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METHODS FOR TESTING SMELL RESPONSE IN FISH

By

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Introduction

Behaviour studies in the laboratory on the reactions of cod to smell stimuli from different bait organisms have been carried out since 1973.

The main methods and some of the results are described in Solemdal and Tilseth (1974).

The present paper describe new methods and improvements of those used earlier.

The results are mainly data from a large number of smell preference tests, ranking different marine organisms according to their ability to elicit bite response of the cod.

Different chemical fractions of a bait organism were tested in preferance test, in order to find the active components of the bait.

On the basis of the results referred in this paper, the development of an artificial bait is in progress.

Material and Methods

1) Experimental fish

Seventyfive cod (Gadus morhua) of the Norwegian coastal population, 40-75 cm in length, were collected in November 1973. The fish were divided in three groups of 25 and fed on herring (Clupea harengus), squid (Illex illhosus) and capelin (Mallotus villosus), respectively. Twenty cod were caught at Little Fisher Bank in the North Sea in December 1974. They were brought to the Institute of Marine Research, Bergen by the research vessel "G.O.Sars". The fish were not fed. In August 1974 thirty, 40-50 cm, cod of the Arcto-Norwegian population were caught at two localities west of Spitsbergen by R/V"G.O.Sars" and brought to the institute. The fish were not fed. A fourth group of cod were caught at Vikanes in the vicinity of Bergen. Fifty I group cod were collected in September 1973. The cod were divided into five groups of ten fish each and fed on herring, squid, mackerel (Scomber scombrus) and mussels (Mytilus edulis). Another thirty cod of the same age group were caught at the same locality in September 1975. This group were fed on euphausiids, mainly Meganyctiphanes norwegica.

2) Experimental equipment

a) Feeding tanks

The big cod were fed in concrete tanks of 2 x 4 x 2.5 m. The I group cod were fed in five 1 x 1 x 0.5 m aquria and one 1 x 2x1.5 m PVC tank. The fish were fed each second day.

b) Experimental tanks

The smell preference experiments from bait organisms were done with the big cods in the large circular concrete tank as previously described (Solemdal and Tilseth 1974). Experiments on the smell preferanse of bait and extracts of bait were carried out with small cods in a cylindrical PVC tank 2 m across, 50 cm

deep. The tank is divided into ten compartments by ten PVC dividers 50 x 50 cm. Water is supplied to the tank through the center of the wall in the perifery of each compartment. The rate of flow to each compartment can be regulated by valves. The outlet of water is through a central standing pipe. The flow rate was set at 500 ml/minute through each compartment. A similar design is described by Kleerekoper (1967).

c) Bait bags

The bait was introduced to the fish in bait bags. These were made of double gauze (Tubinette H56) reinforced with fine meshed seine netting. In the large circular tank they were filled with 100 grams of bait cut in small pieces, while in the PVC tank they contained 40 grams. Extracts was made of Meganyctiphanes norwegica and pumped out into the PVC tank through bait bags filled with cotton. The flow rate was 5 ml/minute, and the exsperiment lasted for 30 minutes.

The behaviour of the small cod was recorded continually and divided in three categories:

I The number of fish swimming into the compartments of the bait bags.

II The number of fish touching the bait bag with their snout or barbel.

III The number of fish biting in the bait bags.

The number of preference tests with different bait organisms are given in Table 1. Tests on fish conditioned on specific bait organisms are given separately.

3) Preferance tests on extracts

In order to learn more about the nature of the active chemical compounds of the bait organisms a series of preferanse tests on small cod conditioned to the smell and taste of Meganyctiphanes norwegica (krill) was performed. The numbers of cod in each test varied from 6 to 10.

Table 1. Number of preferanse test on cod with different bait organisms.

Bait	No. of tests	Bait	No. of tests
Illex illhosus	86	Themisto sp.	15
" " x)	193	Scomber scombrus	21
Clupea harrengus	69	" " x)	13
" " x)	114	Arenicola marina	6
Mallotus villosus	111	Mytilus edulis x)	13
" " x)	93	Oxliver	5
Euphausids	230	Calanus finmarchicus	58
Pandalus borealis	35		

x) Preferanse tests with cod fed the specific bait.

a) Preparation of extracts

Extracts was made of 100 grams (wet weight) of krill. The animals were homogenized in 200 ml destilled water. The homogenate was sentrifuged and the supernatant divided in two equal parts. 80 ml was used as control extract, the other 80 ml was fractionated. The control extract was diluted with filtered sea water to 150 ml. The volume of the end product after fractionation was also adjusted to 150 ml with filtered sea water. In the smell preferance tests with small cod the control extract and the fractionated extract were pumped into the PVC tank at the same time in separate compartments.

Six fractions were made and these were tested against the control extract.

Fraction 1. 80 ml of the extract was heated and boiled for 15 minutes. After cooling to room temperature the precipitate was removed by sentrifugation.

Fraction 2. 80 ml of the extract was mixed mith 160 ml of 96% ethanol. The precipitate was discharged after sentrifigation. The ethanol was removed from the supernatant with a rotary evaporator.

Fraction 3. 80 ml of the extract was treated with 10 ml of CH_2Cl_2 two times. The organic phase was separatet from the water soluble phase and the residue of CH_2Cl_2 removed on a rotory evaporator.

Fraction 4. The pH of the 80 ml extract from krill was pH 7.8. 6N HCl was added until precipation, this occured at pH6, the pH was further lowered to pH 4. The precipitate was removed by centrifugation and the pH of the supernatant adjusted to pH 8 by 6N NaOH.

Fraction 5. When adding 6N NaOH dropwise to 80 ml of the extract precipiation occured at pH 10. The precipitate was removed by sentrifugation and the pH of the sumpernatant adjusted to pH 8 by adding 6N HCl.

Fraction 6. After precipitating 80 ml of the extract with 160 ml ethanol the supernatant was treated with 10 ml 0.75N H_2SO_4 followed by 10 ml 10% Na_2W_{04} $2H_2O$. The precipitate was removed by centrifugation.

RESULTS

Smell preferance tests on unfed cod

Fifteen individually tagged cod of the Arcto-Norwegian population did not show any marked preference for the smell of their prey organisms, Calanus finmarchicus and Themisto sp. which turned out to be the only stomach content by the time of capture. The mean results of 18 tests is presented in Fig. 1. Each vertical line represents the mean values of the bite response on the bait bags of each fish. All fish except one showed bite response. The figure also shows that all fish except one gave the highest bite response on the bait bag containing krill.

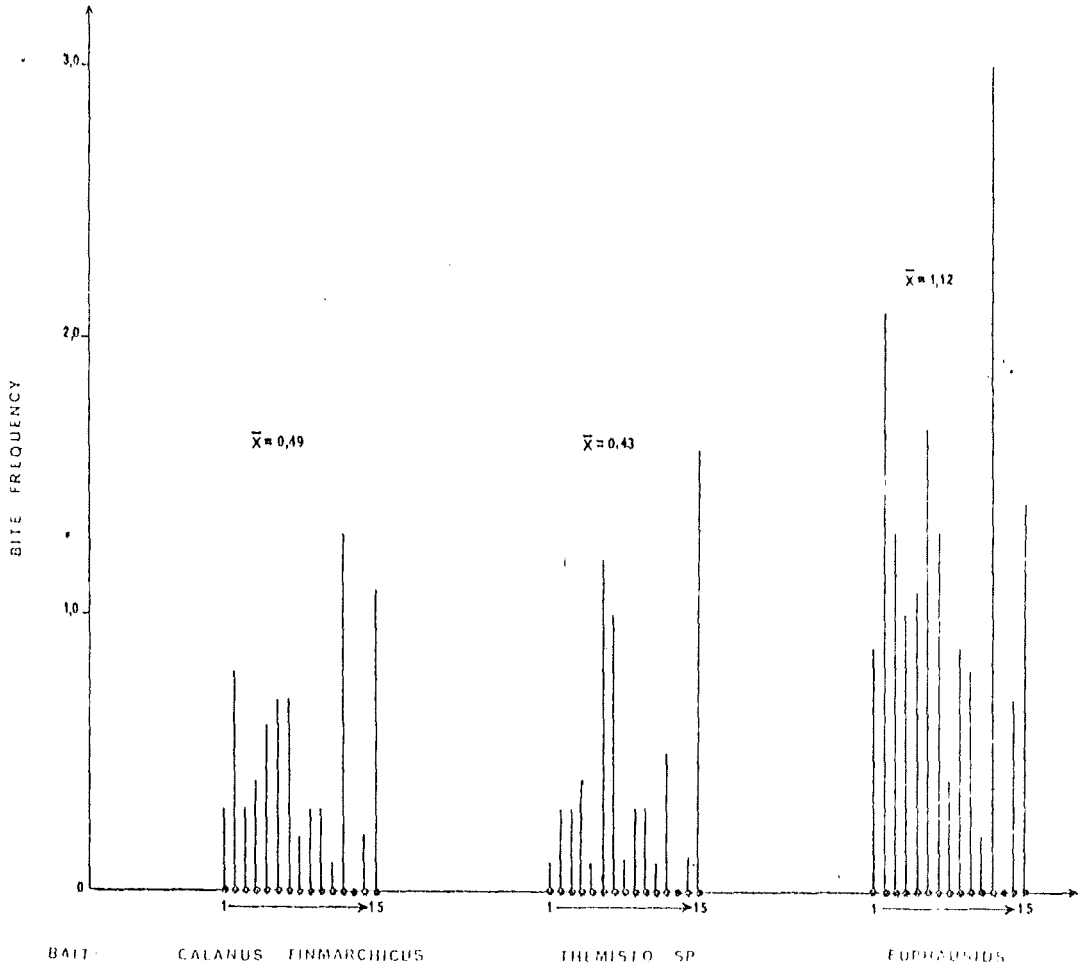

Fig. 1. The response of fifteen individually tagged Arcto-Norwegian cod. Number of bites/tests of each fish on the different bait bags is given as the mean of 18 test.

A similar test was performed with cod captured in the North Sea. Stomach analysis showed that they had eaten benthos organisms and small fishes including mackerel. Ten cods were run in a serie of seven preference tests where they were exposed to the smell of mackerel, common mussel and krill. The results is presented in Table 2 as the mean bite frequencies.

Table 2. The mean bite frequencies of 10 cod in seven smell preferanse tests on bait bags containing krill, common mussel and mackerel.

Bait bags	Krill	Common mussel	Mackerel
Mean bite frequency	0.54	0.51	0.17

Effectiveness of odors from different bait organisms

The results from all the preference tests, shown in Table 1, was treated together, and the result is presented as bite frequencies of cod on the different bait bags. The bite frequencies of fish conditioned on different bait organisms by feeding is treated separately (Fig. 2). The results shows that the odor from bait bags containing the crustaceans Meganyctiphanes norwegica and Pandalus borealis elicted the highest bite frequencies in cod. The influence of conditioning by feeding, on the frequenses of bite is clearly shown. These prey organisms was not used as food for these experimental fish.

Effectiveness of different fractions of extracts of krill

The results from the preference tests on small cods on fractions of extracts of krill against a control extract is presented in Table 3. Only the bite response, which is considered to be the most important behaviour pattern is presented. The table gives the percentage of bites in bags containing fraction and bag containing control extract. The results from these tests shows that the chemical attractants is being reduced when the extract from krill is boiled, extracted with organic solvent, the pH lowered to pH 4 and in a less extent when the pH is increased to pH 10.

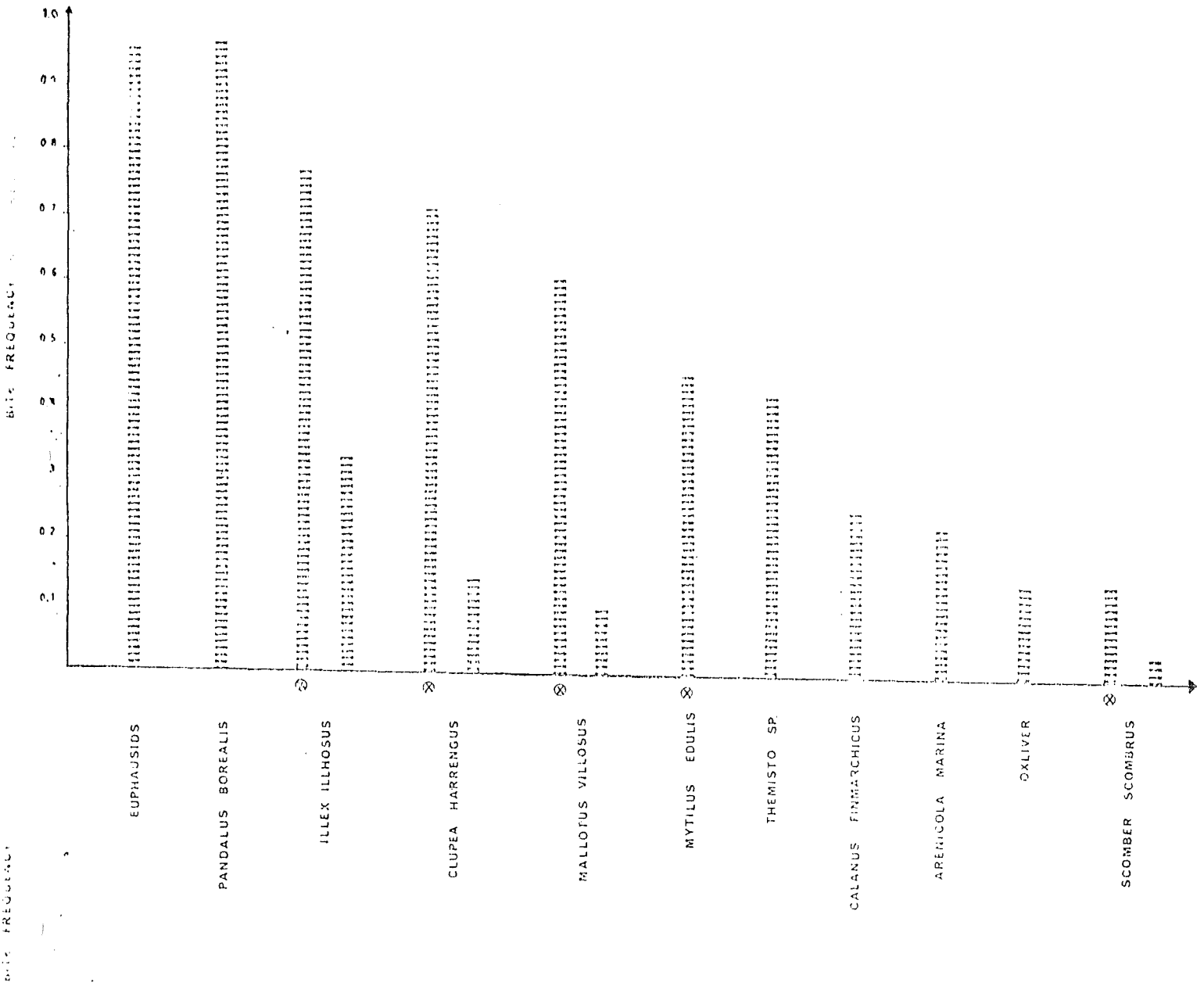

Fig. 2. Bite frequensies of cod on bait bags containing different baits. x) The response of cod conditioned by feeding on the specific bait. The figure represents the mean bite frequensies of totaly 3955 observations.

The pH of extracts of krill is normally pH7.8. The chemical attractants elicting bite response in cod was not altered when the extract was protein precipitated with moderate volumes of ethanol, but the response was significantly redused when the extract was protein precipitated in an acid solution with sodium-tungsten.

Table 3. Bite response of small cod. Percentage of bites in bait bag containing fraction and bag containing control.

	Percentage of bite response		
	Fraction		Control
1	Boiled	28	72
2	Protein precipitated	54	46
3	CH_2Cl_2 extracted	15	85
4	pH4 precipitated	16	84
5	pH 10 precipitated	42	58
6	Super protein free fraction	5	95

DISCUSSION

In presenting the results, we have emphacized the bite response. This behaviour pattern, under laboratory condition is obviously more discriminatory in choice experiments between different odors present in the tank at the same time, than a general food searching behaviour, such as exploratory feeding behaviour described by Steven (1959) and Haynes et al., (1966). Some of our fish have been adapted to laboratory conditions for a long period of time. This could change the natural selectivity for food odors (Tester et al., 1954). The effect of conditioning by feeding is clearly demonstrated in Fig. 2. However, in spite of this the cod prefered the odors of crustacea. The results of the preferance tests on cod directly brought in from the sea not conditioned by feeding showed the very same strong preferanse for odors of krill (Fig. 1 Table 2). One of our working hypotheses was that cod could develop smell and taste preferance for one or more pray organisms (Solemdal and Tilseth 1974). The present paper indicate that cod have probably developed a preferance for the odors of prey organisms of some crustacea, in particular euphausiids and deep water shrimps. This prey organisms is frequently found in the stomach content of cod in Arctic areas (Brotzky 1931, Brown and Cheng 1946).

Cod are easy to handle in the laboratory and as demonstrated in the present paper easily conditioned to food when fed only one food organism. These advantages were utilized when testing the nature of the chemical attractants of extracts of krill responsible for eliciting bite response in cod. The cods were in these tests used as assay organisms to detect removals or alterations of the attractants in extracts of krill. The result of our study so far indicate that the attractants most probably consists of a mixture of compounds. Some compounds seems to be extractable in organic solvent, and the effect of the krill estract was reduced when boiled. This effect was also found by Tester et.al., (1954). Precipitation with ethanol did not alter the effect of the extract, but a following precipitation with sodiumtungsten dramatically reduced the stimulatory effect of the krill extract. Manipulations with the pH of the extract showed that possibly some of the attractant has amphoteric nature. Laboratory studies on eels by Hashimoto et al., (1968) and Konosu et al., (1968) shows that a mixture of amino acids were effective attractants. These papers also shows the influence om multiple components attributing to the effectiveness of natural baits. Our study also shows that amino acids alone could not stand for the total attractive effect of the extract since they were never removed from none of the fractions. These finding are also in agreement to those of Sutterlin (1975) who tested the chemical attraction of marine fishes in their natural habitat.

Summary

1) The present paper demonstrate that cod in a choice situation is most attracted to the smell of the crustaceans Meganyctophanes norwegica and Pandalus borealis compared to other bait organisms. The bite respnce is also best stimulated by the crustaceans.

2) Cod were used as an assay animal to study the nature of chemical attractants.

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