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Alarm Substances

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In accordance with the definition proposed by VON FRISCH, 1941[1], we call substances which communicate the presence of danger, 'alarm substances' (Schreckstoff), provided that they are produced by members of the same species. We define the communication of danger by observing the responses of animals to the substance under investigation. These responses to alarm substance may be either attack, as in ants and bees[2], or more commonly flight. Odour-dependent escape reactions have been described in several animals, and may be caused either by the repellent odour of an enemy, or by a specific alarm substance given off by an individual of the same species when attacked.

Many lower animals flee from the repellent odour of their enemies. Knowledge of these odours may be innate or learned, depending on the species. The mussel *Pecten*[3], the snail *Nassa mutabilis*[4] and limpets of the lower tidal zone[5] show an escape response to starfish and also to extracts prepared from the starfish. Limpets of the upper tidal zone, above the limit of the starfish, do not show this response. *Patella* also reacts with an escape response to the extract of the predaceous snail *Murex*. The freshwater snail *Physa fontinalis* makes defensive movements when it contacts the worm *Glossosiphonia* or even when touched with a glass tube which had been in contact with the worm[6]. *Paroctopus bimaculatus* gets very excited if water is offered to it from a tank in which its main enemy, the marine eel *Gymnothorax mordax* is living[7]. Many fish also recognise their enemies' odour and respond to it in a certain way. Coho and spring salmon (*Oncorhynchus kisutch* and *O. tshawytscha*), swimming upstream, may even turn when they smell odour of mammalian skin[8]. The repellent effect may be due to the *L*-serine of the skin[9]. The European minnow, *Phoxinus laevis* Ag., reacts in a predictable manner to the odour of a pike. The minnow stops swimming and sinks slowly when the pike's odour is perceived. It then remains immobile on the substrate. This behaviour may show the following variations: single fish behave differently from whole schools of fish, blind fish differently from intact fish. Blind, single fish react the most strongly of all to the odour, whereas intact fish in a school respond very little to it[10]. It would appear that the visual perception of members of a school causes a reduction in responsiveness to olfactory stimuli[11]. Certain rattlesnakes react by coiling about in a certain way to the attack of the king snake, which specializes in feeding on other snakes. The rattlesnake will also display this behaviour if it is placed in a terrarium in which a king snake has previously lived, thus indicating that it recognizes the predatory snake's odour. Even rattlesnakes from a region in which the king snake is not found show this reaction, proving that the reaction is innate[12]. Some mammals recognise their enemies by their odour, for instance deer show an escape reaction to the odour of man, the golden hamster shows a defensive posture to the odour of the polecat[13].

Quite different from escape reactions which are elicited by the repellent odour of the enemy are those reactions which are elicited by the release of an alarm substance from an animal attacked by a predator. This alarm substance serves to communicate the presence of danger to members of the same species. For example, an alarm reaction was described by KEMPENDORFF[14] in the South American water snail *Helisoma nigricans* Spix. This snail crawls along the bottom and buries it-

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self in the mud if members of its species are crushed in the immediate vicinity. Earlier, in 1938, VON FRISCH[15] described a fright reaction (Schreckreaktion) in the European minnow, *Phoxinus laevis* Ag., which was studied intensively by himself[1,15,16] and his students SCHUTZ[17] and PFEIFFER[18–23]. EIBL-EIBESFELDT[24] and HRBACEK[25] described a similar reaction, evidently convergent, in tadpoles of the European toad, *Bufo bufo* L., which was investigated by KULZER[26], also a student of VON FRISCH. These fright reactions of fish and tadpoles will be discussed here.

Alarm substance and fright reaction in fish and tadpoles. VON FRISCH[15] discovered the fright reaction in the minnow, *Phoxinus laevis* Ag. When doing field experiments with these fish to investigate whether fish can localize a sound source, he marked one minnow by severing the *nervus sympathicus* near the tail, thus producing a darkening of the skin caudad to the incision. When this fish was introduced into the school, the school rapidly retreated and became very frightened. On another occasion, VON FRISCH released a struggling fish which had become jammed under the rim of a metal feeding tube. As it swam away, the rest of the school became alarmed and fled. In a number of field experiments near the shore of a lake, a small feeding table was placed with a tube through which chopped earthworms could be dropped on to the table. The minnows soon learned of this source of food and within some days were sufficiently conditioned for experiments. In these experiments the fish were first enticed to the feeding tray and 5 min later test substances were introduced through the tube. Effective test substances induced a fright reaction within 30–60 sec. Fish which had assembled at the feeding tray fled a short distance in confusion, then crowded together and retreated. Confidence returned after a variable interval of hours or days.

For accurate observation and quantitative work VON FRISCH[1] kept some experimental minnows in aquaria. These were furnished with a hiding place, a food tube, a food wire, running water, and generally ten minnows per aquarium. The fish had to be conditioned to the experimental tank until they no longer fled when a person approached the tank but remained near the tube in expectation of food. This conditioning time varied from three days to three weeks, but averaged about ten days. The number of fish near the feeding area was noted at intervals of 15 sec for 5 min periods after each feeding. The introduction of the test material always occurred 1 min after the introduction of food. The fright reaction in the aquarium was similar to the reaction observed in the field. The latent period after introduction of an effective test substance varied from 30 sec to 1 min or occasionally even up to 5 min. The alarm substance reached the fish before the fright reaction occurred, since VON FRISCH noted that an increased rate of respiratory movements preceded the fright reaction. The increased respiratory movements caused an increased flow of water through the nose.

VON FRISCH[1] distinguishes seven different intensities in the fright reaction. These are arbitrary stages and any intermediate phase may occur, from the most intense reaction, where all fish are suddenly frightened and hastily flee, to a scarcely visible intimidation. These categories, however, permit useful quantitative evaluation of the data and have also been used in the same or a very similar way by VON FRISCH's students.

+++ Most intense reaction with sudden fright and rapid swimming into the hiding place, followed by an immediate emergence and rapid swimming around the tank, avoiding the feeding place for a long time.

++ Intense reaction as above, but fish do not leave the hiding place. Sometimes, after several minutes, some fish return to the feeding place and timidly snatch bits of food before quickly retreating.

+ Clearly frightened; the school retreats towards or more often into the hiding place but quiets down within 5–10 min and then approaches the feeding area more or less confidently. Sometimes the fish remain longer in the hiding place but without showing extremely timid movements while there.

(+) Only slightly frightened with somewhat confused and excited swimming; often dense crowding together and retreating to the bottom but soon quieting down if nothing further happens; occasionally retreating towards the hiding place.

± Intimidated; less confident and more cautious at the feeding tube; less frequent crowding together and retreating to the bottom; no retreat towards the hiding place.

$\underline{\underline{\pm}}$ Slightly uneasy with some crowding together in the area of the feeding tube, but the reaction soon disappears and the fish never leave the feeding area.

– No reaction.

The above description made for *Phoxinus* applies equally well for many other species of schooling fish, but some behave in a very different way. The tench *Tinca vulgaris* Cuv., and the crucian carp *Carassius carassius* (L.), swim excitedly with their heads against the bottom and with their bodies at an angle of about 60° to the substrate. This behaviour disturbs the mud and debris and conceals these fish in turbid water in their natural habitat. Some bottom fish, *Gobio fluviatilis* (L.) and *Nemacheilus barbatulus* (L.), become motionless and thus may avoid their enemies. This type of reaction is better developed in the adult than in the young fish. Some fish, like *Esomus lineatus* E. Ahl, flee to the water surface where they crowd together and jump out of the water[17]. The hatchet fish, *Carnegiella*

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strigata (Günther) which normally swim close to the water surface will leave the surface and form a dense school in the middle of the tank if frightened by alarm substance.

KULZER[26] studied the fright reaction of the tadpoles, both in the field and in the laboratory. In 23 field experiments a crushed tadpole was brought near to a tadpole school, and a strong fright reaction resulted. The animals closest to the extract became disturbed and swam into deeper water, while the neighbouring animals turned or tried to avoid the place occupied by the crushed tadpole. The orientated, parallel-swimming, schooling pattern was disturbed. The method in the tadpole experiments was analogous to that applied in the minnow experiments. After extract had been poured into the tank, some animals immediately left the feeding area; all animals left within a few minutes. The tadpoles swam towards the bottom or to the corner of the tank opposite the feeding place. The effect of the extract diminished after 20 min and the tadpoles then began to return slowly to the feeding area. The reaction was scored in a similar way to the method employed by VON FRISCH[1] for *Phoxinus*. In addition some experiments were photographed. The reactions were consistent.

The origin of the alarm substance. Since the minnow is extremely sensitive to auditory stimuli[27], the sense of hearing was first suspected by VON FRISCH[1] as a possible receptor in the fright reaction. It was, however, shown that unhurt nervous minnows did not frighten great schools in a lake, and that narcotized and dead fish were a potent source of the fright reaction. Thus auditory cues were ruled out. In other experiments small pieces of minnows or even filtered extract produced the fright reaction, thus proving that no visual stimulus was necessary for inducing the fright reaction. The extract of a minnow proved to be extremely potent in the initiation of a fright reaction. VON FRISCH named the effective chemical *Schreckstoff* (alarm substance, substance d'alarme) and the reaction *Schreckreaktion* (fright reaction, reaction d'effroi). In a series of further tests it was shown that the alarm substance is released only from injured skin[1]. Dead minnows with undamaged skin were ineffective. Equally ineffective were pieces of their stomach, gut, liver, spleen, and muscle. Only injured skin produced strong fright reactions. The age of the minnow and its habitat had no effect on the fright reaction. Skin from different areas of the body was compared and no differences between dorsal skin, containing much melanin and yellow pigment, and the ventral skin, containing much guanin and red pigment, could be found. Superficial injury of the skin showed that the epidermis contains the alarm substance, but did not eliminate the corium as an additional source.

In the tadpole various parts of the body were tested[26]. It was found that strong reactions occurred when either the skin of the dorsal, or the skin of the ventral side or the skin from the tail was presented to the tadpoles. Experiments with viscera were negative. Experiments were also made to determine if the alarm substance was released only when the skin was injured or if it could be secreted actively, as suggested earlier[25]. Individuals were caught with a net and rubbed very lightly while they were held in a petri dish with 3 ml water. After 30 sec the water was tested. Out of 23 tests 17 ended with a strong reaction, 6 with a visible one. Then individuals were caught with a glass without damaging their skin, and the water was tested in five experiments: all ended negatively. In other experiments the tadpoles were stimulated additionally by an electrical shock: no reaction occurred. This proves that the alarm substance is not actively secreted, but is released by any small mechanical injury (Figure 1).

From a careful histological comparison of fish species which produce alarm substance and species that do not, the presence of a certain type of club cell in the epidermis could be associated with the presence of the alarm substance[19]. Teleost epidermis shows two types of secretory cells: the mucus cells (Schleim- or Becherzellen) and the club cells (Kolbenzellen). The former open on to the epidermal surface and pour out their mucus. The latter vary in distribution and appearance

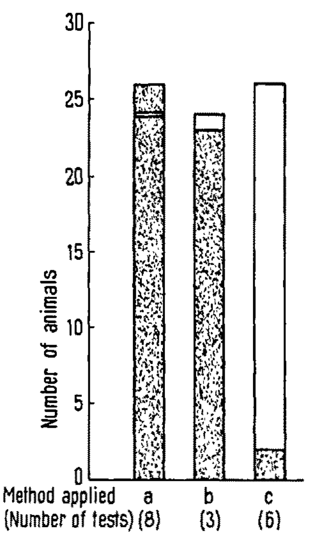

Fig. 1. Experiments demonstrating that the alarm substance in tadpoles is not released actively, but released if the skin is only slightly damaged. Each column represents an average of several experiments. (The number of experiments is indicated under each column.) a, tadpoles caught with a petri dish; b, tadpoles caught with a petri dish and stimulated with electricity; c, tadpoles caught with a net. The height of each column represents the number of animals near the feeding place before the experiment, the blackened part of each column indicates the number of animals at the feeding place 1 min after the experiments. In (a) after adding the solution more individuals were found at the feeding place than during the time prior to the addition of the solution (modified from KULZER[26]).

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in different fish species. In some bottom-living fish, *Anguilla, Carapus, Motella*, club cells have been described which are mucus-producing (Schleimzellen) and are either sloughed off or empty their secretion on to the epidermal surface. In *Phoxinus* and fish which do have an alarm substance, the club cells are never connected with the epidermal surface. These club cells are distinct from mucus-secreting cells in their general morphology and staining reaction. In the mucus cells the nucleus lies peripherally, whereas it lies in the centre of the club cells of those fish which have an alarm substance. Injury to the skin releases the contents of these cells and only in this way does the alarm substance reach the body surface. Strong evidence for connecting club cells with alarm substance was found in comparisons of extracts from samples of the skin which contained different numbers of cells. Histological studies showed that barbel epidermis of the carp and some catfish contains no club cells or only a few very small ones, while the body epidermis of these species is abundantly supplied with these cells. Fright reaction occurred to body skin, but did not occur to barbel skin. It has been suggested that those club cells which are associated with the production of the alarm substance be termed *Schreckstoffzellen* (alarm substance cells)[19]. The characteristics of these cells are shown in Table I and Figure 2.

Analogous studies have been started with tadpoles, but are still in progress.

The effectiveness of the alarm substance. A standard procedure for the preparation of the skin extracts was developed by VON FRISCH in 1938 and applied in all later experiments with fish. A 0.2 g sample of fresh skin, equivalent to the skin of a 7 cm long *Phoxinus*, is cut with scissors 150 times and diluted in 200 ml water. It is shaken at 5 min intervals for 30 min and filtered. From this standard (normal or 'n') extract appropriate dilutions are then made. In any test 100 ml of liquid are poured through the feeding tube during 45 sec. In VON FRISCH's experiment with *Phoxinus* 83% of 101 tests were positive at a dilution of 1:50; 74% of 117 tests at a dilution of 1:100; and 41% of 17 tests at a dilution of 1:500. Aquarium hatched minnows, *Phoxinus laevis* Ag.[17] and *Tribolodon hakonensis taczanowskii* (Steindachner)[19], still respond to a dilution of 1:50000. The extract from 1/100 mm² of skin equal to 0.002 mg skin of a 3 cm minnow is sufficient to repel a school from the feeding place in a 14 l aquarium[17]. The actual concentration reaching the fish is considerably less than that of the extract poured into the tank. So the absolute potency of the extract cannot be obtained in this way, but it serves as a satisfactory method. The standardisation of the skin extract has been based on the degree of dilution which causes the same fright reaction.

Water in which wounded tadpoles lived, produced a fright reaction when tested on schools of tadpoles. KULZER[26] weighed the skin, cut it into fine pieces with scissors, diluted it in 3 ml water, and tested it. He found that 3 ml water which contained only 1.7 mg of dorsal skin caused a very strong fright reaction. Ventral skin seemed to be less effective, and 3.3 mg of ventral skin were necessary to produce the same reaction.

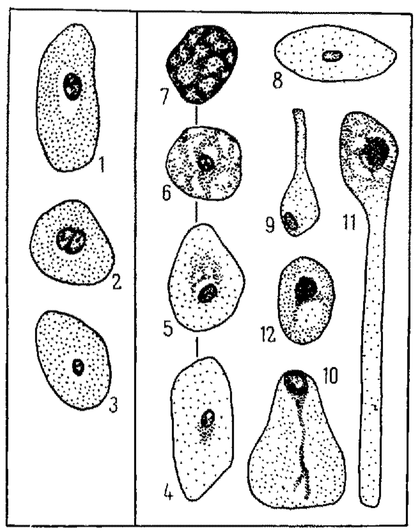

Fig. 2. Diagram of the different kinds of 'club cells'. 1, alarm substance cell of *Phoxinus*; 2, *Amiurus* (= *Ictalurus*); 3, *Kryptopterus*; 4–7, development of mucus cells of *Fierasfer* (= *Carapus*); 8, mucus cell of *Polypterus*; 9, *Motella*; 10, club cell of *Petromyzon*; 11–12, mucus cells of *Anguilla* (from PFEIFFER[18,19]).

Table I. Characteristics of the different types of 'club cells' (c)

	Fish	Phoxinus and other Ostariophysi	Fierasfer	Polypterus	Motella	Anguilla	Petromyzon
Morphology	c contacts inner border of epidermis	—	—	+	—	+	+
	c contacts surface of epidermis	—	—	+	+	+	—
	c is an immature mucus cell	—	+	—	—	—	—
	c is oval or roundish, not club shaped	+	+	+	—	—	—
	nucleus centrally located, not on cell periphery	+	+	+	—	—	—
	outer cell contents homogeneous	+	+	+	+	—	—
	c height is greater than 1/2 height of epidermal layer	·	—	—	—	+	—
	c height is less than 1/4 height of epidermal layer	·	—	+	+	—	—
Function	c is mucus secreting	—	+	+	+	+	?
	c contains alarm substance	+	—	—	—	—	—

Using 0.3 mg or less of dorsal skin (or 1.0 mg or less ventral skin) resulted in no responses.

The chemistry of the alarm substance. VON FRISCH[1] found that skin from dead minnows kept for three days in water at 16° C was as effective as fresh skin, but skin kept for six days was much less effective. Boiling the extract for 1 or 5 min did not affect the potency of the skin extract, while boiling for 10 min reduced its effectiveness to 20%.

HÜTTEL[28] suggested that the alarm substance was a purine (or pterin) -like substance. Purines and pterins are concentrated in xanthophores[29] which are most numerous in the dorsal skin. However, ventral skin was as effective in eliciting the fright reaction as dorsal skin[1]. The claim of PURRMANN[30] that ichthyopterin, isolated from fish skin[31], was identical with the alarm substance, has not been substantiated. SCHUTZ[17] showed that synthetic ichthyopterin[32] was not identical with the alarm substance. Also the ventral skin contained much less than 10% of the ichthyopterin than did the dorsal skin[31]. It was pointed out that the alarm substance of the minnow was not volatile although very soluble in water. So it is an odour for fish, but cannot be smelled by man.

We also know very little concerning the chemistry of the alarm substance from the tadpoles. It was conjectured that the alarm substance was identical with the poisonous secretions of the toad[25]. However, parotic extracts were tested and found to be less effective than was the skin extract[26]. It was shown that the alarm substance can be boiled without losing its effectiveness. KULZER[26] tested a series of substances. Some were not effective while others produced some response. *Bufotoxin* and *γ-bufotoxin* were very effective. Their effectiveness could not be distinguished from that of the skin extracts. 2 mg of the substances were diluted in 2 ml of water. This solution could be very diluted and still retain its effectiveness. Paper chromatograms of skin extracts showed similar distributions for both the alarm substance and the bufotoxin. In addition absorption spectra of both substances were similar, but these results did not prove that both substances were identical.

The detection of the alarm substance and the influence of vision on the reaction. In the minnow[1], as well as in the tadpole[26], the alarm substance is detected only by the olfactory sense, and the gustatory sense is not involved in the reaction. The nervus olfactorius was removed and the animals were tested after recovering. Operated fish and tadpoles never responded to the alarm substance. In sham-operated controls the roof of the skull was opened, folded back, then closed again without injuring the nervus olfactorius. The reaction of these animals was not measurably reduced.

The fright reaction in blind minnows was studied by VON FRISCH[1]. Blind fish did not school, and when exposed to the alarm substance they swam to the bottom of the tank and showed fright movements; however, each fish reacted as an individual, and there was no group response. Visual induction of the fright reaction was also shown in some other experiments. Three minnows with the olfactory organ destroyed were associated in a tank with three unoperated fish and tested with the alarm substance. The unoperated fish promptly showed the expected fright reaction and within a few minutes the operated fish were also alarmed and the entire school showed the effects of the fright reaction for several days. When minnows which were showing a fright reaction were moved to another tank and placed with fish which were not affected by the alarm substance, the behaviour of the latter group depended on the number of frightened fish introduced into their midst. If relatively few fish were introduced the resident fish were not disturbed, but produced a quieting effect on their alarmed associates. If the introduced fish were equal or greater in number, the resident fish became disturbed. SCHUTZ[17] placed two aquaria side by side and arranged the interiors as mirror images of each other. The same number of fish was placed in each tank. The fish of one tank were exposed to the alarm substance and the responses were observed in both tanks. Two series of experiments were done, one where each tank had a hiding place, and one without hiding place. When the frightened fish retired to the hiding place, the fish in the other tank could not see them, and consequently did not show a reaction. But when no hiding place was provided and a strong reaction was induced, so that the frightened fish swam hastily about their tank, then the fish in the neighbouring aquarium showed a typical fright reaction. In contrast to the visual induction of the fright reaction in fish, the tadpoles did not respond to fright movements of their associates. KULZER[26] placed two aquaria side by side and poured alarm substance in one tank, observing the tadpole schools in both. Only the tadpoles to which the alarm substance was offered fled. Later it was shown that the other school also reacted to the alarm substance.

The ontogeny of the fright reaction. VON FRISCH[1] had assumed that the fright reaction of fish was innate, since it was so similar in fish from different geographic origins and different habitats. SCHUTZ[17] and PFEIFFER[23] demonstrated this. Various species of cyprinids were hatched in tanks and tested for the presence of alarm substance and for their ability to show the fright reaction at various times during their development. Recently-hatched fish showed no fright reaction. The alarm substance was present in the skin much earlier

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than their first reaction. Also schooling occurred earlier than the first reaction. It was found that the date of the first reaction was different in different species and did not depend on the absolute age in days, but on the relative age or stage of development. Thus fish which were kept in cold water developed the reaction later than those which were kept in warm water. Table II shows the species studied.

KULZER[26] studied the ontogeny of the fright reaction in tadpoles. The newly-hatched tadpoles immediately reacted to the extract of their companions which were the same age. Before metamorphosis the intensity of the reaction became maximal. The tadpoles during metamorphosis responded to the alarm substance as long as they continued to come to the feeding place in the aquarium. Also the skin of little toads contained an effective alarm substance.

The variation in the fright reaction. Under seemingly identical conditions, VON FRISCH[1] in his many experiments with *Phoxinus laevis* Ag. sometimes obtained positive results at dilutions of 1:500 while on other occasions the fish did not even react to undiluted extract. The great variability in the response was also confirmed by SCHUTZ[17] and PFEIFFER[18–23], and this is the reason why valid conclusions can only be based on large numbers of experiments. VON FRISCH[1] found it necessary to perform 438 tests with schools of *Phoxinus* in order to establish the nature of the reaction and the source of the alarm substance. In his experiments it became apparent that several factors contribute to this variability. Fish tested at different seasons (Figure 3), fish from different waters (Figure 4), males and females, and different individuals from healthy schools all have approximately the same content of alarm substance in their skin and the same reaction. Temperature has also been ruled out as a source variability in the results. The variability in the results depends on the readiness of the school to react. It has been observed that fish from the same catch, after the same treatment react to the same test substance in a totally different fashion. Some contributing factors to these individual differences have been found. It is obvious that the readiness to respond generally decreases with repeated testing. The result may be expected, since the fish are always fed after the alarm substance is poured into the tank

Table II. The age at which various cyprinids develop schooling, the alarm substance, and the fright reaction (data from SCHUTZ[17] and PFEIFFER[23])

Species	Age of fish in days: Earliest reaction to the alarm substance	Earliest age at which alarm substance has been found[a]	Earliest schooling activity
Brachydanio rerio (Ham.-Buch.)[c]	28	20	20
Richardsonius balteatus (Richardson)[c]	42	28	
Phoxinus laevis Ag.	51	38	26
Leuciscus leuciscus (L.)	59		31
Rhodeus amarus (Bl.)	40[b]	0[b]	0[b]

[a] Earlier stages have not been studied. [b] Indicates the number of days after emergence from the mussel, where the initial development takes place. [c] Fish were hatched at 26° C.

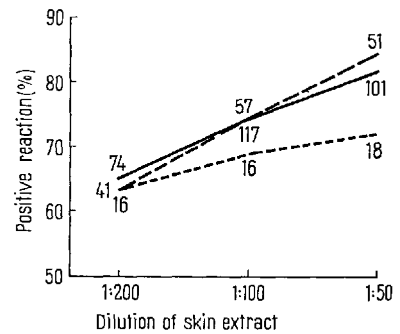

Fig. 3. Effect of the season on the number of positive reactions at various extract concentrations. —— all experiments; ----- experiments performed in April, May, June; ······ experiments performed in October, November, December. Figures on the lines indicate the number of tests for each point (from VON FRISCH[11]).

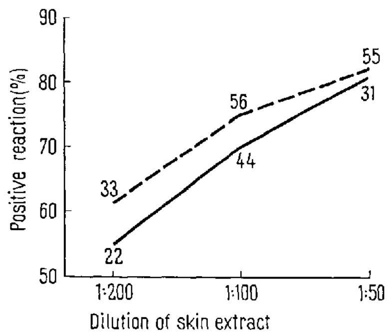

Fig. 4. Effect of the origin on the number of positive reactions at various extract concentrations. Upper line, fish from the river *Isar* (where pike are common); lower line, fish from the little lake *Deininger Weiher* (where predaceous fish are absent with the exception of a few trout). Figures on the lines indicate the number of tests for each point (from VON FRISCH[11]).

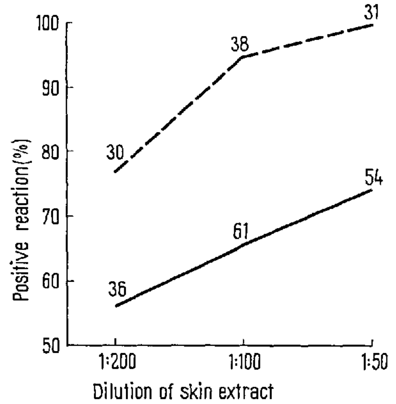

Fig. 5. Effect of the tank size on the number of positive reactions at various extract concentrations. Upper line, 25 l aquarium; lower line, 150 l aquarium; figures on the lines indicate the number of tests for each point (from VON FRISCH[11]).

and this will bring about a conditioning to it. So comparisons must be based on first reactions. The size of the tank also modifies the results, as might be expected, since the dilution is greater in larger tanks (Figure 5). Fish show stronger responses when they have lived in aquaria for two weeks compared with fish that have only been in an aquarium for a few days (Figure 6). Schools which require the longest period for habituation to the tank, show the strongest response to the alarm substance (Figure 7). Since schools which become habituated more slowly have been in the tanks longer, there are two possible reasons for the greater responses: it may well be that timid fish always respond more strongly to the alarm substance. On the other hand, a school requiring a longer time to habituate could require a greater familiarity with its surroundings and become more susceptible to any disturbances, such as the alarm substance. Fish which are in poor physical condition show little or no response to the alarm substance. The readiness to react disappears earlier than the desire to feed. VON FRISCH[1] could reduce the effectiveness of the skin by starvation. After 16 weeks of starvation the potency of the skin was only 25% of the normal value.

In the tadpoles HRBACEK[25] supposed that the reaction is dependent on the kind of food and on the duration of time in the aquaria. KULZER[26], however, demonstrated that the food has no influence on the reaction, and that the duration of captivity in the aquaria has no influence on the responsiveness of the tadpoles. In contrast to fish, the reaction of the tadpoles depends on the water temperature. In warm water the animals react more vigorously to the alarm substance (Figure 8). In addition, fish often become resistant to the alarm substance after a few tests, while this is not the case with tadpoles. The normal responsiveness to alarm substance is present within a day after a test. However, when tested repeatedly within a few hours, the intensity of the reaction also decreases in the tadpoles.

The distribution of the fright reaction. The fright reaction was found by VON FRISCH[1] in four other cyprinids, but not in the perch and other non-Ostariophysi. SCHUTZ[17] studied 14 European and 5 Asian cyprinids and found the fright reaction in all. He also found it in the loaches (Cobitidae) and in three out of six species of the Characidae. He did not find any reaction in about 15 species of non-Ostariophysi. Some 250 experiments were performed between 1957 and 1959 at Naples,

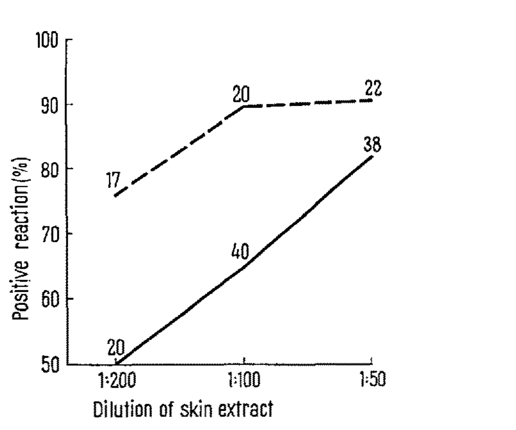

Fig. 6. Effect of the time spent in the tank on the number of positive reactions at various extract concentrations. Upper line, schools more than 14 days in the tank; lower line, schools less than 7 days in the tank. Figures on the lines indicate the number of tests for each point (from VON FRISCH[11]).

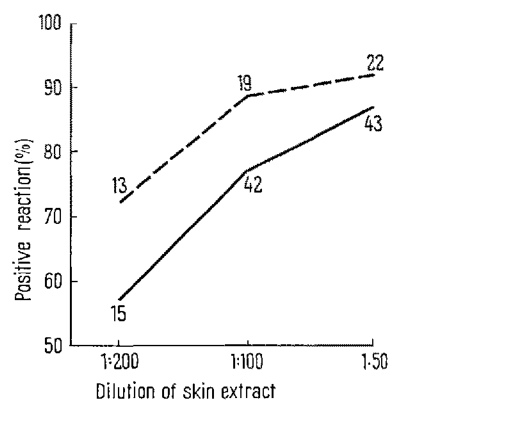

Fig. 7. Reactions to extracts of various concentrations by fish which required 3 days or less for taming (lower line) and fish which required 10 days or more for taming (upper line). Figures on the lines indicate the number of tests for each point (from VON FRISCH[11]).

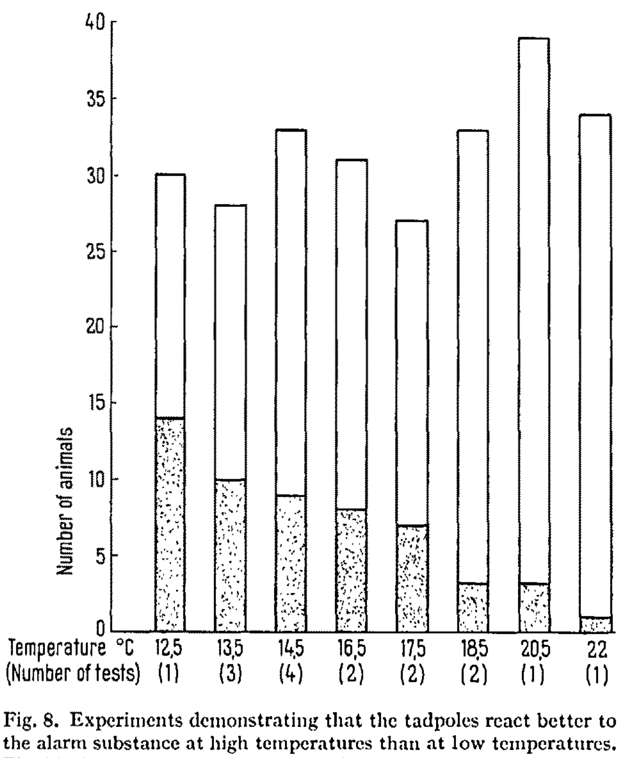

Fig. 8. Experiments demonstrating that the tadpoles react better to the alarm substance at high temperatures than at low temperatures. The blackened part of each column indicates the number of individuals at the feeding place $^1/_2$ min after adding the alarm substance. The numbers under the columns indicate the temperature, and the number of experiments for which each column represents an average (modified from KULZER[26]).

Arcachon and Helgoland by PFEIFFER[18,19]. These experiments, involving 66 species of marine fish from 32 families, showed that the fright reaction occurs only in groups having certain taxonomic affinities. None of these species, all non-Ostariophysi, has the fright reaction. One of the few marine cyprinids, *Tribolodon hakonensis taczanowskii* (Steindachner) was studied, and it was found that this fish does have the fright reaction as well as its relatives in fresh-water. So we can conclude: the fright reaction exists only in the Ostario-

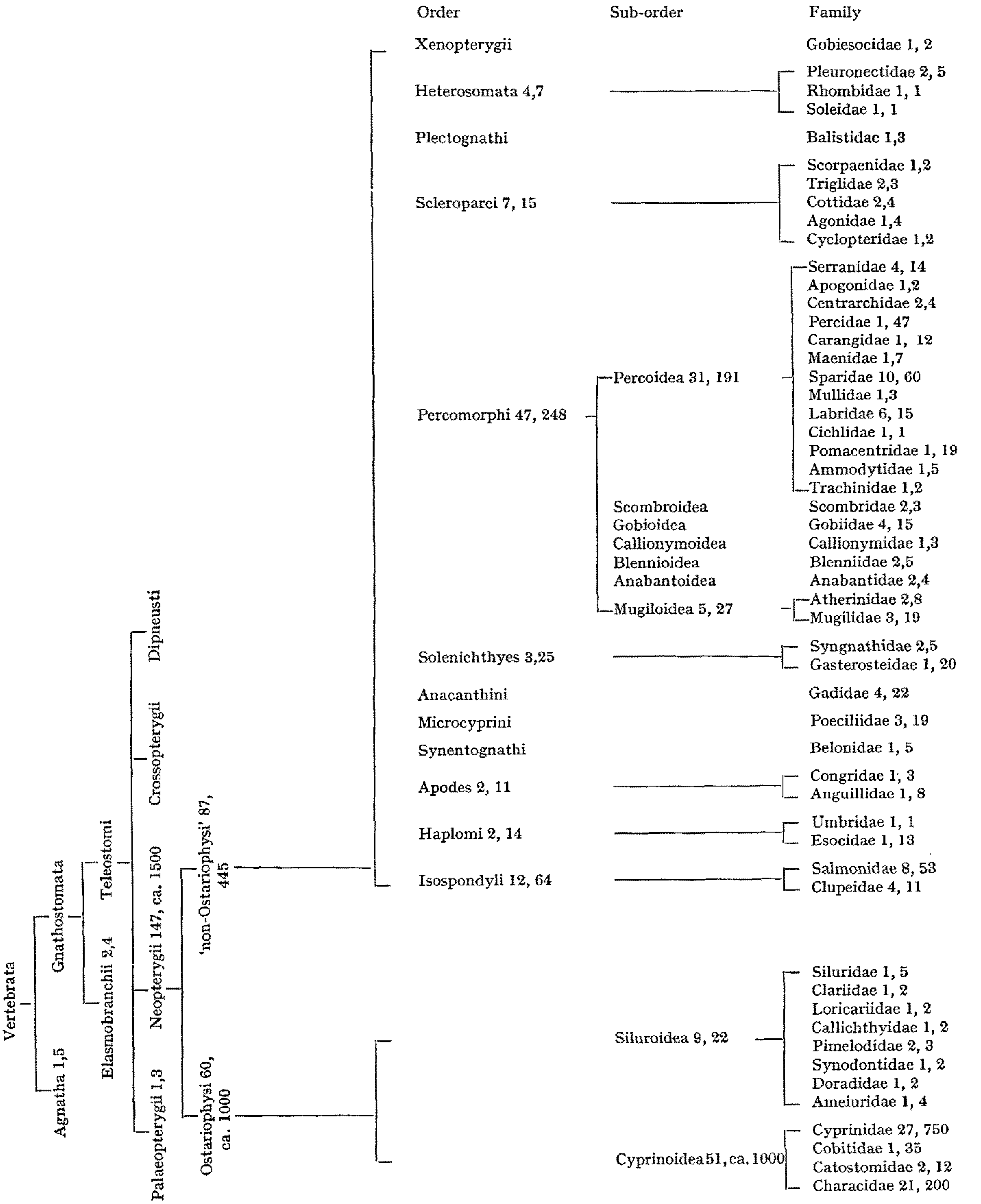

Fig. 9. The taxonomic distribution of the fright reaction and the alarm substance in fish. Both are limited to the Ostariophysi. The fish orders and fish families studied to date are listed. The two numbers following the familial or ordinal names refer to the number of species studied and the number of experiments respectively.

physi and is associated with this group of fish and not with any particular habitat. Since solitary catfish also show the fright reaction[18,19,22], one may conclude that social life is not necessarily connected with the fright reaction. Recently the fright reaction was studied in six species of North American cyprinids and also found in suckers (Catostomidae)[20,23]. It is of interest that the predatory northern squawfish, *Ptychocheilus oregonense* (Richardson), filling the niche of the pike in British Columbia, does have the fright reaction. However, there are some Ostariophysi which evidently do not have the fright reaction. It is absent in some groups of the Characidae[22]. It may be noted that these species are comparatively recent in origin.

The extent of the fright reaction should be known in many species in order to be able to judge the importance of this reaction in the life of fish. The occurrence of the fright reaction is therefore tabulated here. To date, 91 species (44 families) of non-Ostariophysi, and 60 species of Ostariophysi have been studied by VON FRISCH and his students SCHUTZ and PFEIFFER (Figure 9). All non-Ostariophysi evidently lack the fright reaction; the species are listed elsewhere[19]. Since there is no list summarizing the Ostariophysi studied to date, such a list is presented here (Table III). The list shows that the reaction is certainly absent in the *Serrasalminae* and *Mylinae*, and that it may be absent in some other groups of *Characidae*, like the *Hemiodontinae* and *Nannostominae*. These studies are still in progress and no definite conclusions can yet be given. Table IV shows the geographical distribution of the Ostariophysi studied.

Since the reaction occurs in the Ostariophysi and in the Cyprinoidea as well as in the Siluroidea, it can be concluded that the reaction has evolved prior to the

Table III

- Ostariophysi
  - Cyprinoidea
    - Cyprinidae
      - (from Europe)
        - *Alburnus bipunctatus* L.
        - *Alburnus lucidus* Heck.
        - *Carassius carassius* L.
        - *Cyprinus carpio* L.
        - *Gobio fluviatilis* L.
        - *Idus melanotus* Heck.
        - *Leucaspius delineatus* Sieb.
        - *Leuciscus erythrophthalmus* L.
        - *Leuciscus leuciscus* L.
        - *Leuciscus rutilus* L.
        - *Phoxinus laevis* Ag.
        - *Squalius cephalus* Heck.
        - *Telestes agassizi* Heck.
        - *Tinca vulgaris* Cuv.
      - (from Asia)
        - *Brachydanio albolineatus* (Blyth)
        - *Brachydanio rerio* (Ham.-Buch.)
        - *Danio malabaricus* (Jerdon)
        - *Esomus lineatus* E. Ahl
        - *Tanichthys albonubes* L.
        - *Tribolodon hakonensis hakonensis* (Günther)
        - *Tribolodon hakonensis taczanowskii* (Steindachner)
      - (from North America)
        - *Couesius plumbeus* (Agassiz)
        - *Hybognathus hankinsoni* Hubbs
        - *Mylocheilus caurinum* (Richardson)
        - *Ptychocheilus oregonense* (Richardson)
        - *Rhinichthys cataractae* (Val.)
        - *Richardsonius balteatus* (Richardson)
    - Cobitidae
      - (from Europe)
        - *Nemacheilus barbatulus* (L.)
    - Catostomidae
      - (from North America)
        - *Catostomus catostomus* (Forster)
        - *Catostomus macrocheilus* Girard
    - Characidae
      - (from South America)
        - *Aphyocharax rubropinnis* Pappenheim
        - *Astyanax bimaculatus* (L.)
        - *Carnegiella marthae* Myers
        - *Carnegiella strigata* (Günther)
        - *Gasteropelecus sternicla* (L.)
        - *Gymnocorymbus ternetzi* (Boulenger)
        - *Hemigrammus armstrongi* Schultz
        - *Hemigrammus ocellifer* (Steindachner)
        - *Hyphessobrycon cardinalis* Myers and Weitzmann
        - *Hyphessobrycon innesi* Myers
        - *Pristella riddlei* (Meek)
        - *Thayeria obliqua* Eigenmann
        - *Anoptichthys jordani* Hubbs and Innes
        - *Metynnis roosevelti* Eigenmann
        - *Mylossoma argenteum* (Ahl)
        - *Mylossoma aureum* (Agassiz)
        - *Pygocentrus piraya* (Cuv.)
        - *Serrasalmo rhombeus* (L.)
        - *Chilodus punctatus* Müller et Troschel
        - *Hemiodus semitaeniatus* Kner
        - *Poecilobryon trifasciatus* (Steindachner)
        - *Poecilobrycon unifasciatus* (Steindachner)
      - (from Africa)
        - *Phenogrammus interruptus* (Boulenger)
  - Siluroidea
    - (from South America)
      - *Acanthodoras spinosissimus* (Eigenmann)
      - *Corydoras palaeatus* (Jenyns)
      - *Loricaria parva* Boulenger
      - *Microglanis poecilus* Eigenmann
      - *Pimelodella chagresi* Schultz
    - (from North America)
      - *Ameiurus nebulosus* (Le Seur)
    - (from Asia)
      - *Clarias spec.*
      - *Kryptopterus bicirrhis* (Cuv. and Val.)
    - (from Africa)
      - *Synodontis nigriventris* David

▬▬ Fright reaction present
═══ Fright reaction probably absent
—— Fright reaction certainly absent

Table IV
Geographical origin and number of species of Ostariophysi studied

	Europe	Asia	North America	South America	Africa	Total
Cyprinidae	14	7	6	—	—	27
Cobitidae	1	—	—	—	—	1
Catostomidae	—	—	2	—	—	2
Characidae	—	—	—	22	1	23
Siluroidea	—	2	1	5	1	9
Total	15	9	9	27	2	62

divergence of these groups. This would indicate an evolution of the alarm substance and the fright reaction in the Oligocene or earlier. It also can be concluded that the Characidae which do not have the fright reaction must have lost it secondarily. This conclusion is supported by the fact that the species which lack the fright reaction are phylogenetically young. The intensity of the fright reaction can be thought of as the product of the amount of alarm substance in the fish skin and the responsiveness of the fish (Figure 10).

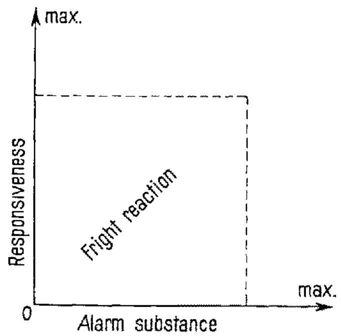

Fig. 10. The fright reaction can be thought of as the product of both, the amount of alarm substance in the skin, and the ability to respond to it (a function of the CNS). If one or both factors are zero, there is no observable reaction. If both factors are maximal the reaction observed will be also maximal.

If one value is zero there is no reaction. Recently many tests were made with the blind Mexican cave fish *Anoptichthys jordani* Hubbs and Innes, and it was found that this fish does not react to its own skin extract and also does not react to the alarm substance of its relative *Astyanax bimaculatus* (L.). However, it was found that the blind cave fish does have the alarm substance. *Astyanax*, which has both the alarm substance and the fright reaction, also reacts well to extracts from the cave fish. The alarm substance cells of the cave fish have been described previously[19]. It is very probable that the cave fish can perceive the alarm substance with its well-developed nose, since it immediately searches for food when the extract is poured into its aquarium. The loss of responsiveness is probably caused by a change in the central nervous system. I think that the loss of the fright reaction in the cave fish is of no disadvantage since there are no predators in these Mexican caves.

The fright reaction of fish has been repeatedly reviewed and often misinterpreted: it was first discovered in the minnow *Phoxinus laevis* Ag. by VON FRISCH[15], and not in the percomorph fish *Tilapia macrocephala* by NOBLE[33] as cited by HASLER[34,35]. NOBLE[33] himself cites VON FRISCH[15], and writes: 'in *Tilapia macrocephala*, the same reaction occurs but only in juvenile fish'. Since the observation was not followed by experiments and since many hundreds of tests with percomorph fish were negative, I would conclude that NOBLE's fish responded to an optical or mechanical stimulus. Such reactions have been described repeatedly in young cichlids[36–38] and young pike[39]; they seem to be widespread in young fish[40].

We do not know the species distribution of the fright reaction in the tadpoles. In some preliminary tests the fright reaction was not found in tadpoles of the frog species *Rana esculenta, R. temporaria, R. pipiens,* and *Hyla arborea*. It might be confined to those species of tadpoles which live in groups, such as those of *Bufo bufo*.

The interspecific effectiveness of the alarm substance. The tests of VON FRISCH[1,15] and his students[17–23] have shown that a marked fright reaction may be induced by the alarm substance from a different species. Strong reactions occur between closely related species, even if they are very widely separated geographically. Within the Ostariophysi, there is evidently a strong similarity in the alarm substance. However, there are differences, and the strongest response is always obtained with the alarm substance from the same species. Interspecific reactions bear a definite relation to the taxonomic position of the species. SCHUTZ[17] has shown very clearly that interspecific reactions are especially strong in taxonomically closely related species. Some interfamilial tests with *Cyprinidae* and *Catostomidae*[23], as well as *Cyprinidae* and *Characidae*, showed that the alarm substance frightened the other family, even if the reaction was never as strong.

KULZER[26] and SCHUTZ[17] studied the effect of the minnow alarm substance on tadpole schools and the effect of the tadpole alarm substance on minnow schools. Tadpoles did not react to the minnow alarm substance[26], and minnows did not react to the tadpole alarm substance. Minnows also did not react to bufotoxin and γ-bufotoxin[17].

[33] G. K. NOBLE, The American Naturalist *73*, 113 (1939).
[34] A. D. HASLER, J. Fish. Res. Bd. Can. *11*, 69 (1954).
[35] A. D. HASLER, *The Physiology of Fishes* (Editor M. E. BROWN, New York 1957).
[36] G. K. NOBLE and B. CURTIS, Bull. Amer. Mus. Nat. Hist. *76*, 1 (1939).
[37] G. P. and J. M. BAERENDS, Behaviour Suppl. *1*, 1 (1950).
[38] E. and P. KUENZER, Z. Tierpsychol. *19*, 56 (1962).
[39] F. GOETHE, Z. Tierpsychol. *2*, 314 (1939).
[40] Recently W. A. SKINNER, R. D. MATHEWS, and R. M. PARKHURST, Science *138*, 681 (1962), reported an alarm reaction in *Atherinops affinis* (Ayres). According to this report this fish should show an alarm reaction to ether- or methanol-extract from members of its own species, and to extract from the following species: *Phanerodon furcatus* (Girard), *Hypomesus pretiosus* (Girard), *Engraulis mordax* (Girard), and *Clupea pallasi* (Val.). From the observation that *Atherinops* reacts less strongly to the extracts from these species than to the extract from members of its own species, SKINNER et al. conclude that the reaction is species specific! – SKINNER et al. do not mention the number of their experiments. The work of SCHUTZ[17] and PFEIFFER[18], which has demonstrated the absence of the fright reaction in *Telmatherina, Atherina, Mugil,* and the absence of alarm substance in Clupeidae, is not cited.

The biological significance of the alarm substance and the fright reaction. VON FRISCH[1] postulated that such a widespread reaction must have a profound biological significance for the fish. The group of fish which shows this reaction, the Ostariophysi, is in general social, lacking defensive structures, and tends to be non-predaceous. VON FRISCH showed that damage to a minnow's skin by the sharp teeth of a pike was sufficient to elicit the fright reaction and thus to protect other members of the school. It was shown that the alarm substance was released even when a minnow swallowed a smaller minnow, although in contrast to the pike, minnows have only pharyngeal teeth[20,23]. In his field experiments VON FRISCH noted repeatedly that minnows which became alarmed in the region of the feeding tube subsequently fed much less readily there than in the nearby areas. The fright reaction changed the behaviour of the fish considerably. Not only did they flee, but their vigilance was also increased and they were particularly wary of areas where they had been frightened. SCHUTZ[17] noted an additional aspect of the value of the fright reaction. Very young minnows possessing the alarm substance in their skin failed to react to it until they were about forty days old. An older fish after eating or injuring a very small individual will be alarmed, swim away, and avoid the school of small fish. Thus the small juveniles are protected from the cannibalistic attacks of the adults. VERHEIJEN[41] recently criticised the assumption that the alarm substance reduces intraspecific predation. He based his conclusions on the observation that some freshly collected minnows swallowed their smaller companions without being frightened, but did react to a concentrated extract in a following test, and writes: 'obviously the large minnows succeeded in swallowing the smaller ones without liberating alarm substance'. It is, however, more probable that the amount of the liberated substance will be much smaller than the concentrated skin extract subsequently tested. In addition it may well be that the threshold of the experimental fish had been lowered so that they could not react to the very dilute substance released by swallowing, but could react to the very concentrated extract. Further, 'freshly collected' fish are not at all good for such tests. The fish should be well habituated to the aquaria before they are tested[1]. Actually it was shown earlier that the alarm substance is really liberated when a minnow swallows a smaller minnow[20,23], and it is equally clear that this amount is very small. The very few aquarium observations by VERHEIJEN[41] cannot be considered valid for the situation in the field.

On the basis of existing evidence, we assume that the fright reaction of the Ostariophysi, as well as that of the tadpoles, is an important insurance against predation. Of course we do not consider it the only insurance! The alarm substance does not protect the individual but protects the school, and it is logically of the greatest value in species exhibiting social life[42]. The schooling tendencies of many Ostariophysi and that of the tadpoles of *Bufo* become more understandable with the demonstration of this reaction. The fright reaction probably has contributed markedly to the biological success of the Ostariophysi, the order containing two thirds of all species of freshwater fish.

Zusammenfassung. Die Ergebnisse der deutschsprachigen Arbeiten von VON FRISCH und seinen Schülern über die Schreckreaktion der Fische und Krötenkaulquappen werden vergleichend zusammengefasst und englisch sprechenden Lesern zugänglich gemacht.

Unter *Schreckstoff* verstehen wir mit VON FRISCH eine Substanz, die Artgenossen vor Gefahr warnt, als *Schreckreaktion* bezeichnen wir das durch den Schreckstoff ausgelöste Verhalten. Durch Feindgeruch bewirkte Fluchtreaktionen und Verhaltensweisen unterscheiden sich von der Schreckreaktion durch das Fehlen des Schreckstoffes und seiner sozialen Motivierung.

Bei der Schreckreaktion der Fische und Krötenkaulquappen handelt es sich um eine Konvergenz. In beiden Fällen stammt der Schreckstoff aus der Haut, wird nur bei Verletzungen frei und geruchlich wahrgenommen. Während Fischhaut überall gleich wirksam ist, ist die Rückenhaut der Kaulquappen doppelt so schreckstoffreich wie ihre Bauchhaut. Fische entwickeln die Fähigkeit zur Schreckreaktion erst im Alter von ein bis zwei Monaten; Kaulquappen reagieren unmittelbar nach dem Schlüpfen auf Schreckstoff. Bei Fischen wird die Schreckreaktion optisch übertragen, bei Kaulquappen nicht. Bei Wiederholung der Aquariumversuche mit Schreckstoff werden Fische wesentlich schneller abgestumpft als Kaulquappen. Die Chemie des Schreckstoffs der Fische und Kaulquappen ist unbekannt. Nur bei den Fischen kennen wir die Herkunft des Schreckstoffs und die Verbreitung der Schreckreaktion. Die Schreckreaktion ist auf die Fischordnung *Ostariophysi* beschränkt und innerhalb dieser Ordnung sehr weit verbreitet. Der Schreckstoff stammt aus den «Kolbenzellen» ihrer Epidermis, für die der Name *Schreckstoffzellen* vorgeschlagen wurde. Die biologische Bedeutung der Schreckreaktion ist bei Kaulquappen wie bei Fischen Schutz vor grösseren Verlusten durch räuberische Überfälle.

[41] F. J. VERHEIJEN, Naturwissenschaften *49*, 356 (1962).

[42] W. PFEIFFER (unpublished) recently studied four groups of *Ostariophysi*. The fright reaction and alarm substance cells are present in Anostominae, Glandulocaudinae and Gyrinocheilidae, but absent in Rhamphichthyidae.