abundance as described below. These observations were integrated for the

entire water columns at 0.5 nmi intervals with a minimum S_v threshold of $-70 \text{ dB re } 1 \text{ m}^{-1}$, and only observations collected between sunrise and sunset were included to control for effects of vertically migrating species.

Acoustic estimation of abundance

Acoustic backscatter measurements were partitioned to species abundances based on the size and species distribution of organisms observed in the surface and midwater trawl collections. Size and species composition from trawl catches were combined with size and species-specific scattering properties to proportionally allocate the acoustic backscatter observed to each species in the catch. Prior to this allocation of acoustic backscatter, trawl catches were corrected for net selectivity in order to account for the size and species-specific likelihood of capture in each of the two net systems (De Robertis et al., 2017a). Target strength relationships from the literature were used to estimate the acoustic scattering from each species in the trawl catch (for methods and target strength relationships used in this study, see De Robertis et al., 2017b). The proportion of total areal backscatter contributed by each species was then calculated along acoustic transects using species and size composition from the nearest trawl. Determination of abundance based on this method of proportional allocation requires high confidence in the acoustic parameters of each species, and thus was limited to those species for which there was high confidence. For this study, this included Arctic cod, saffron cod, Pacific herring, and capelin, which accounted for 92% and 79% of the abundance of organisms in 2012 and 2013, respectively. For all other species, estimates of proportional allocation were done, however abundances were not.

Environmental sampling

Water column properties were measured using CTD casts conducted at each grid station during the surveys. To describe conditions among sites, we included temperature, salinity, dissolved inorganic nitrogen (DIN), and chlorophyll. Due to the high variability between surface and seafloor conditions, temperature, salinity, and DIN values from the surface and approximately 5 meters off the seafloor were used to investigate the variability across sites. Chlorophyll was integrated over the water column, as it was commonly low or the signal was dominated by a subsurface chlorophyll max 10 to 30 m below the surface, and thus would not be well represented by independent surface and bottom measurements. To further account

for the vertical structure of the water column, a mean stratification value (a unit-less transformed measure of the pycnocline depth) was included (Danielson et al., 2017).

In order to associate fish with environmental conditions, measurements of fish abundances by species and by length class (in fish m⁻²) calculated along acoustic trackline in 0.1 nmi intervals were grouped according to nearest CTD station and averaged in each year. This resulted in a total of 100 stations in 2012 and 103 stations in 2013, each of which had a unique CTD cast to account for water column properties.

Statistical analyses

All analyses were done in R (v3.6.1) using the Vegan package (Oksanen et al. 2011). A Mantel test was initially conducted to determine whether surface and bottom water conditions were not in fact independent and thus would allow us to remove additional variables from further analyses. While statistically significant, the correlation was relatively weak ($r = 0.325$, $p = 0.001$ and so temperature, salinity, and DIN from the surface and bottom were included in all tests. Environmental variability was investigated first using unconstrained ordination methods to find patterns among stations. Principal Component Analysis (PCA) was used to identify which environmental variables drive the greatest differences between sites and subregions (northern Bering Sea, southern Chukchi Sea, and northern Chukchi Sea) through the reduction of the original dataset into linear combinations of the environmental variables. Due to the difference in variance among the different environmental measures, the PCA was performed using the correlation matrix. A Monte Carlo permutation test was used to determine significance of principal components. A permutation of analysis of variance (perMANOVA) was conducted on the environmental variables to test for differences among geographic regions (northern Bering Sea, southern and northern Chukchi Sea). To constrain the source of any regional variability found, a test of multivariate homogeneity of group dispersion (disper) was conducted to test for differences in variance among regions.

To investigate the role of these environmental parameters in species distribution among the key pelagic fishes, a Canonical Correspondence Analysis (CCA) was conducted. A Detrended Correspondence Analysis (DCA) of the fish abundances by site was first performed, and found a primary axis length of 2.8, indicating relative heterogeneity

of the species abundance dataset. Of the constrained ordination methods, CCA uses Chi-squared distance and weighted linear mapping and thus would better represent the unimodal nature of the species distributions across the environmental gradients rather than other ordination methods which assume a linear response. CCA relates the community structure based on the species abundances to the variability in the water column conditions. Using this method, stations are ordinated based on the composition of species abundances along axes which represent linear combinations of the environmental variables.

In addition to performing a CCA on the abundances by species, a similar analysis was done using the abundance by length for Arctic cod, the dominant pelagic fish in both years. A data matrix containing abundance per 1 cm length bins (bin centers from 2 - 16 cm) was used to correlate the sites with the size structure of Arctic cod and examine their positions along environmental gradients. The significance of the individual variables in both CCAs was determined based on Monte Carlo permutations ($n=1000$). For both CCAs, the environmental variables were standardized by column and fish abundances (both by species and by length) were normalized by row to examine variability based on relative abundance.

Results

Comparing Sample to Population

In total, 189 surface trawls and 65 midwater trawls were conducted over the two field seasons (100 surface and 21 midwater in 2012, 89 surface and 44 midwater in 2013). Trawl catches were dominated by small fishes (80% <12.1/9.3 in 2012/2013). Arctic cod, saffron cod, Pacific herring and capelin accounted for 98.2% of abundance (77.1% of biomass) in 2012, 51.9% (4.5% of biomass, Cantrawl) and 85.5% (18.5% biomass, Marinovich) of abundance in 2013. Jellyfish were a small portion of the catch by abundance, but a large portion of the catch by weight, accounting for 22.7% of the biomass in the 2012 midwater trawls, 75.3% in the 2012 surface trawls, 91.8% in the 2013 midwater trawls, and 74.5% in the 2013 surface trawls. *Chrysaora melanaster*, a large scyphomedusa, was 96.5% of all jellyfish by weight and was prevalent throughout the survey area. Capelin, herring, and jellyfish dominated the abundance in trawl catches in the southern region of the survey area, while Arctic cod, jellyfish, and capelin were dominant in the northern Chukchi. Saffron cod were most

commonly found in nearshore sampling locations in the southern Chukchi Sea.

The proportion of backscatter from a given species was closely resembled by the catch by abundance due to the low target strength of jellyfish, and thus their limited contribution to the overall acoustic backscatter even in areas of high jellyfish biomass (De Robertis et al, 2017a). Small fishes were the primary contributors to the backscatter allocated to fishes in the survey area, driven by scattering from Arctic cod, saffron cod, capelin, and herring. In both years, greater than 96% of the 38 kHz backscatter exhibited a 38/120 kHz frequency response consistent with fish. Backscatter was the highest in the northern Chukchi Sea, between 69 and 71 N, where juvenile Arctic cod were found to dominate the trawl catches and backscatter in both years (Figure 1). Backscatter averaged over the survey area was higher in 2013 than 2012, with the greatest increase in the northern Chukchi. Backscatter in the southern Chukchi and the northern Bering was also higher in 2013, areas where catches were dominated by herring (Table 1).

The four species of Arctic cod, saffron cod, Pacific herring, and capelin were found to be the primary scattering organisms during both surveys, accounting for 69.6% of the backscatter from fish in 2012 and 76.5% in 2013. In the Chukchi Sea, Arctic cod averaged 3.5 cm in fork length, with few fish greater than 6.5 cm in 2012 and none in 2013. In the northern Bering Sea, larger Arctic cod (> 6.5 cm) were found in both years, with fish up to 17 cm caught in 2013. In the common areas sampled across both years, Arctic cod were 2.7 times more abundant in 2013 than in 2012 (Figure 2).

Saffron cod were abundant in coastal areas, primarily in the southern Chukchi Sea. Saffron cod were 4 times more abundant in 2013 than in 2012, though like Arctic cod, most of the saffron cod observed in the Chukchi were small, with a mean length of 3.8 cm in both 2012 and 2013 (Figure 3). Larger fishes were captured in the northern Bering where abundances were lower. Capelin distribution was patchy throughout the survey area in both years, with relatively low but even abundances throughout the survey region. Pacific herring were the most abundant fish in the northern Bering Sea, and were 4.6 times more abundant in 2013 than 2012. Contrary to the other three species, Pacific herring were smaller in the

northern Bering Sea (primarily < 10 cm) compared to the Chukchi Sea, and larger herring were most abundant in the southern Chukchi Sea. In both years, distribution of Pacific herring in the Chukchi Sea was low and patchy.

Environmental variability across stations was strongly driven by temperature and salinity conditions. Three significant principal components were determined, explaining 76.35% of the variation among sites (Table 2). PC1 (37.03% of the variation between sites) had the strongest loadings from bottom temperature (-0.922), surface temperature (-0.884), and bottom salinity (0.88). Variability in water conditions among the second axis was driven by water conditions representing a stratified water column. PC2 explained 24.18% of the variation with the largest loadings from mean stratification (-0.87), surface salinity (0.783), and integrated chlorophyll (0.659). PC3 (15.14%) was primarily loaded by surface and bottom DIN (0.81 and 0.48) (Figure 4). The three subregions of the survey were found to differ significantly from each other based on the perMANOVA analysis of the environmental variables ($p = 0.024$, $F = 3.78$, $R^2 = 0.018$, $n = 201$). The results of the dispersal analysis were not significant ($p = 0.172$, $F = 1.77$), thus indicating that the significance between groups can be attributed to differences in location in multivariate space and not differences in variation within each subregion.

From acoustic abundance estimates of the dominant pelagic species, Arctic cod, saffron cod, and Pacific herring appeared to be spatially separated in both years of surveys, with Arctic cod distributed primarily in the northern Chukchi Sea, saffron cod in the southern Chukchi, and herring in the northern Bering Sea (Figure 2). These observations are reinforced by the CCA analysis. Two significant axes were found, representing 20.06% of the variation between sites, 87.19% of which was explained by the environmental variables. The variation explained was primarily attributed to CCA-axis 1 (15.93%, $p < .001$, Table 3). As was observed in the PCA of the water column variables, the strongest correlations of variables in the CCA were with bottom salinity (-0.91), bottom temperature (0.88), and surface temperature (0.80). Scores of the four species along the CCA-axes reflects well the observed distribution in the trawl catches; Arctic cod is most closely associated with sites in the northern Chukchi Sea in the portion of CCA-axis 1 associated with low temperatures and

high bottom salinity. Herring and saffron cod align with warmer temperatures and lower salinities found at the northern Bering Sea and southern Chukchi stations, yet are separated along CCA-axis 2 where saffron cod are associated with higher DIN and stratification. Capelin are central within the ordination space, representing their patchy distribution throughout the entire survey area with no strong environmental associations (Figure 5).

A second CCA conducted on the length-frequency distribution of Arctic cod showed size-dependent environmental associations. Size classes were well separated along the one significant axis (CCA-axis 1, $p = .001$) explaining 10.07% of the variation in the data which was most strongly correlated with bottom salinity (0.89), bottom temperature (-0.74) and

surface temperature (-0.52), similar to both the environmental PCA and constrained ordination of the species data (Figure 6). CCA-axis 2 ($p = 0.086$) accounted for 3.57% of the variation. While the model was found to be significant, the total proportion explained across all axes was only 17.58%. Along CCA-axis 1, lengths < 9.5 cm had positive scores associated with cooler temperatures and higher salinities, while lengths > 9.5 had strong negative scores associated with lower salinities and higher temperatures (Figure 7). Within the smaller size classes (< 9.5 cm), variation occurred along CCA-axis 2, with larger Arctic cod having higher scores associated with increased stratification (0.45) and decreased surface temperature (-0.57).

Table 1: Mean backscattering observed at 38 kHz with a frequency response consistent with 'fish' by year and survey area, modified from De Robertis et al., 2017. The common area refers to the 1° by 1° geographic cells that were adequately sampled in both surveys. N. Bering: south of 66 N; S. Chukchi: between 66 and 69 N; N. Chukchi: north of 69 N.

Year	N. Bering s_A (m^2 nmi $^{-2}$)	S. Chukchi s_A (m^2 nmi $^{-2}$)	N. Chukchi s_A (m^2 nmi $^{-2}$)	Common area s_A (m^2 nmi $^{-2}$)
2012	40.6	35.8	88.0	69.4
2013	92.2	57.0	267.2	163.0

Table 2: Summary table of results of the principal component analyses conducted on the water column properties. The first three principal components were found to be significant. Component loadings of all 8 environmental variables are included; temperature (Temp), salinity (Sal), dissolved inorganic nitrogen (DIN), mean stratification (MeanStrat), and integrated chlorophyll (IntChla). Bottom and surface measurements for temperature, salinity, and DIN are indicated by '.bot' and '.surf'.

Principal Components					Component Loadings			
	Eigenvalue	% Var.	Cum. % Var.	P-value		PC1	PC2	PC3
PC1	2.963	37.03	37.03	<.001	Temp.surf	-0.884	0.008	-0.007
PC2	1.935	24.18	61.21	<.001	Sal.surf	0.21	0.783	-0.366
PC3	1.211	15.14	76.35	0.001	DIN.surf	0.026	0.306	0.811
PC4	0.714	8.92	85.27	1	Temp.bot	-0.922	0.187	0.174
PC5	0.525	6.56	91.846	1	Sal.bot	0.88	0.045	-0.226
PC6	0.386	4.82	96.67	1	DIN.bot	0.652	0.023	0.483
PC7	0.159	1.98	98.65	1	MeanStrat	0.211	-0.87	0.191
PC8	0.108	1.34	100	1	IntChla	0.208	0.659	0.26

Table 3: Summary of CCA model results between abundance by species and water column properties.

Total Inertia	2.2914		
Sum of constrained eigenvalues	0.5724		
	CCA 1	CCA 2	CCA 3
Eigenvalues	0.365	0.094	0.067
Cum. % Var.	15.93	20.06	23.01
P-value	0.001	0.003	0.264

Figure 1: Integrated 38 kHz backscatter with a frequency response consistent with 'fish' along survey trackline in 2012 (left) and 2013 (right). Size and color correspond to the observed backscatter. The 50, 100, 150, and 200 m depth contours are shown as dark grey lines, and 500, 1000, 1500, and 2000 m depth contours are shown in light grey.

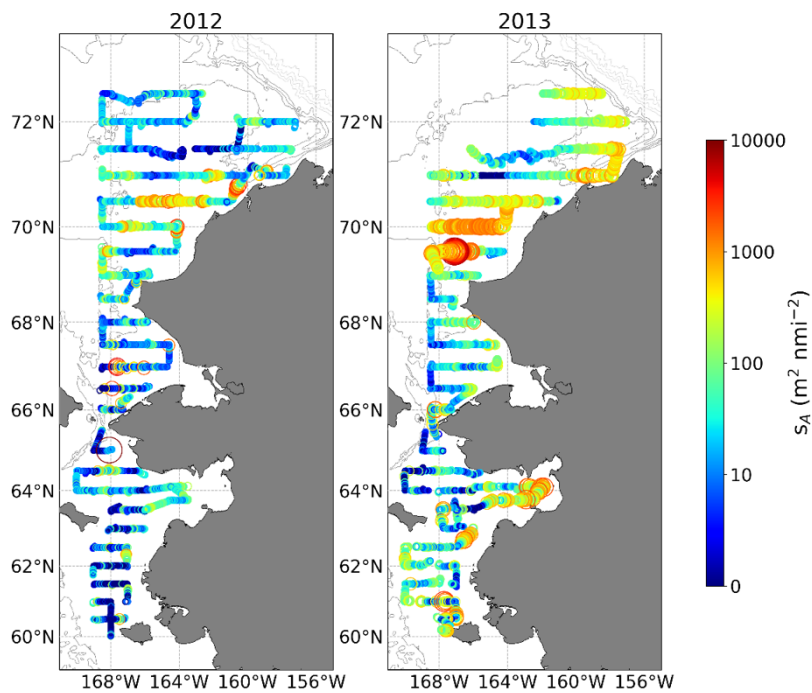


Figure 2: Density in fish m^{-2} of Arctic cod (a, b), saffron cod (c, d), capelin (e, f), and Pacific herring (g, h) in 2012 and 2013 along the survey trackline. Abundances were calculated from proportional allocation of backscatter based on nearest trawl catches. The 50, 100, 150, and 200 m depth contours are shown as dark grey lines, and 500, 1000, 1500, and 2000 m depth contours are shown as light grey lines.

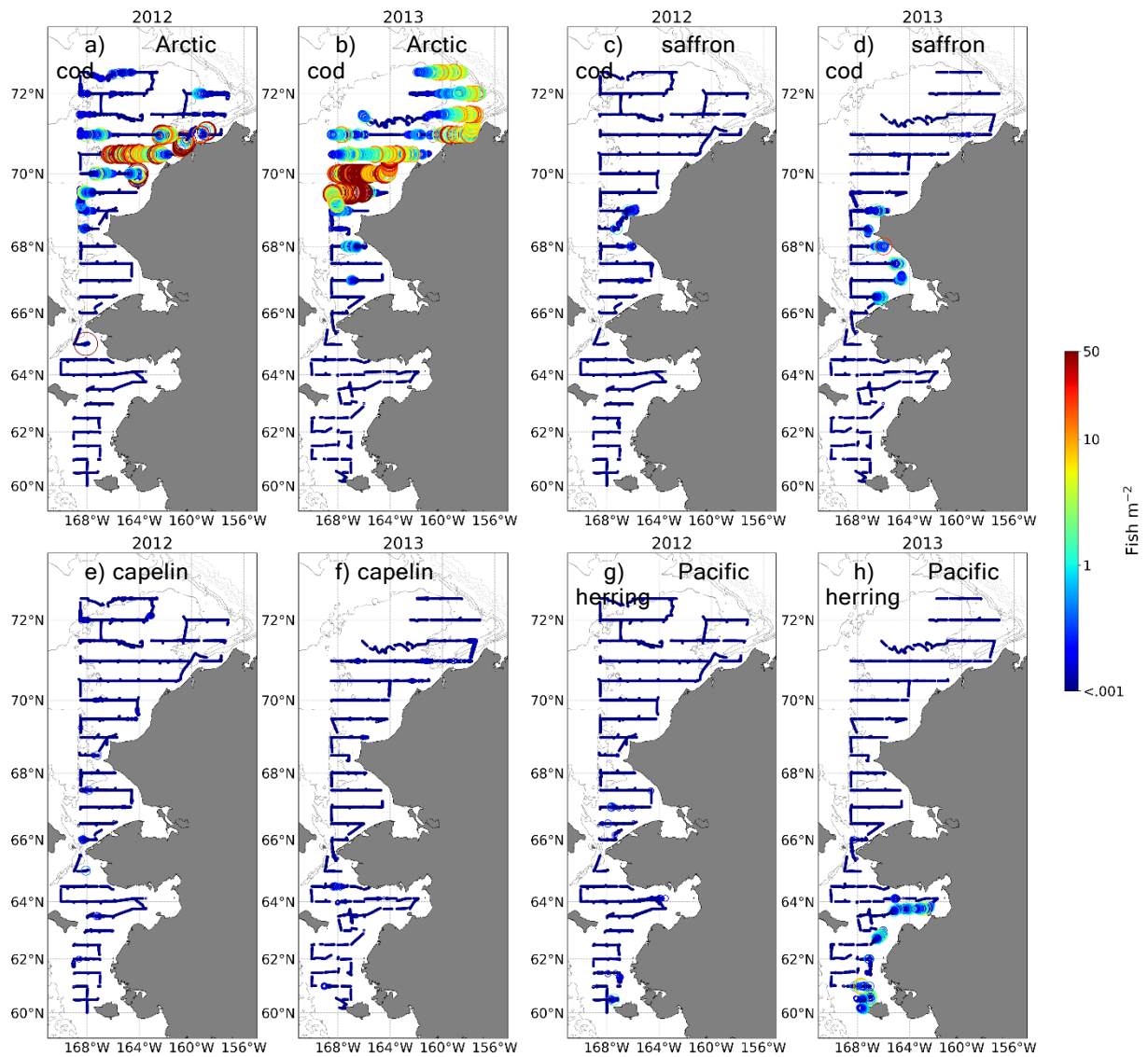


Figure 3: Length-frequency distributions of Arctic cod (a, b), saffron cod (c, d), capelin (e, f) and Pacific herring (g, h) in 2012 and 2013. The distribution for the northern Chukchi Sea is shown as the solid black line, and the combined distribution of the southern Chukchi and northern Bering Sea is indicated by the dashed line. Only data from common areas sampled in both years are included.

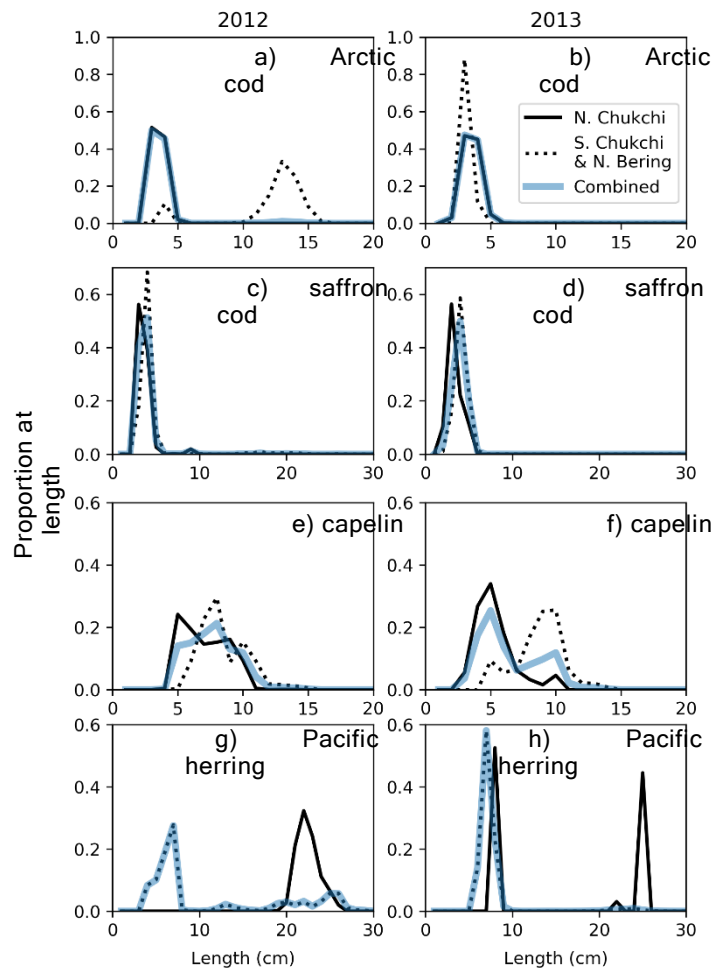


Figure 4: Biplot of the principal component analysis (PCA) of the water column properties. Survey sub-region of is indicated by color for each site. Environmental variables are temperature (Temp), salinity (Sal), DIN (DIN), mean stratification (MeanStrat), and integrated chlorophyll (IntChla). Bottom and surface measurements for temperature, salinity, and DIN are indicated by '.bot' and '.surf'.

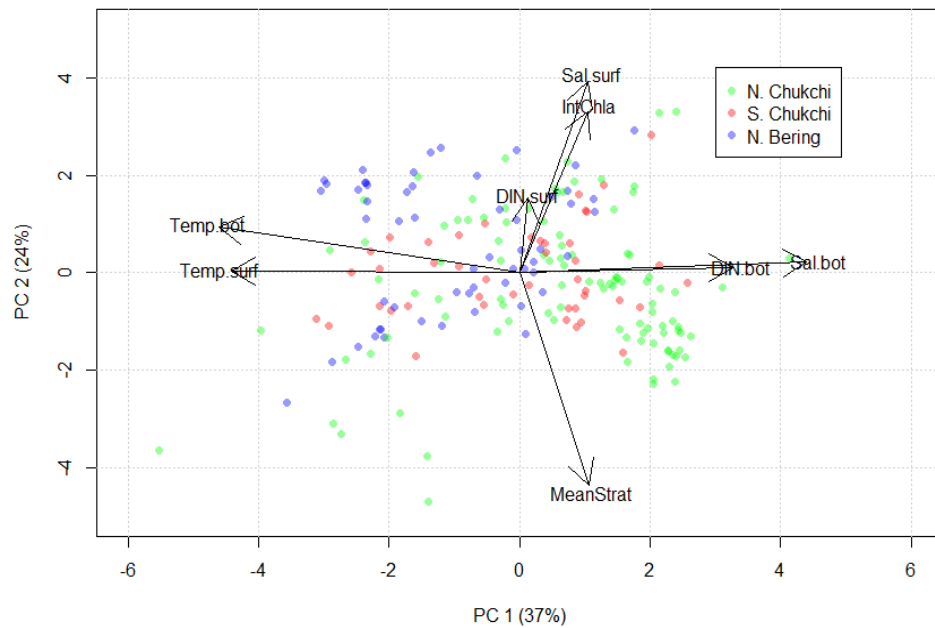


Figure 5: Triplot of the best canonical correspondence (CCA) model, showing the relationship between the major pelagic species Arctic cod (ACod), saffron cod (SCod), capelin (Cap) and Pacific Herring (Herr) and the water column properties previously explored in the PCA. Sub-region of the survey area is indicated by color for each site. Water column properties are labeled as indicated in Figure 4 above.

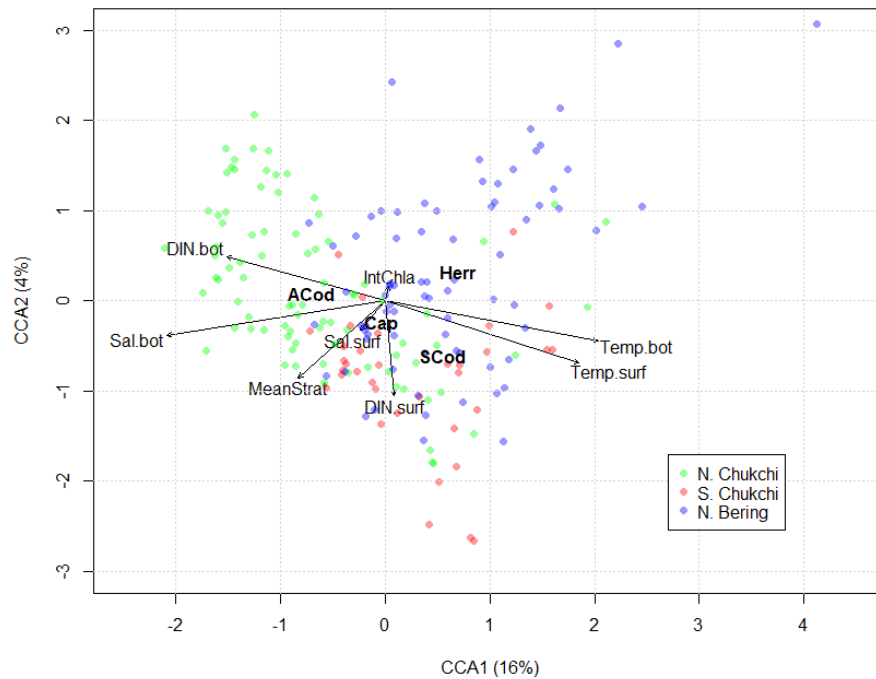


Figure 6: Histogram of scores for each 1 cm length bin along the CCA-axis 1.

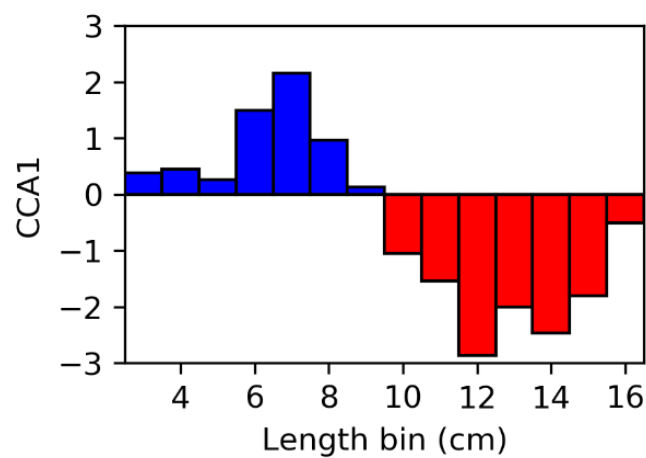
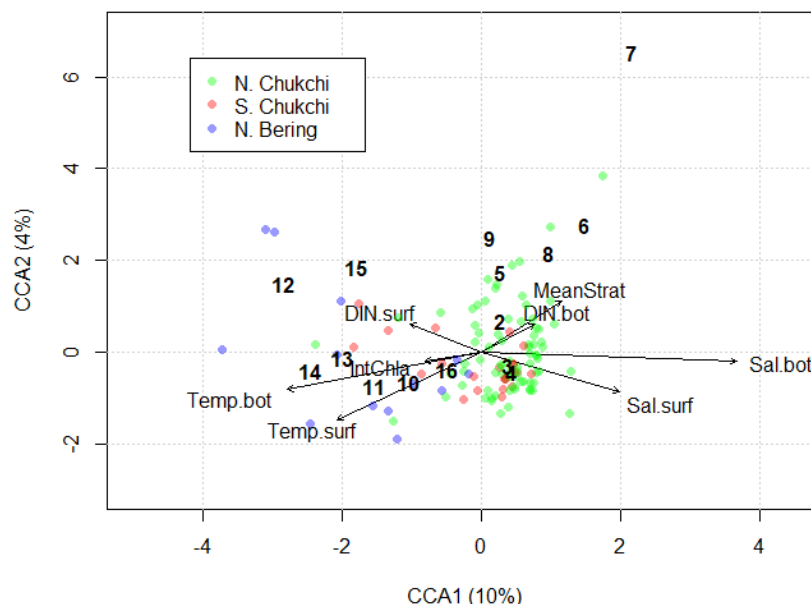


Figure 7: Triplot of the best canonical correspondence (CCA) model, showing the relationship Arctic cod size and the water column properties previously explored in the PCA. Size classes are indicated by the bold numbers, representing the center value of each 1 cm length bin. Sub-region of the survey area is indicated by color for each site. Water column properties are labeled as indicated in Figure 4 above.



Discussion

Acoustic trawl surveys of the northern Bering and Chukchi Seas in 2012 and 2013 indicated that the primary pelagic species were Arctic cod, saffron, cod, capelin, and Pacific Herring, confirming previous observations from bottom-trawl and ichthyoplankton surveys of these species as dominant fishes in the Pacific Arctic Region. Most of the fish caught in the survey were small (< 10 cm). Overall, these four species accounted for greater than 70% of the backscatter in the survey area.

Fish abundances were highest in the northern Chukchi Sea, where age-0 Arctic cod were found in high density. Capelin were more broadly distributed throughout the survey area than the other species, while saffron cod were predominantly found in the southern Chukchi Sea and Pacific herring in the northern Bering Sea. Overall, these spatial distributions were consistent between years. Larger Arctic cod, saffron cod, and capelin were found in the northern Bering Sea compared with the Chukchi, while the largest Pacific herring captured in both years were found in the northern Chukchi. All four species were more abundant and acoustic backscatter was higher in 2013 than in 2012.

A wide range of temperatures (-1.96 to 12.32 °C) and salinities (19.98 to 34.48 PSU) were observed among the CTD stations. As indicated

in the PCA analysis, most of the variability in the environment can be explained by temperature and salinity, with some spatial distinction between subregions of the survey area. The structure of the temperature and salinity variables, which represent the strongest loadings along PC1 and PC2, reflect the expected common water masses of the PAR. In summer, we would expect to see either a single or two-layer water column structure as winter water from the shelf is flushed north and replaced with warmer water from the Bering Sea. This winter water is either displaced entirely throughout the water column or overlaid with seasonal inputs of fresher water.

Typically, water column conditions in pelagic surveys are described as a function of mean water column conditions due to the diel vertical migration and large vertical range of many fish species. However, here we find that the surface and bottom water conditions can diverge significantly when highly stratified, and thus including them independently within the PCA is more appropriate. As indicated by PC1, when temperatures are cold the salinities are likely to be high, driven by the presence of this high salinity winter water still on the shelf. However, along PC2, surface salinity appears to be relatively independent of surface temperature and has an inverse relationship with stratification. This aligns with observations that the surface water mass

overlaying the winter water can be either fresh and cold as a result of recent ice melt, or fresh and warm, reflecting runoff conditions. When fresh, this upper layer lens stabilizes the water column in a highly stratified two-layer system. In both circumstances it appears that integrated chlorophyll increases as surface salinity increases, likely driven by increased water column mixing and recycling of nutrients into the surface waters. While the analysis of multivariate homogeneity of group dispersion indicated that differences in subregions are the result of position in ordination space, there does appear to be high variability in the northern Chukchi sites due to the presence of both these warm and cold fresh surface water conditions. The presence of recent ice melt is apparent in the large number of the northern Chukchi sites exhibiting positive scores along PC1 and negative scores along PC2, indicating colder temperatures at both the surface and bottom but increased stratification due to low surface salinity.

Species abundances and environmental measures indicated that there was a significant though weak relationship between species and water column conditions, however the representation of stations and species along the two significant CCA axes accurately reflect the spatial distribution observed in the trawls survey and from the acoustic estimates. Compared with saffron cod and herring, Arctic cod were much more abundant in the northern Chukchi where lower temperatures were also observed. The scores of saffron cod and herring among the stations further emphasize their high abundances in the southern Chukchi and northern Bering, respectively, and indicate a potential difference of preferred habitat based on their position along CCA-axis 2, strongly correlated with stratification. Pacific herring appear to be present in more dynamic well-mixed sites in the northern Bering Sea compared to the stable environment of Kotzebue Sound in the southern Chukchi where Saffron cod are found. Capelin appear to have low scores along both axes, indicative of their patchy yet widespread distribution.

Based on laboratory observations, when food availability is high, temperatures of 2-8 °C provide the greatest growth potential for juvenile Arctic cod, who exhibit a maximum growth rate from 7 - 8 °C and high rates of lipid accumulation in 0-9 °C conditions (Laurel et al., 2017). Laboratory studies have shown that Arctic cod have a cold-adapted physiology that promotes both high growth and rapid lipid allocation at lower temperatures compared to other gadids such as saffron cod (Laurel et al.

2016). The occurrence of these Arctic cod in colder water may be evolutionarily advantageous, as their growth advantage may establish clear habitat partitioning from saffron cod and other pelagic competitors. At the same time, the consistency in their spatial distribution in both years and high variability of conditions in the northern Chukchi Sea suggests that their distribution may be somewhat insensitive to high variability in local environmental conditions.

The relationship between size class and the environment indicated that smaller age-0 Arctic cod appear more likely to be found at stations where colder and saltier winter water is still present, while larger fish were captured in warmer conditions. In both years, few age-1+ Arctic cod were present in the survey region, though those that were caught in trawl samples were found primarily in the northern Bering Sea. Estimates of reproductive potential of the adult Arctic cod population in the survey region indicate that it is unlikely that the adults are capable of producing enough eggs to yield the number of age-0 fish observed in the acoustic-trawl surveys (Marsh et al., 2019). This suggests the age-0 population in the northern Chukchi likely originates elsewhere and possibly moves out of the northeastern Chukchi Sea as they age. One potential hypothesis is that this fish either follow or are passively advected with the winter water mass in which the eggs are spawned, which continues to flush off of the Chukchi shelf into late summer and fall before a decrease in transport occurs and ice returns to the shelf. Driven by wind and the Pacific-Arctic oceanic pressure gradient, transport through the Bering Strait and across the Chukchi shelf is typically to the north in the annual mean (Woodgate et al., 2005). This physical forcing may result in large-scale advection of fish during the summer, when flow rates on the shelf are highest (Woodgate, 2018). This may serve as a mechanism to drive this age-0 community off of the Chukchi shelf to deeper water along the continental slope into the Arctic basin where Age-1+ fish assemblages have been observed at depth (Parker-Stetter et al., 2011). While there may be a metabolic benefit during this juvenile period to be in warmer conditions to accelerate growth, both the limited competition and conservation of energy through passive transport may be more advantageous for this age-0 population.

Continued loss of sea ice and warming has the potential to negatively impact Arctic cod growth and survival in this region if this winter water mass does serve as a nursery for age-0 Arctic cod. Further investigation of how the

environment influences their abundance and distribution is necessary to better predict ecosystem changes with further climate change. Observation from these 2012 and 2013 integrated surveys established a baseline of observations, but further exploration of year-to-year variability is required to understand the potential impacts of a changing climate on the system. Additional years of large-scale pelagic observations in the U.S. Chukchi Sea are necessary to further establish correlations between water conditions and late-Summer fish distribution. Further insight may lead to the ability simplify predictions of fish population dynamics through modeled or remotely observed datasets of temperature and salinity. Lack of correlation between water mass properties and distribution would suggest other mechanisms (e.g., behavior) may play dominant roles in determining fish distributions.

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