

Functional consequences of structural differences in stingray sensory systems. Part I: mechanosensory lateral line canals

Laura K. Jordan^{1,*}, Stephen M. Kajiura² and Malcolm S. Gordon¹

¹Ecology and Evolutionary Biology, University of California at Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA 90095, USA and ²Biological Sciences, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA

*Author for correspondence (ljordan@ucla.edu)

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SUMMARY

Short range hydrodynamic and electrosensory signals are important during final stages of prey capture in elasmobranchs (sharks, skates and rays), and may be particularly useful for dorso-ventrally flattened batoids with mouths hidden from their eyes. In stingrays, both the lateral line canal and electrosensory systems are highly modified and complex with significant differences on ventral surfaces that relate to feeding ecology. This study tests functional hypotheses based on quantified differences in sensory system morphology of three stingray species, *Urobatis halleri*, *Myliobatis californica* and *Pteroplatytrygon violacea*. Part I investigates the mechanosensory lateral line canal system whereas part II focuses on the electrosensory system. Stingray lateral line canals include both pored and non-pored sections and differ in branching complexity and distribution. A greater proportion of pored canals and high pore numbers were predicted to correspond to increased response to water flow. Behavioral experiments were performed to compare responses of stingrays to weak water jets mimicking signals produced by potential prey at velocities of 10–20 cm s⁻¹. Bat rays, *M. californica*, have the most complex and broadly distributed pored canal network and demonstrated both the highest response rate and greater response intensity to water jet signals. Results suggest that *U. halleri* and *P. violacea* may rely on additional sensory input, including tactile and visual cues, respectively, to initiate stronger feeding responses. These results suggest that stingray lateral line canal morphology can indicate detection capabilities through responsiveness to weak water jets.

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Key words: batoid, elasmobranch, hydrodynamic, prey detection, *Urobatis halleri*, *Myliobatis californica*, *Pteroplatytrygon violacea*.

INTRODUCTION

Batoid elasmobranchs (skates and rays) are a morphologically diverse and highly successful group that locate and capture prey in a mouth located on the opposite side of the body from their eyes. Therefore, they typically rely upon non-visual cues, including hydrodynamic and electric signals, to detect and localize prey items, which are often buried. In the present study we investigate how the ventral mechanosensory lateral line canal system can facilitate prey capture in these dorso-ventrally flattened fishes with differing sensory morphologies.

The mechanosensory lateral line canal system is highly modified in batoids relative to other elasmobranchs; it is distributed over both the dorsal and ventral body surfaces and contains both pored and non-pored canals as well as superficial neuromasts, vesicles of Savi and spiracular organs (Garman, 1888; Chu and Wen, 1979; Maruska, 2001; Jordan, 2008). The subdermal network of ventral pored canals extends toward the wing tips and pelvic fins whereas the non-pored canals surround the mouth (Garman, 1888; Chu and Wen, 1979; Maruska, 2001; Jordan, 2008). The ventral canal system is thought to be important in locating signals produced by prey (Maruska, 2001). The pored canals enable detection of water flow while non-pored canals allow a heightened mechanotactile sensitivity of skin displacement (Maruska and Tricas, 2004).

Surprisingly little is known about how elasmobranchs utilize the information gathered by their lateral line system. Teleost fishes use mechanosensory systems for rheotaxis, predator avoidance, hydrodynamic imaging, prey detection, schooling and locating

conspecifics (reviewed by Coombs and Montgomery, 1999). Rheotaxis has been documented in sharks (Hodgson and Mathewson, 1971; Peach, 2001; Gardiner and Atema, 2007), and feeding responses can be elicited from stingrays presented with weak water jets, such as those produced by bivalves (Montgomery and Skipworth, 1997). However, detection capabilities have rarely been related to interspecific structural differences in sensory anatomy. This study compares the detection capabilities of three stingray species with significant differences in lateral line canal morphology to test functional predictions based on structural differences.

Behavioral responses to water flow are compared among three Eastern Pacific stingray species that vary in ventral lateral line canal type and distribution. The bat ray, *Myliobatis californica*, possesses a high proportion of pored canals and extensive canal branching, resulting in a high pore number [60.5±0.9% and 919±66 pores (Jordan, 2008)]. By contrast, the round stingray, *Urobatis halleri*, and the pelagic stingray, *Pteroplatytrygon violacea*, possess lower proportions of pored canals (36.2±0.3% and 39.9±1.2%, respectively) with reduced branching and lower pore numbers [110±5 and 104±2, respectively (Jordan, 2008)]. We predict that *M. californica* will exhibit greater response to water jet signals than the other species. We will also test whether the extension of pored lateral line canals toward the wing tip determines the extent of the lateral search area sensitive to water flow. These canals extend to 90% of the disc width (DW) in *M. californica* and less than 70% in *U. halleri* and *P. violacea* (Jordan, 2008). We predict that the

broad disc and accompanying pored canal extension of *M. californica* will confer an advantage for water flow detection.

MATERIALS AND METHODS

Experimental animals

Twenty-five round stingrays *Urobatis halleri* Cooper (12 females, 13 males; DW=9.5–24.0 cm) were collected by seine net in Catalina Harbor, Santa Catalina Island, CA, USA, during the summers of 2006 and 2007. Six pelagic stingrays, *Pteroplatytrygon violacea* Bonaparte (five females, one male; DW=49.5–60.0 cm), were collected by longline on the NOAA Ship *David Starr Jordan* during a juvenile shark survey in waters surrounding the Channel Islands, CA, USA. Fourteen bat rays, *Myliobatis californica* Gill (five females, nine males; DW=26.5–38.5 cm), were collected with large hand nets while SCUBA diving in Ripper's Cove, Santa Catalina Island, CA, USA, during the summers of 2006 and 2007. Both juvenile and adult *U. halleri* were caught; however, all *P. violacea* were adults and all *M. californica* were juveniles.

Rays were held at Wrigley Marine Science Center (WMSC) on Santa Catalina Island, CA, USA (33°30'18.52" N, 118°30'36.32" W) in 2.4 m diameter, 1.0 m deep outdoor fiberglass tanks with flow through ambient seawater ranging from 18–25°C at 35 p.p.t. with a turnover rate of fresh seawater approximately every 4 h. Species were housed sequentially in the holding tank with a maximum of 20 round stingrays, six pelagic stingrays or six bat rays in the tank at one time. Individual rays were weighed, measured, identified and inspected for external parasites or injuries. Ectoparasites were removed and any minor injuries from capture and handling showed signs of healing prior to testing. Rays were tested in behavioral trials only after normal feeding was observed in the holding tank, usually within one week after capture. Rays were held for a total of 3–5 weeks and were released at the capture location upon completion of trials. All work was done according to approved IACUC protocols at both the University of Southern California and the University of California, Los Angeles, USA.

Study design

The experimental tank (Fig. 1A) was identical to the holding tank and was fitted with the experimental apparatus consisting of a 1 × 1 m

acrylic plate with 6 mm holes fitted with plastic tubing underneath (Fig. 1B). Four water jets were connected to a peristaltic pump (Cole Parmer 7553-70, Masterflex easyload head 7518-50, Vernon Hills, IL, USA) to allow control of water velocity. Gang valve switches connected to the tubing allowed the experimenter to direct seawater pumped from a bucket, containing seawater identical to that in the tank, through one jet at a time. Also on the plate were four dipole electrodes, with a 1 cm dipole separation distance, connected to underwater electrical cables and a signal generator (see Kajiura and Holland, 2002). The plate was also fitted with tubing attached to a syringe used to inject squid/anchovy rinse as an odor signal at the center of the plate to encourage rays to begin searching for food. Edges of the plate were fitted with a flap that was buried in sand at the bottom of the tank to ensure a smooth ramp onto the plate. Two 'obstacles' or PVC weighted mesh pouches were placed against the edges of the tank near corners of the plate (Fig. 1A). These served to encourage rays following the side of the tank to swim toward the middle where they could encounter the odor signal to begin the trial.

Behavioral experiments

Prior to each trial, food was withheld for 1–2 days until rays showed sufficient motivation to search for prey mimicking signals in the experimental tank. Water flow velocities were chosen to simulate signals from potential prey; clam excurrent flow velocities have been measured at 6–14 cm s⁻¹ (Price and Schiebe, 1978). Water jet velocities used for experiments with *U. halleri* and *M. californica* were approximately 10 cm s⁻¹ whereas those used for *P. violacea* were increased to 20 cm s⁻¹ measured at the excurrent opening of the tubing. This difference was due to limited access to the latter species, which exhibited no response to jets encountered at 10 cm s⁻¹ in preliminary trials. Velocity was increased to improve the likelihood of observing feeding responses for subsequent analyses and comparisons.

Individual rays were moved into the experimental tank with large hand nets and allowed to acclimate for at least 1 h before beginning a trial. During this time the tank was covered with shade cloth, water flow was terminated and aeration was achieved with large weighted airstones, which were removed prior to the start of the trial to limit confounding sensory signals. The tank was uncovered, the water

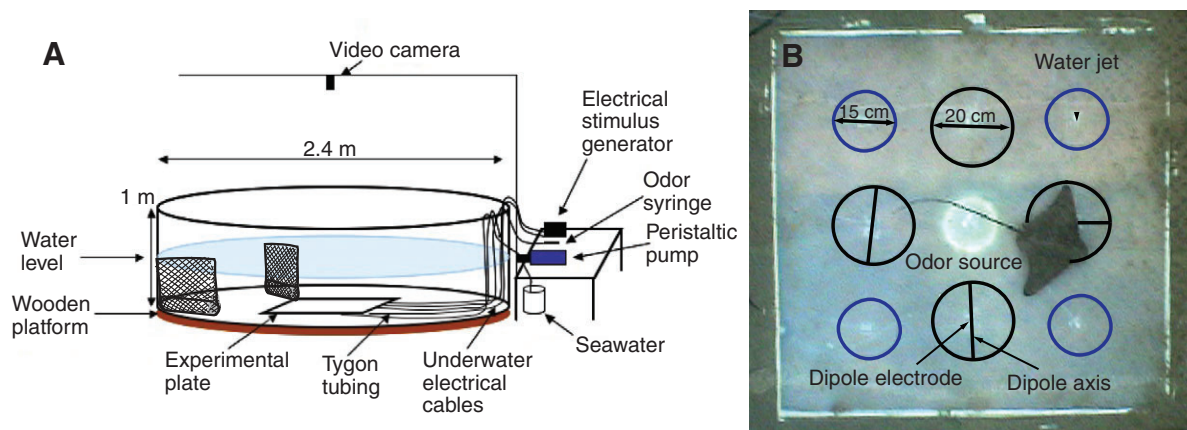


Fig. 1. Experimental set-up. (A) Eight foot diameter tank with the experimental plate centered on the floor and two weighted mesh obstacles with one end against the tank wall and the other end near corners of the plate. Underwater electrical cables and polyethylene tubing connected the plate to the stimulus generator, peristaltic pump and odor syringe and were covered with sand along the tank floor. Experimental trials were filmed by remotely activating the video camera positioned directly over the center of the plate (see Study design). (B) Experimental plate apparatus consisting of four dipole electrodes with 20 cm diameter reference circles and dipole axes indicated, four water jets with 15 cm diameter reference circles and an odor source at the center of the plate equidistant from electrodes and jet sources.

temperature and salinity were measured and the video camera was turned on and slid into place above the plate in the center of the tank immediately before each trial began. Trials took place only during daylight hours to film without artificial lighting. Both *U. halleri* and *M. californica* actively forage during the day, and *P. violacea* is regularly caught on longline during daylight hours (L.K.J., personal observation).

Motivation for feeding was confirmed by observing active searching behavior on or near the experimental plate after introduction of a food odor at the start of each trial. One water jet was activated at a time in random order, and responses were recorded on miniDV tapes at 30 frames s^{-1} using a Sony DCR-PC109 mounted on a track over the tank (Fig. 1A). Electrical responses were tested during the same trials; however, there is no indication that signal order influenced the results. Food rewards were occasionally placed on the plate at an activated water jet or electrode to encourage continued search behavior. Responses when food was present were not used in analyses. Similarly, any responses from rays swimming >1 cm above the plate were not analyzed. Height above the plate was determined from the video with comments by the observer on the side of the tank and confirmed by underwater video at the level of the plate during some trials. Motivation to search for food frequently remained high for the entire hour of the trial. In some trials, however, rays appeared to lose interest or motivation. Behavioral changes indicating disinterest included burying in the sand, altering swimming trajectories away from the experimental plate and rejecting food items offered as rewards. Unless searching and feeding behavior resumed upon reintroduction of an odor source, subsequent encounters with sensory signals were ignored and the trial was ended.

Individual rays participated in up to seven 1-hour experimental trials and were exposed to both water jet and electrical signals. Preliminary trials where rays were exposed to water jet signals with food rewards, and no responses to active jets without food were observed, were considered training and were not analyzed. Individuals varied in the amount of training time required, with *U. halleri* generally requiring more than the other two species. After trials began, rays were fed only in the experimental tank and were tested with 1 or 2 days in between trials. After completing each trial any visible food fragments were removed from the tank and fresh seawater was added. Up to four trials with different individuals were conducted on the same day. It was not possible to drain existing seawater from the tank between consecutive trials on the same day because the plate covered the drain at the bottom of the tank; however, every night the plate was removed and the tank was allowed to completely turnover with fresh seawater.

Video and image analysis

Video sequences of each encounter with a water jet were captured and broken into individual frames using iMovie HD 06 software (Apple Inc., Cupertino, CA, USA). The frame in which the ray contacted the jet was analyzed using ImageJ software (NIH: <http://rsb.info.nih.gov/ij>).

In total, 1566 (441 *U. halleri*; 640 *P. violacea*; 485 *M. californica*) water jet encounters were analyzed and compared. Linear measurements were used to quantify the location of the contact point with the jet source: the midline of the body was drawn from the tip of the rostrum to the base of the tail and the distance from the contact point at a right angle to the midline of the body was measured (Fig. 2). Behavioral responses were ranked from 0 to 5; 0=no response when passing over jet; 1=very slight pause, where swimming movements are altered immediately following contact

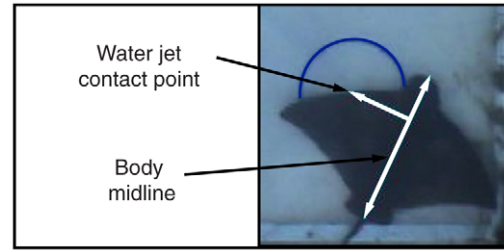


Fig. 2. Example measurements on the video frame at the point of contact with the water jet. The body midline was drawn and the distance to the contact point was measured at a 90 deg. angle.

with the jet; 2=slight pause, where swimming is altered with a braking response without a full stop; 3=turn, where swimming trajectory is considerably altered (>10 deg.) after contact with the jet; 4=pause, where swimming is briefly stopped but no attempt to bite is observed; and 5=bite response, where the ray stops, turns to position the mouth over the jet and actively bites at the jet source (see Movie 1 in supplementary material). We refer to positive responses including all response levels greater than zero. Passes over active jets were excluded if the pectoral fin was actively flapping or undulating above the water jet and thus raised in the water column instead of horizontal along the plate passing directly over the excurrent. While mechanosensory systems can be used to detect flow at considerable distances from the source, these passes were discarded to be deliberately conservative and consistent in our comparisons and to ensure that the velocities encountered were similar to those measured.

Responses of the three species were compared by trial and by position of the contact point on the body. All statistical analyses were performed using SPSS version 15 and 16 (SPSS Inc., Chicago, IL, USA) with models nesting multiple observations for each individual within each species. Variation in body size was controlled for in statistical comparisons by centering the mean body size for each species. A repeated-measures Poisson regression was used to compare the positive response rates of individuals across trials. The distribution of jet encounters along the anterior edge of the body was analyzed using a Poisson regression. Trends in the total number of encounters with increased distance from the midline of the body were compared between species. Similarly, trends in the numbers of total encounters, positive and no responses were compared within species. Differences for all tests were considered significant at $P<0.05$.

RESULTS

All three stingray species successfully acclimated to experimental conditions and readily searched for food during trials. Individuals of both *M. californica* and *P. violacea* typically began active search and response patterns during their first experimental trial whereas individual *U. halleri* often required two–three exposures to the experimental tank before they ventured onto the experimental plate. Only trials where rays attempted to feed on the plate are included. Responses to water jet signals showed more variation within than between individuals with distinct patterns by species.

Response rate by trial

Bat rays, *M. californica*, displayed a significantly higher response rate [repeated-measures Poisson regression, $P<0.01$ in 2 degrees of freedom (d.f.) test] to water jet signals with a positive response to

Table 1. Comparison of response rate to water jet signals by individuals of each species across multiple trials

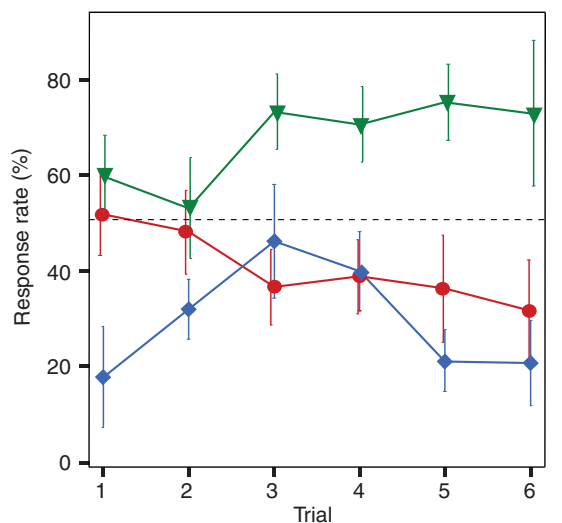
Parameter	Estimate
Intercept	-0.492*** (0.096)
<i>U. halleri</i> vs <i>M. californica</i>	-0.386* (0.170)
<i>P. violacea</i> vs <i>M. californica</i>	-0.63* (0.245)
Body size	-0.21 (0.112)

Estimates from the repeated-measures Poisson regression are shown with s.e.m. given in parentheses. The estimate for the intercept represents the reference species, *Myliobatis californica*, and the magnitude and sign of the estimates for interspecific comparisons show that the rates for both *Urobatis halleri* and *Pteroplatytrygon violacea* are significantly lower relative to *M. californica* (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

over 50% of water jet encounters per trial (Table 1; Fig. 3). When individual *M. californica* responded to water jet signals, they were more likely to have a higher-level response (≥ 3) or bite ($=5$) than the other two species (Table 2). Pelagic stingrays, *P. violacea*, had the largest number of jet encounters analyzed, yet the lowest percentage of positive responses.

Response rate by contact position

All three species encountered water jets significantly more frequently in the head region, close to the midline of the body, with a declining number of encounters toward the wing tip (Fig. 4A–C). The Y -intercepts of the total encounter trend lines differ significantly between the three species (Table 3). Species with a larger wingspan, *P. violacea* and *M. californica*, share a similar negative slope with far more encounters near the midline (Table 3). A more similar distribution of total jet encounters across the wingspan was seen in *U. halleri* (Fig. 4A). Positive and no response trends are not



<i>U. halleri</i>	22 (53)	17 (77)	13 (88)	9 (69)	7 (80)	5 (74)
<i>P. violacea</i>	6 (41)	6 (82)	6 (117)	6 (155)	6 (109)	5 (119)
<i>M. californica</i>	14 (51)	13 (67)	13 (92)	13 (104)	11 (101)	5 (54)

Fig. 3. Positive response rate to water jet signals averaged for individuals by trial for each species. Responses for *Urobatis halleri* are represented in red circles, *Pteroplatytrygon violacea* in blue diamonds, *M. californica* in green triangles. Error bars represent \pm s.e.m. The numbers of individuals tested per trial are shown with the total number of observations in parentheses. The horizontal broken line represents a positive response rate of 50%; only *M. californica* consistently respond above this level.

significantly different at the midline for *U. halleri*; however, positive responses decreased more quickly with increased distance from the midline whereas the no response trendline declines at the same rate as total encounters (Table 3; Fig. 4A). Individual *P. violacea* responded on average to only 25% of jets encountered at the midline (Fig. 4B), with no significant difference between total and no response Y -intercepts (Table 3). By contrast, *M. californica* showed a relatively high positive response rate in the head region with 68% of encounters at the midline resulting in positive responses (Fig. 4C). This is the only species for which positive responses outnumbered no response encounters for a considerable percentage of the wingspan. The trends in all three lines decrease at a similar rate with increasing distance from the midline in *M. californica* (Table 3).

The relative search area within the body surface was expected to correspond with the extension of pored lateral line canals toward the wingtip; however, positive responses were occasionally recorded for *U. halleri* and *P. violacea* past the distal extent of the lateral line canals. These accounted for only 9% of all positive responses for each species (Fig. 4A,B).

A positive correlation between body size and encounter rate of jet signals is apparent both within and between species (Pearson correlation for all stingrays $R = 0.54$, $N = 1566$, $P < 0.0001$). This pattern is consistent with the increased probability of a larger object encountering a point within a confined space. Higher encounter rates did not correspond with higher positive response rates. The side of the body where the jet was contacted and the jet number (1–4) were not found to significantly influence responses.

DISCUSSION

This study is the first to relate variation in stingray lateral line canals with functional differences indicated by behavioral responses. Predictions of detection capabilities based on sensory morphology are largely supported, suggesting strong links between form and function. From an evolutionary standpoint, this relationship is not surprising as it is energetically expensive for organisms to maintain sensory systems beyond those necessary for their success (Niven and Laughlin, 2008).

Response rate

Response rates to water flow signals varied between these three species as predicted by lateral line canal morphology. In *M. californica*, the large proportion of pored canals, high degree of branching and resulting high pore number (Jordan, 2008) corresponded to increased responsiveness to jet signals. Furthermore, *M. californica* displays the highest rate of positive responses across trials (Fig. 3), and is most likely to actually bite at the water jet source (Table 2). These results support the hypothesis that this species is particularly sensitive to weak water jets, such as those produced by clams, their most common prey (Talent, 1982; Barry et al., 1996; Gray et al., 1997). Sensitivity to water jets probably facilitates location of buried clams as these rays glide over the surface of the substratum and select areas for active foraging. Large bat rays regularly dig pits from tens of cms to nearly 1 m deep to unearth their prey (L.K.J., personal observation). This behavior probably requires a great deal of energy for blowing out and pushing away overlying sediments. Potential prey signals should, therefore, be carefully evaluated to ensure a successful capture of the desired prey prior to digging. High response and bite rates indicate that *M. californica* is both highly sensitive and interested in investigating the source of the water jet.

The response rates for *U. halleri* and *P. violacea* at 50% or below correspond to their lower proportion of pored canals and reduced

Table 2. Overall water jet response percentages by species

Species	Total encounters	No response (%)	Response>0 (%)	Response≥3 (%)	Response=5 (%)
<i>U. halleri</i>	441	60	40	25	12
<i>P. violacea</i>	640	68	32	15	7
<i>M. californica</i>	485	39	61	45	27

No response and responses >0 are percentages of the total number of encounters. Response ≥3 and =5 are percentages of all positive responses.

Table 3. Variation in total jet encounters and response type with location of contact point on the body

Parameter	Total	<i>U. halleri</i>	<i>P. violacea</i>	<i>M. californica</i>
Intercept	5.137*** (0.081)	3.754*** (0.120)	5.342*** (0.082)	4.094*** (0.131)
Y-intercept 1 vs 3	-0.681*** (0.121)	0.702*** (0.150)	0.208 (0.106)	1.043*** (0.154)
Y-intercept 2 vs 3	0.413*** (0.106)	0.076 (0.182)	-1.285*** (0.149)	0.615*** (0.167)
Y-intercept 1 vs 2	-1.094*** (0.113)	0.626*** (0.164)	1.493*** (0.142)	0.428** (0.132)
Slope 1 vs 3	0.144*** (0.027)	-0.044 (0.028)	0.058* (0.029)	-0.033 (0.038)
Slope 2 vs 3	-0.047 (0.027)	-0.119** (0.037)	0.160*** (0.036)	-0.055 (0.041)
Slope 1 vs 2	0.192*** (0.025)	0.075* (0.034)	-0.102** (0.032)	0.022 (0.034)
Distance from body midline	-0.280*** (0.020)	-0.091*** (0.022)	-0.385*** (0.023)	-0.247*** (0.032)

The 'total' column compares the linear trends of the total number of water jet encounters by percentage distance from the midline (see Fig. 4) between the three species, 1=*Urobatis halleri*, 2=*Pteroplatytrygon violacea*, 3=*Myliobatis californica*. Columns labeled with species names compare the linear trends for the total number of jet encounters, positive responses and no responses with percentage distance from the midline (1=total encounters, 2=positive responses, 3=no response, within each species; see Fig. 4). Estimates from the Poisson regression are given with s.e.m. in parentheses. The intercept estimate shown uses 3 as the reference for each column with the exception of the 1 vs 2 comparisons (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

secondary canal branching relative to *M. californica* (Jordan, 2008). Other explanations for decreased responsiveness in these species may relate to feeding ecology. It is possible that water jet signals are less important to these species because they are less representative of common prey; however, all rays were trained to associate water jets with food in order to standardize recognition of these isolated signals regardless of typical prey. Furthermore, common prey of *U. halleri* includes mollusks, crustaceans and polychaete worms (Babel, 1967; Valadez-Gonzalez et al., 2001), which bury shallowly in soft substrates and may create weak transient flows similar to clams when circulating water for filter feeding and respiration. The extensive non-pored canals surrounding

the mouth of *U. halleri* however may provide tactile information more important in locating and capturing their primary prey, stomatopods, amphipods and shrimp (Jordan, 2008). During behavioral trials, individuals were occasionally observed to pause on contact with inactive jets or the odor tube source, when odors were not being injected. These responses may imply an increase in attention to tactile signals from the holes in the plate and were observed relatively rarely for *P. violacea* and *M. californica*.

Common prey of *P. violacea* include teleost fish and cephalopods (Wilson and Beckett, 1970). It is possible that their low response rate to water jets is due to differences in the hydrodynamic structure of the signal created by the jet relative to wakes created by

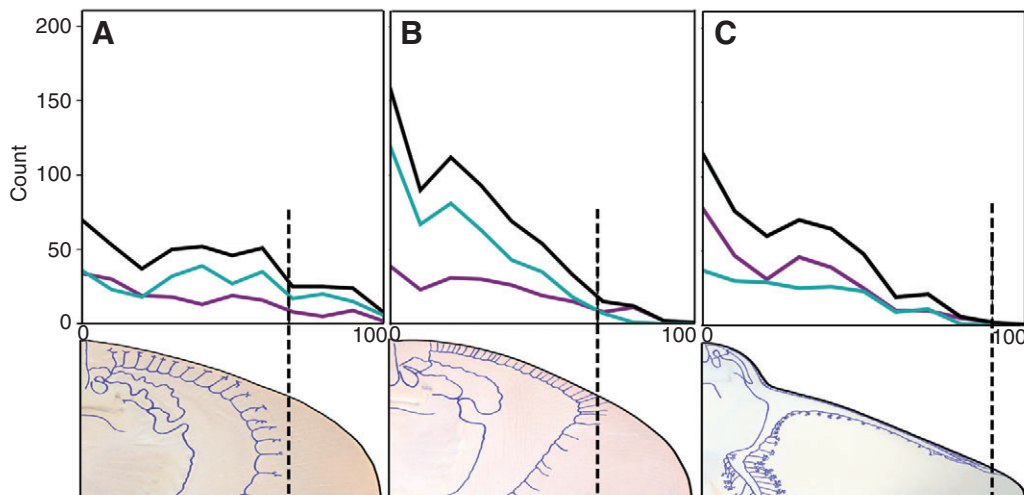


Fig. 4. Response variation with water jet contact point. (A) *Urobatis halleri*, (B) *Pteroplatytrygon violacea*, (C) *Myliobatis californica*. The black line indicates total encounters, the purple line, positive responses and the blue line, no response. The Y-axis shows the count from 0 to 200. Y-intercepts and slopes of these linear trends are given in Table 3. Representative diagrams of the anterior ventral lateral line canals are modified from Jordan (Jordan, 2008), with the X-axis scaled from the 0% at the body midline to 100% at the wing tip. The average extent of the lateral-most canal is indicated for each species by the vertical broken line.

swimming fish and squid. Nevertheless, these rays were trained in the same way as the other two species and they quickly learned to feed on the bottom of the tank and to search for food on the experimental plate. The occurrence of positive responses suggests that *P. violacea* could detect the water jets, associate them with food and exhibit biting behavior similar to those of the other species. Response rates for *P. violacea* declined after trial 4 (Fig. 3). The drop in the positive response rate could result from learning to focus on both visual and water flow signals before eliciting a feeding response. There were several instances where these rays appeared to visually track food as it was placed in the tank. On occasion they swam up toward the food before it had touched the water. This suggests that these rays are highly sensitive to visual signals and probably have a visual field that extends above their head as do other batoids (McComb and Kajiura, 2008). Food pieces that lingered in the water column before falling to the plate were occasionally enfolded with the wings and ingested before hitting the bottom, further indicating use of visual signals to locate the food mid-water. Furthermore, individual rays showed increased motivation and responsiveness after the experimenter provided visual signals mimicking those when food rewards were given. These examples indicate learning and memory as well as integration of multiple sensory signals to determine behavior.

Body position

Contact points with the jet were distributed non-randomly along the body. All three species tended to encounter the jet more frequently in the head region. This could be a result of training the animals to search for food on the plate and alter their swimming pattern to center themselves over the jets to increase the likelihood of encountering food. This trend may also be exaggerated by the exclusion of passes over the jet when wings were actively flapping or undulating, as amplitude of fin beats increases with distance from the midline leaving distal portions of the wing more likely to be raised above the plate surface (L.K.J., personal observation). Patterns of positive responses show marked differences by species. Round stingrays, *U. halleri*, were just as likely to respond or not respond to jets contacted in the head region (0–20% distance from midline); however, as jets were encountered farther toward the wing tip positive responses became less common (Fig. 4A). By contrast, *P. violacea* and *M. californica* showed nearly opposite trends with no response more common along the length of the wingspan in the former and positive responses in the latter (Fig. 4B,C). The pored lateral line canals in *M. californica* have an order of magnitude greater number of pores than those of the other two species (Jordan, 2008). Along the anterior edge of the body these consist of very short branches off the main canal typically with only 1–3 pores per branch, which may improve localization of jet signals. Alternatively, *U. halleri* has greater secondary branching though a similar pore number to *P. violacea* with almost no secondary branching [see fig. 3 and table 2 in Jordan (Jordan, 2008)]. While pore number, branching and distribution of lateral line canals are quantified in these species (Jordan, 2008), neuromast morphology, number and density are unknown. Unlike teleosts, elasmobranchs have a continuous bed of neuromasts throughout the main lateral line canal (Johnson, 1917; Tester and Kendall, 1969; Hama and Yamada, 1977; Maruska, 2001). Interspecific differences in neuromast morphology may impose additional influences on the sensitivity and resolution of these systems. Dorsal lateral line pores located on the leading edge in the head region of all three species may contribute to flow detection as the jet stream is displaced by the body; however, there is no indication that contributions to

detection are significant in this study as *P. violacea* has significantly more dorsal pores yet a weaker behavioral response than *U. halleri* (see Jordan, 2008).

In contrast to our prediction, both *U. halleri* and *P. violacea* can respond to signals encountered beyond the extension of the lateral line canals. It is possible that jet flow was funneled medially by the distal wing and then detected by the lateral line system or that the velocity was great enough to stimulate cutaneous touch receptors on the wing. Furthermore, weak electric fields created by the moving seawater might be detected by the electrosensory system. Montgomery and Skipworth determined that the voltage gradient generated by the weak water jets used in their study could be 1 nV cm^{-1} , which was considered below the electrosensory detection threshold of elasmobranchs (Montgomery and Skipworth, 1997). Kajiura and Holland (Kajiura and Holland, 2002) and part II of the present study (Jordan et al., 2009) have since demonstrated sensitivity well below 1 nV cm^{-1} for species of sharks and rays; thus, this possibility cannot be ruled out. Unfortunately there was no reasonable way to prevent this byproduct of flow in seawater. However, if electrical stimuli were important in directing these responses, all three species should show a more similar response level as seen for electrical responses (see Part II, Jordan et al., 2009). Therefore, cutaneous receptors or funneling may contribute to the ability to detect weak water jets, although less effectively than with direct use of the lateral line canals.

Behavioral experiments are by nature highly variable. Behavioral responses are important in terms of ecological context of sensory function; however, they provide only indirect measures of actual sensitivity. Reduction of potential disparity between sensation and response was accomplished by ensuring motivation to search for food and training to direct the search onto the experimental plate. Aside from increasing encounter rate, differences in body size do not appear to significantly affect these results.

Conclusion

These experiments relate lateral line canal morphology to detection capabilities indicated through behavioral responses. The hypothesis that the proportion of pored canals, degree of branching and resulting high pore number in *M. californica* correspond with increased response rate to hydrodynamic signals is supported. The prediction that the extension of the lateral line toward the wing tip will increase lateral search area is supported in that *M. californica* has the largest range across the disc width where positive responses outnumber no response encounters; however, both *U. halleri* and *P. violacea* could respond positively to water jets contacted beyond the extent of the pored canals. These results suggest that additional tactile and visual sensory input can be important to *U. halleri* and *P. violacea*, respectively. The integration of sensory signals and their role in determining behavior are rich areas for future research. An increased understanding of functional consequences of differences in sensory morphology can be applied to species where little ecological information is available and toward improving the design and specialization of artificial sensory systems.

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