

FEEDING AND SOCIAL BEHAVIOUR
IN BROOK TROUT: APPLIED AND
THEORETICAL IMPLICATIONS

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ABSTRACT

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Feeding and Social Behaviour in Brook Trout: Applied and Theoretical Implications.

The feeding behaviour of brook trout

Salvelinus fontinalis (Mitchill) has been studied by an operant conditioning technique. Feeding rate showed a positive correlation with the duration of food deprivation, and size of the food reinforcement. It decreased as the frequency of reinforcement was reduced. Ecological significance of these results is discussed.

Daily food intake was recorded for periods of 2-3 months. The daily energy intakes of 23g.- and 40 g.- fish were estimated to be 78 and 160 calories, respectively. These values are compared with basal and maintenance metabolic levels reported in the literature.

Hierarchy in small groups of fish was measured both by agonistic interactions and by feeding order. Maintenance of dominance and dominance hierarchy were both found to be susceptible to experimental modification.

Preliminary tests on the behavioural effects of DDT and methoxychlor are reported.

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INTRODUCTION

Most studies of the effects of pollutants on fishes have used the simplest of physiological criteria; that is, the use of bioassays to determine the TLm (median lethal tolerance limit) (Sprague, 1971). However, there has been ⁱⁿ the last few years a shift in emphasis and researchers are now finding that the effects of various pollutants below the TLm can be expressed in subtle ways. Although these dosages might cause only slight changes in the values of physiological characteristics or small changes in behaviour, they may have profound ecological consequences for the long-term survival of a species. The need for more research into the sub-lethal effects of pollutants is even more apparent when proposed 'safe' levels are based on an arbitrary fraction of the lethal level e.g. 1/10 the 96-hour TLm (Sprague, 1971).

Weir and Hine (1970) demonstrated impairment of a classically-conditioned avoidance response to an electric shock of goldfish in a shuttle-box apparatus after exposure to concentrations of metallic ions well below the accepted 'safe' level. The lowest concentrations at which behavioural effects were observed, for example 1/1570 of the TLm for lead, correspond to the limits of sensitivity obtained when measuring the physiological effects of the same materials. Warner et al. (1966).

using a similar experimental paradigm, found deleterious effects at 1/25 and 1/200 the 96-hour TLm for the insecticides toxaphene and tetraethyl pyrophosphate, respectively.

Jackson et al. (1970) showed a detrimental effect upon the conditioned avoidance response of Atlantic salmon parr (Salmo salar) and brook trout (Salvelinus fontinalis) exposed to a sublethal concentration of D D T. On the other hand, by re-positioning the escape opening of Jackson's apparatus, Hatfield and Johansen (1972) obtained an improvement in the conditioned avoidance response after exposure to DDT. The different results of these two studies illustrate some of the problems of using the conditioned avoidance response. Pavlovian conditioning of fish, particularly when shock is used to shape or condition the response, may also have the disadvantage of putting the experimental animal under undue stress; for example, in a study of this type by Anderson and Prins (1970) a large proportion of both control and experimental fish died soon after the experiment.

An alternative approach has been to use unconditioned avoidance behaviour in an "open field" or "free choice" situation. Sprague et al. (1965) demonstrated avoidance by Atlantic salmon parr of waters containing sublethal concentrations of zinc, copper and

3

equitoxic copper-zinc mixtures. Hansen (1969), using sheephead minnows (Cyprinodon variegatus), and Hansen (1972) with mosquitofish (Gambusia affinis), have shown avoidance by unconditioned fish of various sublethal concentrations of pesticides, including DDT, endrin, Dursban, malathion, Sevin and 2,4-D. Although this technique is of considerable value it tends to provide a measure of the ability of fish to detect a pollutant rather than a measure of interference with its normal behavioural patterns.

Kleerekoper (1973) has recently presented another method of studying behavioural pathology in fishes. Essentially, his studies have shown that very small changes in the velocity and angle of turning occurred in goldfish exposed to sublethal concentrations of copper ions. These studies are particularly noteworthy in their sophistication, sensitivity and minimal manipulation of the experimental animal. However, the method does have some drawbacks. It seems difficult to interpret these changes in locomotor orientation under experimental conditions in terms of adaptive significance to the fish. Also, the technique requires elaborate and expensive hardware, such as on-line computer, thereby limiting its widespread use by other workers.

It is clear, therefore, that there are no

well-defined satisfactory criteria for measuring the behavioural effects of pollutants. The intent of the present study was to develop reliable quantitative means to describe normal patterns of behaviour of obvious adaptive importance, for example feeding and schooling behaviour, and subsequently to study the effects of pollutants on these patterns. To this end, studies of the feeding behaviour of brook trout were designed to utilize an operant conditioning paradigm. This type of paradigm rewards rather than punishes responses and would appear to be less stressful on the animal than classical conditioning. Furthermore, it provides a quantitative measure of both feeding effort and food intake which can be readily automated in this type of experiment. Although in a number of psychological studies the feeding responses of fish have been measured, interest has been centered on learning rather than on the feeding behaviour itself. There have been only two previous studies using operant conditioning as a tool to investigate feeding behaviour. In their first study, Rozin and Mayer (1961) showed that goldfish modify their feeding behaviour according to their calorific needs; that is, if their food is diluted with a non-nutritive component the fish increase their food intake to compensate. In addition they showed a direct relationship between ambient temperature and the food

intake of the fish. In a later study (Rozin and Mayer, 1964) it was shown that the day-to-day food intake of individual fish was rather stable and when the effort required to receive food reward was increased (i.e. more than one response was required to obtain a food reward or reinforcement) the fish showed an increase in the number of responses to compensate.

No previous studies have been reported on the operant conditioning of trout and therefore there was no information on such basic variables as the schedules of reinforcement (i.e. the ratio of responses to food rewards), the size of the reinforcement, the duration of the feeding trial and time between trials (i.e. the effect of deprivation). The present study included investigation of these variables.

In another experimental series some aspects of normal social behaviour in brook trout were examined. Kruzynski (1972), in earlier work in this laboratory, presented photographic evidence which indicated that brook trout on a diet containing 2 mg. of methoxychlor/kg. body weight/day showed a marked increase in fish to fish distance over an 18-day period. Eddy (1925) also showed that sublethal doses of chlorethane and potassium cyanide suppressed aggregating in two species of fish

(Ameiurus melas and Schilbeodes insignis). However, as Kruzynski's study was apparently the only report of an effect of a pesticide on schooling behaviour, it was decided to try to develop methods of measuring spacing and hierarchy in trout groups so that these effects might subsequently be examined.

MATERIAL AND METHODS

Experimental Stock

Brook trout Salvelinus fontinalis.

(Mitthill) used in all experiments, were obtained from the Bury Fish Hatchery, Bury, Quebec. The majority of the fish were about 18 months old at the date of purchase. The average body weight was 23 grams, (ranging between 20g. - 40g.) and the total body length was 14 to 16 centimeters. The fish were transported under cool, dark conditions and were held in one hundred and twenty-five litre polyethylene tanks provided with a continuous flow of water.

Water

The water used in all laboratory conditions was dechlorinated by a charcoal filter and equilibrated with air. Periodic titrations by the azide-modification of the Winkler determination (American Public Health Ass., 1960) showed that oxygen was at saturation. Water temperature was maintained at $11\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. The other characteristics of the water are outlined in Appendix I. A photoperiod of 14/10 hours light/ dark was provided to all fish in the laboratory (Westinghouse warm white, fluorescent lights).

Rations

The fish in the holding tanks were main-

tained on Purina Trout Chow.

The experimental diet used in all conditioning experiments was prepared from a blend of minced, dried and pulverized beef liver, beef heart and Ewos Trout Chow (Ewos F.48 Sweden) in a ratio of 2:1:1. This powder was sieved through a number 50 sieve (Endecotts Ltd., London). To nine parts of the powder was added one part of guar gum (Nutritional Biochemicals Corp., Cleveland). The food was finally prepared for dispensing by syringe by adding to one part of this mixture three parts of distilled water by weight. This final preparation had a total water content of 75.5%. The food was oven-dried at 70° C. to constant weight and analysed (Table 1).

Table 2 shows the calorific equivalent of the various constituents of the food based on calorific values and digestibility coefficients estimated by Phillips (1972). Thus, the total calorific value of the food is 873 cal./g. food.

In all the schooling behaviour experiments, the fish were fed on a diet described by Kruzynski (1972). It was prepared from the same meat powder described above, but the guar gum was replaced by gelatin and the water content reduced to 64.2% so that the food was solid rather than liquid.

Table 1. Proximate analysis of oven-dried food.

Constituent	% composition	Method of determination
protein	58.50	Kjeldahl(Griffen, 1927)
ether-soluble extract (lipid)	7.78	Labconco-Goldfisch Fat Extraction Apparatus Model 35003
ash	8.03	1200° C for 90 min. (Lindberg Hevi-duty Model 59844)
nitrogen-free extract (carbohydrate)	25.56	by difference

Table 2. Caloric equivalent of experimental food based on wet weight. (calculations shown in Appendix III)

Constituent	% composition of food	Calorific value (Kcal/g. food)	Calorific contribution (cal/g wet weight)
protein	14.3	3.9	558
fat	2.1	8.0	168
carbohydrate	6.7	2.2	147

Experimental Designs

Feeding studies of individual fish:

The fish were readily conditioned to feed by manually advancing the syringe plunger (p.21) thus 'baiting' the bar (p.19). Most fish learned to obtain food by operating the bar within one day if the feeder was left in the tank and remained conditioned throughout the experimental period of about 3 months.

3 All experiments were carried out with fish individually housed in 15-litre stainless steel tanks (33.6 cm. x 24.0 cm. x 24.0 cm.). Stainless steel stand-pipes maintained a water depth of 18 cm. Each tank was covered by diffusion covers of the type used in fluorescent light fixtures. Each tank was supplied with dechlorinated water, the characteristics of which are shown in Appendix I, at a rate of about 250ml/min. This gives a 99% replacement time of approximately $4\frac{1}{2}$ hours. (Sprague, 1972).

When fish were taken from the holding facilities no anaesthetic was used. The fish were randomly chosen from the holding tank, wrapped in damp paper toweling, weighed, measured and placed in the experimental tank. The fish were acclimated to these tanks for at least one week prior to an experiment.

At the beginning of each feeding trial the feeder was gently lowered into the tank, with the back

of the unit against one end of the tank. In this way, the bar was only exposed to the fish through the hole in the shield. The sensitivity of the bar was adjusted so that the response cycle could not be initiated by water movement against the bar nor could a spurious response be obtained by the fish striking the shield with its snout or tail. On the other hand, the setting was not so insensitive as to require too great a pressure to be applied by fish on the bar. In practice the sensitivity was roughly adjusted by tapping the shield and finely set by observing the fish while feeding. Once set, it was usually sufficiently stable so as not to require adjustment during the course of an experiment, i.e. over several weeks. The tanks were arranged in a straight row. Thus, since only one feeder was used in all experiments, it could be easily lifted from one tank following a feeding trial and transferred to the fish in the adjacent tank to initiate its feeding trial.

In the first (effect of deprivation) experiment, the food was delivered via teflon tubing (1.5 mm. I.D. x 2.3 mm. O.D.) connected to a blunt syringe needle (no. 18) attached to the bar. In later experiments this was replaced by a rigid butyrate tubing (3.0mm. I.D. x 4.5mm. O.D.) which passed through a 7 mm. hole in the bar. The latter modification increased the mechanical isolation of the food delivery from the operant

response. In this way a good spatial- temporal contiguity between response and reinforcement was maintained while the number of false responses caused by a fish accidentally striking the bar while feeding at the tube was reduced. The wider bore and rigidity of the butyrate tubing also increased the precision. In the deprivation experiment, the pumping of the extremely viscous food gave rise to considerable back pressure in the system. This was partly taken up by elasticity of the tubing. However, the subsequent recovery gave rise to some oozing of food between reinforcements.

The size of the food reinforcements used in all experiments except the first, are described in Table 3. Since each reinforcement was quite small, the mean sizes shown were based on twenty samples of ten reinforcements each; that is, 200 reinforcements were collected to determine each mean size shown.

Table 3 . Quantities of food dispensed as reinforcements,
in the conditioned feeding experiments by a
Harvard syringe pump (model 901).

Gear setting	Dispensing time (seconds)	Mean reinforcement weight (mg.)	Standard deviation (mg.)
5	1	2.36	0.04
6,	1	0.97	0.04
7 ,	1	7.67	0.14

The light incorporated into the response bar and the overhead light which switched as soon as a response was registered provided the secondary reinforcement. This is routinely included in operant conditioning experiments to ensure instantaneous reward of a response. It is particularly important when there is a delay in presenting the food reward which was the primary reinforcement in these experiments.

Careful precautions were taken to eliminate extraneous disturbances in the laboratory throughout this study and particularly during an experiment. The floor and walls were carpeted. The frame supporting the experimental tanks was rested on 5cm. thick rubber blocks. And the experimental area was partitioned from the rest of the laboratory. Once the feeder was transferred into a tank the experimenter normally left the area until the feeding trial was terminated, except when making a visual check on feeding behaviour. The one-hour feeding trials were terminated by manually turning the circuitry off. In the later experiments the fifteen-minute feeding sessions were all automatically ended by a timer (Marktime, Ottawa). In either case, once the circuitry was turned off the feeder was immediately removed from the tank.

In the first experiment (effect of deprivation), the fish were each given a feeding trial

commencing with the feeder being lowered into the tank and the circuitry turned on for one hour. Once the circuitry was off, the feeder was transferred to provide the next fish with its trial. All experiments began at 10 A.M. and the same feeding order was maintained in this way throughout so that each fish would always receive its trial at the same time of the day. In all experiments, except the first which investigated the effect of the inter-trial period (deprivation period), the trials were run every 72 hours. This period was considered sufficient to allow complete digestion and excretion (gut clearance) of the food eaten during the feeding trial; Phillips et al. (1965) has shown a gut clearance time for brook trout at 11°C of 63 hours. In this way the influence of one feeding trial over the next (i.e. 'carry-over') would be minimized. Furthermore, in order to balance the experiment, a droplet of the ration was weighed and fed to each fish in such a way that all fish on an experiment were supplemented to receive the same amount as that obtained by the fish which ate the most from the feeder that day.

In preliminary experiments with pollutants, conditioned fish were exposed to the insecticide p.p. DDT (Aldrich Chemical Corp., N.J.). The DDT was diluted with acetone and infused through teflon tubing by the syringe pump described earlier. By pumping the toxicant at a

particular rate, relative to the concentration of the DDT and maintaining a constant inflow of water (250 ml./min) to the experimental tank the desired concentration of exposure was obtained (i.e. either 100, 40, 20, 10 p.p.v.). All exposures were for 24 hours. Feeding behaviour was monitored for at least 6 days (2 feeding trials) following exposure to DDT. At no time before, during or after these experiments were the live fish handled.

Schooling behaviour

The fish were randomly chosen from the holding tank. They were anesthetized with tricaine methanesulphonate (MS 222, Sandoz), weighed, measured and cold-branded (Mighell, 1969) on the dorsal surface so that each fish could be individually identified in the 8 mm. film, and then placed in the experimental tank. The fish ranged from 23 to 57g. in weight. During an experiment they were fed 2% average body weight/day.

Initially quantitative data were obtained on the distribution and behaviour of a number of groups of six to nine trout by using time-lapse cinematography. Following these observations an attempt was made to reproduce Kruzynski's (1972) result. A group of nine brook trout was used in this experiment. They ranged in weight from 28.3g. to 49.1g. and were fed under conditions at which Kruzynski reported the most marked effect. Photo-

graphic observations were made for twenty-three days throughout the daylight period. To evaluate the effects of methoxychlor (City Chemical Corp., N.Y.) on schooling behaviour, the insecticide was incorporated into the food so that each fish received 2 mg. methoxychlor/kg. body weight/day. Water temperature was maintained at 11 ± 1 °C. The water velocity was checked by timing the movement of a small float over a known distance. Photographs were taken at rates ranging from one frame per second to one per seven minutes. The film was later examined on a film editor. Direct visual observations could also be made without disturbing the fish.

Feeding behaviour of a group of fish:

The feeding behaviour of a group of brook trout was examined in an attempt to identify the social status of each member of a group. The defense of, approaches to, and feeding at a single food source were all used to analyse the status of each fish. At the same time, daily observations of agonistic behaviour were made to establish a dominance hierarchy based on counts of aggressive and submissive displays. Six fish ranging in weight from 22.0 - 32.7g., were randomly selected from the holding area, cold branded on their dorsal surface and placed in the experimental tank. During this 5-day experiment information was collected by time-lapse cinematography in

the range of 1 frame per second to 1 frame every 7 minutes as well as with every bar press throughout the daylight period. Personal observations were also recorded on cassette tape recorder and later transcribed. The feeder was available to the group during the entire experiment and provided a 2.4mg. food reinforcement for every bar press. A fish which fed from and defended the feeder continually for at least 12 hours was described as the dominant (α) fish. The feeding hierarchy was determined by confining each dominant fish as it came to express itself. This procedure was continued until there were only two unconfined fish. Subsequently, all these fish were again released in the tank and the hierarchy was determined once more in the same way.

APPARATUS

Feeding Behaviour of Individual Fish

The operant conditioning apparatus was based on a design used by Ames (1967) and particularly on the subsequent modification described by Holmes and Bitterman (1969). Both require that the fish press a lever or bar to obtain its food. The feeder is shown in Fig. 1 and a block diagram of the recording and programming circuitry is shown in Fig. 2. The sequence of events following a bar-press was as follows: The movement of the bar was transferred to the transducer (Etco, type MT18 phonograph cartridge) by the lever mechanism shown in Fig. 1. The transducer converted the mechanical movement into an electric pulse which was then amplified. This pulse was then shaped to ensure that a single pulse of uniform size was obtained irrespective of the duration of the bar press, so that even if the bar was held down no other pulses were passed on and the bar stayed deactivated until released again. This single pulse activated timer #1 which in turn energized and held relay #1 for a predetermined period of time (2 sec. in the deprivation experiment and 0.5 sec. in all other experiments). When relay #1 switched, the response counter (type 54/4 c, Counting Inst. Ltd., England) advanced by one unit, the rotary solenoid (B.P.O. Type 3000 Uniselect) advanced by one contact

and relay #2 was activated. When energized, relay #2 dimmed the overhead light and simultaneously switched on the bar light for the period of time determined by timer #1. Meanwhile, each response activating relay #1 also moved the cumulative recorder pen one step upward, 0.25 mm. The paper drive of this pen recorder (Gerbrands Co.) moved at a speed of 60cm./hr. to provide a time base. A typical cumulative curve is shown in Fig. 3. This graph plots the number of responses on the ordinate against time on the abscissa and provides the most convenient representation of the behaviour observed in this study. Thus, a steep slope indicates a rapid rate of response while a flatter one a slower rate. When relay #1 was energized the rotary solenoid was also activated so that the moving arm of the switch advanced to the next contact. There were 25 contacts on this switch. They were either all connected to the timer controlling the food pump to provide continuous reinforcement (CR), i.e. one reinforcement for every response, or connected to provide a fixed ratio (FR) schedule of reinforcement; for example, to provide an FR6 schedule, every 6th contact was connected to the reinforcing circuitry. This segment of the circuitry was controlled through timer #2 (Hoagland Co., Redbank, N.J.) which was set at 5 sec. in the deprivation experiment and 1 sec. for all other experiments. This timer activated

and held in relay #3 which advanced the reinforcement counter by one unit and activated the reinforcement recorder so that the recorder pen made a blip on the cumulative curve. Relay #3 also activated relay #4 which in turn energized the solenoid clutch of the syringe pump (Harvard Apparatus, model 901) for the period of time determined by timer #2 and drove the syringe plunger to provide the food reinforcement. The system employed two reset loops shown in Fig. 2. Loop #1 reactivated the bar so that another response could be elicited once the period set by timer #1 expired. Since the reinforcement dispensed over the time set by timer #2 was longer than that of the response associated timer #1, loop #2 ensured that responses elicited following reactivation of the bar, but while food was still being dispensed, did not advance the unisector. In this way, the response and reinforcement cycles did not overlap.

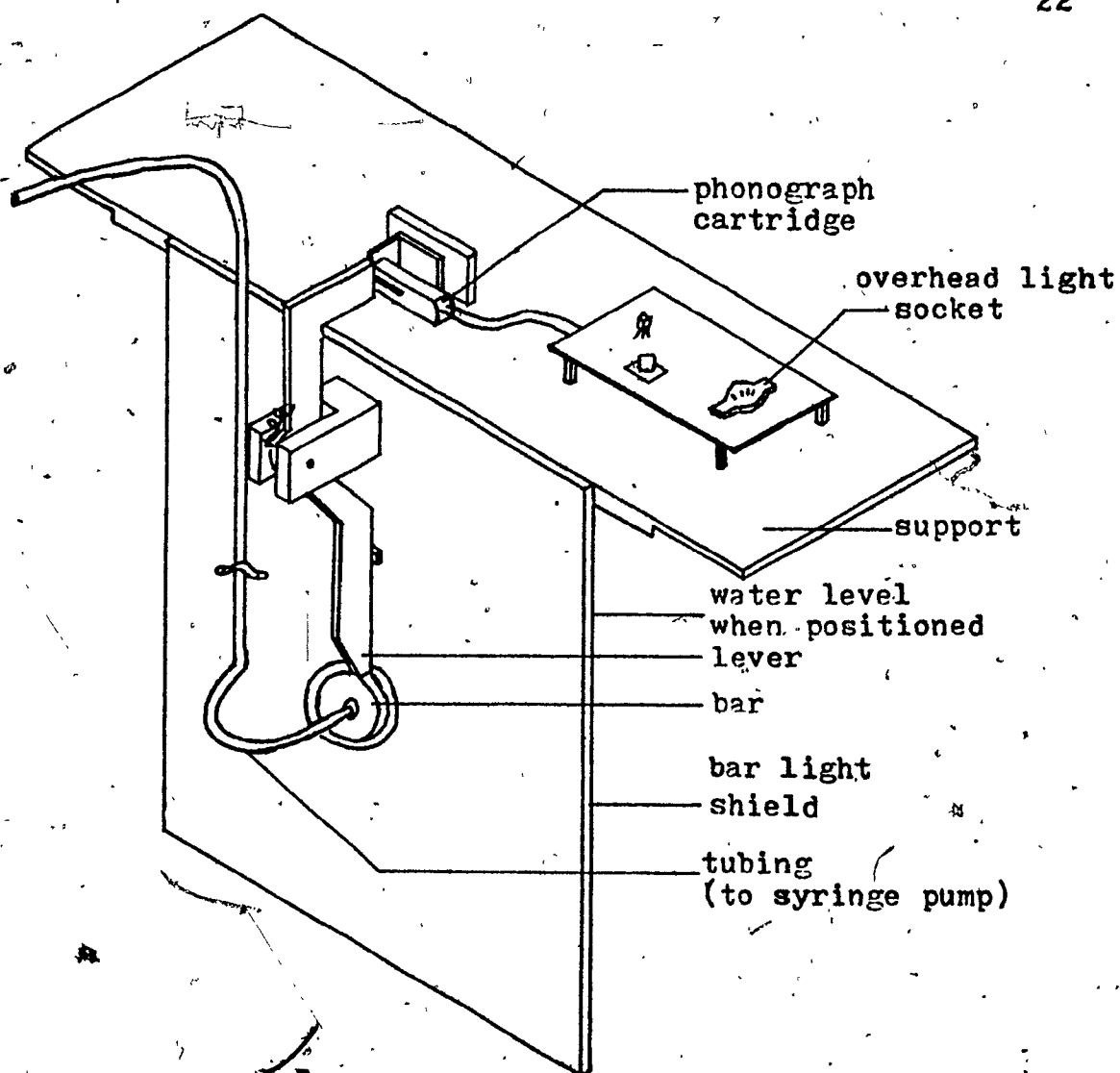


Figure 1 : Diagram of feeder used in conditioned feeding experiments (Scale 0.38:1.00).

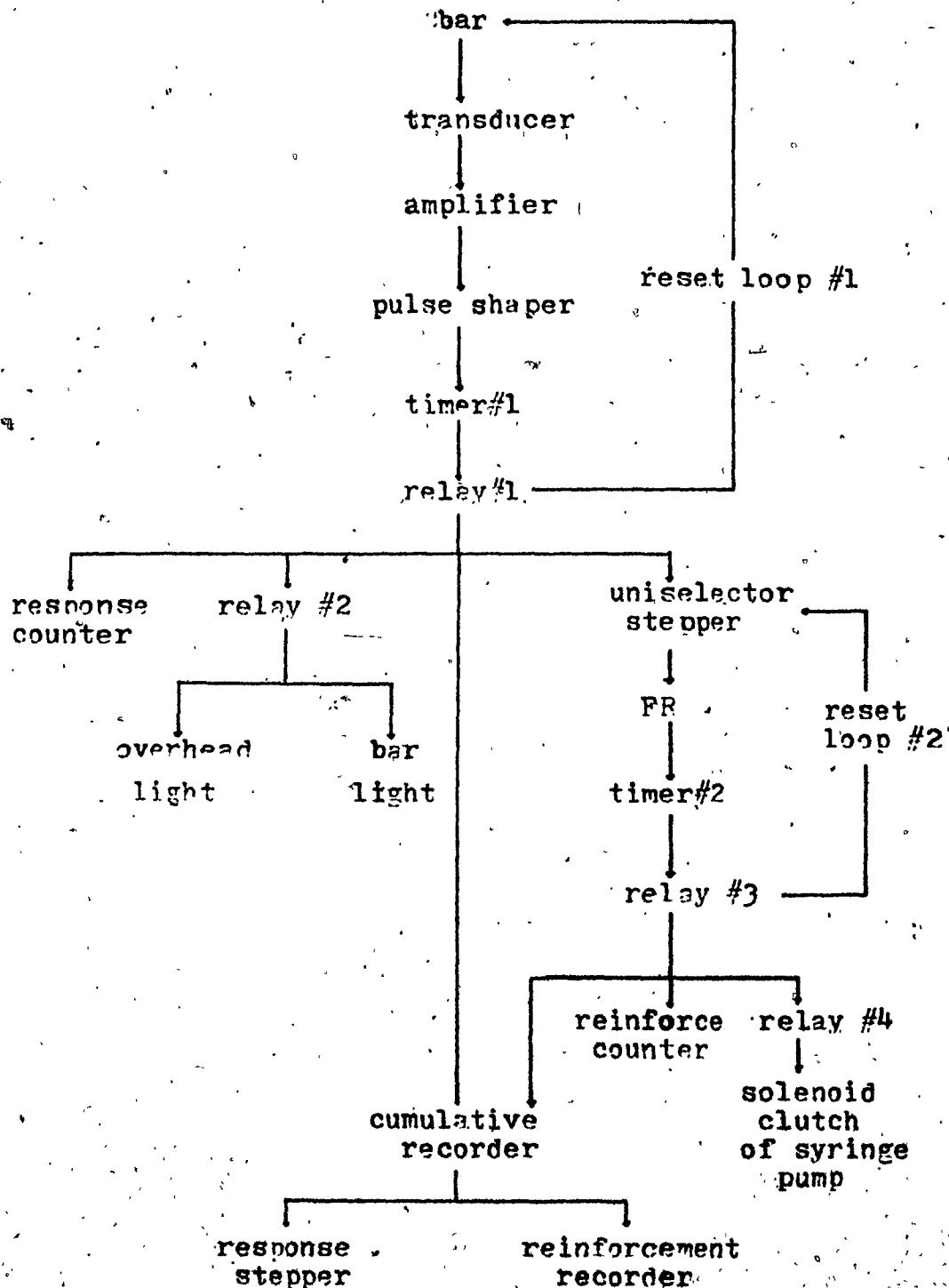


Figure 2. Block diagram illustrating the recording and programming circuitry of the apparatus used in the feeding experiments.

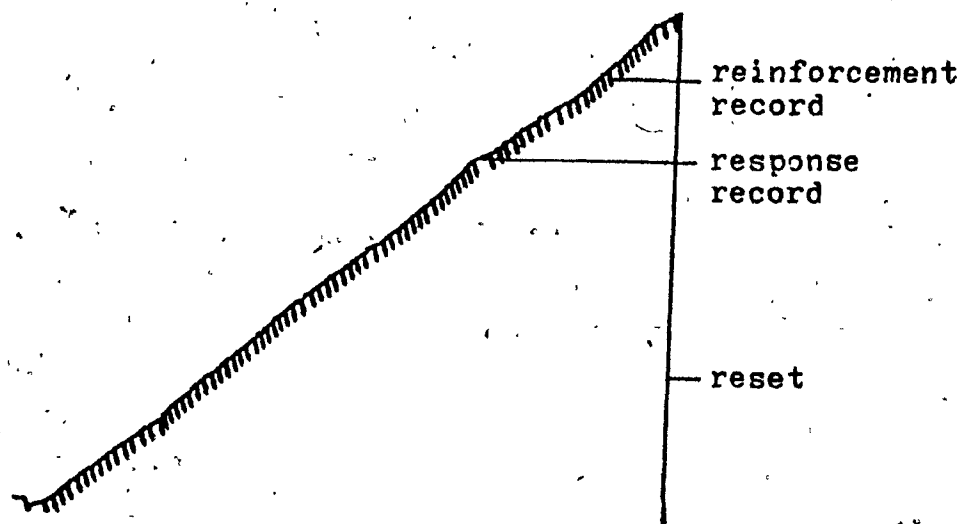


Figure 3: Typical response curve obtained from a fish fed on FR3 with 2.4 mg. reinforcements over the 15 minute feeding trial. Paper drive 60 cm./hour, upward movement of the pen at the rate of 4 responses/m m.

Schooling Behaviour

The experimental tank (Fig. 4) was constructed as described by Kruzynski (1972). Essentially, it was a doughnut-shaped, moulded fiberglass tank (90cm. O.D. x 60cm. I.D.) the bottom of which was filled-in and leveled out with plaster of paris coated with polyester resin. Water depth was maintained at about 4 cm. and was continually supplied at a rate of one litre per minute. A current of water was maintained at about 12cm./sec. by a pump (Cole Palmer Ltd.). The pump motor was controlled by a variable transformer (Variac, type 116B) which in turn controlled the water velocity. As shown in Fig. 4 the water was drawn up vertically from the bottom of the tank and expelled horizontally at the outlet about 15 cm. from the inlet. In this way a continuous current was established. Plastic screening restricted fish from the area of the water inlet and outlet. The tank was illuminated by fluorescent lights along the outer perimeter of the tank and by incandescent lights along the inner perimeter. The fish were prevented from jumping out by a sheet of plexiglass fixed vertically around the edge of the tank to a height of about 45 cm. The photoperiod throughout the experiment was kept at 14/10 hours day/night.

Activities were monitored chiefly by a super-8 format movie camera (Minolta D6) mounted approxi-

ately 1 metre above the tank. A time-lapse circuit (Burns and Summers, 1969) provided single-frame exposures of Kodak Tri-X film taken at predetermined intervals.

An electric clock was mounted in the center of the tank so that the time of day was included into each film frame.

The entire unit was enclosed to reduce extraneous disturbances.

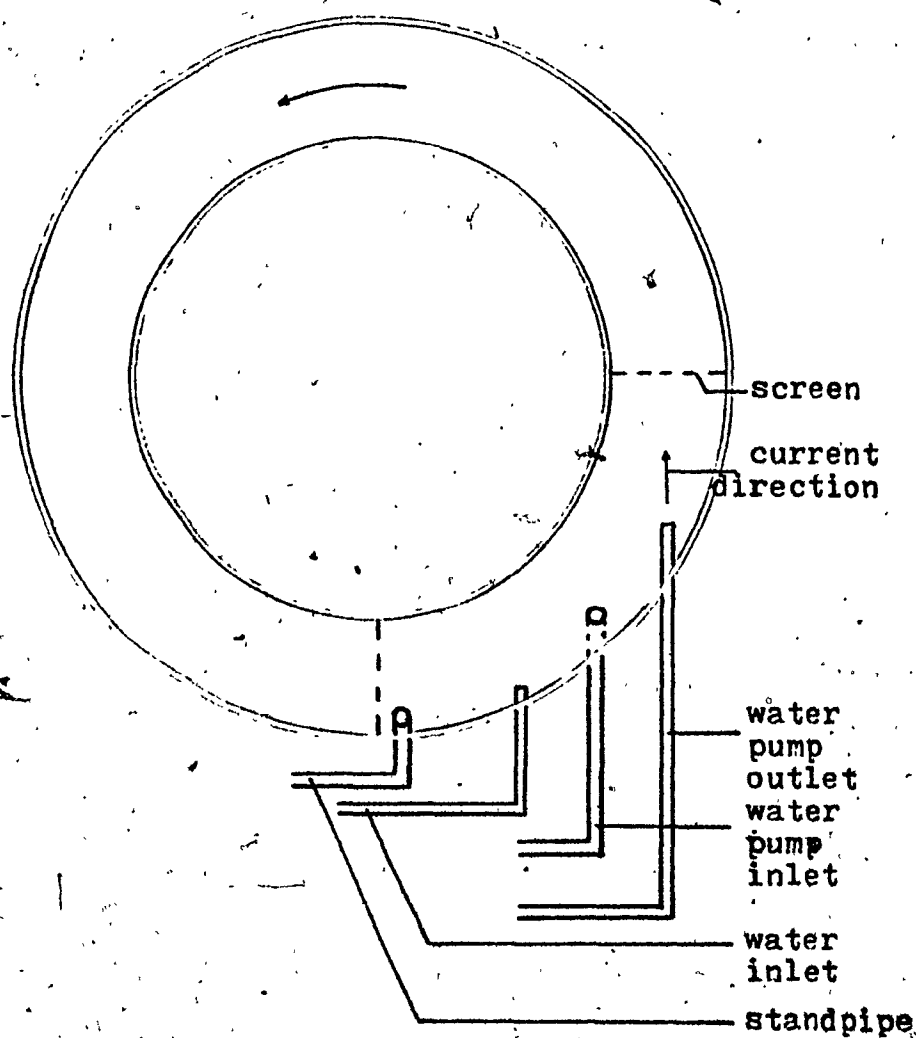


Figure 4 : Diagram of tank used in group studies
(scale 0.11 : 1.0.).

Feeding Behaviour of a Group of Fish

The experimental tank used in this study was a 730-litre polyethylene tank (180cm. x 90 cm. x 45 cm.). It was supplied with dechlorinated water at a rate of one litre per minute. Water temperature was maintained at $12 \pm 1^{\circ}\text{C}$. A support was provided at one end of the tank to hold the feeder shown in Fig. 1. The area behind the feeder was screened-off so that the fish could not pass behind it. The tank was illuminated with fluorescent lighting (40 W.) having 12/12 hours day/night photoperiod. The camera and time-lapse circuitry used here was identical to that used in the study of fish schooling behaviour. The camera was mounted at about 45° angle to the tank and included the front of the feeder and a clock in each frame of film.

RESULTS

Feeding Behaviour of Individual Fish

There is abundant evidence, both direct and indirect to demonstrate the reality of the operant conditioning of feeding behaviour i.e. that the fish learned to associate pressing the response key with the presentation of food and thereby pressed the bar to obtain food. In a normal feeding trial the trout began to actively strike the bar with its snout within a few moments after presentation of the feeder. This was done in one of two characteristic fashions: by positioning itself directly in front of the bar, at right angles to it, and repeatedly moving forward and backward, the fish struck and ate from the feeder; the second technique was to slowly cruise back and forth along the shield striking the bar and feeding on passing. Several fish were also exposed to an extinction procedure; that is, they were presented with the feeder as usual, however, the food reward was withheld. This procedure is routinely used to determine the nature of the conditioned response in operant conditioning experiments. Under these conditions the fish began pressing at a usual rate but by the end of the one-hour trial the rate of response had almost dropped to nil. For example, in three fish the rates of response per minute was 6.9, 5.6 and 4.3 under normal conditions.

but dropped to 2.0, 0.5 and 1.1 respectively after an hour of extinction conditions. It can, therefore, be concluded that responding by the fish was concomitant on receiving food for its effort. Furthermore, the compensation responses observed following a change in the schedule of reinforcement (Fig. 5), or in the deprivation period (Table 4) show that the fish had been conditioned to manipulate the feeder according to their needs.

Effect of changes in feeding interval (deprivation):

A long-term study was performed to determine the effects of different feeding intervals, i.e. 24 and 48 hours, between one-hour periods of access to the feeder. Food was provided on a schedule of continuous reinforcement. Table 4 summarizes the mean rates of response of each fish before and after the change in the period of deprivation. These rates were significantly different ($P < 0.001$) and although significant fish-to-fish differences were also found ($P < 0.001$), there was no evidence of interaction ($P > 0.05$); that is, each fish responded in a similar fashion to the change in the duration of the food deprivation period. This phenomenon obviously indicates a compensation reaction.

Table 4. The rates of response per minute in one-hour feeding trials of trout on a schedule of continuous reinforcement following different periods of deprivation (data shown in Appendix II).

Fish designation	24-hour deprivation <u>1/</u> (responses/minute)	48-hour deprivation <u>2/</u> (responses/minute)	Percentage increase
2	3.7	6.9	86.5
3	2.6	5.6	115.4
4	2.7	6.0	81.8
5	1.9	4.4	76.0

1/ Mean values of 14 daily feeding trials

2/ Mean values of 22 feeding trials

Effect of varying the ratio of reinforcement:

The effects of changes in the ratio of reinforcement on the feeding rate of the fish were examined in this experiment. All fish were given 2.4 mg. of food per reinforcement, the treatments differed only in the ratio of reinforcements to responses (bar presses). The analysis of results shown in Table 5 demonstrates significant fish-to-fish differences ($P < 0.025$) and highly significant treatment effects ($P < 0.001$). There is no evidence of interaction between these two variables ($P > 0.05$), indicating consistent responses by the fish to each treatment. Unfortunately, the order of presentation of treatments did not permit a separate estimate of the effects of time, although by inspection, there appears to be no trend. The mean rates of responses were 19.58, 13.76 and 14.80 responses per minute for FR3, FR6 and FR12 ratios of reinforcement, respectively. Comparisons among treatment means by the Student-Newman-Keuls procedure (Sokal and Rohlf, 1969) showed that the rates of response on FR3 and FR6 were significantly different ($P < 0.05$) from the rate obtained when fish were fed on an FR12 ratio of reinforcement.

In a second part of this experiment the same ratios of reinforcement were employed but the size of reinforcement was approximately three times greater

Table 5 . Rate of response (per minute) as influenced by different schedules of reinforcement with 2.4 mg. reinforcements. (data is shown in Appendix II)^{1/}

Fish	Schedules of reinforcement		
	FR 3	FR 6	FR 12
7	21.96	19.13	15.23
8	15.73	19.13	13.00
9	21.03	18.00	16.16

^{1/} Values quoted are the means of three feeding trials.

Table 6 . Rate of response (per minute) as influenced by different schedules of reinforcement with 7.7 mg. reinforcement, (data is shown in Appendix II)^{1/}.

Fish	Schedule of reinforcement		
	FR 3	FR 6	FR 12
2	15.0	8.7	11.1
4	22.0	17.4	19.7
6	14.7	11.4	11.0

^{1/} Values quoted are the means of four feeding trials.

(7.7 mg. reinforcement). The findings are outlined in Table 6. The analysis indicated significant fish-to-fish differences ($P < 0.001$), treatment effects and time course effects ($P < 0.025$). However, the interactions were not significant ($P > 0.05$). The treatment means were 15.96, 12.94, 12.49 for the ratios FR3, FR6 and FR12, respectively. Tests based on the Student-Newman-Keuls procedure indicated no significant differences ($P > 0.05$) between the rates of response on FR6 and 12 but both were significantly lower ($P < 0.05$) than the rate of response obtained when fish were reinforced on an FR3 ratio. These findings are in contrast to those observed in the first part of this experiment in which the distinction was not between FR3 and the higher two ratios but between FR12 and the lower two ratios. Thus, when presented with large reinforcements, the fish responded more slowly to infrequent rewards and did not make a distinction between the two more frequent rates of reinforcement. On the other hand, with smaller reinforcements the fish distinguished between these two more frequent rates of reward.

Effect of varying the size of reinforcement:

In this experiment, unlike the previous ones, a single ratio (FR.6) of reinforcement was used with different sizes (7.7 mg., 2.4mg. or 1.0 mg.) of reinforcement. The results shown in Table 7 indicate

highly significant fish-to-fish differences ($P < 0.005$) as seen in all other experiments. While the time effect was not significant ($P > 0.05$) the treatment effects (i.e. size of reinforcement) were significant ($P < 0.025$). However the data are complicated by significant interaction effects ($P < 0.05$). Whereas fish responded to changes in the size of reinforcements, at an FR6 schedule of reinforcement, in different ways. This may simply indicate that there are fish-to-fish differences in the level of performance on each size of reinforcement. Using the Student-Newman-Keuls procedure, no significant differences were obtained between the rates of response for a 7.7 mg. and 2.4 mg. reinforcement and between a 2.4 mg. and 1.0 mg. reinforcement ($P > 0.05$) on the other hand there is a significant difference between the rates of response for the largest (7.7 mg.) and smallest (1.0 mg.) sizes of reinforcement; ($P < 0.05$). This result is difficult to interpret because of the significant interaction effects. However it is consistent with the earlier experiments; that is one would predict a higher rate of response for a larger size of reinforcement, provided that the difference between the amounts of reinforcement were large enough.

Table 7. Feeding rates of fish on FR6 with three sizes of reinforcement, (data shown in Appendix II)^{1/}

Fish number	1.0 mg. reinforcement	2.4 mg. reinforcement	7.7 mg. reinforcement
1	7.7	11.2	11.9
3	12.1	12.4	17.0
5	12.8	15.5	14.1

^{1/} Values quoted are the means of three feeding trials.

Effect of simultaneously varying the size
and ratio of reinforcement :

The present experiment investigated the effect of using schedules balanced against the size of reinforcement so that a similar number of responses provided a similar amount of food. The findings are summarized in Table 8. Fish-to-fish differences were once again large but there was no interaction with the treatment ($P > 0.05$). Although there were significant treatment effects, no specific differences between treatment means could be established using studentized range tests. As expected, the rates of response under these balanced conditions were much closer to one another. It may therefore be said that a frequent small reinforcement (CR. 1.0mg) elicits an identical rate of response from the fish as an infrequent large reinforcement (FR8, 7.7mg), the difference between these treatment means being very small. Similarly, a moderate rate and size of reinforcement (FR 3 , 2.4mg.) elicited an identical rate of response to a frequent small reinforcement (CR. 1.0mg.). However, the fish tended to show a lower rate of response on the infrequent large reinforcement (FR 8, 7.7mg.) when compared to the moderate reinforcement (FR 3, 2.4mg.). Consequently, although these values were not significantly different, the trend shows that the moderate reinforcement

Table 8. Rates of response (per minute) of fish presented with balanced schedule and sizes of reinforcement which provide an average of 8.9, 9.4 and 9.2 mg/10 presses for schedule C.R., F R 3, and F R 8 (data shown in Appendix II) 1/.

Fish	Schedule and Size of Reinforcement		
	C.R., 1, 0 mg.	F.R. 3, 2.4 mg.	F.R. 8, 7.7 mg.
1	5.9	4.6	-
2	11.5	14.0	-
3	---	16.7	14.9
4	---	12.6	11.5
5	21.4	----	21.1
6	13.7	---	12.8

1/ Values quoted are the means of three feeding trials.

(FR 3, 2.4 mg) resulted in a rapid rate of response by the fish, the frequent small reinforcement (CR, 1.0 mg) resulted in the next best rate and an infrequent large reinforcement (FR 8, 7.7 mg) with the slowest rate of response. This sequence of events may have been expected from the previous study where, when the treatments (i.e. size and frequency of reinforcement) were unbalanced, fish reinforced on different schedules and sizes of reinforcement performed at different rates. The earlier results showed that the rates of response were influenced by both the rate and size of reinforcement. The present findings demonstrate that when these factors are balanced with each other, the resulting rates of response reflect this balanced condition. It seems clear that within limits, a high frequency of reward can compensate for a small quantity and similarly a low frequency of reinforcement can be compensated for by a sufficiently high reward. It is interesting to note, however, that within the range of values used in this experiment the highest rate of response resulted from a moderate reward reinforced relatively frequently. Since this experiment used an alternating series of treatments in a switch-back design it was possible to check for carry-over effects i.e. where the value of one treatment varies depending on the treatment it has been previously exposed to. This was

tested on the same data as shown in Table 8. There was no evidence that the results of the treatments were influenced by the order of presentation ($P > 0.05$).

Effects of DDT on feeding pattern:

These tests used seven fish which had been conditioned in earlier experiments and were feeding solely by this means for over ninety days. As several studies in the literature have reported behavioural effects of DDT on fish, it was felt that this was a good material to use for preliminary trials. The fish were treated by the method described earlier. One conditioned fish was exposed to each of the following concentrations of DDT and in the following order: 100 ppb, 40 ppb and 20ppb. They were each found dead within the 24-hour treatment period. Of four fish treated with 10 ppb DDT, one died within 24 hours, another dropped from an average feeding rate of 17.8 responses/minute in the two feedings prior to the treatment to 3.0 responses/minute just after the treatment, to <1 /minute 48 hours later and was found dead 96 hours after the start of the treatment. The feeding behaviour of the two other fish was apparently unaffected. In the two feedings prior to treatment they averaged 10.9 and 17.4 responses/minute while in the two sessions following treatment they averaged 10.3 and 15.8 responses/minute.

Maintenance requirements of brook trout:

One of the more important additional benefits obtained from the present operant conditioning studies was an accurate estimate of the total food intake. In addition to the precise dispensing method, the extruded food was cohesive and resisted disintegration in water. Furthermore, the close proximity of response and reward sites ensured minimal exposure to the water and rapid consumption. Thus, there was virtually no food wastage and the recorded number of reinforcements represented the actual food intake of the fish. Table 9 details the weight changes of the fish over the experimental period. If only wet weight changes are considered, an average gain of less than a gram in 3 months, the fish appear to have been supplied with a maintenance ration; that is, "the ration at which an animal would neither gain nor lose body mass under the particular set of environmental conditions" (Warren, 1971). When the utilizable daily calorific intake is calculated (Appendix III) the energy provided by the food (Table 10) is markedly lower than values reported (or estimated from their weights and nutritive composition) in the literature for the "maintenance" requirements of trout. The daily

Table 9. Analysis of the fish used in the conditioned feeding experiments (determined by the methods outlined in Table 1).

Fish number	Original wet weight (g)	Final wet weight (g)	Weight change (g)	Ether ^{1/} extract (%)	Water content ^{2/} (%)
1	25.1	26.5	+1.4	0.65	80.12
2	20.8	23.4	+2.6	1.55	64.07 ^{3/}
3	25.5	25.5	0.0	0.52	80.54
4	37.2	40.0	+2.8	1.86	78.71
5	22.7	20.0	-2.7	0.55	80.24
6	39.7	41.3	+1.6	1.66	78.90
7	23.9	22.0	-1.9	-	-
8	23.3	22.7	-0.6	0.87	80.02
9	24.1	21.8	-2.3	1.36	79.90

^{1/} Includes lipid, etc. expressed as % final wet body weight

^{2/} Expressed as % final wet body weight.

^{3/} Blood loss observed as fish was sacrificed.

Table 10. Estimated daily energy utilization based on observed food intake and body fat catabolism of the fish used in the conditioned feeding studies (constants used and calculations are shown in Appendix III).

Fish number	Experimental period (days)	Utilizable value of daily food intake (calories)	Proposed additional body fat $\frac{1}{2}$ (grams)	Total energy from fat (calories)	Daily energy from fat	Average total daily energy $\frac{2}{2}$ (calories)
1	88	63	0.8643	9146	104	167
2	73	102	0.5415	5117	70	172
3	74	84	0.8874	8386	113	197
4	69	155	0.8260	7806	113	268
5	79	72	0.7383	6977	88	160
6	88	166	0.9477	8956	102	268
7	-	-	-	-	-	-
8	78	72	0.7199	6803	87	159
9	88	79	0.5755	5438	61	140
$\frac{1}{2}$ Additional body fat = 4 % body weight - observed % body weight of fat						
$\frac{2}{2}$ Average available daily energy = value of food + value of fat						

maintenance requirements of each fish in the present study (is) about 78 calories for a 23 g. trout and 160 cal. for a 40 g. fish unless these values were supplemented by catabolism of body stores.

When the fish were analysed for moisture and fat content it was found that, in comparison with typical values obtained from growing brook trout (Phillips et. al. 1965) of 3.2- 4.4% body fat (wet weight) and 74.0- 79.0 % body moisture, with fat plus water representing 80-83% total weight, the composition of the experimental fish was slightly different. The body fat of the fish in the present study averaged only about 1% of the wet weight, while the moisture content was about 80% (Table 9). This tends to support the idea of an inverse relationship between body fat content and moisture content as also noted by Phillips et. al. (1965). Consequently, it seems possible that the experimental fish were supplementing their daily energy needs by fat catabolism.

Thus, the calculated maintenance energy requirements based on food intake alone could be considered underestimates of the true total energy requirements of the fish. The extent to which such catabolism of fat may supplement the dietary energy can be seen if the assumption is made that prior to the experiment the body fat may have been 4% of wet weight, a typical value for growing trout. The calorific supplement due to fat metabolism may now be introduced into the calculation of the daily energy budget (Table 10). The calculations are shown in Appendix III. The estimated daily energy utilization now changes from about 78 calories when food energy is only considered to about 170 calories for a 23 g. fish and from 160 calories for a 40 g. fish to 268 calories. However, even these values are still low compared with most of the values reported in the literature.

Schooling Behaviour

The groups of brook trout observed in an annular tank maintained themselves against the current as a 'compact school' (Kruzynski, 1972) occupying a relatively small portion of the tank. They held positions with no more than 'swimming clearance' (Breder, 1959) between each other. These distances were

so close that they could not be measured accurately from the film.

Although most of the fish observed in the groups studies appeared to be of equal status, two or three fish held positions about 15-20 cm. upstream from the group. Among these fish, one always was positioned with at least its head slightly ahead of the other one or two. This fish will be termed the α fish. Two different types of aggressive display were initiated by the α fish when a fish attempted to pass ahead of it. In the first type the α fish would 'nip' (Hoar, 1951) at this displaced fish in which case the fish usually returned immediately to its former position. The second type of display was as follows: The two fish would hold stationary positions parallel to one another and head to tail about two centimeters apart for several seconds. The pair would then rotate in synchrony for several more seconds making no apparent motions toward each other and maintaining the distance between each other. Finally, one would quickly turn toward the other and nip at its flank. The attacked fish would either turn towards its antagonist, nip it and continue circling or it would immediately retreat. In either case, sooner or later, the challenger would terminate this display pattern by retreating. The display did not usually last more than

10 to 15 seconds, the α fish was always the one challenged and no change of position was ever observed to occur; that is, the α fish always returned to its position with the challenger retreating to its place in the group.

When the α fish was removed overnight to another tank no other fish was seen to take its place and it resumed its former position and status when returned to the group. Such displays were never observed between other fish in the group.

Effect of dietary methoxychlor on schooling behaviour:

Following frame by frame examination of the film with a film editor it was clear that the spatial organisation of the group remained substantially the same before, during and after treatment with methoxychlor. Representative sampling of the daily variation was obtained by the following procedure. The area occupied by the group was measured on the film by selecting frames at 24-hour intervals (at 3 p.m.) for the last twenty days of the study. The area measured as the smallest rectangle which enclosed the group for the first ten days was $298.7 \pm 10.6 \text{ cm}^2$ ($\bar{Y} \pm \text{S.E.}$). For the last ten days it was $316.1 \pm 10.1 \text{ cm}^2$ ($\bar{Y} \pm \text{S.E.}$); there was no significant difference ($0.4 > P > 0.2$) between these values. The dominant and subordinate individuals were also clearly identified on the film. There were no changes seen in the

social structure of the group throughout the period of the experiment and no changes in individual behaviour were evident.

Feeding Behaviour of a Group of Fish

During any one experimental period it was found that a single fish fed from and defended the feeder. Apart from the occasional dash to the feeder made by one or two other fish, its dominance was total. This relationship was so definite as to make counting of the frames of film unnecessary. Table 11 indicates the status of each fish in the group which was elucidated by successively restraining in individual cages, the dominant fish as they came to express themselves.

Table 11: Status of fish in the group as measured by dominance of feeder in the first experimental session.

<u>Status</u>	<u>Fish no.</u>	<u>wt. (g)</u>	<u>Length (cm.)</u>
First	1	30.0	16.0
Second	7	30.5	15.5
Third	5	32.7	16.5
Fourth	6	22.0	14.0
Fifth	8	23.5	14.5
Sixth	10	24.0	15.0

It should be noted that the above order differed from that based on the observations using 'nipping' as a criterion. During the first few days of the experiment, prior to the removal of the fish which first fed from the feeder, a large proportion of the interactions

took place between the α fish (no. 1) and two other fish (nos. 7 & 8). On the basis of their aggressive behaviour these fish were classed as of higher status than the others. However, no. 7 ranked second and no. 8 fifth as determined by successive removal of the dominant fish. Furthermore, no. 5 fish which was never seen to attack any of the other fish and which was, in fact, the subject of attacks by fish found subsequently to have a lower status in Table 11, turned out to rank third in his ability to dominate the feeder.

Since the earlier experiment using the annular tank indicated that the pattern of dominance was apparently quite stable, it was anticipated that a similar result would have been obtained in the group feeding trials. However, manipulation of the social structure by caging the dominant fish and later reforming the group again and re-determining the hierarchy showed changes in the status of three of the six fish: no. 7 fish formerly second ranking became the α fish while nos. 1 and 5 now ranked second and third, respectively.

DISCUSSION

Feeding Behaviour of Individual Fish

Operant conditioning in brook trout:

Rozin and Mayer (1964) have compared the effects of various fixed-ratios of reinforcement on the performance of goldfish conditioned to 'bar press' for food pellets. Their fish showed compensatory responses to changes in frequency of reward similar to those found in a large number of experiments with pigeons and rats (c.f. Ferster and Skinner, 1957). Thus, during a one-hour feeding session, as the fish were placed on larger ratios of reinforcement which provided less frequent food rewards, the animal compensated to maintain its food intake by responding at higher rates. Figure 5 illustrates a logarithmic transformation of their findings where the ordinate represents the food intake attained as a percentage of that obtained on a continuous reinforcement schedule and the abscissa represents the fixed ratio of reinforcement schedule. The theoretical relationship is plotted to demonstrate the slope of the line obtained for constant response rates independent of the ratio of reinforcement. Compensation occurs if the observed rate of response at each 'FR' schedule is higher than the theoretical value. It can be seen that goldfish showed

considerable compensation. In the present study, fish provided with a 2.4mg. reinforcement showed compensation within the range of 'FR' schedules studied although the degree of compensation was below that of fish given a larger (7.7 mg.) reinforcement. Goldfish, given a 20mg. reinforcement, showed a greater compensatory response over a wider range of 'FR' schedules. From curves in Fig. 5, it seems likely that the degree of compensation for fixed-ratio schedules of reinforcement in fish depends on the size of the reinforcement given.

The smaller degree of compensation and steeper slope of the compensation curve of trout compared with that of goldfish may also reflect a species difference. It should be noted that goldfish are tropical fish having no stomach and are continuous feeders. Given continuous access to a feeder, goldfish feed at an evenly-distributed rate over a 24-hour period. On the other hand, trout are rarely found in water over 20°C (MacCrimmon and Campbell, 1969), have stomachs and are discontinuous feeders. Table 12 shows the feeding of a trout with continuous access to the feeder. It is clear that it did not spread its feeding responses evenly through time as was reported for goldfish by Rozin and Mayer (1964). Consequently, the differences between goldfish and trout shown in Fig. 5 may result from the fundamental differences in the type of

ecological niche occupied by these species. Trout may show a lower rate of compensation than goldfish in that they may not adapt to lower levels of food availability by feeding at a much faster rate, as goldfish do, but simply adapt to a lower food intake.

Highly significant fish-to-fish differences appear to be a consistent feature in operant conditioning experiments using fish. They are not only evident in the present studies but also in the studies of Rozin and Mayer (1964) and Gonzalez et al. (1967). Consequently, the technique of using each fish as its own control provides the most reliable experimental control.

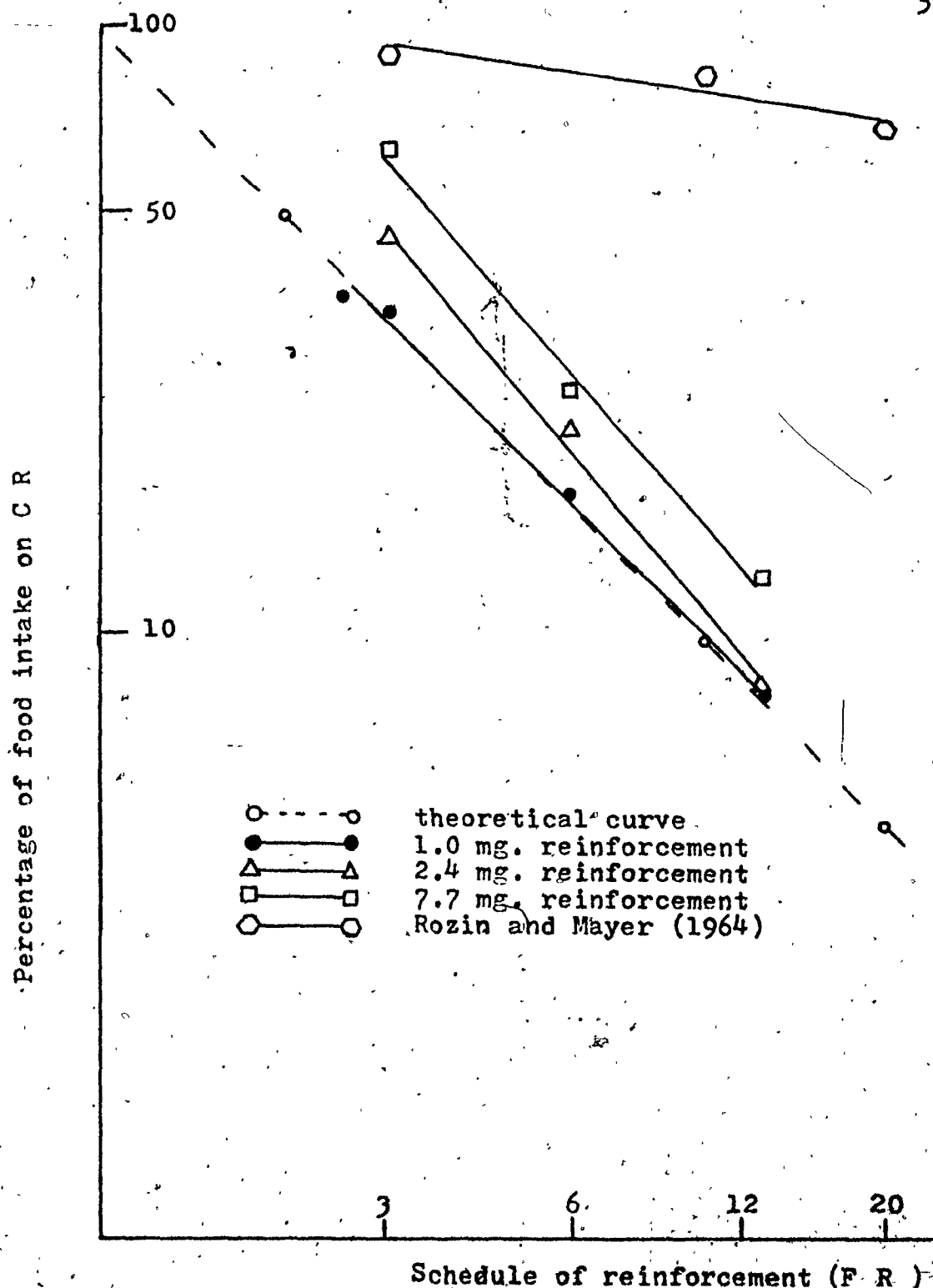


Figure 5 : Food intake on fixed-ratio schedules of reinforcement compared to that consumed on continuous reinforcement (C R).

Table 12. Pattern of feeding of a single trout given continuous access to feeder over 14 days.^{1/}

Date	Time of day at start of feeding	Approximate duration of feeding (min.)
Sept. 7th	12:25	180
Sept. 8th	-	-
Sept. 9th	-	-
Sept. 10th	-	-
Sept. 11th	-	-
Sept. 12th	10:00	15
Sept. 13th	07:00	30
	11:15	15
	19:40	30
Sept. 14th	13:05	60
Sept. 15th	10:15	30
Sept. 16th	09:30	50
Sept. 17th	17:00	60
Sept. 18th	11:00	10
Sept. 19th	13:15	60
Sept. 20th	17:00	60
Sept. 21st	19:00	40

^{1/} 06:00 to 19:00 hours light photoperiod.

Ecological implications:

The findings provide experimental data which can be used to test models of predatory or feeding behaviour of fishes. One such model (Fig. 6) designed to allow prediction of feeding rates of a monophagus species has recently been presented by Colgan (1973). Essentially, he suggests that deprivation is an independent variable which influences gastric volume and, at a slower rate, systemic needs which are the two vectors determining the state of hunger of the fish. Hunger, in turn, influences the probability of eating a presented item. In the present experiment food palatability was high and food was not rejected: the probability of eating a presented item was thus unity. The food intake was supplemented so that all the fish received the same amount of food as the maximum obtained by a fish in the experiment from the feeder alone. Therefore, the state of hunger of all fish, within any one set of experimental conditions, was the same. The analog of the encounter interval of Fig. 6 is the interval between reinforcements and the effort which the fish puts into searching for food can be measured by the rates of response recorded. Feeding rate (F) would therefore equal the encounter or reinforcement rate since the probability of eating (P) is unity. Colgan pointed out that this model may also explain why the effect of

different deprivation periods is best seen when the time available for feeding is limited. Under these conditions there would be increased food searching and a greater probability of encountering prey resulting in an increased feeding response. Obviously, the level of hunger is higher at the beginning of a feeding bout and is reduced as feeding goes on: therefore, the state of hunger of the fish would be most obvious at the beginning of a feeding period. This hypothesis was supported by the present finding as well as those by Moore (1941), Tugendhat (1960) and Beukema (1968). The rate of feeding in the 15-minute trials generally did not change during the feeding trial whereas in the 60 minute trials the rate tended to decrease. Furthermore, Colgan's model suggests that feeding rate is also influenced by another independent variable i.e. 'prey properties'. In the present studies the food represented a prey whose properties of taste, size and availability could all be experimentally manipulated to vary the rate of response.

In fact, the experiments using both fixed ratios of reinforcement and different sizes of reinforcement demonstrated that the findings were as predicted by Colgan (1973). When the relative availability of the 'prey' increased (i.e. at higher ratios of reinforcement)

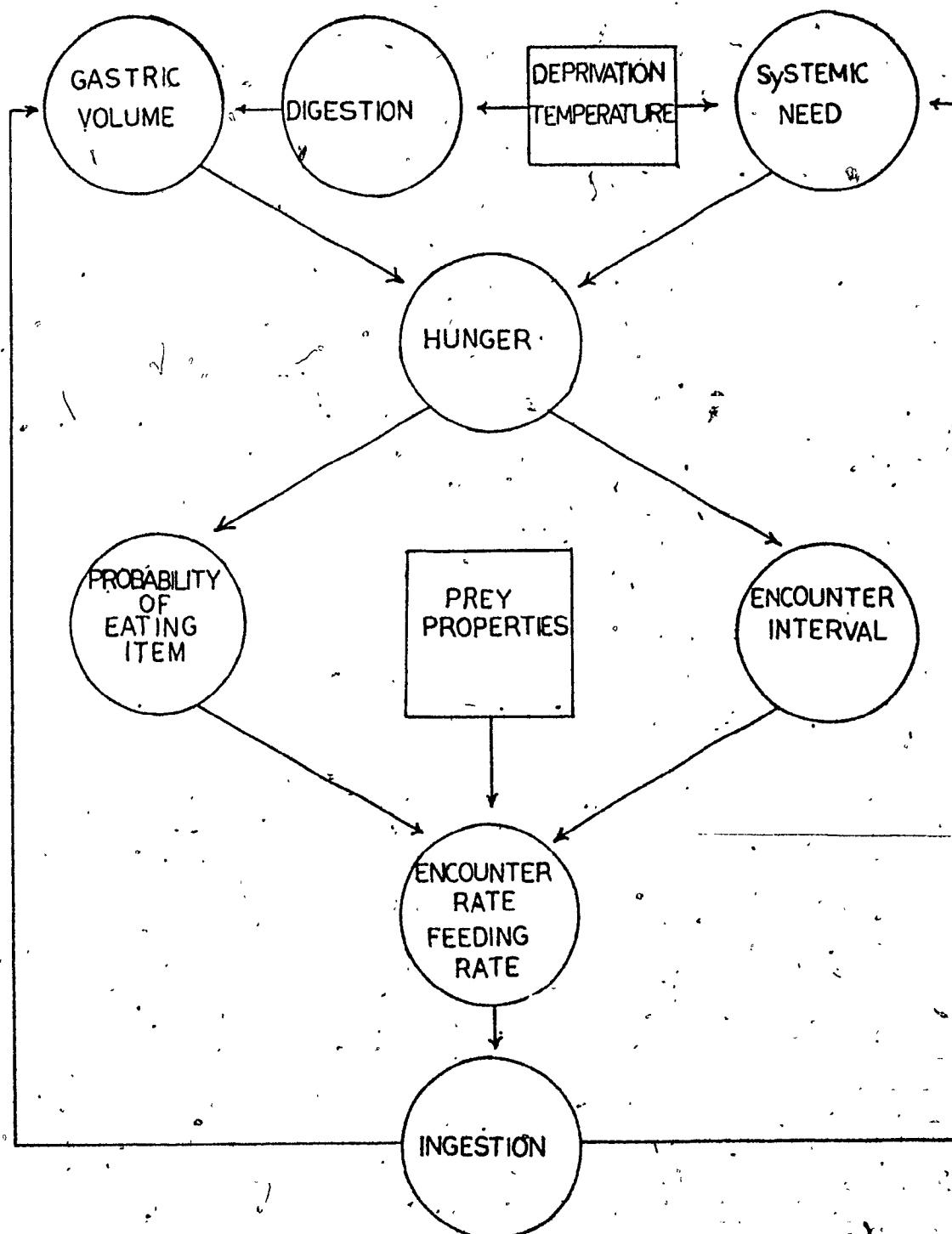


Figure 6: Diagram illustrating a model of the motivational analysis of fish feeding behaviour (after Colgan, 1973).

or the size of the 'prey' increased, the feeding rate increased.

Ware (1972) has found that the searching behaviour of rainbow trout (Salmo gairdneri) waned when food captures did not exceed a particular rate. Similarly, in the present studies, feeding behaviour waned under a low or infrequent rate of reinforcement. As the feeding habits of these two species is similar, this feeding behaviour may represent a general homeostatic mechanism balancing food intake against energy output in trout; that is, this feeding pattern may be adapted to minimise energy expenditures in the search of food, unless there is a high probability of a net energy gain.

This behaviour seems nonadaptive in that it incorporates a positive feedback loop which might ultimately lead to starvation. If food shortage leads to reduced searching activity which lowers further the amount of food consumed the viability of the organism is endangered. However, other responses might be elicited as starvation increases to get out of this cycle. For example, depletion of fat stores may once again stimulate appetitive activities. If a predator reduces its feeding activity when the prey is in short supply, this will also tend to prevent the total elimination of the prey with ultimate benefits to its own population (Wynne-Edwards, 1962).

Figure 7 demonstrates that the relation between prey availability and rate of response is also a function of prey size. Whereas, the response rate is high for a readily available small prey, the rate for a larger prey is more sensitive to availability. Thus, while the rates of responses for a 2.4mg. prey were not significantly different when it was available once every three or six responses, the rates were different at these levels of availabilities when the prey was 7.7 mg.

The last experiment examined the influence of prey size on feeding behaviour. Figure 8 illustrates the relation between prey or reinforcement size and the feeding rate. It is clear that, at the availability of one reinforcement for six responses, the rate of response was directly related to the size of reinforcement. Raymond, et al. (1972) have also shown similar behaviour in goldfish conditioned to swim a runway (trough) for a food reward. They found that the fish swam faster for a larger reward but more slowly for a smaller reward. Ware (1972) stated that feeding behaviour was predominately determined by visual feedback in trout and therefore prey size influenced the feeding responses visually only. This mechanism seems very unlikely under the present feeding conditions where the prey all possessed the same degree of conspicuousness and likely difficult to discriminate visually. However the

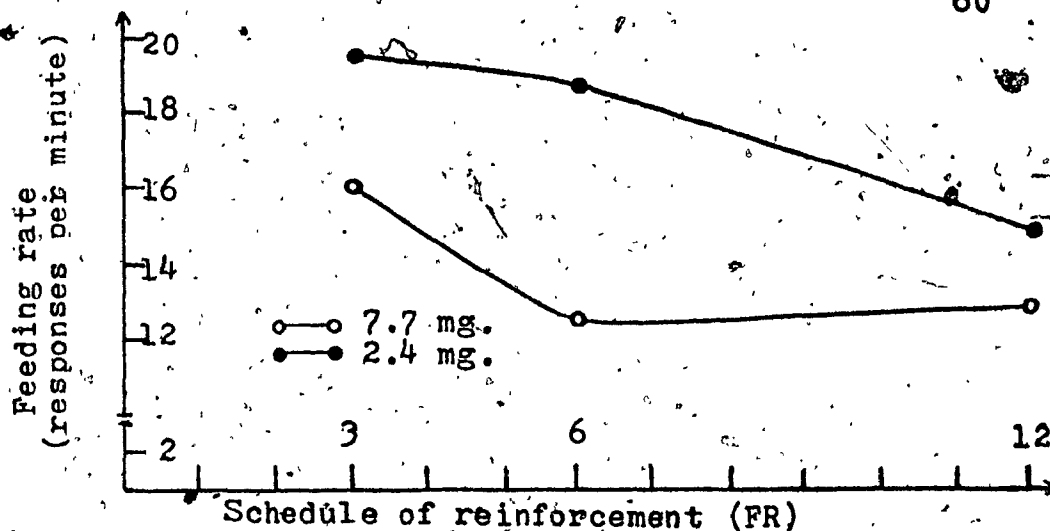


Figure 7: Changes in the feeding response of conditioned brook trout on an FR3, FR6 or FR12 schedule of reinforcement given either a 2.4 mg. or 7.7 mg. reinforcement (based on data shown in Table 5 and Table 6).

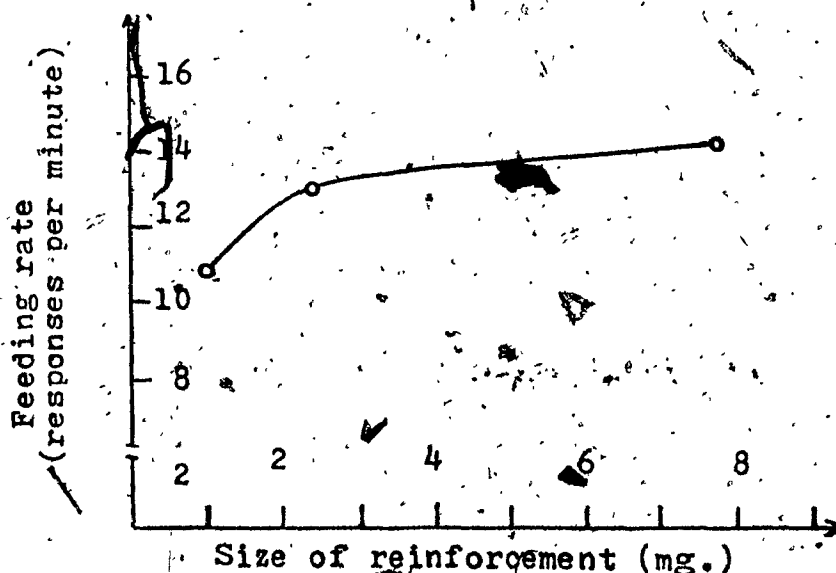


Figure 8: Changes in the feeding response of conditioned brook trout on an FR6 schedule of reinforcement given either a 1.0 mg., 2.4 mg. or 7.7 mg. reinforcement (based on data shown in Table 7).

fish clearly were able to distinguish prey 6.7 mg. different in size. Consequently, not only can feeding behaviour in trout be modified by the visual characteristics of the food, but it seems likely that other sensory loops may exist; for example, there may be both a response to swallowing and feedback from proprioceptors of the gut as suggested by Hamilton (1965).

Psychological implications :

This conditioning study also provides information which is relevant to a controversy about the similarity of the learning process throughout the vertebrates. On one hand, Bitterman (1965) Gonzalez and Bitterman (1967) and Lowes and Bitterman (1967) studying goldfish contended that fish, in contrast to higher vertebrates, notably rats and pigeons, showed the so-called 'reinforcement principle; that is, if trained to work for one size of reinforcement, their performance rate was not altered if the reward size was reduced. This principle presupposes that once nervous connections are made for the conditional response, these connections cannot be modified. On the other hand, Setterington and Bishop (1967) and Mackintosh (1971), Mackintosh and Cauty (1971) and Raymond et al. (1972) argue that fish respond in essentially the same way to reinforcement size as do higher vertebrates and do not exhibit the 'reinforcement

principle.

The present study supports the latter argument. It was clearly shown that trout modified their rates of response according to the size of reinforcement dispensed; that is, a faster rate for a large reinforcement and a slower rate for a smaller reinforcement, irrespective of the order of presentation (Fig. 8).

Maintenance requirements of brook trout:

Brown's (1946) experiments on maintenance requirements of brown trout (Salmo trutta) are a classic study which have served as a baseline for many other workers. For example, her data have been used to develop parameter values for recent mathematical models of fish nutrition by Paloheimo and Dickie (1966) and Hastings (1969). By converting her data to daily energy requirements of trout (Appendix III), Brown's data would predict a daily energy requirement (at 11.5° C) of about 635 calories for a 23g. trout and about 736 calories for a 40 g fish. These values are considerably higher than the values obtained in the present study (78 cal./day for a 23 g fish and 160 cal./day for a 40 g fish) even when fat catabolism was assumed to have occurred (170 cal./day for a 23 g fish, 268 cal./day for a 40 g fish). On the other hand, Brocksen (1966) obtained data for cutthroat trout (Salmo clarki) which give values similar to those found in the present study. His

graph predicts a calorific need for 'no growth' of 172 cal./day for 23 g fish and 300 cal./day for a 40 g fish at 8.5°C (Appendix III). It would be expected that the daily energy requirements at the temperature of the present studies ($11 \pm 1^{\circ}\text{C}$) would be higher. In fact, as previously mentioned, they were lower. It is also interesting to consider the estimates obtained from the respiration studies of Beamish (1964). His data predict a basal respiration rate of 26.18 ml oxygen/day and 55.2 ml/day for 23 g and 40 g fish respectively at 10°C. If we use the reasonable value for the calorific equivalent of oxygen of 4.8 cal./ml. these respiration rates indicate daily basal calorie requirements of about 125 calories and 265 calories/day (Appendix III).

It can be seen that the calorie intake of the fish in the present study at "maintenance level" is well below even that predicted to be the basal requirement i.e. that of a fish extrapolated to zero activity. Even when allowance is made for energy obtained from the catabolism of internal fat stores the estimated caloric requirements are still no greater than the values predicted by Beamish (loc.cit.) as basal. However, as the fish in the present study were clearly at a routine rather than basal level of metabolism the present results suggest, therefore, that maintenance levels of food and basal energy

requirements have together been overestimated by many of the earlier workers. There are several possible explanations. It seems likely that in group feeding trials in the laboratory the fish may be faced with the stresses of social interaction and activity which may elevate their metabolic requirements. Warren and Davis (1967), in a study comparing trout in a stream with those in the laboratory, found the latter more active than the former. Furthermore, in a recent study, Sparks et al. (1972) showed that subordinate fish were more susceptible to toxic doses of zinc than dominant ones. They also noted similar findings for other groups of animals and suggest that the stress of social interaction was responsible for this phenomenon. In the present study the fish were isolated in individual tanks. Another major problem in some of the earlier work has been the underestimate of energy losses in unconsumed or undigested food as well as losses of nutrients into the surrounding water when the food was not immediately consumed. Although it is unlikely that these losses could be totally eliminated, the present system minimized this source of error.

With respect to the respiration studies of Beamish (loc.cit.) several sources of error may be cited which might bias his data upward. Halsband (1953) showed that even slight movement of the pectoral fins of a fish

in a respirometer had a significant effect upon its rate of oxygen consumption. Furthermore, O'Hara (1971) showed that the respiratory rate of fish in a respirometer was greatly elevated initially and suggested a minimum period of 24 hours for acclimation to the apparatus. Beamish began measurements 16 hours after the fish were placed in the respirometer. From observations made during the present study it seems likely that the duration of the stress following handling of trout during weighing, measuring, or transfer from the living tank to a respirometer has been underestimated. For example, when fish which had been feeding with the operant conditioning feeder daily for two weeks were weighed in a manner designed to minimise stress as previously described, they all stopped feeding. It took from 3 to 6 days for them to resume their normal pattern. Kinne (1961) has also examined the effects of various methods of fish handling for the purpose of weighing and measuring, and has also shown considerable changes in feeding behaviour following the handling, even for very short periods, of the desert pupfish (Cyprinodon macularius). If such brief handling and return to their normal living tank is sufficiently stressful to totally inhibit feeding for several days it seems unlikely that a 24-hour period is long enough to lower the metabolic rate to normal levels particularly when they are simultaneously

being transferred to a new environment.

Consequently, values currently used to estimate basal caloric needs may include a metabolic contribution due to a high stress environment. Before more definitive statements could be made, it would be necessary to perform further experiments to determine precisely the proportion of caloric requirements supplied by endogenous catabolism when trout are fed at "maintenance" levels. Since the calculation of the nutritional budget was not a primary aim of the present study this aspect was not followed up in detail. Nevertheless the data presented point out the need for further nutritional studies to provide a better understanding of the metabolic requirements and energy budget of trout.

Effect of DDT on feeding pattern :

If operant feeding behaviour is to have general application in testing sub-lethal effects of pollutants it is obviously desirable to have a feeding rate which remains relatively constant from trial to trial but which, at the same time, is sensitive to stressing agents. In addition, the choice of reinforcement schedule and size of reward should develop a rate of response which is susceptible to experimental manipulation. For example, if the rate were high relative to the maximum rate at which a fish was capable of working or to the theoretical maximum

of the apparatus, the system would be less sensitive to upward shifts and more sensitive to downward shifts of rate. Also, a rate of response either too high or too low might place the animal on a nutritional level which could mask the effects of a variable (e.g. an insecticide) being investigated; for example, it has been shown that the sensitivity of brook trout to the insecticide methoxychlor differs with the amount of food intake (Oladimeji, personal communication). The principal aim of the earlier experiments was to clarify precisely how the fish would respond to changes in the quantity and availability of food in order to establish conditions which would provide the desired properties of sensitivity coupled with low variability. The conditions selected in the DDT trial, an FR3 schedule of reinforcement with a reinforcement size of 2.4 mg., appeared optimum within the range of conditions tested in these earlier experiments.

The results of the trials with DDT illustrate several points: first the concentrations chosen in this experiment (10-100ppb) were based on those used by other workers in investigating the behavioural effects of sublethal doses of DDT on brook trout. These earlier values ranged from 20-300 ppb. (Anderson, 1968, Anderson and Peterson, 1969, Anderson and Prins, 1970, Jackson *et al.* 1970). In the light of the present results, i.e. 100%

mortality within 24 hours with concentrations of 20 ppb. or more, as well as from trials performed later in this laboratory which indicated a 96-hour TLM of less than 10 ppb., it is difficult to understand how brook trout survived the concentrations used by the workers cited. In the present study the DDT was delivered with the inflowing water so that its concentration in the tank should remain constant over the 24-hour treatment period (the manufacturers' reported error of the syringe pump is $\pm 0.1\%$). In the studies referred to above the DDT was simply dissolved in acetone and added to the tank only once at the beginning of the treatment.

Although the data are very limited, the results obtained at the concentration of 10 ppb. suggest that feeding behaviour may be rather insensitive to treatment with DDT. At this near-lethal concentration, the fish either showed no change in its feeding pattern or died soon after the treatment. This "all-or-none" effect may indicate that the dosage-response curve of feeding rate to DDT is extremely steep. This would be consistent with the dosage-mortality curve for trout exposed to DDT which shows a very narrow margin between high mortality and high survival (Marking, 1966).

Schooling Behaviour

It was hoped that long-term observations, recorded on film, would provide quantitative data on fish-to-fish distances. These could be analyzed by nearest-neighbour methods (Clark and Evans, 1954) which have been widely used to describe groups of fish (Breder, 1959; Whitney, 1969; Moss and McFarland, 1970).

By maintaining a shallow water depth (4 cm.), it was expected that the two-dimensional photographs taken overhead would be sufficient to describe the school. However, this procedure was not entirely satisfactory since some vertical separation of the fish occurred. Furthermore, the horizontal distances between fish were extremely small and could not be accurately determined on film. On the other hand, the analysis of agonistic interactions provided information on the social hierarchy. These observations supported Newman's (1956) concept of a 'despotic dominance subordination' relationship amongst brook trout. In his study 10 of 12 groups of four fish showed no change in hierarchy once it became established. In the present study, in two groups of fish the situation was similarly stable even when the α fish was removed and returned several times.

It is difficult to isolate the factors which determine the spacing distance in fish schools.

McFarland and Moss (1967) have listed a number of environmental factors which might influence schooling behaviour including temperature, light salinity and water chemistry. Moss and McFarland (1970) showed that spacing was not influenced by any acceptable level of dissolved oxygen and/or carbon dioxide in the northern anchovy (Engraulis mordax), but it was affected by rapid changes in these factors. In the present study, dissolved oxygen was kept at saturation and it is not likely, therefore, that it played any major role in determining the spacing observed. Furthermore, the pH and other characteristics of the water chemistry of the city water supply did not change at any time during the experiment (Appendix I). Breder (1959) and Whitney (1969) described the effect of light on schooling patterns of many fishes. In general, schools tend to break up in low light conditions but responses to higher light levels are species specific. Gibson and Keenleyside (1966) showed that brook trout become photonegative only at illuminations above 164 foot candles. In the present study the illumination was 130 foot candles. At this intensity the fish form schools but not tight groups to seek each other's shadow.

Methoxychlor appears to be a pesticide which is not particularly effective in inducing behavioural changes in salmonoids. In another study, Hatfield and/

Johansen (1972) exposed Atlantic salmon parr (Salmo salar) to a sublethal concentration of methoxychlor (90 ppb.). They were unable to establish any effect on the learning of a simple conditioned response whereas the insecticides sumithion, abate and DDT were effective at similar concentrations. It may be that the original change in spacing observed by Kruzynski (1972) was caused by something other than the methoxychlor, particularly, since it was only observed in the second part of his second experiment. It is interesting to note that it was also only in this segment of his study that an electric "shock ring" was introduced to his tanks to prevent the fish from drifting in the water current.

Although no changes in the social hierarchy through differential effects of methoxychlor on dominant and subordinate individuals were observed, recent studies by Sparks et al. (1972) have shown that in groups of Bluegills (Lepomis macrochirus) dominant fish survived longer than subordinate ones when exposed to a lethal concentration (32 ppm) of zinc.

Feeding Behaviour of a Group of Fish

This study has placed doubt upon the use of aggressive behaviour as the sole determinant of position in the social hierarchy. Many previous studies of these relationships have usually evaluated status on the basis

of such agonistic displays as 'nipping' i.e. "a frontal display characterized by a darting approach followed by a threat bite" (Hoar, 1951), changes in this display have been used to measure the effects of such factors as population density (Fenderson and Carpenter, 1971) and food deprivation (Moore, 1941). Wootton (1971) has reviewed and found consistency in the various behavioural correlates such as nips and spine raising used to measure aggression in groups of the three-spined stickleback (Gasterosteus aculeatus). However, Miller (1957) and Hinde (1959) warned that no single measure of a behavioural sequence was sufficient to fully describe that behaviour. The present findings, which indicate substantial differences between the hierarchy based on aggressive displays and the feeding hierarchy in brook trout, support this argument. Furthermore, the feeding hierarchy could not be accounted for in terms of body size (see Table 11) as had been suggested, based only on intuitive estimates by Kruzynski (1972) and Newman (1956) for brook trout. There may be a relationship between body size and status in the feeding hierarchy, or willingness to nip and position in the hierarchy. The present technique provides a means of checking these relationships.

Although the observed changes in feeding hierarchy were in contrast to the stability seen in the

maintenance of spatial position within a school, they were not entirely unexpected. Several researchers have demonstrated the effect of experience on various behaviours including predation in rainbow trout (Ware, 1971), aggressiveness in cichlids (Gallagher et al., 1972) and schooling behaviour in Atlantic silversides (Williams and Shaw, 1971).

This difference may be accounted for in at least two ways. In the first case the conditions under which the fish of each experiment were isolated after removal were different. The fish of the first study were completely isolated in separate tanks while in the second study each fish was confined in a mesh cage within the experimental tank i.e. not visually isolated. Also, in the latter case they were isolated for varying periods, from five days for the first dominant fish to one day for the fourth dominant fish. While in the former case the dominant fish was only isolated overnight.

SUMMARY

Feeding Behaviour of Individual Fish

Feeding behaviour in brook trout was investigated by conditioning the fish to press a bar on a feeder (termed a 'response') in order to receive a food reward (termed a 'reinforcement'). By allowing the fish one-hour access to the feeder every 24 and then 48 hours, it was found that the fish doubled their feeding rate to compensate. The effect of changes in the effort required to receive a food reward was studied by varying the response: reinforcement ratio i.e. by using three fixed ratio schedules of reinforcement (FR3, FR6, FR12). In all cases, with either 2.4 mg. or 7.7 mg. reinforcements, the fish responded at faster rates with a more frequent ratio of reinforcement (FR3). The influence of the size of the reward was investigated by dispensing 1.0 mg., 2.4 mg. and 7.7 mg. reinforcements on an FR6 schedule. A direct relationship between the reinforcement size and the rate of reinforcement was found. Both these parameters were varied simultaneously so that the amount of food received after the same number of response was about the same. The fastest rate of response was elicited by a moderate ratio and size of reinforcement (FR3, 2.4 mg.) while frequent small reinforcement (continuous reinforcement, 1.0 mg.) gave a slower rate and an infrequent large reinforcement.

(FR8, 7.7 mg.) resulted in the slowest rate of response. The effect of DDT was found to be lethal within 24 hours at concentrations greater than 10 ppb. At 10ppb, one fish was unaffected while two other fish died within 96 hours. The daily maintenance requirements of the 23g. and 40g. fish in the feeding studies were found to be 78 calories and 160 calories.

Schooling Behaviour

The groups of brook trout studied in an annular tank formed a 'compact school'. Although distances between most fish were small one fish, termed the α fish, was always positioned ahead of the school. Two types of aggressive display were observed to occur between the fish and others of the group. No change in position or status of the α fish was observed. Dietary methoxychlor (2mg/kg. body wt./day) did not influence the spatial distribution or social structure of the school.

Feeding Behaviour of a Group of Fish

The feeder, used in the experiments on the feeding behaviour of individual fish, was presented to a group of six brook trout in a large rectangular tank. The social status of each fish in the group, based on its feeding behaviour, was determined. The aggressive activities observed in the schooling behaviour study were also

observed here. However, the social structure of the group was apparently influenced by the experimental manipulation of the fish.

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CHEMICAL ANALYSIS OF TREATED WATER FOR THE CITY OF MONTREAL

MAY 1972 TO APRIL 1973

The results are expressed in milligrams per litre, except pH and turbidity.

Data supplied by the Municipal Water Works, City of Montreal.

APPENDIX II

Effect of changes in feeding interval (deprivation) on the feeding rate of trout for one hour feeding trials either every 24 or 48 hours.

Feeding interval	Number of responses per feeding trial			
	Fish designation			
24 hours	2	3	4	5
	226	168	158	86
	200	183	240	223
	156	173	190	176
	232	187	120	82
	241	178	138	122
	174	152	167	148
	239	150	152	55
	194	89	154	90
	274	186	123	142
	219	158	134	92
	221	117	187	66
	234	129	158	161
	199	137	135	80
	249	110	158	91
	246	172	167	98
48 hours	341	194	236	209
	348	223	246	222
	438	272	253	214
	360	269	295	233
	361	295	210	257
	350	243	263	150
	401	268	364	264
	380	273	357	216
	435	331	276	193
	413	292	339	191
	412	294	307	220
	351	340	449	323
	444	363	398	276
	483	385	406	295
	430	464	429	329
	417	414	408	291
	400	330	400	285
	430	420	396	296
	438	467	360	294
	476	350	353	319
	474	451	496	298
	456	300	409	330

Effect of varying the ratio of reinforcement, in which a 2.4 mg. reinforcement was dispensed, on the feeding behaviour of individual brook trout 1/.

Date	fish designation			Amount of food eaten by each fish (mg.)
	7	8	9	
April 6	3 341 102	12 250 40	6 168 13	244.8
April 9	3 338 101	12 272 43	6 239 19	242.4
April 12	3 310 93	12 288 45	6 178 13	223.2
April 15	12 200 16	3 225 71	3 272 83	199.2
April 18	12 221 16	3 202 36	3 302 47	112.8
April 21	12 265 20	3 280 26	3 374 105	252.0
April 24	6 287 40	6 365 62	12 276 20	148.8
April 27	6 287 39	6 276 43	12 240 18	103.2
April 30	6 288 41	6 280 43	12 212 22	103.2

1/ Data for each feeding trial of each fish is presented as follows: Schedule (FR3, FR6 or FR 12)
 number of responses in the 15-minute feeding trial
 number of reinforcements in the feeding trial

Effect of varying the ratio of reinforcement, in which a 7.7 mg. reinforcement was dispensed on the feeding behaviour of individual brook trout 1/.

Date	Fish designation			Amount of food eaten by each fish (mg.)	
	2	4	6	no. 2	no. 4 & 6
March 31	12 179 15	6 307 48	3 217 65	276.0	494.0
April 3	12 143 11	6 172 21	3 228 69	299.0	524.0
April 6	12 179 14	6 231 36	3 221 67	276.0	509.2
April 9	12 168 13	6 333 52	3 216 63	178.8	478.8
April 12	3 180 59	12 261 20	6 110 17	448.4	766.6
April 15	3 183 58	12 244 19	6 137 21	440.8	766.6
April 18	3 275 84	12 333 25	6 189 28	638.4	1120.0
April 21	3 260 79	12 340 22	6 246 34	600.4	1044.2
April 24	6 315 48	3 406 123	12 321 21	552.0	934.8

Date	fish designation			Amount of food eaten by each fish (mg.)	
	2	4	6	no. 2	no. 4 & 6
April 27	6	3	12	506.0	881.6
	226 30	371 116	217 16		
April 30	6	3	12	506.0	881.6
	240 37	405 116	157 11		
May 3	6	3	12	483.0	851.2
	246 39	368 112	146 11		

1/ Data for each feeding trial of each fish is presented as follows:

Schedule (FR3, FR6, FR 12)

number of responses in the 15 minute feeding trial

number of reinforcements in the feeding trial

Effect of varying the size of reinforcement, using an FR6 schedule, on the feeding behaviour of individual brook trout 1/.

Date	fish designation			Amount of food eaten by each fish (mg.)
	1	3	5	
April 6	7.7 160 26	2.4 141 23	1.0 218 45	197.6
April 9	7.7 191 30	2.4 152 24	1.0 191 29	228.0
April 12	7.7 135 22	2.4 194 31	1.0 168 26	167.2
April 15	2.4 105 17	1.0 144 23	7.7 182 28	212.8
April 18	2.4 156 23	1.0 202 31	7.7 231 36	273.6
April 21	2.4 224 26	1.0 199 25	7.7 224 34	258.4
April 24	1.0 163 24	7.7 307 48	2.4 244 35	364.8
April 27	1.0 69 10	7.7 316 50	2.4 236 33	380.0
April 30	1.0 117 18	7.7 248 40	2.4 220 31	304.0

1/ Data for each feeding trial of each fish is presented as follows: Size of reinforcement (mg.)
number of responses in 15-minute feeding trial
number of reinforcements in the feeding trial

APPENDIX II cont'd...

Effect of simultaneously varying size and rate of reinforcement on the feeding behaviour of individual brook trout 1.

Date	fish designation						Amount of food eaten by each fish (mg.)
	1	3	5	7	8	9	
March 19	B 75 28	C 253 30	A-A 114 96	C 260 30	A 219 185	B 237 93	228.0
March 22	A 84 56	B 158 63	B 200 75	A 228 195	C 206 24	C 158 19	228.0
March 25	B - -	C 117 14	A 185 158	C 387 41	A 173 152	B 241 94	311.6
March 28	A 104 93	B 210 83	B 191 76	A 312 264	C 224 27	D 263 30	282.4
March 31	B 114 45	C 145 17	A 220 191	C 302 33	A 225 191	B 275 104	250.8
April 3	A 79 68	B 198 78	B 241 93	A 423 338	C 204 25	C 249 29	338.0

1 Data for each feeding trial of each fish is presented as follows:

Schedule (A, B or C)

number of responses in the 15 minute feeding trial

number of reinforcements in the feeding trial

where, A=CR, 1.0 mg.

B=FR3, 2.4 mg.

C=FR8, 7.7 mg.

Daily food energy consumption in feeding behaviour studies of individual fish 1/.

Fish designation	Total food consumption (grams)	Experimental period (days)	Average daily food intake (grams)	Calorific value of daily food intake (calories)
1	6.66	88	0.07	63
2	7.97	73	0.12	102
3	7.20	74	0.10	84
4	12.28	69	0.18	155
5	6.54	79	0.08	72
6	17.71	88	0.19	166
8	6.45	78	0.08	72
9	7.95	88	0.09	79

1/ Based on cumulative data on all feeding studies of individual fish.

Summary of calorific conversion of food constituents.

Constituent	Total available calorific value (Kcal./g. food)	Digestibility coefficient	Energy Available to trout (Kcal./g. food constituent)
Protein	4.35 <u>1/</u>	0.90 <u>1/</u>	(5.65-1.3) (0.90) = 3.9
Fat	9.45 <u>1/</u> ⁹	0.85 <u>1/</u>	(9.45) (0.85) = 8.0
Carbohydrate	4.1 <u>1/</u>	0.86 <u>1/</u>	(4.2) (0.86) = 3.5
Starch	4.2 <u>1/</u>	0.40 <u>1/</u>	(4.2) (0.4) = 1.68
Guar gum	2.5 <u>2/</u>	0.76 <u>2/</u>	(2.5) (0.76) = 1.90

1/ Phillips (1972)2/ Altman and Dittmer (1968)

Determination of calorific value of food used in the feeding behaviour of individual trout experiments.

Constituent	Constituent content in food (mg./g)	Energy Available to trout from food (cal./g food constituent)
Protein	143	557.7
Fat	21	168.0
Nitrogen Free Extract <u>1</u> /	67	147.4
	total=	873.1

1/ Based on the calorific value of carbohydrate and a digestibility coefficient of 0.53 determined from the guar gum and estimated starch content of the food.

Estimated percent composition of Brown's (1946) ration 1/

Component	fat	carbohydrate	protein	ash	water
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Liver	2.82	3.94	14.78	0.96	77.5
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Minced beef	7.10	-	14.69	0.71	77.5
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Overall	5.67	1.31	14.72	0.79	77.5
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1/ Based on typical values for liver and lean hamburger given by Altman and Dittmer (1968).

Calorific conversion estimate of Brown's (loc. cit.) ration.

Constituent	Constituent content in food (mg./g.)	Energy available to trout from food cal./g food constituent
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Fat	56.7	453.60
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Protein	147.2	574.08
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Carbohydrate	13.1	45.85
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Using Brown's (loc cit.) data 40 g.

trout would consume a maintenance ration of 120 mg./g. fish /week or 0.6857 g. wet weight of food/day. Thus the maintenance requirement is 0.6857 g. x 1074 cal./g. = 736 cal/day.

Similarly, a 23 g. fish would consume 180 mg. /g. fish/week or 0.5910 g. wet weight food/day.

Thus the maintenance requirement is $0.5910 \text{ g.} \times 1074 \text{ cal/g.}$
 $= 635 \text{ cal./day.}$

Based on Brocksen's (1966) data for Salmo clarki at an average temperature of 8.5°C , the maintenance requirement was 9.6 cal. food/ Kcal. fish/day (from their Fig. 7). Trout on a low ration have an energy value of 4.22 cal./g. dry weight of fish (Phillips, 1965). Now, a 23 g. fish of 18.5% dry weight = 4.255g. and has an energy value of :

$$4.255 \text{ g.} \times 4.22 \text{ cal/g.} = 17.9561 \text{ k cal.}$$

Again, a 40 g. fish of 18.5% dry weight = 7.4 g. and has an energy value of:

$$7.4 \text{ g.} \times 4.22 \text{ cal/g.} = 31.228 \text{ k cal.}$$

The daily maintenance requirement can be calculated:

$$\begin{aligned} \text{A 23 g. fish, } 9.6 \text{ cal./k cal.} \times 17.9561 \text{ k cal} = \\ 172.4 \text{ cal.} \end{aligned}$$

$$\begin{aligned} \text{A 40 g. fish, } 9.6 \text{ cal./k cal} \times 31.228 \text{ k cal} = \\ 299.8 \text{ cal.} \end{aligned}$$

Calculations based on Beamish's (1964) data for basal energy requirements of brook trout at 10°C. Since his regression formula was nonsensical, values were estimated from his fig. 1. An oxy-calorific coefficient can be used to convert his measurements of oxygen consumed by fish to equivalent amounts in calories of energy resources oxydized. This value is about 4.8 cal./ml. O₂ (Warren and Davis, 1966). Based on Beamish's (loc. cit.) data the respiration rate of a 23 g. trout is 1.5585 ml. O₂ /hour or 37.4 ml. O₂ /day and 3.2857 ml. O₂ / hour or 78.9 ml. O₂/day for a 40 g. fish. When a reasonable R.Q. value of 0.70 (Hastings, 1969) is used, the actual daily rate of respiration is :

For a 23 g. trout- $37.4 \text{ ml.} \times 0.70 = 26.18 \text{ ml.}$

For a 40 g. trout- $78.9 \text{ ml.} \times 0.70 = 55.20 \text{ ml.}$

Now using the oxy- calorific conversion factor the estimated daily basal energy requirements which would be predicted are;

For a 23 g. trout - $26.18 \text{ ml.} \times 4.8 \text{ cal./ml.} = 122.8 \text{ cal.}$

For a 40 g. trout - $55.20 \text{ ml.} \times 4.8 \text{ cal./ml.} = 265.0 \text{ cal.}$