REACTION OF TUNA TO STIMULI, 1953

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United States Department of the Interior, Douglas McKay, Secretary
Fish and Wildlife Service, John L. Farley, Director

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by

Albert L. Tester
Professor of Zoology
University of Hawaii

Heeny Yuen
Fishery Research Biologist
Pacific Oceanic Fishery Investigations
Honolulu, T. H.

and

Michio Takata
Research Fellow
University of Hawaii

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INTRODUCTION

During 1951 and 1952, studies of the response of tuna (mostly "little tunny", *Euthynnus affinis*) to stimuli were conducted in tanks and ponds of the Hawaii Marine Laboratory at Coconut Island, Oahu, under contract (I6fw-13331 and I6fw-18564) between the University of Hawaii and the U. S. Department of the Interior, Fish and Wildlife Service, Pacific Oceanic Fishery Investigations. The results of these studies (Special Scientific Report: Fisheries Nos. 71, 91, and 130) indicated that the most promising line of attack on the immediate practical problem--discovering a means of attracting tuna to within reach of a fishing vessel at sea--was in the field of chemo-reception. It was found that the tuna in the pond responded positively and often violently to extracts of tuna and other fish flesh, viscera, etc.

In June 1953 another contract (14-19-008-2126) was negotiated to pursue these studies further. The testing of materials in the pond was to be continued. Sea tests were to be conducted to observe the response of "wild" tuna not only to extracts, but also to visual stimuli such as lures of various sizes, shapes, and colors, used either alone or in conjunction with extracts.

This report includes the results of this third study. Although the work was conducted mostly from June to October 1953, sea tests extending from January 29 to November 13, 1953, are included.

ACKNOWLEDGMENTS

As in past years, troll fishing to stock the ponds with tuna was conducted under the skillful direction of Mr. Lester Zukeran, skipper of the University of Hawaii research vessel *Salpa*. Mr. Eugene Nakamura and Mr. Royden Ikeda, students, assisted in both fishing and sea testing with this vessel. Mr. Charles Nakamoto, assistant to the Hawaii Marine Laboratory, again was responsible for the feeding and care of the captive fish.

We are again indebted to Mr. Raoul Pantaleoni of E. I. Du Pont de Nemours & Company, to Dr. Ernst T. Theimer of van Ameringen-Haebler, Inc., to Mr. George H. Zirkel of P. R. Dreyer, Inc., and to Mr. R. E. Horsey of Sindar Corporation for supplying many of the chemical compounds which were tested in the pond. In particular, we are grateful to Mr. Fritz Jermann of Hawaiian Tuna Packers Ltd. for preparing most of the extracts which were tested at sea.

The staff of the Pacific Oceanic Fishery Investigations cooperated to the utmost. Special thanks are due Mr. O. E. Sette, Director, and Mr. D. L. McKernan and Dr. W. F. Royce for advice and assistance. Not only were the two research ships *Charles H. Gilbert* and *Hugh M. Smith* made available for sea testing, but also many of the staff members conducted or assisted in conducting the sea tests, including D. L. McKernan, J. Slipp, D. Yamashita, H. Mann, and T. Otsu.

The senior author, who directed the investigation, assumes responsibility for the compilation of this report and for the analysis and interpretation of the data which are included. Most of the sea testing was undertaken by the second author; most of the pond testing was undertaken by the third author.

POND AND TANK EXPERIMENTS WITH EXTRACTS OF FOOD AND OTHER MATERIALS

Catching and Establishing the Fish

Two little tunny (Euthynnus affinis), originally established during the summer of 1952 in the large Pond No. 5 at Coconut Island (Tester et al. 1954) survived the winter and formed the nucleus of a new population which was established during July and early August 1953. To the two survivors were added 12 tunny and 9 yellowfin (Neothunnus macropterus) at the end of June. As in the previous year, the fish were caught by trolling with the Salpa and were transported in the vessel's livewell to Pond No. 5. Of the 21 fish introduced to the pond only one, a tunny, failed to become established, that is, to start feeding. This great success, compared with that of previous years, is attributed to the presence of the two survivors, which acted as leaders of the new fish.

Mortality among the established tunny was moderate between July 1 and October 1, 1953: 5 of the 13 died, leaving 8 which have survived to the time of writing (January 1954). Although the 9 yellowfin appeared to be in good condition at the time they started feeding, all had died by the middle of September. The reason for this mortality, which took place irregularly throughout the period, is unknown.

Two tunny originally established during the summer of 1952 in a concrete tank (Tester et al. 1954) survived the winter and were used in experiments during the early summer. One jumped from the tank and died on July 19, 1953. The other, which was partially blind, died 3 days later.

Feeding the Fish

From April 25 to July 17, 1953, the tunas in the pond were fed exclusively on frozen squid from the mainland. They were fed 4 times a week (Tuesday, Thursday, Saturday, and Sunday) at a rate of about 2 pounds of squid per fish per week. Following July 17, 1953, they were fed daily for 1 week on squid, 1 week on skipjack flesh, 1 week on frozen shrimp from the mainland, and thereafter 4 times a week on skipjack flesh. The change in diet was associated with experiments (to be discussed later) probing the possibility of food-conditioned responses.

The tunny in the tank were fed on tuna flesh 4 times a week throughout 1952 and until they died in 1953.

Materials and Testing Procedures

One hundred and sixty-nine experiments were conducted during the summer of 1953, 162 in the pond and 7 in the tank. Of these, 94 involved extracts of "natural" foods and the other 75 involved solutions or suspensions of chemical materials. Pertinent data on these tests are included in the appendix to this report. The tests are numbered in sequence with those presented in appendix I of Tester et al. (1954). They are referred to by number in the sections which follow, e.g. #495.

"Natural" foods from which extracts were made included skipjack (Katsuwonus pelamis) flesh, skipjack viscera, skipjack blood, dolphin (Coryphaena hippurus) ovaries, anchovy (Stolephorus purpureus), hammerhead shark (Sphyrna sp.) flesh, mainland squid, mainland shrimp, and marine plankton. The materials were prepared in a similar manner: fish, squid, or shrimp flesh was chopped either by hand or in a grinder, placed in a Waring Blendor and macerated after adding tap water, further diluted with water, and allowed to stand for several hours in a refrigerator. Either the whole extract was tested, or the clear portion was separated by decanting or centrifuging and tested. The procedure was the same for blood and plankton except that the initial chopping was not necessary. A self-digested skipjack viscera preparation supplied by Hawaiian Tuna Packers Ltd. was also used in preliminary tests. In some preparations 95-percent ethyl alcohol, instead of water, was added prior to maceration in the Blendor.

The miscellaneous chemical materials were dissolved or suspended in water before testing, using Tergitol and/or mechanical emulsifier when necessary.

Tank and pond tests were conducted in a similar manner to that of last year (Tester et al. 1954). The only important change involved the pond: the observation tower was moved from the western end to the center of the north side (fig. 1). In brief, testing was conducted as follows: the fish were timed and counted in an attraction area during five 3-minute control periods (2-minute periods in the tank); the test material was introduced from the top of the tower into the attraction area through a stream of water which flowed constantly; and the fish were timed and counted during ten subsequent 3-minute test periods. "Timing" consisted of recording on an electric clock operated by a push switch the number of seconds spent by one or more individuals of the tunny school in the attraction area. The "attraction area" was a portion of the pond in front of the tower which was marked off by two pieces of string stretching across the pond; it was approximately 39 by 75 feet in area. "Counting" consisted of recording on a hand counter the number of passes made by each fish in the attraction area while the fish was moving in either a "down" or "up" (easterly or westerly) direction. Materials introduced through the pipe leading from the top of the tower remained in the attraction area for at least 15 minutes and then gradually dispersed. The time and direction of dispersal was governed by the strength and direction of the weak tidal current. Usually a period of at least 30 minutes was allowed to elapse between the end of one experiment and the start (control conditions) of the next to enable introduced materials to disperse or to become diluted to a level well below the threshold of response.

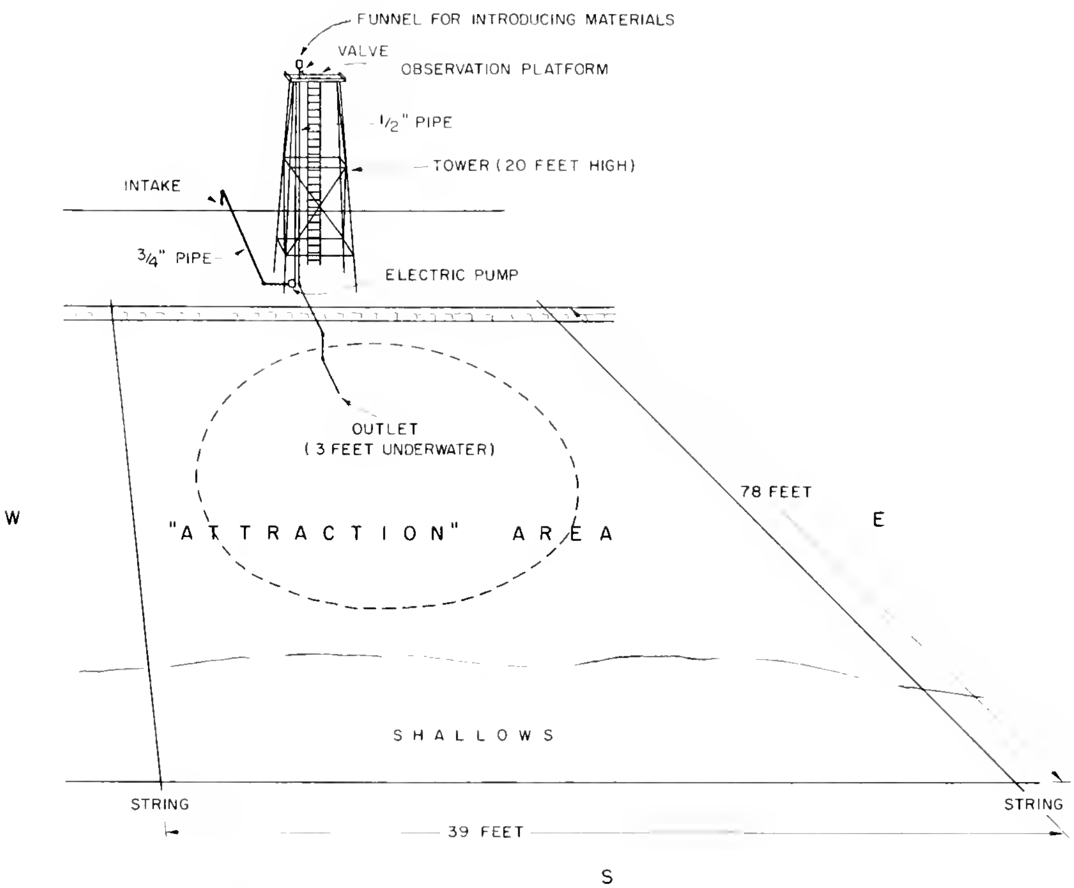

Fig. 1.--Diagram of Pond No. 5 at Coconut Island showing the observation tower, attraction area marked off with string, and the pipes, pump, and funnel used to introduce test materials to the pond.

In addition to obtaining quantitative data in the manner described above, the observer also recorded his visual impression of the strength of the response in one of five categories indicated as "-", an apparent repulsion; "0", no response; "X", a weak positive response; "XX", a moderate positive response; and "XXX", a strong positive response.

Quantitative data were obtained only with the tunny. Yellowfin were also present in the pond, but no attempt was made to measure their reactions because of their erratic behavior. It might be noted, however, that when they did enter the attraction area during an experiment, their response was similar to that of the tunny except that it was generally less pronounced for a given stimulus.

Response to Extracts of "Natural" Food Substances

Although the tunny in Pond No. 5 had been fed exclusively on mainland squid for about 2 months prior to July 1, 1953, before their diet was changed they showed a positive response to extracts of skipjack viscera (#362, but not #357, 358, 363, 368), skipjack flesh (#359, 360, etc., but not #367), skipjack blood (#383), dolphin ovary (#377), anchovy (#384, but not #396), hammerhead shark flesh (#393, but not #373), mainland shrimp (#374, 375), mainland squid (#370, 376, etc.), and marine plankton (#387). The two tunny in the concrete tank, which had been fed exclusively on tuna flesh, showed a positive response to extracts of skipjack flesh (#378, 389), anchovy (#385), mainland shrimp (#382, 386), mainland squid (#380), but not to an extract of hammerhead shark flesh (#392).

In general, the response in the pond was weak and extremely variable as compared with that obtained with extracts of tuna flesh, viscera, etc. in the summer of 1952. To produce responses with tuna flesh extract it was necessary to use about five times as much material as was used in the summer of 1952 (about 100 g. as compared with about 25 g. of flesh). However, the amount used was not as much as was necessary during the spring of 1953 (about 350 g.), when the two surviving tunny were particularly unresponsive (Tester et al. 1954).

The fact that the squid-fed tunny did respond to such a wide variety of extracted food substances (even to plankton in one experiment) indicated that they were not conditioned to the smell or taste of the particular food which was being fed. However, at times, it seemed that the response of the pond tunny to squid extract was more pronounced than that to extracts of other substances.

It was decided to undertake a series of experiments involving three test substances (extracts of squid, skipjack flesh, and shrimp), each to be tested on each of three days per week (Tuesday, Wednesday, and Thursday), the tests to run for 3 consecutive weeks. Prior to and during each successive test period the food was to consist respectively of squid, skipjack flesh, or shrimp. Each week one of these foods was to be fed to the fish at 4 p.m. each day from Thursday (after the Thursday tests were made) to the following Wednesday. The order of testing was to change each day, so that no two tests would appear in the same order on successive days of each week (Latin square design).

Two departures from the plan were made. Due to an error, the same order of testing was used in two of the three days in Week 1. A fourth substance was added to the three primary tests, namely, a blend (equal parts) of squid, skipjack flesh, and shrimp extract. Thus two tests were made in the morning and two in the afternoon of each day. The series either started or ended with the blend, which was either preserved or not preserved with sodium bisulphite. A summary of the results is given in appendix I (#398 to 409; #414 to 425; and #436 to 447). It will be noted that in each test the same method of preparation and the same amount of material was used (100 g. extracted with 2,000 ml. water).

For each test the strength of the response according to the observer's visual impression and also the strength of the response as measured quantitatively is given in table 1. The variate for the latter, which was used for statistical analysis, may be explained by means of an example. In the experiment conducted with an extract of skipjack flesh on Thursday of the second week, when the fish had been fed skipjack flesh (see table 1, $E_2D_3F_2$), the mean time spent by the fish in the attraction area was 7.2 seconds during control periods and 28.6 seconds during test periods, with a mean difference of 21.4 seconds. The mean number of "passes" in the attraction area was 4.4 during control periods and 15.2 during test periods, with a mean difference of 10.8 passes. The differences in both time and count reflect the response, although their relative value as a measure of response is difficult to assess. The two were added, giving 32.2; rounded off, giving 32; and increased by 20, giving 52. The last step, adding 20, was included because in some cases negative differences were obtained, i.e., the fish spent relatively more time and/or made more passes in the attraction area during control than during test periods.

Order of testing is not indicated in table 1. Inspection of the data indicated no consistent trends in response with order of testing within days: on some days the first substance tested gave a high response whereas on other days the last substance tested gave a high response. Similarly, inspection of the data indicated no significant differences between the preserved and non-preserved blend. Accordingly the results were analyzed in toto according to three criteria of classification:

(1) Extract -- E_1-squid; E_2-skipjack; E_3-shrimp; E_4-blend

(2) Days -- D_1-Tuesday; D_2-Wednesday; D_3-Thursday

(3) Food (= Weeks) -- F_1-squid; F_2-skipjack; F_3-shrimp

Table 1.--The response of tunny to four extracts (E) on three days (D) in each of three weeks during which they were fed three different foods (F). For further explanation see text.

Food	Days	Extract E_1 Squid	E_2 Skipjack	E_3 Shrimp	E_4 Blend	Total
F_1 squid	(D_1 Tue.)	44(X)	49(XX)	21(X)	62(XXX)	176
	(D_2 Wed.)	29(X)	30(XX)	29(X)	57(XXX)	145
	(D_3 Thurs.)	35(X)	52(XXX)	12(0)	41(XX)	140
		108	131	62	160	461
F_2 skipjack	(D_1 Tue.)	43(XX)	49(XX)	57(X)	24(X)	173
	(D_2 Wed.)	27(X)	56(XX)	34(X)	23(X)	140
	(D_3 Thurs.)	9(0)	52(X)	32(X)	31(XX)	124
		79	157	123	78	437
F_3 shrimp	(D_1 Tue.)	44(XX)	36(XX)	48(X)	27(X)	155
	(D_2 Wed.)	28(X)	32(XX)	36(X)	51(XXX)	147
	(D_3 Thurs.)	5(X)	26(X)	10(0)	23(0)	54
		77	94	94	101	366

The analysis is as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square
F	2	405.723	202.861
D	2	1304.889	652.444**
E	3	995.334	331.778*
FxD	4	467.944	116.986
FxE	6	2240.499	373.416*
DxE	6	968.000	161.333
FxDxE	12	1139.167	94.930

where * indicates an F value for which P is less than 0.05, and ** indicates an F value for which P is less than 0.01, in all cases using the second order interaction as the error term.

There are no significant differences among the mean responses to the different foods which were fed. However, as shown in the following values of the grand means, there is a general decrease from Week 1 (F_1) to Week 3 (F_3): 38.4, 36.4, 33.2. In view of the results which follow, this trend, if real, is more likely related to time than to food. Of necessity, time (weeks) and food are confounded in the design.

There are highly significant differences in the mean responses of the fish between successive days of the week. As shown by the grand means for successive days, there is a progressive decrease in response from D_1 (Tuesday) to D_3 (Thursday): 42.0, 36.0, 27.3. The decrease is consistent within weeks, as is apparent from the lack of any significant interaction involving days in the analysis of variance table, or as may also be seen from the data of table 1. It may be shown that the regression of response on days is highly significant (mean square for regression is 1290.667**) and accounts for most of the variation between days. A study of tidal heights, direction of tidal current, and weather at the time of each test revealed no apparent relationship to account for the regression. It is most likely due to progressively increasing fatigue or a dulling of response with successive testing. It may reflect a learning process--the fish can be deceived into a feeding reaction by the extract but become less responsive when they find that no food is available[1/]. Although the fish were allowed to rest on Saturdays and Sundays, the regression may have been carried over from one week to the next, particularly if it involved a progressive dulling of response or a learning process, thus accounting in part at least for the decrease in response between weeks noted in the preceding paragraph.

There are significant differences in the responses to the various extracts. The grand means for squid, skipjack flesh, shrimp, and blend extracts are respectively as follows: 29.3, 42.4, 31.0, 37.7. According to these results the skipjack flesh extract gave the greatest response and the squid extract the smallest response, with the blend slightly greater than the mean of the other three. Although heterogeneity is indicated, it may not reflect differences in response between the substances _per se_, but rather an interaction between the extracts and the food fed (no significant differences between the grand means for extracts are indicated if the FxE interaction is used as the error term).

There is a significant first order interaction between extracts and foods (or weeks). This indicates that the fish responded differently to the four extracts when they were fed on different foods (or, in different weeks). The nature of the differential response may be seen from the following summary, which gives the mean response according to both food and extract:

1/ This has been suspected to occur between successive tests within days on other occasions.

Food (Weeks)	Extract			
	E_1(squid)	E_2(skipjack)	E_3(shrimp)	E_4(blend)
F_1(squid)	36.0	43.7	20.7	53.3
F_2(skipjack)	26.3	52.3	41.0	26.0
F_3(shrimp)	25.7	31.3	31.3	33.7

It is unfortunate that the effect of food cannot be separated from the possible effect of the time component (Weeks), although it is difficult to see how the latter, if a regression, could lead to a significant interaction with extract. The inclusion of the blend extract data has contributed to the interaction. However, the interaction is still significant if these data are excluded from the calculations. The response to squid extract was greatest when the fish were being fed squid. The response to skipjack extract was greatest when the fish were being fed skipjack. The response to shrimp extract was greatest when the fish were being fed skipjack, but it was greater when they were fed shrimp than when they were fed squid. The differential response indicates a degree of conditioning of the fish to a particular food substance. In part, this may explain the weaker responses to skipjack flesh and viscera extracts during July 1953 as compared with the summer of 1952, for in 1953 they were being fed squid whereas in 1952 they were being fed tuna flesh.

On completion of the experiments on conditioning the tunny were fed skipjack flesh three times a week. Additional experiments indicated no significant difference in response between extracts of light red (#469, 473, 478, 484) and dark red (#470, 472, 480, 482) skipjack flesh.

Alcohol extracts of skipjack flesh seemed to elicit a much weaker response than aqueous extracts (see #475 to #489). This was the case when the alcohol extracts were tested without further processing and also when they were evaporated to dryness and redissolved in water. The reason may be that the "attractant" was only partially extracted with alcohol; this was suspected to be true in some of the preparations used in 1952-53 (Tester et al. 1954).

Response to Chemical Compounds

The following chemicals in solution or suspension were tested during the summer of 1953:

Adenylic acid, muscle (#517)
Alanine, alpha DL (#450)
Amyl caproate (#505)
Amyl cinnamate (#372)
Anethol (#427)
Anisic aldehyde (#365)
Arginine monohydrochloride, L (#428)
Asparagine, DL (#426)
Aspartic acid, L (#453)
Aspartic acid, DL (#463)
Astrotone (#515)
Bologna flavor (#411)
Betaine (#474)
Blood fibrin (#523)
Butter flavor (#413)
Butyric acid, alpha amino n (#455)
Cadaverine dihydrochloride (#500)
Calcium pantothenate, D (#493)
Casein (#512, 524)
Casein hydrolysate (#522)
Chlorophyll (#459)
Cholic acid (#496)
Ionine C-1 (#506)
Isoeugenol (#410)
Lactulbumin (#521)
Leucine, DL (#430)
Liver extract concentrate (#499)
Liver fraction "L" (#497)
Lysine monohydrochloride (#466)
Lysine monohydrochloride, L (#451)
Mercaptan, phenyl ethyl (#516)
Methionine (#390)
Musk ambrette (#503)
Musk ketone (#498)
Musk xylol (#504)
Norvaline, DL (#456)
Ornithine monohydrochloride, DL (#468)
Phenylalanine (#491)
Phenyl ethyl alcohol (#509)
Proline, L (#467)
Pyridoxine hydrochloride (#462)
Riboflavin (#510)
Serine, DL (#429)
Sodium bisulphite (#449)

Citral (#371)	Testosterone (#501)
Creatinine (#495)	Threonine, DL (#461)
Eucalyptol (#395, 513)	Threonine, DL, with allothreonine (#460)
Glutamic acid hydrochloride, L (#519)	Thiamine hydrochloride (#492)
Glutamic acid monohydrate, DL (#452)	Tonquin musk (#511)
Glutaric acid, alpha keto (#431)	Tryptophane (#434)
Gluten (#525)	Tryptophane, DL (#458)
Glycyl-glycine (454)	Tyrosine, DL (#490)
Guanine (#435)	Uracil (#494)
Guanine hydrochloride (#464)	Urami (#465)
Hexahydrobenzoic acid (#518)	Viosterol (irradiated ergosterol) (#507)
Histidine (free base) (#448)	Vitamin A, crystalline (#514)
Histidine monohydrochloride, DL (#432)	Yeast hydrolysate (#520)
Hydroxyproline (#433)	Xanthophyll oil (#457)
Ionine, alpha methyl (#508)	

There was no positive response to any of the above substances. In a few (#365, 413, 498, 503), activity simulating a response was noted, but this may have been caused by extraneous factors or it may have been a curiosity about or sensing of some of the strong smelling materials. In others of a bright orange color (#510) or a dark green color (#459) there was an avoiding or repellent effect which was obviously visual in origin.

Discussion

In view of the results given in a preceding section which indicate that the response to extracts of "food" substances may be partially conditioned by the particular type of food fed, it seems worthwhile to speculate further on the part which conditioning may play in causing all responses in the pond. The so-called "attractant" to tuna may be a complex of substances which are present in one grouping or another, and to a greater or lesser extent, in extracts of many marine animals such as fish, squid, shrimp, or even plankton. Certainly the tuna in the pond have become accustomed to being fed on dead material--so much so that they ignore live bait in the pond. Possibly they have associated the act of feeding with the smell or taste of this "attractant" which is released into the water from the cut-up food material when it is thrown to them at feeding time. Thus they may exhibit a conditioned feeding response when stimulated by this "attractant" which is present in the extract. This would be a possible explanation of why they respond to extracts of one material when another is being fed. In addition, a further conditioning to a particular substance or complex of substances in a particular food organism could conceivably take place. If so, this would explain why they show a heightened response to extracts prepared from the particular food substance which they are being fed. It was hoped that even if this were so, wild fish at sea might be naturally "conditioned" to a smell given off by the whole, live organisms on which they feed, particularly if the organisms were occasionally injured during the feeding process and gave off attractive body juices. More information on the part played by conditioning, natural or otherwise, might be obtained from pond experiments if the tunny would feed on live bait. As they will not do this (unless the bait is caught and thrown to them), the only other obvious way to settle the question is to present the extracts to wild fish at sea and to study their response.

POND EXPERIMENTS WITH EDIBLE LURES

As the tuna in the pond were accustomed to being fed with materials thrown to the surface by an attendant, they showed a strong visual response to objects which suddenly appeared on the surface and also to persons who approached the edge of the pond. This conditioned behavior made it difficult to perform meaningful experiments in the field of visual and visual-chemical stimulation (Hsiao and Tester 1954). Inedible objects such as leaves falling to the surface or stones thrown to the surface would attract the fish if they were within about 50 feet of the object (the tunny have a keen sense of vision). They would dash after such objects, snap at them, and sometimes take them into their mouths but, as nearly as could be observed, would then reject them. It would have been possible to devise experiments to study the response to inedible objects of different materials, buoyancy, size, shape, and color to see if there was any preference, but this was not done for fear that if these artificial lures were made sufficiently attractive to be taken by the fish they might be eaten and would cause their death. To avoid this possibility edible lures were used. A series of 33 pond experiments were conducted over the period from August 18 to October 29, 1953. These are discussed in the following sections.

Materials and Methods of Preparation

In view of the practical problem of attracting tuna to the stern of a fishing vessel, there was need for an edible material which was low in cost, easily prepared, and at the same time both visually and chemically attractive to the fish. In other words it was desired to create a lure which was attractive in shape, size, color, and movement in order to visually attract the fish from a distance, and which at the same time was edible and sufficiently palatable to induce them to want more. Three basic materials were tested: (1) gelatin capsules, (2) macaroni, and (3) agar gelatin.

The gelatin capsules (Eli Lilly & Co., No. 000) were about 1 inch long when closed, and consisted of two parts, the body (about 7/8 inch) and the cap (about 5/8 inch). They gradually softened in water, eventually collapsing and dissolving after several minutes. Unsuccessful attempts were made to impart motion to the whole capsule. A slow motion was imparted to the open-ended body or cap when floating on the surface by (a) gluing pellets of calcium carbide inside the closed end, (b) gluing pellets of "Bromo-seltzer" inside the closed end, and (c) stuffing the body or cap loosely with cotton wool (to form an adsorption surface) and filling with 95-percent alcohol (extract of skipjack flesh) before throwing to the surface of the water. The motion of the first two depended on gas production on contact with sea water for the propelling force; that of the third depended on the affinity of alcohol for water. The motion was very slow, of the order of about 6 inches per second or less. The carbide-filled capsules were not used in the pond for fear of killing the fish.

Some of the gelatin capsules were coated with a sticky glue (plastic in acetone) and were then dusted with aluminum powder (Sirius Commercial Aluminum Compound) to make them silvery in appearance. Others were coated with a gummy concentrate of skipjack extract (boiled to a residue) giving them a brownish color. Still others were coated with both the aluminum and the concentrate. They were tested both with and without the motion imparted by the chemicals.

The precut macaroni ("Royal" or "Golden Grain") was cooked in water, in concentrated skipjack extract, and in concentrated anchovy extract, in some cases both with and without preservative (about 2 percent sodium bisulphite). The cooked pieces were about 3/8 inch in diameter and 1-1/2 inches long.

The amount of extract used in cooking each small batch was adjusted so that all was absorbed by the time the macaroni was cooked to the right consistency. The pieces were then removed from the pot with a spatula and dried on a screen until they were slightly sticky to the touch. Before being used some were coated with corn starch to keep them from sticking together; others were coated with aluminum powder to give them a silvery appearance. Some were plugged at both ends with glue or with small pieces of cotton wool so that they tended to float when thrown to the surface; others, not plugged, would slowly sink.

Agar (common Japanese variety) was purchased in long strips (about 11 x 1 x 1 inches). Both "colorless" and red varieties were used. It was boiled with water (300 ml. to one strip), concentrated skipjack extract, and concentrated anchovy extract, both with and without the addition of aluminum powder and both with and without preservative. The boiled solution was poured into flat pans and set in the refrigerator. It was then removed and sliced into strips approximately 2 x 1/4 x 1/16 inches. Strips thicker than 1/16 inch tended to sink too rapidly; those thinner than 1/16 inch tended to float on the surface of the water.

Method of Testing

In the first experiment, the edible lures were thrown to the fish in the pond from ground level after the school had gathered in front of the observer. As the fish were visually attracted to the observer as well as to the lures this method was abandoned. In all subsequent experiments the lures were thrown to the pond from the tower when the tunny were away from but approaching the attraction area. Generally the fish did not see the splash of the lures as they hit the water, but saw the lures themselves on entering the attraction area. Observations were made of the number of lures which sank before the fish approached (classified as "not seen"), the number presumably visible to the fish but ignored ("ignored"), the number taken into the mouth but rejected or spat out ("rejected"), and the number taken and eaten ("eaten"). Observations were made also on the behavior of the fish, e.g., whether they showed excitement and whether they returned to the attraction area after the lures were presented. In later experiments the response was indicated in the same categories as were used in the experiments with chemical stimulation (0 to XXX).

Each experiment took a considerable period of time (usually about 30 minutes); it could be performed only when the tunas were cruising regularly up and down the pond. Only a few lures (usually three with one observer or up to eight with two observers) could be presented to the fish at one time; if more were used it was difficult to keep track of the ultimate fate of each.

Results

Four experiments were performed with gelatin capsules, using about 12 capsules in each and presenting them two or three at a time to the fish. In the first, the capsules were coated with aluminum powder, plugged with cotton, and motivated with alcohol extract. In the second, they were coated with aluminum powder and extract concentrate, plugged with cotton, and motivated with alcohol extract. In the third, they were coated with aluminum powder and extract concentrate, and motivated with Bromo-seltzer. In the fourth, they were coated with extract concentrate only, and motivated with Bromo-seltzer. In the first experiment, the fish were attracted to the capsules, took them into their mouths, but then rejected them. In the other three experiments they were attracted to and ate all the capsules. Thus it seems that all preparations were visually attractive to the fish, but only those coated with extract concentrate were actually eaten. The small amount of motion imparted to the capsules by the alcohol and Bromo-seltzer appeared to play little or no part in attraction.

In 14 experiments performed with macaroni lure preparations there was no noticeable difference in response to materials which were (1) either preserved in sodium bisulphite, or not; (2) either plugged with cotton wool, or not (although more of the latter sank before being observed by the fish); and (3) either cooked in concentrated skipjack extract or cooked in concentrated anchovy extract. The data grouped into four main categories are included in table 2.

It is noteworthy that the pond fish consumed 54 percent of the plain macaroni lures; however, these were usually eaten only towards the start of a series of experiments. The results suggest that coating with aluminum powder increases the visual attractiveness of the lure, that coating with extract increases its chemical attractiveness or palatability, and that a combination of the two gives the best response, with 95 percent of the lures being eaten. However, only in the comparison of "with aluminum and extract" versus "plain" is the difference in response statistically significant[2].

In 13 experiments with agar lure preparations, there was no noticeable difference in response between preparations with or without the preservative nor between preparations boiled in concentrated skipjack or anchovy extract. Plain red agar strips seemed to be more attractive than plain colorless agar strips, but the difference was not statistically significant. The results grouped in four categories are included in table 2.

2/ Testing the ratio (ignored plus rejected): (eaten) yields an adjusted Chi-square of 13.8 with one degree of freedom (P less than 0.01).

Table 2.--Summary of the response of tunny to pieces of cooked macaroni and strips of agar in several experiments

Lure and preparation	Number of pieces				Total	Percent eaten
	Not seen	Ig-nored	Re-jected	Eaten		
Elbow macaroni:						
Plain	(7)	2	9	13	24	54
With aluminum	-	-	4	10	14	71
With extract	(1)	1	3	7	11	64
With aluminum and extract	(4)	1	1	40	42	95
Agar strips:						
Plain	-	3	6	10	19	53
With aluminum	-	2	5	2	9	22
With extract	(2)	1	-	22	23	96
With aluminum and extract	(3)	1	3	11	15	73

The best response, with 96 percent of the lures being eaten, was obtained with agar strips cooked in concentrated extract. As they assumed a brownish color, they may have been visually as well as chemically more attractive than plain colorless agar. The addition of aluminum did not seem to increase the attractiveness of the agar preparations.

A series of tests was conducted to determine if there were any differences in response to (a) macaroni cooked in concentrated skipjack extract and coated with aluminum powder, and (b) agar cooked in concentrated skipjack extract and impregnated with aluminum powder. In both cases the preparations, part of a large quantity used in sea tests, were preserved with 1 percent sodium bisulphite. The lure preparations were presented alternately to the fish, eight pieces at a time. The pairs of tests were repeated several times throughout the morning, and again throughout the afternoon of the same day. The results are shown in tables 3 and 4.

In the morning series (table 3) the tunny ate 69 percent of the macaroni lures and 100 percent of the agar lures. In the afternoon series (table 4) they ate only 10 percent of the macaroni lures but still ate 100 percent of the agar lures. It is obvious from the data, and it was still more obvious from observation, that the pond fish showed a decided preference for the agar lures after the first pair of tests. The percentage of macaroni lures which was eaten gradually decreased with successive testing. In the last three pairs of tests in the afternoon some of the pieces of macaroni were taken into the mouth, but all were rejected; on the other hand all agar strips, if seen, were eaten. Apparently a learning process was involved. In general, the overall reaction (speeding, surfacing, splashing, return to area, etc.) was more pronounced with the agar than with the macaroni lures; the difference was more noticeable in the morning than in the afternoon tests.

Table 3.--Results of experiment (#32--a.m.) with macaroni and agar preparations, eight pieces of each presented alternately in a series of tests

Lure and preparation	Test No.	Number of pieces: Not seen	Ig-nored	Re-jected	Eaten	Total	Percent eaten	Reaction
Elbow macaroni:	1	(8)	-	-	-	-	-	XX
	3	-	-	-	8	8	100	XX
plus aluminum and	5	-	-	1	7	8	88	XX
	7	-	1	-	7	8	88	XX
extract	9	-	5	-	3	8	38	X
	11	(pieces not visible due to re-flection of sun)					-	XX
	13	-	1	-	7	8	88	X
	15	(8)	-	-	-	-	-	
	17	-	4	-	4	8	50	XX
	18	-	3	-	5	8	62	X
	19	-	5	-	3	8	62	X
Totals		(16)	19	1	44	64	69	
Agar strips:	2	-	-	-	8	8	100	XXX
	4	(1)	-	-	7	7	100	XX
plus aluminum and	6	-	-	-	8	8	100	XX
	8	-	-	-	8	8	100	XX
extract	10	-	-	-	8	8	100	XX
	12	-	-	-	8	8	100	XX
	14	-	-	-	8	8	100	XX
	16	-	-	-	8	8	100	XX
Totals		(1)	-	-	63	63	100	

Table 4.--Results of experiment (#33--p.m.) with macaroni and agar preparations, eight pieces of each presented alternately in a series of tests

Lure and preparation	Test No.	Number of pieces: Not seen	Ig-nored	Re-jected	Eaten	Total	Percent eaten	Reaction
Elbow macaroni:	1	-	2	3	3	8	62	X
	3	-	3	4	1	8	12	X
plus aluminum and	5	-	4	3	1	8	12	X
extract	7	-	2	6	-	8	0	X
	9	-	4	4	-	8	0	X
	11	-	6	2	-	8	0	X
Totals		-	21	22	5	48	10	
Agar strips:	2	-	-	-	8	8	100	XX
	4	-	-	-	8	8	100	X
plus aluminum and	6	-	-	-	8	8	100	X
extract	8	(1)	-	-	7	7	100	X
	10	-	-	-	8	8	100	X
	12	(2)	-	-	6	6	100	X
Totals		(3)	-	-	45	45	100	

Discussion

The pond tests showed that tunny held in captivity would eat edible preparations such as gelatin capsules, pieces of macaroni, and strips of agar gelatin, and that these were more avidly consumed if they were made chemically attractive, or palatable, with concentrated extracts of skipjack or anchovy. In some cases, the relative number eaten seemed to depend, in addition, on the visual attractiveness of the lures.

The pond fish showed a distinct preference to agar preparations over macaroni preparations after the first pair of trials: a learning process seemed to be involved. The reason for the preference is uncertain, but it probably involved a difference in smell, taste, or texture of the materials. Although the two preparations were basically different in composition (macaroni is a starch and agar is a sulphuric acid ester of galactan), both were thoroughly impregnated with the same concentrated extract of skipjack flesh, and presumably should have had a similar smell or taste. Perhaps the considerable difference in texture between the macaroni and agar preparations was responsible for the choice: the strips of agar were softer, more pliable, and more "jelly-like" than the pieces of macaroni.

Although there was no assurance that sea fish would respond in a similar manner to the pond fish (which were accustomed to feeding on dead, non-motile food), it was considered worthwhile to conduct sea tests with edible lures. Unfortunately, attempts to induce appreciable motility in edible lures have been unsuccessful to date. As will be discussed later, motility may be an important factor in the successful use of either edible or inedible lures to attract tuna at sea.

SEA TESTS WITH EXTRACTS

Preparation of Material

For the most part the extracts used in the sea tests were prepared in 50-gallon drums by Mr. F. Jermann of Hawaiian Tuna Packers Ltd. The materials are briefly described as follows:

(1) Skipjack viscera, ground, steam-cooked, water added, filtered, preserved with 2 percent sodium bisulphite (demijohns - 1/20/53).

(2) Yellowfin flesh, ground, extracted with water, filtered, and preserved with 2 percent sodium bisulphite (Drum No. 1 - 2/18/53).

(3) Yellowfin flesh, as above (Drum No. 2 - 2/24/53).

(4) Yellowfin flesh, as above (Drum No. 3 - 3/4/53).

(5) Skipjack viscera, ground, extracted with water, preserved with 2 percent sodium bisulphite (Drum No. 4 - 3/13/53).

(6) Skipjack viscera, as above (Drum No. 5 - 5/11/53).

(7) Skipjack viscera, ground, steam-cooked, water added, filtered, and preserved with 2 percent sodium bisulphite (Drum No. 6 - 6/20/53).

(8) Slipjack viscera, ground, frozen into 5-pound blocks (various dates).

All materials were tested on the captive tunny in the pond, in some cases both before and after sea tests. The responses in the pond were generally weak and variable due to the unresponsive condition of the two surviving tunny during the late winter and spring of 1953. However, all substances did produce a response in at least one out of several tests (Tester *et al.* 1954).

The extracts in the demijohns and drums were used either as prepared or they were diluted to various strengths on board ship. The frozen material was thawed and extracted with sea water on board ship for at least 1 hour before use.

In addition to the above, two tests were performed with anchovy extract prepared on board by crushing the live bait in buckets of water, allowing the preparation to settle, and then decanting the supernatant liquid.

Methods of Testing

The methods of testing the response of sea fish to the extracts were many and varied. In some cases the extract was poured from buckets from the side or stern of the ship as it described an arc or circle near or around a flock of birds and, presumably, a school of tuna. In the majority of the tests, the tuna school was attracted to the stern with live bait, chumming was stopped, and the extract was poured over the stern into the group of feeding fish. In others, when chumming ceased, the extract was poured in varied quantities and dilutions from the port and/or starboard sides forward of amidships. In still others, it was pumped in a stream or spray from amidships; the pump was controlled from the flying bridge where the fish could be seen.

Results

In all, 16 tests were performed with skipjack or yellowfin extract and 2 with anchovy extract, with several trials per test in some cases. The tests were performed mostly on schools of skipjack or on mixed schools of skipjack and yellowfin, skipjack and tunny, or skipjack and frigate mackerel. In addition two tests were conducted on a pure school of frigate mackerel and one on a school of dolphin.

The results were either negative or inconclusive. Unchummed schools could not be raised by an arc or circle of extract. Chummed schools could not be held at the stern by introducing the extract in small quantities by either bucket or pump.

In a few tests, on pouring in a large quantity of extract after the school had been chummed to the stern and chumming was stopped, a few fish were seen jumping in or passing through the material. It could not be determined whether they were responding to the extract or were chasing stray baitfish. The latter seems to be the more likely explanation.

Discussion

In view of the results outlined above, and others to be reported below in which extracts were used in combination with edible lures, it may be concluded with reasonable certainty that the local skipjack will not respond to extracts of skipjack viscera, yellowfin flesh, and anchovy. It seems likely that this conclusion also applies to local tunny and yellowfin, which showed a response in the pond but not at sea. However, the sea tests on tunny and yellowfin were few in number and were conducted on schools in which they were mixed with skipjack. There is the possibility that they might respond to the extracts if schooled alone.

No sea tests were conducted with extracts of squid and shrimp, both of which might be considered to be a more natural food than skipjack, yellowfin, or anchovy, and therefore might be expected to elicit a response even though the other materials failed. There were several reasons for not using extracts of squid and shrimp, including the lesser availability and higher cost of these materials. The chief reason was that the pond fish had responded well to tuna flesh extracts even though they had been fed exclusively on squid for a period of 2 months. This indicated that they had not been conditioned to any particular food item, but rather were responding to an "attractant" which was generally distributed throughout all food items. It was assumed that this might also be the case with the fish in their natural environment, and that if they responded at all, they would respond (almost) equally well to extracts of tuna, anchovy, shrimp, squid, etc.

The negative results obtained in the sea tests indicate that the sense of smell plays little or no part in the feeding of skipjack, and perhaps other species of tuna as well, in their natural environment. They further indicate that the pond fish had learned to associate the smell of the juices of cut-up food material with the act of feeding and thus exhibited a conditioned response to the extracts, as already discussed in a previous section.

SEA TESTS WITH INEDIBLE LURES

Attention was next directed at a study of the response of tuna at sea to objects which might be visually attractive and which could perhaps be used as chum either alone, with extracts, or with live bait.

Materials

The following materials were prepared and tested (others were prepared, but there was either no opportunity for testing them or it was obvious that no further information would be obtained by testing them):

(1) aluminum foil: 3/8-inch squares; used dry (floating) or wet with water and Tergitol (slowly sinking).

(2) tin strips: approximately 1/4 x 2 inches; cut from tin cans (one test) or tin plate (several tests).

(3) tin trangles: approximately 2 inches across, with concave sides (cut from tin plate from which can tops had been stamped out).

(4) mica flakes: "artificial snow".

(5) calcium carbide pellets: approximately 1/4- to 1/2-inch pieces of irregular shape.

Three "drag lure arrays" were prepared and used: (1) an 8-foot iron bar to which were attached small strips of tin with wire leaders; (2) a 10-foot iron bar, V-shaped, to which were attached tin strips, highly polished sport-fishing spoons with hooks, or rubber fish with or without hooks; (3) two 8-foot 1/2-inch pipes connected at one end to a "T" and bent into a V-shape, to which were attached leaders and lures of various kinds. Extracts could be supplied to this last array through a 1/2-inch garden hose (75 feet long) connected to the "T". The extract was dispersed through holes drilled in the arms of the V-shaped pipe.

Methods of Testing

In all tests with inedible objects, the fish were first chummed to the stern with live bait, chumming was stopped, and the materials were thrown to the feeding fish and the response noted. In later experiments a quantitative measure of response was employed: (a) the fish were chummed to the stern, a few were caught by the fishermen, and chumming was stopped although the fishermen continued fishing--the time (in seconds) from the cessation of chumming to the disappearance of the fish was recorded, as well as the number of fish caught during this period; (b) the school was then re-chummed to the stern with live bait, a few were caught, chumming with live bait was stopped and chumming with the objects was started--again, the time to the disappearance of the fish and the number of fish caught during this period were recorded.

In several experiments extracts were introduced at the same time as the inedible lures were presented to the fish.

The drag lure arrays were towed behind the ship at various distances (up to about 75 feet) both in chasing a school without using chum and also after having chummed a school to the stern. In some experiments extracts were released either from the stern or side of the ship, or in the case of the third drag lure, through the garden hose by siphoning action.

Results

Some 40 experiments were conducted with inedible objects and drag lures, including those with and without the use of extract. Typical results for some of those in which the response was measured are included in table 5.

There was no noticeable response to the strips of red cellophane or the mica flakes. The squares of aluminum foil produced a momentary attraction to the chummed fish, but would not hold them.

The tin strips and triangles, which gyrated and flashed in the water as they sank, were temporarily attractive to the fish. When a school was chummed to the stern with live bait, individual tuna could be momentarily lured from the stern to amidships by chumming with strips or triangles of tin from the latter location. This was repeated on several occasions. However, having investigated the flashing tin, the skipjack would return to the stern to feed on live bait. Of 57 skipjack caught from one school during such experiments, 2 had one strip of tin apiece in the stomach. In some tests, when chumming with live bait was replaced by chumming with tin strips from the stern, the fish remained with the ship for a slightly longer period than when no chum was used, but they followed the strips of tin as they sank and therefore kept beyond the reach of the poles.

The calcium carbide pellets gave off bubbles of acetylene gas when thrown into the water, along with a milky precipitate which streamed behind the pellets as they sank. This visual stimulus seemed to attract the fish and hold them for a short period of time. However, no pellets were found in the stomachs of fish which were caught, and no dead or distressed fish were noted in the fishing area after chumming with this active chemical.

The addition of extracts when chumming with visual lures did not seem to cause any added excitement or attraction.

The lure array, either without or with the addition of extract, did not seem to attract the schools when towed near them. However, it was difficult to keep up with the schools when the array was being towed; usually the schools managed to keep ahead of the ship. As shown by data included in table 5, towing the lure array close to the stern after the fish had been chummed with live bait did not seem to hold them for any additional period of time beyond that of the controls.

Discussion

Obviously little success was achieved in attracting and holding schools of tuna with inedible lures of the types which were used. The observation that the tuna will respond to some extent to shiny objects such as strips of tin, silvery objects such as squares of aluminum foil, and effervescing objects such as wet calcium carbide pellets is of interest and possible value in devising other artificial lures. The results suggest that the sense of vision is of major importance in the feeding of tuna in their natural environment.

SEA TESTS WITH EDIBLE LURES

Materials

The preparation of edible lures, consisting basically of gelatin capsules, macaroni, and agar, has been described in a previous section. There was no opportunity of testing the gelatin capsules at sea. Several tests were performed with the non-motile, edible, macaroni and agar preparations. In addition experiments were performed with dead anchovy, cut-up preserved herring, and strips of skipjack flesh, both without and with the addition of extract.

Methods of Testing

In one series of experiments with macaroni and agar preparations the method of testing was similar to that finally adopted with inedible lures, namely, to establish a control time (to disappearance of fish) and count (of fish caught) after chumming with live bait ceased, and to determine a test time and count while chumming the previously live bait-chummed fish with the edible lure. In a second series of experiments direct chumming of the schools with edible lures was attempted. When possible, this was followed by chumming with live bait to see if the school would respond.

With the other experiments (dead anchovy, herring, etc.) the materials were used as chum in some cases after the tuna had been chummed to the stern with live bait and in others without chumming with live bait.

Results

The results of the first series of experiments with macaroni and agar preparations are included in table 5. On comparing the timing and catch under control and test conditions, there is no indication that the edible lures were attractive to the fish.

The second series of experiments, involving direct chumming with the edible lures, was undertaken following a suggestion that the skipjack might be temporarily conditioned to feeding on live bait and, for that reason, might not feed on the edible lures when they were subsequently presented.

Attempts were made to chum ten different schools with edible lures during October and November 1953 with negative or inconclusive results. While attempting to chum three skipjack schools and one dolphin school with macaroni and agar preparations, fish were caught on the trolling jigs soon after chumming began, but there was no assurance that this was related to the chumming. In all other cases except one, the results were negative. For the most part, the schools were "wild". Of nine tuna schools, one was chummed to the stern of the vessel with difficulty using live bait, two failed to respond to live bait, two disappeared before live bait could be used, and for the remaining four, no live bait was available.

The one exception might be described in more detail. A large school of 17- to 25-pound skipjack was chummed in five different passes with one or a combination of the following edible lures: macaroni cooked in skipjack extract and coated with aluminum, macaroni cooked in skipjack extract but uncoated, macaroni cooked in water and coated with aluminum, agar cooked in skipjack extract and impregnated with aluminum, macaroni cooked in anchovy extract and coated with aluminum, and cubes (3/4-inch) of skipjack flesh. On each pass the skipjack were attracted to the stern and, in all, 67 of them were caught with pole and jig. Live bait was not used; none was available. However, it was suspected that the school of skipjack was feeding on opelu (Decapterus) and that each time the vessel approached the school the opelu sought refuge under it, thus attracting the skipjack to within reach of the poles. Opelu were spat up by some of the fish which were caught, and were also found in the stomachs. Moreover, some of the fishermen reported having seen the opelu in the water. None of the stomachs which were examined contained edible lures.

In earlier experiments, after skipjack had been chummed to the stern with anchovy, chumming was stopped, and dead anchovy were thrown to the feeding fish. They exhibited continued feeding activity for a time, but followed the dead fish down as they sank. Attempts to chum skipjack schools with strips of skipjack flesh failed. The strips were cut to include the silvery skin on one side, and were about the same size as the anchovy used as chum.

Table 5.--Time (in seconds) and catch (in numbers of fish) during control and test conditions when testing inedible lures, lure arrays, and edible lures (for explanation see text)

Experiment No.	Lure	Control		Test	
		Time	Catch	Time	Catch
S52	Mica flakes	30	2	30	0
S53	Mica flakes plus extract	18	0	15	0
S54	Tin strips	43	4	75	0
S56	Tin strips	-	-	190	0
S49	Calcium carbide	20	0	60	0
S50	Calcium carbide plus extract	25	0	115	2
S57	Lure array (3) with spoons	20	0	20	0
S58	Lure array (3) with spoons and extract	25	1	30	0
		-	-	35	0
S59	Lure array (3) with rubber fish	45	2	36	0
S60	Macaroni plus extract plus aluminum, plugged with cotton	100	14	110	7
S61	Macaroni plus extract plus aluminum	45	5	40	1
		-	-	43	4
S62	Agar strips plus extract plus aluminum	30	1	30	0
		-	-	45	1

Discussion

Failure to attract and hold the schools of tuna with non-motile edible lures perhaps was to be expected in view of the fact that present fishing methods (for skipjack) depend on the use of live rather than dead bait. However, in view of the success in pond tests, it was hoped that the fish at sea would respond, to some extent at least, to the extract-impregnated lures. This hope was not realized. Possibly some response of interest might be noted if the experiments with direct chumming (without the preliminary use of live bait) were repeated under more favorable circumstances, e.g., during the regular summer fishing season when skipjack schools are plentiful and when they respond well to live bait.

There is the possibility that tuna in other parts of the world, particularly those species which will take dead bait on longline gear, might respond to the macaroni or agar preparations. Dr. W. M. Chapman, Director of Research of the American Tunaboat Association, has informed us (verbally) that in the California fishery yellowfin have responded to plain cooked macaroni on occasion. With fish such as these, a positive and consistent response might be obtained if the extract-impregnated, silver-colored macaroni or agar preparations were used.

SUMMARY AND APPRAISAL OF RESULTS

The main object of this investigation has been to study the response of tuna to chemical and visual stimuli in the hope that the information so gained can be utilized in developing a substitute for live bait.

In the past 2 years numerous experiments have been conducted on the response of captive little tunny to chemical stimuli. A screening of over 100 known chemical compounds ranging from simple inorganic to complex organic materials and including several reputed fish attractants has been undertaken. Apart from an apparent sensing of some of the strong-smelling aromatics and an avoidance of some of the highly-colored dyes, the results have been negative. It seems unlikely that further screening of chemicals will produce any worthwhile information.

It has been found that the captive tunny respond with a vigorous feeding reaction to extracts of "food" substances such as skipjack and other fish (flesh, viscera, blood), squid, and shrimp. The response to these several substances was obtained regardless of which one was being fed to the fish as a subsistence diet. However, there was an indication of a slightly better response to a particular extract when the substance from which it was prepared was being used as food. This observation, together with the fact that the fish were being fed on dead, cut-up materials, suggested that the captive fish may have learned to associate the smell of the juices exuding from the cut-up food with the act of feeding and that the response to extracts was entirely conditioned by the nature of the food. This would presume that the so-called "attractant" in the extracts is a substance (or substances) common to the body juices of fish, squid, shrimp, and other forms. As the captive tunny will not forage for live bait occurring in the pond, but rather must be hand-fed, it is unlikely that further information on the question of conditioning can be obtained from pond experiments.

It was hoped that even if there was a partial conditioning of the pond fish, tuna in their natural environment might be naturally "conditioned" to associate the smell of living injured or uninjured prey with the act of feeding, and that they would therefore respond to the extracts. This hope was not realized. A sufficient number of sea tests on skipjack schools were conducted to warrant the conclusion that the fish showed no appreciable response to extracts of skipjack flesh and viscera, extracts of yellowfin flesh, and extracts of live bait. Unfortunately no tests were conducted on "pure" schools of little tunny (the species tested in the pond) or yellowfin (which are known not only to eat dead bait on longlines but to respond occasionally to chumming with cut bait). Also, no sea tests were conducted with extracts of squid and shrimp, both of which might be considered a more natural food than skipjack or yellowfin flesh. Nevertheless the negative results obtained from such sea tests as have been conducted indicate that the sense of smell plays little part in the feeding of tuna in their natural environment.

A study was made of the response of tuna at sea to a variety of inedible lures which were used as chum, both with and without extract, after the fish had been initially chummed to the stern of the ship with live bait. There was a momentary response to shiny objects such as strips of tin, to silvery objects such as squares of aluminum foil, and to effervescing objects such as calcium carbide pellets. Similarly there was a momentary response to dead baitfish. The addition of extract had no apparent effect. These results indicate that the sense of vision plays a much greater role in feeding than the sense of smell. However, neither these visual lures nor a "drag-lure array" (designed to simulate a school of live bait) towed behind the vessel was successful in holding the fish at the stern.

The captive tunny in the pond were used to test a variety of edible lures in which the qualities of visual attractiveness and palatability were combined. Gelatin capsules, pieces of macaroni, and strips of agar treated with concentrated extract were eaten more frequently than when they were not treated with extract. In some of the edible lures, visual attractiveness was increased by coating or impregnating with aluminum powder. In a series of experiments in which the captive tuna were presented alternately with macaroni pieces and agar strips, both cooked in concentrated extract and made silvery with aluminum, the fish showed a preference for the agar. The preference may have been associated with the texture of the material. It became more pronounced with successive testing, suggesting that a learning process was involved.

Sea tests with these edible lures gave negative or inconclusive results. Unfortunately the tests were made during the autumn, when skipjack schools were both scarce and erratic in their behavior. Several of the schools which failed to respond to the edible lures also failed to respond to live bait. The experiments with the more promising substances (particularly with agar strips impregnated with concentrated extract and aluminum powder) should be repeated during the regular summer skipjack season. If they again fail to attract our local skipjack schools, which are notoriously difficult to chum with live bait, it does not necessarily follow that they will also fail to attract skipjack, yellowfin, or other tuna species elsewhere.

In view of the lack of success in attracting local skipjack schools to the stern of a vessel and holding them there with the many materials and combinations of materials which have been used, we may ask what quality of attraction is missing. The missing quality is probably motion--the rapid motion of a living fish or squid, or the rapid motion of a lure trolled through the water. It may be that motion is a prerequisite to attraction by artificial lures. Other qualities of likely importance are size, shape, appearance, texture, and taste of the lures.

Imparting motion to a small object (say, 2 to 4 inches in length and 1/2 to 1 inch in diameter) is a difficult although not impossible task. One method is to use the energy of a chemical reaction and the principle of jet propulsion. Once a satisfactory "motor" has been devised, other problems concerning buoyancy, shape, speed, nature and direction of movement, and fabrication of a self-propelled lure may be tackled. In fabrication the problem of cost looms large, but may be dismissed pending the development of successful experimental models. The problem of making the lure attractive in appearance, texture, and taste does not seem to offer such formidable difficulties as that of imparting motion.

An investigation of methods of imparting motion to small objects is presently underway. If a promising motile lure can be devised, it will be tested on schools of skipjack at sea.

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APPENDIX

A summary of experiments conducted in tank (T) and pond (P) by Tester (ALT) or Takata (MT), with reaction classified as positive (X to XXX), no response (0), or negative (-), over the period July to September 1953. All experiments, except one, conducted on little tunny.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
357	7/1	Skipjack viscera, 250 ml. (50 lb. extracted with water, heated, filtered, and decanted --HTP--6/20/53).	P	ALT/MT	0	No indication of attraction--yellowfin only timed and counted.
358	7/1	Skipjack viscera, 500 ml. (Same as #357).	P	ALT/MT	0	Possibly a slight attraction, but not certain.
359	7/1	Skipjack flesh, 1,000 ml. (250 g. extracted with 2,250 ml. water--whole extract--7/1/53).	P	ALT/MT	XX	Speeding and feeding; jacks also reacted.
360	7/1	Skipjack flesh, 1,250 ml. (Same as #359).	P	ALT/MT	XXX	Very good reaction.
361	7/2	Skipjack flesh, 500 ml. stock extract. (equivalent to about 50 g. flesh).	P	ALT/MT	X	Mostly speeding.
362	7/2	Skipjack viscera, 500 ml. (Same as #357).	P	ALT/MT	X	Slight speeding and slight feeding.
363	7/2	Skipjack viscera, 500 ml. (Same as #357).	P	ALT/MT	0	No noticeable reaction.
364	7/2	Skipjack flesh, 1,000 ml. stock extract. (equivalent to about 100 g. flesh).	P	ALT/MT	X	Slight speeding and turning back; yellowfin also reacted slightly.
365	7/2	Anisic aldehyde C. P., 8 ml.	P	ALT/MT	0	Slight speeding in 1st and 2nd periods, but whether or not it was due to material is uncertain.
366	7/6	Skipjack flesh (100 g. extracted with 3 liters water for 3 hours--whole extract).	P	ALT/MT	X	Mostly speeding with little feeding.
367	7/6	Skipjack flesh, 1 liter stock extract.	P	ALT/MT	0	Possibly a slight reaction in 1st period, but not certain--pump turned off except for introducing material.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
368	7/6	Skipjack viscera (100 g. extracted with 3 liters water--whole extract--7/2/53)	P	ALT/MT	0	Possibly sensing in 1st period, but no noticeable reaction.
369	7/7	Skipjack flesh (265 g. extracted with 4 liters water overnight--filtrate).	P	ALT/MT	X	Slight reaction with speeding; jacks also reacted slightly.
370	7/7	Squid (180 g. extracted with 3 liters water overnight--filtrate).	P	ALT/MT	XX	Speeding and feeding by tunny and yellowfin.
371	7/7	Citral, 10 ml.	P	ALT/MT	0	No indication of sensing.
372	7/7	Amyl cinnamate, 10 ml.	P	ALT/MT	0	
373	7/8	Hammerhead shark flesh (130 g. extracted with 1,500 ml. water overnight--filtrate).	P	MT	0	Jacks reacted slightly in 2nd period.
374	7/8	Shrimp, 1 liter (250 g. extracted with 2 liters water overnight--filtrate).	P	MT	XX	Definite reaction with feeding.
375	7/8	Shrimp, Same as #374	P	MT	XX	Good feeding reaction; yellowfin and jacks also reacted.
376	7/8	Squid, 1,500 ml. (135 g. extracted with 2 liters water overnight--filtrate).	P	MT	X	Mostly speeding; yellowfin reacted with tunny and independently; jacks also reacted slightly.
377	7/9	Dolphin ovary (177 g. extracted with 2,800 ml. water overnight--whole extract).	P	MT	X	Slight reaction with speeding.
378	7/10	Skipjack flesh, 500 ml. stock extract.	T	ALT/MT	XXX	Good reaction with speeding, turning, and feeding.
379	7/10	Skipjack flesh, 1 liter stock extract.	P	ALT/MT	X	Speeding with some feeding.
380	7/10	Squid, 500 ml. (135 g. extracted with 2 liters water--filtrate--7/7/53).	T	ALT/MT	XXX	Violent reaction, much more so than #378; there was much splashing and feeding.
381	7/10	Squid (100 g. extracted with 1,500 ml. water--filtrate--7/7/53).	P	ALT/MT	X	Much speeding, little feeding, and little turning.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
382	7/10	Shrimp (50 g. extracted with 500 ml. water--centrifuged immediately--centrifugate cloudy).	T	ALT/MT	X	Sensing and speeding
383	7/13	Skipjack blood (130 ml. diluted to 1,000 ml. with salt water--7/10/53).	P	ALT	X	Mostly sensing and speeding but with some returning and swirling; yellowfin reacted with tunny.
384	7/14	Anchovy, 1,000 ml. (120 g. extracted with 1,500 ml. water overnight--whole extract).	P	ALT	X	Very slight but positive reaction; yellowfin and jacks also reacted slightly.
385	7/14	Anchovy, 500 ml. (Same as #384).	T	ALT	X	Slight reaction only at beginning.
386	7/14	Shrimp (50 g. extracted with 900 ml. water filtrate).	T	ALT	X	Slight excitement with feeding reaction.
387	7/14	Plankton (35 g. extracted with 600 ml. water--plankton was collected outside Kaneohe Bay).	P	ALT	X	Sensing with some swirling and turning back.
388	7/14	Squid (67 g. extracted with 1,000 ml. water overnight--whole extract).	P	ALT	XXX	Great excitement, feeding and turning back.
389	7/14	Skipjack flesh, 500 ml. stock extract 7/9/53.	T	ALT	XX	Good reaction with swirling and feeding.
390	7/15	Methionine, 0.5 g.	P	ALT	O	Fish remained deep.
391	7/15	Skipjack flesh, 1,000 ml. stock extract--7/9/53.	P	ALT	XX	Speeding, surfacing; some returning, but no feeding; yellowfin reacted with tunny and also independently.
392	7/15	Hammerhead shark flesh (90 g. extracted with 500 ml. water --whole extract--7/13/53).	T	ALT	O	Slight speeding when they sensed the material but no reaction thereafter; no timing.
393	7/16	Hammerhead shark flesh (150 g. extracted with water--filtrate--7/15/53).	P	ALT	XX	Tunny, yellowfin, and jacks reacted in first 3 periods. This was a good reaction compared with former results obtained with this material.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
394	7/16	Skipjack flesh, 1,000 ml. stock extract--7/9/53.	P	ALT	X	Tunny and yellowfin reacted slightly.
395	7/16	Eucalyptol--U.S.P., 6 ml.	P	ALT	0	
396	7/16	Anchovy (100 g. extracted with 1,000 ml. water--whole extract--7/13/53).	P	ALT	0	Practically no reaction; could the eucalyptol tested just prior to this test be responsible?
397	7/16	Squid (100 g. extracted with 1,000 ml. water--whole extract--7/13/53).	P	ALT	X	Weak reaction by tunny and yellowfin
398	7/21	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	X	Tunny and yellowfin acted together and independently; fish were still excited and milling after the test.
399	7/21	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	XX	Better reaction than #398; yellowfin reacted in 9th period.
400	7/21	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	X	Very slight reaction by tunny and jacks at first, but it didn't last.
401	7/21	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	XXX	Excellent reaction by tunny, yellowfin, and jacks in first 5 periods.
402	7/22	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract--material smelt bad).	P	ALT	XX	Tunny reacted immediately and yellowfin later; no feeding observed but turning, swirling and speeding for both.
403	7/22	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract)--smelly?	P	ALT	X	Sensing by tunny in 1st period; slight excitement by yellowfin which didn't last.
404	7/22	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract)--smelly.	P	ALT	X	Definite speeding with some attraction for both tunny and yellowfin.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
405	7/22	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	XXX	Strong reaction with great excitement and swirling; yellowfin reacted slowly.
406	7/23	Blend of squid, skipjack and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	XX	Very good initial reaction by both tunny and yellowfin.
407	7/23	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	X	Slight reaction by tunny and yellowfin
408	7/23	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	XXX	Excellent reaction with swirling, returning and feeding; yellowfin and jacks reacted with tunny in 7th period.
409	7/23	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT	0	Practically no excitement--a slight swerving only at first.
410	7/27	Isoeugenol, 10 ml.	P	MT	0	
411	7/27	Magna spice concentrated bologna flavor, 10 ml.	P	MT	0	
412	7/27	Blend of squid, skipjack and shrimp (1,000 ml. stock solution--100 g. equiv. with 2% sodium bisulphite--kept at room temperature).	P	MT	XX	Speeding with some feeding by both tunny and yellowfin.
413	7/27	Imitation butter flavor, alcohol soluble, 10 ml.	P	MT	0	Slight repellent effect in 1st period? Slight feeding by tunny and yellowfin on what seemed to be oil in material which floated on surface.
414	7/28	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT/MT	X	Slight reaction with circling and returning.
415	7/28	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT/MT	XX	Definite reaction in 1st and 2nd periods by tunny and yellowfin with slight speeding.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
416	7/28	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XX	Both tunny and yellowfin reacted in first 3 periods, but no noticeable reaction thereafter.
417	7/28	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract--added 2% sodium bisulphite).	P	MT	X	Erratic behavior.
418	7/29	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slight reaction with turning and speeding; yellowfin sensed material but did not react; jacks reacted in 1st period.
419	7/29	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XX	Good reaction with turning, swirling, dashing, surfacing and feeding; one yellowfin reacted slightly.
420	7/29	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Weak but definite reaction with slight feeding in 1st period only.
421	7/29	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slow reaction--no noticeable speeding; good reaction with feeding by yellowfin.
422	7/30	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	O	Slight sensing and slight speeding in 2nd period only.
423	7/30	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Weak reaction with some feeding, turning, and speeding; yellowfin sensed material but did not react.
424	7/30	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slight reaction with some turning and speeding 1st period only.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
425	7/30	Blend of squid, skipjack and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XX	Good reaction with turning, surfacing and feeding; yellowfin and jacks sensed material but did not react.
426	7/31	D L Asparagine, 2 g.	P	MT	O	
427	7/31	Anethol, 5 ml.	P	MT	O	
428	7/31	L Arginine monohydrochloride, 2 g.	P	MT	O	
429	7/31	D L Serine, 3 g.	P	MT	O	
430	7/31	D L Leucine, 2 g.	P	MT	O	
431	8/3	Alpha keto glutaric acid, 3 g.	P	MT	O	
432	8/3	D L Histidine monohydrochloride, 2 g.	P	MT	O	
433	8/3	L Hydroxyproline, 1 g.	P	MT	O	
434	8/3	L Tryptophane, 1 g.	P	MT	O	
435	8/3	Guanine, 2 g.	P	MT	O	
436	8/4	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Weak reaction with some returning but no feeding.
437	8/4	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XX	Good reaction with returning, surfacing and feeding in 1st period only; sensing but no reaction by jacks.
438	8/4	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XX	Reaction in 1st period only, but speeding through 5th period; slight speeding by yellowfin.
439	8/4	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract)--smelt bad.	P	MT	X	Very weak reaction in 1st period only.
440	8/5	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XXX	Very good reaction with turning, surfacing, and feeding initially but did not last.

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
441	8/5	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	XX	Good reaction in 1st period only.
442	8/5	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slight reaction with some feeding by 2 tunny early in 1st period.
443	8/5	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slight reaction in 1st period only; fish confined most of their activities to shallow water.
444	8/6	Squid (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT/MT	X	Speeding and returning but no feeding.
445	8/6	Skipjack flesh (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT/MT	X	Slight reaction in 2nd period and slight feeding in 3rd period.
446	8/6	Shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT/MT	0	Slight sensing in 1st period only.
447	8/6	Blend of squid, skipjack, and shrimp (100 g. extracted with 2,000 ml. water overnight--whole extract).	P	ALT/MT	0	No noticeable reaction at any time--merely a slight speeding.
448	8/7	Histidine (free base), 2 g.	P	MT	0	Fish swimming deep.
449	8/7	Sodium bisulphite, 2 g.	P	MT	0	
450	8/7	D L Alpha alanine, 2 g.	P	MT	0	
451	8/7	L Lysine monohydrochloride, 2 g.	P	MT	0	
452	8/7	D L Glutamic acid monohydrate, 1 g.	P	MT	0	
453	8/7	L Aspartic acid, 2 g.	P	MT	0	Most of the tunny did not come within the influence of the material during the early periods.
454	8/10	Glycyl-glycine, 1 g.	P	MT	0	
455	8/10	Alpha amino n butyric acid, 2 g.	P	MT	0	
456	8/10	D L Norvaline, 1 g.	P	MT	0	
457	8/10	Xanthophyll oil, 10 ml.	P	MT	0	

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac- tion	Remarks
458	8/10	D L Trypotophane, 1 g.	P	MT	0	
459	8/10	Chlorophyll, 2 g.	P	MT	-	Repellent effect, especially during first 2 periods.
460	8/11	D L Thronine (with allothre-onine), 2 g.	P	MT	0	
461	8/11	D L Threonine, 1 g.	P	MT	0	
462	8/11	Pyridoxine hydrochloride, 1 g.	P	MT	0	
463	8/11	D L Aspartic acid, 2 g.	P	MT	0	
464	8/11	Guanine hydrochloride, 1 g.	P	MT	0	
465	8/11	Uramil, 2 g.	P	MT	0	
466	8/12	D L Lysine monohydrochloride, 2 g.	P	MT	0	Tunny remained at south end of pond for the first 5 periods.
467	8/12	L Proline, 1 g.	P	MT	0	
468	8/12	D L Ornithine monohydrochlo-ride, 1 g.	P	MT	0	
469	8/14	Skipjack flesh (100 g. pale flesh extracted with 2,000 ml. water overnight--whole extract)	P	MT	0	No noticeable res-ponse.
470	8/14	Skipjack flesh (100 g. dark flesh extracted with 2,000 ml. water overnight--whole extract)	P	MT	XX	Good reaction with speeding.
471	8/17	Skipjack flesh (100 g. extrac-ted with 2,000 ml. water--whole extract--8/14/53).	P	MT	X	Very slight reac-tion.
472	8/18	Skipjack flesh (100 g. dark flesh extracted with 2,000 ml. water overnight--whole extract)	P	MT	X	Mostly speeding which lasted through the 5th period; violent reaction and feed-ing by <u>Abudefduf</u>.
473	8/18	Skipjack flesh (100 g. pale flesh extracted with 2,000 ml. water overnight--whole extract)	P	MT	XX	Good reaction with much speeding and little feeding.
474	8/18	Betaine, 5 g.	P	MT	0	

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
475	8/18	Skipjack flesh, 100 g. equiv. (600 g. extracted with 2,100 ml. 95% ethyl alcohol and centrifuged; residue extracted again with 1,000 ml. alcohol; centrifugate evaporated to a viscous mass by heating; and concentrate redissolved in water).	P	MT	X	Some turning back and much speed-ing--speeding lasted throughout experiment.
476	8/18	Skipjack flesh, 200 g. equiv. (same as #475).	P	MT	O	Very slight speed-ing up.
477	8/19	Skipjack flesh, 100 g. equiv. (same as #475)	P	MT	O	Slight sensing in 1st period only.
478	8/19	Skipjack flesh (100 g. pale flesh extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slow reaction--no speeding.
479	8/19	Skipjack flesh, 200 g. equiv. (same as #475)	P	MT	O	No noticeable re-action.
480	8/19	Skipjack flesh (100 g. dark flesh extracted with 2,000 ml. water overnight--whole extract)	P	MT	XX	Good reaction with some feeding.
481	8/20	Skipjack flesh (residue from exp. #475 extracted with water --centrifugate).	P	MT	O	
482	8/21	Skipjack flesh (100 g. dark flesh extracted with 2,000 ml. water overnight--whole extract).	P	MT	X	Slight reaction with some turning and speeding.
483	8/21	Skipjack flesh, 200 g. equiv. (400 g. extracted overnight with 95% ethyl alcohol--centrifugate).	P	MT	O	Possibly slight repellent effect.
484	8/21	Skipjack flesh (100 g. pale flesh extracted with 2,000 ml. water overnight--whole extract)	P	MT	X	Some turning and feeding; this re-action was slight-ly stronger than that obtained with the dark flesh tested previously (#482).

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
485	8/21	Skipjack flesh, 200 g. equiv. (400 g. extracted overnight with 95% ethyl alcohol, centrifugate evaporated by heating to viscous mass, and concentrate redissolved in water).	P	MT	X	Turning and speeding--speeding lasted throughout experiment.
486	8/21	Skipjack flesh (200 g. extracted overnight with 2,000 ml. water--whole extract).	P	MT	X	Turning and speeding.
487	8/24	Skipjack flesh (200 g. extracted with 400 ml. water--centrifugate--8/21/53).	P	MT	X	Slight reaction, but much speeding.
488	8/24	Skipjack flesh (200 g. extracted with 200 ml. water and 200 ml. 95% ethyl alcohol--centrifugate--8/21/53).	P	MT	0	Slight speeding in 1st period only.
489	8/24	Skipjack flesh (200 g. extracted with 400 ml. 95% ethyl alcohol--centrifugate--8/21/53)	P	MT	0	Slight speeding in first 2 periods but no noticeable reaction.
490	9/1	D L Tyrosine, 2 g.	P	MT	0	
491	9/2	Phenylalanine, 0.5 g.	P	MT	0	
492	9/2	Thiamine hydrochloride, 2 g.	P	MT	0	
493	9/2	Calcium pantothenate (dextrorotatory), 1 g.	P	MT	0	
494	9/3	Uracil, 1 g.	P	MT	0	
495	9/3	Creatinine, 2 g.	P	MT	0	
496	9/4	Cholic acid, 2 g.	P	MT	0	Erratic behavior.
497	9/4	Liver fraction "L", 2 g.	P	MT	0	Material dark brown in color.
498	9/4	Musk ketone, 2 g.	P	MT	0	Sped just after passing through material in 1st period--sensing (?).
499	9/4	Liver extract concentrate 1:20, 2 g.	P	MT	0	Material dark brown in color, but fish did not seem to notice it.
500	9/8	Cadaverine dihydrochloride, 0.5 ml.	P	MT	0	
501	9/8	Testosterone U.S.P., 0.5 g.	P	MT	0	

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
502	9/8	Skipjack flesh, 500 ml. (4,500 g. extracted with 22,500 ml. water in reefer--supernatant--9/2/53).	P	MT	XX	Good reaction in 1st period with turning and speeding--speeding lasted through 8th period.
503	9/8	Musk ambrette, 2 g.	P	MT	0	Reacting to floating leaves; tunny followed leaves as they were blown out of area.
504	9/8	Musk xylol, 2 g.	P	MT	0	
505	9/11	Amyl caproate, 4 ml.	P	MT	0	Fish sped through area on one pass for no apparent reason, but no noticeable reaction to material.
506	9/11	Ionone C - 1, 5 ml.	P	MT	0	
507	9/11	Viosterol (irradiated ergosterol), 10 ml.	P	MT	0	
508	9/11	Alpha methyl ionone, 5 ml.	P	MT	0	
509	9/11	Phenyl ethyl alcohol, 5 ml.	P	MT	0	
510	9/17	Riboflavin--5--phosphate diethanolamine 2 g.	P	MT	-	Definite repellent effect from this dark orange material.
511	9/17	Tonquin musk synth., 1 g.	P	MT	0	
512	9/17	Casein (nutritional Biochemicals Corp.) 5 g.	P	MT	0	
513	9/18	Eucalyptus oil, U.S.P., 70/75, 2 ml.	P	MT	0	
514	9/18	Crystalline Vitamin A (alcohol), 10 mg.	P	MT	0	
515	9/18	"Astrotone" B R, 2 ml.	P	MT	0	
516	9/18	Phenyl ethyl mercaptan, 2 ml.	P	MT	0	
517	9/18	Adenylic acid (muscle), 0.1 g.	P	MT	0	
518	9/18	Hexahydrobenzoic acid, 2 ml.	P	MT	0	
519	9/21	L Glutamic acid hydrochloride, 5 g.	P	MT	0	
520	9/21	Yeast hydrolysate (enzymatic), 5 g.	P	MT	0	

Exp. No.	Date 1953	Substance and preparation	Tank or pond	Observer	Reac-tion	Remarks
521	9/21	Lactalbumin, 5 g.	P	MT	0	
522	9/21	Casein hydrolysate (enzy-matic), 5 g.	P	MT	0	
523	9/21	Blood fibrin, 5 g.	P	MT	0	
524	9/21	Casein (General Biochemicals, Inc.), 5 g.	P	MT	0	
525	9/21	Gluten, 5 g.	P	MT	0	