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EXPLORING THE TACTILE AND GUSTATORY CAPACITIES OF FISH FINS

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ABSTRACT

Animals use appendage-based sensation to modulate behaviors ranging from locomotion and feeding to reproduction and object manipulation. Sensation is fundamental to many tetrapod limb functions, yet it has until recently remained largely uninvestigated in the paired fins of fishes, the limb homologues. Sensory feedback via fins can provide important information on the surrounding environment as well as on a fish's physical interactions within it. This thesis explores the capacity of fish fins to function as multimodal sensory devices in touch and taste. In chapter two, I investigate whether membranous fins may function as touch sensors. I show that fin ray afferents innervating the pectoral fin of the pictus catfish (*Pimelodus pictus*) are sensitive to pressure and light surface brushing, thus expanding the known known sensory repertoire of paired fins. In chapter three, I investigate touch and potential texture encoding mechanisms in the pectoral fins of the round goby (*Neogobius melanostomus*). I show that fish can sense coarse tactile features of their near range physical environment via fins with similar morphology and response properties to other vertebrates. In chapter four, I investigate the distribution of taste buds across the paired fins of several pomacentrids (damselfishes). While damselfish exhibit fewer taste buds than many other benthic species, I show taste buds are found at localized densities of up to 200/mm^2 and exhibit variation in their distribution across species and fins that likely reflect differences in the functional demands for chemical sensation. In chapter five, I synthesize these results more broadly and identify future research directions that will further elucidate the morphology and structure-function relationships that underpin fins as multimodal sensors.

CHAPTER ONE: INTRODUCTION

Animals use appendage-based sensation to modulate behaviors ranging from locomotion and feeding to reproduction and object manipulation. Appendages are remarkably diverse in form and function and exhibit sensory systems well adapted to meet the functional demands associated with a given environment. Mechanosensation is an import source of feedback across all animals (Kung, 2005), allowing the detection of an animals' own body movements and positions as well as of the physical characteristics of the environment. Through touch for example, the fingers of primates or the whiskers of rodents can gather information on the geometry (i.e. shape, size, and orientation) and surface features (i.e. roughness) of contacted objects (Diamond et al., 2008; Lederman and Klatzky, 1993; Saal and Bensmaia, 2014). Appendages such as the antennae of insects can also function as chemosensors, utilizing chemical cues in the surrounding air or water to detect food, suitable habitat, conspecifics, and predators (Hallberg and Skog, 2010; Hansson and Stensmyr, 2011; Schneider, 1969). Sensory feedback gathered from an appendage can often be integrated with that of other sensory pathways (i.e. vision) to facilitate the successful completion of complex behaviors (Dickinson et al., 2000; Sherman and Dickinson, 2004; Sober and Sabes, 2005).

Sensation has been shown to be fundamental to many tetrapod limb functions, yet it has until recently remained largely uninvestigated in the paired fins of fishes, the limb homologues. Fish fins serve diverse functions, often involving locomotion but also including posture, respiration, feeding, and brooding (Gibb et al., 1994; Gosline, 1994; Green et al., 2011; Higham, 2007; Künzler and Bakker, 2000; Taft et al., 2008; Westneat, 1996). While fin movements have been studied extensively, less is known about capacities of fins to function as sensors and the morphology that underpins their sensory roles. Innervated with sensory nerves and specialized

endings, fins are capable of providing proprioceptive input on fin ray position and movement that have important implications during locomotion (Aiello et al., 2016; Aiello et al., 2017; Williams IV et al., 2013). Fishes are also commonly observed to contact the bottom substrate, plants, or other animals using their body and fins (Bardach and Case, 1965; Flammang and Lauder, 2013; Gosline, 1994). While the tetrapod somatosensory system exhibits fine tactile sensitivity capable of resolving contacted surface features via a diverse set of mechanoreceptors (Johnson, 2001; Manfredi et al., 2014; Weber et al., 2013), it is unknown whether membranous fish fins can provide feedback on the external environment via touch. As a direct link to the near-range environment, feedback from fins on touch-related events and surface features, such as texture, could have important implications in locomotion, navigation, stabilization, and burying behaviors.

Fishes also use sophisticated chemosensory systems to detect the abundant dissolved chemical compounds in their environment. Unlike the taste buds of other vertebrates, fish taste buds are not only located within the oropharyngeal region, but can also be found across the body and fins in some species (Atema, 1971). While taste buds within the oral cavity typically determine food palpability, extraoral taste buds can facilitate the detection of distant chemical sources. Catfishes, for example, can locate a stationary remote food source using taste alone (Bardach et al., 1967). The gustatory system of fishes is adaptable as the abundance and distribution of taste buds vary across ecologically different fish species and often relates to feeding habits and habitat (Fishelson and Delarea, 2004; Gomahr et al., 1992; Harvey and Batty, 2002; Kiyohara et al., 1980). Studies from a limited number of species have shown that benthic fishes typically exhibit more extraoral taste buds compared to that of midwater or pelagic fishes (Davis and Miller, 1967; Gomahr et al., 1992). Living close to the bottom, demersal fishes are

often nocturnal, inhabit turbid water, and may feed on cryptic prey where vision may not be as effective and the external gustatory system is therefore of increased importance for detecting food.

This thesis examines fish fins as multi-modal sensory structures by combining behavior, anatomy, and physiology to investigate 1) the capacity of fins to sense touch, 2) whether fins can encode coarse textural features of contacted surfaces, and 3) the distribution patterns of extraoral taste buds across the fin surface. To investigate whether fins exhibit similar tactile capabilities and utilize the same design principles observed in touch sensitive limbs of other vertebrate taxa, I focus on the fins of benthic species. Bottom dwelling fishes maintain a close physical connection to the substrate and often exhibit gross morphological adaptions to their fins that presumably enhance substrate associated behaviors where tactile feedback would be beneficial. To investigate how fin sensory morphology varies across species and between fins, I focus on the distribution patterns of extraoral taste buds. Based on the location of taste buds within the outer layers of the epidermis, I can use immunohistochemical approaches to map the sensory morphology across an entire appendage. I examine the density and spatial distribution patterns of taste buds in the paired fins of several pomacentrids (damselfishes) to examine how sensory morphology relates to damselfish ecology and fin function. Damselfish are an ecologically and behaviorally diverse group of fishes that are well studied and easily obtained thus facilitating their research. Furthermore, damselfish primarily live on coral reefs which is an environment where the utility of extraoral taste buds has not been previously investigated, thus broadening the known distribution of taste buds across fishes.

In my second chapter, I investigate the capacity of membranous fish fins to sense touch. While a key feature of the tetrapod somatosensory system is the tactile perception of object

movement over the skin, it is unknown whether membranous fins may function as passive touch sensors in the absence of extensive fin movement. To examine whether fin ray afferents can respond to tactile stimuli, I examine the pectoral fins of the pictus catfish (*Pimelodus pictus*), a species that lives in close association with the benthic substrate. Kinematic analysis showed these fishes hold their pectoral fins protracted near the ventral margin of the body during steady swimming as well as at rest; a position conducive for use as passive sensory surfaces. Immunohistochemistry reveals that the pectoral fins are highly innervated with sensory fibers a subset of which terminate in putative mechanoreceptors. We find that pectoral fin ray afferents of *P. pictus* provide sensory feedback in response to tactile stimulation even in the absence of fin ray bending. These data demonstrate that touch can be sufficient to produce an afferent nerve response in a fin not specialized as a substrate probe, thus expanding the known sensory repertoire of paired appendages in fishes.

In my third chapter, I investigate touch and potential texture encoding mechanisms in pectoral fins of a bottom dwelling fish. Given the sensitivity to touch shown in fins, it was of interest whether fins exhibit similar tactile capabilities and characteristics observed in touch sensitive limbs of other vertebrate taxa. Texture perception and its neural encoding mechanisms have traditionally been studied in the primate hand, yet animals of all types interact with a variety of surfaces. Beyond the simple sensation of contact, feedback from fins on how contacted surfaces feel (i.e. roughness, slipperiness, etc.) could be important for habitat selection as well as in a variety of other behaviors such as substrate associated locomotion, navigation, posture, and burying. As a model for investigating the functional capacities of touch in fins, I studied the round goby (*Neogobius melanostomus*), a bottom dwelling species that lives on substrate types of varying roughness and whose pectoral fins frequently contact the bottom. Analysis shows that

the receptive field sizes of fin ray afferents are small and afferents exhibit response properties to tactile motion that are consistent with those of primates and other animals studied previously. Results from a rotating drum stimulator show that afferents respond to periodic stimuli (coarse gratings) when scanned across their receptive fields with a phase locked response to the temporal frequency of the stimulus. These data demonstrate that round gobies can have the capability to sense coarse tactile features of their near range physical environment with fins.

In my fourth chapter, I investigate how fin sensory morphology varies across species and fins by mapping the spatial distribution of extraoral taste buds. It has been hypothesized that pelagic species and/or fishes inhabiting clear water depend more on vision, olfaction, and mechanoreception rather than taste during feeding behaviors and thus should exhibit fewer extraoral taste buds. I explored the paired fins of damselfish (family Pomacentridae) to test this hypothesis as well as to investigate how sensory morphology relates to fin function and ecology. Using immunohistochemical techniques, I identified putative taste bud receptors in the paired fins of three damselfish species: ambon damselfish (*Pomacentrus amboinensis*), neon damselfish (*Pomacentrus coelestis*), and the blue green chromis (*Chromis viridis*). While damselfishes exhibit similar distribution patterns to other species, I found that the density of taste buds across the paired fins is less than that of many benthic fishes. *C. viridis* exhibits the highest mean density of taste buds across both the pectoral and pelvic fins, which may be an adaptation to feeding solely on zooplankton. The results presented here suggest that extraoral taste buds are more widespread and utilized among fishes than previously thought and that the functional demands for gustatory feedback via fins can be found in a myriad of habitats and environmental conditions.

In my fifth chapter, I synthesize the results from previous chapters and identify future research directions that will expand upon our current knowledge of fins as multi-modal sensors. This thesis has shown through electrophysiology that fish fins can provide feedback on touch and contacted surface features. Limiting form and function interpretations of the fin ray mechanosensory system however, has been the inability to 1) map mechanosensors across the fin and 2) match the activity patterns of a given receptor to its morphology. It is also clear that fins can function in gustation with the density and spatial distribution of taste buds serving as examples of ecomorphological adaptations to the functional demands associated with a fish's ecology. Outstanding questions remain however, regarding the function of extraoral taste buds on fins during feeding and their possible role in touch sensation. Traditionally viewed through the lens of locomotion, fins simultaneously function as adroit devices for movement and sophisticated sensors capable of providing multi-modal feedback. By investigating the multiple sensory capacities of fins, we open new windows into their sensory functions that can inform the morphology, behavior, and evolution of fishes more broadly.

CHAPTER TWO: TOUCH SENSATION BY PECTORAL FINS OF THE CATFISH *PIMELODUS PICTUS*

Abstract

Mechanosensation is fundamental to many tetrapod limb functions, yet it remains largely uninvestigated in the paired fins of fishes, limb homologues. Here we examine whether membranous fins may function as passive structures for touch sensation. We investigate the pectoral fins of the pictus catfish (*Pimelodus pictus*), a species that lives in close association with the benthic substrate and whose fins are positioned near its ventral margin. Kinematic analysis shows that the pectoral fins are held partially protracted during routine forward swimming and do not appear to generate propulsive force. Immunohistochemistry reveals that the fins are highly innervated, and we observe putative mechanoreceptors at nerve fiber endings. To test for the ability to sense mechanical perturbations, activity of fin ray nerve fibres was recorded in response to touch and bend stimulation. Both pressure and light surface brushing generated afferent nerve activity. Fin ray nerves also respond to bending of the rays. These data demonstrate for the first time that membranous fins can function as passive mechanosensors. We suggest that touch-sensitive fins may be widespread in fishes that maintain a close association with the bottom substrate.

Introduction

The paired fins of fishes, which are homologues to the limbs of tetrapods, serve diverse functions, often involving locomotion but also including defense, station holding, respiration and other roles. While fin movements have been studied extensively, little is known about the

sensory roles of fins or their relations to behavior. Past research on the mechanosensory abilities of fins has focused on a few select species and functions. The pectoral fins of dogfish (*Scyliorhinus canicula*) are sensitive to pressure and ventral flexion of the fin (Lowenstein, 1956). The filamentous pelvic rays of hake (*Urophycis chuss*) (Bardach and Case, 1965) and the finger-like pectoral fin rays of sea robins (*Prionotus* sp.) (Bardach and Case, 1965; Silver and Finger, 1984), which are used as highly mobile appendages during foraging, respond to tactile and proprioceptive (i.e. movement and position of the fin rays) stimuli. The membranous pectoral fins of more typical ray finned fishes have also been shown to function in a sensory capacity. The fin rays of bluegill sunfish (*Lepomis macrochirus*), a model species for the study of fin-based propulsion, respond to fin ray deflections and have putative mechanoreceptor structures associated with nerves that run along the fin rays from base to distal tip (Williams IV et al., 2013). The ability of *L. macrochirus* to interpret the movement and position of their fins, a form of mechanosensation distinct from the sensation of surface touch, is important for navigating complex environments (Flammang and Lauder, 2013) and modulating rhythmic fin movements (Williams and Hale, 2015). While a key feature of the tetrapod somatosensory system is the tactile perception of object movement over the skin, it is unknown whether membranous fins may function as passive touch sensors in the absence of extensive fin movement.

Fishes that commonly interact with the substrate (i.e. demersal or benthic fishes) provide a rich context in which to investigate the prevalence and functional significance of touch-sensitive fins. Feedback from the bottom, contacted objects or water movement could modulate locomotor, orientation and feeding behaviors. Fishes living at the sediment–water interface,

including many species of catfishes, gobies and blennies, often exhibit robust and broad pectoral fins (Gosline, 1994), which may function in part to increase surface area for sensory feedback.

Here we explore fins as passive sensory surfaces by examining the movement and touch sensation of the membranous pectoral fins of the pictus catfish (*Pimelodus pictus*), a bottom dwelling species native to low-visibility regions of the Amazon River (Ibarra and Stewart, 1989; Sioli, 1984). The leading-edge fin ray of their pectoral fin is modified into a hardened spine with serrated dentations along both edges (Figure 2.1) (Alexander, 1966). Associated in some species with a poison gland, the spine can be locked into place when threatened, to prevent ingestion (Bosher et al., 2006; Halstead et al., 1953; Wright, 2009). Given this morphology, the pectoral fins are presumed to serve a primarily defensive role (Reed, 1924; Reed and Lloyd, 1916), yet they still retain soft rays connected by a membrane behind the leading-edge spine. *Pimelodus pictus* provide an interesting comparison to the bluegill sunfish, whose pectoral fins are composed entirely of soft rays and act as the primary propulsive structure

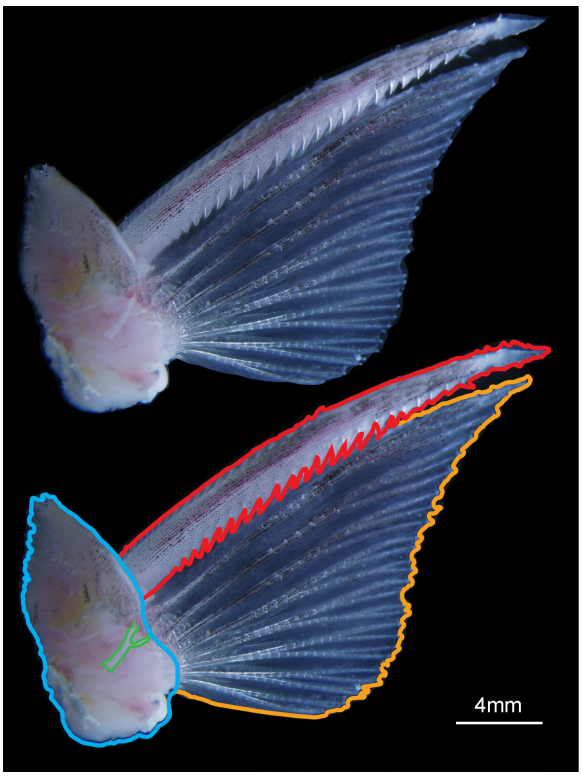

Figure 2.1: Pictus catfish (*Pimelodus pictus*) pectoral fin and girdle (right fin; dorsal view). At bottom, elements are outlined for clarity: a portion of the pectoral girdle (blue), fin ray afferent (green), leading-edge spine (red) and soft fin rays (yellow). Presumed to function in a primarily defensive role, the pectoral fins retain soft rays connected by a membrane behind the leading-edge spine. Prior to electrophysiology, the dermal layer and connective tissue of the pectoral girdle are removed to expose fin ray afferents (shown in green: bottom) innervating the base of the fin rays. Scale bar: 4 mm.

for low speed locomotion and midwater station holding (Gibb et al., 1994; Jones et al., 2007; Peng et al., 2007). In bluegills, fin ray bending associated with propulsion is accompanied by the phasic generation of action potentials by fin ray afferents (Williams IV et al., 2013). The spined structure and presumed nonpropulsive function of catfish pectoral fins would seem to limit the mechanical stimulation of the rays via bending during locomotion. However, given the fins' close proximity with the substrate, these fishes may be able to feel the immediately local environment in the absence of extensive ray bending.

To address whether fins may be acting as touch sensors, we combine behavior, neuroanatomy and physiology to examine *P. pictus* pectoral fin movement and response to touch. We describe positioning of the pectoral fins during swimming, and examine the neural architecture of the fin rays and membrane through antibody staining of nerve fibres and sensory endings. Additionally, we determine mechanoreceptive capabilities of the catfish pectoral fin by exerting touch and bend stimulations of the fin while recording from afferent nerve fibres. By addressing fin sensation in a different functional morphology and behavioral context from previous work, this investigation aims to increase our understanding of the diversity of pectoral fin mechanosensation. In addition, investigations of such systems will help to elucidate how mechanosensory surfaces are organized in biological systems and through the evolution of fins and limbs. In the applied realm, fins and their associated sensory morphology may provide insight into the design of engineered sensory membranes, particularly for use in aquatic environments.

Materials and Methods

Animals:

Pimelodus pictus were obtained commercially and housed in 40L aquaria at the University of Chicago (Chicago, IL, USA) under seasonal day:night light cycles with a water temperature of 258C. Fish used for immunocytology and physiological experiments were euthanized in a 0.5 g/L solution of MS-222 (tricaine methanesulfonate, Sigma-Aldrich, St. Louis, MO, USA) in water.

Pectoral fin movement during swimming behavior:

To understand how *P. pictus* position their pectoral fins during free rhythmic swimming behaviors, fish (n = 5) were filmed in a 20.4 x 20.5 cm tank over a mirror angled at 45°, which allowed for the simultaneous recordings of the lateral and ventral views. Fish ranged in size from 5.0 to 7.9 cm (mean = 6.2 cm, SD = 1.18 cm) standard length and required several tail-beat cycles to swim the length of the experimental tank. While a fish swam freely in the tank, video was recorded at 250 frames/s with a Basler A504 K high-speed video system (Basler Vision Technologies, Ahrensburg, Germany) and XCAP software (Epix, Buffalo Grove, IL, USA). For analysis, we restricted swimming trials to swimming during which the fish was moving rhythmically forward without turning and during which all parts of the fish were at least two pectoral spine lengths away from the walls of the tank. For four of the five individuals, we selected the first five bouts of swimming behavior that met our criteria and analyzed the first full tail-beat cycle from each bout. For an additional individual, behavior consisted of a single extended swimming bout, so we analyzed consecutive tail-beat cycles of that one bout. Pectoral fin and tail angles relative to the body axis were measured for each frame of the tail-beat cycle by selecting seven landmarks on the ventral view image of the fish, with a customized digitizing program in MATLAB (MathWorks, Natick, MA, USA). The tip of the snout and the midpoint of

the body between the pelvic fins represented the body axis. The tail axis was represented by the midpoint of the body between the pelvic fins and the fork of the caudal fin. The distal tip and insertion point of the leading-edge pectoral fin ray were marked on both the left and right pectoral fins. Angles were compared at each eighth of the tail-beat cycle, including the beginning and end of the cycle, for a total of nine time points per cycle. Lateral view videos were used to assess whether there was dorsoventral movement of the fin during swimming. This was done through visual inspection of video that was digitized.

Neuroanatomy of pectoral fins:

Antibody staining methods were modified from Thorsen and Hale (2007) and Svoboda et al. (2001). Four fish were stained and imaged. Fins were preserved in 4% paraformaldehyde in phosphate-buffered saline (PBS), then dehydrated by stepping into 100% methanol at 48C for long-term storage through PBS : methanol ratios of 3 : 1, 1 : 1 and 1 : 3. Before staining, fins were rehydrated by stepping up into PBS through PBS : methanol ratios of 1 : 3, 1 : 1 and 3 : 1. To permeabilize tissues, fins were incubated in deionized water for 60 min at room temperature, then in acetone at -20°C for 7 min. After rinsing in de-ionized water, fins were incubated in collagenase for 60 min then rinsed in PBS. Fins were blocked in 10% bovine serum albumin (BSA) in PBS containing 0.1% Tween-20 (PBST). Fins were incubated at 4°C in 10% BSA in PBST with both primary antibodies simultaneously. To visualize nerves, the primary antibody was a monoclonal antibody to a neurofilament associated protein (3A10, Developmental Studies Hybridoma Bank, Iowa City, IA, USA). For putative mechanoreceptors, a monoclonal antibody to cytokeratin 20 was applied (ab76126, Abcam Inc., Cambridge, MA, USA). Cytokeratin 20 has been used in human skin as a marker for Merkel cells, which are specialized mechanoreceptor

cells associated with afferent nerves [21]. Each primary antibody was used at a concentration of 1 : 100. After 2 days, fins were rinsed in PBST for approximately 5 h, then incubated in 10% BSA in PBST containing both secondary antibodies simultaneously. The secondary antibody to visualize nerves was goat antimouse antibody conjugated to rhodamine (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). For putative mechanoreceptors, the secondary antibody was goat antirabbit IgG (H þ L) conjugated to fluorescein (A-11012, Invitrogen Molecular Probes, Eugene, OR, USA). Each secondary antibody was used at a concentration of 1 : 100. Fins were removed from secondary antibodies after 1 –2 days and stored in PBS at 4°C until they were imaged. Pectoral fins were imaged using a Zeiss 510 confocal microscope (Thornwood, NY, USA). Images of the ventrolateral surface of the fin were captured as epithelial pigmentation on the dorsomedial surface made nerve structures difficult to visualize.

Physiology of fin ray afferents in response to mechanical stimulation:

After exposure to a lethal dose (0.5 g/L) of MS-222, the pectoral fin and associated musculature of the pectoral girdle were excised from the body. To immobilize the fin for electrophysiology, pins were inserted through the muscle of the pectoral girdle to the bottom of a dish lined with Sylgard (Dow Corning, Midland, MI, USA). Fin rays were clamped down at their proximal ends using small diameter tubing to prevent additional movement. The membrane between each fin ray was cut to isolate individual rays for mechanical stimulation. To maintain responsiveness for electrophysiology, the dish was filled with extracellular solution prepared according to the methods of Masino and Fetcho (2005) and refreshed periodically throughout the experiment.

Electrophysiology methods followed Williams IV et al. (2013). To make electrodes, borosilicate glass capillaries (GC150F-7.5 1.5 mm OD, 0.86 mm ID, Harvard Apparatus, Holliston, MA, USA) were pulled using a Flaming/Brown micropipette puller (model P97, Sutter Instrument Co., Novato, CA, USA) and firepolished using a microforge (MF-830, Narshige, East Meadow, NY, USA). Electrode diameter ranged from 10 to 30 mm. Electrodes were filled with extracellular solution and mounted on an Axon Instruments CV-7B headstage (Molecular Devices, Foster City, CA, USA). Prior to electrode attachment, afferent nerves on the dorsomedial surface were exposed via dissection of surrounding dermal layer, muscle and connective tissue (Figure 2.1). This orientation provided for more reliable nerve recording, as afferents were more readily accessible with the dorsal fin surface facing up. Multi-unit extracellular recordings were obtained with a Multiclamp 700B amplifier in current-clamp mode. Voltage recordings were digitized with a DigiData 1440A digitizing board (Molecular Devices) and acquired using pClamp 10 software (Molecular Devices). Video of the stimuli was recorded at 125 frames/s using a Basler high-speed video camera and synched with the electrical recordings using an external signal.

Pectoral fin rays were exposed to mechanical stimulation designed to examine afferent responses to ray bending, pressure applied perpendicularly to the ray and surface brushing along the fin ray surface (Figure 2.2). Stimuli were performed via an actuator mounted on a voice coil positioning stage (VCS10-023-BS-01-M, H2 W Technologies Inc., Valencia, CA, USA) that was controlled by a programmable driver (Intelligent Servo Drive IDM640-8EI, Technosoft, Canton, MI, USA). The SOMLAB software system (developed by John F. Dammann III, University of Chicago) was used to generate stimuli. To separate the effect of movement distance from velocity, we applied step-and-hold and ramp-and-hold stimuli, respectively. Both types of

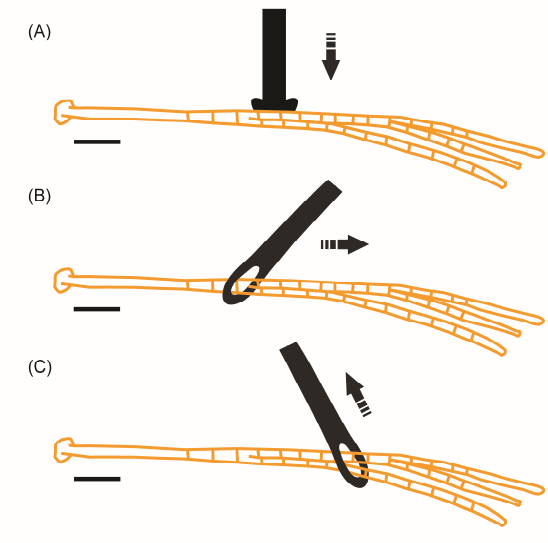

Figure 2.2: Touch and bend stimulation applied to the fin rays. Mechanical stimulation was generated via a probe connected to a linear actuator. (A) Fin rays were exposed to pressure perpendicular to their dorsal surfaces via the flat head of a pin (1.4 mm diameter). (B) To create a brush stroke, a single fin ray was threaded through the eye of a needle. The probe (i.e. the needle) was positioned so that movement occurred directly along the proximodistal axis of the ray to generate the sensation of object motion over the fin. (C) The distal end of a fin ray was threaded through the eye of a needle and clamped to prevent slip. Fin rays were deflected towards their proximal end to generate ray bending. Scale bar is 1 mm.

stimulus consisted of an initial displacement followed by a period of stasis until the probe was withdrawn. During step-and-hold stimuli, the velocity was held constant while the displacement distance varied. Conversely, for ramp stimuli we used five different velocities while the final displacement distance was held constant. The hold period allowed for the examination of afferent activity while the fin rays were held in a fixed deflected position. Multi-unit recordings were taken from the right pectoral fin of five individuals ranging in size from 7.2 to 8.8 cm (mean = 7.56 cm, SD = 0.69 cm) standard length, with three to four repetitions of each stimulus type.

Afferent responses to pressure were assessed using the blunt head of a pin (1.4 mm diameter) attached to a force transducer (MLT1030/A; ADInstruments, Colorado Springs, CO, USA). Once positioned perpendicular to the dorsal fin ray surface, we applied a randomly presented series of step-and-hold (0.12, 0.3, 0.6, 1.2, 1.8 mm) and ramp-and-hold (1.2, 2.4, 12, 24, 48 mm/s; final amplitude of 1.2 mm) stimuli. Data from the force transducer indicate that these indentations imparted on average, a force equivalent to approximately 0.5, 1.5, 3, 6 and 9 g onto the fin ray, respectively. To prevent fin ray damage the hold period was limited to 3 s and the bottom of dish in which these experiments occurred was lined with Sylgard, a highly

compliant material. We also exposed fin rays to light surface brushing by passing the eye of a needle along the length of a ray. The probe, positioned near the middle of a fin ray, moved towards the distal tips precisely in the direction of the rays' proximodistal axis via a randomly presented series of extensions at different distances (0.6, 1.2, 2.4, 3.6, 4.8 mm at 2 mm/s) and velocities (0.6, 1.2, 2.4, 3.6, 4.8 mm/s; final brushing distance held constant at 2.4 mm). In an effort to examine afferent responses to fin ray bending individual fin rays were connected to the actuator and exposed to a randomly presented series of step-and-hold (1.2, 1.8, 2.4, 3.0 and 3.6 mm) and ramp-and-hold (0.8, 1.2, 2.4, 4.8, 9.6 mm/s; final displacement fixed at 2.4 mm) bending stimuli. The hold period during fin ray bending trials was 5 s.

Electrophysiology analysis:

We used MATLAB 7.10.0 (Mathworks) to down-sample (100 - 10 kHz), filter (second-order elliptical filter) and analyze the physiological data. We performed analysis of variance (ANOVA) and paired t-tests on the full datasets. Linear regressions were performed on data from each individual due to variability among fish. Statistical analyses of the mechanical stimulation data were performed using JMP software (SAS, Cary, NC, USA). Spike rate was calculated as the number of action potentials observed divided by the duration of the relevant observation period in seconds. We defined a burst of action potentials as three or more spikes occurring within 50 ms of each other. The first action potential recorded after the start of a stimulus was considered the beginning of a burst. For the purposes of our statistical analyses, trials that did not record a burst of action potential were coded as 0 for burst duration(s) and activity rate during the burst. Spike rate analysis during the hold interval varied across trials. The hold period during pressure trials was limited to a 1.5 s portion of the 3 s hold interval. This 1.5 s period began 1 s

after the cessation of movement, to prevent inclusion of activity in response to the indentation. This portion of the hold interval was compared with a 0.5 s prestimulus baseline. During fin ray bending trials, the hold period was limited to a 3 s portion of the 5 s hold interval. The 3 s portion of the hold period began 1 s after the step onset and was compared to the prestimulus baseline. To identify and sort individual units from our extracellular recordings, we used a modified version of the spike sorting software, Wave_clus (Quiroga et al., 2004). Wave_clus provides an automatic method of spike sorting based on wavelet decomposition and superparamagnetic clustering (SPC). To detect spikes, we set a 1 ms absolute refractory period and used an amplitude threshold five times the standard deviation of the background noise. The minimum cluster size was set to 20 units.

Results

Pectoral fin movement during swimming behavior:

Kinematic analysis focused on steady forward swimming with the goal of determining whether the pectoral fins were actuated rhythmically during this behavior. From simultaneous lateral and ventral views, we observed that pectoral fins were positioned near the ventral surface of the animal, with the distal tip of the fins angled laterocaudally and ventral to the fin base. We found that typical slow swimming included tail-beat frequencies of 4.83+1.25 Hz (mean + SD.) and axial oscillations with peak tail angles of 16° + 9°. In this axial locomotor context, the pectoral fins remained partially protracted over the course of a fin-beat cycle, at a relatively constant protraction angle from the body axis of 35° + 13° (Figure 2.3). One-way analysis of variance (ANOVA) showed no significant difference among pectoral fin angles at each eighth of the tail-beat cycle (ipsilateral fin, $F = 0.09$, $p > 0.9994$; contralateral fin, $F = 0.13$, $p > 0.9974$).

For comparison, ANOVA showed the tail angle varied significantly over the same time points ($F = 40.21$, $p < 0.0001$). At each of these time points, there were no significant differences between pectoral fin angles on opposite sides of the body ($p > 0.2959$). Although we focused on steady forward swimming, we also noted that when turning or encountering the wall of the tank, there was more pectoral fin movement, suggesting possible roles in braking and maneuvering.

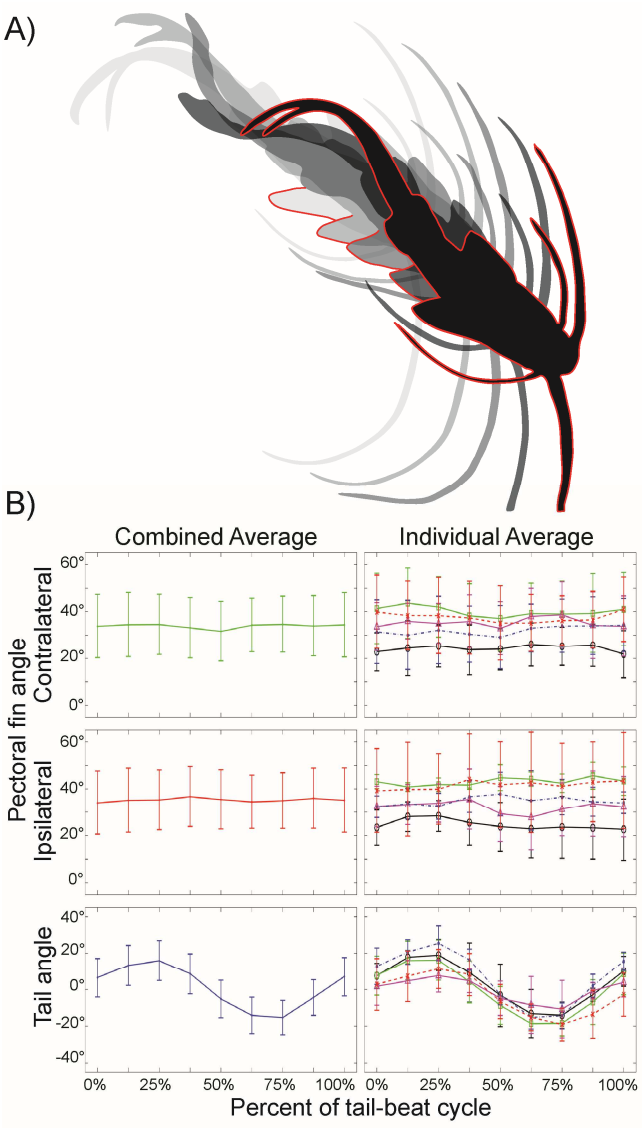

Figure 2.3: Pectoral fin, tail and body positioning of *P. pictus* during tail-beat cycle. (a) Ventral silhouettes taken from movie stills to illustrate the position of the tail and pectoral fins over 0% (light grey; far left), 25%, 50%, 75% and 100% (black with red body outline; far right) of the tail-beat cycle. Each right pectoral fin is outlined in red. Total length of tail-beat cycle shown was 0.114 s. (b) Mean pectoral fin and tail angles over the tail-beat cycle. Left column shows the combined mean angles of five fish over a total of 25 tail-beat cycles. Right column shows mean angles for five fish each over five tail-beat cycles, with each fish represented by a different line and symbol. While the tail shows large oscillations indicative of thrust production, the pectoral fins show no regular movement pattern over the tail-beat cycle. Error bars are 1 SD above and below mean.

Mechanosensation of pectoral fins:

The pectoral fin rays and membrane of *P. pictus* are heavily innervated, presumably by sensory fibres since these are non-muscular structures. Generally, nerves ran parallel to the fin rays (Figure 2.4) and were observed from the base of the rays to near the distal tips. Individual processes branched from the nerves as they travelled distally. It is still unclear whether each process corresponds to a different neuron or if there are multiple branches associated with each cell. Structures staining with an antibody to cytokeratin 20, a mechanoreceptor marker, were also present throughout the fin. In places, these structures were closely associated with nerve endings (Figure 2.4), often near sites of nerve branching. We attribute the lack of a visible afferent association with all the putative Merkel cell structures shown to imaging limitations due to depth of field and resolution, as well as possible incomplete antibody staining. Along the length of the leading-edge spine, branching nerve structures were observed between the tooth-like serrations.

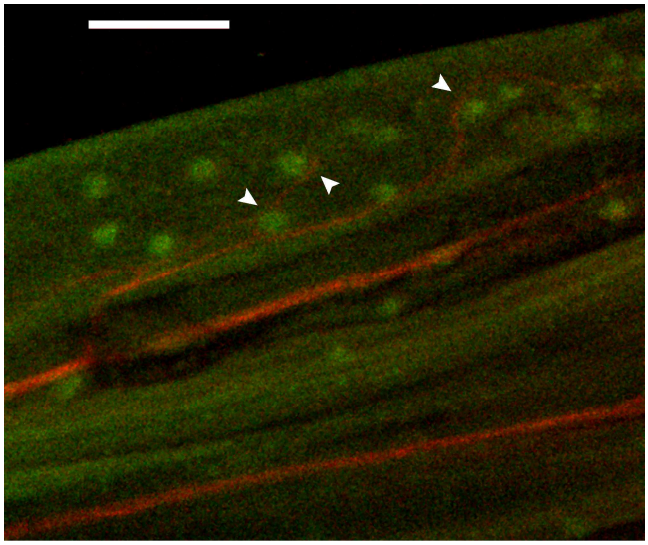

Figure 2.4: Immunostained pectoral fin rays showing nerves and associated putative mechanoreceptors. Nerves (red) run from the base of the fin to the fin rays' distal tips. Structures (green) present along the nerves stain with an antibody to cytokeratin 20, a mechanoreceptor marker. These putative mechanoreceptor cells are present throughout the fin and in places (denoted by white arrows) are closely associated with nerve endings. Scale bar: 200 mm.

Afferent nerve response to mechanical stimulation of pectoral fins:

Pectoral fin ray afferents of *P. pictus* provide sensory feedback in response to tactile stimulation even in the absence of fin ray bending. In response to localized pressure exerted

perpendicular to the dorsal fin ray surface, afferents exhibited bursts of increased spike rate (one-way ANOVA, $F = 15.46$, $p < 0.0005$) at both the onset and offset of contact compared with prestimulus baseline rates. The duration of the burst of activity associated with the initial contact increased with increasing force when assessed across all individuals and steps (one-way ANOVA, $F = 3.71$, $p < 0.0204$; Figure 2.5). While bursts of action potential were consistently recorded at the largest indentation force of 9 g, only a subset of trials recorded bursts at the lowest contact forces of 0.5 g (1 of 21 trials) and 1.5 g (6 of 21 trials). Afferent firing rate also correlated with the rate at which fin ray contact was made. We found that the spike rate (spikes/s) during the initial application of pressure increased with increasing velocity when assessed across all individuals and velocities (one-way ANOVA $F = 9.35$; $p < 0.0002$). Overall, these data suggest that the catfish fin ray afferents are sensitive to pressure exerted perpendicular to the fin ray surface.

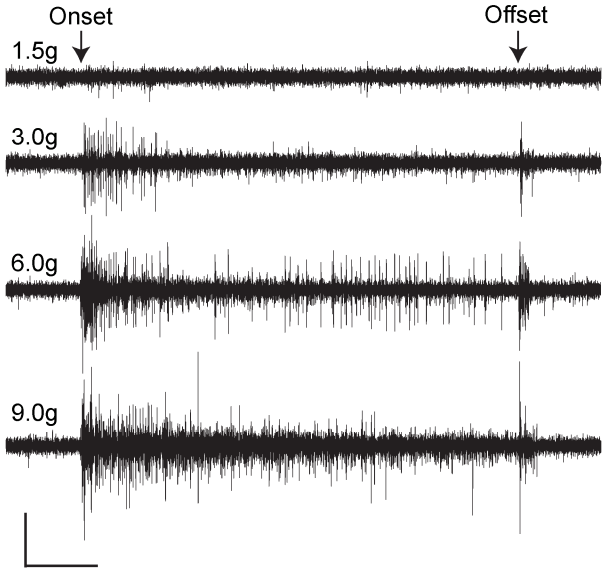

Figure 2.5: Physiological response of fin ray afferents to pressure exerted perpendicular to the dorsal fin ray surface. Responses to 1.5, 3.0, 6.0 and 9.0 g step indentations are shown. Nerve activity reflects the force of indentation. The duration of the burst of activity associated with the initial contact increased with increasing force when assessed across all individuals and forces (one-way ANOVA, $F = 3.71$, $p < 0.0204$). Scale bar: x = 1 s, y = 0.02 mV.

We also found that afferents exhibited a robust response to surface brushing along the proximodistal axis of the fin. During the brush stroke, we recorded an increased spike rate

(Student's t-test, $t = 5.69$, $p < 0.0039$) compared with prestimulus baseline rates and an increase in the number of stimulus-evoked spikes with increasing stroke distances (one-way ANOVA $F = 17.3$; $p < 0.0001$). In four of the five individuals examined in this study, linear regressions show a significant positive correlation exists between afferent activity and brushing velocity (Table 2.1). Spike sorting analysis of 2.4 mm brush strokes conducted at different velocities indicate that as the probe moved along the ray several afferent units were active. We expected to observe the sequential activation of separate units as the probe encountered more distally located mechanoreceptors, but units often showed consistent activity throughout the duration of the 2.4 mm brush stroke (Figure 2.6). As the receptive field of mammalian Merkel cells has been recorded at 2–3 mm [24], we attribute this sustained activity to the probe moving within the receptive field of several sensory endings. Information about stimulus velocity and direction might be encoded by the activation pattern of these discrete afferent units. While we found that the same units were consistently active at all velocities tested, units recorded during a single brush varied in their firing pattern, number of stimulus-evoked spikes and latency times. Despite these differences, however, the spatio-temporal discharge pattern between trials conducted at different velocities was visually similar, further supporting our hypothesis that the pectoral fins of *P. pictus* are capable of functioning as passive touch sensors (Figure 2.6).

Table 2.1: Linear regression results for brush stroke velocity by afferent activity rate (spikes/s)

Individual	n	Slope	y-intecept	r^2	*F*-ratio	*P*-value
1	15	2.70	7.14	0.23	3.9	0.0697
2	20	23.27	21.95	0.89	148.25	<0.0001*
3	20	2.30	4.05	0.5	18.29	0.0005*
4	20	10.12	1.19	0.96	557.74	<0.0001*
5	20	0.91	5.11	0.25	6.11	0.0236*

* Asterisks indicate a significant positive correlation

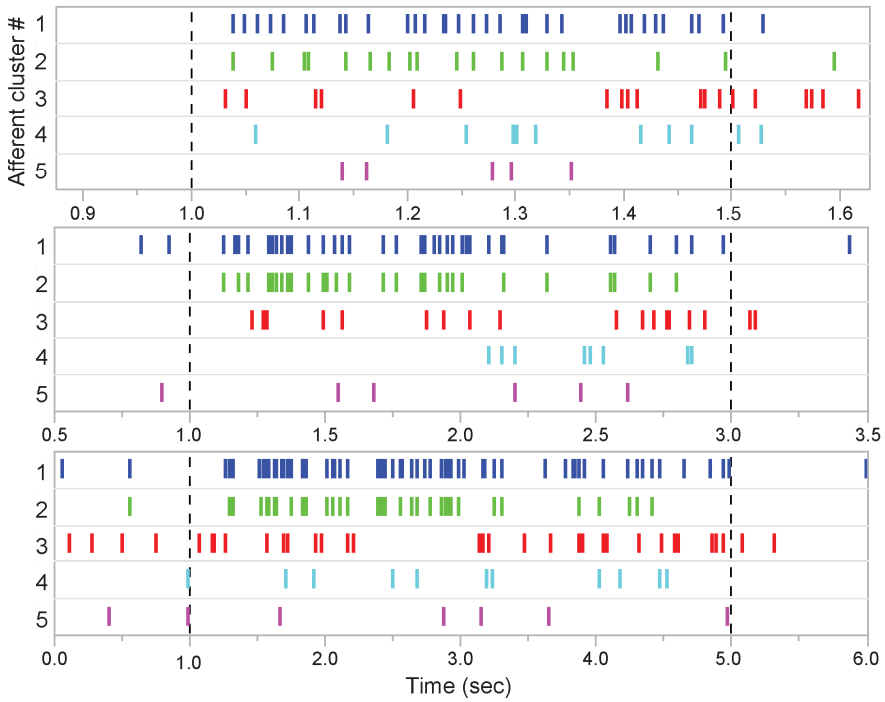

Figure 2.6: Raster plots of spike sorted afferent units in response to light surface brushing. Plots show the identity and timing of spikes corresponding to particular neurons during a 2.4 mm brush stroke conducted at three different velocities: (a) 4.8 mm/s, (b) 1.2 mm/ and (c) 0.6 mm/s. Each row represented by a different color shows spikes from a particular neuron. Dotted lines represent the onset and offset of the brushing stimulus. The spatio-temporal discharge pattern between trials conducted at different velocities is visually similar. The same afferent units are active at all velocities tested, but exhibit variation in their firing pattern, number of stimulus-evoked spikes and latency times.

Analysis of physiological responses to fin ray bending indicates that, similar to the bluegill sunfish, *P. pictus* receive feedback on both fin ray movement and position (Figure 2.7). We observed bursts of increased spike rate during step-and-hold stimuli (one-way ANOVA, $F = 32.39$, $p < 0.001$) at both the onset and offset of ray bending compared with prestimulus baseline rates. Furthermore, nerve activity reflected the amplitude of the bend, as the duration of the burst associated with the initial deflection increased with increasing deflection amplitudes when assessed across all individuals and step amplitudes (one-way ANOVA, $F = 6.45$, $p < 0.0017$). While bursts of action potential were always recorded at step onset of the largest bending

amplitude of 3.6 mm, bursts were only observed in a subset of trials at the lowest bending amplitude of 1.2 mm. Afferent activity also provides feedback on fin ray position as we found that spike rate (spikes/s) during a 3 s portion of the 5 s hold interval of step-and-hold stimuli was significantly higher than prestimulus baseline rates (Student's t-test, $t = 3.65$, $p < 0.0064$).

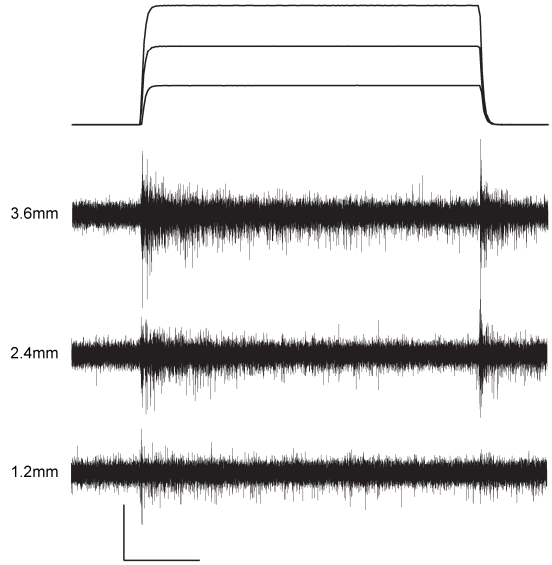

Figure 2.7: Physiological response of fin ray afferents to fin ray bending. Responses to 3.6 mm (top), 2.4 mm (middle), and 1.2 mm (bottom) step-and-hold stimuli are shown. Pectoral fin ray afferents of the *P. pictus* provide proprioceptive feedback on fin ray movement and position. Increasing the amplitude of fin ray bending resulted in an increase in the duration of the corresponding burst of nerve activity at stimulus onset (one-way ANOVA, $F = 6.45$, $P < 0.0017$). Nerve activity (spikes/sec) during the hold interval was significantly higher than baseline rates (students t-test, $t = 3.65$, $P < 0.0064$). Scale bar, x = 1sec, y = 0.02 mV.

Discussion

Exploration of the kinematics and sensory neurobiology of *P. pictus* pectoral fins yields three conclusions: (i) pectoral fin kinematics of *P. pictus* during steady swimming are non-locomotor, with fins held protracted near the fish's ventral margin, conducive for use as passive sensory surfaces; (ii) as in mammalian limbs, *P. pictus* pectoral fins can sense pressure and object motion; and (iii) the functional capacity of the pectoral fin sensory system includes proprioception, pressure detection and the sensation of surface touch, expanding the known sensory repertoire of paired appendages in fishes.

The electrophysiological response to touch that we observed in fin ray afferents of *P. pictus* provides evidence of fin mechanoreception in a largely unexplored context. Bardach and

Case (1965) found that afferents from pectoral fins of searobins (*Prionotus* sp.) respond to touch, and afferents from the pelvic fins of the hake (*Urophycis chuss*) respond to touch and small movement of the rays. However, both of these finger-like fins are used by the fish specifically for probing the substrate and are morphologically specialized for this function, so their sensory abilities could conceivably be unique specializations among fishes. Pectoral fin ray afferents of bluegill sunfish (*Lepomis macrochirus*) provide proprioceptive feedback in response to relatively large fin ray deflections (Williams IV et al., 2013). The results presented here provide the first evidence that touch can be sufficient to produce an afferent nerve response in a fin not specialized as a substrate probe.

As *P. pictus* catfishes are found in low-visibility riverine environments with steady flow (Ibarra and Stewart, 1989; Sioli, 1984), mechanosensation may be a particularly important sensory modality during routine swimming as well as at rest. The pectoral fins' lack of abduction and adduction cycles during forward swimming and ventral body positioning facilitate their potential function as passive scanning surfaces for sensory detection. Sensitivity to surface touch on the fins may provide the fish information about nearby environmental features, including conspecifics, refuges or potential food items located lateral and ventral to the body. Catfish have laterally positioned barbels that provide mechanosensory information (Alexander, 1966; Fox, 1999), like the pectoral fins, in and around the plane of the fish's ventral surface (Figure 2.3a). Pectoral fins probably complement the barbel mechanosensory system by providing information about the region between the barbels and body as the fins are swept along the substrate during swimming.

In addition to their potential for sensing solid objects, pectoral fins could supplement the lateral line system by providing information about fluid flow around the fish. Sensitivity to small

deflections may help pectoral fins stabilize the body or otherwise control body positioning. Additionally, the pectoral fins' position at a distance from lateral line neuromasts on the body wall might allow detection of velocity gradients or vortices. Sensing such hydrodynamic features could aid in complex swimming behaviors. For example, trout have been shown to exploit vortices to reduce energetic costs (Liao et al., 2003a), a behavior that involves synchronizing body movements to vortex shedding rates behind an object in a flow (Akanyeti and Liao, 2013; Liao et al., 2003b). Examining how barbels, fins and neuromasts function together to represent the external mechanical environment would be an interesting case study of mechanosensory integration.

We suggest that the observed sensory receptors in the fin rays are mechanosensory, since they stain with an antibody to cytokeratin 20, a specific marker for the mechanoreceptive Merkel cell in humans (Moll et al., 1995) and in rodents (Moll et al., 1996). Although we cannot match physiological activity to a particular fiber ending, we believe we are labelling all of the afferent fibres in the rays, and based on their distribution and the putative Merkel cell structures, these nerve endings are the most likely candidates for transmitting mechanical stimulus information to the main afferent. Cytokeratin 20 is currently the most specific marker for Merkel cells (Boulais and Misery, 2007), but other methods could help confirm the identity of the cells observed in *P. pictus*. While the size and shape of Merkel cells varies between mammals (Kurosumi et al., 1979), birds (Saxod, 1978), amphibians (Fox and Whitear, 1978) and fish (Lane and Whitear, 1977; Whitear and Lane, 1981), an essential set of morphological criteria exists by which Merkel cells are generally recognized: lobulated nucleus, finger-like protoplasmic protrusions in the part of the cell opposite to the nerve terminal, and dense-core granules in close proximity to the nerve terminal. We propose that future investigations on fin mechanosensation employ electron

microscopy to identify Merkel cells using these aforementioned morphological features (reviewed in Halata et al., 2003; Moll et al., 2005). A full map of these fins' sensory morphology including receptor cells and nerve afferents would also provide insight into the encoding of surface features more broadly.

From an evolutionary perspective, the data presented here suggest that putative Merkel cell structures may have arisen in limb structures before the evolution of sarcopterygians. While the presence of Merkel cells has been previously described in the epidermis of several fish species (Lane and Whitear, 1977), there have been no previous accounts of these mechanosensors in the paired fins of fishes. Whether these sensory structures are primitive to fins or have been secondarily derived from epidermal origins remains unknown. Understanding the function and distribution of this fin-associated sensory morphology also has the potential to inspire membranous sensors for use in engineering contexts. For example, the integration of tactile feedback into the design of underwater robotic devices will facilitate complex tasks involving object manipulation.

Beyond the capacity of fish fins to act as passive mechanosensory surfaces, the results presented here suggest that the pectoral fin ray sensory system has evolved in response to the functional demands associated with a species' ecological niche. Differences in sensitivity to touch among fishes may reflect diversity that exists in mode of life, fin kinematics and behavior. Given that the sense of touch allows the most direct connection with the immediately local environment, we predict that touch-sensitive fins are widespread among fishes that maintain a close association with the substrate, and may be common beyond just benthic and demersal fishes. Fishes that inhabit low-visibility or deep-sea environments, or exhibit a nocturnal mode of life, may also benefit from touch-sensitive fins. The full extent to which fishes use their pectoral

fins for tactile sensation, however, is unknown. Future investigations would benefit from a comparative approach to understanding the function, neuroanatomy and sensory coding dynamics (spatial discrimination of stimuli, receptive field size, etc.) associated with touch-sensitive fins.

CHAPTER THREE: SENSING THE STRUCTURAL CHARACTERISTICS OF SURFACES: TEXTURE ENCODING BY A BOTTOM DWELLING FISH.

Abstract

The texture of contacted surfaces influences our perception of the physical environment and modulates behavior. Texture perception and its neural encoding mechanisms have traditionally been studied in the primate hand, yet animals of all types live in richly textured environments and regularly interact with textured surfaces. Here we explore the generalizability of texture sensation across vertebrates and limb types by investigating touch and potential texture encoding mechanisms in the pectoral fins of fishes, the forelimb homologs. We investigated the pectoral fins of the round goby (*Neogobius melanostomus*), a bottom dwelling species that lives on substrate types of varying roughness and whose fins frequently contact the bottom. Analysis shows that the receptive field sizes of fin ray afferents are small and afferents exhibit response properties to tactile motion that are consistent with those of primates and other animals studied previously. In response to a periodic stimulus (coarse gratings), afferents phase lock to the stimulus temporal frequency and thus can provide information about surface texture. These data demonstrate that fish can have the capability to sense the tactile features of their near range physical environment with fins.

Introduction

Our perception of the natural world is greatly influenced by the tactile recognition of surface features. Through touch we gather information about the microgeometry and material properties of contacted surfaces. Texture perception and its neural encoding mechanisms have

traditionally been studied in the primate hand, yet animals of all types interact with a variety of surfaces. Detail on the roughness of tree bark, the slipperiness of wet stone, or even the material composition of animal skin (i.e. fur, scales, feathers) influences perception of the physical environment and behavior. Here we explore the generalizability of texture sensation across vertebrates and limb types by investigating touch and potential texture encoding mechanisms in the pectoral fins of fishes, the forelimb homologs. Fish provide the opportunity to study appendage-based texture encoding in a very different context from that of previous studies as fishes are phylogenetically distant from primates, inhabit an aquatic environment, and have membranous appendages that are structurally very different from the fleshy arms, hands and digits of primate limbs.

In primates, mechanosensory skin surfaces contain an assortment of distinct afferent populations that function together to encode aspects of tactile sensation including texture. Terminating as either free nerve endings or in specialized mechanosensory cells, afferents can be broadly differentiated according to their functional response properties to sustained adaptation (i.e. slowly vs rapidly adapting) and morphological characteristics such as receptive field size (Johnson, 2001). By integrating afferents that differentially respond to aspects of skin deformation, mechanosensory surfaces are well suited to resolving surface features that span many orders of magnitude. In the primate finger pad system, coarse textural features (on the order of millimeters) are most faithfully encoded in the spatial pattern of activation across slowly adapting type 1 (SA1) afferents that terminate in Merkel cells (Blake et al., 1997; Connor et al., 1990; Connor and Johnson, 1992; Yoshioka et al., 2001). Fine textures (on the order of micrometers), however, are encoded by texture-specific vibrations that produce characteristic temporal spike patterns in rapidly adapting (RA) and Pacinian (PC) afferents (Manfredi et al.,

2014; Weber et al., 2013). SA1 and RA fibers are well suited to conveying the spatial information of contacted surfaces due to their small (3-5mm) receptive field size and dense innervation in locations important for touch (Darian-Smith and Kenins, 1980; Johansson and Vallbo, 1979; Vega-Bermudez and Johnson, 1999).

Here we sought to investigate whether the fins of fish exhibit similar tactile capabilities and use the same design principles observed in touch sensitive limbs of other vertebrate taxa. Touch has primarily been investigated in terrestrial contexts, but aquatic organisms such as fishes are commonly observed to contact the bottom substrate, plants, or other animals using their body and fins. Fin ray afferents have been shown to provide proprioceptive input on fin movement (Aiello et al., 2016; Aiello et al., 2017; Williams IV et al., 2013) as well as sensation of pressure (Hardy et al., 2016). As a direct link to the near-range environment, feedback from fins on how contacted surfaces feel (i.e. texture) could inform a host of behaviors such as substrate associated locomotion, navigation, station holding, and burying. If fish touch sensation is similar to other touch sensitive systems that exhibit spatial resolution of contacted surfaces, we would expect the receptive fields of fin ray afferents will be small (on the order of a few millimeters) and able to respond to coarse textural features of contacted surfaces.

As a model for investigating the functional capacities of touch in fins we studied the round goby (*Neogobius melanostomus*). Found on substrate types of varying roughness (Miller, 1986; Young et al., 2010), these fish rest on and interact with the bottom using their large membranous pectoral fins. Pectoral fins are flexible structures, composed of bony rays held together by a membrane, that deform during motion and during contact with the physical environment. Regions of the goby pectoral fin that touch the substrate were determined. We report here on the receptive field size of associated afferents, characterizing their response to

coarse textures (gratings of varying spatial period) using a custom-built drum stimulator. In addition to a more nuanced understanding of pectoral fin mechanosensation and the sensory role that fins may play, particularly for substrate associated species, this study contributes to our understanding of evolution of appendage based tactile sensation more broadly. By investigating texture perception in a very different context from that of primates we provide insight into the general features of touch that may occur broadly across vertebrates.

Methods

Animals:

Round Goby (*Neogobius melanostomus*) were maintained in 40L aquaria at the University of Chicago (Chicago, IL, USA) under seasonal day:night light cycles with a water temperature of 15-25°C. Individuals used for physiological experiments were euthanized in a 0.5 g l^{-1} solution of MS-222 (Tricaine methanesulfonate, Sigma-Aldrich, St. Louis, MO) in water. All experimental, housing, and euthanasia protocols were approved by the University of Chicago Institutional Animal Care and Use Committee (ACUP Protocol 71589#).

Functional Properties and Physiological Response to Textured Surfaces:

Electrophysiology methods followed Williams IV et al. (2013). Briefly, we recorded multiunit physiological responses from nerves innervating the fin rays on the medial side of the pectoral fin using glass suction electrodes. Fin rays were clamped at their proximal end and the inter-ray membrane was cut to isolate individual rays for mechanical stimuli.

We recorded the response of fin ray afferents to a proximodistal brushing stimulus in order to determine their receptive field size and firing characteristics associated with tactile

motion. The tactile stimulus, performed via an actuator mounted on a voice coil positioning stage (VCS10-023-BS-01-M, H2W Technologies Inc., Valencia, CA), consisted of moving a smooth rod (dimensions: 1mm x 5mm) perpendicular along the length of a given fin ray. The force of fin ray contact was measured at less than 4g. We used the Somlab software system (developed by John F. Dammann III, University of Chicago) to generate stimuli and controlled the actuator using a programmable driver (Intelligent Servo Drive IDM640-8EI, Technosoft, Canton, MI). The stimulus moved 8 mm towards the distal tip of a given fin ray at 5, 10, or 20 mm/sec to examine the effect of speed. Stimuli were repeated 10 times for each experiment with an inter-stimulus duration of 5 sec to prevent adaptation. Multi-unit recordings were taken from the right pectoral fin of seven individuals.

To investigate whether fin ray afferents reliably encode spatial features of coarse surfaces, we recorded nerve activity in response to 3D printed gratings delivered to the medial fin ray surface using a custom-built rotating drum stimulator. Gratings have been used extensively in texture encoding experiments as their repetitive and simple spatial pattern facilitate the analysis of potential encoding mechanisms (Darian-Smith and Oke, 1980; Lederman et al., 1982; Morley and Goodwin, 1987; Yoshioka et al., 2001). The drum consisted of a series of gratings (height = 1mm, width = 2mm) created with the use of a LulzBot TAZ 6 3D printer (ABS, Aleph Objects, Loveland, CO, USA). To examine the ability of afferents to resolve surfaces of varying spatial period, the distance between elements varied between 3, 5, and 7mm. The drum powered by a Faulhaber 2224U012SR DC motor (MicroMo Electronics, Clearwater, FL, USA) rotated at either 20, 40, 60, or 80mm/sec to examine the effect of scanning speed on the afferent response. Each trial began with the drum positioned directly dorsal and in light contact with a fin ray. Regardless of the drum's rotational speed, gratings were presented for 3s

followed by a 5s period of stasis and subsequent return to the original starting position before the start of the next stimulus. Five repetitions of each stimulus were presented to the fin for each speed and grating type. Video of the stimuli was recorded at 125 frames/s using a Fastcam APX RS camera (Photron, San Diego, CA) and synched with the electrical recordings using an external signal.

Physiological Data Analysis:

Data were sampled at 100 kHz, down-sampled to 10 kHz, and analyzed in MATLAB 2017a (Mathworks, Natick, MA, USA). To identify and sort individual units from our extracellular recordings, we used a modified version of the spike sorting algorithm, Wave_clus (Quiroga et al., 2004). Wave_clus provides a semi-automatic method of spike sorting based on wavelet decomposition and superparamagnetic clustering (SPC). To detect spikes, we set a 2.5ms absolute refractory period and used an amplitude threshold 5-times the standard deviation of the background noise. Statistical analyses of the mechanical stimulation data were performed using JMP software (SAS, Cary, NC, USA). We used linear mixed-effect models that consider repeated measures to evaluate our fixed effects (i.e. stimulus speed, grating spatial period) on a variety of response variables. For all such models, we used subject ID as a random factor.

We applied a set of criteria to define the first and last action potential associated with each afferents' receptive field in response to a proximodistal brushing stimuli. First, action potentials within 50ms after onset of the stimulus were excluded from subsequent analyses to eliminate transient effects associated with the initialization of mechanical stimulation. Spikes occurred during this 50ms period in 63 of the 240 stimulus presentations analyzed and typically consisted of just a single action potential. In an effort to exclude non-stimulus evoked activity we

applied a firing rate threshold (mean + SD) based on the ISI of spikes occurring during a 1sec pre-stimulus baseline period. However, as pre-stimulus activity was typically absent we applied an additional firing rate threshold (mean + 5*SD based on the ISI of spikes that occurred during the stimulus. Given the stimulus speed (5, 10, or 20mm/sec) and the duration between the first and last action potential that satisfied these conditions, we calculated the linear size of each afferents' receptive field.

For a subset of afferents that exhibited activity to the sustained indentation of the probe after stimulus offset, we determined the dynamic and static phase of the exhibited slowly adapting response. We defined the dynamic phase of the adaption as the time between stimulus offset and the datapoint in which the moving standard deviation (window length = 3) of the instantaneous firing rate for two consecutive spikes were both below one. The static phase continued immediately from this plateau in the firing rate for two additional seconds. The coefficient of variation (CV), a measure of spike regularity, for each stimulus in the static phase was calculated as the standard deviation of static phase ISIs divided by the mean static ISI.

To calculate the degree to which an afferent's response to textured surfaces matched or was tuned to the stimulus frequency we calculated the vector strength of the response (Goldberg and Brown, 1969). Vector strength, a measure of phase locking between a periodic stimulus and a response, ranges from 0 for uniform firing across all phases of the stimulus to 1 for perfectly synchronized firing at only one phase of the stimulus. We calculated the vector strength of the response for each trial (n = 5 for each combination of scanning speed and spatial period) and compared the mean experimental vector strength value to that expected from firing unrelated to the periodicity of the stimulus. The mean firing rate (spikes/s) of each experimental trial was used to generate 200 Poisson spike trains. We calculated the vector strength of these simulated

spike trains and then randomly and with replacement selected one value from each trial (n = 5 for each for each combination of scanning speed and spatial period) to generate a mean simulated vector strength value. This process was repeated 10,000 times in order to get the distribution of vector strengths arising from the null hypothesis (that there is no phase locking to the grating period).

Results

Receptive Field Properties:

We determined afferents' receptive field size and firing characteristics with a proximodistal brushing stimulus applied to the fin. For all physiological experiments conducted in this study, we targeted the ventral surface of the distal half of fin rays, previously identified as regularly contacting the substrate during routine behavior (Figure 3.1). The probe moved 8mm along a given fin ray at 5, 10, or 20mm/sec. From our multi-unit recordings, we identified afferents (n = 8) whose activity occurred completely within the stimulus area allowing for the determination of receptive field length. Afferents whose activity was cut off by either the start or stop of the stimulus were excluded from the analysis as the true distance with which the afferent could have responded was unknown. Trials at a given speed were repeated 10 times to control for possible variability in the application of the stimulus as well as to investigate these afferents' response consistency. We found that the spatiotemporal discharge pattern and first spike onset were consistent across repeated stimuli, highlighting the precision with which fin ray afferents respond to repeated tactile stimuli (Figure 3.2a).

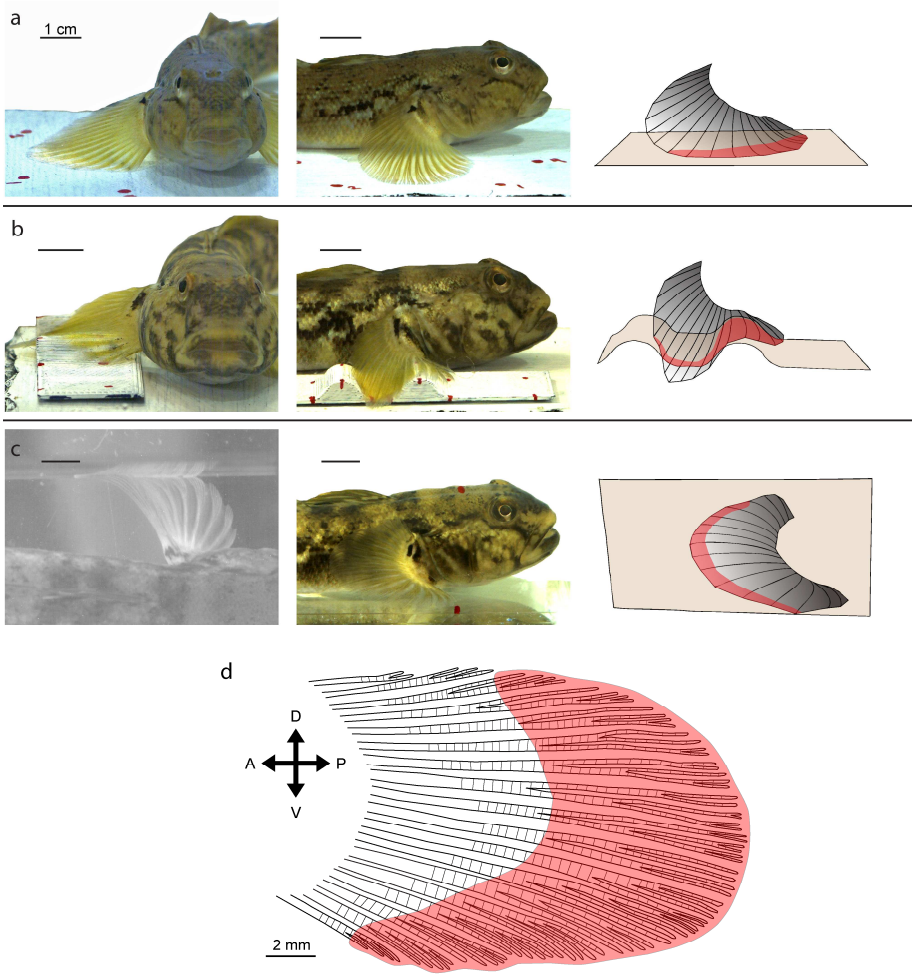

Figure 3.1: Physical interaction between the pectoral fin of *N. melanostomus* and the bottom substrate while at rest on three substrate types. To visualize the interaction between the fin and the substrate 3D models were created of both surfaces using a three-camera stereo setup and the R package StereoMorph (Olsen and Westneat, 2015). (a) Anterior (left) and lateral (middle) camera views of a round goby resting on a flat piece of slate rock (20 x 10cm). Lateral view of a 3D model (right) illustrating the position of the pectoral fin (grey) including each of the 19 fin rays relative to the bottom substrate (tan colored trapezoid). Regions of the pectoral fin observed to touch the bottom are shaded red. Fin ray contact on a flat surface is localized towards the distal tips of trailing edge fin rays. (b) Anterior (left) and lateral (middle) camera views of a pectoral fin resting on a 3D printed bottom consisting of two mounds (5mm high). Camera images and lateral view of 3D model (right) show that the fin is flexible and readily molds to the shape of the bottom substrate. Fin ray contact (red) is more substantial and localized to the distal half of the middle and trailing edge fin rays. (c) Posterior (left) and lateral (middle) camera views of a round goby pectoral fin propped against a vertically oriented pane of glass to mimic how these fishes wedge themselves against and between bottom substrate. Scale bars: 1cm. (D) Illustration showing regions of the pectoral fin marked in red that likely contact surfaces during routine behavior. Scale bar: 2mm.

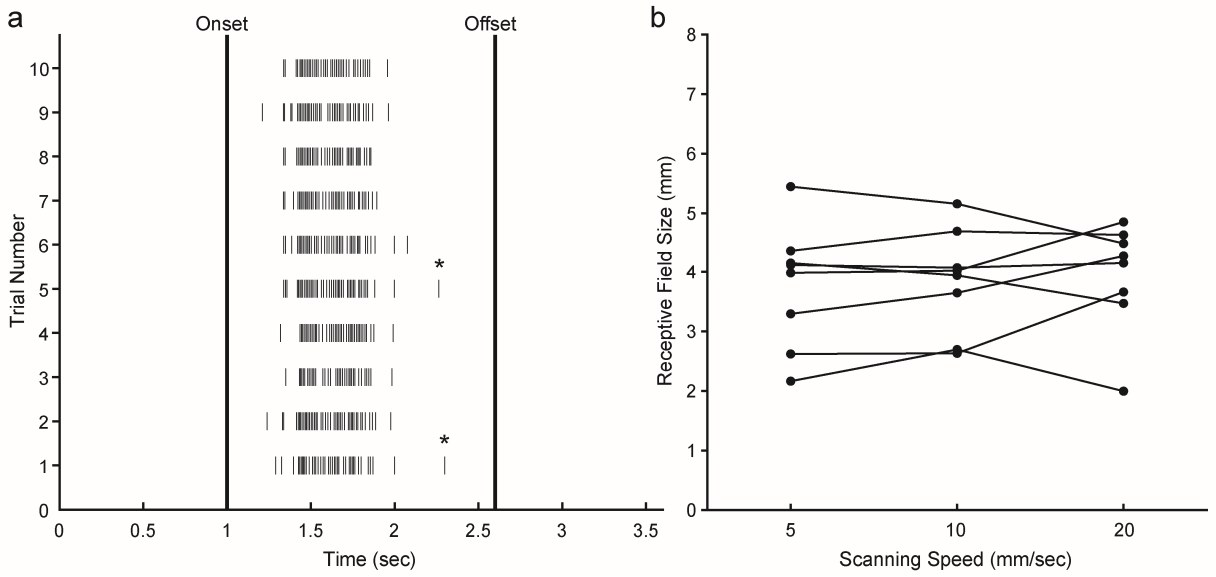

Figure 3.2: Determination of fin ray afferent receptive field size to tactile motion. (a) Spike raster of a representative afferent showing the response to a proximodistal brushing stimulus moving across 8mm at 5mm/sec. The spatiotemporal discharge pattern and first spike onset were consistent among trials. Spikes marked with an asterisk were excluded from analyses based on a firing rate threshold (see Methods). (b) Fin ray afferents have small receptive fields, similar in size to those of the SA1 and RA afferents observed in primates. Given the stimulus speed (5, 10, or 20mm/sec) and the duration between the first and last action potential for each trial, we calculated the linear extent of each afferent's receptive field. The mean receptive field size of afferents ranged from 2.28-5.07mm. Each point represents the mean of 10 trials and points connected by a line belong to the same afferent (n = 8 afferents).

Fin ray afferents exhibited small receptive fields, similar in size to those of the SA1 and RA afferents observed in primates. We found that the mean receptive field diameter of recorded afferents was 2.28-5.07mm (Figure 3.2b). In addition, we asked whether these afferents exhibit response characteristics to tactile motion that are similar to those of mammals. As the scanning speed increased and the duration of the stimulus decreased, we predictably found a significant decrease in the duration of stimulus evoked activity ($F_{2,14} = 74.61$, $P < 0.0001$) and the number of evoked spikes ($F_{2,14} = 64.56$, $P < 0.0001$) (Figure 3.3a,b). Mechanoreceptor firing rates in mammalian systems have been shown to increase as the speed of objects scanned across the skin increases (Essick and Edin, 1995; Goodwin and Morley, 1987; Greenspan, 1992). Similarly, we

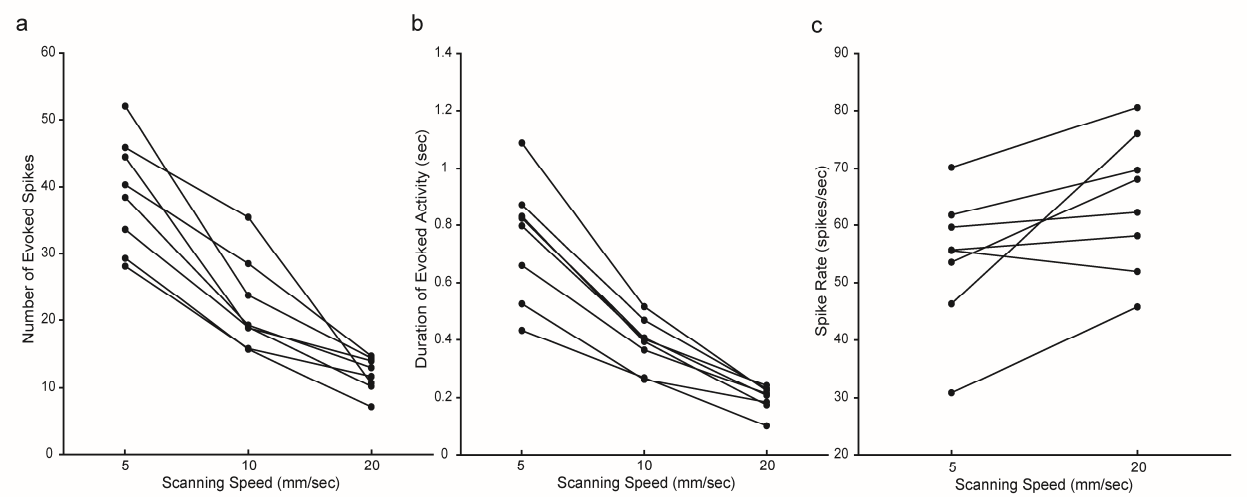

Figure 3.3: Afferent response to a proximodistal brushing stimuli. (a)Mean number of stimulus-evoked spikes versus scanning speed. (b) Mean duration of stimulus-evoked activity versus scanning speed. The number of evoked spikes ($F_{2,14} = 64.56$, $P < 0.0001$) and the duration of stimulus evoked activity ($F_{2,14} = 74.61$, $P < 0.0001$) significantly decreased with increasing stimulus speeds as the probe spent less time in contact with the fin ray surface. (c) Mean firing rate versus scanning speed. Similar to mammalian mechanoreceptors, the firing rate of fin afferents significantly increased as the scanning speed of the probe increased from 5 to 20mm/sec ($F_{1,7} = 7.34$, $P = 0.0302$). As the speed of the probe increased, the faster rate of change in stimulus components (i.e. compression, stretch, indentation) are reflected in the higher discharge rates. Each point represents the mean of 10 trials and points connected by a line belong to the same afferent (n = 8 afferents).

found that the firing rate of these fin afferents significantly increased as the scanning speed of the probe increased from 5 to 20mm/sec ($F_{1,7} = 7.34$, $P = 0.0302$)(Figure 3.3c). As the scanning speed of the probe increased, the faster rate of change in the stimulus components (i.e. compression, stretch, indentation) is reflected in the higher discharge rates.

We also recorded afferents (n = 4 afferents from three fish; 120 total stimulus presentations) that continued to fire well after the stimulus stopped moving indicative of a slowly adapting response to the sustained indentation of the probe. The response, characterized by a sharp decrease in firing rate shortly after stimulus offset followed by an extended duration of tonic firing, is similar to that seen in SA1 units in other systems. When averaged across trials, the

spike rate (spikes/s) during the period prolonged indentation was 13.63 ± 3.71 (mean ± SD). We calculated the coefficient of variation (CV) of the interspike intervals to determine the regularity of their static-phase firing rates. We found that these fin ray afferents displayed mean CVs ranging from 0.35 – 0.58 suggestive of high ISI variability and further support of the functional similarity between the slowly adapting afferents of fish and those observed in mammalian taxa (Iggo and Muir, 1969; Knibestöl, 1975; Wellnitz et al., 2010).

Texture Encoding:

Targeting regions of the fin that routinely contact the bottom, we identified fin ray afferents that exhibit a phase locked response to the periodic structure of coarse gratings. Complete data sets consisting of the response to three sets of gratings (spatial periods of 3, 5, or 7mm) moving at four different speeds (20, 40, 60, 80mm/sec) were recorded from six neurons. Given the spatial periods and the scanning speeds employed, the stimulus temporal frequency (scanning speed/grating spatial period) ranged from ~3 Hz to 27 Hz.

Afferents were responsive to individual gratings as they moved across their receptive field (Figure 3.4). In order to measure the strength of entrainment to the periodic structure of the coarse gratings, we computed the vector strength of the afferent response. A vector strength of zero corresponds to uniform firing across all phases of the grating cycle whereas a value of one corresponds to firing at only one phase location relative to each passing grating. We found that afferents exhibited high vector strength values, consistently above 0.4, regardless of the grating spatial period or its corresponding temporal frequency (Figure 3.5). Furthermore, mean vector strength values for each combination of spatial period and scanning speed were significantly

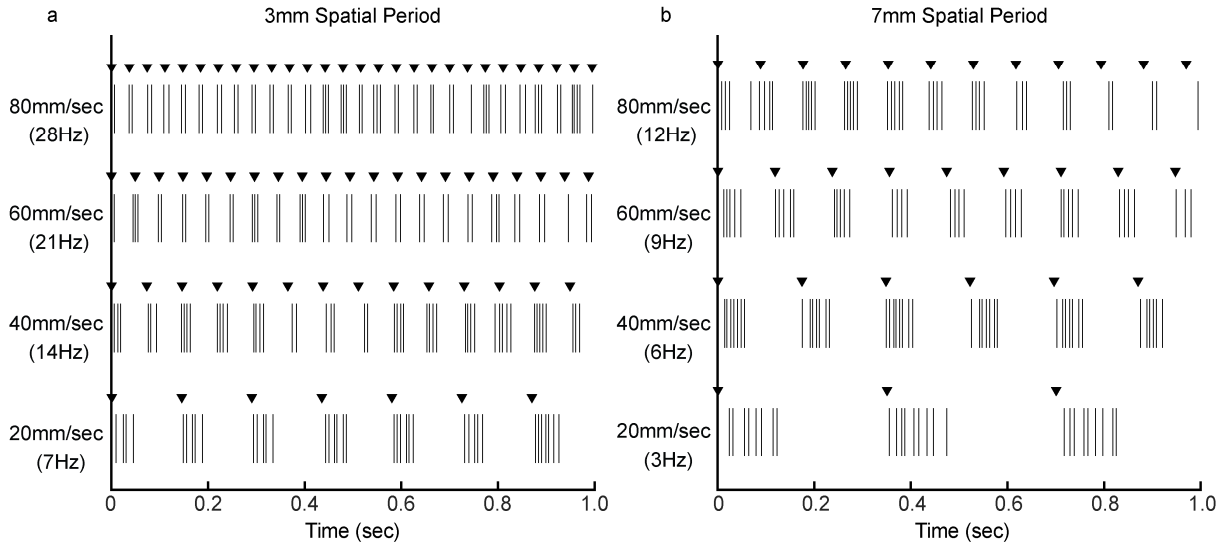

Figure 3.4: Afferent spiking in response to coarse gratings. Each grouping of raster plots shows the representative response of a fin ray afferent to gratings with either an (a) 3mm or (b) 7mm spatial period moving over its receptive field. The scanning speed (20, 40, 60, and 80mm/sec) as well as the corresponding stimulus temporal frequency are indicated to the left of each raster. Arrowheads mark the time separation between successive gratings. The first arrowhead in each plot has been aligned to the first spike associated with a grating discharge. We found that afferents were responsive to each successive grating and that the average number of spikes elicited by each grating significantly decreased as the scanning speed increased from 20 to 80mm/sec (Tukey-HSD, $P \leq 0.0001$).

higher than expected by chance ($P < 0.001$). The spatial period of the grating had a significant effect on the strength of the entrainment ($F_{2,10} = 5.4792$, $P < 0.0247$). For example, as the grating spatial period increased from 3 to 7mm, the mean vector strength of the response when pooled across scanning speeds significantly increased from 0.54 to 0.73 (Tukey-HSD, $P = 0.0197$). Changes to scanning speed however, did not have a significant effect on the magnitude of entrainment in response to gratings with a given spatial period of 3mm ($F_{3,15} = 0.9157$, $P = 0.45$), 5mm ($F_{3,15} = 0.5542$, $P = 0.65$), or 7mm ($F_{3,15} = 2.0402$, $P = 0.15$) suggesting the robustness of the phase locking across a wide spectrum of biologically relevant scanning speeds.

Gratings are well defined if any two of the following stimulus parameters are known: spatial period, scanning speed, and temporal frequency. As the phase locked response provides

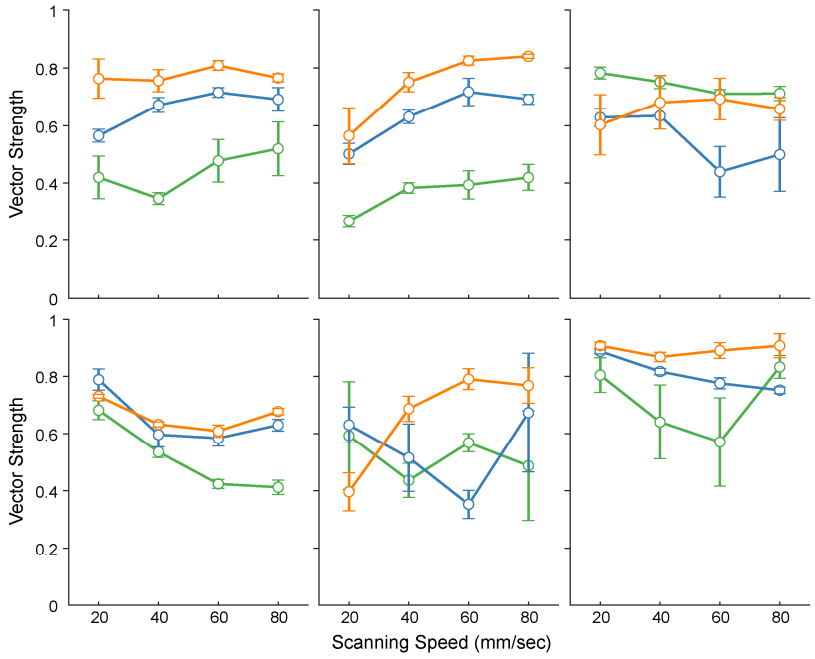

Figure 3.5: The strength of entrainment (i.e. phase locking) to the spatial period of coarse gratings. Vector strength, a measure of phase locking between a periodic stimulus and a neural response, ranges from 0 for uniform firing across all phases of the stimulus to 1 for perfectly synchronized firing at only one phase location relative to each passing stimulus. Each plot shows the response from an individual afferent (n = 6) to gratings with a 3mm (green), 5mm (blue), and 7mm (orange) scanned across the fin at 20, 40, 60, and 80mm/sec. Circles mark the mean (n = 5 trials) vector strength value at a given combination of grating spatial period and scanning speed. Afferents exhibited high vector strength values, consistently above 0.4, regardless of the grating spatial period or its corresponding temporal frequency. Mean vector strength values for each combination of spatial period and scanning speed were significantly higher than expected by chance ($P < 0.001$). The high degree of phase locking provides precise information on the temporal frequency on coarse gratings independent of the grating spatial period or scanning speed. Error bars represent one standard deviation.

reliable information regarding the temporal frequency, we investigated whether mean firing rate or the mean number of spikes elicited per grating ridge faithfully encode either the scanning speed or spatial period. When the spatial period was held constant, we found that changes to scanning speed, and thus the stimulus temporal frequency, had a significant effect on the normalized mean afferent firing rate (spikes/sec) for gratings with a 3mm ($F_{3,15} = 7.4793$, $P = 0.0027$), 5mm ($F_{3,15} = 4.8636$, $P = 0.0147$), and 7mm ($F_{3,15} = 4.2052$, $P = 0.0240$) spatial period

(Figure 3.6a). While we observed a significant increase in the mean firing rate with changes in scanning speed from 20 to 80mm/sec for each grating, the response was invariant to scanning speeds above 40mm/sec. Likewise, we did not observe a significant effect of changes to spatial period when the scanning speed was held constant at 20 ($F_{2,10} = 0.4563$, $P = 0.6462$), 40 ($F_{2,10} = 0.1300$, $P = 0.8795$), 60 ($F_{2,10} = 3.338$, $P = 0.0775$), or 80mm/sec ($F_{2,10} = 0.8523$, $P = 0.4552$)(Figure 3.6).

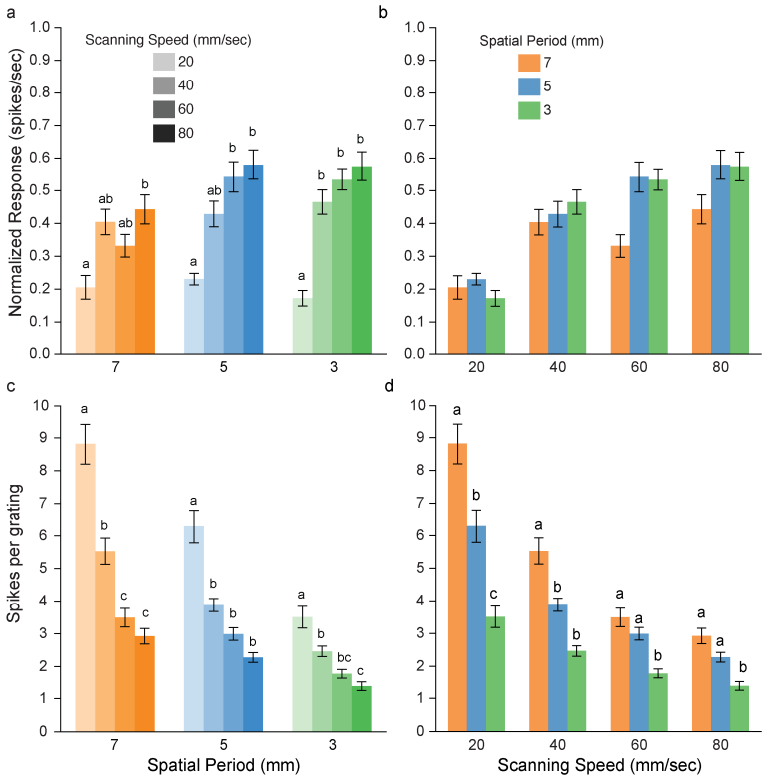

Figure 3.6: Measures of the mean afferent response when either spatial period or scanning speed is held constant. (a) When the spatial period was held constant, changes to scanning speed, and thus the stimulus temporal frequency, had a significant effect on the normalized mean afferent firing rate (spikes/sec) for gratings with a 3mm ($F_{3,15} = 7.4793$, $P = 0.0027$), 5mm ($F_{3,15} = 4.8636$, $P = 0.0147$), and 7mm ($F_{3,15} = 4.2052$, $P = 0.0240$) spatial period. While afferents responded with a significant increase in the mean firing rate with changes in scanning speed from 20 to 80mm/sec for each grating, the response was invariant to scanning speeds above 20mm/sec. (b) The spatial period of the grating did not have a significant effect on the response when the scanning speed was held constant at 20 ($F_{2,10} = 0.4563$, $P = 0.6462$), 40 ($F_{2,10} = 0.1300$, $P = 0.8795$), 60 ($F_{2,10} = 3.338$, $P = 0.0775$), or 80mm/sec ($F_{2,10} = 0.8523$, $P = 0.4552$). (c) The average number of spikes elicited per contact with a grating significantly decreased as the scanning speed increased from 20 to 80mm/sec for each of the three sets of gratings (Tukey-HSD, $P \leq 0.0001$). (d) When the scanning speed was held constant however, only at the lowest scanning speeds of 20mm/sec was spike number significantly different for each grating. Error bars denote the SEM across gratings and scanning speeds.

We determined the average number of spikes produced per contact with a grating by dividing the afferent firing rate by the stimulus temporal frequency. In response to frequencies above 10Hz, afferents fired 1-3 times per contact with a grating. Occasionally, the mean interspike interval closely matched that of the stimulus temporal period (1/temporal frequency) indicating that the afferent fired exactly once as each grating passed over its receptive field. At the lowest frequency we tested however (~3Hz for 7mm spatial period gratings moving at 20mm/sec), the average spike count was 8.82 ± 3.39 (mean $\pm$ SD). When the spatial period was held constant, we found that the average number of spikes elicited per contact with a grating significantly decreased as the scanning speed increased from 20 to 80mm/sec for each of the three sets of gratings (Tukey-HSD, $P \leq 0.0001$)(Figure 3.6c). When the scanning speed was held constant however, we found that only at the lowest scanning speeds of 20mm/sec was spike number significantly different for each spatial period (Figure 3.6d). Taken together, mean firing rate and spike number do not faithfully encode either the grating spatial period or scanning speed across the experimental range of stimuli tested here.

Discussion

From these results we conclude that: 1) pectoral fins can have the capability to respond to the coarse features of textured surfaces and tactile motion, and 2) the morphology and response properties to touch reported here for the pectoral fin of a bottom dwelling fish are consistent with those of mammalian systems studied previously.

The paired fins of fishes, which are homologues to the limbs of tetrapods, serve in diverse behaviors such as locomotion, posture, respiration, feeding, and brooding (Gibb et al., 1994; Gosline, 1994; Green et al., 2011; Higham, 2007; Künzler and Bakker, 2000; Taft et al.,

2008; Westneat, 1996). Previous studies on the filamentous pelvic rays of hake (*Urophycis chuss*)(Bardach and Case, 1965) as well as the membranous pectoral fins of bluegill sunfish (*Lepomis macrochirus*)(Williams IV et al., 2013) and wrasse (Family Labridae)(Aiello et al., 2017) have shown that fin ray afferents provide proprioceptive feedback in response to movement and deflection of the rays. Hardy et al. (2016) reported that afferents innervating the pectoral fin of pictus catfish (*Pimelodus pictus*), a bottom dwelling species, respond to pressure and light surface brushing, thus expanding the known sensory repertoire of paired appendages in fishes to include touch. Given the sensitivity to touch shown in fins, we sought to investigate whether fins exhibit similar tactile capabilities and characteristics observed in touch sensitive limbs of other vertebrate taxa.

Fish frequently contact the bottom substrate, plants, or other animals using their fins. Observations of the round goby on a variety of substrate types indicate that a significant portion of the pectoral fin contacts and molds to the contours of the bottom while at rest (Figure 3.1). Beyond the simple sensation of contact, fin ray feedback on how contacted surfaces feel (i.e. roughness, slipperiness, etc.) could be important for habitat selection as well as in a variety of other behaviors such as substrate associated locomotion, navigation, posture, and burying. When selecting suitable habitat, for example, bottom associated fish often exhibit clear preferences for particular types of substrate such as silt, sand, or pebbles (Gibson and Robb, 2000; Moles and Norcross, 1995). Compared to the terrestrial environment, the aquatic realm imposes unique sensory challenges as the amount of visible light available for animals to utilize rapidly decreases with depth and is negatively affected by the turbidity of water. While fish can utilize their visual and lateral line systems to discern physical features of their immediate surroundings, sensitivity to differences in the material and geometric characteristics of contacted surfaces provides direct

feedback on mechanical characteristics of surfaces and presumably take on a more consequential role in low visibility conditions where other sensory modalities may not be effective.

Physiological analysis of the pectoral fin ray afferent response demonstrates that fins are well suited to encoding coarse textural features of contacted surfaces. We found that a subset of afferents exhibited receptive fields that span 2 - 5mm in length. The presence of small receptive fields within fins suggest their utility for sensing mechanical events with a high degree of spatial resolution. Using a rotating drum stimulator, we found that afferents respond to coarse gratings when scanned across their receptive fields with a phase locked response to the temporal frequency of the stimulus. The degree of phase locking as measured by vector strength was significantly higher than expected by chance for all gratings tested and found to be invariant to changes in scanning speed. Changes in scanning speed while the spatial period of the grating was held constant had predictable effects on the afferent response and showed similarities to the SA response observed by Morley and Goodwin (1987) in the monkey finger pad. Occasionally at high scanning speeds, we observed that the mean interspike interval closely matched that of the stimulus temporal period. This precise match between interspike interval and temporal period in response to coarse gratings has been reported by Darian-Smith and Oke (1980) for SA1, Pacinian, and RA fibers in the monkey finger pad. While these authors reported that multiple discharges per grating were uncommon, Morley and Goodwin (1987) as well as the data presented here show that afferents typically responded to each grating with multiple spikes. This discrepancy between studies could be due to differences in contact force, range of temporal frequencies examined, or grating dimensions.

In this work we present the first investigation of the fin ray sensation of texture, utilizing regular coarse gratings to facilitate matching stimulus features to the afferent response. When

scanned across the skin, the temporal frequency of gratings is dependent on both spatial period and scanning speed. It is therefore possible for gratings with different spatial periods to generate identical temporal periods simply by modulating the scanning speed. Given this fact and lack of response feature (i.e. firing rate, spike number per grating) that alone faithfully encodes either the spatial period or scanning speed across the experimental range of stimuli tested, individual fin ray afferents may not unequivocally encode the spatial structure of gratings. While phase locked responses of individual afferents provide precise information on the temporal frequency, spatial information about contacted surface features likely depends on information signaled across the afferent population. In primates the spatial structure of coarse surfaces is best encoded in the spatial variation of slowly adapting type 1 (SA1) afferent firing rates (Blake et al., 1997; Connor et al., 1990; Connor and Johnson, 1992; Weber et al., 2013; Yoshioka et al., 2001). Calculated as the difference in mean firing rates between fibers with receptive field centers separated by a fixed distance, spatial variation on a scale of ~2mm was found to most closely correlate with ratings of perceived roughness (Connor et al., 1990). Given the high innervation density of the primate hand, this distance corresponds to firing rate differences between adjacent or nearly adjacent receptors (Darian-Smith and Kenins, 1980; Johansson and Vallbo, 1979).

The finding that fin ray afferents have small receptive fields and that their responses are modulated by the periodic structure of coarse gratings provides support for the hypothesis that fish utilize an encoding mechanism similar to that discussed above for primates. To test this hypothesis, it will be necessary to determine the position and density of mechanoreceptors within fins. Touch sensitive membranes of other animals often exhibit localized regions of high sensory ending density that facilitate greater sensitivity and an increased spatial resolution of tactile stimuli (Gentle and Breward, 1986; Gottschaldt and Lausmann, 1974; Johansson and Vallbo,

1979; Sawyer and Catania, 2016). Furthermore, the construction of spatial event plots would facilitate a more in depth look at how features of contacted surfaces are spatially represented across the receptive field. For coarse textures such as gratings and embossed dot patterns scanned across the monkey finger pad, the spatial structure of the stimulus is well preserved in the spatial pattern of activation in SA1 and, to a lesser extent, RA afferents (Connor and Johnson, 1992; Phillips et al., 1992; Weber et al., 2013). Understanding how stimuli are spatially represented across the receptive field of fin ray afferents would better facilitate comparisons with known texture encoding mechanisms.

These data demonstrate that the pectoral fins in the round goby, and we postulate fins in general, can gather information about the coarse structure of contacted surfaces. Fins had traditionally been thought of as primarily motor devices, but a growing body of literature has shown that fins function as sensors capable of providing precise feedback on fin ray position and movement as well as touch related events (Aiello et al., 2017; Hardy et al., 2016; Williams IV et al., 2013; Williams and Hale, 2015). Given previous studies across an ecologically diverse group of fishes showing the ability to encode information about the static and dynamic aspects of mechanosensory stimuli, we hypothesize that this sensitivity to contacted surface features is widespread among fishes. The sensitivity to and resolution of tactile stimuli presumably varies amongst species and among fins as the ecology of each species will influence the functional demands for touch. To understand the spatial resolution of the fin ray sensory system to contacted surfaces, future investigations would benefit from employing fine gratings (i.e. spatial periods closer to 1 mm), embossed dot patterns, or more naturalistic textures that span the range of textures found in the natural environment. The findings presented here of mammalian-like touch in the fins of a fish also suggests that the appendage based sensory apparatus needed for

the tactile exploration of the physical environment arose in vertebrate limbs before the evolution of sarcopterygians.

CHAPTER FOUR: THE ABUNDANCE AND DISTRIBUTION OF TASTE BUDS ACROSS THE PAIRED FINS OF DAMSELFISH

Abstract

The sense of taste in fishes is mediated by taste buds located in the oropharyngeal cavity as well as across the body and fins of some species. The abundance and distribution of extraoral taste buds often reflect adaptations to a particular habitat or feeding behavior. It has been hypothesized that species inhabiting environments where vision is of limited use (i.e. benthic and/or turbid) rely on taste during feeding and should exhibit more extraoral taste buds than open water or surface feeding fishes. To further investigate this idea, we describe the morphology and distribution patterns of taste buds across the paired fins of three distantly related species of damselfish. Damselfish are thought to rely on vision during feeding and inhabit an environment (i.e. shallow, clear and light-rich waters of coral reefs) where the utility of extraoral taste buds has not been previously investigated. Using immunohistochemical techniques, we found pear-shaped receptors across the paired fins whose morphological characteristics are consistent with those of taste buds identified previously. By mapping the full array of taste buds, we show how the functional demands for chemical sensation vary across the paired fins. Taste bud density increased along fin margins and was highest (~200/mm^2) at the distal tips of the elongated leading-edge pelvic fin rays suggesting the importance of this region for chemical sensation. We observed species specific variation in the distribution of taste buds likely reflecting differences in the functional demands for feeding across the fins. These data demonstrate that the paired fins of damselfish are heavily innervated and likely play an important chemosensory role.

Introduction

The chemosensory systems of fishes, which include gustation, olfaction, and the common chemical sense are integral to behavior. The aquatic environment is abundant in dissolved chemical compounds that fish can utilize to detect prey, conspecifics, and predators as well as to navigate. The sense of taste provides important feedback during feeding behaviors and is mediated by taste buds, the peripheral sensory organs for gustation. The taste buds of fishes, unlike those of other vertebrates, are not confined to the oropharyngeal region, but can also be found across the body and fins. Fish fins have been shown to play a significant role in multiple sensory modalities. Innervated with sensory nerves and specialized endings, fins are capable of detecting chemical stimuli (Bardach and Case, 1965; Silver and Finger, 1984) as well as providing mechanosensory input on fin ray position, movement, and touch related events (Aiello et al., 2017; Hardy et al., 2016; Williams IV et al., 2013). The presence of taste buds along extraoral regions of the body has enabled species such as the catfish (*Ictalurus nebulosus*) to use taste as a short-range chemical sense to detect and locate food sources by means of taste alone (Bardach et al., 1967).

Taste buds have been found in all vertebrates except for the hagfishes (Barreiro-Iglesias et al., 2008; Northcutt, 2004). Among these animals, fish exhibit the highest number of taste buds with some species of catfish displaying upwards of several hundred thousand taste buds across the body and fin surfaces (Atema, 1971; Finger et al., 1991). The anatomy, physiology, and innervation of taste buds have been investigated in select species (Davenport and Caprio, 1982; Kapoor et al., 1976; Reutter and Witt, 1993). While morphological variation exists among species and across different regions of the body (Reutter et al., 2000; Reutter and Witt, 1999), the ultrastructure of fish taste buds is well defined. Fish taste buds are pear or onion-shaped

intraepithelial sensory structures consisting of multiple cell types that include gustatory receptor cells, support cells, and basal cells. Both gustatory receptor and support cells have an elongated shape with their apical ends protruding into the oral cavity or external environment via a small pore. Pore diameter (2 – 20 um) as well as the number of cells (5 – 67) within each taste bud varies amongst species (Jakubowski and Whitear, 1990). Each taste bud has a network of nerves located between the disk-shaped basal cells and the basal part of the receptor cells that are derived from either the facial (VII), glossopharyngeal (IX) or vagal (X) cranial nerves depending on its location along the body (Atema, 1971; Finger et al., 1991).

The importance of cutaneous taste buds during feeding is expected to be higher in benthic rather than pelagic species. Studies have shown that that the distribution and density of taste buds across the body and fins of fishes often reflects their ecology and feeding habits. For example, taste bud density is typically lower in planktivorous and surface-feeding cyprinids than in the bottom feeders (Davis and Miller, 1967; Gomahr et al., 1992). Investigations of extraoral taste buds to date have focused primarily on benthic or dermersal fishes such as the silurids (Atema, 1971; Nakamura et al., 2017; Northcutt, 2005; Sakata et al., 2001), cyprinids (Davis and Miller, 1967; Gomahr et al., 1992), mullids (McCormick, 1993), and gadids (Harvey and Batty, 1998; Harvey and Batty, 2002; Kotrschal et al., 1993). Living close to the bottom, these fishes are often nocturnal, inhabit turbid water, and may feed on cryptic or buried prey where vision may not be as effective. It has been hypothesized that pelagic species and/or fishes inhabiting clear water depend more on vision, olfaction, and mechanoreception rather than taste during feeding behaviors and thus should exhibit fewer extraoral taste buds. To further investigate this idea as well as to expand upon our current knowledge of cutaneous taste buds across fishes more broadly we chose to explore the paired fins of damselfish (family *Pomacentridae*).

Quantitative data on the distribution and density of taste buds across the paired fins of fishes is known from a few species (ÇInar et al., 2008; Gomahr et al., 1992; Harvey and Batty, 1998; Harvey and Batty, 2002). Taste buds are not uniformly distributed across a given fin. Instead, it's been shown that taste bud density is higher along fin margins and lower in more interior regions. Along a given fin ray, taste buds are distributed parallel to the long axis of the fin rays and follow existing fin ray branching patterns (Nakamura et al., 2017). The lack of fine spatial detail on how taste buds are distributed within fins has limited form and function interpretations of the fin gustatory system. Previous studies have reported densities from a single or limited number of fin rays with few if any additional quantitative measurements taken along the proximodistal axis of a given fin ray. This study is therefore the first to map and quantify the full array of taste buds in a fish fin in order to determine how the functional demands for chemical sensation vary across the appendage.

Damselfish are an ecologically diverse group of 405 species that inhabit an environment (i.e. shallow, clear and light-rich waters of coral reefs) where the utility of extraoral taste buds has not been investigated. Additionally, as primarily diurnal planktivores, damselfish are thought to rely on vison during feeding. During the day these fishes spend the majority of their time foraging in the water column, but as the light diminishes they move closer to the reef in order to rest overnight under reef shelter (Allen, 1991; Hobson, 1975; Hobson and Chess, 1978). Studies have shown that damselfish possess exceptional visual acuity that includes color discrimination as well as the ability to detect ultraviolet and polarized light (Hawryshyn et al., 2003; Mussi et al., 2005; Siebeck et al., 2008). While zooplankton are often transparent as a means to escape predation, their exoskeletons scatter polarized light, which at least some damselfish species can detect to locate their prey (Mussi et al., 2005). Given the importance of vision in these fishes, we

hypothesize that damselfish have fewer extraoral taste buds compared to other species previously examined.

Using immunohistochemical techniques, we identified sensory endings in the paired fins of damselfish that positively label with an anti-calretinin antibody. Previous studies have used calretinin, a calcium binding protein, to detect taste buds in a variety of fish species (Díaz-Regueira et al., 2005; Germanà et al., 2007; Nakamura et al., 2017; Northcutt, 2005; Varatharasan et al., 2009). Although the physiology of these endings has yet to be determined, their anatomy, consistent with descriptions of taste buds in other species, and positive association with calretinin suggests their identity as taste buds. To investigate how sensory morphology relates to damselfish fin function and ecology, we calculated the density and spatial distribution of these putative taste buds across the paired fins of three distantly related species of damselfish (based on a recent phylogeny by Frédérich et al. (2013)). In addition, we compare variation in the distributions of these receptors among fins and to other species known to exhibit chemosensory abilities via fins.

Here we describe the morphology and distribution of taste buds across the paired fins of three species of damselfish: Blue green chromis (*Chromis viridis*), neon damselfish (*Pomacentrus coelestis*), and the ambon damselfish (*Pomacentrus amboinensis*). These species vary in their position on the reef (substrate associated vs. midwater), diet, and gross fin morphology. *C. viridis* are zooplantivores found in large aggregations high in the water column above coral heads. *P. coelestis* are omnivores foraging close to the bottom amongst coral rubble on variable amounts of zooplankton and benthic algae. *P. amboinensis* are omnivores feeding primarily on benthic algae near coral outcrops. Among the species investigated here, we hypothesized that *P. coelestis* would exhibit the highest density of taste buds across the paired

fins given their close association to the substrate. While *C. viridis* feed almost exclusively on zooplankton they do so in high in the water column where vision can be expected to be of primary importance. In contrast, *P. coelestis* are omnivores foraging in visually complex environments near the bottom where light levels and water turbidity are more likely to be negatively impacted and the relative importance of gustatory feedback can be expected to more critical. As the first study to investigate taste buds in the paired fins of a coral reef species, the data here suggest that taste buds on fins may be more widespread amongst fishes than previously reported.

Methods

Animals:

Specimens were purchased through the aquarium trade and maintained in separate aquaria as part of a 1200L saltwater flow-through system at the University of Chicago (Chicago, IL, USA). Individuals used for immunohistochemical experiments were euthanized in a 0.5 g l^{-1} solution of MS-222 (Tricaine methanesulfonate, Sigma-Aldrich, St. Louis, MO) in water. All experimental, housing, and euthanasia protocols were approved by the University of Chicago Institutional Animal Care and Use Committee (ACUP Protocol 71589#).

Neuroanatomy of damselfish paired fins:

Antibody staining methods were modified from Thorsen and Hale (2007) and Svoboda et al. (2001). Both pectoral and pelvic fins from three individuals of each species were stained and imaged. Fins were preserved in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight at 4°C. To permeabilize tissues, fins were incubated for 24 hours at 4°C in PBS

containing 1.0% Triton X-100. Fins were then blocked at room temperature in 10% normal goat serum (NGS) in PBS containing 0.1% Tween-20 and 0.5% Triton X-100 for 1 hour.

Fins were incubated at 4°C in blocking solution with both primary antibodies. Nerves were stained with the use of mouse monoclonal anti-acetylated tubulin (aat, Sigma-Aldrich) at a final concentration of 1:250. Receptor cells within taste buds were stained with the use of the monoclonal antibody, 7697, directed against calretinin (Swant Antibodies, Bellinzona, Switzerland) at a final concentration of 1:1000. After 48 hours, fins were rinsed 3 times for 30 min each with PBS and then incubated at 4°C in blocking solution containing both secondary antibodies. We used a goat anti-mouse IgG (H+L) cross-adsorbed, Alexa Fluor 546 (Thermo Fisher Scientific) and a goat anti-rabbit IgG (H+L) cross-adsorbed, Alexa Fluor 647 (Thermo Fisher Scientific). Both secondary antibodies were used at a final concentration of 1:250. Fins were removed from secondary antibodies after one to two days, rinsed three times for 30 min each with PBS, and stored in PBS @ 4°C until they were imaged.

Taste Bud Density and Distribution Analysis:

Both sets of paired fins (n = 3 individuals for each species) were imaged using a Caliber I.D. RS-G4 confocal microscope (Rochester, NY, USA). Z-series stacks of 3um thickness were taken of the fin rays and associated fin membrane. The location and number of taste buds was determined using the "spot" detection feature in Imaris 9.0.1 software (Bitplane AG). It should be noted that taste bud counts reported here are conservative estimates that only include clearly visible and well-defined taste buds. It is probable our analysis missed those deeper in the tissues as well as those at the distalmost tips where despite strong anti-calretinin activity the boundaries of individual taste buds could typically not be identified.

The density and distribution of receptors were quantified within 5 contiguous regions of interest (ROI) along the proximodistal axis of each fin ray using a custom MATLAB script (Mathworks, Natick, MA, USA). As fin rays were each of a different length, each ROI spanned vertically 20% of fin ray length and extended laterally to a point equidistant to the adjacent ray (Figure 4.1). Receptors within each ROI were counted and a density measurement (cells/mm^2) calculated. On the pectoral and pelvic fin, fin rays # 1 and 2 were combined into a single ROI to capture the full extent of leading-edge innervation. Similarly, the last two rays of the pectoral fin were combined to capture the full extent of innervation on the trailing edge of the fin. In addition to these leading and trailing edges as discussed above, we selected pectoral fin rays #5, 8, 10, and 13 of *P. amboinensis,* #6, 9, 11, and 15 of *C. viridis,* #5, 8, 10, and 14 of *P. coelestis* as well as pelvic fin ray #4 and 6 for taste bud density calculations.

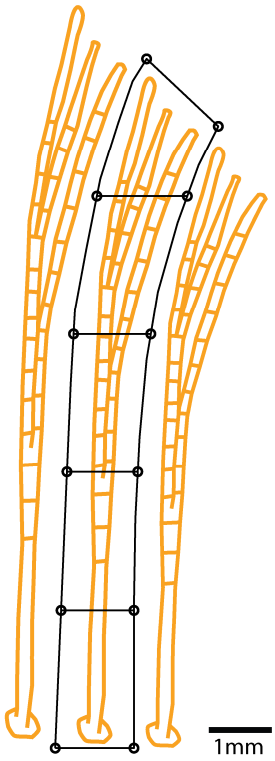

Figure 4.1: Methodology to determine the spatial distribution of extraoral taste buds. Taste buds were identified using the "spot" detection feature in Imaris image analysis software (Bitplane, UK). Density estimates were then calculated within 5 contiguous ROI's along the proximodistal axis of a given fin ray using a custom MATLAB script. Each ROI spanned vertically 20% of fin ray length and extended laterally to a point equidistant to the adjacentmost ray

Results

Gross Fin Morphology:

We examined the external fin morphology of each species in order to account for potential differences among species in the spatial distribution and density of taste buds. The gross morphology of the pectoral and pelvic fin was consistent across species with few notable differences. Pectoral fins had similar ray counts (17-18) in all three species. In these species, the

pectoral fins' leading edge consists of two unbranched rays: the uppermost ray is rudimentary in comparison to fin ray #2 which is much more robust and extends the full length of the leading edge. The trailing edge pectoral fin ray in each species is also unbranched. Other rays branched along their lengths, with the pectoral fin rays of *C. viridis* and *P. coelestis* routinely branching at least twice while the fin rays of *P. amboinensis* branch once. The pelvic fin of each species is composed of a total of 6 fin rays including a robust leading-edge spine that extends halfway along the leading edge. The first soft ray of the pelvic fin (fin ray #2) is the longest and extends posteriorly well beyond the rest of the fin rays. In all three species, this fin ray branches approximately half-way along its length. In *P. coelestis* and *P. amboinensis* the leading-edge bifurcation of this ray, distal to the branchpoint, terminates in a filamentous tip whereas the trailing edge segment retains the classical morphology of more typical soft bony rays. In *C. viridis* however, both sister rays distal to the branchpoint are robust, filamentous, and extend past the margin of the trailing rays.

Taste Bud Morphology:

Immunolabeling identified calretinin-positive endings across the pectoral and pelvic fins of all three species examined. The morphological characteristics of these newly identified bulbous endings in damselfish are consistent with those of taste buds identified previously in other studies. Located at or in close proximity to the epidermal surface, these putative taste buds are small (~15 μm diameter) and protrude to the external surface via a pore of ~ 4 um diameter (Figure 4.2A). Each taste bud is a composite structure consisting of ~ 6 - 10 elongated pear or onion-shaped receptor cells (Figure 4.2B, C). Immunolabeling with a general neuronal marker revealed an organized network of sensory fibers that extend distally within each ray following fin

ray branching patterns. Nerve fibers exit the rays to innervate the inter-ray membrane, a subset of which terminate in expanded tufts at the base of each taste bud (Figure 4.2D). In some locations, we observed nerve fibers innervating multiple taste buds (Figure 4.2E). This finding, along with the observation that nerve bundles run alongside aligned taste buds, suggests that multiple taste buds may operate as a functional unit. While not shown here, we also found taste buds on the dorsal, anal, and caudal fins, further suggesting the importance of chemosensory feedback from the fins of damselfish.

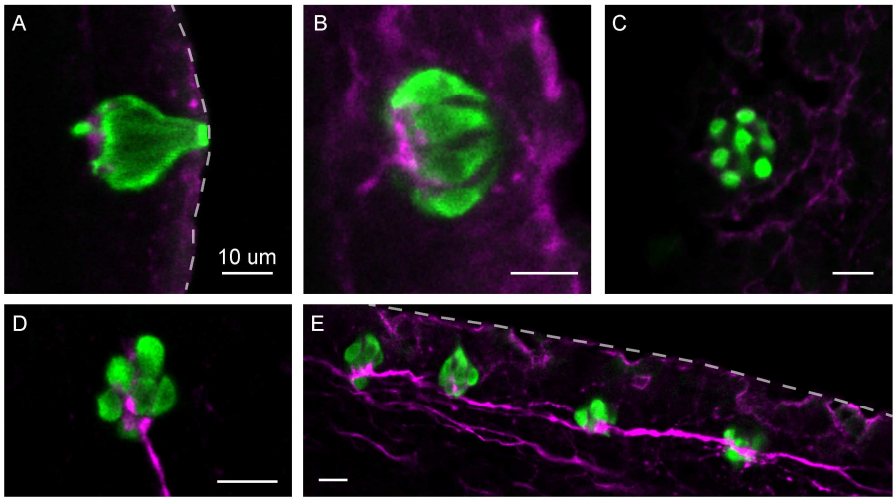

Figure 4.2: Morphology of putative taste buds in the paired fins of damselfish. A) Located at or in close proximity to the epidermal surface, taste buds (anti-calretinin, green) are small (~15-25 µm diameter) pear-shaped sensory structures that extend to the external surface via a 3-5 um diameter pore at their apical end. B) Taste buds are composite structures composed of multiple cell types including the elongated receptor cells shown here. C) The number of these calretinin-positive receptor cells within each taste bud varies from ~ 6 - 10. D) Nerve fibers (AAT, magenta) terminate in an expanded nerve plexus at the base of each taste bud. E) We often observed nerve fibers innervating multiple taste buds suggesting that along a given fin ray taste buds may operate as a functional unit. Dashed lines mark the edge of the fin. Scale bars: 10um.

Patterns of taste bud distribution:

Despite differences between species and fins, we identified several patterns regarding how taste buds are generally distributed across the paired fins of damselfish. Taste buds are

distributed parallel to the long axis of the fin rays **(**Figure 4.3). In proximal, non-branched regions of a fin ray, taste buds are arranged in a column along the proximodistal axis. As fin rays branch distally the column of taste buds also branches. Taste buds were located on or in very close proximity to the fin rays themselves with very few receptors distributed within the inter-ray membrane. On leading and trailing edge fin rays, taste buds are localized to the exterior edge of the rays whereas on central fin rays (i.e. fin rays #5 – 13) taste buds can be found throughout a given ray with no apparent localization to a particular edge.

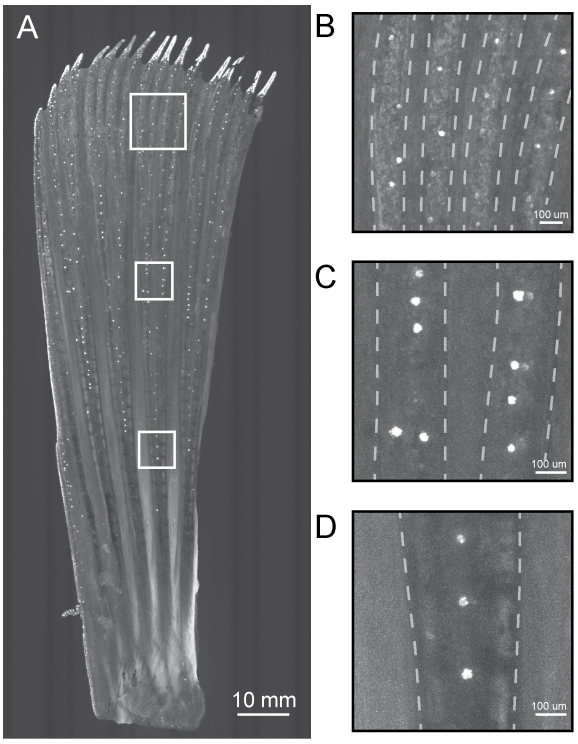

Figure 4.3: Distribution of taste buds within damselfish fins. A) Greyscale image of calretinin fluoresce showing taste buds across the leading-edge pectoral fin rays of the neon damselfish (*Pomacentrus coelestis)*. Boxes show regions enlarged in B-D. Taste buds are distributed parallel to the proximodistal axis of a fin ray. B-D) We found that taste buds were located on or in very close proximity to the fin rays themselves. Taste buds were rarely observed within the inter-ray membrane. As such taste buds in proximal, non-branched regions of a given fin ray are linearly arranged in a column. However, as fin rays branch distally this single row of taste buds observed proximally branch to follow existing fin ray branching patterns. Dashed lines mark the outline of the fin rays. Scale bars: 10mm in A; 100um in B-D.

On the pectoral fin, we found the highest taste bud densities along the margins of the fin such as the leading and trailing edges (Figure 4.4). Excluding these areas, we found a prominent proximodistal gradient along a given fin ray with few receptors observed proximally and much higher densities found distally. Relatedly, taste bud density was lowest in proximal regions of central fin rays (i.e. fin rays #5 – 13). On the pelvic fins, taste buds were most prominent along

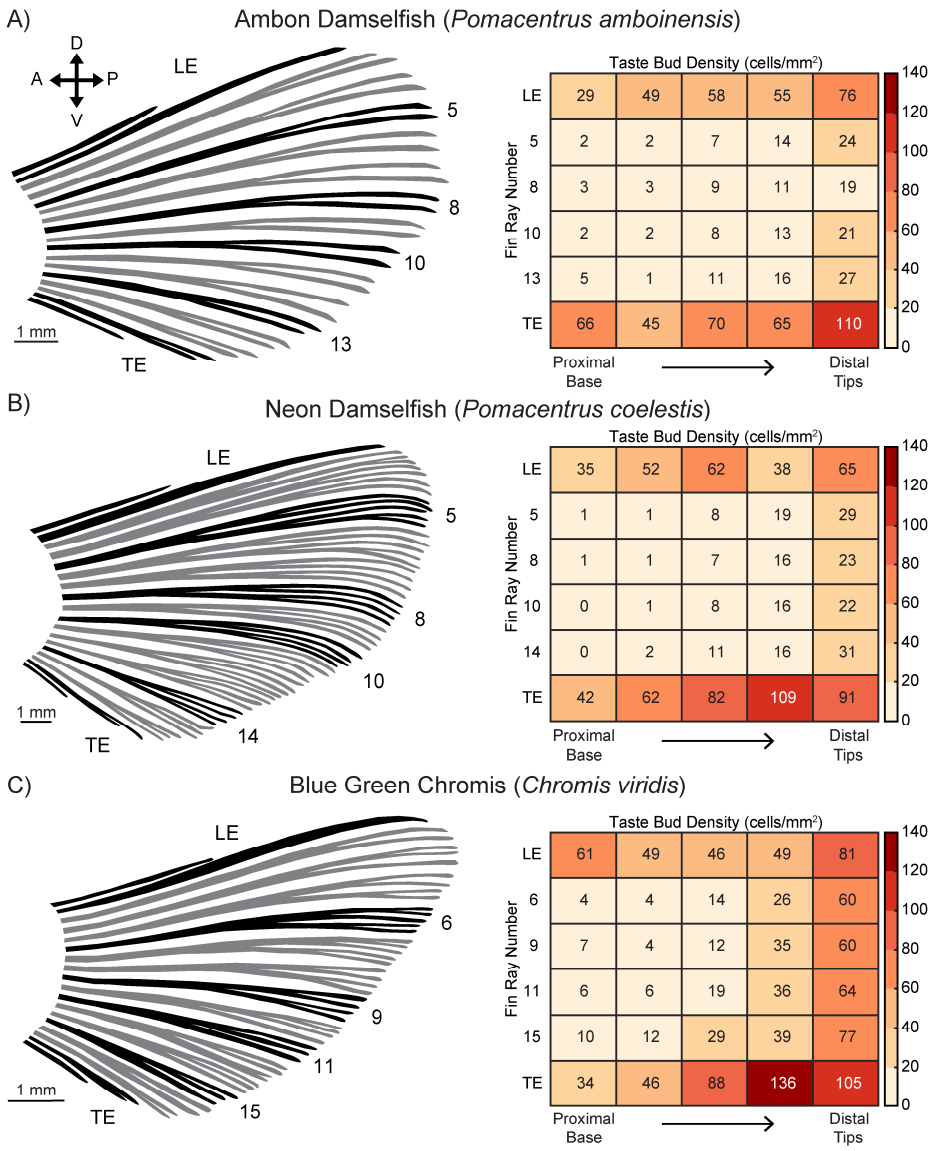

Figure 4.4: Illustration of damselfish pectoral fin morphology and taste bud distribution. Left: Illustration of the pectoral fin from A) *Pomacentrus amboinensis*, B) *Pomacentrus coelestis*, and C) *Chromis viridis*. Fin rays selected for taste bud analysis are colored black and labeled. Right: Heatmaps show the density of taste buds along fin rays of interest. Each row shows data collected from a given fin ray. Each cell shows data collected from a given region of interest (ROI). As fin rays were each of a different length, each ROI spanned vertically 20% of fin ray length and extended laterally to a point equidistant to the adjacentmost ray. The mean (n = 3 individuals) taste bud density (cells/mm^2) for each ROI is marked numerically and is also represented by color (dark red = higher abundance; light orange = lower abundance), as indicated in the key. We find that damselfish pectoral fins are densely innervated on the margins (i.e. edges) of the fin with few taste buds located centrally. Among species, *C. viridis* exhibits the highest mean density of taste buds with a notable increase found in the distal tips of middle fin rays.

the margins with a significant concentration found on the leading edge (Figure 4.5). As noted previously, the first soft ray of the pelvic fin (fin ray #2) extends posteriorly well past the margin of the rest of the fin. We found that the distal tips of these elongated rays were heavily innervated with upwards of 200 taste buds/mm^2, suggesting the importance of this region for gustatory stimuli detection. Taste bud density along the trailing edge of the pelvic fin was much lower than that of the leading edge and consistently lower than that of the pectoral fin trailing edge.

Species Comparisons:

In contrast to our initial hypothesis, *C. viridis* has the highest mean taste bud density for both the pectoral fin (40.65 ± 20.17; mean ± SD) and the pelvic fin (48.44 ± 20.56; mean ± SD). *C. viridis* also has the highest mean density for any particular region of interest with 192 taste buds/mm^2 found in the distalmost ROI of pelvic fin ray #2 (Figure 4.5C). This density is approximately double that of comparable regions found in *P. coelestis* and *P. amboinensis,* which exhibit only a single filamentous distal extension of the fin ray (Figure 4.5A,B).

We observed species-specific differences in the density of taste buds across the paired fins. On the pectoral fin, we found that densities on the leading and trailing edges were fairly consistent across species (Figure 4.4). However, *C. viridis* has a two to three-fold increase in taste bud density on the distal tips of central fin rays (i.e. fin rays #6-15) compared to identical fin regions in the other species. On the pelvic fin, we found that the leading-edge fin rays (fin rays #1 and 2) have much higher densities than the rest of the fin (Figure 4.5). While all species show a reduction in taste bud density from the leading to trailing edge of the pelvic fin, this trend

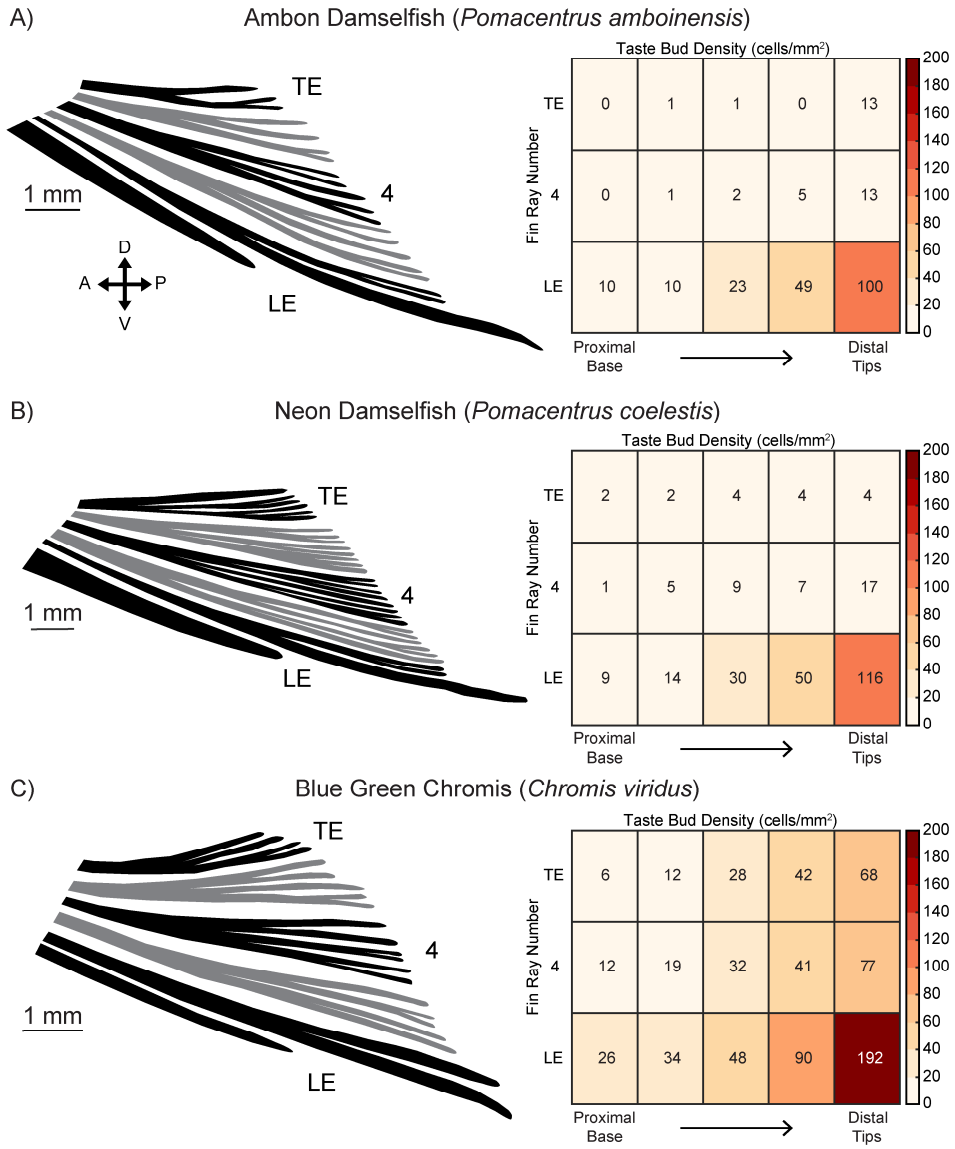

Figure 4.5: Illustration of damselfish pelvic fin morphology and taste bud distribution. Left: Illustration of the pectoral fin from A) *Pomacentrus amboinensis*, B) *Pomacentrus coelestis*, and C) *Chromis viridis*. Fin rays selected for taste bud analysis are colored black and labeled. Right: Heatmaps show the density of taste buds along fin rays of interest. Each row shows data collected from a given fin ray. Each cell shows data collected from a given region of interest (ROI). As fin rays were each of a different length, each ROI spanned vertically 20% of fin ray length and extended laterally to a point equidistant to the adjacentmost ray. The mean (n = 3 individuals) taste bud density (cells/mm^2) for each ROI is marked numerically and is also represented by color (dark red = higher abundance; light orange = lower abundance), as indicated in the key. Pelvic fins are heavily innervated with taste buds found primarily along the edges of the fin. However, in contrast to the pectoral fin, taste buds exhibited a marked decrease along the pelvic fin chord from the leading to the trailing edge. The distal tips of pelvic fin ray #2 exhibit the highest innervation in all three species indicating this regions importance in sensation. As a possible adaption to feeding primarily on zooplankton, *C. viridis* exhibit two filamentous distal extension of this fin ray leading to approximately double the innervation density in the region.

was more pronounced in *P. amboinensis* and *P. coelestis*. In these species taste bud densities decreased tenfold from the leading to trailing edge whereas in *C. viridis* the reduction was approximately threefold. The high degree of innervation of the paired fins of *C. viridis* suggest that the fins serve a more vital role in gustation than do those of *P. coelestis* and *P. amboinensis*.

Discussion

From these results we conclude that: 1) the paired fins of damselfishes are heavily innervated with taste buds and likely are important for chemosensation, 2) damselfishes exhibit fewer extraoral taste buds on fins compared to benthic fishes, and 3) the functional demands for gustation in damselfishes varies across fins and among species.

The abundance of taste buds on the paired fins of damselfishes suggests that these regions are important for chemosensation. By distributing taste buds across the fins, fishes can greatly increase the available surface area for chemical detection. Fins also function as locomotor devices that move fluid and sweep through it and as such the presence of taste buds on fins likely serves to enhance the likelihood of detecting chemical cues, particularly when holding station in low flow environments. Fishes must not only be able to detect relevant chemical stimuli, but also to locate them in space. Fishes with widely dispersed taste buds can more easily compare the strength of chemical stimuli arriving at different points along the body and fins in order to localize the source of a chemical (Harvey and Batty, 2002). This ability to spatially discriminate relevant chemical cues allows catfish, for example, to efficiently locate a food source with minimal energy expenditure using the sense of taste alone (Bardach et al., 1967).

The distribution patterns of taste buds found here in the paired fins of damselfishes are similar to those of other species previously examined, suggesting a common set of design

principles by which taste buds are arranged on fins. Unlike the fins of benthic species such as catfish that are more likely to come into physical contact with potential food items while foraging, we argue that taste buds on the fins of damselfishes facilitate taste at a distance. As such the spatial distribution of taste buds across the paired fins reflects regions most likely to contact oncoming chemical compounds. On both the pectoral and pelvic fin of the three species examined here, we found the highest densities along fin margins and much lower densities within interior regions of the fin. As damselfishes utilize their pectoral fins as the primary propulsors during swimming (Hale et al., 2006), the spatial distribution of taste buds appears to be correlated with regions optimally exposed to water flow and most likely to first come into contact with chemical stimuli. It has also been hypothesized that this distribution takes advantage of the thinner hydrodynamic boundary layer over the edges of the fin which serves to enhance the likelihood of chemical stimuli reaching a given taste bud (Harvey and Batty, 1998; Harvey and Batty, 2002). We found that each species examined here has a robust distal extension of pelvic fin ray #2. While the precise nature of how damselfish pelvic fins function during behavior is unknown, it is clear that these fins are heavily innervated. Given that the pelvic fins are positioned in line with the body axis, taste buds distributed along the leading edge, similar to that of the pectoral fin, are optimally located to detect oncoming chemical stimuli. Furthermore, when fully extended below the body, the pelvic fin ray extensions along the leading edge increase the depth of the animal up to ~40% thus expanding the area for chemical sensation over a greater depth of the water column.

The results presented here from the paired fins of three damselfish species provide further support for the hypothesis that pelagic and/or visual predators typically have fewer extraoral taste buds than benthic, nocturnal, and/or fishes inhabiting turbid water. While previous studies

that report on the density of taste buds from a single or limited number of locations along the paired fins limit comparisons among species, it is clear that damselfishes exhibit fewer taste buds than many other species. Harvey and Batty (1998) show that the paired fins of cod (*Gadus morhua*) are heavily innervated with spot densities of up to 700/mm^2 on the pectoral fin and a mean density of 439/mm^2 for the first two pelvic fin rays. Harvey and Batty (2002) provide comparative quantitative data on the abundance and distribution of cutaneous taste buds in five species of gadoid fishes. These authors found that densities along the pectoral fin leading edge were typically less than 100/mm^2 and that mean densities on the pelvic fin rays ranged from 42 – 398/mm^2 across species. Gomahr et al. (1992) report data on ten cyprinid species with measurements taken from a single location at the base of fins. Densities of up to 150 and 132/mm^2 were found on the pectoral and pelvic fins respectively with the more benthic fishes typically exhibiting the most taste buds. While most regions of the damselfish fin exhibit fewer than 50 taste buds per mm^2, we find that densities at the distal tips of leading-edge pelvic fin rays are comparable with the fins and even barbels of many species. For example, the barbels of catfish and goatfish probe the substrate in search of food with taste bud densities reaching over 200/mm^2 at their distal tips (Kiyohara et al., 2002; Sakata et al., 2001). The high innervation density relative to the rest of the paired fins and comparable innervation to that of barbels, classically thought of as highly specialized structures for taste sensation, suggest that the modified extensions of the pelvic fin rays may serve as a specialized sensory device. Taken together, it is likely that body and fin regions from fishes inhabiting a diversity of environments and environmental conditions possess taste bud at densities previously thought confined only to fishes and structures specialized for taste.

We found that *C. viridis* exhibited the highest density of taste buds across the paired fins and hypothesize that this may be a specialization for feeding almost exclusively on zooplankton. *C. viridis* form large stationary aggregations high in the water column above coral heads while foraging. As obligate coral dwelling damselfish, *C. viridis* localize to particular coral heads and as such rely on the current to deliver them planktonic foods. While interpretations of the relationship between sensory morphology and ecology are limited here due to low species sampling, we hypothesize that the increased density of cutaneous taste buds observed in this species better facilitate the detection of food items upstream allowing these fishes to properly orient to them. Once prey appear within visual range, *C. viridis* utilize a low-suction feeding mode to capture their prey (Coughlin and Strickler, 1990). Compared to *P. coelestis* and *P. amboinensis*, the increased density of the two heavily innervated filamentous extensions of pelvic fin ray #2 also likely increases the ability of *C. viridis*' to detect chemical cues at lower concentrations. Future behavioral work that investigates how the pelvic fins are actuated during the different phases of feeding (i.e. detection, localization, and prey capture) will further tease apart the function underpinning the distribution of this sensory anatomy.

To further tease apart the relationship between fin sensory morphology and fish ecology, future comparative work that more broadly samples across Pomacentridae will be needed. While *P. coelestis* and *P. amboinensis* are found close to the bottom and thus might be expected to rely less on vision given that visual cues are often limited by highly complex habitats like coral reefs, these fishes exhibited lower taste bud densities across the paired fins. Almost all damselfishes have been classified as either herbivores, planktivores, or omnivores that consume both filamentous algae and small animal prey. As omnivores, the diet of these *P. coelestis* and *P. amboinensis* includes variable amounts of benthic algae that we hypothesize lower the demands

for gustation via fins. The abundance and spatial distribution of taste buds in herbivorous damselfish that feed exclusively on algae would provide useful information on the utility of extraoral taste buds across the range of damselfish diets. Based on the results presented here, we hypothesize that taste bud densities across the paired fins are highest for planktivores and lowest for the herbivores.

While taste buds provide feedback that has obvious implications during feeding, extraoral taste buds may also function in other contexts. Damselfish are known to utilize chemical alarm cues elicited through mechanical damage from the skin of conspecifics as well as diet cues released upon defecation by a predator to assess the risk of predation (Ferrari et al., 2017; Lönnstedt and McCormick, 2011; McCormick et al., 2019). Chemical cues are also known to influence orientation and settlement behaviors among fishes (Atema et al., 2002; Døving et al., 2006; Lecchini et al., 2005). It has largely been assumed that these types of chemical cues are detected solely by the olfactory systems of fishes, but their chemical composition and identify is still unknown (Ferrari et al., 2010; Mitchell et al., 2017). In teleosts, olfactory receptors are limited to the olfactory organs located in the dorsal part of the snout which transmit chemical information via the olfactory cranial nerve (I). While anatomically distinct, the olfactory and oral gustatory systems of fishes are known to detect similar types of chemical stimuli (*i.e.,* amino acids, bile salts) at comparable concentrations (Caprio, 1977; Hara, 1994). Therefore, extraoral taste buds may supplement the olfactory system in detecting some of these aforementioned chemical cues. To date however, no study has examined the electrophysiological response of taste buds on fins and comparisons within the same species between oral and extraoral taste buds on other body regions are few (Kasumyan, 1999; Ogawa and Caprio, 2010; Shamushaki et al., 2008). Interestingly, these studies have shown that extraoral taste buds in fishes with a developed

extraoral taste system are more sensitive and have a wider effective spectrum to amino acids than their oral counterparts. Extraoral taste buds may also play a functional role in mechanosensation. It has been suggested that mechanical displacement of the taste bud may deflect microvilli of the basal Merkel-like cells or alternatively, free nerve endings within or surrounding the taste buds thus allowing the detection of tactile stimuli (Ogawa et al., 1997; Zachar and Jonz, 2012). Future experiments combining cell labeling techniques and intracellular physiological recordings will be necessary to test the chemosensory and mechanosensory abilities of taste buds on fins.

We predict that cutaneous taste buds are more widespread and utilized among fishes than previously thought. Coral reef fishes such as damselfish inhabit an environment where the external gustatory system has not been previously investigated. Classically, it has been hypothesized that pelagic and/or sight feeding fishes rely less on gustation during feeding given their lack or limited number of extraoral taste buds. The results presented here suggest that fishes inhabiting a myriad of habitats and environmental conditions may utilize chemosensory feedback from taste buds across the body and fins. While damselfish, like many other diurnal reef fishes, are thought to rely on vision during feeding, feedback from extraoral taste buds across the paired fins can complement and function together with the other sensory systems to maximize feeding efficiency. Looking at the diet and paired fin morphology of other coral reef fishes, it is likely that taste buds on fins are abundant in many genera. Future investigations should take a comparative approach to understand the full extent of taste buds across fishes as well as the ecological factors that influence the placement and number of these receptors on particular regions of the body and fins.

CHAPTER FIVE: DISCUSSION

During my dissertation, I have combined studies of behavior, anatomy, and physiology to examine the capacities of fish fins as sensors. A diversity of fishes live in complex habitats where feedback on the surrounding environment and their interactions within it can modulate behavior. In my second chapter, I found fin ray afferents within the membranous pectoral fin of a bottom dwelling fish are sensitive to touch. In my third chapter, I found that fish can sense coarse tactile features of their near range physical environment via fins with similar morphology and response properties to other vertebrates. Taking advantage of established immunohistochemical approaches to labeling the gustatory sensory morphology of fishes, I examined the spatial distribution of taste buds across the paired fins of damselfish. In my fourth chapter, I found via mapping taste buds across the entire fin surface that sensory morphology of the gustatory system is adaptable and relates to primary fin function and damselfish ecology. As a conclusion to this thesis, I discuss my results more broadly and propose future experiments that will further elucidate the morphology and structure-function relationships that underpin fins as multimodal sensors.

The results from chapters two and three show that the fin ray sensory system of select species is well equipped to provide feedback on touch events and contacted surface features. As both chapters investigated the pectoral fins of substrate associated species it remains to be seen whether the observed afferent response represents a specialized condition for contact in benthic species or a general feature of all fins. In addition to these broader electrophysiological studies, future comparative work on mechanoreceptor topography is necessary to understand how fins and perhaps even particular fin rays may be adapted for touch. Given the varying frequencies

between which the fins of benthic and non-benthic fishes touch the substrate, I hypothesize that the fins of benthic fishes are more densely innervated to meet the increased functional demands for touch. Additionally, mechanoreceptors in other vertebrates are often concentrated in regions of great functional significance for tactile sensitivity such as the finger pads of primates (Johansson and Vallbo, 1979), the nose of moles (Sawyer and Catania, 2016) or the bill tip of birds (Gentle and Breward, 1986; Gottschaldt and Lausmann, 1974). It is unclear whether mechanoreceptors in fins are similarly localized to particular regions of the fin such as the distal tips of fin rays where touch events may more readily occur. Understanding the function and distribution of fin-associated sensory morphology also has the potential to inspire membranous sensor placement for use in engineering contexts. The integration of tactile feedback into the design of underwater robotic devices can facilitate complex tasks involving object manipulation or substrate associated locomotion.

A major limitation to interpreting the form and function of fin ray sensory systems is the inability to match the activity patterns of a given receptor to its morphology. Current approaches utilizing spike sorting techniques to analyze multiunit physiology of fin ray afferents have shown their capacity for proprioception and touch sensation, but the physiological significance of different receptor endings is currently unknown. Simultaneously integrating confocal imaging and electrophysiological techniques with ethologically relevant manipulations of the fins would facilitate the one to one mapping of sensory morphology and function. The fin ray sensory system is capable of both slowly and rapidly adapting responses to mechanical stimuli, indicative of a diversity of mechanosensitive cell types (Aiello et al., 2016; Aiello et al., 2017; Hardy et al., 2016; Williams IV et al., 2013). To date free nerve endings and putative Merkel cells have been found in membranous pectoral fins (Hardy et al., 2016; Williams IV et al., 2013), but it is unclear

whether other types of touch receptors are present, what aspects of the tactile response each type of sensory structure is responsible for, and whether putative Merkel cells in fins respond to stimulation consistent with other taxa. Hindering progress on these questions has been the sparsity of established mechanoreceptor antibody markers in fish and associated challenges to mapping these receptors in whole mount fin preparations. However, through a more targeted account of fin sensory morphology we can begin to inform the evolutionary histories of sensory endings across vertebrate appendages and the functional demands arose to meet.

The results from chapter four show that the utility of taste buds on fins is likely more widespread across fishes than previously expected. Extraoral taste buds have been hypothesized to be largely confined to fishes inhabiting benthic environments or turbid water where vision is of limited use. Previous studies have shown that taste bud density and topography can generally can be predicted from a species proximity to the bottom, but exceptions to the rule indicate that other ecological and behavioral considerations must be investigated. For example, Gomahr et al. (1992) found that among cyprinids the minnow (*Phoxinus phoxinus*) exhibited relatively high taste bud densities across the body and fins despite being found mid-water in clear creeks and lakes. Similarly, data on the paired fins of damselfishes presented in chapter four indicate that these fishes exhibit numerous extraoral taste buds. Damselfish live in an environment (i.e. clear, shallow waters near coral reefs) and employ a feeding strategy (i.e. rely on vision during foraging) not previously expected to benefit from extraoral taste buds. Therefore, in addition to habitat type, research needs to look more broadly at varying feeding modes and even behavioral ecology to understand the utility of extraoral taste buds.

Complicating our understanding of taste bud topography on fins and their distribution across fishes is the lack of studies detailing the function of extraoral taste buds. While these

receptors almost certainly contribute to feeding behaviors, it is largely unclear whether oral and extraoral taste buds are functionally equivalent. For example, do taste buds located in the mouth respond to similar types of stimuli and at comparable concentrations than those taste buds across the body and fins? Given the hypothesized need to facilitate taste at a distance, extraoral taste buds on fins may be more sensitive and respond to a broader range of chemical stimuli than those in or near the oral cavity. To date however, no study has examined the electrophysiological response of taste buds on fins, but comparisons within the same species between oral and extraoral taste buds on other body regions suggest that such functional differences do exist (Kasumyan, 1999; Ogawa and Caprio, 2010; Shamushaki et al., 2008). Future comparative studies that target the physiology of taste buds from a variety of oral, body, and fin regions together with measurements on taste bud density will provide a more complete picture of how fishes of varying ecology are adapted to detecting chemical stimuli.

Taste buds have also been postulated to function in touch. A number of species have shown that the gustatory nerves (VII, IX, and X) respond not only to chemical, but also to tactile (touching, sliding, brushing, and water current) stimuli (Davenport and Caprio, 1982; Kiyohara et al., 1985; Ogawa et al., 1997). While the majority of fibers within these nerves respond to either chemical or mechanical stimulation, bimodal fibers have also been found. It remains to be seen however, whether the taste buds themselves or possibly the nerve fibers surrounding these receptors are the source of this mechanical sensitivity. It has also commonly been suggested that the basal cells of taste buds which have synaptic contacts with nerve terminals and contain serotonin might function as mechanoreceptors (Nakamura et al., 2017; Sakata et al., 2001; Toyoshima et al., 1984; Zachar and Jonz, 2012). As mentioned previously, the integration of imaging and electrophysiology with fine tactile manipulations of individual taste buds on fins

has the potential to facilitate testing their possible mechanosensory functions. Taste buds as multimodal sensory structures that exhibit both chemosensory and mechanosensory capacities would open up exciting avenues of future research regarding sensory integration and the role of extraoral taste buds in non-feeding behaviors.

REFERENCES

Aiello, B. R., Stewart, T. A. and Hale, M. E. (2016). Mechanosensation in an adipose fin. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 283, 20152794.

Aiello, B. R., Westneat, M. W. and Hale, M. E. (2017). Mechanosensation is evolutionarily tuned to locomotor mechanics. *Proceedings of the National Academy of Sciences, USA* 114, 4459-4464.

Akanyeti, O. and Liao, J. C. (2013). The effect of flow speed and body size on Karman gait kinematics in rainbow trout. *Journal of Experimental Biology* 216, 3442-3449.

Alexander, R. M. (1966). Structure and function in the catfish. *Journal of Zoology* 148, 88-152.

Allen, G. R. (1991). Damselfishes of the world. Melle, Germany: Mergus.

Atema, J. (1971). Structures and functions of the sense of taste in the catfish (*Ictalurus natalis*). *Brain, Behavior and Evolution* 4, 273-294.

Atema, J., Kingsford, M. J. and Gerlach, G. (2002). Larval reef fish could use odour for detection, retention and orientation to reefs. *Marine Ecology Progress Series* 241, 151-160.

Bardach, J., Todd, J. and Crickmer, R. (1967). Orientation by taste in fish of the genus Ictalurus. *Science* 155, 1276-1278.

Bardach, J. E. and Case, J. (1965). Sensory capabilities of the modified fins of squirrel hake (*Urophycis chuss*) and searobins (*Prionotus carolinus* and *P. evolans*). *Copeia*, 194-206.

Barreiro-Iglesias, A., Villar-Cerviño, V., Villar-Cheda, B., Anadón, R. and Rodicio, M. C. (2008). Neurochemical characterization of sea lamprey taste buds and afferent gustatory fibers: Presence of serotonin, calretinin, and CGRP immunoreactivity in taste bud bi-ciliated cells of the earliest vertebrates. *Journal of Comparative Neurology* 511, 438-453.

Blake, D. T., Hsiao, S. S. and Johnson, K. O. (1997). Neural coding mechanisms in tactile pattern recognition: the relative contributions of slowly and rapidly adapting mechanoreceptors to perceived roughness. *Journal of Neuroscience* 17, 7480-7489.

Bosher, B. T., Newton, S. H. and Fine, M. L. (2006). The spines of the channel catfish, *Ictalurus punctatus*, as an anti-predator adaptation: an experimental study. *Ethology* 112, 188-195.

Boulais, N. and Misery, L. (2007). Merkel cells. *Journal of the American Academy of Dermatology* 57, 147-165.

Caprio, J. (1977). Electrophysiological distinctions between the taste and smell of amino acids in catfish. *Nature* 266, 850-851.

ÇInar, K., ŞEnol, N. and Kuru, N. (2008). The distribution of taste buds in *Garra rufa*. *Anatomia, Histologia, Embryologia* 37, 63-66.

Connor, C. E., Hsiao, S. S., Phillips, J. R. and Johnson, K. O. (1990). Tactile roughness: neural codes that account for psychophysical magnitude estimates. *Journal of Neuroscience* 10, 3823-3836.

Connor, C. E. and Johnson, K. O. (1992). Neural coding of tactile texture: comparison of spatial and temporal mechanisms for roughness perception. *Journal of Neuroscience* 12, 3414-3426.

Coughlin, D. J. and Strickler, J. R. (1990). Zooplankton capture by a coral reef fish: an adaptive response to evasive prey. *Environmental Biology of Fishes* 29, 35-42.

Darian-Smith, I. and Kenins, P. (1980). Innervation density of mechanoreceptive fibres supplying glabrous skin of the monkey's index finger. *Journal of Physiology* 309, 147-155.

Darian-Smith, I. and Oke, L. E. (1980). Peripheral neural representation of the spatial frequency of a grating moving across the monkey's finger pad. *Journal of Physiology* 309, 117-133.

Davenport, C. J. and Caprio, J. (1982). Taste and tactile recordings from the ramus recurrens facialis innervating flank taste buds in the catfish. *Journal of Comparative Physiology A* 147, 217-229.

Davis, B. J. and Miller, R. J. (1967). Brain patterns in minnows of the genus Hybopsis in relation to feeding habits and habitat. *Copeia*, 1-39.

Diamond, M. E., Von Heimendahl, M., Knutsen, P. M., Kleinfeld, D. and Ahissar, E. (2008). 'Where' and 'what' in the whisker sensorimotor system. *Nature Reviews Neuroscience* 9, 601-612.

Díaz-Regueira, S. M., Lamas, I. and Anadón, R. (2005). Calretinin immunoreactivity in taste buds and afferent fibers of the grey mullet *Chelon labrosus*. *Brain Research* 1031, 297-301.

Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M., Kram, R. and Lehman, S. (2000). How animals move: an integrative view. *Science* 288, 100-106.

Døving, K. B., Stabell, O. B., Östlund-Nilsson, S. and Fisher, R. (2006). Site fidelity and homing in tropical coral reef cardinalfish: are they using olfactory cues? *Chemical Senses* 31, 265-272.

Essick, G. K. and Edin, B. B. (1995). Receptor encoding of moving tactile stimuli in humans. II. The mean response of individual low-threshold mechanoreceptors to motion across the receptive field. *Journal of Neuroscience* 15, 848-864.

Ferrari, M. C., McCormick, M. I., Allan, B. J. and Chivers, D. P. (2017). Not equal in the face of habitat change: closely related fishes differ in their ability to use predation-related information in degraded coral. *Proceedings of the Royal Society B: Biological Sciences* 284, 20162758.

Ferrari, M. C., Wisenden, B. D. and Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology* 88, 698-724.

Finger, T. E., Drake, S. K., Kotrschal, K., Womble, M. and Dockstader, K. C. (1991). Postlarval growth of the peripheral gustatory system in the channel catfish, *Ictalurus punctatus*. *Journal of Comparative Neurology* 314, 55-66.

Fishelson, L. and Delarea, Y. (2004). Taste buds on the lips and mouth of some blenniid and gobiid fishes: comparative distribution and morphology. *Journal of Fish Biology* 65, 651-665.

Flammang, B. E. and Lauder, G. V. (2013). Pectoral fins aid in navigation of a complex environment by bluegill sunfish under sensory deprivation conditions. *Journal of Experimental Biology* 216, 3084-3089.

Fox, H. (1999). Barbels and barbel-like tentacular structures in sub-mammalian vertebrates: a review. *Hydrobiologia* 403, 153-193.

Fox, H. and Whitear, M. (1978). Observations of Merkel cells in amphibians. *Biologie Cellulaire* 32, 223-232.

Frédérich, B., Sorenson, L., Santini, F., Slater, G. J. and Alfaro, M. E. (2013). Iterative ecological radiation and convergence during the evolutionary history of damselfishes (Pomacentridae). *The American Naturalist* 181, 94-113.

Gentle, M. J. and Breward, J. (1986). The bill tip organ of the chicken (*Gallus gallus var. domesticus*). *Journal of Anatomy* 145, 79.

Germanà, A., Paruta, S., Germanà, G. P., Ochoa-Erena, F. J., Montalbano, G., Cobo, J. and Vega, J. A. (2007). Differential distribution of S100 protein and calretinin in mechanosensory and chemosensory cells of adult zebrafish (*Danio rerio*). *Brain Research* 1162, 48-55.

Gibb, A., Jayne, B. and Lauder, G. (1994). Kinematics of pectoral fin locomotion in the bluegill sunfish *Lepomis macrochirus*. *Journal of Experimental Biology* 189, 133-161.

Gibson, R. and Robb, L. (2000). Sediment selection in juvenile plaice and its behavioural basis. *Journal of Fish Biology* 56, 1258-1275.

Goldberg, J. M. and Brown, P. B. (1969). Response of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: some physiological mechanisms of sound localization. *Journal of Neurophysiology* 32, 613-636.

Gomahr, A., Palzenberger, M. and Kotrschal, K. (1992). Density and distribution of external taste buds in cyprinids. *Environmental Biology of Fishes* 33, 125-134.

Goodwin, A. and Morley, J. (1987). Sinusoidal movement of a grating across the monkey's fingerpad: representation of grating and movement features in afferent fiber responses. *Journal of Neuroscience* 7, 2168-2180.

Gosline, W. A. (1994). Function and structure in the paired fins of scorpaeniform fishes. *Environmental Biology of Fishes* 40, 219-226.

Gottschaldt, K.-M. and Lausmann, S. (1974). The peripheral morphological basis of tactile sensibility in the beak of geese. *Cell and Tissue Research* 153, 477-496.

Green, M. H., Ho, R. K. and Hale, M. E. (2011). Movement and function of the pectoral fins of the larval zebrafish (*Danio rerio*) during slow swimming. *Journal of Experimental Biology* 214, 3111-3123.

Greenspan, J. D. (1992). Influence of velocity and direction of surface-parallel cutaneous stimuli on responses of mechanoreceptors in feline hairy skin. *Journal of Neurophysiology* 68, 876-889.

Halata, Z., Grim, M. and Bauman, K. I. (2003). Friedrich Sigmund Merkel and his "Merkel cell", morphology, development, and physiology: review and new results. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists* 271, 225-239.

Hale, M. E., Day, R. D., Thorsen, D. H. and Westneat, M. W. (2006). Pectoral fin coordination and gait transitions in steadily swimming juvenile reef fishes. *Journal of Experimental Biology* 209, 3708-3718.

Hallberg, E. and Skog, M. (2010). Chemosensory sensilla in crustaceans. In *Chemical communication in crustaceans*, (eds. T. Breithaupt and M. Thiel), pp. 103-121. New York, NY: Springer.

Halstead, B. W., Kuninobu, L. S. and Hebard, H. G. (1953). Catfish stings and the venom apparatus of the Mexican catfish, *Galeichthys felis* (Linnaeus). *Transactions of the American Microscopical Society* 72, 297-314.

Hansson, B. S. and Stensmyr, M. C. (2011). Evolution of insect olfaction. *Neuron* 72, 698-711.

Hara, T. J. (1994). Olfaction and gustation in fish: an overview. *Acta Physiologica Scandinavica* 152, 207-217.

Hardy, A. R., Steinworth, B. M. and Hale, M. E. (2016). Touch sensation by pectoral fins of the catfish *Pimelodus pictus*. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 283, 20152652.

Harvey, R. and Batty, R. (1998). Cutaneous taste buds in cod. *Journal of Fish Biology* 53, 138-149.

Harvey, R. and Batty, R. (2002). Cutaneous taste buds in gadoid fishes. *Journal of Fish Biology* 60, 583-592.

Hawryshyn, C., Moyer, H., Allison, W., Haimberger, T. and McFarland, W. (2003). Multidimensional polarization sensitivity in damselfishes. *Journal of Comparative Physiology A* 189, 213-220.

Higham, T. E. (2007). Feeding, fins and braking maneuvers: locomotion during prey capture in centrarchid fishes. *Journal of Experimental Biology* 210, 107-117.

Hobson, E. S. (1975). Feeding patterns among tropical reef fishes. *American Scientist* 63, 382-392.

Hobson, E. S. and Chess, J. R. (1978). Trophic relationships among fishes and plankton in the lagoon at Enewetak Atoll, Marshall Islands. *Fishery Bulletin* 76, 133-153.

Ibarra, M. and Stewart, D. J. (1989). Longitudinal Zonation of Sandy Beach Fishes in the Napo River Basin, Eastern Ecuador. *Copeia* 1989, 364.

Iggo, A. and Muir, A. R. (1969). The structure and function of a slowly adapting touch corpuscle in hairy skin. *Journal of Physiology* 200, 763-796.

Jakubowski, M. and Whitear, M. (1990). Comparative morphology and cytology of taste buds in teleosts. *Jahrbuch für Morphologie und mikroskopische Anatomie. 2. Abteilung, Zeitschrift für mikroskopisch-anatomische Forschung* 104, 529-560.

Johansson, R. S. and Vallbo, A. (1979). Tactile sensibility in the human hand: relative and absolute densities of four types of mechanoreceptive units in glabrous skin. *Journal of Physiology* 286, 283-300.

Johnson, K. O. (2001). The roles and functions of cutaneous mechanoreceptors. *Current Opinion in Neurobiology* 11, 455-461.

Jones, E. A., Lucey, K. S. and Ellerby, D. J. (2007). Efficiency of labriform swimming in the bluegill sunfish (*Lepomis macrochirus*). *Journal of Experimental Biology* 210, 3422-3429.

Kapoor, B., Evans, H. E. and Pevzner, E. (1976). The gustatory system in fish. In *Advances in Marine Biology*, vol. 13 (eds. F. S. Russel and M. Yonge), pp. 53-108. London, New York, NY: Academic Press.

Kasumyan, A. (1999). Olfaction and taste senses in sturgeon behaviour. *Journal of Applied Ichthyology* 15, 228-232.

Kiyohara, S., Hidaka, I., Kitoh, J. and Yamashita, S. (1985). Mechanical sensitivity of the facial nerve fibers innervating the anterior palate of the puffer, *Fugu pardalis*, and their central projection to the primary taste center. *Journal of Comparative Physiology A* 157, 705-716.

Kiyohara, S., Sakata, Y., Yoshitomi, T. and Tsukahara, J. (2002). The 'goatee'of goatfish: Innervation of taste buds in the barbels and their representation in the brain. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269, 1773-1780.

Kiyohara, S., Yamashita, S. and Kitoh, J. (1980). Distribution of taste buds on the lips and inside the mouth in the minnow, *Pseudorasbora parva*. *Physiology & Behavior* 24, 1143-1147.

Knibestöl, M. (1975). Stimulus-response functions of slowly adapting mechanoreceptors in the human glabrous skin area. *Journal of Physiology* 245, 63-80.

Kotrschal, K., Whitear, M. and Finger, T. E. (1993). Spinal and facial innervation of the skin in the gadid fish *Ciliata mustela* (Teleostei). *Journal of Comparative Neurology* 331, 407-417.

Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature* 436, 647-654.

Künzler, R. and Bakker, T. C. (2000). Pectoral fins and paternal quality in sticklebacks. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267, 999-1004.

Kurosumi, K., Kurosumi, U. and Inoue, K. (1979). Morphological and Morphometric Studies with the Electron Microscope on the Merkel Cells and Associated Nerve Terminals of Normal and Denervated Skin. *Archivum histologicum japonicum* 42, 243-261.

Lane, E. B. and Whitear, M. (1977). On the occurrence of Merkel cells in the epidermis of teleost fishes. *Cell and Tissue Research* 182, 235-246.

Lecchini, D., Planes, S. and Galzin, R. (2005). Experimental assessment of sensory modalities of coral-reef fish larvae in the recognition of their settlement habitat. *Behavioral Ecology and Sociobiology* 58, 18-26.

Lederman, S. J. and Klatzky, R. L. (1993). Extracting object properties through haptic exploration. *Acta Psychologica* 84, 29-40.

Lederman, S. J., Loomis, J. M. and Williams, D. A. (1982). The role of vibration in the tactual perception of roughness. *Perception and Psychophysics* 32, 109-116.

Liao, J. C., Beal, D. N., Lauder, G. V. and Triantafyllou, M. S. (2003a). Fish exploiting vortices decrease muscle activity. *Science* 302, 1566-1569.

Liao, J. C., Beal, D. N., Lauder, G. V. and Triantafyllou, M. S. (2003b). The Kármán gait: novel body kinematics of rainbow trout swimming in a vortex street. *Journal of Experimental Biology* 206, 1059-1073.

Lönnstedt, O. M. and McCormick, M. I. (2011). Chemical alarm cues inform prey of predation threat: the importance of ontogeny and concentration in a coral reef fish. *Animal Behaviour* 82, 213-218.

Lowenstein, O. (1956). Pressure receptors in the fins of the dogfish *Scylliorhinus canicula*. *Journal of Experimental Biology* 33, 417-421.

Manfredi, L. R., Saal, H. P., Brown, K. J., Zielinski, M. C., Dammann III, J. F., Polashock, V. S. and Bensmaia, S. J. (2014). Natural scenes in tactile texture. *Journal of Neurophysiology* 111, 1792-1802.

Masino, M. A. and Fetcho, J. R. (2005). Fictive swimming motor patterns in wild type and mutant larval zebrafish. *Journal of Neurophysiology* 93, 3177-3188.

McCormick, M. I. (1993). Development and changes at settlement in the barbel structure of the reef fish, *Upeneus tragula* (Mullidae). *Environmental Biology of Fishes* 37, 269-282.

McCormick, M. I., Ferrari, M. C., Fakan, E. P., Barry, R. P. and Chivers, D. P. (2019). Diet cues and their utility for risk assessment in degraded habitats. *Animal Behaviour* 152, 19-28.

Miller, P. (1986). Gobiidae. In *Fishes of the North-eastern Atlantic and Mediterranean*, vol. 3 (eds. P. J. P. Whitehead, M. L. Bauchot, J. C. Hureau, J. Nielsen, and E. Tortonese), pp. 1019-1085. Paris, France: United Nations Educational, Scientific and Cultural Organization.

Mitchell, M. D., Bairos-Novak, K. R. and Ferrari, M. C. (2017). Mechanisms underlying the control of responses to predator odours in aquatic prey. *Journal of Experimental Biology* 220, 1937-1946.

Moles, A. and Norcross, B. L. (1995). Sediment preference in juvenile Pacific flatfishes. *Netherlands Journal of Sea Research* 34, 177-182.

Moll, I., Kuhn, C. and Moll, R. (1995). Cytokeratin 20 is a general marker of cutaneous Merkel cells while certain neuronal proteins are absent. *Journal of Investigative Dermatology* 104, 910-915.

Moll, I., Paus, R. and Moll, R. (1996). Merkel cells in mouse skin: intermediate filament pattern, localization, and hair cycle-dependent density. *Journal of Investigative Dermatology* 106, 281-286.

Moll, I., Roessler, M., Brandner, J. M., Eispert, A.-C., Houdek, P. and Moll, R. (2005). Human Merkel cells – aspects of cell biology, distribution and functions. *European Journal of Cell Biology* 84, 259-271.

Morley, J. and Goodwin, A. (1987). Sinusoidal movement of a grating across the monkey's fingerpad: temporal patterns of afferent fiber responses. *Journal of Neuroscience* 7, 2181-2191.

Mussi, M., Haimberger, T. J. and Hawryshyn, C. W. (2005). Behavioural discrimination of polarized light in the damselfish *Chromis viridis* (family Pomacentridae). *Journal of Experimental Biology* 208, 3037-3046.

Nakamura, T., Matsuyama, N., Kirino, M., Kasai, M., Kiyohara, S. and Ikenaga, T. (2017). Distribution, innervation, and cellular organization of taste buds in the sea catfish, *Plotosus japonicus*. *Brain, Behavior and Evolution* 89, 209-218.

Northcutt, R. G. (2004). Taste buds: development and evolution. *Brain, Behavior and Evolution* 64, 198-206.

Northcutt, R. G. (2005). Taste bud development in the channel catfish. *Journal of Comparative Neurology* 482, 1-16.

Ogawa, K. and Caprio, J. (2010). Major differences in the proportion of amino acid fiber types transmitting taste information from oral and extraoral regions in the channel catfish. *Journal of Neurophysiology* 103, 2062-2073.

Ogawa, K., Marui, T. and Caprio, J. (1997). Bimodal (taste/tactile) fibers innervate the maxillary barbel in the channel catfish. *Chemical Senses* 22, 477-482.

Olsen, A. M. and Westneat, M. W. (2015). StereoMorph: an R package for the collection of 3D landmarks and curves using a stereo camera set-up. *Methods in Ecology and Evolution* 6, 351-356.

Peng, J., Dabiri, J. O., Madden, P. G. and Lauder, G. V. (2007). Non-invasive measurement of instantaneous forces during aquatic locomotion: a case study of the bluegill sunfish pectoral fin. *Journal of Experimental Biology* 210, 685-698.

Phillips, J., Johansson, R. S. and Johnson, K. O. (1992). Responses of human mechanoreceptive afferents to embossed dot arrays scanned across fingerpad skin. *Journal of Neuroscience* 12, 827-839.

Quiroga, R. Q., Nadasdy, Z. and Ben-Shaul, Y. (2004). Unsupervised Spike Detection and Sorting with Wavelets and Superparamagnetic Clustering. *Neural Computation* 16, 1661-1687.

Reed, H. (1924). The morphology and growth of the spines of siluroid fishes. *Journal of Morphology* 38, 431-451.

Reed, H. D. and Lloyd, T. J. (1916). The Nature of the Spines in Catfishes. *Transactions of the American Fisheries Society* 45, 202-206.

Reutter, K., Boudriot, F. and Witt, M. (2000). Heterogeneity of fish taste bud ultrastructure as demonstrated in the holosteans *Amia calva* and *Lepisosteus oculatus*. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 355, 1225-1228.

Reutter, K. and Witt, M. (1993). Morphology of vertebrate taste organs and their nerve supply. In *Mechanisms of taste transduction*, (eds. S. A. Simon and S. D. Roper), pp. 29-82. Boca Raton, FL and Ann Arbor, MI: CRC Press.

Reutter, K. and Witt, M. (1999). Comparative aspects of fish taste bud ultrastructure. In *Advances in chemical signals in vertebrates*, (eds. R. E. Johnston, D. MÏller-Schwarze, and P. W. Sorensen), pp. 573-581. New York, Boston, MA, Dordrecht, London and Moscow: Kluwer Academic and Plenum.

Saal, H. P. and Bensmaia, S. J. (2014). Touch is a team effort: interplay of submodalities in cutaneous sensibility. *Trends in Neurosciences* 37, 689-697.

Sakata, Y., Tsukahara, J. and Kiyohara, S. (2001). Distribution of nerve fibers in the barbels of sea catfish *Plotosus lineatus*. *Fisheries Science* 67, 1136-1144.

Sawyer, E. K. and Catania, K. C. (2016). Somatosensory organ topography across the star of the star-nosed mole (*Condylura cristata*). *Journal of Comparative Neurology* 524, 917-929.

Saxod, R. (1978). Development of Cutaneous Sensory Receptors Birds. In *Development of Sensory Systems*, (ed. M. Jacobson), pp. 337-417. Berlin, Heidelberg: Springer.

Schneider, D. (1969). Insect olfaction: deciphering system for chemical messages. *Science* 163, 1031-1037.

Shamushaki, V. J., Abtahi, B., Kasumyan, A., Kenari, A. A. and Ghorbani, R. (2008). Taste attractiveness of free amino acids for juveniles of Persian sturgeon Acipenser persicus. *Journal of ichthyology* 48, 124-133.

Sherman, A. and Dickinson, M. H. (2004). Summation of visual and mechanosensory feedback in Drosophila flight control. *Journal of Experimental Biology* 207, 133-142.

Siebeck, U., Wallis, G. and Litherland, L. (2008). Colour vision in coral reef fish. *Journal of Experimental Biology* 211, 354-360.

Silver, W. L. and Finger, T. E. (1984). Electrophysiological examination of a non-olfactory, non-gustatory chemosense in the searobin, *Prionotus carolinus*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 154, 167-174.

Sioli, H. (1984). The Amazon: limnology and landscape ecology of a mighty tropical river and its basin. Dordrecht: Springer Science & Business Media.

Sober, S. J. and Sabes, P. N. (2005). Flexible strategies for sensory integration during motor planning. *Nature Neuroscience* 8, 490-497.

Svoboda, K. R., Linares, A. E. and Ribera, A. B. (2001). Activity regulates programmed cell death of zebrafish Rohon-Beard neurons. *Development* 128, 3511-3520.

Taft, N., Lauder, G. and Madden, P. (2008). Functional regionalization of the pectoral fin of the benthic longhorn sculpin during station holding and swimming. *Journal of Zoology* 276, 159-167.

Thorsen, D. H. and Hale, M. E. (2007). Neural development of the zebrafish (*Danio rerio*) pectoral fin. *Journal of Comparative Neurology* 504, 168-184.

Toyoshima, K., Nada, O. and Shimamura, A. (1984). Fine structure of monoamine-containing basal cells in the taste buds on the barbels of three species of teleosts. *Cell and Tissue Research* 235, 479-484.

Varatharasan, N., Croll, R. P. and Franz-Odendaal, T. (2009). Taste bud development and patterning in sighted and blind morphs of *Astyanax mexicanus*. *Developmental Dynamics* 238, 3056-3064.

Vega-Bermudez, F. and Johnson, K. (1999). SA1 and RA receptive fields, response variability, and population responses mapped with a probe array. *Journal of Neurophysiology* 81, 2701-2710.

Weber, A. I., Saal, H. P., Lieber, J. D., Cheng, J.-W., Manfredi, L. R., Dammann, J. F. and Bensmaia, S. J. (2013). Spatial and temporal codes mediate the tactile perception of natural textures. *Proceedings of the National Academy of Sciences, USA* 110, 17107-17112.

Wellnitz, S. A., Lesniak, D. R., Gerling, G. J. and Lumpkin, E. A. (2010). The regularity of sustained firing reveals two populations of slowly adapting touch receptors in mouse hairy skin. *Journal of Neurophysiology* 103, 3378-3388.

Westneat, M. W. (1996). Functional morphology of aquatic flight in fishes: kinematics, electromyography, and mechanical modeling of labriform locomotion. *American Zoologist* 36, 582-598.

Whitear, M. and Lane, E. B. (1981). Fine structure of Merkel cells in lampreys. *Cell and Tissue Research* 220, 139-151.

Williams IV, R., Neubarth, N. and Hale, M. E. (2013). The function of fin rays as proprioceptive sensors in fish. *Nature Communications* 4, 1729.

Williams, R. and Hale, M. E. (2015). Fin ray sensation participates in the generation of normal fin movement in the hovering behavior of the bluegill sunfish (*Lepomis macrochirus*). *Journal of Experimental Biology* 218, 3435-3447.

Wright, J. J. (2009). Diversity, phylogenetic distribution, and origins of venomous catfishes. *BMC Evolutionary Biology* 9, 282.

Yoshioka, T., Gibb, B., Dorsch, A. K., Hsiao, S. S. and Johnson, K. O. (2001). Neural coding mechanisms underlying perceived roughness of finely textured surfaces. *Journal of Neuroscience* 21, 6905-6916.

Young, J. A., Marentette, J. R., Gross, C., McDonald, J. I., Verma, A., Marsh-Rollo, S. E., Macdonald, P. D., Earn, D. J. and Balshine, S. (2010). Demography and substrate affinity of the round goby (*Neogobius melanostomus*) in Hamilton Harbour. *Journal of Great Lakes Research* 36, 115-122.

Zachar, P. C. and Jonz, M. G. (2012). Confocal imaging of Merkel-like basal cells in the taste buds of zebrafish. *Acta Histochemica* 114, 101-115.