T H E U N I V E R S I T Y O F M I C H I G A N

SCHOOL OF NATURAL RESOURCES

Department of Fisheries, Forestry, and Wildlife

Final Report

THE CHEMICAL SENSES OF FISHES

(Period: May 1, 1963 - December 31, 1971)

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ORA Project 018990

supported by:

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INTRODUCTION

The basic mechanism of the receptor function of smell and taste in all vertebrates is less understood than sight, hearing, and the mechanical senses. Fishes, as prototype vertebrates, should be good subjects to elucidate not only these receptor functions but, because of their simpler brain pattern, they should also lend themselves to experiments destined at unravelling some aspects of the central nervous system organization of smell and taste and the behavior associated with this organization. This rationale guided the formulation of research projects undertaken under this grant which dealt largely with the chemical senses of fishes. In its final phases some work was undertaken, for comparative purposes, also on the chemical senses of aquatic insects. The overall significance of the research lies in its comparative aspects shedding light on the senses of smell and taste in the vertebrates, including man.

This report will consist of three parts. First comes an article solicited from the principal investigator by the editors of the forthcoming book Perspectives of Chemoreception by Marine Organisms (Academic Press, Inc.). It embodies the major portion of the research supported by this grant and presents it in relation to other work on the chemical senses of fishes, hence in a suitably broader framework (Bardach & Villars, in press).

This broad treatment of the chemical senses of fishes is followed by Part B, a résumé of certain work during the last two years of grant support that was not adequately emphasized in the above-mentioned article. The final and

third portion of the report is a list of the 20 articles published in scientific journals as the result the work of research associates, graduate assistants, and technicians fully or partially supported by grant funds.

PART A

SMELL AND TASTE IN FISHES

I. INTRODUCTION

In this chapter the anatomy of fish chemoreceptive systems and their capabilities will be briefly described. The role of the chemical senses in the behavior of fishes will be discussed as well as deficiencies in the information presently available and the need for future research.

The basic mechanism of the receptor function of smell and teste in all vertebrates is less understood than sight, hearing, touch and the lateral line. However, the importance of the chemical senses in the lives of aquatic animals becomes more apparent each year. Among fishes, taste is restricted primarily to aspects of food finding and feeding, but smell, while it may serve for food detection and location, is also important in reproductive and social behavior.

Fish can smell volatile substances in great dilution, such as oil of cloves or phenols and their derivatives. They smell and taste various amino acids (Sutterlin & Sutterlin, 1971; Bardach, et al., 1967) thus demonstrating some functional overlap between their olfactory and gustatory domains. Various reactions to specific smells such as to home streams, home sites, species mates, enemies, reproductive partners, and young have been demonstrated but the nature of the compound or compounds involved in these reactions remains unknown.

Differences between smell and taste are both physiological and anatomical. Smell responses at their most acute levels can be triggered by a few molecules in a fish's nasal chamber (Teichmann, 1962); while taste thresholds of fishes,

far lower in certain species than those of man (Glaser, 1966), are in the

micromolar range and to be effective require millions of molecules per cm^3

of a stimulant to bathe the aminal's taste sensors on its body or in its

mouth.

Smell and taste are also clearly distinguished by the anatomy of their

sensors. Smell sensors are the ciliated receptor cells in the olfactory

epithelium on the lamellae in the nasal pit (Holl, 1965), whereas the taste

cells have their microvilli gathered in taste buds and spread over the body,

concentrated on filamentous fins or barbels and/or distributed selectively

in oral and gill cavities. The nerves that connect these sensory transducers

to the brain are the first cranial or olfactory nerve and the taste fibers

of cranial nerves VII, IX, and X, respectively.

Psychophysical tests in man have established the four basic taste quali-

ties and furnished the description of smell categories such as fruity, minty,

etc. Since such descriptions cannot be obtained from animals, the capabili-

ties of their chemoreceptors are best described by listing the kinds of com-

pounds they detect and by testing for specificity of receptors and/or nerves.

For instance, Konishi and Zotterman (1963) found seven taste fiber types in

the carp which react either to single taste substances or to a combination

of them. Konishi, et al. (1966), observed some taste fibers in the sea

catfish (Plotosus) reacting to a specific stimulus emitted by a prey; he

called them Nereis fibers. Also tasted are some larger compounds, customarily

not associated with taste, such as glycerophospholipids, (Konishi & Zotterman,

1963; Bardach, et al., 1967).

A common chemical sense has been postulated, especially for aquatic animals, by some workers (Parker, 1922); its existence has been denied by others (Buddenbrock, 1952). It may rely for its functioning on numerous free nerve endings in the epidermis which are suggested to subserve pain sensation by Whitear (1971a), a function not inconsistent with the perception of some chemical stimuli. Recent investigations also by Whitear (1965, 1971b) indicate that a "common chemical sense" in fishes, at least could rely altogether or in part on small single chemosensory cells in the epidermis which are spinally innervated. It could also rely on the chemical sensitivity of lateral line receptors. These have been shown to respond to monovalent cations by Katsuki, et al. (1971). It remains to be elucidated to what extent the animals actually use the chemical information that reaches their central nervous system from the lateral line, a set of sensory structures hitherto not considered to respond to chemicals but rather to act as a touch sense at a distance.

It is possible then that fishes possess as many as five different chemosensory systems: (1) olfaction, (2) taste buds consisting of several taste cells which are cranially innervated sensors, in the mouth and in many species also on their bodies, (3) single taste cells on their bodies as well as perhaps in their oral cavities which are spinally innervated and may have a lesser gamut of sensitivities than the taste buds, (4) free nerve endings, and (5) the lateral line system, which has a limited range of capabilities to sense chemicals. As little is known about the latter two putative chemosensory systems they have been mentioned in this introduction for completeness' sake but they will not be discussed any further.

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II. ANATOMY OF CHEMOSENSORY STRUCTURES

A. SMELL

1. The Nose

In order to bring dissolved or dispersed stimulants to the olfactory receptors in the nasal pit, which has no connection with the mouth (except in lungfishes), certain mechanisms have evolved among fishes that serve to transport water through the nose.

A stream of water may be led to a fish's nose through a groove enhanced by the configuration of the head, as in the hammerhead shark (Sphyrna), and in many rays, as in the guitarfish (Rhinobatos) (Figure 1). A flap of skin and/or connective tissue is further instrumental in funneling the water through the relatively open nasal depression with its olfactory epithelium arranged in folds or lamellae. This is again found in elasmobranchs as well as among a great many teleosts such as the Elritze (Phoxinus) (Figure 1) and other minnows and the pike (Esox).

Other fishes employ hydraulic devices: A sac (as in the lamprey, Petromyzon, Figure 1) or sacs are filled and emptied by the respiratory movements of head and branchial skeleton so that water is driven over the olfactory folds. The anatomy of such hydraulic exchange mechanisms was described by Burne (1909) but their flow dynamics are not well understood. One may suspect an incomplete exchange of water to occur in many cases and with it a stimulus may linger or be repeated beyond its significant time span. Such

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a contingency would have to be dealt with by central mechanisms and feedback organization. These are likely to come into play because elaborate integration provisions exist in the olfactory bulb and antidromic impulses have been observed in the olfactory tract.

Fishes with the best developed sense of smell have the most numerous olfactory folds; the eel (Anguilla) and the bullhead (Ictalurus) (Figure 2, top right) are pertinent examples. The elongated, closed nasal pits may have moveable intake tubes (eel). Water is drawn through the nose by an intricately patterned movement of cilia (Figure 1). The flow can be speeded up or slowed down either in both nasal chambers or in one alone, as in the bullhead. Intake tubes that can be moved voluntarily and independent ciliary control of one or the other nasal chamber may serve to enhance detection of stimulus concentration differences on the right as opposed to the left side of the fish. It should be noted that there are cilia on the supporting cells of every fish nasal epithelium; these help to propel the stimulus—carrying boundary layer of water over the mucus-covered sense cells. The role of the mucus in the transduction of the stimulus is unknown.

The skipjack tuna (Katsuwonis pelamis), a fast swimmer that strongly reacts to food smells (Bardach & Todd, 1970), employs all three mechanisms (as do many fishes) for propelling the water through the nose: there is a groove on each side of the forehead, leading to the incurrent naris; cilia create a vortex pattern over the lamellae and hydraulic sacs assist in the ventilation of the nose (Gooding, 1963).

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The elaboration of various nasal mechanisms such as moveable nasal intake tubes and a large number of olfactory folds are certain measures of olfactory acuity. Compare, for instance, the nose of the nearly anosmic sticklebacks (Gasterosteidae) (Figure 2) with that of the bullhead or the eel (Figure 2). The relative importance of the various senses in a fish's life is further illustrated by a look at the relative sizes of the various centers in the brain of the stickleback and bullhead, respectively (Figure 2, bottom). Atema, et al. (1970), have suggested, on the basis of behavior observations of these two animals, that fishes with a well developed smell sense have more variable and "plastic" behavior than those that depend primarily on sight.

Aside from the number of folds in the nose, the arrangement of sensory in relation to supporting epithelium also differs among different fishes. As one would expect, the more acute the animal's sense of smell, the larger the areas covered with sensory as opposed to supporting epithelium. In the pike (Esox) for instance, a fish that does not rely greatly on smell, the folds are low and few and there are sparse, small circular patches of sensory tissue on and between them. In the salmon (Figure 2), a silver stained planar section of the nose shows numerous dark islets of olfactory cells among hillocks of mucus secreting supporting cells. Figure 2, depicting the olfactory fold of an eel, shows that mucus as well as supporting cells occur in the patches of olfactory epithelium. The ultrastructure of fish olfactory cells is reviewed in Kleerekooper (1969); its treatment is outside the realm of this article.

2. The Olfactory Bulb

The vertebrate sense of smell, like sight, is characterized by peripheral integration and convergence, that is by a reduction in numbers of higher order conducting neurons in the olfactory tract as compared to the number of axons of the primary or sensory neurons in the olfactory nerve proper that lead to the olfactory bulb. In the burbot, for instance, Doeving (in Kleerekooper, 1969) estimated 10^7 olfactory cell axons lead to the bulb. There they become reduced a thousand fold to provide the tract with about 10^4 fibers. Great variety exists in the topography of fish brains, especially in the spatial relation of the bulb to the brain and the nose, respectively; some fishes have long, others short olfactory nerves, with corresponding differences also in the lengths of their olfactory tracts. Figure 3, prepared by Professor Uchihashi illustrates these variations for Japanese fishes. Such information is of value to neurophysiologists who may seek easy access to primary or higher order smell neurons. The table also supports the suggestion by Aronson (1968) that a long olfactory tract as opposed to a long olfactory nerve permits faster conduction of olfactory information. Such an arrangement would benefit fishes with a well developed taste system on their bodies because it would permit better integration and coordination of impulses received in the two systems; the long tracts among the cyprinid and gadid fishes—both with many external taste buds—are cases in point (Figure 3).

The neuro-anatomy of the olfactory bulb of fishes resembles that of other vertebrates. From outward to inward there is a layer of olfactory fibers, and then a layer where these interlace with dendritic structures of mitral cells

in the distinct aggregations of the glomeruli, and finally layers of external and internal cells from which arise the olfactory tract fibers. Distinction is possible between afferent and efferent fibers in the bulb. In the center of the bulb there is an olfactory ventricle.

Detailed functional analysis of the fish olfactory bulb is lacking; it is assumed to "sort out" stimuli and to begin to categorize them for further elaboration in the brain. It is also likely to be an important way station in the regulation of primary neuron function through antidromic impulses. The latter has been discussed by Doeving (1966), and others.

3. Olfactory Brain Centers

The olfactory bulb connects with the forebrain through the olfactory tract, which consists of lateral and medial bundles. The separation between lateral and medial fibers is maintained in their connections with the fore-brain; the medial bundle goes to dorsal medial forebrain and the lateral bundle to the lateral forebrain (Figure 4). Preserving the separation of connections might be of significance in preserving any coding occurring in the olfactory bulb.

There is no one accepted terminology to discuss the various areas of the forebrain; possible homologies with higher vertebrates are still debated. Schnitzlein (1968) defined six major nuclear regions of the telencephalon— dorsolateral, dorsal, dorsomedial, ventromedial, ventral, and a central area.

Both the role of the forebrain as an olfactory center and the neural development of the forebrain vary greatly among fishes. The well developed

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olfactory system of cartilaginous and bony fishes is reflected in the degree

of elaboration of the anterior portion of the forebrain in the area where

olfactory tract fibers enter. However, many bony fishes show another direc-

tion of forebrain development—namely elaboration of the caudal dorsolateral

area. This development can occur without there being significant increase

in connections with the olfactory system; at the same time, however, caudal

forebrain enlargement is accompanied by a great increase in thalamic and

hypothalamic connections (Aronson, 1963). Such an enlarged posterior forebrain

seems to serve as a higher order integrating center for non-chemosensory

receptor systems. Behavioral studies of forebrain-ablated animals support

this speculation (Bernstein, 1961).

In sharks olfaction and the lateral line are the primary sensory systems.

However, they show very little neural interaction with one another. In

teleosts the primary connections from olfactory areas of the forebrain are

with the thalamic and hypothalamic areas providing the neural network for

olfactory-gustatory interaction (Herrick, 1905). This network further connects

with efferent branchial motor nerves facilitating effective feeding behavior

(Ariens-Kappers, et al., 1936).

B. TASTE

1. Taste Buds and Their Distribution

Vertebrate taste buds show much basic similarity; in fishes clusters of

a dozen to twenty cells in the epidermis are gathered into a structure that

resembles a closed flower bud on a stalk of connective tissue; the latter

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leads the taste nerve through the dermis. Some of these cells are taste cells usually with one large microvillus at their apex, others are supporting cells with shorter, more numerous apical protuberances. They are underlain by cuboid, oval, or flattened basal cells. Recent electromicroscopy (Graziadei & de'Han, in press) suggests that frog taste buds may have both afferent and efferent connections and thus, that their function could be centrally modulated. When one cuts a taste nerve the taste buds degenerate; when the nerve regenerates taste buds form again. The dynamics of taste cell induction require elucidation even though their natural turnover has been studied (Beidler, 1963).

Some fishes have taste buds all over their bodies with even those on their tails being innervated by a branch from a cranial nerve. Consequently one may find among fishes very peculiar ramifications of cranial nerves, especially in the Ramus recurrens of the facial nerve discussed in detail by Freihofer (1963). Taste buds innervated by this and other specially modified cranial nerves and located on the fish's body surface, fins or other appendages such as barbels or oral cirri, may greatly exceed in number those that are located in the mouth and gill cavities. For instance, in a 22 cm yellow bullhead (Ictalurus natalis) we found about 175,000 taste buds on the barbels and the body as opposed to 20,000 in the mouth and pharynx (Atema, 1969). The codfish family shows a similar prevalence of body taste buds, compared to oral ones.

Aside from their distribution, taste buds may occur in clusters, in patches, or singly. They may be elevated on an oral papilla (Figure 5) or sunken in pits, even in openings among scales (Figure 6). Consequently, their

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sensory apices may emerge slightly above the surface of the skin (Figure 5)

or be in a depression. We doubt that these varying anatomical appearances

reflect functional or physiological differences. However, the occurrence of

taste buds on appendages and on the body of fishes—a unique feature among

vertebrates—enables the species so endowed to use taste for navigation and

as a distance sense, that is to detect and locate food (see below) as well as

to decide whether or not to ingest and then swallow it.

2. Taste Nerves and Taste Centers in the Central Nervous System

  a. Spinally innervated taste sensors

The searobins and their relatives (Triglidae) have peculiarly modified

pectoral fins (Figure 7). These have limited but pronounced taste capabilities

(Scharrer, et al., 1947; Bardach & Case, 1965). Taste stimuli may be perceived

by the abundant free nerve endings such as can also be found around taste

buds (Figure 5) but it is more likely that they are mediated by special sensory

cells on the fin rays. The latter seems likely because Whitear (1965) described

small methylene blue staining cells in the scaleless skin of many fishes,

including searobins. The cells have certain properties, a microvillus, pecu-

liar granules, lobate nuclei, that implicate a taste function for them (Whitear,

1970); they are, however, singly too small for investigation with present

electrophysiological techniques.

The tasting appendages of searobins are innervated by spinal nerves from

ganglia that resemble in size certain anterior brain centers (Figure 7); the

ganglia represent a truly remarkable specialization of the vertebrate central

nervous system, reminiscent of the sacral ganglion of Jurassic sauropod reptiles. Nerves emanating from them go largely tailward, in the spinal cord, an anatomical fact corroborated by behavior observations: upon very local and selective stimulation of the taste sensors on the fins, the fish begins to dig and move its tail, while the same stimulus applied selectively to the lips leads to gulping and swallowing (Bardach & Case, 1965).

b. Cranially innervated taste sensors

The central connections of cranial gustatory fibers are in the dorsal medulla; there is some separation of information from fibers of external and internal taste buds. Those emanating from buds in the mouth and on the lips and, if present, on the head barbels and body travel in cranial nerve VII and end more anteriorly in the dorsal medulla. Fibers from the pharnyx, gills, gill arches, and gill rakers belong to cranial nerves IX and X and terminate in the caudal medulla.

From the above two sensory centers in the medulla fibers connect to motor areas, also within the medulla. Development of these connections suggests that gustatory information may be primarily used to direct reflex actions of lips, jaws, and gills. Further connections of taste centers of the medulla go through fibers to the secondary gustatory nuclei located at the base of the cerebellum and optic tectum and thence to the hypothalamus where they allow integration of gustatory information with other sensory information— visual, olfactory, lateral line, and inner ear.

In fish which utilize gustatory information more extensively these pathways are extremely well developed; the primary ganglia in the medulla show increased size and complexity, in some cases resulting in the development of gustatory lobes. The anterior lobe is called the facial lobe receiving external information, and the caudal (or vagal) lobe receives mostly oral taste fibers.

The pattern of development of gustatory centers reflects characteristics of species behavior and the extent of use of gustatory information. In the cod, for example, vagal lobe development is very elaborate. Distinct centers receive information from the mouth (median portion of vagal lobe) and from the taste buds on the body (lateral vagal lobe). Direct connections to motor centers allow rapid precise movements (Ariens-Kappers, et al., 1936). In contrast, the catfish shows elaboration of both vagal and facial lobes as well as interactions between gustatory and olfactory centers. In addition, the catfish's use of tactile information is reflected in gustatory connections with those centers.

III. PHYSIOLOGY OF SMELL AND TASTE

A. THRESHOLDS AND EFFECTIVE STIMULANTS

So-called odor specialists such as the silk moth are so sensitive that one or two molecules of its pheromone suffice to elicit a reaction, but this sensitivity falls off drastically when the compound is slightly altered. Fishes, while also employing sex pheromones, show a far wider range of olfactory capabilities than insects; they are therefore considered to be odor generalists like other vertebrates. This does not imply lack of sensory acuity, however, because fishes respond to highly diluted or dispersed scents.

In a remarkable test small eels learned to associate phenylethyl alcohol, a constituent of rose oil, with the eel heaven-like reward of being allowed access to a dark tube. So dilute was the scent to which they reacted (2.8×10^{-18} or nearly 2×10^3 mol/cm^3) that there could not have been present more than two to several odorant molecules at one time in the 1-2 mm^3 nasal capsule (Teichmann, 1959). Other volatile substances to which fishes have been trained and found to respond at low concentrations are: eugenol, citral, and 1-methanol (Teichmann, 1962).

Similar dilutions can be assumed to elicit smell triggered responses under more natural conditions: The dispersal reactions of Elritzen to "Schreckstoff" (fright substance) (von Frisch, 1941) can be called forth by a dilution of skin extract of approximately 2×10^{-11}. In view of the fact that the nasal chambers of a minnow of 5 cm length is far smaller than that of an eel of about 20 cm—the size of those employed by Teichmann—one may again assume

that relatively few molecules of the substance(s) in the Schreckstoff extract are involved in the reaction.

Schools of skipjack tuna (Katsuwonis pelamis), a fish usually considered to feed by sight, would engage in frantic food search upon the introduction into their round, 10 m diameter, 75,000 liter tank of ca 100 cc of colorless salt water in which one specimen of their customary food fish had been rinsed for several minutes (personal observation). The dilution can hardly be calculated but it may approach that just mentioned for the trained eels and the untrained, frightened minnows.

Taste thresholds of fishes are far higher; Glaser (1966) used serial dilution techniques and conditioning to ascertain the taste acuity of the minnow Phoxinus (Table I). The fish, while endowed with extra-oral taste buds has far less of them than a bullhead or a hake (Urophycis). To ascertain whether summation of impulses from very numerous taste sensors would lower the threshold, we tested the quinine sensitivity of anosmic bullheads; it lay around 1×10^{-4} molar, two orders of magnitude higher than for smell. Cystein hydrochloride, a substance not tested by Glaser (ibid.) but one which we knew to be a good taste stimulant was still tasted by anosmic fishes at 1×10^{-6} molar. With its sense of smell intact the fish would sense a dilution of 1×10^{-7} molar.

Certain substances, then, are both smelled and tasted by the fishes and distinction between smell and taste substances seems to be less pronounced among them than in higher vertebrates, a phencmenon that is not unexpected considering their aquatic existence (Bardach, et al., 1967). Among

the chemicals that elicit both taste and smell responses cysteine is approached by naive bullheads while quinine is avoided. Quinine stimulated taste buds, but depressed spontaneous discharges in the olfactory tract. Cysteine stimulation evoked increases in the discharges of both smell higher order and taste secondary neurons.

The substances presently known to evoke responses of both smell and taste sensors of fishes are listed in Table II. Even salt solutions affect both chemical senses, leading one to suspect that salts, in the aquatic domain, may play an important chemosensory role. Yet the behavioral significance of the sensory overlap between smell and taste and its functional correlations in the central nervous system have not been investigated.

Smell and taste overlap need not be restricted to food stimuli although this seems to be the case in the bullhead where the smell system is primarily involved in social behavior. In the Pacific salmons, amino acids some of which are likely to be smelled as well as tasted, as they are in the Atlantic salmon (Sutterlin & Sutterlin, 1970, 1971), are also implicated in smell-triggered avoidance behavior. Idler, et al., 1956, tested mammalian skin rinses in which there was valine, leucine, isoleucine, cystine, and 1 serine. The latter was the most potent constituent in eliciting a temporary cessation of upstream migration by representatives of all five species of Oncorhynchus; the reaction supposedly protects fish against predation by bears, seals and the like (Idler, et al., 1956).

Another function of low molecular weight amino-compounds is to attract predatory fish to their prey. Kleerekoper, et al. (1959), isolated from the

water that bathed two species of trout and a perch, respectively, various amines and amino acids. Especially one of them called "amine F" attracted the sea lamprey and marine predators alike.

Schreckstoff, mentioned above as triggering dispersal of minnows and occurring in the club cells of Ostariophysean skin is a soluble substance or mixture of substances of unknown nature, but likely of small size (Schutz, 1956); it is smelled because severance of the olfactory nerve abolishes the reaction.

Given the broad spectrum of sensitivity of the vertebrate nose and the barely fathomed redundancy and variety of responses of its individual neurons (Lettvin & Gesteland, 1960; Nanba, et al., 1965), one suspects that biologically meaningful scents—triggering feeding as well as social or reproductive behavior—are not single compounds but mixtures of several of them, each at its own level.

Food smells do, in fact, seem to represent specific mixtures of different compounds. At the aquarium in Oita (Japan) we measured the acceleration in swimming speed of captive yellowtail (Seriola quinqueradiata) upon presentation of food scents. The only significant speed-up, from about 1 to 5 m/sec. was observed when a rinse of the fish's customary food, a species of Stolephorus, was released. Rinses from other genera of fishes, of synthetic scents of mollusks, as well as presentation of several pure compounds, amino acids, and trimethyl amineoxide (TMO) produced no changes in swimming speed.

Even man recognizes, by smell, different species of freshly caught fish—the reader can prove this to himself by sniffing at a fresh carp and comparing

its scent to that of a pike, an eel or a trout. Fish can do the same (Göz, 1941) and they can also recognize by smell individuals of their own species (Todd, et al., 1967; Todd, 1971). Individual, and probably species recognition, as suggested by the yellow-tail observation presented above, are more likely to rely on signature, tune-line mixtures of several compounds than on a particular single one. However, single compounds in mixtures may also be important as in the avoidance by salmon of serine, among other amino acids, and the amine F, prey attractant, in a welter of other related low molecular weight compounds. Combination of signature tune and single attractant characteristics of scents would go a long way to explain the uncanny smell performances of fishes. We still need to unravel what the social and food scents might be, a fascinating exercise in its own right but also one with interest to sport and commercial fishing industries.

B. ELECTROPHYSIOLOGICAL CORRELATES OF SMELL AND TASTE

1. Smell

There are fewer elctrophysiological studies of fish than of mammalian chemical senses even though it is technically possible to place electrodes at any level in the fish smell and taste systems. In fact, as Nanba, et al., 1965, say, fishes are very suitable experimental animals to study smell. "Dosage control of stimulants and their application is easier in water than in air, and fishes have a broad spectrum of olfactory sensitivities. In addition they can learn easily to distinguish between different smells." Electrophysiological investigations of fish smell and taste are on the increase.

Electrode monitoring in the olfactory system of fishes has been concentrated on the epithelium and its sensory cells and on the fibers of the tract, for the obvious reason that these provide the easiest access and permit clearer recording than is possible from the extremely fine fibers of the nerve or the complex of cells in the bulb.

Slow potential changes in the olfactory epithelium of fishes were shown to occur on smell stimulation (Shibuya, 1960); they are somewhat faster in fishes with a pigmented olfactory epithelium (eel) than in the absence of pigments. The former also has a keener sense of smell. Upon stimulation and coincident with the slow potential phenomena (EOG), one also observes changes in spike discharges from olfactory cells (Shibuya, 1960). However, largely because of the difficulties of recording from single primary sense cells, the dynamics of olfactory cell function in all vertebrates are incompletely understood. Recent electrophysiological studies of fish olfactory epithelia deal mainly with ascertaining the range of sensitivites of selected species, especially to amino acids (Suzuki & Tucker, in press; Sutterlin & Sutterlin, 1971).

Recordings from single units of the fish olfactory tract (Doeving, 1966; Nanba, et al., 1965) give information on neural events after considerable integration and elaboration of information has taken place in the bulb. Nanba, et al., summarize their results from Abramis brama and Carassius auratus as follows:

(1) A fiber exhibits different patterns of activity when different odors are employed (temporal coding).

(2) Different fibers respond to the same odor in different ways (spa-
     tial coding).

(3) The response to the stimulus depends not only on the nature of the
     odor but also upon its concentration, the turbulence of the water
     stream acting on the mucosa and the spontaneous background activity
     acting on the fiber. However, the effect of an odor was consistently
     reproducible provided the other parameters did not change.

(4) Many fibers discharge in rhythmic bursts, spontaneously, or on
     stimulation with an odor. This rhythmic activity persists after
     dissection of the central end of the olfactory tract.

(5) Most fibers exhibit adaptation following continuous stimulation
     with the same odor.

(6) The position of receptors belonging to the fiber being recorded can
     be determined by mechanical stimulation of discrete areas of the
     mucosa. If other receptors are so stimulated, lateral inhibition
     results.

Surface and depth recordings were secured from the forebrain of sharks

by Gilbert, et al., 1964. They found changes in wave patterns and potentials

upon perfusion of the nose with fish and crab extracts as well as with amino

acids, and Kleerekoper's "amine F." Further details on physiological aspects

of fish olfaction can be found in Kleerekoper, 1969, who also compares electro-

physiological investigations of fish smell with those done on other verte-

brates, especially amphibians and mammals.

2. Taste

Electrophysiological correlates of taste responses in fishes, like those

of smell, are generally similar to those of other vertebrates. Yet some cer-

tain peculiarities of the fish taste sense have been shown by electrophysio-

logical means.

(1) Cranially innervated taste sensors (taste buds) show a greater

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spectrum of responses than spinally innervated ones (free nerve endings and/or small presumptive chemo-sensory cells). Spinal taste nerves especially do not respond to sugars (Table III).

(2) Oral taste receptors of carp respond to gaseous CO_2 and CO_2 dissolved in water (Hidaka, 1970). Thus they seem to be involved in the regulation of respiration.

(3) Fish possess distinct taste sensors for dilute as well as more concentrated electrolytes (Konishi, 1967).

(4) Fish are suspected to taste "special substances" emanating from prey (Konishi, 1966; Konishi & Zotterman, 1963); some of these may be glycerophospholipids. The matter is discussed in detail in Bardach and Atema (in press).

(5) Fine discrimination of taste seems to be enhanced by fishes possessing seven or more taste fiber types which respond to single compounds or groups of compounds (Konishi & Zotterman, 1963, their Table I p. 219).

The following additional characteristics of taste are probably not restricted to fish even though they were demonstrated through electrophysiological experiments with them. Different species of fishes possess different numbers of fibers that respond to basic taste substances, for instance 79 out of 114 carp taste fibers responded to sugars (Konishi & Zotterman, 1963) while we found only 5 out of 51 comparable sweet fibers in the bullhead.

Such fiber distributions are, no doubt, the genetic endowment of a species that has evolved through food preferences or restrictions. These adaptations are comparable to olfactorily triggered innate food preferences of neonate snakes (Burghardt, 1970), even though they are not as striking. They also exist in the smell domain of fishes (Suzuki & Tucker, in press; Sutterlin & Sutterlin, 1971).

Anatomical, behavioral, as well as electrophysiological observations

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point to taste as being in many fish species a more discriminatory sensory

modality than in other classes of vertebrates. We offer here, as additional

evidence for this tenet the integrated recordings of responses from bullhead

barbel taste fibers to three relatively similar substances (Figure 8).

All records from several taste fibers of fishes also show mechanoreceptor

responses and close associations exist between taste and touch center in the

fish's medulla. Some taste fibers are also sensitive to peripheral heating or

cooling. Taste appears to be relatively more important in fishes than it is

in the remaining chordates.

IV. BEHAVIOR

A. FEEDING BEHAVIOR

All fish use the chemical senses in feeding, albeit to a varying extent.
In highly visual fishes, these senses only test the food immediately prior to
ingestion. Whatever the senses involved, feeding behavior shows a stereo-
typed sequence of behavioral components. Wunder (1927) defined three stages:
(1) alert, (2) locate, and (3) control; Atema's (1971) similar classification
expanded the control component, to wit: (1) arousal (alert), (2) orientation
and search (locate), (3) food uptake into the mouth, and (4) food ingestion.
Chemical sensing can mediate any or all of these steps, partially or
completely.

1. Arousal

Arousal to the presence of food is the necessary first step in the
feeding sequence. A variety of behavioral responses can be indicative of the
animals having become alerted to the presence of food; among them changes in
respiratory patterns, swimming patterns, activity levels or barely perceptible
tensing of the body or fins. Arousal by a chemical component of the food has
been demonstrated in a number of studies. Hobson (1963) noted that dogfish
sharks (_Musculatus_ _canis_) only approached bait from downstream, indicating
that a chemical stimulus rather than visual or auditory one is involved,
since the latter would extend equally in any direction. Tester (1963) demon-
strated changes in activity of sharks in response to water extracts from

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damaged and undamaged prey as well as to blood and some other chemical compounds.

Parker and Sheldon (1913) demonstrated that cutting the olfactory tract or blocking the nares of dogfish prevent their response to bags of food which previously excited them. They also noted that the catfish (Ameirus = Ictalurus) does not respond to food when its olfactory tract is cut, but with a normal olfactory system it shows the ability to notice and locate a food packet even with an impaired gustatory system (removed barbels). Moray eels (Gymnothorax) are unable to detect or locate food when their nares are plugged (Bardach, et al., 1957). Killifishes (Fundulus heteroclitus) are also unable to recognize food when their nares are stitched shut (Parker & Sheldon, 1913).

Kleerekoper and Mogenson (1963) noted that the characteristic increase in the activity of lampreys following the introduction of food extract (or water which had contained live specimens of the lampreys normal prey) does not occur if the olfactory apparatus is blocked. Likewise, the searobin and the hake require an intact olfactory system to become alert and begin a search (Bardach & Case, 1965). Some response of the gustatory fins was seen in response to a food extract in anosmic animals but no search ensued.

Pipping (1926, 1927) examined the response of fish to food odors in relation to the morphology of their olfactory organ. Animals with no other method but forward progression to irrigate the olfactory epithelium show less response to food extracts than those with a highly developed nasal apparatus, such as an elongated nasal chamber and pronounced anterior and caudal

openings, as well as accessory sacs or cilia to move the stimulus over the olfactory epithelium.

Although most work indicates that olfaction mediates arousal, some fishes are capable of recognizing the presence of food without their olfactory apparatus, presumably through their gustatory system. Bardach, et al. (1965), and Atema (1971) demonstrated that the yellow bullhead (Ictalurus natalis) shows essentially normal feeding behavior with its olfactory epithelium cauterized and even feeds essentially normally with its forebrain removed. With the experimental removal of the forebrain there is some increase in latency of arousal, possibly attributable to the general role of the forebrain in arousal, as opposed to an olfactory role of the forebrain (Atema, 1969).

Leopard sharks, Triakis semifasciata, with both nares blocked by paraffin, still show increased locomotor activity and zest for food upon the introduction of whole squid (Carney, personal communication). Normal sharks were present during the observations. Therefore the normal fish might have aroused the deolfacted ones; yet the activity of normal sharks under other conditions do not trigger activity in the test fish.

2. Food Search

The mode of food location shows a wider diversity of sensory dependence than the other steps of feeding behavior. In a significant portion of fishes, the primarily visual ones, there is usually little or no involvement of chemosensory information in the localization of foods. For example, the

killifish which Parker and Sheldon (1913) described show such emphasis on visual stimuli that they will chase water droplets down the side of an aquarium.

Fishes with lesser vision depend upon their chemical senses to locate food, some upon olfaction and some on gustation. Parker (1914) observed the searching pattern of dogfish which had one naris blocked. The handicap led the fish to circle continually to the side of the intact organ, instead of swimming its normal figure eight pattern. Interestingly, the fish required no longer to locate the food source than an unencumbered subject.

Gustation seems to play a primary role in the orientation and location of food for a number of fishes, which show taste buds over the body and fins (Ictalurus and other catfishes, some minnows, blind cavefishes, hake, tomcod, and other gadoid fishes). Schemmel (1967) in discussing the adaptation of Astyanax to cave dwelling, noted that the blind cave dweller had developed a more extensive gustatory innervation of the jaw and ventral region than his river dwelling cousin. He suggested that this more extensive gustatory sense serves to better locate, rather than to detect their food. In the bullhead, there is no behavioral evidence for the necessity of olfactory information during search. In experiments conducted in tanks of up to 5 m in length, there seems to be little role for olfaction in any ∽∽pect of the bullhead's feeding behavior. Unilateral destruction of gustatory receptors, however, results in circling similar to that seen in the dogfish after occlusion of one nostril (Figure 9) (Bardach, et al., 1965).

3. Orientation

Nares as well as taste sensors are bilaterally symmetrical; the latter, as in the case of the bullhead, where they are most concentrated on the tips of the barbels, could permit true tropotaxis to occur (comparison of chemical concentration between two points). Similarly, the distribution of taste buds over the entire body, from snout to tail would make it possible for a fish to detect a fore-aft difference in stimulus concentration (Figure 10). We noted that bullheads find the source of a chemical faster and with less deviation from a straight path in still, as opposed to flowing, turbulent water (Figure 11). It appears therefore that certain fishes, under certain conditions rely on a tropotactic mode of gustatory searching.

Olfactory tropotaxis has been questioned by Kleerekoper (1969). It is, in fact, difficult to conceive how a fish could detect absolute concentration differences in the streams of water through its two nares (except for the extreme case of the hammerhead shark with its widely separated nares). Rather, a mechanism might operate whereby the fish through reflexes attempts to equalize the sensory inputs it receives through both nares. This would effectively orient the fish toward the source of the odor. Temporary enhancement of discrepancies of input from right and left nares respectively would assist in the discrimination necessary to achieve stimulus equalization.

Two such mechanisms have been noted: the first involves inhibition of one olfactory bulb while the nares connected to the opposite one are stimulated, and vice versa (Hara & Gorbman, 1967). The second is the differential flow controlled by the epithelium itself. Dr. Chen in our laboratory

demonstrated that stimulation with a food substance of one nasal chamber of a bullhead slows down the flow of water through the opposite chamber (Figure 12). The difference in flow is caused by differences in the ciliary beat in the two chambers. Transection of the anterior commissure or unilateral severing of a tract abolishes the reaction which must therefore be mediated by efferent fibers.

Another explanation of the manner in which fishes orient to food scents might involve phobotaxis, the tendency to change the angle of deviation in response to changes in stimulus concentration. Kleerekoper (1967a,b) demonstrated, with Scyliorhinus and Mustelus diplodus, that the angle of deflection in their swimming paths decreases with the presence of natural food odors. Teichmann (1959) noted a clear phobotactic response in the blind eel (Anguilla anguilla) to an odor conditioned to be associated with food.

Rheotaxis is yet another pattern of response possibly employed in food location. The presence of a current not only facilitates the delivery of the stimulus but it can apparently provide a directional cue also. Kleerekoper (1967a) showed that a 10% flow difference between two compartments allows a fish to localize an odor source in the compartment from which it emanated. Odor alone isn't sufficient and no rheotaxic behavior is noted in the absence of a food odor. It is noteworthy in this context that the blind cavefish (Amblyopsis) shows positive rheotaxis to currents of 2-7 cm/sec (Poulson, 1963). The fish may rely entirely on the lateral line in its food search or it may employ both nose and lateral line combined in the manner suggested above. In the food-poor environment of caves this would be a time and energy saving procedure.

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4. Food Uptake and Ingestion

Testing of food palatability relies on gustation (Bardach and Atema, 1972) as is suggested when moray eels reject quinine treated anchovies (Bardach, et al., 1957), sharks reject deodorized squid (Tester, 1963), and yellowtail immediately spit out denatured food pellets (Bardach, personal observation).

Taste cells and mechanoreceptors lie close to one another on lips and appendages, as well as in the mouth, and their centers in the central nervous system also allow close comparison between what food feels and tastes like. Ablation studies emphasized that touch and taste qualities are important and that food uptake and ingestion proceeds in a sequence of reflexes.

Ablation studies also support the concept of separation of function between the facial and vagal gustatory lobes. In bullheads, ablation of the facial lobe blocks food uptake into the mouth only, while vagal ablation blocks food ingestion (Atema, 1971). The muscles involved are not impaired since other jaw action, such as yawning, appears normal; also impairment is specific to the complex behavior trains of either food uptake or ingestion. Atema interpreted these results as evidence that external taste buds mediate food uptake while internal gustation mediates ingestion. A fish with its facial lobes intact and its vagal one impaired will pick up food morsels and immediately spit them out again, only to pick them up again; one fish persisted in doing so for over an hour (Atema, 1971).

B. REPRODUCTIVE BEHAVIOR

Chemosensory information plays a role in a number of aspects of fish behavior; in parental·protection and mating, in homing to spawning sites, and in various nonreproductive social behaviors, including individual recognition and territorial behavior, species recognition and schooling, as well as in the recognition of predators and of stress and danger signals.

1. Homing

The role of olfaction in the homing of fishes has been extensively studied. The best known instance of homing is that of migratory salmon, which will travel across an ocean in their life cycle. Many other fish species also show cyclic return to a specific site; for instance wild brown trout, suckers, charr, sculpin, and rainbow trout. Other nonmigratory fish will demonstrate homing if displaced ·from their normal locale; among these are longear sunfish, certain gobies, green sunfish, largemouth bass and white bass (Kleerekoper, 1969).

Harden Jones (1968) differentiated between the maintenance of a home position and the return to a home area either after being displaced, or during cyclical migrations. He noted that the role of the senses in the maintenance of a home position has not been carefully studied, while much recent work deals with the problem of the homing response. Thorpe (1956) proposed vision to be of major importance in the maintenace of a location in the home area. Exceptions might be Protopterus and the blind cave fish (Thines, 1955). Hasler (1966) also included in his hypothesis of salmon homing, a

role for vision, not only in the final recognition of the spawning site but also in recognition of points along the upstream migration of the salmon and in orientation during the ocean phase of travel. He hypothesized that the evening and night rest spots at points along the downstream movement of the fingerlings, serve to visually imprint upon them the landmarks at which they rest. In their later return home they use the imprinted signposts for orientation.

But the most extensively supported theories hold that olfactory information is necessary for upstream migration. The question of whether a stream could in fact be recognized by its chemical components was tested by Hasler and Wisby (1951). They trained blind minnows to discriminate between the waters from two forks of a stream. Not only were the fish capable of performing this task, but the qualities of the stream remained such, over time, that the discrimination could still be made. They also noted that similar training was successful with salmon fingerlings.

Other behavioral data also suggest chemical cues in the homing response. Migrating salmon could be removed from a stream and tested for their response to their homestream and other streams. Idler, et al. (1961), and Fagerlund, et al. (1963), demonstrated that these salmon increased their swimming speed and dispersed their shoals upon introduction of their homestream water, but not upon introduction of an equal amount of stream water from another spawning site.

The nature of the chemical homing stimuli was examined. They were found to be volatile, dialyzable, heat labile compounds, destroyed by active

charcoal or by storage (Idler, et al., 1961; Hasler & Wisby, 1951; Creutzberg, 1961).

The olfactory sense is the major receptor of this chemical input. Hasler not only noted that the ability of minnows to discriminate between streams depends upon an intact olfactory apparatus but he also cites various field studies on returning salmon which indicate that blocked or cauterized nares, or severed olfactory tracts greatly impair the ability of the damaged fish to correctly reach their spawning grounds (Hasler & Wisby, 1957; Wisby & Hasler, 1954; Kleerekoper, 1969).

A different approach to the study of the role of olfaction in salmon homing was taken by a group of researchers at the University of Washington in Seattle. By making electroencephalic recordings from the olfactory bulbs of salmon, caught during their upstream migration, they were able to obtain a physical measure of the salmon's response to various stream waters. They demonstrated increased bulb activity in response to stimulation by waters from the spawning site but not from other streams (Hara, et al., 1965; Ueda, et al., 1967) (Figure 13).

Further studies using this technique have attacked the problem of how homestream odor is remembered. Various, as yet inconclusive, studies have attempted to block the memory for homestream waters or change the fishes' original preference to that of another spawning site (Oshima, et al., 1969; Gorbman, 1971).

In order to explain how salmon swim ceaselessly many hundred miles up-stream an olfactorily triggered rheotactic response has been suggested

(Kleerekoper, 1969). The homestream odor triggers it and if the fish makes an improper turn into the wrong stream its drive to swim upstream ceases with the absence of the "scent" of its spawning site. It would then be carried back downstream to a place where it will again encounter its homestream odor and begin its ascent anew. No role in homing has been suggested for gustatory senses.

2. Mating

Mating pheromones have been reported in certain fish. In one species of gobies, Bathygobius soporator, small quantities of seawater holding a gravid female are sufficient to elicit courtship displays by the male (Tavolga, 1956). In the gurami (Colisa calla), a similar phenomenon was demonstrated. Even though visually isolated, water from a female fish triggered the bubble-nest blowing in the male which is preliminary to courtship (Mainardi & Rossi, 1970). In the shad also, a substance from the female has been implied in triggering courtship behavior in the male (Roule, 1931).

Similarly, chemicals are sometimes used in the discrimination between sexes. Seitz (1940) noted that the male Haplochromis multicolor mouths the urogenital region of other fish of the same species, and suggested that such behavior might assist in the discrimination between sexes. McGinitie (1939) demonstrated the role of chemicals in such discrimination with the blind goby, Typhlogobius californiensis. A pair of these fish will reside together in burrows created by a shrimp. A pair will remain relatively tranquil in their burrow, but if another blind goby enters, a vicious fight ensues. If the

intruder is a male the resident male attacks; if a female enters, the female

attacks. The following simple experiment showed that this recognition was

based on chemical cues. A cellophane bag containing either a male or a

female goby was placed in the burrow and a hole punched in it such that the

water could flow out, that information in the water alone was sufficient to

inform the resident pair of the sex of the intruder. The appropriate resident

would then attack the bag.

Other research has demonstrated that chemicals attract the female to the

male in the lamprey (Roule, 1931), in a blenny (Blennius pavo (Eggert, 1931),

and in a glandulocaudine fish (Nelson, 1964). In the salmon the opposite is

believed to occur; the female attracts the male (Belding, 1934). Strong cir-

cumstantial evidence for female sex pheromones that attract the males also

comes from certain deep sea fishes (many ceratioid anglerfishes and Cyclothone

microdon). Their males must attach themselves to the larger females before

reaching maturity; aside from various other attributes of sexual dimorphism,

the olfactory organs of the two sexes differ markedly, as described by

Marshall (1971): "After metamorphosis, the males become rapidly macrosmatic.

When fully developed each olfactory organ contains a stack of broad sensory

lamellae, which are open to the sea through two wide nostrils. The olfactory

organs of female ceratioids (and Cyclothone) are reduced to minute proportions.

Matching this extreme dimorphism, the olfactory nerves, bulbs and forebrain

are strongly developed in males but regressed in females." Finally, it has

been suggested that pheromones from one male stimulate courtship in other

males (Leiner, 1930; Losey, 1969). The conclusions have been questioned,

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however (Bardach & Todd, 1970).

The olfactory rather than the gustatory sense is suspected of handling all chemical information in courting, primarily because of the low levels of chemicals involved. Tavolga (1956) demonstrated that deolfacted gobies no longer respond to the female odor. The sensory system involved has not been critically investigated in the remainder of the mating pheromones studies.

In some cases, however, gustation might play a role. As mentioned above, Seitz (1940) observed mouthing by Haplochrimis multicolor in sex discrimination, an activity that could involve gustatory rather than olfactory cues. Gouramis (Colisa) use their long filamentous pelvic fins to stroke each other during their courtship (Forselius, 1951). These fins, although primiarly tactile, also bear tastebuds (Scharrer, et al., 1947; Steinbach, 1950). This might suggest some role for gustation in their mating.

5. Parental Behavior

The role of chemical senses, appears to be significant in the care of the young in some species of fish. Especially cichlids recognize their own young, pay them ample attention, and in some cases frequently mouth them.

Kühme (1967) demonstrated chemical recognition of young jewel fish by their parents, which will select a water source that contains their young over other water outlets (Figure 14).

Myrberg (1966) showed a similar behavior in Cichlasoma nigrofasciatum, a highly visual fish. He maintained that later parental recognition is a visual learning phenomenon which depends upon earlier, instinctive

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chemosensory recognition. McGinitie (1939) noted that parent blind gobies

will not eat their young while other gobies will, suggesting some specific

recognition based on nonvisual—probably chemical—cues. Künzer (1964) also

demonstrated recognition of their own young by a dwarf cichlid.

It is unclear whether these parent fish smell or taste their young;

olfaction would be suspected but the frequent mouthings of the young by many

cichlids has given rise to the suggestion that gustatory information may play

a role (Myrberg, 1966). Certainly gustatory information would be expected to

allow discrimination by the parent between the young and food; perhaps it is

also used to discriminate between their own and another brood.

C. SOCIAL BEHAVIOR

1. Species Recognition

The significance of chemical cues in the recognition of one fish by

another has been reported only recently (Todd, et al., 1967). Chemicals can

serve for recognition of conspecifics as in the phenomenon of schooling, and

for the recognition of other species. Fright pheromones (Schreckstoff) exist

which are utilized in signaling the need for escape. Furthermore fish can

recognize individuals within their own species, which implies that they

possess more elaborate social behavior than one normally gives them credit for.

a. Species recognition and schooling

The degree to which individuals within a species will aggregate covers a

wide spectrum. Some fish are hermits who remain alone and will not tolerate

others of their species while others show highly uniform movements; they move in schools which appear like the movements of one, not of many fish. Groupings of fish can be elicited by external factors, such as food or light, or they may be based upon mutual attraction (Allee, 1931). In a very loose sense, the term schooling includes any grouping of fish which is based upon mutual attraction.

The most significant sensory system in schooling is the visual one. Impairment of vision dissolves schools even in fishes which otherwise show little use of vision. A partial list of fishes which have been demonstrated to depend upon vision to school is found in Atz (1953). Other senses, however, are also involved in schooling, albeit to a far lesser extent. Menidia menidia and Alosa pseudoharengus will still swim within a school for a few seconds when blinded, although the response is not maintained much longer (Shaw, 1970). Though not thoroughly investigated, it has been postulated that schools may be kept coherent by tactile, chemical, auditory, and lateral lines clues; some of the species listed by Atz (1953) in this regard are: Ameiurus = Ictalurus, Plotosus, Tilipia, Pneumatophorus, and Rutilus.

While not having a pronounced schooling response, Wrede (1932) demonstrated that Phoxinus will prefer to enter one compartment in a three compartment maze which had previously held a species mate. Hemmings (1966) revealed a similar preference among cichlids, and Kühme (1963) demonstrated some chemical stimuli in the schooling of young jewelfish. Keenleyside (1955) showed a similar phenomenon for the rudd, Scardinius erythrophthalmus, and ascertained that it is specific to members of the same species. He then deolfacted

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the experimental fish and the preference disappeared. These studies suggest

that olfaction can provide an initial fish-to-fish attraction and maintain

individuals in proximity to one another.

In some fishes schooling can be maintained in the absence of olfaction.

Bullheads will still show the same aggregating-schooling behavior with their

olfactory tracts cut. Forebrain ablation which affects the central connec-

tions for the olfactory system, in contrast to deolfaction which just elimi-

nates the nose, also seems to have some effect on schooling behavior (Aronson,

1963). The effect is not so much a decline of, but rather a change in aggre-

gation, suggesting a regulatory, modifying role for the forebrain such as

intimated by Aronson and Kaplan (1968). After forebrain ablation schooling

behavior is more reflexlike and stereotyped than it was before (Berwein, 1941;

Jansen, 1933; Shaw, 1970).

A possible role for taste in fish aggregations might be related to the

detection of external conditions. Increases in CO_2 content disperses aggre-

gations in cichlids (Baerands and Baerands von Roon, 1950) and variations in

pH of the water can increase or decrease their aggregations (Hemichromis)

(Noble & Curtis, 1959).

b. Species recognition and fright reaction

Related to schooling and aggregation is the effect of chemical signals

which result in dispersal. The fright reaction was first noted by von Frisch

(1941) in the minnow Phoxinus which shows strong schooling behavior. The

presence of an injured minnow would cause a "fright reaction" in the rest of

the school, a wash of the extract of pieces of such an injured minnow would effectively scatter a group of minnows that had gathered to feed. The school would disperse, gather together at a distance from the source of the alarming odor, and flee.

The nature of the alarm reaction was clearly defined. The fact that an extract was effective eliminated visual, tactile, or lateral line cues. In a series of tests it was demonstrated that skin, particularly the epidermis, contains the alarm substance (Schreckstoff).

Alarm pheromones and reactions are widespread throughout the Ostariophysian fishes, including the Cypriniformes and also Siluriformes that inhabit the marine as well as fresh-water environments. In some of the most recently evolved characins the alarm pheromones are absent, although they are Ostariophysi; it has been suggested that the ability to produce the pheromone has been secondarily lost in this group (Pfeiffer, 1963). Within the order Cypriniformes, the alarm pheromone from one species will elicit an alarm reaction in other species. The strongest reactions occur between closely related species, even when they are widely separated geographically. The interfamilial responses are only slight (Schutz, 1956), which suggests that the chemical structure of the pheromone probably varies from species to species, being least similar in the most widely separated groups.

Steven (1959) reported the presence of the alarm pheromone and reaction in a non-Ostariophysian fish, the atherinid, Hepsetia stipes (Atherinomorus stipes). Skinner, et al. (1962), described an alarm reaction in another atherinid, the top smelt, Atherinops affinis. Rosenblatt and Losey (1967)

failed to find the pheromone, or reaction, in the top smelt and suggested

that Skinner, et al., described an alarm reaction caused by experimentally

induced stress, possibly resulting from mechanical disturbances.

The source of the alarm pheromone in the Ostariophysian fish appears to

be similar throughout the group. Superficial injury of the skin shows that

the epidermis contains the alarm substance (von Frisch, 1941). There is a

particular type of club cell in the epidermis which is unique to those spe-

cies which produce alarm pheromones. According to Pfeiffer (1960) these club

cells only release their contents to the body surface upon injury. The

barbel epidermis of the carp and some catfish contains no club cells or only

a few very small ones, while the body epidermis of these species is abundantly

supplied with these cells. The fright reaction is elicited by body skin, but

not by barbel skin (Pfeiffer, 1963). Very little work has been done in ascer-

taining the chemistry of alarm pheromones. Boiling the substance for 5 min-

utes does not affect its potency, but boiling continued for 10 minutes reduces

its effectiveness to about one-fifth of its normal level (von Frisch, 1941).

It has been suggested that the alarm substance is a purine or pterin-like

substance (Hüttel, in Pfeiffer, 1963). These substances are concentrated

only in xanthophores which are most numerous on the dorsal surface. Since

ventral skin is an equally effective source of the alarm pheromone, Hüttel's

suggestion is most likely incorrect (Pfeiffer, 1963).

The alarm substance for Phoxinus is not volatile although extremely

soluble in water (Schutz, 1956). Despite this fact the pheromone is detected

by the fish's sense of smell. When the olfactory nerve is separated, Phoxinus

is not able to detect its presence.

Von Frisch (1941) and Kulzer (1954) demonstrated that the reaction is olfactory, not gustatory. Deolfaction of minnows or tadpoles effectively blocks the reaction.

The effect of the fright substance on schooling was discussed by Thines and Vandenbussche (1966) who noted that there is a diurnal variability in schooling intensity. This variability is reflected in a corresponding variation in the sensitivity of the school to alarm substances.

2. Individual Recognition

The role of olfaction for individual recognition within species has been studied by Göz (1941). He demonstrated that a member of the species Phoxinus was capable of discriminating, after training, between individuals of its own species.

The possible significance of individual recognition in social behavior was first noted by Todd, et al. (1967). They chose a fish which shows a highly complex social pattern, and well developed chemosensory system, the yellow bullhead. The bullhead, like Phoxinus, shows the ability to discriminate between individuals of his species. Todd demonstrated that the olfactory system is necessary for this recognition to occur. The substance which allows recognition was first thought to be either in the slime or in the urine. But slime alone could be used to condition fish to recognize one another. Thus, the skin contains the chemicals necessary for individual recognition just as it contains the source of Schreckstoff (Bardach & Todd, 1970).

Individual recognition could be affected by experience. It has been

observed that a stable community could be upset by submitting one member to

some external stress. For example, if the dominant fish of a three-fish com-

munity is removed and placed in a tank with another larger fish, and conse-

quently severely mauled in several fights, his status is changed upon return

to his own community. In contrast, a fish who is just removed and later

returned does not show a status change. Similarly, testing the effect of

such stress on the recognition of one by another fish indicates that a

trained fish no longer recognizes a certain other individual after it is sub-

jected to a losing battle.

The question remains unresolved of whether the skin odor changes as a

result of a losing battle, or whether some stress odor is superimposed which

counter balances a previously positive odor response. Evidence that these

responses are olfactory was demonstrated by deolfaction of the fish, which

subsequently were unable to recognize known tank mates.

In communities of fish, certain territorial boundaries can be observed.

If one member of a community is removed, the introduction of his odor alone

is sufficient to elicit many of the same behavior traits in the other tank

mates, as if the individual himself were present. For example, a very domi-

nant fish would cause three other tank mates to respond by hiding in their

shelters. When it was removed, and later water from its tank poured into the

community tank, almost immediately the residents responded by fleeing and

hiding.

Todd observed that four deolfacted fish, when placed in a large community

tank took an excessively long time to establish some rank order. They fought excessively among themselves as if they had difficulty learning to recognize each other. He noted that when they finally began to establish a community there was some regeneration of the sensory cells in the nares (Todd, 1968).

Later, observations were made of some already established communities in which the members were deolfacted after some residence time together. In these cases no clear disorientation of the tank mates resulted, suggesting that perhaps previously learned patterns did not require olfactory information for maintenance of adequate communal behavior.

Observations were made by Atema (1969) of the effect of deolfaction, forebrain removal, and degustation upon the interaction among bullheads. His results indicate that taste plays no role in social behavior among bullheads. They are known to explore one another with their highly gustatory barbels, but this is probably a tactile response.

Both deolfaction and forebrain removal show effects upon aggressive interactions. Deolfaction enhances aggression in many cases as does forebrain removal. However, it is also noted that these same operations do not cause this effect consistently. The results are variable enough to suggest that other natural factors, as yet unexplored, strongly affect aggression and communal interaction among the fish.

They also permitted comparison with comparable phenomena in other vertebrate classes (Atema, et al., 1969) and led us to conclude that each succeeding step in vertebrate evolution has been one toward more behaviorial plasticity, as reflected in the forebrain development that finally produced

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the human neocortex. However, considerable differences in behavior exist

within each vertebrate class and within smaller taxa; many groups include

both species that have developed stereotyped behavior and others with plastic

behavior, correlated respectively with visual or olfactory dominance in their

central nervous system. This dualism can be shown clearly in the fishes,

where stickleback and bullhead can be taken as extreme examples.

V. FUTURE RESEARCH ON FISH CHEMICAL SENSES

A. FISH AS SUBJECTS FOR BASIC SMELL AND TASTE RESEARCH

Details of smell and taste stimulus transduction processes are unknown. Fish lend themselves well for the electrophysiological probing of all the components of their smell and taste systems including the appropriate portions of their brains. If the brain correlates of fish smell and taste senses were to become the areas of concern for a number of researchers interested in nervous and sensory system evolution, one should proceed to devise simple and reliable stereotactic instruments for the fish brain.

Furthermore, the relatively long olfactory nerve of certain fishes, e.g., the gar (Lepisosteus), invites investigations of the basic conduction characteristics of fine unmyelinated fibers in the vertebrate nervous system. Also, the olfactory epithelium of fishes is more accessible than that of land vertebrates.

The peculiarly wide array of fish forebrain development, from being essentially a series of smell ganglia in some and a set of elaborate integration centers in other species invites work on questions of comparative brain development, even to throw light on evolution of the highest primate brain centers.

Taste is generally considered a simpler sense than smell, at least its gamut is far more restricted. Basic taste processes are, therefore, somewhat better understood and workable models exist for some of them (Beidler, 1954). Attempts have been made to extract from taste buds of mammals certain substances

instrumental in the binding of a taste stimulus to its sensory substrate and, therefore, in eliciting the initial biophysical event with which sensation begins (Dastoli & Price, 1966; Price, 1969). Fishes with barbels and well developed external taste buds (most Ictalurid catfishes) would be a far better experimental animal for taste biochemistry and biophysics than mammals. Their thin barbels carry thousands of taste buds and they therefore have a far greater proportion of sensory in relation to neutral skin tissue than the mammalian tongue. The large amount of nonsensory skin on the tongue was a serious obstacle in, and led to criticisms of, the just mentioned extraction experiments.

Spinally innervated taste sensors in high concentrations on the free fin rays of searobins (Triglidae) make them suitable for electrophysiological research. Considerable interest exists in this variant of taste sensors in contrast to taste buds proper, as the investigation of their capabilities may be relevant to an understanding of congenital taste anomalies in man in which normal taste bud development is lacking.

B. ECOLOGICAL IMPLICATIONS OF RESEARCH WITH FISH SMELL AND TASTE CAPABILITIES

Emphasis should be placed on determining the nature of effective stimuli that elicit feeding behavior and certain social behavior traits of fishes. Certain scents that trigger feeding seem to contain one or more low molecular weight substances. Presently available, especially chromatographic techniques of chemical analysis would make possible the detailed description of compounds and mixtures that lead to feeding responses, with resulting practical applications to fish management.

Similarly, as the slime of fishes is widely involved in social behavior

we should try to characterize the chemical nature of the compounds in slime

by detailed analysis of the mucus, including the histochemistry of the skin

cells most likely involved in the production of these substances.

Some research interest might also lie in the apparent greater overlap

between smell and taste reactions in fishes as compared to terrestrial verte-

brates. Beyond categorizing further which materials are both smelled and

tasted one would wish to gain insight into the central coordinating mechanisms

of these two related sensory modalities. The fish nervous system can serve

well to elucidate them, certainly with the use of a fish stereotactic instru-

ment, already mentioned. Here again, understanding of how smell and taste

work together in food search, food finding, and ingestion is bound to have

practical applications to fish husbandry.

As one surveys the species of fish on which experiments and observations

that deal with their chemical senses were made, one notes the relatively small

number of them and the fact that far more of them reside in fresh than in

salt water. Neither fact is surprising; first, most biologists live in or

near population centers, inland—hence the emphasis on freshwater forms.

Secondly, marine fish are often more delicate, and, thirdly, once a certain

animal has shown some interesting attributes or proved itself to be hardy and

suited to laboratory life it is this animal which will be employed in preference

to others in many aspects of research. In the piscine realm the minnow

Phoxinus (Elritze) and the goldfish are good examples of these restrictive

characteristics.

However, the living resources of the sea are increasingly important in maintaining and improving the quality of human nutrition. At the same time we are also polluting the seas to an ever greater extent. Some chronic pollution effects are believed to work through interference with the chemical senses of aquatic animals, either by impairing sensory performance or as mimics of natural stimuli (Blumer, 1970). More emphasis must be placed on finding out what role the chemical senses play in the lives of many of the important marine fishes. For instance, what are the taste and smell capabilities of commercially important herbivores, planktonivores, and piscivores. Such knowledge, together with other more basic information on their chemical senses (e.g., the role of olfaction in arousal to food, and more generally, the role of the olfactory forebrain) would help to eliminate one serious bottleneck in mariculture, the economically sound feeding of many fish species.

Another critical area in which the chemical senses are known to play a role is that of mating and parental care. Less information exists on it than on feeding behavior although it is likely to be as critical for performing sound mariculture with many species, as is detailed knowledge of the role of smell and taste in feeding.

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Table I. Comparison of taste thresholds of man (after von Skramlik, 1948) with those of the minnow Phoxinus (from Glaser, 1966)

| Substance | Capabilities in Mole/1 | | Superiority of the Fish Over Man |
	Man	Minnow	
Raffinose	---	1/245760	
Sucrose	1/91	1/81920	900-fold
Lactose	1/16	1/2560	160-fold
Glucose	1/13	1/20480	1575-fold
Galactose	1/9	1/5120	569-fold
Fructose	1/24	1/61440	2560-fold
Arabinose	1/13	1/15360	1182-fold
Saccharin	1/9091	1/1536000	169-fold
Quinine hydrochloride	1/1030928	1/24576000	24-fold
Sodium chloride	1/100	1/20480	205-fold
Acetic acid	1/1250	1/204800	164-fold

Table II. Compounds to which smell and taste organs of fishes respond
electrophysiologically with notations on behavioral reactions
to them

Compound	Fish	Smelled Behaviorally	Tasted Behaviorally	Source(s)
L-Alanine	Bullhead	?	?	1
L-Cysteine	Bullhead	X	X	1, 3
L-Glutamine	Bullhead	?	?	1
L-Methionine	Bullhead	X	X	1, 3
L-Proline	Atlantic Salmon	?	X Does not initiate feeding	2
L-Serine	Bullhead	?	?	1
Sodium Chloride	Bullhead	X	X	1, 3
Potassium Chloride	Bullhead	?	?	1
Hydrochloric Acid	Bullhead	?	?	1
Acetic Acid	Bullhead	X	?	1
Quinine HCl	Bullhead	X	X	1, 3

1. Bardach, et al., 1967.
2. Sutterlin & Sutterlin, 1970, 1971.
3. Bardach and coworkers, unpublished.

Table III.   Comparison of electrophysiological taste responses of hake and tomcod fins as opposed to those of the searobin*

	Hake or Tomcod (taste buds; cranial innervation)	Searobin (no taste buds; spinal innervation
Acetic acid	+	+
Sodium chloride	+	+
Sucrose**	+	O
Quinine hydrochloride	+	+
Cysteine	+	+
Homocysteine	?	O
Methionine	O	O
Cystein hydrochloride	+	+
Cystic acid	?	?
Glutathione	+	+
Aspartic acid	+	O
Alanine	+	+
Serine	O	O
Leucine	O	+
Glutamic acid	+	O
Tryptophane	?	?
Tryptamine	?	?
Phenylalanine	+	+
Glycine	+	O

*From Bardach, et al., 1967; Bardach & Case, 1965.
**Note especially absence of reactions to sugar in the spinal fibers.

Figure 1

Gross anatomy of the fish nose indicating mechanism for the perfusion of

nasal chamber.

a) Sea Lamprey (_Petromyzon marinus_)

    note single opening, folds of mucosa and nasophyryngeal pouch; after

    Kleerekoper and Van Erkel, 1960

b) Hammerhead Shark (_Sphyrna_)

    after Tester, 1963

c) Guitarfish (_Rhinobatos_)

    after Kleerekoper, 1969

d) Minnow (_Phoxinus_)

    after Teichmann, 1962

e) Eel (_Auguilla_)

    after Teichmann, 1962

f) Mullet (_Mugil_)

    after Burne in Kleerekoper, 1969

Figure 2

Aspects of fish olfactory organs.

a-g) Schematic representations of numbers and arrangements of lamellae in

olfactory rosette

after Teichmann, 1954

a) Stickleback (<u>Gasterosteus</u>)

b) Pike (<u>Esox</u>)

c) Rainbow Trout (<u>Salmo</u>)

d) Perch (<u>Perca</u>)

e) Minnow (<u>Phoxinus</u>)

f) Bullhead (<u>Ictalurus</u>)

g) Eel (<u>Anguilla</u>)

h + j) Brains of Stickleback and Bullhead respectively showing sizes of

olfactory (bottom) as opposed to optic portions (center).

Note relatively large optic lobe in h and large olfactory one in j.

i) Planar section through olfactory folds of a salmon; showing fields of

olfactory cells (Bodian silverstain).

k) Distribution of cell types on an olfactory lamella of the eel.

top

1) olfactory epithelium

2) indifferent epithelium

3) median raphe

bottom Detail of area, above

1) receptors

2) supporting cells

3) ciliated cells

4) basal cells

5) goblet cell

6) clubshaped secretory cells

7) olfactory vesicle or cell with sensory hairs

Figure 3

The relative lengths of olfactory nerves and tracts in certain Japanese

fishes.

after Uchihashi, 1953

List of Families, by numbers: 1 - 6 Clupeidae; 7 Engraulidae; 8, 9 Sal-

monidae; 10 Plecoglossidae; 11 Osmeridae; 12 Plotosidae; k3 Siluridae;

14 Bagridae; 15 - 22 Cyprinidae; 23 Cobitidae; 24 Anguillidae; 25 Congridae;

26 Muraenesocidae; 27 Ophichthidae; 28 Muraenidae; 29 Fistularidae;

30 Hemiramphidae; 31 Exocoetidae; 32 Scombresocidae; 33 Sphyraenidae;

34 Mugilidae; 35 Channidae; 36 - 38 Scombridae; 39 Coryphaenidae; 40, 41 Car-

angidae; 42 Leiognathidae; 43 Trichodontidae; 44 Oplegnathidae; 45 Branchio-

stegidae; 46 Priacanthidae; 47, 48 Percichthyidae; 49 Serranidae; 50 Sciaeni-

dae; 51 Sillaginidae; 52, 53 Sparidae; 54 - 55 Pomadasidae; 56 Embiotocidae;

57 Labridae; 58 Tetrodontidae; 59 Hexagrammidae; 60 Platycephalidae;

61, 62 Gobiidae; 63 Gobioididae; 64 Uranoscopidae; 65 Ammodytidae; 66 Stich-

aeidae; 67 - 70 Pleuronectidae; 71, 72 Gadidae

Figure 4

Schematic cross section through the fish forebrain, after Schnitzlein, 1968.

Figure 5

Distribution and anatomy of fish taste buds.

a) Distribution of taste buds on the skin of an adult catfish, Ictalurus
    natalis.

    Scale of Grid = 1 cm. Each dot represents 100 taste buds. (Number of
    dots per square = number of taste buds per mm^2). Solid black = more than
    15 taste buds per mm^2.

b) Distribution of taste buds on head, lip and maxillary barbel.

    A.) 1:1 representation of taste buds (linear enlargement 5x)

    B.) enlargement of the parts of the maxillary barbel (10x)

    C.) photograph of the distal end of the maxillary barbel (15x)

    1) maxillary barbel

    2) inflow naris

    3) outflow naris

    4) nasal barbel

    5) pore of lateral line canal

    6) supporting cartilage

    7) branch of cranial nerves V and VII

    8) taste bud

    9) mucus with protruding taste hairs

    10) eye

c) Distribution of the taste buds in the mouth and pharynx. Numbers indi-
    cate taste buds per mm^2 for that area. High concentrations on ridges are
    shown by arrows and attached numbers.

d) Innervation of tastebuds and surrounding skin in the barbel of a Sturgeon
    from Lagler, Bardach and Miller, 1962

e) Top

    Tastebud on barbel of Bullhead

    Bottom

    Cross section through barbel of Bullhead

f) Elevated taste bud on lingual papilla of a Searobin

e and f authors photo

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Figure 6

Taste buds between scales of the Guppy; two round holes are observed inter-

rupting the scale pattern and each represents the pore of a taste bud. The

tiny bulb-like structures seen inside these holes correspond to the tips of

microvilli which characterize the free portion of a taste cell. Courtesy

P. Graziadei (magnification as supplied 9600x).

Figure 7

The spinally innervated taste system of Searobins (Triglidae).

top

The Searobin (Prionotus carolinus) showing its 3 anterior, movable

pectoral fin rays on which taste sensors are located

bottom

Anatomical details of Searobin brain and anterior spinal cord

after Morrill, 1899

OLF = olfactory tract

OP. L = optic lobe

CBL = cerebellum

I, II, III = spinal nerves

G = large ganglion of spinal nerve III, innervating free rays

NFR = nerves of fin rays

Figure 8

Comparison of integrated responses of several Bullhead fibers to cysteine and

related compounds.   See text for explanation.   Time constant - 0.02 sec;

Recorder speed - 5mm/sec equals one chart division.   After Fujiya and

Bardach (1966).

Figure 9

Photographed swimming path of a Bullhead unilaterally deprived of taste buds

(white portion of fish in sketch).

Method of obtaining record is illustrated in Figure 10 and described in

Bardach, et al., 1967b.

Figure 10

Swimming path of a blinded Bullhead with fluorescent plastic platelet af-

fixed on head, searching for chemical stimulus release point and photographed

in ultraviolet light; stimulus has fluorescin in it so as to show its dis-

tribution. Method is described in Bardach, et al., 1967b.

Figure 11

a) Swimming path of a Bullhead towards release point of a taste stimulant in

   still water where substance will spread concentrically. Note almost

   straight path.

b) Swimming path of a Bullhead towards a release point of a taste stimulus in

   flowing water; insert shows uneven stimulus distribution. Note that the

   path is more complex than in a).

Figure 12

a) Method of obtaining time record of water flow through nares in fishes.

Top insert shows micropipette directed at incurrent nares; appearance of

a nonstimulant dye at posterior nares as monitored with binocular micro-

scope determines flow-through time.

b) Record of unilateral flow control in Bullhead nares upon stimulation with

acetic acid achieved through differential ciliary action.

Figure 13

Electroencephalographic response of the olfactory bulb to home water. EEG

readings were taken from the olfactory bulb of adult migratory salmon shortly

after they had arrived at their spawning sites. The lower tracings (indi-

cated by a, b, and c) are the actual EEG readings; the upper (indicated by

A, B and C) are integrated tracings of those EEG's. The top two (A and a)

are in response the salmon's home stream; the next two (B and b) are in re-

sponse to waters nearby the spawning site; and the last two are responses

to water from a source unrelated to the home stream   (after Ueda, et al.,

1967).

Figure 14

Experimental arrangement for testing pheromones from young cichlids; after

Kühme, 1963.

PART B

CHEMOSENSORY PHYSIOLOGY AND BEHAVIOR OF FISHES AND AQUATIC INSECTS

Résumé of certain work on the chemical senses of aquatic animals not emphasized in Part I.

A. In the last two years of grant support chemical senses both of fishes and aquatic insects were investigated. Electrophysiological as well as anatomical and behavior aspects of smell and taste of these two higher groups of aquatic organisms were studied. Emphasis in the work on fishes was placed on:

1. Central structures, terminal points, and functions of the sense of taste in catfish. In particular, the effects of central taste ablation on the extra- and intra-oral specific taste systems in the bullhead were studied. It was found by selective ablation of brain components that the two gustatory inputs (facial taste system and vagal taste system) of catfish have distinctly different functions. The facial taste system operates in accurate localization of food and also controls the "pick-up" reflex (ingestion). The vagal taste system controls the swallowing reflexes; and

2. The central taste structures of catfish, specifically the anatomical relationships between the two distinct taste systems and the tactile system. It was found that all the taste nerves are joined by tactile sensory and partly motor components from the periphery to the brain. Shortly after nerve trunks enter the brain, however, the different components diverge and each system collects in different nucleus.

The following abstract further details these findings:

Structures and Functions of the Sense of Taste in the Catfish, Jelle Atema, <u>Brain, Behavior and Evolution,</u> Vol. 4: 273-294 (1971).

Altered behavioral reactions of the bullhead, _Ictalurus natalis_, were observed following selective ablation of the CNS nuclei of either the vagal and/or facial taste systems. It was found that the facial nerve (VII) subserved all the taste buds on the body skin, lips, and anterior portion of the mouth and constitutes a single system. The other taste system is innervated by the vagal and glossopharyngeal nerves (IX and X) and contains all the taste buds on the posterior part of the mouth and gill arches. The interaction between the two taste systems became evident when selective ablations were performed removing either the entire sensory area of the facial lobe or the entire sensory area of the vagal lobe in the dorsal medulla oblongata. In the former case catfish were found to be unable to localize food accurately and to pick it up; in the latter case fish were unable to swallow, but had no difficulty in localizing or picking up the food. Thus it was concluded that the two sensory (taste) inputs have distinct functions. The facial system operates in accurate localization of food and also initiates the initial pickup of food in combination with tactile inputs. The vagal system performs the final discrimination of food particles and also controls the swallowing reflex.

Also investigated were:

3. Sensitivity levels of fish trained to respond to known concentrations of various amino acids. Comparisons were made for both smell and taste systems capabilities to detect amino acids at very low concentrations. Examples are: L-Methionine: Taste = 1×10^{-7} M; Smell = 1×10^{-8} M; and L-Cysteine: Taste = 1×10^{-5} M; Smell = 1×10^{-7} M.

4. Bullhead olfactory neural responses to various concentrations of

amino acids and other chemicals were investigated. It was found that

bullheads are differentially sensitive to the smell of various amino

acids, as intimated by Bardach, et al., 1967, and corroborated in the

following table which suggests that the smell spectrum of fishes

varies from species to species probably as genetic adaptation to

specific food **scents**, as demonstrated by Burghardt, 1970, for neonate

snakes.

B. In view of the known high olfactory sensitivities of fishes on the

one hand and insects on the other hand, and in view of an overall thrust of

the research towards chemical perception in the hydrosphere, it appeared of

interest to embark on some comparative investigations of the chemical senses

of fishes and insects, respectively. Led by anatomical considerations (well

developed palpi and antennae) the predaceous diving beetle, Cybister

fimbriolatus fimbriolatus, was selected as the first aquatic insect to be

investigated.

1. In comparisons of structures and functions of the antennae and palpi

of predaceous diving beetles, it was found that the beetles can detect

a distant food source (smell) and also determine its palatability

(taste) with either labial palpi, maxillary palpi, or antennae. In

addition, it was found that the antennae are the principal organs

mediating orientation towards a previously detected food source.

2. Anatomical characteristics of the receptors borne by the maxillary

AMINO ACID SENSITIVITIES OF FISH OLFACTORY SYSTEMS,
ASCERTAINED ELECTROPHYSIOLOGICALLY AND LISTED IN ORDER
FROM GREATEST TO LEAST EFFECTIVENESS

Salmon (Salmo salar)[1] 10^{-4} M test Solutions	White Catfish (Ictalurus catus)[2] 10^{-3} M test Solutions	Yellow Bullhead (Ictalurus natalis)[3] 10^{-4} M test Solutions
L-Alanine	L-Glutamine	L-Cysteine
DL-Alanine	L-Methionine	L-Methionine
L-Histidine	L-Alanine	L-Glutamine
DL-Serine	L-Asparagine	L-Leucine
Methionine	D-Methionine	L-Alanine
L-Cysteine (10^{-5} M)	L-Cysteine	L-Serine
Glycine	L-Serine	D-Methionine
DL-Leucine	L-Aspartic Acid	L-Cystine
L-Threonine	L-Leucine	L-Glutamic Acid
DS-Phenylalanine	L-Glutamic Acid	L-Proline
DL-Threonine	D-Alanine	
DL-Valine	L-Histidine	
L-Arginine	D-Aspartic Acid	
D-Alanine	L-Threonine	
DL-Isoleucine	L-Valine	
D-Threonine	D-Asparagine	
D-Amino Butyric Acid	D-Isoleucine	
L-Proline	D-Serine	
Taurine	L-Proline	
L-Hydroxyproline	D-Leucine	
5 Amino Valeric Acid	D-Glutamine	
	D-Proline	
	Glycine	

[1]Sutterlin & Sutterlin, 1971.
[2]Suzuki & Tucker, in press.
[3]Morrow, Univ. Mich., unpbl.

palpi were examined; electronmicroscopy studies showed three basic

types of receptors present on the maxillary palpi:

    a. Sensilla trichoidea
    b. Sensilla coeloconica
    c. Sensilla basiconica

The sensilla basiconica were found to be present in fields on the tip

of the palpus; they could be differentiated into three types:

    a. Corolla shaped
    b. Bolbos shaped
    c. Circumvallate

More recently it has been also ascertained that fields of sensilla

placoidea are present on both the palpi and antennae of these beetles

The latter receptors are virtually identical with those mediating

olfaction in the honey bee, a strictly terrestrial insect.

3. Relationship of flight with mating behavior in predaceous diving

beetles. It was noted from observations when collecting the animals

these two activities coincided. The relation is suspected to alter

a dispersive mechanism. Congregation of these beetles in flight

suggests that a pheromone might both be emitted and detected while

they are in the air. Pheromone communication in reproductive behav-

ior has been well studied and substantiated for numerous terrestrial

insects; more than likely it also acts in the aircalphase of many

aquatic insects; details remain to be investigated.

4. Electrophysiological responses of the maxillary palpi of predaceous

diving beetles to stimulation by chemicals representative of the four

basic taste modalities showed that sodium chloride was the most

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efficacious stimulant. In addition it was found that sodium chloride

also mildly stimulated the beetles to initiate searching behavior.

Further details expanding the above brief summary can be found on the

abstracts of several pertinent areas below:

Feeding Behavior of the Predaceous Diving Beetle, Cybister fimbrilatus
fimbriolatus (Say). Bioscience, Vol. 20, No. 20, Pg. 1111, 1970.
G. H. Johnson and W. K. Jakinovich, Jr.

In contrast to numerous reports in the literature and the popular name of

the insects, it has been found that these beetles are not predaceous at all,

but actually scavengers. They are particularly sensitive to dead animal mate-

rial but will not attack live smallfish, insect larvae, snails, or daphnia.

In addition, they appear to be entirely dependent upon chemical clues for the

detection and location of food.

Electron Microscopic Studies on the Palpi of Cybister fimbriolatus
fimbriolatus (Say). F. Yasuzumi, S. Yamaguchi, and G. H. Johnson.
Z. Zellforsch., Vol. 117, 476-484, 1971.

A maxillary palpus of C. f. fimbriolatus approximately 2.5 mm in length

consists of four segments. It is covered with chitinous cuticular elements

which are made up of a set of hexagonal plates which interlock regularly. A

small number of microsetae are found, each projecting from a punctule. Sen-

silla basiconica are particularly abundant in field at the tip of the palpus.

Two types are readily apparent, one surrounded by a thick wall and one not.

The former are referred to as "circumvallate sensilla" and the latter as "naked

sensilla." Cross sectional examination of the sensilla basiconica reveals

that the outer segments of the distal processes of sensory cells extend into

the central projections of the circumvallate sensilla. No neural elements

innervate the naked sensilla.

Flight Behavior of the Predaceous Diving Beetle, Cybister fimbriolatus fimbriolatus (Say). G. H. Johnson. Coleopterists' Bulletin, Vol. 26, No. 1, 1972.

Beetles caught during flight mated when placed in aquaria, beetles caught from the water did not. Their swarming pattern also suggests an airborne pheromone to be involved in mating and the entire behavior pattern to be a dispersive one destined to expand existing habitats.

Contributions to the Sensory Physiology of Predaceous Diving Beetles. The Function, Structure, and Neural Responses of the Maxillary Palpi of the Predaceous Diving Beetel, Cybister fimbriolatus fimbriolatus (Say). Gerald H. Johnson. Ph.D. Thesis, The University of Michigan, 1971.

Behavior experiments after various ablations of oral appendages on beetles ascertained the functional capabilities of the chemoreceptors borne on the maxillary palpi, as well as the antennae and labial palpi. Beetles were capable of detecting fish flesh at a distance, eventually locating, determining palatability, and feeding as long as a single antenna or either type of palpus was intact. Beetles deprived of all palpi and antennae in no way reacted to chemical stimuli. Ablations of both palpi and antennae were found to increase detection time for food scents significantly. Only antenna ablations were found to increase location time significantly.

Scanning electronmicroscopy revealed three basic types of sensilla present on the maxillary palpi: basiconica, trichoidea, and coeloconica. Dense fields of sensilla basiconica were found on the tips of the apical segments of the maxillary palpi. Numerous sensilla coeloconica were found to be widely distributed on all segments of the palpi. A few small groups of sensilla trichoidea were found on the apical segments. Three types of sensilla

basiconica were distinguished and described as circumvallate, bolbos, and corolla shaped.

The internal structures of the sensilla basiconica were examined by static electronmicroscopy. Neural elements were found innervating only the circumvallate sensilla basiconica. Cross sectional examination just proximal to a field of the sensilla basiconica revealed many clusters of distal neural processes, each cluster enclosed within a scolopale. Each scolopale, in turn, was surrounded by a trichogen cell and tormogen cell. Ciliary structures were observed in the distal processes of the sensory cells.

Electrophysiological examination indicated neural responses to chemical, as well as mechanical stimulation of the sensilla basiconica of the maxillary palpi. Distilled water and chemicals in low concentrations induced low levels of neural response. Sodium chloride solutions in the range of .1M to 1.0M induced the greatest neural activity in the sensilla basiconica. Responses were also elicited by the two sugars tested, sucrose and trehalose. Responses of lower intensity were obtained with acetic acid and quinine hydrochloride stimulating solutions. Acetic acid stimulation at .1M concentrations evoked responses characteristic of injury.

C. Overall summary, with asides on the relevance of the research undertaken.

The human forebrain evolved from the primitive forebrain of fishes, the human sense of smell, less overtly important in human behavior than sight or hearing is till significant in social and individual behavior; it is the most

poorly understood of the sensory modalities, both central and peripheral as pertains to its basic functions as well as in its behavioral contexts. In the quest of gaining insight into these unknown parameters of the chemical senses, including genetic malfunction and disease aspects of smell and taste, it is legitimate to delve back in time, as it were, and look at smell and taste and its evolution throughout the vertebrate series, beginning with the fishes. This was the overall intent and thrust of the nine years of research summarized here:

In its anatomical phase it elucidated structural aspects of sense organs and pinpointed similarities and differences between adaptations to the aquatic as opposed to the aerial portions of the biosphere. Brain anatomy was connected to social behavior and correspondences elucidated between fishes and primates.

In its neurological phases it dealt with forebrain function and evolution, detailing feeding reflexes of lower vertebrates, permitting assessment of various phases of foodgetting (alert, search, detection, testing, ingestion, and swallowing) that have application to general models dealing with hunger and satiation.

In its neurophysiological phase substantial insights were gained into the gamut of taste sensations in vertebrates—more multi-modal than anticipated—into the function of taste mediated through structures other than taste buds—of importance to problems of genetic or traumatic taste agnosia in man. In the domain of neurophysiology of fish olfaction, our research revealed substantial, specific, and clonal adaptive specialization which may be of

comparative significance even to inquiries into the diversity of smell capabilities of the human family, should it ever become relevant or interesting to undertake them.

In its behavior phase, it emphasized the important and unique role of the chemical senses of aquatic organisms to such aspects of their lives as feeding, mating, and social interactions, including aggression. The overriding role of individual scents was revealed particularly in the latter context. Certain correspondences to primate behavior were uncovered suggesting a very basic relation, in various vertebrate groups, between smell, individual recognition, and aggression.

Serendipitous spin-offs emerged in other areas as well, leading to the inception of biosphere-management, i.e., pollution-oriented research projects by former workers on this research grant: the role of man-made, water-voided chemicals and their interference with sensory performance (e.g., detergents) and the effects of pheromone analogues animal on behavior (e.g., certain soluble portions of oils) are cases in point. These effects are subtle and chronic but destructive in the long run nevertheless; we would not understand them and therefore be unable to counteract them if we did not engage in basic research on comparative aspects of the nervous and sensory systems of all organisms in the biosphere which have become our responsibility in this latter day of human domination of the globe.

PART C

LIST OF PUBLICATIONS COMPRISING WORK SUPPORTED BY
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