EFFECTS OF CHEMICALS ON A SCHOOLING FISH, KUHLIA SANDVICENSIS [1,2]

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Recent widespread interest in the reactions of fish and other aquatic vertebrates to chemical substances has prompted us to place on record certain significant results which have accrued during the preliminary phases of an investigation directed toward dispersing schooling fish by chemical stimuli. This study was undertaken to find, by judicious selection and testing, whether or not certain chemical compounds in exceedingly dilute concentrations would evoke reactions in fish which would alter the sensory bonds between them, thus effecting the desired dissolution of the school. Although much additional research is yet to be accomplished on this problem, the results thus far point toward promising leads and will, we anticipate, be of considerable value to other workers engaged in closely related lines of investigation.

A chemo-sensory approach to the problem of dispersing fish in schools appears justified on the basis of (1) the role of vision in the formation, maintenance, and dissolution of fish schools as demonstrated by Parr (1927, 1931), Breder (1929, 1942, 1951), Bowen (1931, 1932), Breder and Nigrelli (1935), Johnson (1939), Schlaifer (1940) and others, and (2) the chemical sensitivity of fish as indicated by the early studies of Parker (1912), and a host of others up to the present day. It is clear in regard to vision that whatever may be the total forces which weld and maintain the fish school, dissolution of the school occurs when light intensity falls below a certain threshold value. Moreover, many experiments with blinded fish of species which school under normal conditions indicate that schooling fails to occur under these altered circumstances. For these reasons one of the specific objectives of this study is to discover chemical compounds capable of inducing amblyopia. As regards the chemical sensitivity of fish a search is underway to segregate from promising chemicals those which evoke the irritating or repelling responses desired. Upon this background of information further tests are being conducted with (1) closely related molecules with different substituents and (2) structurally analogous compounds. Finally, we hope to combine active parts of molecules into substances which may increase the intensity of response.

A survey of the literature indicates that the reaction of fish to chemical substances has not been a fertile field of investigation except insofar as certain specific piscicides, insecticides and pollutants are concerned. The history, use and effectiveness of piscicides have been reviewed recently by Krumholz (1948), Solman (1950) and Smith (1950). These authors stress rotenone as the most effective fish poison

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for use in fisheries work. Hubbs (1930) found nascent oxygen to be extremely toxic to fish, and Behrens and Hikiji (1933) discovered that central respiratory stimulants (cardiazole, camphor, picrotoxin, lobeline and rotenone) were generally toxic to fish although there is no strict structural parallelism. Throughout recorded history certain plants have been known to contain substances toxic to fish. Some of these are discussed by Fickendey (1911), Chopra (1941) and Bianco (1943). Very scant information exists on the active compounds or mode of action on fish of any of these piscicides.

Many of the newer insecticides and weed killers which are applied over extensive areas have posed problems in fish conservation. The toxicity of DDT to fish has been discussed by numerous workers, among them Ginsberg (1945, 1948) and Linduska and Surber (1948). Dilute concentrations of pyrethrum (Bandt, 1933), phenol larvicides (Knowles, Parker and Johnson, 1941), and 2,4-D (Harrison and Rees, 1946) have also been found destructive to fish.

The reaction of fish to chemicals as mediated through the olfactory sense has received considerable attention particularly since the experimental results obtained by Parker (1911, 1913), Parker and Sheldon (1914) and others who differentiated general chemical sensibility in fish into the separate receptors, stimuli and physiological effects of smell, taste and the common chemical sense. Recent studies by Hasler and Wisby (1949) have demonstrated that conditioned fish can detect phenol at concentrations of 0.0005 p.p.m. That odoriferous substances may evoke an alarm response has been demonstrated by Von Frisch (1941) who proved that the injured skin of the European minnow, *Phoxinus laevis,* gave off a substance, perceived through the olfactory epithelium, which initiated a fright response among its "school mates." Hüttel (1941) found this substance to be purine- or pterine-like. Unfortunately no quantitative data are available for comparatively assessing the response. Because of the extreme sensitivity of fish to odors, stimuli mediated through the olfactory sensations may well play an important role in the life of fish, serving to guide them to feeding grounds, to direct migrating fish to their homing areas, and to warn fish about toxic substances present in the environment (Walker and Hasler, 1949). In regard to the latter point responses of fish to pollutants have provided a considerable amount of the literature relating reactions of fish to chemical substances, especially those perceived through olfaction. The effect of phenolic substances on fish has been reviewed and extended recently by Jones (1951), toxicity levels for fish in waters polluted with hydrocyanic acid and other industrial by-products have been examined by Daugherty and Garrett (1951), and fatal concentrations of zinc for trout have been recently determined by Goodman (1951). Such responses by fish to pollutants have indicated their usefulness in the bioassay of chemical by-products (Hart, Doudoroff and Greenbank, 1945; Hasler and Wisby, 1949; Daugherty, 1951).

It is abundantly clear from the literature that the three chemical senses as described for fish by Parker (1912) are susceptible to different types of stimuli and that there is great disparity in thresholds for stimulation, especially between olfactory sensation and those of taste and the common chemical sense. Although there is no comparative information available for fish on this disparity in threshold of stimulation, tests have been conducted with humans which provide much data germane to our investigation. Katz and Talbert (1930) measured the intensities of stimulation of 55 different substances for odor, eye irritation and nasal irritation in human subjects. They found that trinitro tertiary butylxylene (artificial musk) was the most powerful as an odor and was observable at 0.00005 p.p.m., while phenacyl chloride was the most powerful eye irritant, observable at 0.0083 p.p.m., and was also the most powerful nasal irritant, discernible at 0.021 p.p.m. It is interesting to note that the irritant receptors of the human eye are two or three times as sensitive as those in the mucous membranes in the nose, but are nevertheless 160 times less sensitive than the olfactory apparatus. At this stage in the development of a comparative physiology of the chemical senses we are not permitted to expect fish to respond to stimuli at the same threshold levels as do humans; nonetheless, it may be instructive to deduce possible proportionate threshold intensities on the part of fish from the meager data available. The maximum detectable dilution of a chemical substance in fish mediated through the olfactory receptors appears to be approximately 0.0005 p.p.m. (Hasler and Wisby, 1949). Thus, if the proportionate threshold intensities of minimal stimuli for the general chemical senses of humans and fish are somewhat comparable, we would expect that the eyes of fish might possibly be irritated by a suitable compound in a concentration as dilute as 0.08 p.p.m., and that the common chemical sensory receptors in the skin might be irritated similarly by a concentration as dilute as 0.3 or 0.4 p.p.m. These estimates provide us with a point of departure and a frame of reference for the quantitative aspects of this study.

Too little information is available to point definitely to any of the chemical senses as demanding our sole attention toward the solution of the problem. The greater sensitivity of olfactory receptors holds the possibility that certain chemicals in very dilute amounts might induce the desired response. However, none of the responses attributable to such chemicals appears to evoke behavior which would disperse schooling fish. In general, prior studies on the physiology of taste in fish have indicated that these stimuli evoke positive reactions to food, whereas negative or defensive reactions are mediated through the common chemical sense. Recent anatomical and physiological studies of the fishes *Prionotus* and *Trichogaster* indicate that the difference between the common chemical sense and taste can only be based on the innervation and the presence or absence of taste buds (Scharrer, Smith and Palay, 1947). Thus, the reaction of the animal cannot safely be used as a criterion for precise differentiation of stimulus-response patterns for these two senses. Nonetheless, the sensations resulting from stimulation of the receptors of the common chemical sense are more closely allied to the sense of pain (Moncrieff, 1944) and thus should provide the desired behavior. Since vigorous and defensive actions are the types sought in this study, the bulk of our tests involve chemicals eliciting this type of response which may be generally considered to be mediated through the free nerve endings. These, although we are not certain for this species, are presumably located over the surface of the body.

Very little information is available concerning repellent action of chemicals on bony fish. While conducting experiments on Mexican blind characins Breder and Rasquin (1943) found that acetic acid, 10 per cent, and ammonium carbonate were repellent. At the onset of World War II extensive investigations were conducted to develop a suitable shark repellent, and cupric acetate as well as other acetates was found fairly effective (Burden, 1945; McBride and Schmidt, 1943). Further analysis indicated that acetic acid was the active repelling agent when these substances were dissolved in water, but no suitable volumetric information is available. Since the War the same material was tested and found effective for sharks and several species of bony fish in Australian waters (Whitley and Payne, 1947).

Selection of Chemicals

The basic considerations for selecting chemicals capable of dispersing schools of fish were (1) that they be grossly irritating to fish rather than merely toxic or narcotic, and (2) that they be rapid in action, reaching, in our experiments, a maximum effectiveness within a period of two or three minutes. In searching for suitable chemicals we were aware that substances which would elicit these reactions in other groups of animals might not be effective likewise for fishes. However, as a point of departure it was assumed that reactions to chemicals by other well-studied groups of animals would be helpful. Accordingly, the kinds of information which led to the initial choice of chemicals for testing were (1) actions of insecticides and insect repellents, (2) general knowledge of mammalian toxicants, stenches, irritants and lachrymators, and (3) previous results with chemical piscicides and fish repellents. After preliminary tests with substances in these categories it soon became evident that most of the desired chemical properties were obtainable in the group of irritant poisons classified usually as lachrymators and skin irritants. These substances probably impart their effect to fish through receptors of the common chemical sense as conceived by Parker. The selection of test chemicals was thus further narrowed to the classes of substances known to possess these properties from experimental results obtained for other animals, chiefly insects and mammals. Lest this system of selection miss chemicals or classes of chemicals which might be irritating to fish, but not to other animals thus far studied, other chemicals considered possibly effective have been included in the testing program.

The property of irritation is generally accorded to a chemical by the presence of characteristic groups in the molecular structure. However, an attempt to classify known irritants according to such active groups reveals many significant exceptions, so that *a priori* such a scheme does not appear very promising. Nonetheless, the many relations between structure and irritant power seem to demonstrate the anlage of a skeleton on which a classification will almost certainly be built. Other factors, such as those which control the amount of irritant reaching the sensory receptors (vapor pressure in air, and solubility in water), are certainly important contributors to the efficacy of stimulation. Upon these qualifications presumably active chemicals of the following groups have been selected for the testing program: mercaptans, sulfides, thiocyanates, isocyanates, isothiocyanates, phenols, highly halogenated compounds, and certain classes of unsaturated compounds. Those with lower molecular weight were also given priority in testing because of the greater solubility expected.

Classification of chemicals tested

A preliminary classification of the chemicals tested thus far follows. It will be noted that certain of the substances listed have properties which fit them into more than one category, and thus make them doubly recommended for testing.

a. *Insecticides and insect repellents*

Several insecticides and insect repellents have been tested. With regard to their stimulation of the common chemical sense in man these substances would fit into no single classification, but would run the gamut from bland to mildly irritating. We have explored the action of the following insecticides:

n-butyl carbitol thiocyanate (Lethane B 71)
chlorinated camphene (Toxaphene)
diethyl-*p*-nitrophenyl thionophosphate (Parathion)
1, 2, 3, 4, 5, 6-hexachloro-cyclohexane 92% gamma isomer or benzene hexachloride (Lindane or Gammexane)
1, 2, 3, 4, 10, 10-hexachloro-1 : 4, 5 : 8-diendomethano-1, 4, 4a, 5, 8, 8a-hexahydronaphthalene (Aldrin)
isobornyl thiocyanoacetate 80%, remainder related thiocyanoacetates (Thanite)
lauryl thiocyanate (Loro)
1, 2, 4, 5, 6, 7, 8, 8a-octachloro-4, 7-methano-3a, 4, 7, 7a-tetrahydroindane 60% with related dicyclopentadiene derivatives (Chlordane)

The following insect repellents were tested:

dimethyl phthalate
2-ethyl hexanediol-1, 3 (Rutgers 612)

From the meager structural evidence on insecticidal action, highly chlorinated organic molecules appear effective as nerve poisons, organic thiocyanates exert rapid depressant effects, and narcotic vapors such as carbon disulfide impart anaesthetic action. Thus tests were made on structurally related compounds. Halogenated compounds tested were:

alpha-chloronaphthalene
p-chlorophenyl isocyanate
2, 3-dichloro-1, 4-naphthoquinone
2, 4-dichlorophenol
p-fluorobenzonitrile
hexachlorobutadiene
phenacyl bromide
phenacyl chloride
sodium hypochlorite (Chlorox)
sodium pentachlorophenate
thiocyanic acid 5, 5-trichloro amyl ester
trifluoroacetic acid

Cyanates, thiocyanates and isothiocyanates examined were:

allyl isothiocyanate
ammonium thiocyanate
barium thiocyanate
phenyl isocyanate
phenyl isothiocyanate
potassium cyanate
potassium thiocyanate

The actions of other organic sulfur compounds noted were:

allyl thiourea
1, 2-ethane dithiol
ethyl mercaptan
hexamethylene dithiol
potassium ethyl xanthate
β, β'-thiodipropionitrile

The following compounds, analogous to repellents, were also tested:

dibutyl phthalate
cyclohexanol

b. *Substances irritating or toxic to mammals*

A few compounds known to be toxic to mammals and several rated as repulsive or irritating have been tested. These were:

allyl isothiocyanate
allyl mercaptan
barium thiocyanate
caffeine
carbon disulfide
copper salts (copper chloride, sulfate and acetate)
diethylamine
dimethyl sulfate
ethyl mercaptan
isovaleric acid
methyl strychnine
Parathion
phenacyl bromide
phenacyl chloride
phenyl isocyanate
phenyl isothiocyanate
phenol
piperidine
pyridine
quinine
skatole
sodium cyanide
sodium pentachlorophenate
strychnine
thallium sulfate

c. *Piscicides and fish repellents*

As mentioned previously, relatively scant attention has been given to the subject of reactions of fish to chemicals except for certain special purposes. The same or similar compounds found effective as piscicides or repellents by others were tested. These were:

acetic acid
benzoyl peroxide
cupric acetate
cupric chloride
cupric sulfate
ethyl mercaptan
hydrogen peroxide
phenol
rotenone (Fish-Tox)
sodium hypochlorite (Chlorox)
4-tertiary butyl catechol

d. *Special irritants*

The following chemicals were used to test the effects of active oxygen as a possible irritant:

sodium perborate
sodium peroxide

Reactions to reduced oxygen tension were tested by adding sodium bisulfite to the water.

Methods

Preparation of solutions

Solutions of solids were prepared by weighing, and of liquids by volumetric measurement to give the required concentration (20 p.p.m., 10 p.p.m., etc.) when diluted and mixed in the known volume of sea water in the test aquaria. Solutions were made up in sea water to a volume of approximately 100 cc. Water soluble substances presented no difficulty, but for insoluble substances it was necessary to add about one drop of a dispersing agent before emulsifying with a hand homogenizer. In the early phase of the work Aerosol OT and Triton B 1956 were used as emulsifiers and dispersers for chemicals. Later a systematic search for a suitable general disperser for insoluble substances in sea water was made. Fifty commercial products submitted by the producing companies were tested for their ability to disperse mineral oil in sea water. Efficiency and general desirability were judged on the basis of the amount of foaming and the degree of precipitation or creaming after set intervals. As a result of these tests "Tergitol" Dispersant NPX was considered the most suitable under our conditions of testing. However, we are aware that a good disperser for mineral oil in sea water may not be the most satisfactory for other substances in the same medium. Final decision on emulsifiers and chemical dispersers will be made by testing them with specific compounds found to arouse the desired response in the test animals.

For preparation of solutions more dilute than 10.0 p.p.m. a definite volume of a more concentrated solution was taken, then an aliquot was introduced into the test aquaria. Compounds were screened initially at 20.0 p.p.m., and chemicals which were definitely effective at that concentration were tested at lower values. Although the common chemical sensory receptors of fish theoretically should be stimulated with appropriate chemicals at a maximum dilution of 0.3 to 0.4 p.p.m., the minimum dilution value of 20.0 p.p.m. was selected for initial screening to assure response by the fish if the substance had but slight stimulating value. Although such substances may exert no effect on fish in greater dilutions, final appraisal of the molecular structure for all active chemicals may disclose certain common characteristics which would lead us to more effective irritants and repellents, and ultimately aid in framing a structural basis of activity.

The minimum time of testing was two minutes, and observations were generally concluded at the end of that time unless the fish responded to the chemical. Although this brief interval may seem inadequate for results of such tests, it is in line with the objectives of the problem—to find a rapidly acting substance capable of dispersing schooling fish.

Apparatus

The initial screening of chemical substances was conducted in aquaria of 50 liters capacity containing a school of four or five small fish, *Kuhlia sandvicensis,* each ranging from 30 to 60 millimeters in total length. The outer surface of the rear glass side of each aquarium was painted black to effect greater contrast with the silvery fish on movie film. An electrically driven stirrer was mounted in the corner of each tank to hasten the dispersal of chemical substances throughout the tank. For recording the speed of action an electric clock with an enlarged second hand was mounted above each tank. Overhead a battery of three Photoflood lamps in aluminum reflectors illuminated the contents of each aquarium.

The introduction of the chemical substance and the subsequent response on the part of the fish were permanently recorded on 16 mm. black-and-white movie film. Visual observations on behavior were also recorded. The movie record was made to detect subtle differences in reaction difficult to describe verbally or to remember for subsequent comparison.

Results

Inactive chemicals

The following list of chemicals elicited no observable response at a concentration of 20.0 p.p.m.:

acetonitrile
allyl thiourea
ammonium thiocyanate
barium thiocyanate
benzoid acid-*o*-(alpha mercaptoacetamido)
benzoyl peroxide
catechol
chlorinated camphene (Toxaphene)
alpha-chloronaphthalene
p-chlorophenyl isocyanate
cupric chloride
cupric sulfate
cyclohexanol
cyclohexyl-4-amino phenol HCl
gamma-chlorobutyronitrile
2-3-dichloro-1, 4-naphthoquinone
diethylamine
dimethyl phthalate
dioxane
ethylene oxide
ethylene oxide polymer
p-fluorobenzonitrile
hexamethylene dithiol
hydrogen peroxide
hydroquinone
β-β'-iminodipropionitrile
indole-3-acetic acid
methyl acrylate
methyl strychnine
mica (500 mesh)
morpholine
1, 2, 4, 5, 6, 7, 8, 8a-octachloro-4-7-methano-3a, 4, 7, 7a-tetra hydroindane (Chlordane)
piperidine
potassium cyanate
potassium ethyl xanthate
potassium thiocyanate
pyridine
Rutgers 612
skatole
sodium perborate
sodium peroxide
thallium sulfate
β, β'-thiodipropionitrile
triethanol amine
urea

Active chemicals

Active chemicals and the degree of response by the test animals at various dilutions are summarized in Table I.

Effects of particle size and true solution

Since many of the effective chemical compounds were insoluble in sea water and had to be dispersed therein in the form of an emulsion, it was necessary to know something about the relation of droplet size to the magnitude of the response evoked in the fish. Although no data are available relating the intensity of response by fish to the comparative number of sensory receptors stimulated by substances dissolved or suspended in water, we are probably permitted to assume *a priori* that the magnitude of response varies directly with the number of receptors stimulated. Thus, when fish are exposed to dilutions of relatively insoluble substances in the neighborhood of 0.1 p.p.m. it is quite possible that the number of molecules in solution or the number of particles in suspension bear significantly on the intensity of the response.

To illuminate this question for purposes of preliminary evaluation and judgment of the most potent compounds tested, the effect of particle size and true solution on the response to one of the most irritating chemicals discovered (allyl isothiocyanate) was measured. Three preparations were made. First, the dispersing agent, "Tergitol" Dispersant NPX, was added to the chemical as was sea water, followed by simple emulsification in a hand-operated homogenizer. Second, the chemical was placed in isopropyl alcohol to form a true solution to which "Tergitol" Dispersant NPX and sea water were added before emulsifying in a homogenizer. Third, a true solution of the chemical was formed by adding sea water and warming until the insoluble phase had disappeared. Droplet size of the first two emulsions was measured microscopically using an ocular micrometer. In the first suspension the droplets were quite variable in diameter, averaging 4.5 μ, whereas in the second suspension they were of quite uniform size, averaging 3.0 μ.

Tests were conducted as described previously using dilutions of 0.1 p.p.m. for each of the three preparations. No detectable difference in response was discerni-

TABLE I

*Summary of the responses of Kuhlia sandvicensis to various dilutions of chemical irritants. The symbol *** denotes a violent reaction, ** a medium reaction, * a slight reaction, and — no discernible reaction. See text for details.*

Chemical	Emulsifier	Dilution in p.p.m.						
		20.0	10.0	2.0	1.0	0.2	0.1	0.05
Acetic acid	None	*						
Acrylonitrile	None	*						
Allyl isothiocyanate	None	***			***	***	**	—
Allyl mercaptan	Tergitol NPX	*						
Benzene	Triton B 1956	*						
n-Butyl carbitol thiocyanate	None	***			—			
Caffeine	Aerosol OT	*						
Carbon disulfide	None	*						
Catechol (derivative)	Aerosol OT	*						
alpha-Chloronaphthalene	Aerosol OT	*						
Cupric acetate	None	*						
Dibutyl phthalate	Aerosol OT	*						
2,4-Dichlorophenol	Dioxane and Triton B 1956	**						
Diethyl-*p*-nitrophenyl thionophosphate (Parathion)	Aerosol OT	**						
Dimethyl sulfate	None	*						
2, 4-Dinitronaphthol-ammonium salt	None	*						
Du Pont PB-70	Dioxane and Triton B 1956	*						
1, 2-Ethane-dithiol	Triton B 1956	**						
Ethyl mercaptan	None	***		—	—			
Hexachlorobutadiene	Aerosol OT and Triton B 1956	*						
1, 2, 3, 4, 5, 6-Hexachlorocyclohexane, 92% gamma isomer (Lindane)	None	***			*			

Table I—*Continued*

Chemical	Emulsifier	Dilution in p.p.m.						
		20.0	10.0	2.0	1.0	0.2	0.1	0.05
1, 2, 3, 4, 10, 10-Hexachloro-1:4, 5:8-diendomethano-1, 4, 4a, 5, 8, 8a-hexahydronaphthalene (Aldrin)	None	***		—	—			
Hydroquinone	None	*						
2-Hydroxy-3-cyclo-hexyl-1, 4-naphthoquinone	Benzene and Aerosol OT	**		*				
Isobornyl thiocyanoacetate (Thanite)	None	***	***	***	***	*		
Isovaleric acid	None	*						
Lauryl thiocyanate (Loro)	None	***						
Methyl methacrylate	None	**						
Phenol	None	***		**				
Phenacyl bromide	Ethyl alcohol and Tergitol				***		**	—
Phenacyl chloride	Ethyl alcohol and Tergitol				***		**	—
Phenyl isocyanate	Aerosol OT	**						
Phenyl isothiocyanate	None	***	***		*			
Potassium thiocyanate	None	*						
Quinine	Ethyl alcohol and Tergitol		*					
Rotenone (Fish-Tox)	None	**			—			
Sodium bisulfite	None	**	—					
Sodium cyanide	None	***		***	*			
Sodium hypochlorite (Chlorox)	None		***	***		—		
Sodium pentachlorophenate	None	**						
Strychnine	Ethyl alcohol and Tergitol		*					
4-Tertiary butyl catechol	Aerosol OT	**	—					
Thiocyanic acid 5, 5, 5-trichloro amyl ester	Tergitol		***	**				
Trifluoro acetic acid	None	*						

ble with the two suspensions, indicating, possibly, that differences in particle size of the magnitude mentioned do not make any appreciable difference in the reception and response to stimuli. However, the true solution did evoke a discernibly greater activity, thus possibly implying that in such dilutions molecular or near-molecular size of the irritant contributes to more widespread stimulation of available receptors.

Discussion

Intensity, nature and rapidity of responses

Behavior patterns of *Kuhlia sandvicensis* in response to chemical stimulation, mediated primarily through the common chemical sensory receptors, permitted analysis by three separate criteria: (1) the intensity of response (slight, medium or violent, as indicated by the asterisks in Table I), (2) the nature of the response (the particular nervous receptors stimulated and the sensations aroused), and (3) the intervals between bodily contact with the chemical substance and the first discernible reaction. Precise quantitative analyses of the intensity of responses are impossible to achieve, but satisfactory estimates were made on a background of many tests evoking a wide variety of behaviorisms. Slight responses elicited by mild irritants were manifested by rapid mouth movements, efforts to avoid the substance, and vertical swimming, first to the surface, then down, repeated rapidly. These reactions to slight irritants occurred invariably, together with other activity, in responses rated as medium and violent, and were usually the prelude to these intensified reactions. A medium response was denoted by various patterns of behavior all more intense than that indicated for slight response, but in no case did the substances arouse violent reaction. Rapid swimming vertically and about the aquarium, gulping at the surface, jerky motion, etc., were types of responses that rated this designation. Extreme behavior of several characteristic types was designated as violent response. Very erratic and rapid swimming, often leaping out of the aquarium, paralysis, head shaking, blindness and death within two or three minutes were classified in this category.

The mode and place of action of chemicals on fish are not simple to define. The widespread distribution of common chemical sensory receptors on the general body surface, eyes, nasal capsules and the mouth, coupled with the fact that the complexes of chemical senses are not well differentiated for fish, behavioristically speaking, as they are for mammals, precludes the expectation of precise patterns of response to particular stimuli. Excluding the chemical senses of taste and smell, we would still expect different modalities of the common chemical sense in fish just as are found in humans, where different sensations are experienced from substances attacking the eyes, the nose or the throat. An analysis of the responses by fish to chemicals known to arouse particular sensations in other animals bears out this supposition. For example, well known general skin irritants for man such as phenol and allyl isothiocyanate apparently stimulate all free nerve endings on the body making it impossible to pinpoint a site of irritation because of the violent reaction evoked. Also, the very potent lachrymators, phenacyl bromide and phenacyl chloride, arouse intense sensations resulting in violent head shaking and early indications of blindness, precisely the mode of response expected for substances irritating primarily to the eyes. Sodium cyanide and thiocyanic acid 5, 5, 5-trichloro amyl ester, substances related to respiratory poisons for insects (Brown, 1951), and the reducing agent, sodium bisulfite, caused fish to stay at the surface where they gulped air and showed other typical signs of suffocation. Both tetanic and flaccid paralytic effects were noted for certain compounds tested. A clear example of tetanic paralysis wherein the operculum was fixed rigidly at an acute angle on the head, the fins held stiffly outward, and the body bent in a convulsive manner resulted from the introduction of isobornyl thiocyanoacetate. This substance is known to act as a narcotic for insects but induces paralysis in mammals. Ethyl mercaptan, of which little is known neurophysiologically except for its effect on the olfactory receptors, also induced a severe tetanic paralysis in fish. Notable among those substances inducing flaccid paralysis was thiocyanic acid 5, 5, 5-trichloro amyl ester, which, in addition, destroys the equilibrium. The slowly moving fish gradually leave the surface, where they also exhibit symptoms of suffocation, and swim upside down before they eventually become moribund on the bottom of the tank.

Characteristic responses which appeared to differentiate between stimulation of taste receptors and of the common chemical sense were aroused by testing quinine and strychnine. These substances, according to Scholl and Munch (1937), are two of the most bitter substances stimulating the human taste, strychnine being about three times as bitter as quinine. Brucine, about three to four times as bitter as strychnine, was not available for testing. Test fish shook their heads violently and made "spitting" motions for a few seconds after the initial contact with the alkaloids, then, exhibiting no irritation, settled down to normal schooling behavior. Quinine actually evoked a more intense and more lengthy response than strychnine.

Comparative responses of fish and other animals to chemical irritants

Good examples of the highly selective nature of the sensations aroused by chemicals on different organisms are clearly shown in the list of inactive chemicals and in the table of active compounds. Although there was some relationship between chemicals irritating to fish and those having a similar effect on other animals, there were many and significant exceptions.

Exceptions in the ranking position of the chemicals tested thus far were the high molecular weight thiocyanates which had little or no effect on the olfactory and common chemical sensory receptors of mammals, and may even have a relatively low order of toxicity, but are classed here as some of the most effective irritants to fish. A possible explanation for this disparity in reactivity may be associated with the medium in which the animals exist, air in the case of mammals and water in the case of fish. In the former, where air transport is necessary, the relatively low vapor pressure would seem to be a plausible explanation of the lack of response. In the latter, greater reactivity for fish probably results from relatively greater solubility, an assumption which awaits confirmation. Substances of this character emphasize the necessity of an empirical search through a wide variety of chemical types for compounds which, although not irritating to other animals, may arouse intense sensations in fish.

An appreciable number of the chemical groups tested were selected because they elicit a profound and often violent response in man, but we have found them rather ineffective as fish irritants. Such chemicals as most mercaptans, certain amines, some nitriles and skatole fell into this category. For an explanation of much of this disparity in reaction we concur with Moncrieff (1944) that in man conditioned reflexes are important, resulting from associating many of these odors with unpleasant experiences or with substances which we learn to recognize as toxic. Significant also, we suspect, is the fact that most of these substances have a low solubility in sea water, thus having a sub-minimal concentration for reaction, whereas nearly all have an appreciable vapor pressure.

Substances which were found to have about equal effect in irritating fish and mammals fall generally into the groups of intense skin irritants and lachrymators, examples of which are allyl isothiocyanate, phenacyl chloride and phenacyl bromide. Noteworthy among these generally effective compounds is the prevalence of sulfur, which lends support to the point stressed by Moncrieff (1944) that this element imparts skin-penetrative power, thus increasing the vulnerability of the free nerve endings. Our results also emphasize the importance of the bond type, whether it be ionic or covalent, in governing the effect of irritants. For example, all thiocyanates tested were effective irritants in the organic covalent form, but the ionic salts of ammonium, barium and potassium thiocyanate were ineffective. Conversely, covalent cyanides or nitriles proved ineffective, but the cyanide ion belonged with the group of irritants.

Substances found most effective in repelling sharks and certain bony fishes (Burden, 1945; Whitley and Payne, 1947), cupric acetate and acetic acid, were only mildly irritating to our test species and show little promise of satisfying the objectives of this study.

It was pointed out previously that too few data were available in the literature to formulate conclusions on comparative stimulus-response activity in the chemical senses between humans and fish, but that the ratio of response to strength of stimulus for the complex of chemical senses in humans was rather well known, especially since the researches of Katz and Talbert (1930). A hypothetical working parallelism for the chemical senses in fish was established on the basis of the maximum detectable dilution of a chemical substance in fish mediated through the olfactory receptors, based on the results of Hasler and Wisby (1949). We calculated, in accord with the proportional stimulus-response data for the chemical senses in man, that the eyes of fish might possibly be irritated with suitable chemicals at a dilution of about 0.08 p.p.m., and that the common chemical sensory receptors over the remainder of the body might be stimulated at a maximum dilution of about 0.3 or 0.4 p.p.m.

Our data indicate that both phenacyl bromide and phenacyl chloride, known to be powerful lachrymators and to induce behavior in our experimental animals suggestive of intense irritation of the eyes, were quite effective in a dilution of 0.1 p.p.m. and ceased to be discernibly effective at 0.05 p.p.m., precisely fitting our hypothesis. The most effective general irritant, allyl isothiocyanate, evoked observable non-localized responses at the level indicated for the two lachrymators just discussed. The next most effective general skin irritant, isobornyl thiocyanoacetate, failed to arouse discernible sensations at dilutions below 0.2 p.p.m.

Solubility of organic compounds in sea water

Our results have pointed to the fact that the distribution of the reacting substances in sea water, either in the form of a true solution or a colloidal sol, is basic to stimulus-response investigations of this type. These data, although only suggestive in nature, seemed to indicate that finely dispersed particles, particularly in the less concentrated solutions, were not as effective for the stimulation of the free nerve endings over the general body surface as molecules in a true solution. Clouding this aspect of the work is the near absence of solubility data for organic compounds in sea water. However, we can extend what is known about the solubility of these compounds in pure water in an effort to predict their behavior in sea water. It is to be expected that solubility will be generally less in sea water because of the "salting out" effect. Moreover, we would expect in any group of organic compounds having closely related structural composition that the solubility would be greater for those of lower molecular weight and of greater polarity. Considerations such as these guided our selection of compounds.

Observations on the use of chemical dispersing agents with rather insoluble compounds indicated, because of the persistence of a separate phase of emulsion droplets in very dilute solutions where the concentration was well below the amount capable of existing in true solution, that the film of emulsifying agent surrounding each droplet may inhibit the attainment of equilibrium between the two phases of solvent and solute. Because of this, caution must be used in selecting and using chemical dispersing agents when it is desired to achieve maximum molecular concentration of the solute.

General conclusions of a preliminary nature

Although generalizations at this stage of development in this project may be somewhat premature, certain aspects appear reasonably clear. An evaluation of the behavior of the test schools in response to the active chemicals definitely indicated that substances perceived only through the human chemical receptors as odors or tastes did not arouse sensations capable of dispersing schooling fish, but that substances which stimulate human receptors for the common chemical sense, such as general skin irritants, lachrymators and nerve poisons, were effective. Thus future work will be directed toward securing more potent irritants and toward developing synergistic reactions with those already proven effective. A listing of the types of compounds expected to be most effective, based on our analysis of eighty-seven chemicals tested thus far, and placed in order of probable importance, includes multi-halogenated organic compounds, organic thiocyanates, organic isothiocyanates and halogenated ketones. Revisions of this list may be expected as further tests are made within each group, as our list of groups tested is extended, and as other species of schooling fish are used as test animals. We wish to point out again that, although the results of this investigation have been secured by testing and analyzing the reaction of fish to 87 judiciously selected compounds, these appraisals are considered preliminary because of the vast amount of work yet to be done along the lines suggested by this phase of the investigation.

The authors are indebted to many chemical companies which have offered chemical dispersing agents and chemical samples for testing and greatly appreciated advice.

Summary

1. The objective of this study was to find, by judicious selection and testing, whether or not certain chemical compounds in exceedingly dilute concentrations would arouse sensations in fish which would alter the sensory bonds between them, thus effecting dispersal of schooling fish.

2. The basic considerations in selecting chemicals capable of dispersing schools of fish were (1) that they be grossly irritating to fish rather than merely toxic or narcotic, and (2) that they be rapid in action, reaching, in our experiments, a maximum effectiveness within a period of two or three minutes.

3. A preliminary classification of the 87 compounds tested included insecticides and insect repellents, substances irritating or toxic to mammals, piscicides and fish repellents, and special irritants. Suitable chemical dispersers were added to the relatively insoluble compounds. Tests were made at dilutions of 20.0, 10.0, 2.0, 1.0, 0.2, 0.1, 0.05 p.p.m.

4. The intensity, nature and rapidity of response to introduced chemicals were recorded by observation and by motion pictures. Although a clear perceptual pattern of the mode and site of action of certain chemicals known to arouse localized sensations in terrestrial animals was not definitely discernible, certain reactions suggested that special areas were intensely stimulated. General skin irritants (phenol, allyl isothiocyanate) strongly stimulated the entire body; lachrymators (phenacyl chloride, phenacyl bromide) seriously irritated the eyes and impaired vision; some general nerve poisons (isobornyl thiocyanoacetate, ethyl mercaptan) induced tetanic paralysis; whereas another (thiocyanic acid 5, 5, 5-trichloro amyl ester) resulted in flaccid paralysis; and respiratory impairment was brought about by a reducing agent (sodium bisulfite) and by probable respiratory poisons (sodium cyanide, thiocyanic acid 5, 5, 5-trichloro amyl ester).

5. Examples of the highly selective nature of the sensations aroused by chemicals on different organisms were clearly demonstrated. High molecular weight thiocyanates which have little or no effect on the common chemical sense of mammals are highly effective irritants for fish. Others (mercaptans, certain amines, some nitriles, skatole), exceedingly irritating to man, appear to have little or no effect on fish. Substances classed as intense skin irritants or as lachrymators (allyl isothiocyanate, phenacyl chloride, phenacyl bromide) for man appear to be equally effective for fish. Underlying reasons for such disparities and similarities in reaction were suggested.

6. Certain characteristics of molecules appear to be significant in predicting success for irritants, and thus contribute toward a framework for a theory establishing relations between chemical constitution and physical properties on the one hand and aggressive action on the other. Increased aggressiveness with sulfur in the molecule was noted, and the bond type, ionic or covalent, apparently exerts a governing effect with irritants as shown by contrast between the very effective covalent thiocyanates and the ineffective ionic thiocyanate salts.

7. A striking proportionate parallelism seems to exist for stimulus-response reactions of all the chemical senses between humans and the fish used for these tests.

8. Results thus far indicate that substances perceived as odors and tastes for humans do not arouse sensations capable of dispersing fish in schools, but that general skin irritants, lachrymators and nerve poisons may be capable of doing so. Types of compounds which now seem most effective for dispersing fish in schools are multi-halogenated organic compounds, organic thiocyanates, organic isothiocyanates and halogenated ketones.

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