

Results and observations. The normal blood glucose level in the control fish was found to be 63.3 ± 11.7 mg/100 ml (Table). The level showed a fall as the starvation commenced. A direct correlation was found to exist between the blood glucose level and period of starvation, i.e. the level went on falling as the period of starvation was prolonged. Though the changes in blood glucose and NPN level (38.9 ± 7.7 mg/100 ml) in the control group fish were statistically not significant by the end of the 10th day of starvation ($P > 0.5$), nevertheless a fall in the two values was observed. This depletion of the 2 constituents of the blood became highly significant on the 30th day ($P < 0.01$) of starvation. The values of glucose and NPN levels in the blood of starving *Clarias batrachus*, on the 150th day, the last day of the experiment, were 32.07 ± 9.1 mg/100 ml and 20.5 ± 4.8 mg/100 ml respectively. It was calculated that a fall of 49.3% and 47.3% had occurred in the blood glucose and NPN levels respectively, by the end of the 150th day compared with the control values.

Discussion. A perusal of the results obtained (Table) clearly shows that marked depletion in the blood glucose and NPN levels occurred throughout the period of 149 days of starvation. AL-GAUHARI⁶ did not observe any change in blood glucose level of the fish *Clarias lazera*, even after 4, 5, 6 and 7 months of fasting period. However, HANNA¹² noted a fall of 60% in the glucose level of the same species after 7 months of starvation, although the value did not change during the first 4 months. PHILLIPS et al.⁵, on the contrary, observed a decrease in blood glucose level of the fish *Salvelinus fontinalis* in the first 3 days, after which the level was almost steady. Similarly the blood glucose level of *Gadus morhua* declined from 108 mg/100 ml to 72 mg/100 ml in the first 37 days but remained at this level after 51 days at 6.5°C¹³. Surprisingly, the blood glucose level appeared unaffected, even after 3 weeks of starvation in *Myxine glutinosa*¹⁴. KIERMEIR⁴ observed that several species of fresh water teleosts, both active and sluggish, maintained their normal blood glucose levels during long periods of starvation. Interestingly, the active fish showed a very gradual decrease, while the sluggish ones exhibited a remarkable constancy.

BENTLEY and FOLLETT¹⁵ observed a definite fall in the blood glucose level of the lamprey after 5 months of starvation. SUNDARARAJ et al.¹⁶ noticed a definite and continuous fall in the blood glucose level of the Clupeoid fish *Notopterus notopterus*, but only after 48 h of starva-

tion, during which a hyperglycemic peak was found after 24 h, while HOCHCHKA and SINCLAIRE¹⁷ did not notice any change in the blood glucose level of *Salmo gairdnerii*, even after 14 days of starvation, though its liver glycogen had fallen considerably.

Studies on blood NPN level are not many⁸, a slight rise in serum NPN level following 3–4 days of starvation was found in the eel *Anguilla japonica*. However, the present study shows that there was a parallel fall in blood glucose and NPN levels of the fish *Clarias batrachus* during starvation, the fall appeared to be very prominent as compared with the control values.

Conclusions. From the foregoing discussions it is evident that contradictory results have been obtained by various authors, on the blood glucose and NPN levels of fishes. It appears strange that many workers did not find any change in the blood glucose level of several species, even after quite long periods of starvation, since starved animal should ordinarily show little or more depletion in its blood glucose level. However, the present observations, like those of many workers, show that a definite fall in the blood glucose and NPN values, from the control levels of 63.3 ± 11.7 mg/100 ml and 38.9 ± 7.7 mg/100 ml to 32.0 ± 9.1 mg/100 ml and 20.5 ± 4.8 mg/100 ml respectively occurred by 150th day of starvation.

Zusammenfassung. Untersuchungen über das Verhalten des Blutzuckers nach längerem Nahrungsentzug bei Fischen.

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¹⁶ B. I. SUNDARARAJ, M. KUMAR, P. V. NARSIMHAN, M. R. N. PRASAD, T. A. VENKITASUBRAMANIAN and J. MALETHY, *Indian J. exp. Biol.* 4, 1 (1966).

¹⁷ P. W. HOCHCHKA and A. C. SINCLAIR, *J. Fish. Res. Bd. Can.* 23, 975 (1962).

¹⁸ I express my indebtedness to Dr. R. S. TANDON, under whose directions the work was carried out. I am also thankful to State C.S.I.R. for the financial support, and the authorities of the University of Lucknow for the laboratory facilities.

The Ability of Cod (*Gadus morhua*) to Orient Towards a Sound Source

The acoustic perception of the direction of a sound wave (sound localization) in fish is theoretically problematic^{1–3}, but there is quite a lot of evidence that several sharks⁴ and some teleosts^{5–8} possess this sensory ability. However, for several reasons, either incomplete experimental designs or inadequate sound fields caused by improper boundary conditions, almost none of these experiments provided a really decisive proof for acoustic localization at several meters from the sound source, e.g. no specific alternative explanations have been investigated⁹.

The present experiments¹⁰ meet the requirements to answer the following questions: Is cod able to determine the direction of a sound? If so, is the labyrinthine system involved or the lateral-line system (hypothesis of VAN BERGHEIJK¹)?

Under a raft in the middle of an almost circular fjord (local depth 35 m; 'diameter range' 300–400 m) a round netting cage, altitude 17 cm, was suspended at a depth of

¹ W. A. VAN BERGHEIJK, in *Marine Bio-Acoustics* (Ed. W. N. TAVOLGA, Pergamon Press, New York 1964).

² E. SCHWARTZ, *Fortschr. Zool.* 21, 121 (1973).

³ A. N. POPPER and R. R. FAY, *J. acoust. Soc. Am.* 53, 1515 (1973).

⁴ D. R. NELSON, *Diss. Univ. of Miami, USA* (1965).

⁵ K. OLSEN, Working Group for fishing Technology, 8th IF meeting (Lowestoft, England 1969, mimeo), p. 10.

⁶ K. OLSEN, *Coun. Meet. Int. Coun. Explor. Sea* (1969; mimeo B20).

⁷ A. SCHUIJF, J. W. BARETTA and J. T. WILDSCHUT, *Neth. J. Zool.* 22, 81 (1972).

⁸ A. N. POPPER, M. SALMON and A. PARVULESCU, *Animal Beh.* 21, 86 (1973).

⁹ In experiments where *unconditioned* oriented responses to sound are studied NELSON⁴ (partially), OLSEN⁶ and POPPER et al.⁸; this is probably irrelevant.

¹⁰ The services and the generous material support offered by direction and staff of the Havforskningssinstitutt, Bergen (Norway), is gratefully acknowledged. The Netherlands Organization for the Advancement of Pure Research (Z.W.O.) financed travels to Norway (twice) and to Scotland for this project (R 88-34).

4.75 m (top). By means of netting screens in the cage, a radial corridor was made with an entrance near the circumference and with an ending in the centre. A cod, length about 30 cm, was reinforced to perform a closed track through the corridor (operant). When the cod swam in the corridor, a transient free switched, interrupted pure tone of 75 Hz (18 dB re 1 μ bar) was presented, when it was in the correct position and orientation to make a choice out of 4 possible sound directions (I, II, III and IV numbered counterclockwise). For this object 4 sound projectors were hanging in a circle (radius 5.3 m) around the centre of the cage each 60° apart. The outermost stimulus alternatives I and IV were 90° to the right and the left, respectively, of the fish when leaving the corridor.

The stimulation ended when a response of the cod was observed, or otherwise the observation period ended after 4.5 sec equal to 3 intervals (sound 0.75 sec plus pause 0.75 sec). Subsequently the fish was rewarded at the feeding place that was in line with the active sound projector. A directional response consisted of stopping locomotion with the pectoral fins (this pattern could be absent), followed by suddenly turning and swimming straight towards the lateral netting wall. Typically it was a correct choice: an oriented response towards the source. The responses were observed through a periscope and classified by an independent observer not aware of the stimulus direction. After the judgement, the fish was rewarded by the trainer. The 4 remotely operated, acoustically transparent food dispensers¹² were mounted in a cylindrical frame resting on the cage. The food consisted of feet of periwinkles. A cylinder of opaque PVC sheet (acoustically transparent) around the netting cage concealed the locations of the sound projectors from the eye of the fish.

Table I. Example of a stimulus response matrix

Response towards		I	II	III	IV
Stimulus type	I1	6	0	0	0
	I2	5	1	0	0
	II1	1	5	0	0
	II2	0	7	1	0
	III3	1	1	5	0
	III4	0	2	3	1
	IV3	0	0	0	6
	IV4	0	0	1	5

Table II.

Type: minus left pars inferior N = 14				Type: control animal N = 44					
	A ^a	B	C	D		A	B	C	D
I	1	1	1	1	I	5	5	1	0
II	0	0	2	1	II	0	6	4	0
III	4	0	0	0	III	0	1	12	0
IV	2	1	0	0	IV	0	0	3	7

^a A = response towards I, etc.

During the observation series, the locations of the sound projectors (the pair 1 and 2) in the directions I and II could be interchanged and/or similarly the pair at the other side of the raft (3 and 4 in the directions III and IV). This procedure provided a test whether discrimination is based on inherent minute differences between the sound projectors (intensity, timbre). The choices of the fish can thus be classified in 4 × 8 different categories depending on the stimulus type.

Two subjects were conditioned successfully after about 30–70 trials (2 to 3 days). Table I presents the stimulus response matrix for cod G4. For various reasons, it was not possible to obtain a fixed number of observations in each row.

Since interchanging the positions in a pair of sound projectors was rather laborious, each of the four possible configurations was kept unaltered during a variable number of trials (range: 7–18), but the stimulus directions were always selected at random.

There were no indications for a conditioning on source specific cues¹² in the sequence of response observations, taken in the order of occurrence. Moreover, randomness could be accepted¹² for the sequence of successes and failures (runs test): a necessary condition for the presentation in a contingency table. Small sample tests showed that it is admissible to join the bold printed frequencies in Table I in common categories with equal stimulus directions (comparison of 2 binomial populations¹³ for each direction). Similar results were obtained in another subject (G1); however, responses for stimuli from direction I had to be excluded from statistical analysis in G1, because it visited food dispenser I too little. If all categories are pooled by a similar procedure, a 4 × 4 table is obtained (3 × 4 in G1).

The discrimination of the bearing of the sound sources by the intact cod G4 is evident (binomial test for the dichotomy success and failure with the pessimistic null hypothesis $H_0: P\{\text{success}\} \leq 0.5$ for each considered direction; by combination of these tests H_0 is rejected if the sum S of all successes, irrespectively of the directions, assumes large values; in G1, the worst case, the outcomes were: $S = 25$, $p = 0.01$). Measurements of the acoustic pressure and the particle velocity confirmed the absence of specular reflections¹¹ (echoes; acoustic isotropy).

After severing^{14,15} the nerve roots innervating the pars inferior of the left labyrinth in cod G4, the ability to discriminate the directions of sound was abolished (upper Table II) although the lateral-line system was still functioning. The prompt responses observed before the operation persisted, but a rapid extinction of performing the operant was observed. In the well-trained cod G1, all the procedures of the surgery¹⁵ were carried out with the exception of the severing of the nerve roots. After the operation, the discrimination results were at least as good as before the operation (control experiment; Table). It is concluded that cooperation of both labyrinths is involved in directional hearing. The results contradict the unverified theory of VAN BERGEIJK¹.

¹¹ M. A. VAN ARKEL, W. MAASSE and A. SCHUIJF, *Experientia* 29, 642 (1973).

¹² A. SCHUIJF, in preparation.

¹³ E. L. LEHMANN, *Testing Statistical Hypotheses* (Wiley, New York 1959), in particular p. 143.

¹⁴ The author would like to express his gratitude to Prof. S. DIJKGRAAF who developed and performed all the necessary operations (see ref. ¹⁵) for this study.

¹⁵ S. DIJKGRAAF, *Experientia* 29, 737 (1973).

That cod probably possesses directional hearing has previously been stated^{5,16}. CHAPMAN¹⁶ has proved that sound perception in cod depends on the angle between the directions of a tone and a masking noise. Recently¹⁷ it has been shown that the saccular microphonics in the haddock (*Melanogrammus aeglefinus*) exhibit a directional sensitivity, as has been expected since long¹⁸. The explanation of directional hearing is sought in the direct stimulation of the labyrinthine maculae^{19,4,7} (not via a link with the swimbladder). However, in cod, indirect stimulation of the saccular otoliths is likely²⁰. ENGER et al.¹⁷ therefore suggest that stimulation of the maculae by re-radiated sound from the swimbladder is avoided (minimized) by proper orientation of (part of) the hair cells (insensitive to radial displacements emanating from the swimbladder).

Zusammenfassung. Im freien Schallfeld wurde in einer Belohnungsdressur beim Kabeljau (*Gadus morhua*) akustische Lokalisation nachgewiesen. Die Entfernung

des Fisches von der Schallquelle betrug 5,3 m ($f = 75$ Hz). Ausschaltversuche zeigen, dass das Seitenorgansystem nicht wesentlich an der Schallrichtungsunterscheidung beteiligt ist.

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Einfluss von ATP in einer holidischen Diät auf *Myzus persicae* (Sulz.) (Aphidina)

Bei der Bearbeitung stoffwechselfysiologischer Probleme gewinnt die Ernährung der Versuchstiere mit einer holidischen (voll-synthetischen) Diät immer grössere Bedeutung. Die bei phloemsaugenden Insekten verwandten Nährlösungen orientieren sich an Phloemsaftanalysen diverser Pflanzen^{1,2} und enthalten allesamt Verbindungen der Stoffklassen Kohlenhydrate, Aminosäuren, Vitamine sowie Spurenelemente^{3,4}. Als Mass für die Güte der Diät wird ein möglichst hohes Gewicht nach definierter Lebenszeit, kurze Ontogenie und hohe Reproduktionsrate gewählt. Mit der phloemsaugenden Aphide *Myzus persicae* ist erstmalig der Nachweis gelungen, dass ATP in der Nahrung auch für phytophage Insekten von Bedeutung ist. Als Diät (kurz: ST-Diät) wurde eine speziell für diese Spezies optimierte Nährlösung⁵ verwendet. Dieser Diät zugesetztes ATP (hier als Adenosin-5'-triphosphorsäure Dinatriumsalz) bleibt auch nach 10 Tagen bei 20°C, 65% RLF und Dauerlicht gut nachweisbar (Boehringer Test, enzymatische Reaktion). Für die Versuche wurden an jungen Rettichpflanzen gezogene Virgines in Käfigen mit entsprechenden Diäten eingeschlossen. Nach je 24 h wurden die geborenen Larven gezählt und die Mütter in einen neuen Käfig überführt. Nach 4 Tagen hatten die an einer Diät mit 1,11 μM ATP saugenden Mütter ein um 9,4% höheres Gewicht als Mütter an der ST-Diät. Täglich wurden an der ATP-Diät 5,7 Larven/Imago (= 120%), an der ST-Diät 4,5 (100%) geboren. 11 Tage alte, auf den entsprechenden Diäten geborene Mütter zeugten bei einmaligem Diätwechsel

nach 6 Tagen bis zu diesem Zeitpunkt auf der ST-Diät 2,6 Larven/Imago (100%), auf der ATP Diät 6,4 (245%). Die Larven erreichten ihr höchstes Körpergewicht bei 1,11 μM ATP in der Diät im Bereich von 0,44 bis 2,22 μM (Tabelle I).

Das Optimum liegt etwas über den im Phloem festgestellten ATP-Mengen: für 18 verschiedene Laubbäume wurde bis zu 0,59 μM ⁶, im Läuseerüsselexsudat von *Salix* im Mittel 1,68 μM ⁷ und für die Monokotyle *Yucca* 1,22 μM ATP⁸ gemessen. Die ATP-Konzentration ist im Sommer wesentlich geringer als im Frühjahr⁶, steigt jedoch im Herbst wieder an⁸. Eigene Diätwahlversuche zeigen, dass ATP wie auch bei der blutsaugenden Tsetsefliege⁹ als Phagostimulans in erhöhter Konzentration von Bedeutung ist. Vielleicht bevorzugt *M. persicae* einen relativ hohen ATP-Gehalt (vergleiche hierzu auch die geringe Populationsdichte der Aphiden im Sommer, hohe im

¹ J. S. KENNEDY und T. E. MITTLER, Nature, Lond. 171, 528 (1953).

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Tabelle I. Beeinflussung des Körpergewichtes alatoider (geflügelt werdender) und apteroider (flügellos bleibender) Morphen 6 Tage alter *M. persicae* Larven durch ATP-Gaben in der holidischen Diät

Gewicht % Morphé	μM ATP in der Diät						
	0,00	0,35	0,44	1,11	1,67	2,22	4,45
Alatoid	100,0	104,4	110,9	111,1	109,4	106,0	102,6
Apteroïd	100,0	105,0	107,0	110,6	108,1	109,7	104,4

Die mittleren Körpergewichte aller in 4 Tagen geborener Larven sind prozentual auf das Körpergewicht von Larven auf ATP-freier Diät bezogen.