TULANE STUDIES IN ZOOLOGY

Volume 14, Number 2 June 12, 1967

STUDIES ON THE BIOLOGY OF THE FEEDING REACTION IN *GOBIOSOMA BOSCI*

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Abstract

In the naked goby, *Gobiosoma bosci*, an inhabitant of oyster reefs, a feeding reaction was induced with dialyzed oyster extracts. Certain amino acids and amines with similar molecular structure also induced feeding. Basically they have straight chains, 2-5 carbon atoms, and no group interfering with the area of the nitrogen atom. Dialyzed and chromatographed oyster extracts were bitten by gobies in areas corresponding with knowns of alanine, aspartic acid, glutamic acid, glycine, and an unknown. The unknown, which was heat stable, received most of the response with alanine next. Gobies responded to water in which oysters had been held and to water from an oyster reef, but not to sea water. Feeding by *Gobiosoma bosci* is divided into two phases. The first seems to be following a volatile compound, possibly an amine, to the oyster. Feeding itself is induced by a heat-stable unknown, with lesser contribution by certain amino acids.

Hasler (1957) and Teichmann (1962) summarized much of the evidence demonstrating that some species of fishes are attracted to food by chemical perception. However, the compounds perceived by fishes for orientation to food seem to be largely unknown (McBride et al., 1962).

There are two cases where the type of compound is known that produces a specific response in a fish without training: Amines are utilized by lampreys for orientation to prey (Kleerkoper and Mogensen, 1963), and the amino acid serine produces a fear response in salmon (Idler et al., 1956). One recent study (Bardach and Case, 1965) investigated some sensory responses to tissue extracts. A study by Steven (1959) claimed to show feeding responses caused by some compounds.

We have been studying response of the small naked goby, *Gobiosoma bosci* Lacépède to certain smells from the Atlantic oyster, *Crassostrea virginica* (Gmelin). This fish lives in the interstices of oyster reefs. Injured or killed oysters on some reefs always attract this fish (Hoese, 1964). With this in mind we set out to find the compound, or compounds, from oysters that attract fish and cause them to feed.

Methods

Oyster extracts were prepared from four or five 2-5 inch oysters by pulverizing oysters in a blender, dialyzing the liquid, and

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concentrating the extracts by about a factor of 10 by evaporation. One to 50 ml of extract were chromatographed as paired spots or narrow bands 3-12 inches in length on full or halfsheets. The extracts were chromatographed on Whatman grades 3 & 17 paper generally as outlined by Berry et al. (1962). The two main solvents used were N-butanol—50% acetic acid (2:1) and water-saturated phenol. The chromatographs were dried 1 or 2 days. The paper was then cut perpendicular to the solvent front dividing the spot chromatograms into 2 sections and the band chromatograms into 2 one inch strips and one longer strip. One strip from each chromatogram was placed in the aquarium with the fishes and the second developed with 0.2% ninhydrin or aniline acid phthalate reagent. The remainder of the strip chromatogram was used for further testing or elution of the bands. In some cases the strips were removed from the aquaria, dried and stained. It was found that the bands had become diffuse, but that the centers of the bands were generally detectable. Duplicate 2-dimensional chromatographs were run, one used for testing, the other stained. The areas selected by the fishes were located by placing rulers beside the strips. Comparison of the intensity of the bands and spots to the intensity of stained known compounds suggested that each band contained between 1 & 20 μ of amnio acid.

Oysters were collected from a single intertidal reef off the Port Aransas Causeway. Water was taken from this reef, evaporated or distilled at different pH levels, and chromatographed. Oyster water was prepared by holding four to eight 3-5 inch oysters in 2 liters of sea water for 24 hours or longer, evaporating or distilling and chromatographing. Control water was collected from open bay or local Port Aransas waters and was held for the same period without oysters.

Gobies were seined from an artificial pond at Live Oak Point 3 miles NNE of Fulton, Texas. There are few oysters in the pond, and the gobies feed mainly on polychaetes, so they were not conditioned to oyster meat. The behavior of the pond gobies was not different from that of gobies which had been conditioned to oysters.

Fourteen to 20 *Gobiosoma bosci* that had been held in a 32 liter aquarium were used for tests. The fish were starved at least 7 days to enhance the reaction and were replaced after having been exposed to a maximum of ten compounds. Filter paper soaked with 2cc of usually saturated solutions of compounds was introduced into the aquarium in 5 minute trials. This produced a maximum dilution of 1 part in 161, 250. A positive response was one where each individual bit (or tasted) the paper more than once at each encounter. Control filter paper soaked in sea water rarely produced a bite and never a second one. A total of 62 controls was negative.

Undisturbed gobies in aquaria remained on the bottom. When filter paper or chromatographs were introduced, no increased activity occurred unless they contained extracts or compounds that they recognized. The test was almost always clear-cut and in all cases where an individual bit a particular substance on paper it bit the paper repeatedly. The reaction subsided rapidly so that within 5 minutes control conditions were usually in effect again. A more ideal test could hardly be expected, and perhaps some of the difficulty in previous work has been the use of species which rely less on sense of smell than *Gobiosoma bosci* seemingly does.

The Feeding Response

The feeding response was characterized by rapid movement of a normally quiet, sedentary species and biting of objects in aquaria, including each other. Occasionally an individual would react to the extract with a "jerk" or rapid quiver of the whole body. Sometimes they would bow the head upward and rise up on their pelvics. Then they became excited and proceeded to search the aquarium. Oyster extracts introduced into aquaria in dialyzing membrane made into "bags" always attracted most gobies in an aquarium to the vicinity of the "bag" where the gobies commenced biting the "bag" as if it were food. Moving the "bag" to other areas of the aquarium caused the fish to move with it. Finding the "bag" was accomplished much the same as described for *Microgadus timcod* (Herrick, 1904).

Control "bags" of sea water got no response although some fish occasionally passed very close. To show that no visual cues were involved, extracts and controls were introduced into an aquarium in total darkness. Five minutes later, when the light was turned on, the majority of the gobies

were clustered around the "bag" with the oyster extract and biting was in progress. However, visual cues can be important. Feeding a group of gobies in one aquarium caused those in adjacent aquaria to rush toward the aquarium where feeding was occurring, probably because they saw the excited activity of fish in the adjacent tank. Naked gobies will chase and eat live, moving food, such as *Daphnia* and adult brine shrimp *(Artemia)*.

Molecular Structure of Compounds Producing Feeding Response

There was considerable molecular similarity between compounds producing a response. Although many of the compounds were probably recognized by the gobies, only those with nitrogen produced a feeding response. Apparently this area of the molecule is most important, because certain groups which could sterically interfere with the amino group inhibit the reaction. For example, methionine produces a response, but S methyl-l-cysteine does not. The latter differs by having one carbon less in the chain and this directs the methyl group towards the amino group. In methionine the terminal methyl group is directed away from the amino group.

The molecules producing feeding are all characterized by being simple, short, and straight-chained with only certain attached groups. Table 1 shows the structural characteristics of molecules causing and not causing feeding. Table 2 lists the positive compounds. The following did not cause feeding: amines—agmatine; acids—acrylic, formic, acetic, propionic, butyric, valeric, succinic, lactic, cyanuric, hydantoin, 2 thiobarbituric; amino acids—arginine HCl, as paragine, p aminobenzoic, 3 aminobutyric, citrulline, cystine, 2, 6 diaminopimelic, glutamine, histidine HCl, isoleucine, lysine, phenylalanine, proline, pyroglutamic, serine, threonine, tyrosine, valine; peptides—glycl-glycine, glutathione; sugars—glucose, mannose, arabinose, rhamnose, galactose; miscellaneous—guanidine, creatinine, hydroxylamine, ammonia, urea, butylurea, indole, creatine.

Quantitative data are presented in Table 2 although separate trials are not statistically comparable, because conditions were seldom the same in each set of trials. The data are included in Table 2 to show that more than one fish was involved and the trials are repeatable. Despite these variables the small molecule of glycine and larger molecules such as 5 aminovaleric acid and methionine appeared less effective than moderate sized ones. Alanine, which is intermediate in size, usually gave the strongest response.

Table 1
Structural characteristics and examples of molecules investigated

COMPOUNDS PRODUCING FEEDING REACTIONS HAVE
2-5 carbon atoms (glycine, alanine, 5 aminovaleric acid)
amino group on carbon 2 terminal or not (alanine, glutamic acid, 2 aminobutyric acid)
amino group on terminal carbon 3 (β alanine), 4 (4 aminobutyric acid, putrescine), or 5 (5 aminovaleric acid)
1 carboxyl group (glycine, alanine)
2 carboxyl groups (glutamic acid, aspartic acid)
no carboxyl groups (putrescine, trimethylamine)
SH group (cysteine)
methylated nitrogen (trimethylamine)
S-CH_3 in 4 position (methionine)
COMPOUNDS PRODUCING FEEDING REACTIONS DO NOT HAVE
6+ carbon atoms (lysine, cystine, diaminopimelic acid)
ureido groups (asparagine, citrulline, glutamine)
rings (histidine, phenylalanine, proline, tyrosine)
branched chains (leucine, isoleucine, valine)
hydroxyl groups (serine, threonine, tyrosine)
no amino groups (formic acid, lactic acid, ammonia)
nitrogen on carbon 3 not terminal (3 aminobutyric acid)
S-CH_3 in 3 position (S methyl-l-cysteine)

Recognition of Constituents in Oyster Extract

Biting of specific areas of paper chromatograph strips occurred repeatedly and most of the bands bitten stained purple with ninhydrin. Four of these bands had Rf values on one-dimensional chromatographs consistent with knowns of alanine, glutamic acid, glycine, and aspartic acid. Much of the biting was centered at an Rf of .60 in phenol and about .35 in butanol-acetic corresponding exactly to our identified spot for alanine. Subsequently fish were exposed to bands of a phenol and butanol-acetic 2-dimensional chromatograph. The bands from the chromatographed extracts selected

by the fishes corresponded to those identified as aspartic acid and alanine, and also to the unknown (Fig. 1).

The 7 most abundant amino acids roughly estimated from chromatographs were (in decreasing order) alanine, glycine, glutamic acid, taurine, arginine, alanine, and aspartic acid. Some of the fish may have bitten β alanine, because it was very close to alanine. They bit only alanine on the 2 dimensional chromatograph.

Most of the biting activity on both 1 and 2 dimensional chromatographs centered at an Rf of about .88-.92 in phenol and .33-.35 in butanol-acetic. Some one-dimensional phenol chromatographed extracts showed a brown proline ninhydrin stain here but some did not. Fish bit the paper there in either case, but the response was much greater when the spot was apparent.

This unknown component was not destroyed by heat or distillation (Table 2). However, boiling oyster extract apparently removed the compound the fish used for orientation and finding the extract then became by chance. Chance also apparently determined finding of the amino acids tested, because the gobies never went directly to the amino acid saturated paper, but began a search pattern.

Basic distillation isolated a compound which did not cause a feeding reaction but attracted about 8 or 9 gobies to the area. This compound had an Rf of about .97 in butanol-acetic. It was absent in acid distillations, and did not stain with ninhydrin.

Response to Oyster Water

Gobiosoma bosci was able to recognize water that oysters recirculated (oyster water) as well as water from an oyster reef. This species did not recognize local Port Aransas water or open Aransas Bay waters. Table 3 shows the response of 20 gobies to 100 cc of various waters introduced into a 32 liter aquarium. Oyster water was collected from 1 liter of water held with 8 oysters for 9 days. Control water was the same sea water held for 9 days without oysters. Further evidence of their recognition is shown in Table 2. *Gobiosoma bosci* seldom bit filter paper soaked in unconcentrated oyster water, but occasionally they showed a fairly strong feeding response (Table 2).

A single compound (Rf .50 in phenol) found in both oyster water and reef water could be collected from basic but not acid distillation. This substance was brown with-

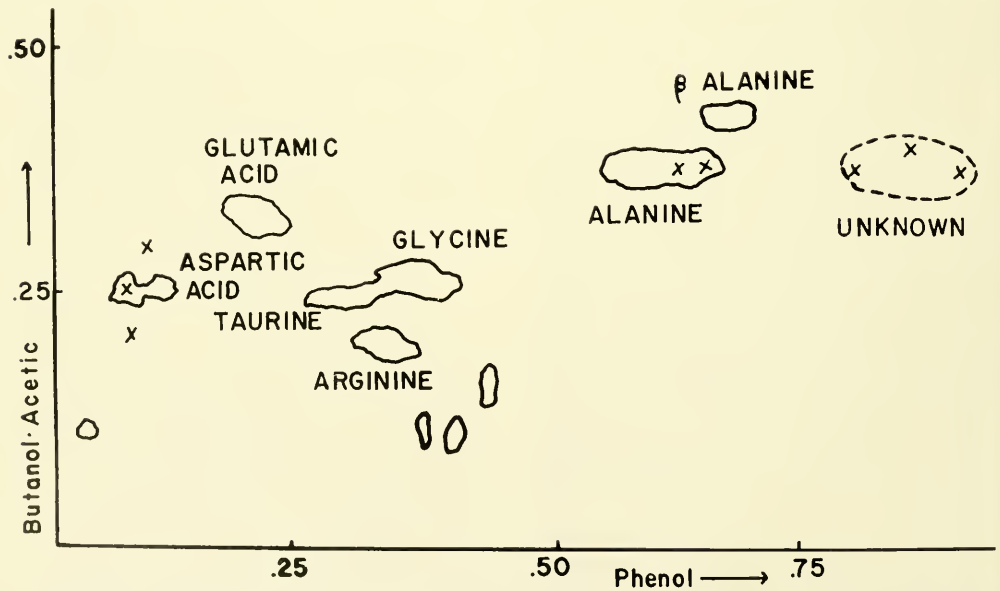

Figure 1. Reproduction of 2-dimensional chromatograph, ninhydrin stained, showing identified amino acids. X marks spots where gobies bit on duplicate chromatograph. Original chromatograph was about 35cm². Unknown area about 2 x 5 cm.

TABLE 2
Number of fish biting filter paper soaked compounds in each time interval

	0-30 sec.	0.5-1 min.	1-2 min.	2-5 min.
Glycine				1
Glycine				1
Glycine			2	1
Glycine				2
DL Aspartic acid	2		5	
"			1	1
"		1	5	
L Glutamic acid	2			
"				2
DL Cysteine			2	
"		1		
DL Alanine	4			
"	4	1		
DL Methionine		2		
"			1	
DL Ornithine		1		
"				1
4 Aminobutyric	1	2	3	2
"	4	1	1	2
Putrescine		1		
"	2			
Trimethylamine		1	2	
"			1	
2 Aminobutyric	2	1		
"			1	1
2, 4 Diaminobutyric			3	2
"		2	1	1
β Alanine		4	1	
"				2
5 Aminovaleric				2
"			1	
Oyster extract		5	4	1
"		6		
Oyster extract		1	1	2
"	4	8	3	1
Boiled oyster extract			8	4
Autoclaved oyster extract	1		3	8
Oyster water (distilled)		1		1
"				2
"		1		
Sea water (62 trials)		All negative		

out staining, but may have been ninhydrin positive. Another compound (Rf .78 in phenol, .48 in butanol-acetic) agreed with our Rf levels of 4 aminobutyric acid except the spot was brown instead of purple. A purple spot (Rf .78 in phenol) was sometimes present in oyster extracts, and once was present in oyster water. The free amino acid content of mollusks is known to vary with environmental factors (Ranke, 1959), which may explain these differences.

Discussion

Simpson et al. (1959) reported alanine, glycine, taurine, glutamic acid, aspartic acid, and arginine, in decreasing abundance, to be the most common free amino acids in *Crassostrea virginica* and our chromatographs indicate roughly the same. All of these except taurine and arginine induce feeding in *Gobiosoma bosci*. Alanine, the most abundant in oysters, induces one of the best feeding reactions, and was bitten more often on oyster extract chromatographs than any but the unknown. The effective amino acids were also found to be the 5 most abundant amino acids in Redfish Bay, Texas water (Park, et al., 1963).

It seems that the reaction involving amino acids is related to the area of the nitrogen atom. The remainder of the molecule is probably unimportant as long as it does not interfere with the amine group. Perhaps interference by highly electro-negative

TABLE 3

Response of *G. bosci* to oyster water. Oys = 100cc water held with oysters. Con = 100cc sea water. Asp 1- = 30cc Aspartic acid solution. Asp 2- 70cc aspartic acid solution. Obs = 5 min observation with no introductions. All trials lasted 5 minutes and were continuous.

		BITES (of any object)	TWITCHES (of any part of body)	RISES (on pelvic fins)
Oys		3	4	8
Con		1	—	—
Obs		1	—	—
Oys		3	4	—
Con		—	—	—
Oys		3	1	—
Obs		—	—	—
Con		—	—	—
Oys		2	2	5
Obs		—	1	1
Asp 1		3	1	2
Asp 2		1	20	1
Con		—	—	—
Oys		7	6	—
Totals	Oys (5 trials)	18	17	13
	Con (4 trials)	1	—	—
	Obs (3 trials)	1	1	1

groups (C=O, OH) or steric interference explains why naked gobies show no recognition to some amino acids. But some molecules with branched chains or more than 5 carbon atoms are ineffective and have none of these characteristics. Perhaps this is explained by a specific compound (our unknown) producing the typical feeding response by fitting completely into a receptor site, with nitrogen producing most of the reaction. Other molecules may fit this space well enough to allow the nitrogen atom to fit in its receptor site, but some with branched chains, or other side groups, or too much length, will not allow the nitrogen molecule to fit completely in its site. Also two separate sites with differing shapes may be involved. The system may be similar to one recently discovered in the house fly (Robbins, et al., 1965) involving several amino acids and the guanosine phosphates. The hypothesis may be further clarified when more compounds and their isomers are tested.

It would seem that food-finding in *Gobiosoma bosci* is divided into two phases. One is following a volatile compound (amine?) to the oyster; the olfactory corridor. The other is inducement of the feeding reaction by amino acids and the heat stable unknown. In some cases this may reach the state of a feeding frenzy. In one instance we introduced alanine into an aquarium with about 20 *Gobiosoma robustum* and a single pelagic postlarva which was 1/2 the size of the largest goby. The postlarva was attacked several times by gobies swimming to the surface, although this behavior had not been observed for several days previously. Where there was no food present gobies would bite algae, pieces of wood, pieces of paper, and each other when induced to feed. Part of this frenzy seems to develop by vision, although it can also occur to some degree in darkness.

It is important to know how many other animals recognize the same compounds. These compounds occur in many prey animals (Ranke, 1959; Simpson, et al., 1959), and animals as diverse as snails and crabs are attracted by smell to oysters as are gobies. We found that alanine and aspartic acid induced feeding in *Bathygobius soporator* and *Gobiosoma robustum,* and our unknown was bitten avidly by *Fundulus grandis* and *Sphoeroides nephelus.* Studies of this sort may be relatively easy, since both the attractant and the feeding inducer are easily isolated by chromatography.

The function of the compound(s) present in oyster water is less easily explained. Perhaps it may be used in orientation to an oyster reef, and a reef has a characteristic smell differing from a grass flat, just as dif-

Figure 2. One dimensional control chromatograph, phenol developed, in aquarium with 14 gobies.

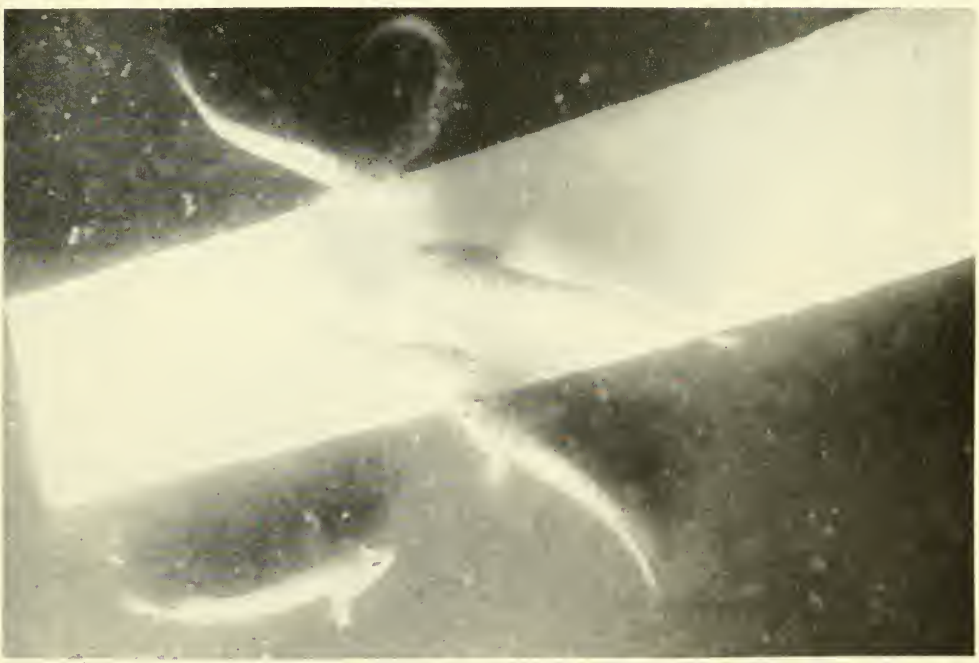

Figure 3. One dimensional oyster extract chromatograph, phenol developed, in same aquarium with 14 gobies. Fish are congregated at an Rf of about .90.

ferent streams may have recognizable smells to migrating salmon (Fagerlund et al., 1963).

Most of the work was done in the laboratory of Dr. Chase Van Baalen without whose valuable help, suggestions and supplies the study would have been impossible. Dr. J. C. Briggs provided aquaria and suggestions and numerous individuals helped collect gobies and oysters. Dr. B. J. Copeland provided some research funds, allowing us to complete the study. Dr. E. D. Lane took Figures 2 and 3.

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