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The detection threshold for odor plume tracking in the smooth dogfish, Mustelus canis

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THE DETECTION THRESHOLD FOR ODOR PLUME TRACKING IN THE SMOOTH DOGFISH, *Mustelus canis*

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DEDICATION

I would like to dedicate this work to the woman who inspired many women, not only myself, to pursue science; Bojan Jennings. And to my parents, who have always supported my quirky curiosity and interest in the ocean.

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THE DETECTION THRESHOLD FOR ODOR PLUME TRACKING

IN THE SMOOTH DOGFISH, *Mustelus canis*

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ABSTRACT

It is commonly believed that sharks have extreme sensitivity to odor and use that to locate their prey. This research provides data on behavioral detection thresholds to food odors in a small shark species, *Mustelus canis*. We analyzed *M. canis* tracking behavior in a binary choice flume designed specifically for testing odor preferences of aquatic animals. Odor was serially diluted until no tracking responses were observed. Results indicate that sharks spent significantly more time in the odor side of the flume, regardless of their individual side bias, until the "squid juice" was diluted several orders of magnitude. For the whole flume the two greatest dilutions (10^{-4}-10^{-5}) did not cause significant choice, for the upstream flume half, all but the greatest dilution (10^{-5}) caused significant odor side preference and for the downstream flume no odor preferences exceeded the significance threshold, except the 10^{-4} dilution which resulted in significant repulsion. Therefore, sharks indeed tracked odor plumes to the upstream odor release area: odor presence on the right or left side of the flume significantly changed their individual random side bias so that they spent up to 75% of their test periods on the odor side. However, this side choice varied significantly with odor dilution. First, the sharks showed a strong response in the upstream flume half where the odor signal is strongest, but no

significant response downstream where odor signals are down to <0.1 % of the source concentration.

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LIST OF ABBREVIATIONS

BU ..... Boston University

MBL ..... Marine Biology Laboratory

WHOI ..... Woods Hole Oceanographic Institution

Introduction

Elasmobranch fishes (sharks, skates and rays) are widely believed to have superior olfactory sensitivities as reflected in commonly quoted statements such as "sharks can smell a drop of blood from a mile away". Indeed, it is common knowledge that fishermen who chum the waters surrounding their boats attract sharks rapidly and in great numbers (Meredith & Kajiura, 2010). There are, however, no data on actual detection distances and odor concentrations required for sharks to track a food odor plume to its source, since it is typically not known when and where the sharks first detected the chum. Tracking involves not only odor detection *per se*, but also the availability of sufficient temporal and spatial information to follow the stream of odor patches that comprise an odor plume (Atema 2012, 1969; Webster 2007). Given this, we were interested in investigating the true behavioral threshold needed to provoke a tracking behavior. We used a small local shark species, *Mustelus canis*.

The basis of many ecological interactions fundamentally depends on an organisms' ability to acquire and interpret relevant information about its' surroundings. Sensory cues with various physical properties carry information some distances throughout different environments. Because of these differences in habitat, each sensory signal is transmitted and received differently depending on the physical constraints. Therefore, organisms have evolved various habitat specific systems and mechanisms of odor detection (Dusenbery, 1992). Among these sensory systems, the chemical senses are pertinent sources of ecological information, both on land, as

well as, in the aquatic environment (Atema, 1987; Wilson, 1970).

Locating food and shelter, avoiding predators, and attracting a mate are universal activities among all organisms and a wide variety of strategies and sensory systems are used to accomplish these goals (Colin, 2003). Chemical stimuli in the ocean are imperative for the survival of the organisms that encounter them. These types of stimuli can lure larval fish to settlement (Gerlach *et al*. 2007), alert and orient animals to prey (Gelsleichter *et al.*, 1999, Zimmer and Butman, 2000), attract a mate (Vickers, 2000), and aide navigation in migration (Vrieze, 2011). Generally, odor dispersal and distribution occurs in a three-dimensional environment is visualized as an 'odor-landscape' (Atema, 1996). The need and ability to recognize and interpret this odor landscape is present throughout all taxa along the phylogenetic tree. It is critical to marine organisms for survival and success to use chemical signals to determine the distance and orientation to the odor source (Webster *et al.*, 2007). This is a challenging task. To appreciate this challenge, understanding the interaction between sensory information, the environment and how this constrains the behavior of all organisms (Kikas *et al.*, 2001).

Many organisms navigate across expansive odor landscapes. Sensory systems have adapted recognition of chemical stimuli that aides in survival and evolutionary success (Døving *et al.* 1977). The notion that animals respond to differences in odor concentration gradients, the change in concentration as a function of distance, is

based on the fact that odor is diluted and diffused gradually away from the source. However, odor dispersal is chaotic and in mixing conditions generated a cascade of increasingly smaller eddies that makeup the odor landscape (Atema 2012). Odor plumes show chaotic intermittency, with the concentration variance several orders of magnitude greater than the concentration mean. Therefore, the spatial concentration gradient can only be determined by averaging the concentration of the plume eddies (Webster, 2007). Nonetheless, odor dispersal and olfaction has been determined to be particularly important to gather information about distant odor sources as chemical signals travel in currents. Therefore odor cues can be transported much further in the marine environment than visual, mechanical or electrical signals (Atema, 2012).

Parker (1911) was the first, to investigate chemical sensitivity in elasmobranch fishes over a century ago. The shark nose will broadly direct the animal using large nostrils and olfactory structures. Anatomy reveals a great surface area of olfactory epithelia (Schluessel *et al.*, 2008) and large volume of the olfactory bulbs (Lisney *et al.*, 2007). Four main classes of chemicals (odorants) are known to be detected by a fishes' olfactory sense: amino acids, gonadal steroids, bile acids and prostaglandins (Hara, 1994). Each of these four chemical classes are detected by different receptors. The shark therefore can use chemical information as a primary method of finding food (Parker, 1914), predator detection (Rasmussen & Schmidt, 1992), and homing and navigation (Gardiner & Atema, 2007). Therefore, based on

the overall evolutionary success of sharks and the expansive environment that many sharks inhabit, they have earned the reputation of being “swimming noses”.

A remaining challenge is to understand how elasmobranchs navigate these odor plumes and the threshold of a stimulant is needed to elicit a behavior. The lateral line is essential in steering the animal along an odor plume, while the olfactory system is needed for odor identification and search motivation (Gardiner and Atema, 2007, 2010). The knowledge of chemosensory thresholds of an animal for a particular chemical cue is important to understand the relationship between chemoreception and ecology forging patterns and their energetic costs (Wilson, 1970, Pearson *et al.,* 1980). The odor landscape of a food source within which food searching behavior occurs depends on the chemosensory threshold (Løkkeborg *et al*., 1995).

Electrophysiological evidence showed that in the lobster dactyl chemoreceptor organs sensitivity threshold to NH_4 is around just over 10^{-6} molar when tested against a 10^{-6} background concentration. When back ground concentration is raised, the detection threshold rises similarly and the entire response function shifts to higher concentrations (Borroni and Atema, 1988). These results demonstrate that chemoreceptors are only sensitive to information greater than the background. Behavioral thresholds leading to plume tracking may differ from physiological thresholds but likely function in the same way. Yellowfin tuna (*Thunnus albacares*)

use a low threshold olfactory sense to respond to differences in mixtures of food odors and could respond to single compound (tryptophan) concentrations of 10^{-11} M (Atema *et al.*, 1980). Thresholds for tracking behavior have not yet been established for elasmobranchs.

Here, we determine the behavioral threshold for tracking food odor plumes in the small benthic-coastal shark species, *Mustelus canis*. Following earlier related experiments (Gardiner and Atema, 2007), we analyzed *M. canis* tracking behavior in a binary choice flume designed specifically for testing odor preferences of aquatic animals. Odor was serially diluted until no tracking responses were observed. We also noted the distance at which tracking started. From these data we can later estimate odor concentrations that led to the start of tracking and the corresponding detection distances in the field.

Methods

Animal collection and husbandry:

Smooth dogfish (*Mustelus canis*) were caught by trawling in Nantucket Sound and purchased from the Marine Biology Laboratory's Marine Resources Center (MBL) in Woods Hole, Massachusetts USA. MBL housed the sharks for less than one month before being brought to our shark lab at the Woods Hole Oceanographic Institution (WHOI) for behavioral testing. At MBL they were fed a diet of local frozen squid

(*Loligo pealeii*) and frozen capelin (*Mallotus villosus*); at WHOI we fed them a diet of frozen squid every other day. This research was done under IACUC protocols 10-017 and 13-019 from Boston University and WHOI, During the entire time in our lab, all animals were healthy, fed well, grew and once even gave birth to three "pups". Dogfish and pups were under the specialty care of a veterinarian. Given their good health and the nature of our work, all animals were returned to the sea at the end of the season.

Throughout the experiments, the sharks were housed in one of two 3-meter diameter, inflatable, soft-sided pools. The sharks were sexed, measured (total length and fork length) and given two colored tags for individual identification using T-bar anchor tags through the dorsal fin (Table 1). To ensure they were motivated to track a food odor, sharks were only tested once feeding in pools became consistent. In the pools, sharks were trained to feed off a 1- meter long wooden dowel. A ~15g piece of freshly thawed squid was treaded with fishing line and attached to the dowel through a small hole. Training them to feed only from the dowel allowed us to reward animals during olfactory trials. Preliminary trials had shown that sharks naturally shy and even jump away from the dowel without consistent exposure to this method of food delivery. Earlier experiments had shown that food reward is essential to motivate animals to track even weakly diluted odors (Jennings, Pace, unpubl. exp.).

Experimental Design

To establish food odor concentration thresholds that result in sharks tracking the odor to the source we gave them a binary choice of two parallel plumes: an odor plume versus a seawater plume. The plumes were identical in turbulence but only one contained food odor. Because individuals can exhibit side bias, we tested each shark twice in a row, once with odor on the right followed by odor on the left, in random order. We preceded this with a "blank" test in which only seawater plumes were presented. Thus a test consisted of three trials. Each animal was tested on different days with five odor concentrations in serial dilutions. To limit the number of animals and to test for possible time effects including learning, we repeated each test series three times with the same individuals: July, August, September, 2013. This allowed us to evaluate the effects of odor concentration, learning and individual variance on their ability to track an odor plume to its source.

Experimental test conditions

All tests were conducted in a 10-meter flume with an 8-meter test area, designed to conduct pair-wise choice experiments with animals freely choosing between water flowing in parallel from two sources (Gardiner & Atema, 2007; Figure 1). This flume provided a unidirectional, semi-laminar flow of sand- filtered and temperature-controlled seawater from Nantucket sound, re-circulated to maintain a flume flow rate of 2.5 cm/s at a water depth of 40cm. Experiments were run in daylight from July through October 2013 during which pool water temperatures varied 20–24^{o} C.

Flume temperature for all experiments was maintained at 18^{o}+/- 1 C regulated with a chiller located externally to the flume lab.

Plume structure and Odor dilution

M. canis is primarily a benthic shark seeking food on the bottom. We therefore released the odor plumes from two bottom sources leading to bottom boundary layer turbulence that travelled down the flume in a cascade of diluting eddies. Odor injection was 40ml/min/source driven by a 2-channel peristaltic pump and oozing from a 4 mm ID nozzle which created minor additional small-scale turbulence. The uniform flow conditions prevented large-scale turbulence so that the plumes did not meander and did not mix until the downstream start box. Regular dye tests were performed to assure flow conditions sufficient to maintain separate plumes (Fig. 1, 2 with a dye plume).

As odor emerges from the source nozzles it mixes with ambient flume water. Plumes can be described as a stream of "flavored" eddies (Atema 1996). Both the source and particularly the bottom boundary layer generate significant small-scale turbulence.

Odor dilution in the plume as it travels downstream can be estimated from electrochemical (Moore & Atema, 1991) and laser-induced fluorescent dye measurement (Webster, 2007). Mean and peak concentrations dilute away from the source exponentially such that 1m downstream from the source the mean plume

concentration is already diluted by 3 orders of magnitude and even the rare high-concentration peaks are 2 orders of magnitude lower than the source concentration (Fig. 1). Further dilution proceeds slowly with distance. As small-scale turbulence breaks up the odor signals they mix and dilute and eventually disappear with time and distance. Simultaneously, molecular diffusion also diminishes leading to mean concentrations downstream that are 4-5 orders of magnitude lower than the source. We estimate a mean signal dilution of 10^{-4} in the middle of the flume and 10^{-5} well downstream.

Odor background

Recirculation during odor trials causes a gradual build-up of background odor. A typical test of 20 minutes (odor plume establishment 6 min + shark test 4 min, repeated twice for side switch) would release 0.8 L of odor in the total 8,000L flume volume, gradually creating a signal-to-background (S/B) ratio at the plume source of 0.8 / 8,000 = 1 / 10,000 at the start of the subsequent test. On most days, 4 tests were performed leading to S/B = 1 / 2,500. On rare occasions all 7 animals were tested on one day leading to an end of the day S/B= 1 / 1,430. The flume's high velocity return flow driven by centrifugal pumps through two 15-meter, 7.5-cm diameter hoses stirred the odor thoroughly upon re-entry into the flume as visualized by dye. This mixing resulted in a uniformly diluted background eliminating the presence of persistent and meaningfully organized odor eddies. Note that on each day only one odor concentration was tested so that the background

was always a fraction of the test concentration and odor signals at the source were 3-4 orders of magnitude above the background. In a study of receptor adaptation, lobsters behaviorally detected food-related odors that were less than one order of magnitude above background (Borroni *et al.,* 1988). *Most important for this study is that the odor plume signals gradually diluted until downstream they could sink into the background.*

Experimental protocol

On the day of testing, a shark was gently lifted by net from its holding pool and placed in the flume. A single trial consisted of a 10-minute habituation period where the shark was free to swim throughout the entire flume with no odor or seawater plume present. Preliminary trials indicated a 10-minute habituation period was sufficient for sharks to display their typical smooth and regular cruising and/or bottom-resting behavior. Following the habituation time, the shark's swimming behavior was video recorded for the next 10 minutes with seawater plumes on both sides to establish the animals' side bias for comparison with its side preference during odor trials.

Following the 10 min seawater control test the shark was gently coaxed into the start box, the most downstream area in the flume with a retractable mesh gate where the shark was held prior to odor testing (Fig. 1). Once the shark was corralled in the start box, the odor and seawater plumes were started using a

peristaltic pump to inject odor and seawater at the rate of 40mL/min. The initial odor side was chosen randomly. As soon as the odor was started, a timer and the video and tracking software were started simultaneously. Separate dye tests had shown that the odor reached the start box after 6 minutes. During this time, the shark remained corralled in the start box. Three minutes into this holding period the shark's gill rate was recorded along with the water temperature of the flume. After 6 minutes the gate was lifted and the shark was again able to freely explore the flume, until it located the source area or until four minutes had passed. During this time, five measures were scored: (1) the flume side, odor/seawater, where the shark came out of the start box; (2) the elapsed time to reach the odor source area (3) the number of bumps or bites at the odor nozzle (4) the number of times the shark circled the odor source area, as well as (5) any notes on unique behaviors. The trial ended when the shark entered the source area (a 10-cm radius around the odor nozzle) where animal were rewarded with a piece of squid from the wooden dowel described above. If the animal was unsuccessful locating the source area, the food was not offered and the trial would conclude after 4 minutes. At the end of a trial, the plume inflows were stopped, the shark was coaxed back into the start box, and the odor and seawater sources were switched for a second test to account for side bias. This second test included the six-minute start box period and the four-minute experimental trial. While the odor was switched the shark was given a 10-minute period to freely swim throughout the flume without plumes injected. Then the animal was lifted our of the flume and put back into the home pool. In sum, each

shark experienced the following test sequence: 10 min acclimation, 10 min (including 4 min recording with control seawater), 6 min start box, 4 min odor test, 10 min swim during odor switch, 6 min start box, 4 min switched odor test. Each shark was tested three times (once in July, August and September) with all 5 dilutions, except for one shark, which took longer to train to the dowel food presentation and could only be tested twice at each dilution. In sum, we tested 7 animals, with 5 dilutions in 3 sequential series of tests; each test consisted of 3 trials: a seawater control and two odor trials in which odor was presented on the left or right side of the flume in random order. In order to keep odor delivery even among tests and to avoid 'contamination' of tubing and test materials, we used only one dilution on any given day and tested on average 4 sharks per day with that dilution. Each shark was tested no more than twice per week.

All trials were recorded using overhead cameras. The video feed was transmitted to two computers where swimming behavior was tracked through software (Ethovision 3.1 and MatLab, MathWorks) (Noldus, The Netherlands) Ethovision was also used to generate additional tracking analysis that cannot be assessed during the trials, including time spent, turn frequency, angle and speed, and swimming speed in each quadrant of the flume.

Stimulus preparation

The food odor stock solution ("squid juice") was made by emulsifying in a blender

50grams of fresh-frozen, thawed local squid mantle (*Loligo paleii*) in 1L of seawater taken from the test flume and coarsely filtering it though paper towel. This stock and four log serial dilutions were tested starting with 100ml of "squid juice" blended with 900mL of flume seawater (recorded as log 0, 2, 3, 4, 5). Serial log dilutions with flume water were made fresh daily to the desired level for the test day,

Statistical Analysis

-Side Bias

Given that each animal will have an inadvertent side bias, odor choice can only be evaluated against this bias. In other words, one wants to learn to what degree the odor presence alters the side bias (B). Thus to measure the odor response (C) one needs to calculate how much more or less time an animal spends on the odor side both when the odor is presented on the right (OR) and on the left (OL) side. This can be attraction (OR-B)>0 or repulsion (OR-B)<0, or no effect (OR-B)=0. The bias (B) can be measured directly in a control seawater test without odor. Note that OR, OL, and B are scored from +50% when odor is on the right to -50% with odor on the left. Thus all three parameters can range from 0-100% of time spent on one or the other side. We base all behavioral analyses on this C-score:

C = (OR-B) – (OL-B) = OR-OL.

Fig. 3 shows this graphically as a left-right dial for a few realistic examples.

-Data analysis

We analyzed several different odor responses.

(1) Time spent on the odor side of the whole flume and on the upstream and downstream halves of the flume using the C-score described above. We analyzed the effects of odor dilution, test series and individual variance with Friedman ANOVA, followed by analysis of the individual factors (odor dilution and test series) using a Wilcoxon test to limit the effect of variance between individuals.

(2) Success rate of locating the odor source and taking a food reward was scored as follows. A trial was considered successful if the sharks' head entered a target area of 10 cm radius around the odor source and took a squid reward from the dowel. Shark that were successful on both the left and right side odor trials scored a 1; if only one trial was successful they scored a 0.5, and if no food reward was collected on either the left or the right the shark scored a 0. Therefore, the maximum score for all trials throughout 5 dilutions and 3 test series would be 15

Results

Individual animals

The seven sharks ranged from 55.5 – 82.5 cm total length and included 5 males and 2 females (Table 1). They are considered older juveniles. To facilitate subsequent analysis of individual performance we ordered the sharks A-G from best to worst based on their subsequent C-score in the whole flume.

Odor side choice (C-score; time spent on odor side)

A two-way ANOVA with dilution and test series as independent variables (Table 2) showed significant effects on time spent on the odor side for the whole flume (F-ratio = 7.2, $p < 0.0001$) and for the upstream flume half (F-ratio = 8.7, $p < 0.0001$), but not for the downstream flume half (F-ratio = 2.3, $p = 0.065$). This may be related to the physics of odor dispersal with weaker odor information farther downstream.

The seven sharks spent significantly more time in the odor side of the flume, regardless of their individual side bias, until the "squid juice" was diluted several orders of magnitude (Fig. 4). For the upstream half of the flume, the results show a simple response function with strong odor choice at the four highest concentrations ($C \sim 50$, i.e. ~75% time spent on the odor side) and no response at the lowest concentration ($C = 2$, i.e. 51% on the odor side). Contrast analysis showed indeed significant differences in odor preference response between dilutions 0-3 and 4-5 for the whole flume and between 0-4 and 5 for the upstream flume half (Table 2A, B). Because odor choice in the downstream flume half was weak ($C = +20$ to -20) and not significant overall (Table 2C), whole flume results were less pronounced than upstream results alone: for the whole flume the two highest concentrations scored $C \sim 40$ (~70%) and the two lowest $C \sim 10$ (~55%, preference not significant).

For the upstream flume half, all but the greatest dilution (10^{-5}) caused significant odor side preference (Table 3, Fig. 4B). For the whole flume the two greatest

dilutions (10^{-4}-10^{-5}) did not cause significant choice and for the downstream flume no odor preferences exceeded the significance threshold, except the 10^{-4} dilution which resulted in significant repulsion (Table 3, Fig. 4). We discuss this curious result later.

The effect of test series and thus experience on odor choice was shown by analyzing results from the greatest dilution that still caused a significant odor preference (Fig 4). For the whole flume this was 10^{-3}. Only in the third test series was this dilution causing significant preference, while in the second series it only approached significance and in the first series there was no choice (Table 3B; Fig. 5B). For the upstream flume half a similar result was seen at dilution 10^{-4} (Table 3D; Fig. 6B). These results suggest that learning may have taken place between the 3 series and that in the best series a tracking threshold upstream is found between dilutions 10^{-4} and 10^{-5}.

In the no-odor tests, side bias across animals and dilutions did not change over test series (Fig. 7A), but individual animals varied in their side bias ranging from 40% (small left bias) to 75% (right bias) with individual variance ranging from SEM=0.25-9.5% (Fig. 7B). Interestingly, the two individuals that had the greatest side bias were also the smallest (male A and female C). [Bias was taken into account by the experimental design which scored the effect of odor as a change from each animal's side bias in the same trial.]

Individual animals also varied in odor preference responses from C=7-37 in the whole flume (Fig. 5C) and C=18-47 in the upstream flume half (Fig. 6C). In both cases, the same animals sored best (smallest male A) and worst (medium-sized male G). Overall there does not appear to be a sex or size effect of odor tracking responses.

Feeding success

Feeding success as measured by the number of squid pieces eaten declined linearly with odor dilution from 40 to 22 out of a maximum of 42 possible with 7 animals in 6 trials (3 test series with 2 trials per test). The Standard Deviation showed an interesting trend with the smallest values at the dilution extremes (Fig. 8A). This suggests that at the strongest odor concentration (dilution 0) all animals did well locating the source area and getting food. The opposite may cause the same effect at the lowest concentration (dilution -5) when the task becomes uniformly difficult. In the middle (dilutions -3 and -4) we find the conditions where invidual animals vary in their response leading to greater variance in success rates.

Combining all 7 animals and 5 dilutions in two tests per trial, the feeding success rate per series increased from 46 out of 70 pieces (66%) eaten in the first experimental trial in July, to 54 (77%) in August and 62 (89%) in September (Figure 8B). The increase of successful trials suggests improvement, possibly as a result of familiarity with the flume and testing conditions.

Individual feeding success (Fig. 8C) in 6 trials at 5 dilutions had a theoretical maximum of 30 and varied from 19 (in the smallest animal, male A) to 29 (largest male D and medium male E each missed only one feeding chance)

Discussion

The goal of this research was to determine the odor dilution threshold at which sharks track an odor plume to the source. Results show first that the sharks indeed tracked odor plumes to the upstream odor release area: odor presence on the right or left side of the flume significantly changed their individual random side bias so that they spent up to 75% of their test periods on the odor side. However, this side choice varied significantly with odor dilution. First, the sharks showed a strong response in the upstream flume half where the odor signal is strongest, but no significant response downstream where odor signals are down to <0.1 % of the source concentration. Second, the sharks' response diminished to zero as the source concentration was diluted: in the upstream flume half the threshold was located between 10^{-4} and 10^{-5} dilution. Individual sharks varied in their odor response: of all animals, the smallest male consistently spent the most time on the odor side, both in the whole flume and the upstream flume half, but had the lowest feeding success rates. The largest male showed one of the weakest odor responses in the upper flume, but had a high success rate of feeding. To interpret these results we need to

consider the structure of odor plumes and the function of the sharks' olfactory responses.

As an odor is released it is most concentrated at the source and, as it disperses, it leaves behind progressively smaller eddies: the plume is a stream of constantly mixing swirls of odor and non-odor patches (Atema, 2012). These eddies mix to produce increasingly smaller amounts of chemical concentration, both the mean concentration and the concentration found in the odor patches, as sketched in Fig. 1. Thus, as an organism swims through an odor plume, from downstream to upstream, it encounters a gradient of chemical patches from weak but broadly distributed to concentrated but sparse. Odor plumes are chaotic and particularly near the source peak concentrations in odor patches are orders of magnitude greater than the mean. Therefore a reliable spatial concentration gradient can only be determined by averaging the concentrations of many local patches, a process that can take minutes and is of little use to fast swimming animals. It is likely that most animals track a plume from patch to patch using fast-adapting receptor cells and sub-second behavioral responses. This was shown in an experiment on bilateral odor responses ("stereo-smelling") with the same shark species used here, which resulted in the interpretation that animals turn into odor patches as they encounter them, thus staying in the plume (Gardiner and Atema, 2010). Losing the plume is costly, as it may take long time to reconnect.

This study has investigated the tracking threshold behavior as displayed in the small common coastal elasmobranch, *M. canis.* All sharks tested recognized and searched for squid juice at the highest concentration, as well as, odor that was diluted up to 1,000x (3 orders of magnitude). These results suggest that sharks have a moderately low threshold to track odors that occur from their prey. When allowed to search the entire flume, the lowest average response ranged between 1,000x to 10,000x (three to four orders of magnitude) (Figure 4, Table 2).

These results, for the smooth dogfish, are consistent with other odor threshold research for other marine organisms. Response thresholds to a squid attractant for Pacific Halibut (*Hippoglossus stenolepis*) was estimated at 10^{-3} and walleye pollock (*Theragra chalcogramma*) at 10^{-4} – 10^{-6} (Davis *et al.*, 2006). For the lobster (*Homarus americanus*), dactyl chemoreceptors maintained the ability to produce a response to ammonium (NH_4^-) as long as the stimulus pulses were just above a background that could be varied from 10^{-6} to 10^{-2} molar (Borroni and Atema, 1988). Lobster chemoreceptor sensitivity was altered by the presence of the background odor. Adapted receptors reset the threshold of the stimulus response at a new concentration level determined by a background concentration. From this we hypothesize that shark sensitivity may be similar.

Food deprivation increased the response threshold in both sablefish (*Anoplopoma fimbria*) (Løkkeborg *et al.*, 1995) and yellowfin tuna (*Thunnus albacares*) (Atema,

1979). In sablefish, after one day of food deprivation, the mean response threshold to total dissolved amino acids were 10^{-8} M and after four days, the sensitivity had increased even further to 10^{-11} M. Likewise in tuna, hunger motivated search behavior to amino acid constituents to a threshold of 10^{-11} M. In both studies, the intensity of behavioral responses also increased with both stimulus concentration and duration of starvation. This suggests that sharks also may be increasingly sensitive to bait odor if food was withheld before testing.

Throughout these experiments, casual observations suggest that sharks may improve in terms of success across the three series tests preformed and analysis in fact did reveal an increase individual sharks' success (Figure 5). This suggests that sharks have the ability to "learn" a trial protocol, and when rewarded positively, sharks will more affectively navigate diluted odor plumes. This finding was consistent with observations from an unpublished experiment that revealed that *M. canis* has the ability to uncouple odor from a food item. It was observed that young dogfish pups would not search an odor plume when no reward was offered (Pace, unpubl.). In earlier experiments with older juveniles and adult *M. canis* we observed the same negative learning.

The current study was performed under laboratory conditions and therefore we can only speculate how odor thresholds may differ in nature under coastal seawater conditions. For example: laboratory seawater was sand filtered, and therefore

potentially contained less odor stimuli than the ocean environment. Nevertheless, the present results suggest that *M. canis* is sensitive to even highly diluted odor. In addition, the physical nature of odor dispersal in the marine environment shows that the odor and the hydrodynamics of turbulent eddies contain information that is inextricably linked (Atema 2012; Gardiner and Atema, 2007). Searching for prey probably involves a multi-modal use of rheotaxis as well as chemotaxis in a positive feed-back loop (Davis *et al.,* 2006; Gardiner *et al.,* 2014). The use of hydrodynamic sensors of the fish lateral line helps direct and navigate the shark towards an odor source, while chemical stimuli are critical to the initiation and motivation of a foraging behavior (Gardiner & Atema, 2007, Vickers, 2000). The current research suggests that the amount of chemical information needed can be diluted by several orders of magnitudes compared to the point source (10^{-5} – 10^{-6}) to (10^{-7} -10^{-8}). It is also worth noting that there was great variation between sharks (Figure 6) and the differing abilities of individuals indicates that the threshold may be much more sensitive some than others.

M. canis commonly inhabits turbulent shallow waters acting as a scavenger and opportunistic predator. In this environment, successfully localizing prey over a long distance using visual stimuli would be limited (Bigelow & Schroeder, 1953). Visual cues in the dark (night) or because prey items are hidden are limited. This may explain why chemical cues are, at least, equally pertinent for prey capture (Gardiner, 2014). In general, fish with higher response thresholds to bait odor have more

restricted searching areas for bait (Løkkeborg *et al*., 1995). Understanding how sensitive these animals are to chemical stimuli may indicate the ranges that benthic elasmobranchs are capable of traveling to feed. In addition, research, currently in review, examines how sensitivity to chemical stimuli in *M. canis* decreases at projected checkpoints of ocean acidification (Dixson *et al.*, In Review). Relating these results to the current study may indicate how the behavior in the smooth dogfish is affected over the next 100 years.

This investigation provides new information about the threshold required to initiate plume tracking of elasmobranch. Further investigation is necessary to determine the nature of chemosensory stimulants and why prey odors are unique in noisy environments, as well as what maintains odors at ecologically meaningful concentrations. Unique mixtures of compounds may also be important, in addition to the mechanisms by which odor is concentrated in plumes; including the physical forms of odors such as lipid droplets, and the turbulent structure of odor plumes.

Tables and Figures

Table 1.

Shark ID	**A**	**B**	**C**	**D**	**E**	**F**	**G**
ID Tag	Black/Yellow	Red/Blue	Red	Green/White	Black	Yellow[2]	Light Blue
Sex	M	F	F	M	M	M	M
Total Length (cm)	55.5	77	62.5	82.5	64	66.5	66
Fork Length (cm)	47	66.5	53	71	54	53	54

Table 2.

ANOVA: Effect of Odor Dilution on time spent on odor side (C-score), across all 7 animals and 3 test series.				
A) Whole Flume	F Ratio	p-value	Contrast: 0-3/4-5 F ratio	p-value
	7.2	**<0.0001**	26.35	**<0.0001**
B) Upstream Half	F Ratio	p-value	Contrast: 0-4/5 F ratio	p-value
	8.73	**<0.0001**	10.36	**0.0018**
C) Downstream Half	F Ratio	p-value		
	2.28	0.065		

Table 3.

ODOR CHOICE **Wilcoxon Results for the Mean C throughout test series 0-3**				
A. WHOLE FLUME, 7 SHARKS, 3 SERIES	Dilution	Signed Rank	p-value	
	1	14.0	**0.016**	
	2	14.0	**0.016**	
	3	10.5	**0.031**	
	4	7.0	0.31	
	5	6.0	0.38	
B. WHOLE FLUME, 7 SHARKS	Dilution	Series	Signed Rank	p-value
	3	1.0	1.5	0.81
	3	2.0	9.5	0.062
	3	3.0	14.0	**0.016**
C. UPSTREAM FLUME, 7 SHARKS, 3 SERIES	Dilution	Signed Rank	p-value	
	1	13.0	**0.031**	
	2	111.5	**<0.0001**	
	3	74.5	**<0.0001**	
	4	68.0	**0.009**	
	5	-2.5	0.93	
D. UPSTREAM FLUME, 7 SHARKS	Dilution	Series	Signed Rank	p-value
	4	1.0	9.50	0.063
	4	2.0	-0.50	0.98
	4	3.0	12.0	**0.047**
E. DOWNSTREAM FLUME, 7 SHARKS, 3 SERIES	Dilution	Signed Rank	p-value	
	1	45.5	0.068	
	2	29.5	0.32	
	3	31.0	0.082	
	4	-52.5	**0.02**	
	5	18.0	0.52	

Figure 1. Flume, plume, odor dilution graphic.

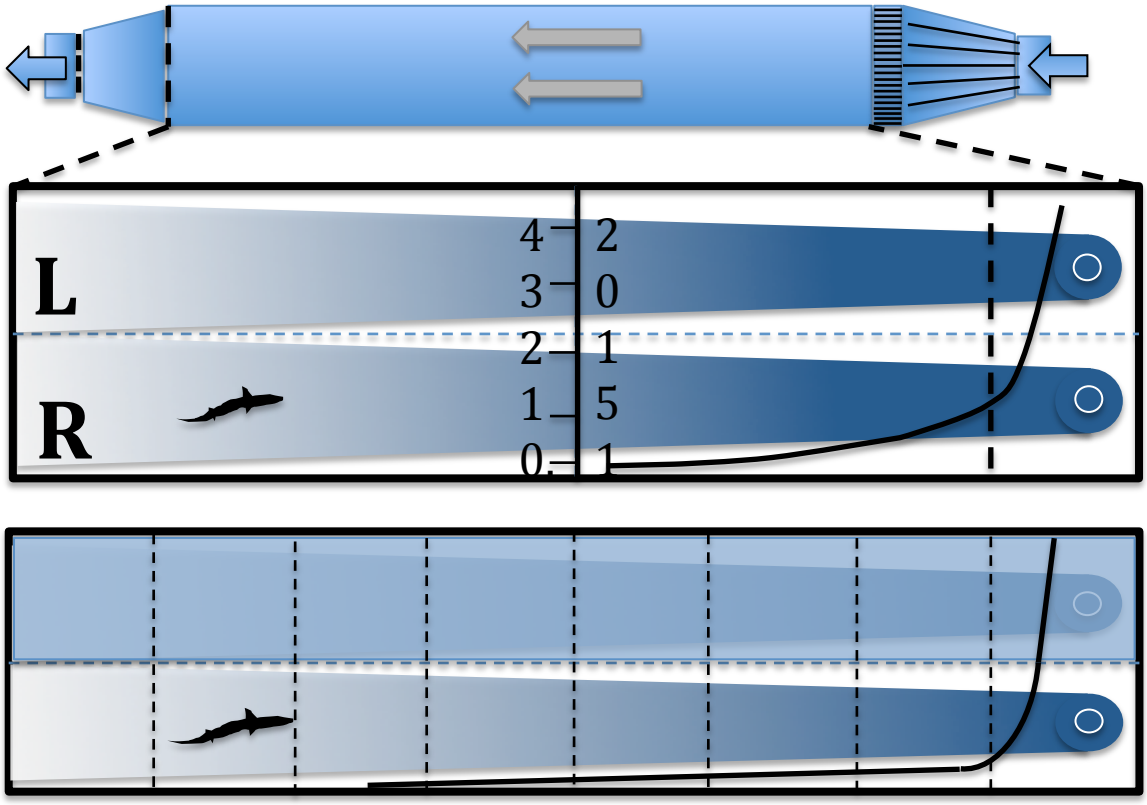


Figure 2. Flume lab with flume, two dye plumes, shark, shark home pool (blue).

Figure 3. C-score examples: calculation of odor response in relation to animal side bias.

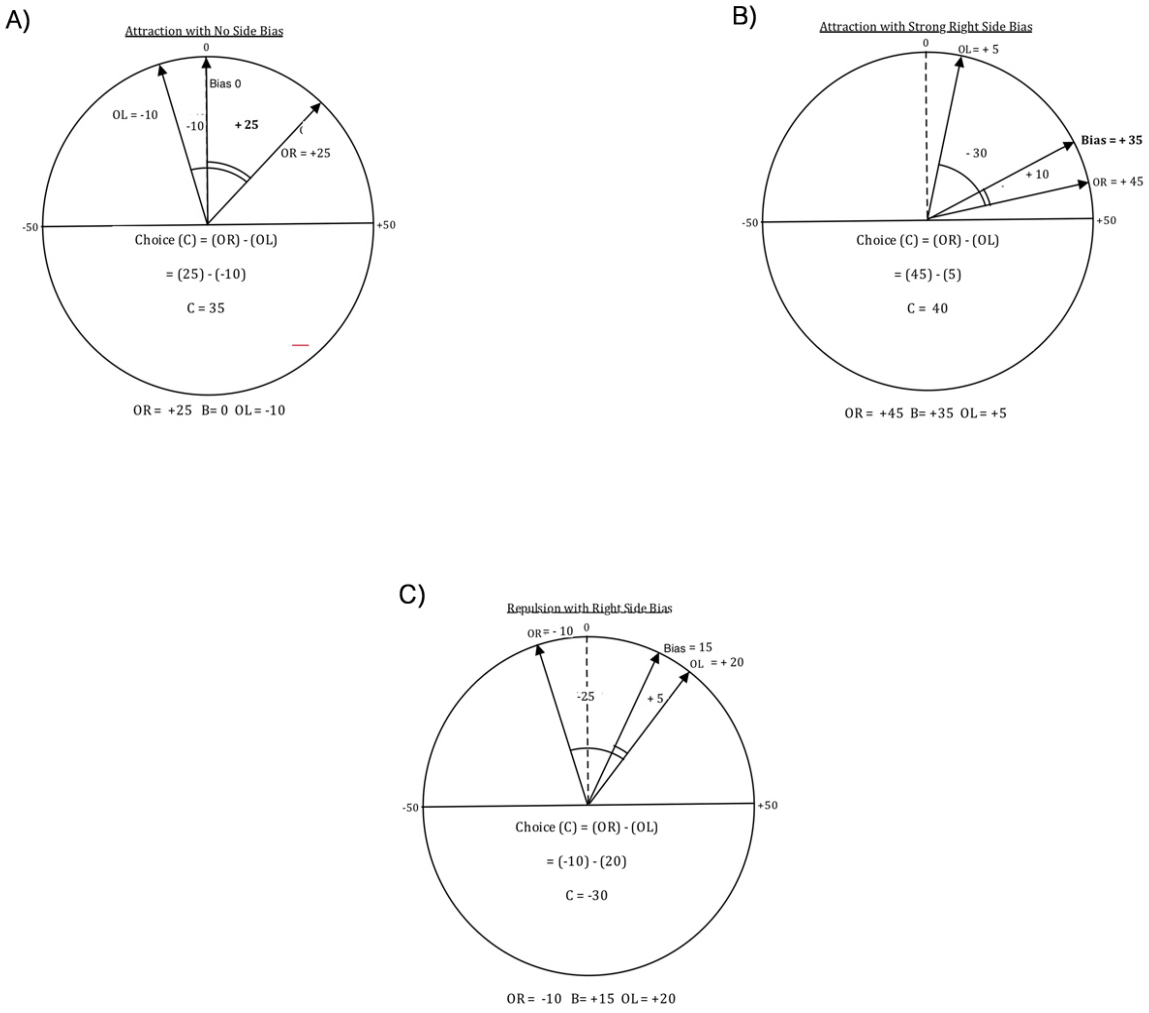
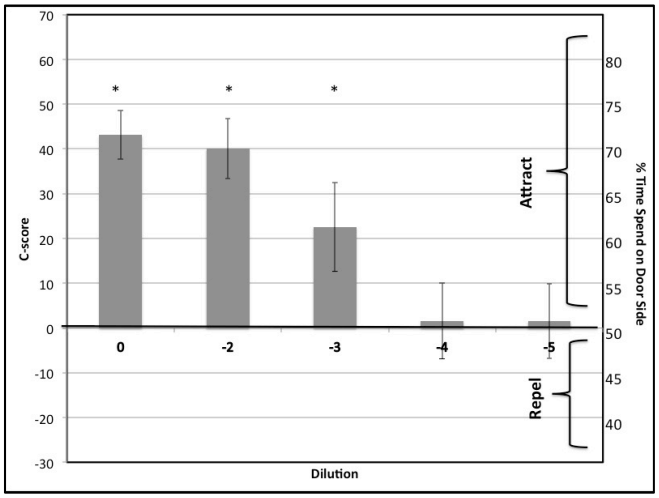
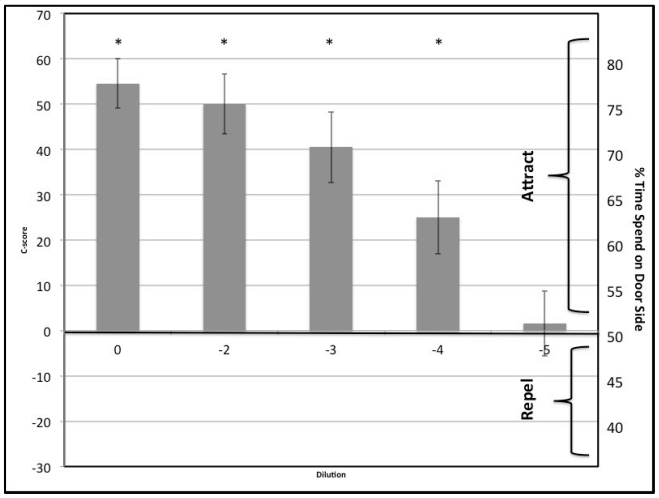


Figure 4. Odor side choice at 5 dilutions: response function and threshold

A) Whole Flume



B) Upstream Flume Half



C) Downstream Flume Half

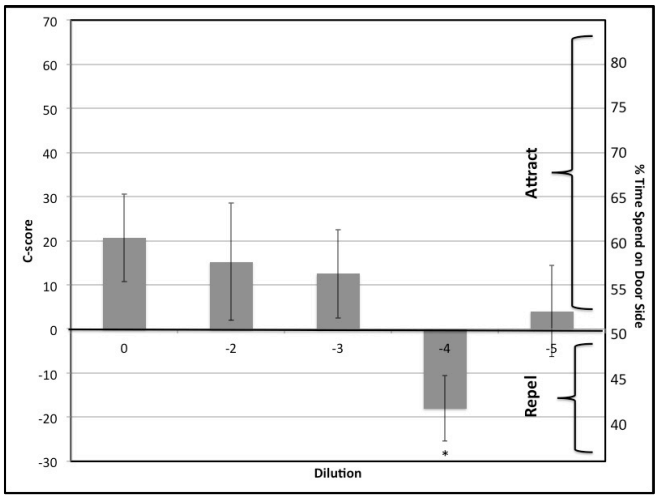
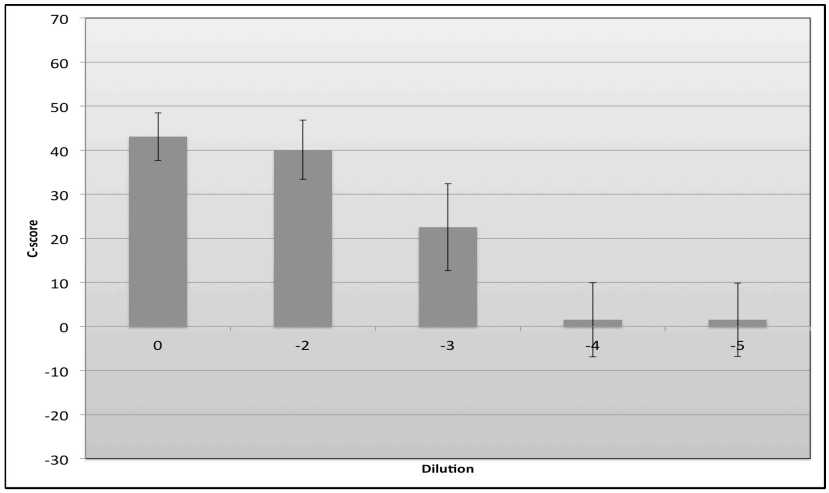
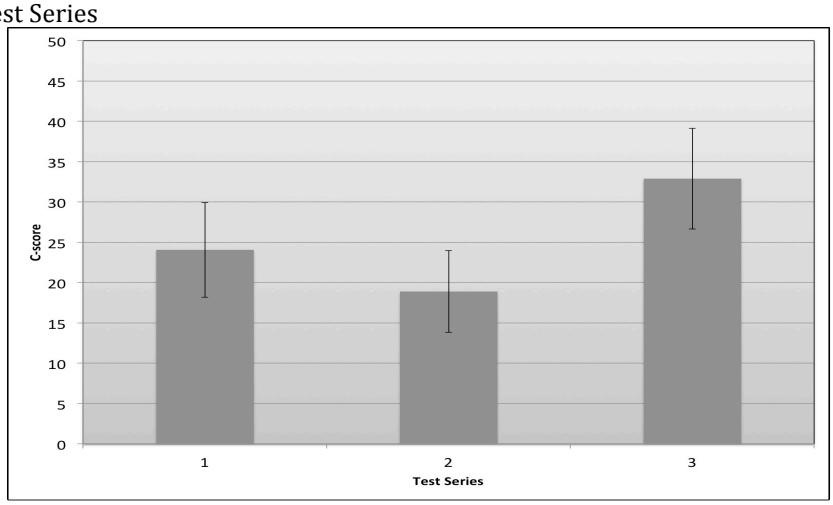


Figure 5. Odor side choice in whole flume, mean C-score (+/-SEM)

A) Dilution



B) Test Series



C) Individual Animals

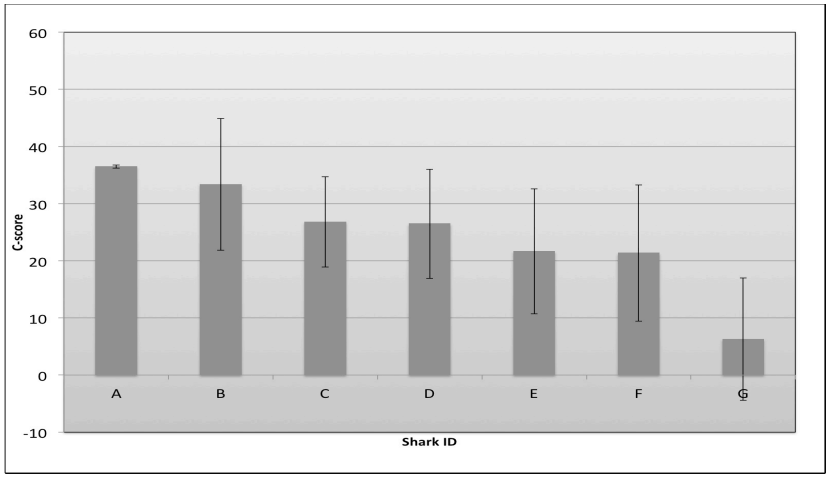
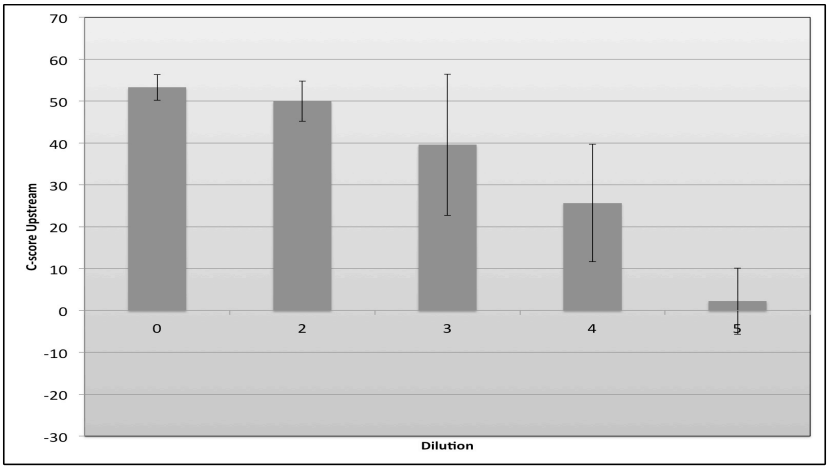
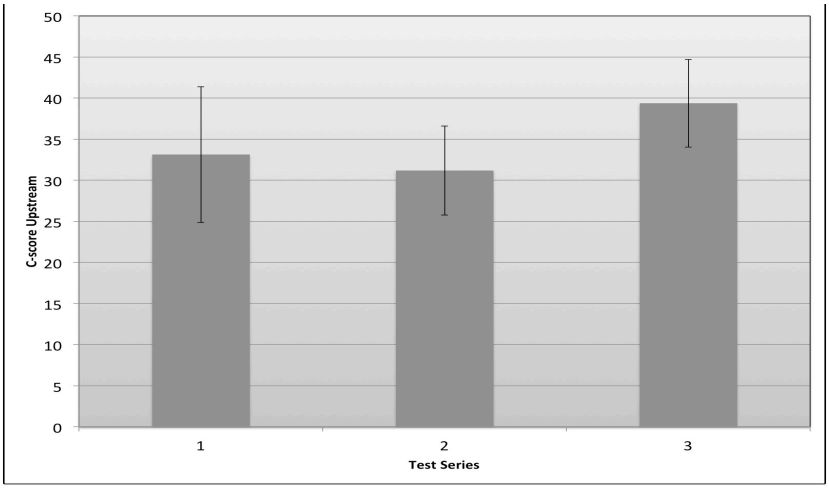


Figure 6. Odor side choice in upstream flume half, mean C-score (+/-SEM)

A) Dilution



B) Test Series



C) Individual Animals

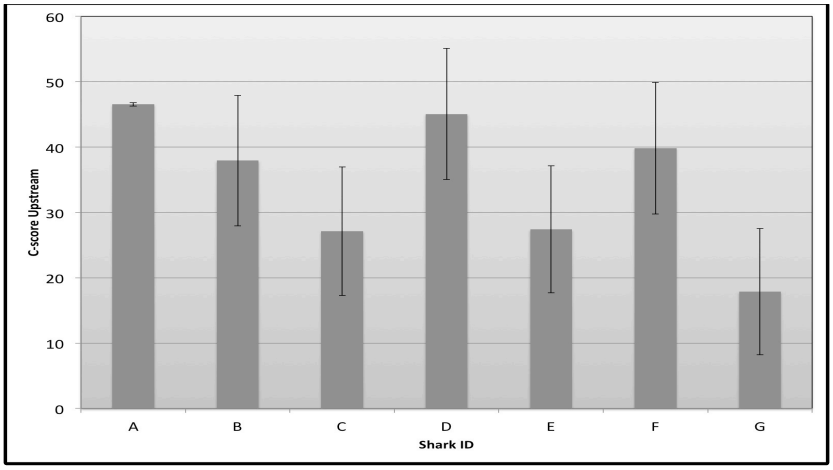
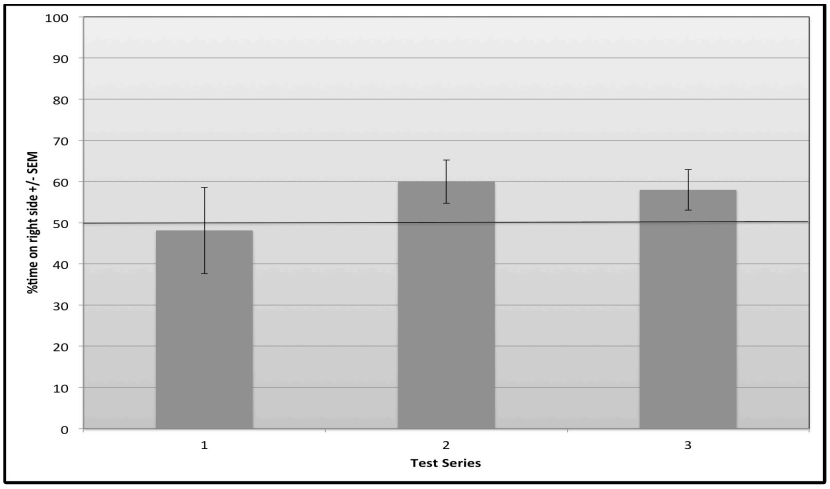


Figure 7. Side bias (Mean right Side Bias, SB +/-SEM) during control seawater tests

A) Test Series



B) Individual Animals

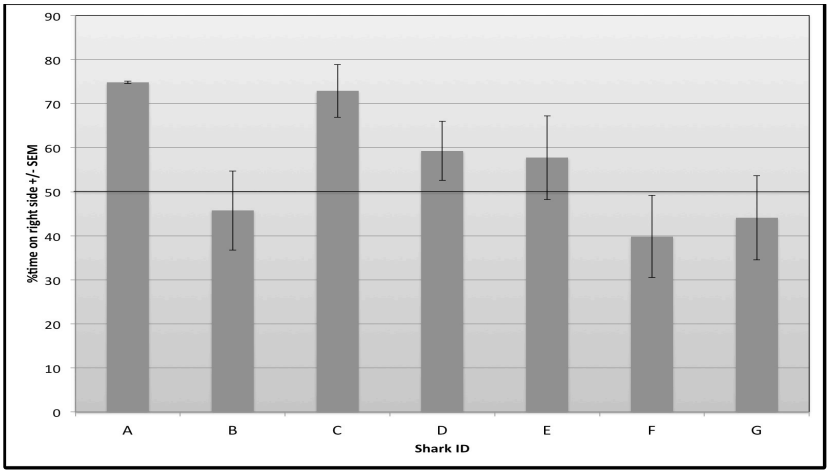
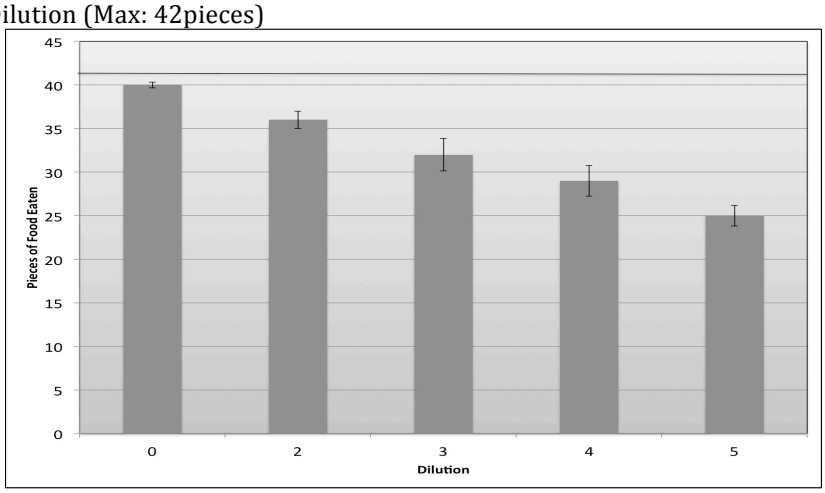
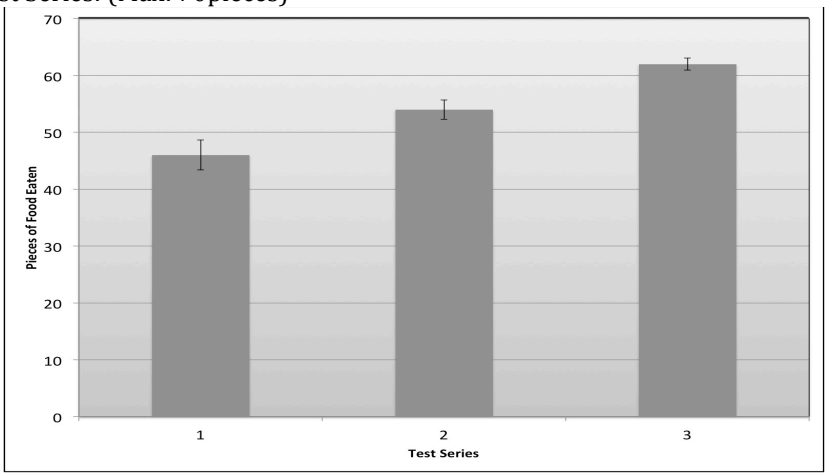


Figure 8. Number (+/- SD) of Pieces of food eaten by all sharks in the 3 series

A) Dilution (Max: 42pieces)



B) Test Series: (Max: 70pieces)



C) Individual Animals (Max: 30pieces)

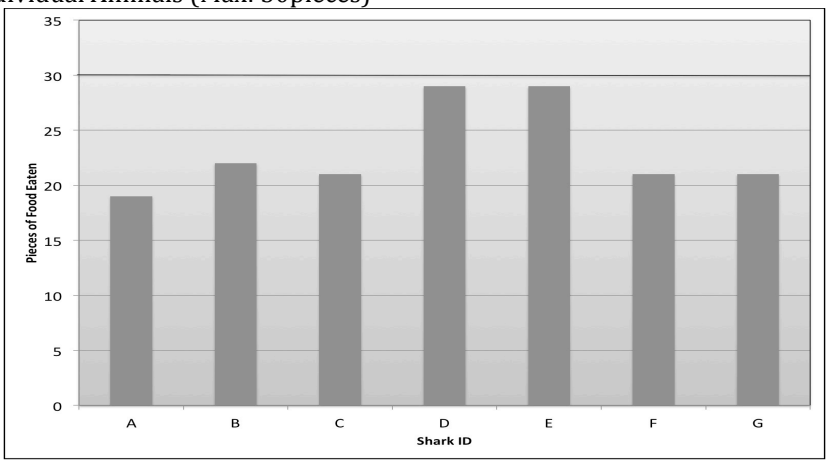


TABLE LEGENDS

Table 1. List of sharks: ID tag, sex, size (total length TL, fork length FL). Post-hoc ID order A-G by odor preference rank (C-score in Fig. 5C).

Table 2. Two-way ANOVA: Effect of Odor Dilution on time spent on odor side (C-score), across all 7 animals and 3 test series. A) Whole flume. B) Upstream flume half. C) Downstream flume half. Bold values indicate significance ($p < 0.05$)

Table 3. Wilcoxon Signed Ranks test for time spent by all 7 sharks on the odor side. A) Whole flume, dilution effect for 3 series combined; B) Whole flume, series effect on dilution 3; C) Upstream flume half, dilution effect for 3 series combined; D) Upstream flume half, series effect on dilution 4; E) Downstream flume half, dilution effect for 3 series combined. Bold values indicate significance ($p < 0.05$)

FIGURE LEGENDS

Figure 1. Flume, plume, odor dilution graphic.
Top. Flume overview. Seawater enters in an upwelling box on the right, passes through a spreader followed by a collimator (diffuser) and then moves uniformly through the 8m test section at 2.5 cm/s downstream, where it passes through a grated gate that can be lifted to open and close the start box; finally the water passes through a retention gate and drops into a return box from where two centrifugal pumps move the water at high velocity back to the upwelling box.
Middle. Two parallel plumes are generated in the source area (upstream white circles); source and bottom boundary layer turbulence are travelling downstream while diluting exponentially away from the source. At one meter downstream (vertical broken line) mean source concentration has been diluted to 1% and maximum peak concentrations are ~5%; at 4 meters the mean and peak concentration are down to 0.1% and 1% respectively. Dilution model based on Webster, 2007. Shark to scale (1m). L and R = left and right side of flume, facing upstream.
Bottom. Representations of plume dilution with and without background build-up (shown on left and right side of flume respectively).

Figure 2. Flume lab with flume, two dye plumes, shark, shark home pool (blue), and Ashley at the controls. At bottom of image: start box with raised gate releasing shark for test. Both odor and seawater plumes visualized with rhodamine-B dye for illustration purposes only.

Figure 3. C-score examples: calculation of odor response in relation to animal side bias. A) no side bias; odor attraction when presented both on the left and right. B)

large right side bias with odor attraction on both sides. C) small right side bias, but repulsion on both sides.

Figure 4. Odor side choice at 5 dilutions: response function and threshold, comparing whole flume response to upstream and downstream halves. A) Whole flume. B) Upstream flume half. C) Downstream flume half. Left axis: C-score; right axis: % time on odor side. Asterisk: C significantly different from zero (Wilcoxon Signed Ranks test $p<0.05$)

Fig. 5. Odor side choice in whole flume (C-score). Effect of A) dilution, B) test series and C) individuals. Individual sharks ordered by whole flume odor choice performance (Fig. 5C). Fig. 5A=Fig. 4A.

Fig. 6. Odor side choice in upstream flume (C-score). A) Effect of odor dilution (in 10-log steps). B) Improvement over time series. C) Individual animal differences; individual sharks ordered by whole flume odor side choice, Fig. 5C).

Figure 7. Side bias (Mean right Side Bias, SB +/-SEM) during control seawater tests. A) Series differences across all dilutions and individuals (N=35). B) Individual differences across all dilutions and series (N=15). SB >50%= more time on right; SB<50%= more time on left.

Figure 8. Source localization and feeding success: pieces of food eaten. A) Effect of odor dilution (in 10-log steps) with a maximum is 42 pieces possible B) Improvement over time series with a maximum is 70 pieces possible. C) Individual animal differences with a maximum is 30 pieces possible; individual sharks ordered by whole flume odor side choice, Fig. 5C).

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