# EFFECTS OF DIETARY METHOXYCHLOR ON BROOK TROUT FED AT DIFFERENT RATES

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## ABSTRACT

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EFFECTS OF DIETARY METHOXYCHLOR ON BROOK TROUT FED AT DIFFERENT RATES.

This study was undertaken to verify, in the laboratory, the effects of dietary methoxychlor on the food maintenance requirements of the brook trout, Salvelinus fontinalis.

Five 30-day experiments were performed during which the fish were held in annular chambers while swimming against a current of 12.2 cm/sec at temperatures between 10 and 12.5°C. The fish were fed artificial diets given at different rates of 0.5, 1.0, 1.5 and 2.0 percent of their weight daily. The test diets contained methoxychlor (33 to 134 ppm) to produce an intake level of 0.67 mg/kg of fish/day.

The results show that at high feeding levels methoxychlor had no appreciable effect on growth but significant reduction occurred at low feeding levels. The maintenance requirements, estimated from wet weight growth curves, were markedly increased by methoxychlor in all experiments. Measurements of fat and residual methoxychlor in whole fish at the end of the experiments indicate that both fat and methoxychlor were related to feeding levels. Haemoglobin contents were slightly higher in methoxychlor-exposed fish at low feeding levels.

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# INTRODUCTION

The purpose of this study was to determine in the laboratory the effects of dietary methoxychlor on the food maintenance requirements of the brook trout, *Salvelinus fontinalis* (Mitchill).

The control of blackflies has two major reasons: in certain areas of North America, blackflies, although not a disease vector to man, are occasionally an almost intolerable nuisance, whereas in Africa *Simulium* is the vector of Onchocerciasis which causes "river blindness" of its victims. DDT has long been used for the control of blackflies (Jamnback and Collins, 1955), but its persistence, and ability to accumulate in aquatic food chains (Macek and Corn, 1970; Hickey, Keith and Coon, 1966) dictated the search for an alternative suitable effective larvicide, yet less persistent and less toxic to other animals.

Travis (1949) reported that methoxychlor could be a suitable substitute larvicide. Kapoor *et al.* (197[illegible]) and Metcalf *et al.* (1971) also indicated that methoxychlor is less toxic to warm-blooded animals, readily degrades in the environment and is stored in fish tissues at much lower levels than DDT. However, there is little information as to what effects sublethal concentrations of methoxychlor might have on fish food organisms, and on the fish themselves.

In 1971, Wallace studied the effects of methoxychlor larviciding on non-target invertebrate fish food organisms of streams near Baie-Comeau, Quebec. His post-treatment samples showed that the impact of methoxychlor was not restricted to only blackfly larvae, but also seriously affected Ephemeroptera, Plecoptera and Trichoptera, suggesting that methoxychlor was harmful to most aquatic invertebrates. Since brook trout are known to feed on such drifting organisms following methoxychlor application, it appears very likely that following methoxychlor applications in a stream the fish would be subjected to an insecticide-contaminated food supply.

A number of studies have been carried out on the effects of various environmental factors on the maintenance requirements of fish, the amount of food consumed to provide for routine metabolism (Brown, 1957). The "scope for growth" defined by Warren (1971) as 'the difference between the energy value of all the food an animal would and could consume and the energy value of all uses and losses of food other than growth, under particular set of environmental conditions' is determined by the quality of the environment. In nature, low concentrations of toxicants that are not immediately lethal may affect fish either directly by impairing its ability to assimilate food, or indirectly by reducing the food supply, thus lowering cropping efficiency.

In either way, there will be a reduced "scope for growth" and a resultant increase in food maintenance requirement.

Maintenance requirements vary with age, size, physiological and environmental factors. There is an abundant literature on the effects of temperature on the maintenance requirements of fishes, which shows an increase in maintenance ration with increasing temperature (Pentelow, 1939; Brown, 1957).

Brocksen and Cole (1972) reported a resultant increase in the maintenance requirements of three species of fish, Bairdiella, Bairdiella icista, orangemouth corvina (Cynoscion xanthulus), and sargo (Anisotremus davidsoni) when they were exposed to salinities outside the normal range of 33 - 37 ppt. Growth, food consumption, food assimilation and respiration were also adversely affected by salinities other than 33 - 37 ppt.

Some studies have been done on the effects of pesticides and other toxicants on the maintenance requirements of fishes. The presence of 0.05 ppb of dieldrin in water was reported not only to have reduced the amount of food the sculpins could consume, but also increased the amount of food necessary to provide for their maintenance from 23.5 cal/kcal/day to 31.5 cal/kcal/day, (Warren, 1971, P. 163). Growth of the cichlid fish, Cichlasoma bimaculatum exposed to 0.2 mg/l of potassium pentachlorophenate while on restricted rations fell behind that of the controls. When they were placed on unrestricted ration; the poisoned fish compensated for the decreased efficiency of energy utilization by consuming more food. Although they lagged behind initially, they caught up before the end of the experiment (Warren, 1971).

Only a few studies have been published on the effects of dietary methoxychlor on fish. Moreover, there is little information as to the levels of accumulation in aquatic fish food organisms. Kruzynski (1972) reported an accumulation of 1.02 to 1.42 ppm of methoxychlor by aquatic insect larvae that were exposed to 0.075 mg/l of methoxychlor for 15 minutes. Mayer et al (1970) reported loss of vigour towards the end of 15-day experiments in rainbow trout Salmo gairdneri exposed to high dose combinations of methoxychlor with DDT and dieldrin. A dilution of blood serum sodium concentration and a speeding up of gonadal development were reported in methoxychlor-fed cutthroat trout (Grant et al, 1969). Kruzynski (1972) showed that the swimming ability of brook trout exposed to dietary methoxychlor in the range of 0.01 to 0.16 mg/kg fish per day was markedly impaired while those that received 1.00 and 2.00 mg per kilogram had increased stamina. Histopathological studies of exposed fish revealed necrosis of the liver and kidney tissues and low red blood cell count. On the other hand he showed that dietary methoxychlor of the range 0.01 to 2.00 mg per kg of fish per day had no significant effect on the growth of brook trout, presumably because all the test groups had a constant food supply of about 2 percent body weight daily.

Recent studies have furnished some information as to the modes by which pesticides are taken up and stored by the fishes. Some authors feel that the mouth is the main route (Mount, 1962), while others maintain that the gills are the primary route of entry (Holden, 1962; Ferguson, Ludke and Murphy, 1966). Understanding the total effect of pesticides on fish includes what happens to the pesticide taken in by the fish. Although chlorinated hydrocarbons have low solubility in water, they are readily absorbed by oils and fats (Holden, 1964). The amount of pesticide accumulated or metabolised by a fish is therefore related to the amount of lipids. Residues may also vary from organ to organ depending on the lipid content of the corresponding organ. Mayer et al. (1970) reported a residue of 774 ppm in adipose tissue of rainbow trout given 3 mg of methoxychlor *per os* in corn oil on alternate days for two weeks; they also noted that feeding of methoxychlor with dieldrin resulted in a higher level of residual methoxychlor. Kruzynski (1972) reported that brook trout fed diet containing from 6.25 to 100 ppm of methoxychlor retained 40% of the administered dosage. Burdick et al. (1968) exposed four brook trout to 0.005 mg per litre of methoxychlor for 7 days and found residues of

142.4 ppm in the oil of one fish that died after four days of exposure.

Stream larviciding for blackfly control has obvious undesirable secondary effects. It destroys a large portion of invertebrate fauna and accumulates in insects and fish. It therefore appears that stream-dwelling fish could be adversely affected by methoxychlor treatment in two ways: their food could be drastically reduced and whatever organisms left would have been contaminated with the insecticide. It was therefore felt necessary to initiate a study of feeding brook trout with different amount of food containing the same amount of methoxychlor primarily to determine the effects of the insecticide on the food maintenance requirements.

Addendum

This thesis was already being typed when some information was received on a recent study undertaken by Wallace (pers. comm. 1973). Wallace reported methoxychlor residues in simuliid larvae caught after treatment of the Chalk River, Ontario, with 0.81 μg/l of methoxychlor (initial concentration in the water) which ranged from 240-2,570 μg/kg. Residues in trichoptera larvae following an exposure, under laboratory conditions, to between 0.075 to 0.1 ppm methoxychlor ranged from 1400 to 1500 ppb.

# MATERIAL, APPARATUS AND METHODS

## MATERIAL

The fish used for this study were yearling brook trout, Salvelinus fontinalis (Mitchill), which were obtained from the Bury Fish Hatchery, Bury, Quebec, from where they were conveyed in plastic bags. It was impossible to obtain fish of uniform size for all experiments; the initial average weight of the fish used in the first experiment was 8.93 g whereas for experiments 2, 3, and 5 they weighed 9.42, 12.57, 20.98 and 18.42 g respectively.

Upon arrival at the laboratory the fish were held in 1400 litre refrigerated plastic tanks (Frigid unit, Ohio Model MT700), equipped with a cooling unit (Min-o-Cool Units, Model BHL909B). The temperature of the water was maintained at 10°C, and the fish were fed daily with Purina trout chow at a rate of about two percent of their body weight. The fish were apparently healthy and less than 1% mortality occurred during the holding period, which lasted about one week prior to the beginning of each experiment.

## APPARATUS

The laboratory was supplied with water (City of Montreal) delivered through plastic (PVC) pipes after it has been dechlorinated in a carbon filter. The chemistry

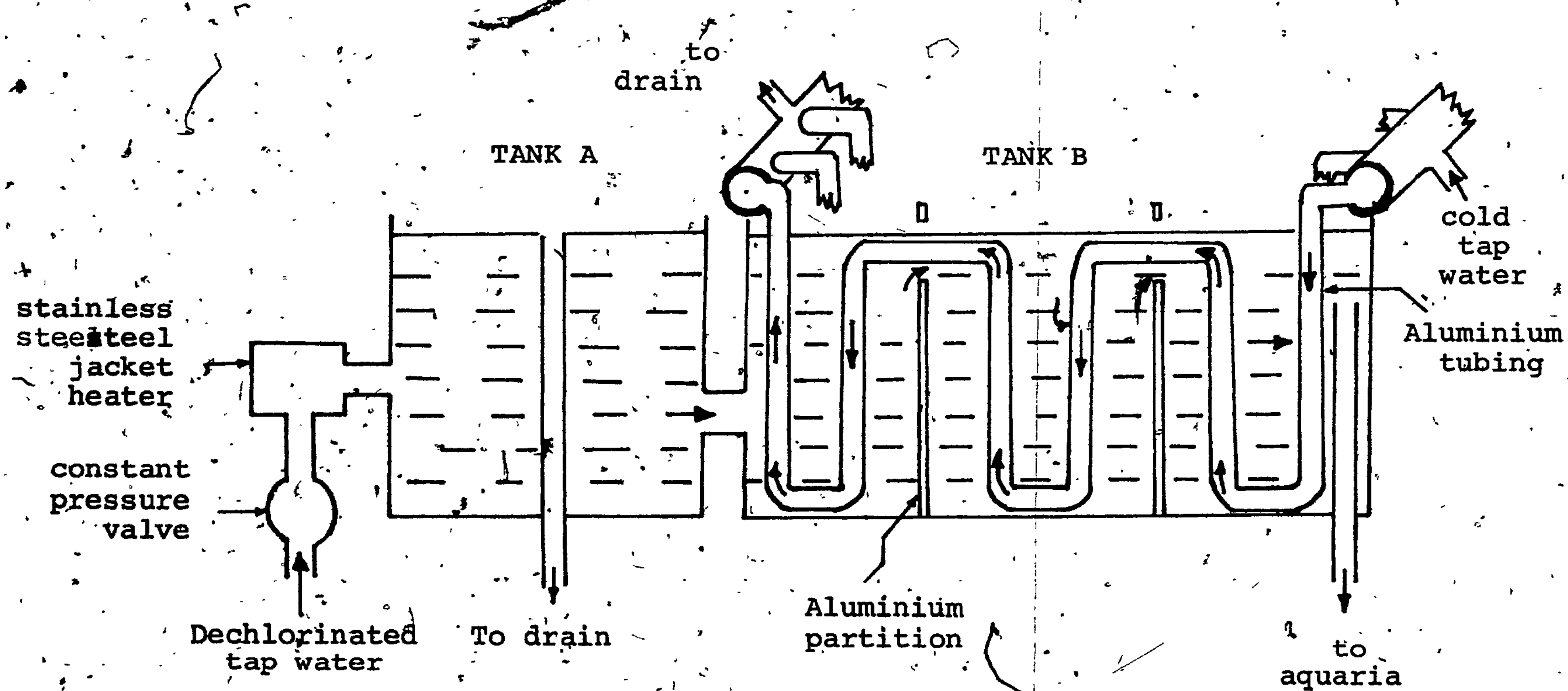



Figure 1. Diagram of a heat exchange unit designed to reduce air supersaturation from closed system water supply destined for fish study aquaria. (Arrows indicate the direction of flow of water).

of the water over the period during which the experiments were performed is presented in Table 1.

Table 1: Chemical analysis of treated water for the City of Montreal (data provided by City of Montreal waterworks).

May 1972 to April 1973 (Mean values)

| Alkalinity $CaCO_3$ (mg/l) | Total hardness (mg/l) | $CO_2$, (mg/l) | pH |
|---|---|---|---|
| 84 | 128 | 0.04 | 7.9 |

Temperature control was achieved in two ways: during the summer, cooling was effected by a chilling unit which reduced water temperature to 12.5°C. ± 1°C. During the winter, the water supply system described by Kruzynski (1972) was modified to prevent the occurrence of supersaturation. The apparatus introduced into the system, illustrated in Figure 1, is a heat exchange unit made up of eight aluminium tubes bent and reunited at both ends by two larger pipes. The tubing assemblage was placed in a plastic box partitioned by two sheets of aluminium in order to achieve a steady sequential flow from one compartment into the other, thus getting a more effective heat exchange.

Incoming cold water was preheated to 13°C by means of a stainless steel jacket heater, then aerated in tank A, from which it flows into tank B holding the heat exchange unit. This water was then cooled by cold tap water flowing through the aluminium coil in the opposite direction, bringing the water temperature to the desired level of 10°C. The dissolved oxygen concentration, measured by the Winkler method (Azide Modification - Standard Methods, 1971) ranged from 9.30 to 11 ppm (about 90 percent saturation).

The test tanks used for these studies were annular growth chambers described by Kruzynski (1972). A flow of water of 925 ml/min. was controlled by predictability flow meters and the water temperature was kept at 10°C in all experiments except the third one which was performed at 12.5°C. Each growth chamber was equipped with an electric motor-driven paddle wheel which produced a current of 12.2 cm/sec., and an electric shock ring behind the standpipe to prevent the fish from resting there. Each test tank was illuminated with a 40-watt light bulb and a 12-hour photoperiod (6 to 18 hr.) was controlled by a time-switch.

## METHODS

### Preparation of the Diet

The artificial diet used for these studies was prepared according to Kruzynski (1972). This diet consisted of a mixture of two parts of beef liver, one part of beef heart and one part of Ewos trout chow (F 169). The liver and heart were ground together in a waring blender, oven-dried and ground to a powder. Methoxychlor emulsion was prepared by dissolving the required amount in 1 ml. of xylene and adding five drops of emulsifying agent (Atlox 3335, Ciba Geigy, Montreal) and making it up to one litre with distilled water. Methoxychlor was introduced into the diet by mixing five parts of the emulsion by weight with five parts (by weight) of warm distilled water, four parts dry powder chow and one part of the binding agent, gelatin. The mixture was poured into a shallow tray, put in the refrigerator where it set and was then cut into small pieces which were frozen until used.

The methoxychlor used, 1-1-1-trichloro 2-2, bis (p-methoxyphenyl) ethane, was 89.5 percent technical grade. Pesticide Reference Standard (Entomological Society of America) and was obtained from City Chemical Corporation, N.Y.

Since the aim of this study was to determine the effect of methoxychlor at different feeding intensity levels (0.5, 1.0, 1.5 and 2.0 percent body weight per day) it was important that all fish be subjected to a similar insecticide intake level even though they received different amounts of food. Determinations of the amount of methoxychlor in the final diet preparations by gas chromatography revealed a concentration of approximately 89 percent of the nominal values. The concentrations of methoxychlor in in the diet were therefore different for different ration test groups and were as shown in Table 2.

Table 2: Concentrations of methoxychlor in various diets given at different rations.

| Ration (percent/body wt.) | Methoxychlor content of diet (ppm) |
|---|---|
| 0.5 | 134.20 |
| 1.0 | 67.13 |
| 1.5 | 44.70 |
| 2.0 | 33.60 |

The concentrations of methoxychlor were so selected as to produce an intake level of 0.67 mg. of methoxychlor per kilogram of fish per day. This level was chosen after Kruzynski (1972) observed that intake levels of 1.00 and 2.00 mg/kg/day have caused kidney and liver damage which migh might also have impaired food utilisation. On the average our fish weighed slightly less, hence a lower daily intake of the insecticide was chosen.

The control diet was also prepared as described previously except that the emulsion consists only of 1 ml. of xylene and 5 drops of emulsifier made up to 1 litre with distilled water.

The diet was analysed for protein and fat content and its composition is shown in Table 3. The protein was determined by the Kjedahl method as described by Griffin (1927); the fat content was determined by ether extraction with a Labconco Goldfish Fat Extractor (Model 35003), using about 3 g of dry material subjected to a four-hour reflux distillation. The ash content was determined by incineration of 1.5 g of food at 1200°C.

Table 3: Proximate analysis (based on dry weight) of the artificial diet used to study the effect of dietary methoxychlor on the maintenance requirements of brook trout.

| | | |
|---|---|---|
| Percentage dry matter | = | 35.82 |
| Protein | = | 67.50 |
| Fat | = | 10.50 |
| Ash | = | 8.09 |

## Experimental Design

At the beginning of each experiment, 30 brook trout of desired size were selected and randomly distributed in each of the six annular growth chambers; two days later the fish were anesthetized in MS 222 (tricaine methane sulphonate), individually marked using the method of Mighell (1968)

(1

blotted dry and weighed to the nearest hundredth of a gram. During the first experiment, subsequent weighings were done at 10-day intervals and the fish ration was adjusted accordingly; in other experiments they were weighed at the beginning, after 15 days of feeding and at the end of the experiment. In all cases fish were not fed 24 hours prior to weighing. At the end of each experiment, all surviving fish (except the samples used for hemoglobin determinations in experiment 4 and 5) were weighed and kept frozen for body composition and residue analysis. Samples of frozen fish from each test group were dried in an oven at 70°C, pulverized, and analysed for fat content using ether extraction as for the diet.

The blood haemoglobin content was measured at the end of experiments 4 and 5 on five fish from each test group. The method used was that of Wintrobe (1961), using a 'cyanmethemoglobin standard' obtained from the Laboratory Centre for Disease Control, Ottawa. The blood samples were obtained by severing the caudal peduncle of anaesthetized fish and collecting the blood in heparinized capillary tubes.

Determinations by the cyanmethemoglobin method were found to be generally slightly lower than the haemoglobin values obtained from determinations based on total iron (Larsen and Snieszko, 1961) hence a correction factor of 1.02 X cyanmethemoglobin values + 0.25 was applied to each of the haemoglobin values obtained. Dete

Determination of Residual Methoxychlor:

Five frozen fish from each of the three experimental groups and one of the three controls were thawed out, blotted dry and homogenized with dry ice in a waring blender. A 20 g sample was weighed out, mixed with 80 g anhydrous sodium sulphate and this was used for the extraction as described by Hesselberg and Johnson (1972).

Residual methoxychlor in extracts from whole fish were determined by gas chromatography using a Hewlett-Packard 5750 Gas chromatograph equipped with an electron capture detector. A glass column (8mm OD, 6mm ID by 76.2 cm long) was packed with 10% stationary phase (4% ov-17 + 6% D.C. QF-1 (F5-1265) fluorosilicone) on 60/80 mesh chromosorb WAW. The conditions of operation were as follows:-

| | |
|---|---|
| Column temperature | 210°C |
| Injection Port temperature | 205°C |
| Detector temperature | 240°C |
| Carrier gas | 4.5 methane 95.5 argon 67ml/min. |
| Retention time | 22 minutes |
| Injection volume | 1µl; the 'solventflush' technique described by Kruppa and Henly (1971) |

was employed to preclude sample hang-up through distillation in the syringe.

The peak areas of the unknown samples were compared to standard curves prepared from samples of known concentrations of the reference standard methoxychlor. The concentrations of methoxychlor in whole fish were then expressed as micrograms/gram (ppm) wet weight of fish.

# RESULTS

The fish readily adapted to the experimental tanks, they were active and readily accepted both the control and the experimental diets. The change over to the diet containing methoxychlor did not cause any change in the behaviour of the fish and no difference in their spatial distribution as was observed by Kruzynski (1972) could be noticed in any of these experiments.

The number of fish that died in the course of each experiment is indicated in Table 4. During the second experiment, a high mortality occurred one day following the backwash of the dechlorinator; the higher loss among one of the control groups (1 percent of body weight ration) remains however unexplained. There appears to have been no significant difference of mortality between the control and the experimental fish which could be attributed to dietary methoxychlor.

## Growth Experiments:

The results of the first growth experiment where no methoxychlor was added to the diet are illustrated in Figure 2. The maintenance rations for this experiment and the following ones were determined by interpolation from curves relating the growth data to the various feeding levels. The results of this experiment indicate that the

Table 4. Mortality observed in yearling brook trout fed either a control diet or exposed to a daily intake of 0.67 mg of methoxychlor/kg of fish during a 30-day period while swimming in annular chambers against a current of 12.2 cm/sec.

| Expt. No. | Temp. of Expt. | Initial average wt. of fish g. | No. of fish at beginning of expt. | Ration Test Groups (as percentage of body wt.) 0.5 | | 1.0 | | 1.5 | | 2.0 | | 2.5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Con. | Expt. | Con. | Expt. | Con. | Expt. | Con. | Expt. | Con. | Expt. |
| 1 | 10°C | 8.93 | 30 | 10 [1/] | | 3 | | 5 | | 3 | | 4 | |
| 2 | 10°C | 9.42 | 30 | | | 20 [2/] | 10 | 7 | 9 | 6 | 8 | | |
| 3 | 12.5°C | 12.57 | 30 | | | 8 | 5 | 2 | 5 | 2 | 5 | | |
| 4 | 10°C | 20.98 | 30 | | | 8 | 9 | 3 | 4 | 9 [3/] | 2 | | |
| 5 | 10°C | 18.42 | 21 | 1 | 0 | 0 | 0 | 0 | 0 | | | | |

1/ The fish in this test group were not fed initially, feeding was begun on the 11th day after 8 fish died.

2/ A high mortality following the backwash of the dechlorination.

3/ Drain pipe was blocked by a dead fish, resulting in a loss of 4 fish in the overflow.

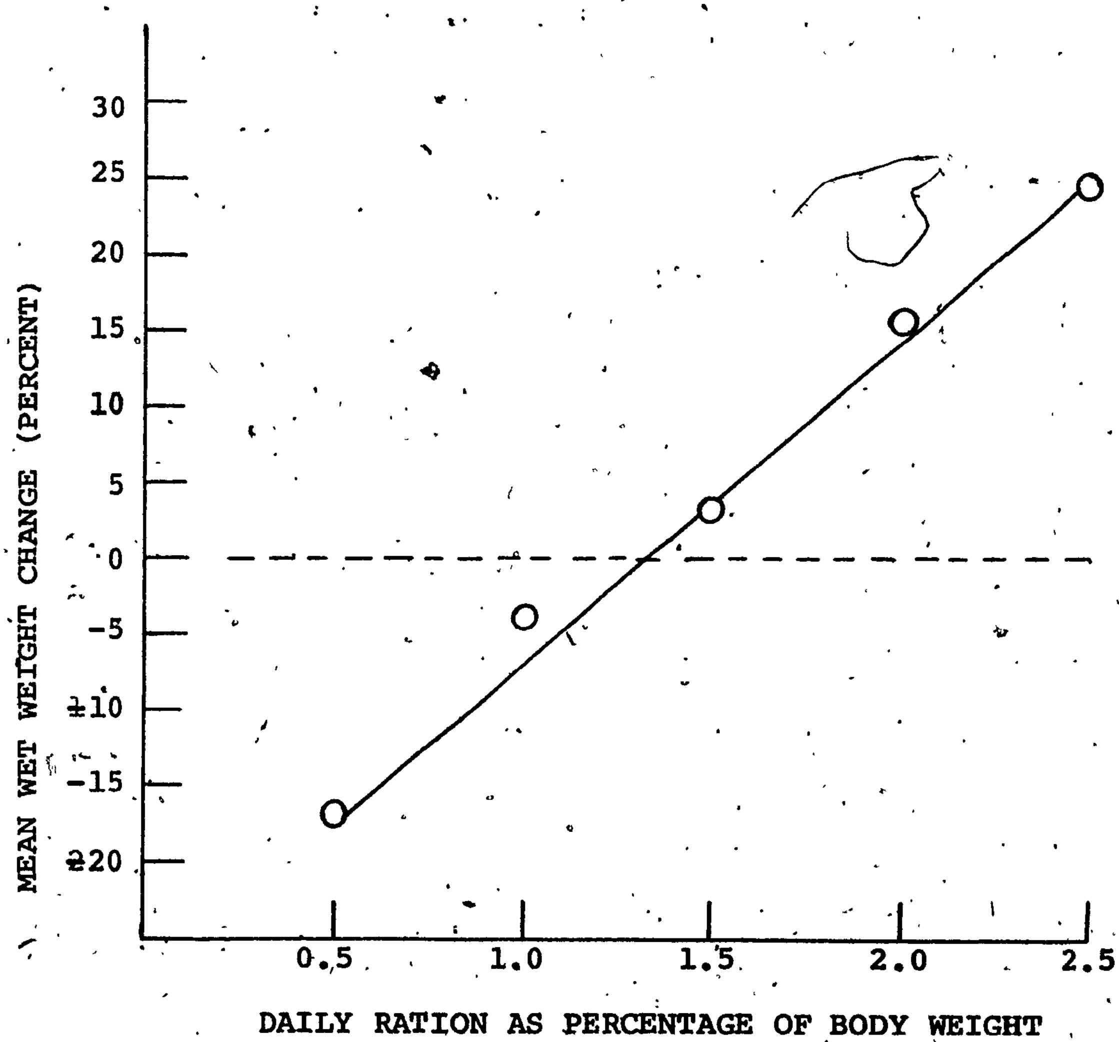



Figure 2. Relationship between the wet weight change of yearling brook trout and their feeding levels while being kept in annular chambers, swimming against a current of 12.2 cm/sec and at 10°C.

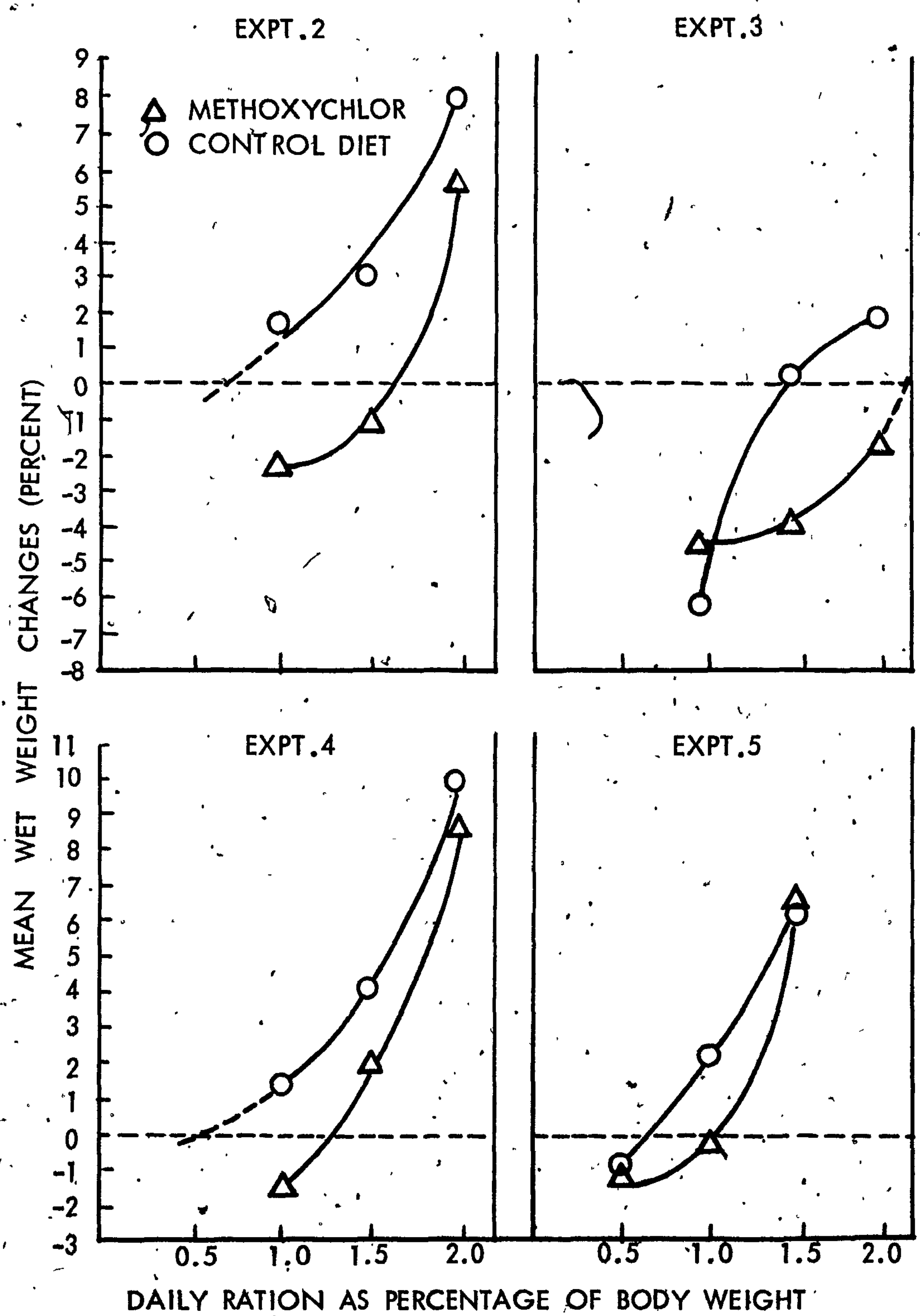



Figure 3. Relationship between the mean wet weight changes and feeding levels of yearling brook trout fed either control diet or exposed to a daily dietary intake of 0.67 mg of methoxychlor/kg of fish/day during a 15-day period (0-15) while swimming in annular chambers against a current of 12.2 cm/sec.

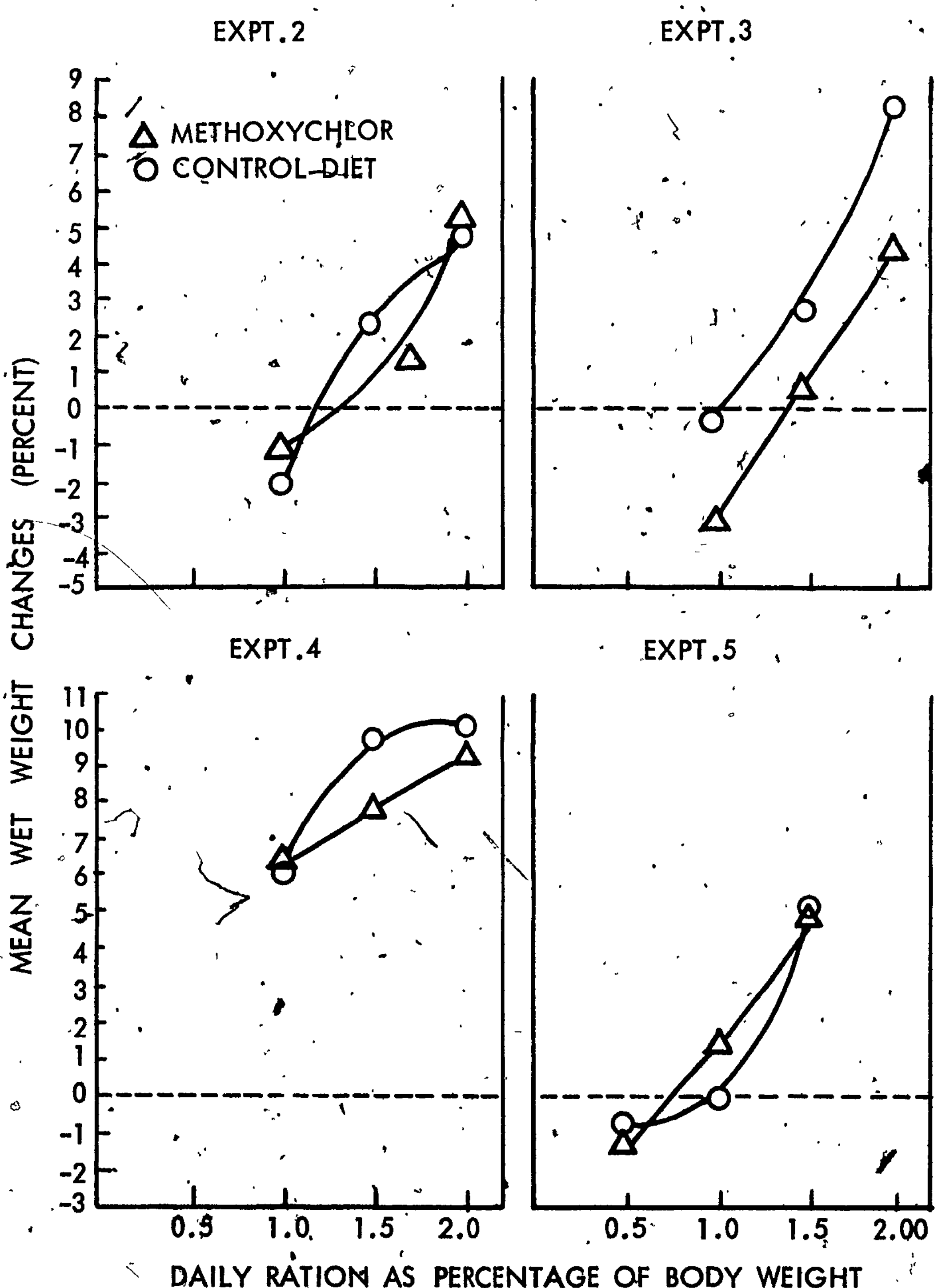
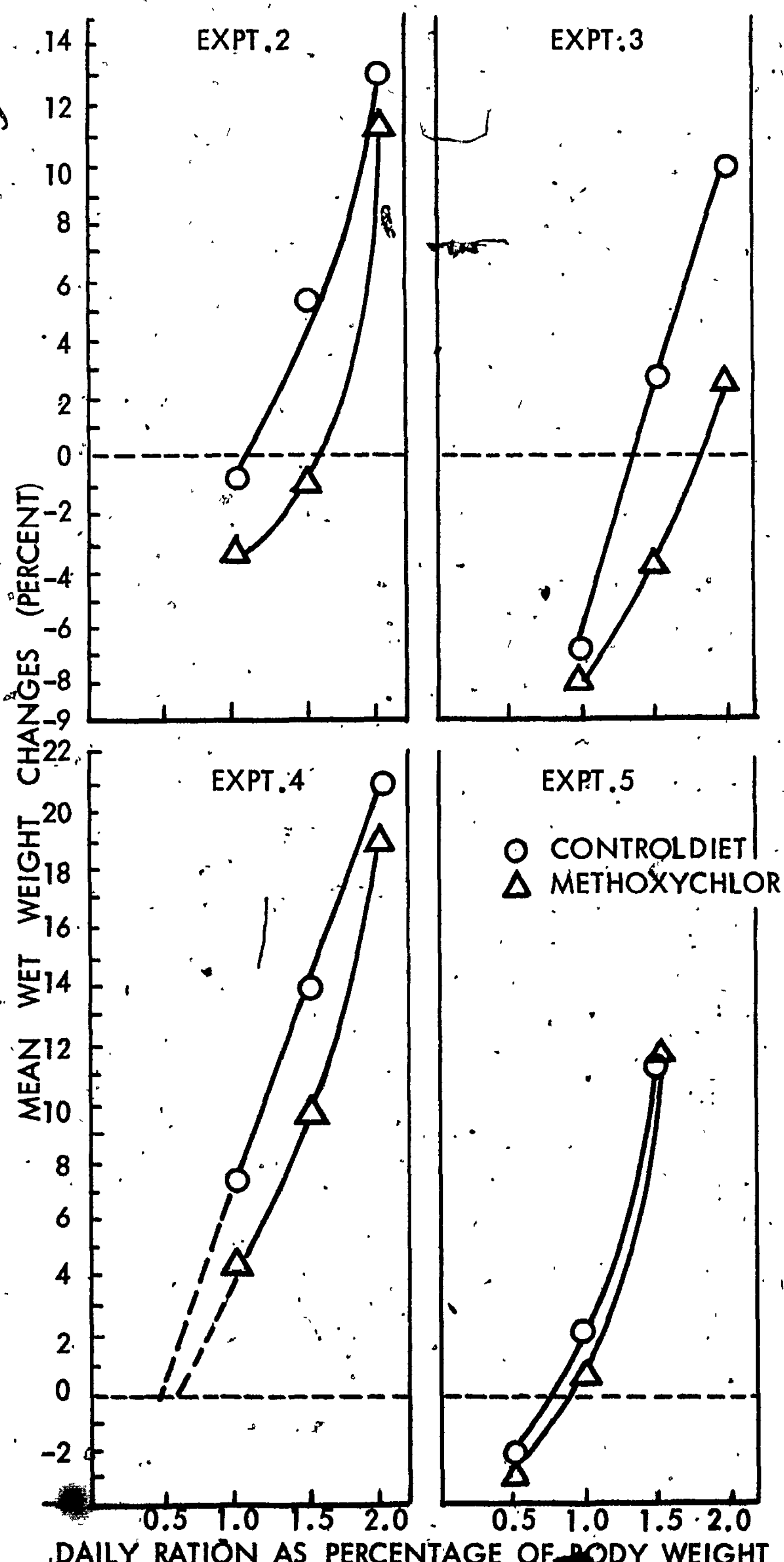



Figure 4. The relationship between the mean wet weight changes and feeding levels of yearling brook trout fed either control diet or exposed to a daily dietary intake of 0.67 mg of methoxychlor/kg of fish/day during the second 15-days (days 15-30) of a 30-day period while swimming in annular chambers against a current of 12.2 cm/sec.

Figure 5. The relationship between the mean wet weight changes and feeding levels of yearling brook trout fed either a control diet or exposed to a daily dietary intake of 0.67 mg of methoxychlor/kg of fish/day during a 30-day period while swimming in annular chambers against a current of 12.2 cm/sec.


EXPT.2
EXPT.3
EXPT.4
EXPT.5
CONTROL DIET
METHOXYCHLOR
MEAN WET WEIGHT CHANGES (PERCENT)
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-9
22
20
18
16
14
12
10
8
6
4
2
0
-2
0.5
1.0
1.5
2.0
DAILY RATION AS PERCENTAGE OF BODY WEIGHT

maintenance ration for yearling brook trout of an average weight of 8.93 g was about 1.3 percent of their body weight per day, a value which served as a basis for the selection of the feeding levels in the following experiments. The absolute amount of food required for maintenance was therefore 13.3 g per kilogram of wet weight of fish per day.

The response of the fish to the experimental diet for 15 and 30 days was measured as mean changes in wet weight computed from the difference between the pooled average weights of all the fish in each test tank at the beginning and the end of each test period. These results were expressed as percentage weight changes.

The effects of dietary methoxychlor on the growth of brook trout are illustrated in Figures 3, 4 and 5 by eye-fitted curves relating the relative growth to the different feeding levels. Fig. 3 represents the results of the first half of the experiments (0 to 15 days), while Figures 4 and 5 show the results of the second period (15 to 30 days) and the entire period respectively. The overall appearance of the results is that methoxychlor can adversely affect growth of brook trout, but the effect varies with feeding intensity.

The control growth curves show that during the first 15 days (Figure 3), the control fish all gained weight in experiments 2, 4 and 5 except at 0.5 percent feeding level in the latter; growth in the third experiment was poor and weight change ranged from -7 to +1.5 percent. Since the activity and metabolic rate of poikilotherms generally increase with temperature, more energy of the food was probably used up at the higher temperature (12.5°C).

During the following period, 15 to 30 days, the growth of the control fish varied from about -1 to +8 percent in experiments 2,3 and 5. However, the growth of the control fish in experiment 4 was much higher (+6 to +10 percent) probably because the rations chosen were relatively too high for the size of the fish.

The control growth curves for the entire period shown in Figure 5 indicate that during experiments 2, 3 and 5 wet weight gains of +10 to +13 percent were attained at the highest feeding level whereas much greater variation was observed at lower levels. Gains of +7 to +21 percent occurred during the fourth experiment.

During the first 15 days of experiments 2, 4 and 5, the methoxychlor-exposed fish experienced weight changes ranging from about -2 to +9 percent (Figure 3), whereas in experiment 3 the growth of these fish was the poorest, and in fact, losses were recorded at all feeding levels. The third experiment was performed at 12.5°C as compared to others carried out at 10°C. Whatever was the factor responsible for lower growth during the experiment, its effect was apparently amplified by the presence of methoxychlor in the diet.

During the second half of the experiments (Figure 4), the growth of the poisoned fish in experiments 2, 3 and 5 varied from about -3 to +5 percent whereas for the fourth experiment much higher gains were observed.

Considering the entire 30-day period, (Figure 5) reveals that in experiments 2 and 5 the growth of methoxychlor-exposed fish was quite similar, ranging from -3 to +11 percent. The growth pattern for experiments 3 and 4 was however different; no weight loss was observed in experiment 4 and the growth was relatively high, reaching 19 percent at the highest feeding level. In experiment 3 the overall growth of the poisoned fish was poor due primarily to marked losses which occurred during the first 15 days.

The comparison of the growth of control and methoxychlor-exposed fish in Figures 3, 4 and 5 reveals one striking similarity: in all four experiments the growth curves of the methoxychlor-exposed fish are always to the right of the control curves. The only exception to this is the second half of experiment 5 (Figure 5) where the growth of the control fish is lower only at the intermediate feeding level. It is therefore very apparent that dietary methoxychlor fed at a rate of 0.67 mg/kg/day has a detrimental effect on the growth of brook trout, the extent of which appears to vary with the intensity of feeding. The pairs of curves for experiments 2, 4 and 5 during the first 15

Table 5. Analysis of variance to compare the effect of dietary methoxychlor (0.67 mg/kg/day) on the growth of brook trout at three feeding levels. (Wet weight changes of individual fish over the periods indicated were used for the computation).

| Duration (days) | Source of Variation | EXPERIMENT 2 Mean Square | Degree of Freedom | Signi-ficance | EXPERIMENT 3 Mean Square | Degree of Freedom | Signi-ficance | EXPERIMENT 4 Mean Square | Degree of Freedom | Signi-ficance | EXPERIMENT 5 Mean Square | Degree of Freedom | Signi-ficance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-15 | Total | 0.533 | 118 | | 0.780 | 152 | | 2.209 | 144 | | 0.904 | 124 | |
| | Between treatments | 1.920 | 5 | | 6.570 | 5 | | 16.380 | 5 | | 7.907 | 5 | |
| | A(Methoxychlor) | 1.632 | 1 | * | 3.674 | 1 | ** | 2.363 | 1 | ns | .290 | 1 | ns |
| | B(feeding levels) | 3.580 | 2 | * | 11.356 | 2 | * | 39.309 | 2 | * | 18.766 | 2 | * |
| | AB(Interaction: Feeding level/ Methoxychlor | 0.404 | 2 | ns | 3.231 | 2 | ** | 0.460 | 2 | ns | .855 | 2 | ns |
| | Within treatments | 0.472 | 113 | | 0.583 | 147 | | 1.699 | 139 | | .610 | 119 | |
| 0-30 | Total | 1.396 | 118 | | 2.252 | 151 | | 4.915 | 144 | | 2.227 | 124 | |
| | Between treatments | 7.510 | 5 | | 20.989 | 5 | | 39.374 | 5 | | 29.062 | 5 | |
| | A(Methoxychlor) | 1.619 | 1 | ns | 14.453 | 1 | ** | 11.462 | 1 | * | .451 | 1 | ns |
| | B(feeding levels) | 17.610 | 2 | ** | 41.413 | 2 | * | 92.585 | 2 | ** | 72.224 | 2 | ** |
| | AB(Interaction: Feeding level/ Methoxychlor | .357 | 2 | ns | 3.833 | 2 | * | .120 | 2 | ns | .204 | 2 | ns |
| | Within treatments | 1.126 | 113 | | 1.610 | 146 | | 3.675 | 139 | | 1.099 | 119 | |

ns non significant $p>0.05$
* significant $p\leq0.05$
** highly significant $p<0.01$

days were similar in that the experimental and the control curves converged at the highest feeding level, with the growth of the treated fish only slightly lower, suggesting that increased rations reduced if not eliminated the impairing effect of methoxychlor on growth. However, the growth curve of the treated fish in experiment 3 during this period (0 - 15 days) was much below those of the control both at the intermediate and highest feeding levels.

The results also suggest that over the 30-day period growth was more affected by methoxychlor at the low feeding levels than at higher ones (see Figure 5, experiments 2, 4 and 5); the contrary however occurred in experiment 4. This pattern was primarily established in the first 15-day period((Figure 3) where the effect of methoxychlor appears much more pronounced than during the second 15-day period.

While the 30-day period growth curves for the methoxychlor-exposed fish are still much below that of the control at the intermediate feeding levels, they are close at the highest feeding levels in all cases except for the third experiment which was performed at 12.5°C.

The analysis of variance carried out to verify possible interaction between the feeding levels and methoxychlor is presented in Table 5. The analysis was computed using the changes in wet weight of the individual fish over the period in question. However, the number of

Table 6. Comparison, using a t-test, of the effect of dietary methoxychlor (0.67 mg/kg wet weight of fish) at different feeding levels on the growth of brook trout held in annular growth chambers for 30 days.

| Expt. No. | Duration (days) | | Mean wet weight changes at corresponding rations (mg) 0.5% | 1% | 1.5% | 2% | t-values at indicated rations 0.5% | 1% | 1.5% | 2% | df. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0-15 | Con. | | +208 | +273 | +607 | | 1.65* | 2.07* | 0.09 | 113 |
| | | Expt. | | -104 | -141 | +598 | | | | | |
| | 15-30 | Con. | | -295 | +101 | +598 | | 0.54 | 0.32 | 0.32 | 113 |
| | | Expt. | | -154 | + 46 | +514 | | | | | |
| 3 | 0-15 | Con. | | -972 | +218 | +276 | — | 1.11 | 3.51** | 2.10* | 147 |
| | | Expt. | | -725 | -520 | -166 | | | | | |
| | 15-30 | Con. | | - 7 | +217 | +1086 | | 1.13 | 0.23 | 3.10** | 147 |
| | | Expt. | | -256 | +168 | +460 | | | | | |
| 4 | 0-15 | Con. | | + 45 | +780 | +1663 | | 1.10 | 0.82 | 0.12 | 139 |
| | | Expt. | | -390 | +488 | +1681 | | | | | |
| | 15-30 | Con. | | +1182 | +2190 | +2600 | | [illegible].27 | 1.07 | 2.00 | 139 |
| | | Expt. | | +1074 | +1806 | +1865 | | | | | |
| 5 | 0-15 | Con. | -178 | +362 | +1068 | | 0.11 | 1.759* | 0.454 | | |
| | | Expt. | -152 | - 61 | +1177 | | | | | | |
| | 15-30 | Con. | -229 | + 27 | +1030 | | 0.48 | 0.63 | 0.45 | | |
| | | Expt. | -343 | +177 | +922 | | | | | | |

*Significant P<0.05
**Highly significant P<0.01

observations for the different groups were not equal; a factorial analysis with unequal replication was used. These results indicate that fish on lower ration were more susceptible to methoxychlor and as such had a lower relative growth. The results of experiment 3 demonstrate the increased susceptibility to methoxychlor with reduced ration most clearly. During the first 15 days of experiment 3, growth was markedly impaired as ration decreased ($P<0.05$). Examination of the data for the entire period show a less marked, but yet significant effect ($P<0.05$)).

The result of a comparison of the effects of dietary methoxychlor on growth of brook trout at the different feeding levels, using a t-test, is presented in Table 6. These results show that methoxychlor impaired growth significantly ($P<0.05$) during the first 15 days at the 1 percent feeding level only in experiments 2, 4 and 5. Comparisons based on mean wet weight changes during the second half of the experiments indicate that the difference in the growth of the control and treated fish was significant only in experiment 3 (see Table 6).

It therefore appears that the growth of methoxychlor-exposed fish was lowered during the first 15 days but approached that of the controls during the second half of all experiments except the third one; this was most clearly demonstrated in experiments 2 and 5. In experiment 2,

Table 7. The maintenance requirements of yearling brook trout fed either a control diet or exposed to a daily intake of 0.67 mg/kg of fish of dietary methoxychlor during a 30-day period while swimming against a current of 12.2 cm/sec.

| Expt. No. and (Temp.) | Initial Average Wt. of Fish (g) | Duration (Days) | Control Maintenance Requirements (MR) (g/kg of Fish) | Maintenance Requirements of Methoxychlor Fish (g/kg of Fish) | Difference Between MR of Control and Methoxychlor Fish | %Difference Between MR of Control and Treated |
|---|---|---|---|---|---|---|
| 1 | 8.93 | 0-20 | 12.0 | | | |
| (10°C) | | 0-30 | 13.3 | - | - | - |
| 2 | 9.42 | 0-15 | 7.8 | 17.2 | 9.4 | 130.6 |
| | | 15-30 | 12.4 | 13.8 | 1.4 | 11.3 |
| (10°C) | | 0-30 | 11.0 | 16.0 | 5.0 | 45.5 |
| 3 | 12.57 | 0-15 | 15.0 | 21.8 | 6.8 | 45.6 |
| | | 15-30 | 10.6 | 14.2 | 3.6 | 34.0 |
| (12.5°C) | | 0-30 | 13.2 | 18.2 | 5.0 | 37.9 |
| 4 | 20.98 | 0-15 | 6.2 | 12.7 | 6.5 | 104.8 |
| | | 15-30 | - | - | | - |
| (10°C) | | 0-30 | 4.4 | 6.2 | 1.8 | 40.9 |
| 5 | 18.42 | 0-15 | 7.2 | 10.8 | 3.6 | 50.0 |
| | | 15-30 | 7.8 | 9.6 | 1.8 | 23.1 |
| (10°C) | | 0-30 | 7.8 | 9.0 | 1.2 | 15.4 |

comparisons of the mean wet weight changes after 15 and 30 days show that methoxychlor-exposed fish at the 1.5% feeding level lost an average of 141 mg during the first 15 days while the controls gained about 273 mg; however, during the second 15 days, the control gained 101 mg and the treated, 46 mg. The same trend was also observed in experiments 4 and 5.

No significant difference between the growth of control and poisoned fish was observed at the highest feeding levels (1.5% in experiment 5 and 2% in others), the poisoned fish putting on nearly as much weight as the controls. The only exception occurred in experiment 3 where the wet weight gain of thecoontrol fish was significantly higher than that of methoxychlor-exposed fish at the 2 percent ration. In this experiment and at this feeding level, the poisoned fish lost an average of 166 mg while the control gained 276 mg during the initial 15 days (Table 5) whereas for the entire 30-day period, weight gains were 295 and 1362 mg for the poisoned and the control fish respectively.

Maintenance Requirements:

The food maintenance requirement data of control and methoxychlor-exposed brook trout were obtained by interpolation from the growth curves presented in Figures 2, 3, 4 and 5 and are shown in Table 7. There was a considerable variation in the estimated maintenance requirement among the five experiments. In control fish, for example, the estimated food maintenance requirements for a 30-day period ranged between 4.4 and 13.3 mg/kg/day, the increasing size of the fish being most probably the main factor of variation. Because of the differences in the size of the fish, temperature of the water and time of the year it appears inappropriate to compare the experiments among themselves and importance should only be given to comparison of control and methoxychlor exposed fish in these experiments.

The effect of methoxychlor on the maintenance requirement of the brook trout is illustrated by the percentage increase in the amount of food required to maintain the treated fish over that for the controls; this difference ranged between 45 and 130 percent for the first 15 days, whereas for the entire period it ranged from 15 to 45%, suggesting that the effect of methoxychlor was more pronounced during the first part of the experiment.

The results therefore suggest that at least under the experimental conditions described, brook trout exposed to a methoxychlor-contaminated diet would see their energy requirement markedly increased. From the proximate analysis of the diet shown in Table 3, the diet would have a calorific equivalent of 1424.54 cal/g wet weight. Based on food intake alone the maintenance calorific requirement of

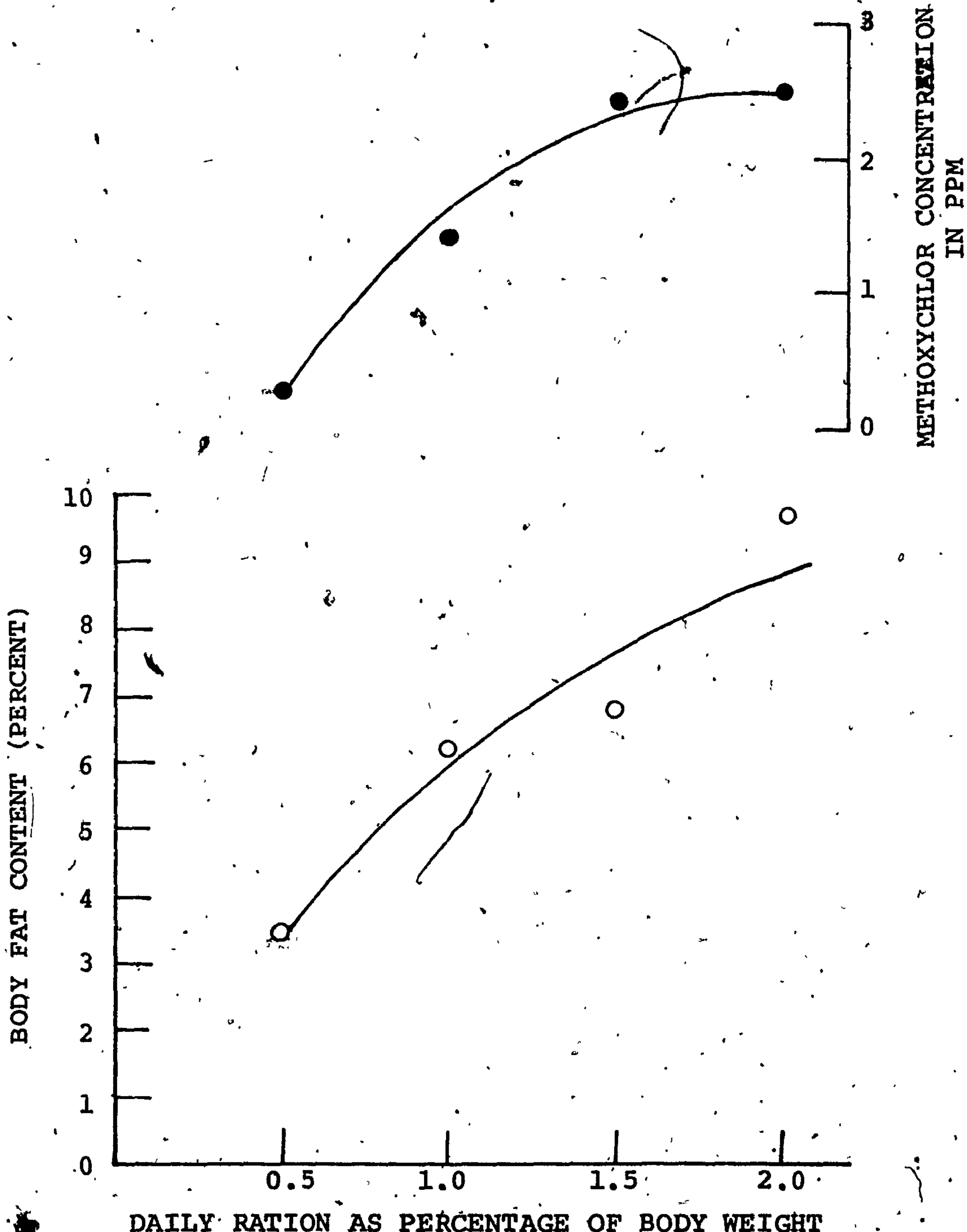



Figure 6. Relationship between feeding levels of brook trout and the resulting body fat and methoxychlor content after 30-day exposure to 0.67 mg/kg/day of methoxychlor. The data represent average values obtained from experiments 2, 3, 4 and 5.

Table 8. Mean blood haemoglobin content (g/100 ml blood) of brook trout which had been fed 0.67 mg/kg body weight fish/day of dietary methoxychlor for 30 days while swimming against a current of 12.2 cm/sec in annular growth chambers at 10°C.

| Expt. No. | Ration (% body wt. | No. of fish sampled | | Mean wet wt. of fish at end of expt. (standard dev.) | | Mean haemoglobin values in g/100 ml blood (standard dev.) | |
|---|---|---|---|---|---|---|---|
| | | Con. | Metho. | Con. | Metho. | Con. | Metho. |
| 4 | 1.0 | 4 | 5 | 19.83(5.49) | 20.15(2.54) | 9.41(.28) | 10.24(.85) |
| | 1.5 | 5 | 5 | 25.45(5.77) | 21.45(1.86) | 9.49(.75) | 9.74(.56) |
| | 2.0 | 5 | 5 | 25.17(3.94) | 23.25(5.25) | 9.51(.68) | 9.48(.63) |
| 5 | 0.5 | 5 | 5 | 16.39( .85) | 16.79(2.02) | 8.77(.93) | 9.00(.27) |
| | 1.0 | 5 | 5 | 18.48(1.07) | 18.81(2.5) | 9.74(.35) | 10.00(.78) |
| | 1.5 | 5 | *5 | 20.27(2.59) | 19.52( .73) | 8.98(.80) | 8.73(.22) |

*Only four were included in the calculation of the means as one was out of the range, possibly due to an error in the determination of haemoglobin.

Table 9: Residues of methoxychlor in, dry weights and fat content of brook trout fed either the control diet or exposed to 0.67 mg dietary methoxychlor/kg fish/day for 30 days while swimming against a current of 12.2 cm/sec in annular chambers.

| Expt. No. (Temp.) | Ration (% Body Wt. | Dry Wt. of Fish as % of Wet Wt. Control | Treated | Methoxychlor[1] Residue in Whole Fish (ppm) | Fat Content of Whole Fish (% of Dry Wt.) Control | Treated |
|---|---|---|---|---|---|---|
| | 1.0 | 22.1 | 19.7 | 1.98 | 3.34 | 4.15 |
| 2 | 1.5 | 20.6 | 19.3 | 3.64 | 7.02 | 4.50 |
| (10°C) | 2.0 | 21.4 | 18.0 | 5.08 | 7.00 | 6.16 |
| | 1.0 | 19.1 | 20.2 | 0.51 | 6.42 | 5.65 |
| 3 | 1.5 | 20.9 | 21.1 | 0.61 | 9.01 | 6.07 |
| (12.5°C) | 2.0 | 22.1 | 20.3 | 1.00 | 9.04 | 9.78 |
| | 1.0 | 23.4 | 23.4 | 1.05 | 5.75 | 9.04 |
| 4 | 1.5 | 22.8 | 22.3 | 0.24 | 22.95 | 9.30 |
| (10°C) | 2.0 | 23.8 | 23.9 | 1.43 | 11.25 | 13.07 |
| | 0.5 | 21.7 | 20.8 | 0.25 | 7.50 | 3.54 |
| 5 | 1.0 | 21.1 | 21.3 | 0.62 | 4.98 | 5.97 |
| (10°C) | 1.5 | 23.5 | 21.8 | 5.23 | 13.69 | 7.38 |

1/ No methoxychlor was detected in any of the control fish control fish in experiments 2, 3, 4 and 5 would be 144, 230, 161, 209 cal/day respectively for the entire 30-day period.

Haemoglobin determinations:

The results of the haemoglobin determinations carried out at the end of experiments 4 and 5 are shown in Table 8. These results indicate that among the control fish, the haemoglobin level has not been materially affected by feeding levels; however, slightly higher values occurred at the lowest and intermediate feeding levels in methoxychlor-exposed fish. At the highest rations the haemoglobin values in the controls and treated fish were closely similar.

Fat content and residual methoxychlor:

The amount of residual methoxychlor in whole body homogenates of fish that survived the 30-day treatment with dietary methoxychlor and the dry weights of both the control and treated fish at the various feeding levels in experiments 2, 3, 4 and 5 are presented in Table 9 together with fat content. Figure 6 represents the relationship between fat composition and concentrations of residual methoxychlor in whole brook trout with the ration. These results indicate that the concentration of methoxychlor stored in the tissue increased with daily ration, this being particularly noticeable at the highest feeding level. The highest levels of methoxychlor residue, 5.08 and 5.23 ppm were obtained at the highest ration in experiments 2 and 5 respectively.

Although the fish were fed different amounts of food they all received the same amount of methoxychlor (0.67 mg/kg/day). These results however suggest the level of storage of methoxychlor is linked to food availability which in turn obviously determines the amount of fat which appears to be fairly closely related to the feeding levels to which the fish had been previously subjected.

The fat content of the fish at different rations show that increase in ration was accompanied by a subsequent increase in body fat (Figure 6). On the average, the fat content of the control fish were higher than that of the treated, except in experiment 4 where the amount of fat and methoxychlor accumulated at the intermediate feeding level were lower than at the other feeding levels, and the fat content of the controls were also lower. The levels of storage of residual methoxychlor presented in Table 9 represent from 1.2 to 24 percent of the administered dose.

The dry weights (expressed as percentage of the wet weight) of control fish, like the observed wet weight changes, were slightly higher than those for the treated fish (Table 9), suggesting that the higher gain in the control must have been due to better tissue elaboration as opposed to accumulation of water.

# DISCUSSION

It has long been established that organochlorine insecticides can persist in the aquatic environment and that they can accumulate in food chains. It is therefore evident that in addition to the absorption of insecticides free in the water, through the gills or otherwise, fish are also subjected to insecticide-contaminated food with the known deleterious effects (Muirhead-Thomson, 1971).

In the present study of the effects of dietary methoxychlor on the growth and food maintenance requirements of brook trout, the insecticide was shown to impair growth, primarily at low feeding intensity at the same time suggesting higher maintenance requirements. Total body fat content increased with food consumption and the residual methoxychlor in body tissues was closely related to the fat content.

## Physiological implications

The comparison of the maintenance requirements values obtained with control brook trout in the present study with those of other workers is difficult because the diet and other experimental conditions are different. Brown (1946b) used laboratory data similar to those used in the present study to show the relationship between the food maintenance requirement and body weight of two-year old brook trout kept in groups of 10 in each aquarium (glass) at 11.5°C and fed minced meat with liver. Extrapolation from Brown's data (1946b, Figure 6) gives maintenance requirement of about 150 mg/g/week for 20 g fish, or a calorific equivalent of about 460 cal/day. In the present study about 160 cal/day was obtained with 20 g fish, an estimate which is lower than Brown's possibly because of the differences in diets and the slightly higher temperature (11.5°C). The calorific value of her diet calculated according to Phillips (1965) was 1070 cal/g (wet weight) whereas our diet was estimated as 1424 cal/g (see Appendix A for details). Brown's higher maintenance values could also be due to the inherent difficulty of collecting unconsumed food as some must have gone into solution. Computations made from Brocksen's (1966) data yielded maintenance energy values of about 247.6 cals/day for cutthroat trout (Salmo clarki) averaging 21 g; these fish were held in laboratory streams at a water velocity of 24 cm/sec at a mean temperature of 8.5°C and fed housefly adults and larvae. Brocksen's values (see Appendix B for details) and those obtained in the present study with fish of about the same size are obviously lower than Brown's.

It therefore appears that feeding experiments have limited value to estimate maintenance requirements. The relation of wet weight changes to rations may vary according to the prevailing experimental conditions such as diets, activity (swimming) and temperature. While Brown's maintenance requirement studies were carried out in aquaria where no current was produced, fish in the present study had to maintain their position against a current of 12.2 cm/sec. Moreover, Phillips (1965) reported that there is an inverse relationship between fat and water content. Accuracy and precision of maintenance requirements obtained by this method may therefore be affected by the fact that the fish may be accumulating water while the fat is being metabolised, resulting in a misleading estimate of wet weight change. Determinations based on dry weights of fish and on calorific values of food consumed may yield more reliable estimates of maintenance requirements. In this study however, wet weight measurements were used but the conclusion reached that methoxychlor increased the food maintenance requirements appears valid since the final dry weights (see Table 9) of the control fish were higher than those of the treated, suggesting that the differences observed between the two are not due to mere water content.

Various workers have shown that environmental stress can increase maintenance requirement through either increased metabolic rate or decreased food conversion efficiency. Brocksen and Cole (1972) reported an increase in the food maintenance requirements of the bairdiella (Bairdiella icista), the orangemouth covina (Cynoscion xanthulus) and the sargo (Anisotremus davidsoni) at salinities outside the normal range of 33 - 37 ppt. Here, salinity other than the optimum was acting as an osmotic stressor and the increase in maintenance requirements was attributed to higher respiratory costs and possible decrease in assimilation efficiency. Chadwick and Brocksen (1969) reported a similar increase of maintenance requirements in sculpins (Cottus perplexus) poisoned with 0.5 μg/loof dieldrin from 23.5 to 31.5 cal/kcal/day. Chapman's results with the cichlid fish, Cichlasoma bimaculatum poisoned with pentachlorophenate was similar to those obtained in the present study in that the growth of fish poisoned with potassium pentachlorophenate lagged behind that of the controls initially, but they later caught up by consuming more food. In the present study, the diet was restricted and as such the reduced impairment observed during the second half of the treatment must have been due to adjustment or adaptation by the fish.

The observed increased cost of maintenance could have resulted from impairment of metabolism as a result of damage to vital organs of tissues by methoxychlor. Kennedy et al (1970) reported liver damages in bluegills (Lepomis macrochirus) exposed to 0.04 mg/l of methoxychlor in ponds. Kruzynski (1972), following almost identical laboratory conditions as in the present study reported damage to liver tissues in brook trout exposed to 2.00 mg/kg/day methoxychlor and shrinkage of kidney tubules in those exposed to

0.125 mg/kg/day of dietary methoxychlor for 43 days.

Methoxychlor has also been known to have some injurious effect on the blood. Eisler (1967), reported lower haematocrits, but no significant difference in the erythrocyte number and haemoglobin level between control puffers (Spheroides maculatus) and those exposed to 30 ppb methoxychlor. However, those exposed to a combination of methoxychlor and methyl parathion or methyl parathion alone for 96 hours had less haemoglobin and lower haematocrits. Kruzynski (1972) also reported a decrease in mean haematocrit values from 41.2% in the control to 34.3% in yearling brook trout exposed to 0.5 mg/kg fish/day of dietary methoxychlor for 43 days; mean red blood cell count of the control and treated fish were 1.15 and 1.08 million/mm$^3$ respectively. Although Kruzynski did not determine the haemoglobin content, it is possible that the decrease in haematocrit might have been accompanied by a corresponding decrease in haemoglobin, which could have resulted in a reduced oxygen-carrying capacity of the blood.

This kind of damage done by methoxychlor and related pesticides to vital organs could culminate in an overall impairment of the performance of the fish. For example, respiration studies undertaken by Brocksen and Cole (1972) suggested that the increase in the maintenance requirement of the three fishes mentioned earlier was due to an increased respiration cost and possible decrease in assimilation efficiency. A decreased swimming stamina similar to that reported by Kruzynski (1972) in brook trout exposed to 0.01 to 0.16 mg/kg/day dietary methoxychlor could be one of the consequences of interference with the respiration of the fish, as less energy is available for such activities.

Studies have shown that a decrease in active metabolism (such as might be caused by damage to vital organs) reduces the 'scope for activity' with a resultant decrease in growth (Fry, 1957). The literature on the effect of methoxychlor on growth is quite inconclusive. Swedberg (1970, in Kruzynski 1972) reported a reduced growth of cutthroat trout (<u>Salmo clarki</u>) exposed periodically at 28 day intervals, to 0.1 and 0.3 mg/l of methoxychlor, whereas those that were fed a diet containing 0.24 and 0.79 mg/kg body wt/day in clean water showed no reduced growth. On the other hand, Kruzynski (1972) reported that dietary methoxychlor in the range of 0.01 to 1.00 mg/kg/day for 33 days had no significant effect on growth of brook trout held at 7.5°C; swim-tested fish fed 2 mg methoxychlor/kg/day for the same period showed a 17.73% wet weight gain as compared to 23.22% in the controls. However, he fed his fish constant daily ration of 2 percent body weight. This substantiates the suggestion, that the effect of methoxychlor on growth observed in this study was more pronounced because the ration was restricted.

The results of Experiment 3 of this study suggests that the impairment of growth by methoxychlor was greater at 12.5°C than at 10°C. Kruzynski (1972) noted a similar effect of temperature on the growth of brook trout exposed to dietary methoxychlor. Comparisons of specific growth rates by him showed that treated fish gained less weight at 10 than at 7.5°C.

Residual methoxychlor:

A limited number of studies have been done on accumulation and breakdown of methoxychlor in vertebrates. Weikel (1959) suggested that methoxychlor is eliminated rapidly from rat liver through biliary excretion whereas Creaven et al (1967) reported that some fish possess drug-metabolising enzymes which can o-dealkylate pesticides as in mammals. Burdick et al (1968) observed in three species of fish that methoxychlor accumulation is less than that of DDT. An evaluation of biodegradability made by Metcalf et. al (1971) from a laboratory 'model ecosystem' suggests a more rapid biodegradability of methoxychlor and also reported that it concentrates to a lesser extent than DDT in mosquito fish (Gambusia affinis). Mayer et al (1970) reported a residue of 100 ppm in adipose tissue of rainbow trout (Salmo gairdneri) fed 0.6 mg methoxychlor on alternate days for 14 days (7 doses in all); in the same experiment fish fed 1 mg DDT/day, had a residue of 173 ppm in adipose tissues. The same authors reported that feeding of dieldrin with methoxychlor to trout resulted in an increase of residual methoxychlor. Ferguson and Binham (1966) concluded that higher mortalities might result from a mixture of insecticides due to differences in the mode of action of the different insecticides causing a greater overall stress. Taylor (1970, in Kruzynski, 1972) reported a whole body residue of 2.65 ppm in a fish that died during blackfly larviciding with methoxychlor in Labrador. Kruzynski (1972) reported that brook trout fed diet containing from 6.25 to 100 ppm of methoxychlor retained 40% of the administered dosage.

In the present study, residual methoxychlor ranged from 0.24 to 5.3 ppm (see Table 9). These values represent about 1-24 percent of the amount presumably ingested by the fish. According to interpolations made from Kruzynski's data (1972, Figure 12) an accumulation of 15 ppm could have been expected at the highest feeding level as compared to the observed 5.23 ppm. Kruzynski's study was performed at 7.5°C and it is possible that the elimination of methoxychlor occurred faster at 10 and 12.5°C at which the present study was performed.

The relation between residual pesticides and the amount of fat in tissue has already been established (Bridges et al, 1963). The present study with brook trout further demonstrates this important phenomenon where residual methoxychlor increased with food consumption although all fish had the same insecticide intake (0.67 mg/kg/day). It appears that higher feeding level promotes greater fat deposition which in turn promotes storage of the liposoluble methoxychlor. It may be that the insecticide taken at the highest feeding levels is stored in fatty tissues and thus rendered innocuous whereas at lower feeding levels, the lower body fat content allowed more free insecticide in the body, thus allowing a greater toxic action which in this study manifested itself in reduced growth.

Although the ability of fatty tissues to store organochlorine insecticides has long been established, the toxicity of insecticide in relation to feeding activity and fat deposition has apparently not received serious attention primarily in the context of the ecological implications of pesticides on the quality and quantity of aquatic food organisms.

Ecological significance:

In the natural environment, stream-larviciding for blackfly control with methoxychlor has two adverse effects: the insecticide kills many non-target organisms and readily accumulates in drifting dead and/or dying organisms. Wallace (1971) reported that field treatments with methoxychlor caused a major drift of blackfly, Ephemeroptera and Trichoptera larvae. Wallace's (pers. comm., 1973) recent studies confirmed his earlier work in that the impact of methoxychlor was not restricted to the blackflies alone but catastrophic decimation of other invertebrates also occurred. Fish are among the non-target organisms affected by stream-larviciding with insecticides when they consume contaminated drifting insects which may kill them (Hatfield, 1969).

Methoxychlor can accumulate in the fish food organisms. Levels of accumulation which were severalfold the concentration in the water have been reported. Wallace (pers. comm., 1973) found methoxychlor residues in drifting simuliid larvae caught after the treatment of the Chalk River, Ontario, which ranged from 240-2,570 μg/kg, although the maximum concentration in the water (0.81 μg/l) was much below the 0.075 mg/l commonly used for ground-level larviciding. Wallace also measured methoxychlor residues that ranged from 1400 to 1500 ppb in Trichoptera larvae following an exposure, under laboratory conditions, to between 0.075

to 0.1 ppm methoxychlor.

It is evident from the above that the net effect of methoxychlor can be a drastic reduction and contamination of the fish food biomass. Such a reduction causes a lower cropping efficiency, coupled with the toxic effect of methoxychlor might culminate in an increased maintenance requirement.

In the present study brook trout were subjected to a daily intake of 0.67 mg/kg of fish of dietary methoxychlor with varying amount of food. At this rate a 20 g fish would receive 0.0134 mg/day or 0.4 mg during the 30-day p period. Wallace's (pers. comm., 1973) values of residual methoxychlor in insect larvae obtained under field conditions are a very valuable reference point to speculate how much methoxychlor a fish could get from contaminated organisms following methoxychlor applications in streams. In nature, a 20 g fish feeding at a daily rate of 5% of its weight on food containing approximately 2.5 ppm methoxychlor would receive a daily dosage of 0.0025 mg which is about five times less than that applied in the present study.

In view of the fact that most of the post-treatment drift occurred within about 120 minutes of the beginning of the treatments, (Wallace, pers. comm. 1973), it is very unlikely that fish would be exposed to the contaminated diet for as long as the 30 day period for which they were exposed in the present study or the 43 days for which Kruzynski (1972) exposed his fish. It is however possible that fish eating large quantities of poisoned drifts may be exposed to higher doses, but for much shorter periods. If Wallace obtained residue levels as high as 2.5 ppm following an initial concentration of only 0.81 μg/l much higher level could be expected in fish food organisms exposed to 0.075 mg/l used in commercial larviciding. A much higher dose than that used in the study administered over a shorter period would have been closer to what operates in the natural environment.

The results of the present study suggest that the residual amount of methoxychlor is linked with the amount of fat in the tissue. A fish that has ample, but contaminated food supply may therefore accumulate a high concentration of methoxychlor. Heavy accumulation of pesticides have been known to lower the resistance of fish to diseases and predators. Schoental (in Cope 1965) found Salmonids were more susceptible to furunculosis and fungus diseases when their bodies contained residues of DDT. This could also be true for methoxychlor as similar chronic effects such as damages to kidneys, reduced growth and slowed reaction to external stimuli have been reported for both insecticides. In nature, when the feeding of a fish is interrupted as a result of scarcity of the food organisms, it is most likely that fat oxidation will support the cost of maintenance. The insecticide stored up in this fat may thus be liberated. It therefore appears that with reduced availability of food, the fish might be more susceptible as the insecticide taken with the diet will not be stored in fat but free to act on susceptible metabolic sites.

It can be appreciated that food availability and quality are among the most important environmental factors that determine growth. A toxicant-induced increase in maintenance requirements of the sort observed in this study and others (Warren 1971 p. 164, Brocksen and Chadwick, 1969) reduces the scope for growth as less energy is available.

This laboratory study suggests that low levels of methoxychlor in food organisms could cause, at least for a short period, serious impairment of the growth of stream-dwelling fish particularly when the food supply is low.

# SUMMARY

Five experiments were carried out to determine the effects of dietary methoxychlor on the food maintenance requirements of the brook trout, <u>Salvelinus fontinalis</u> (Mitchill).

The experimental apparatus were annular chambers equipped with paddle wheels producing a current of 12.2 cm/sec. The temperature of the water was maintained at 12.5°C in experiment 3 and 10°C in others. The fish were fed with an artificial diet containing various amounts of methoxychlor.

The first experiment in which only the control diet (without methoxychlor) was fed to the fish served as a basis for the selection of feeding levels in the other experiments. In experiments 2, 3, 4 and 5, the fish were all subjected to 0.67 mg/kg of fish/day of dietary methoxychlor at the various rations for 30 days. The concentrations of methoxychlor in the diet were therefore different for each ration test groupaand were 134.20, 67.13, 44.70 and 33.60 ppm for 0.5, 1.0, 1.5 and 2.0 percent test groups respectively.

The results suggest that methoxychlor had little effect on growth at high feeding levels but reduced growth at low food intake. The values of the food maintenance requirements were obtained by interpolation from curves relating wet weight changes to the feeding levels during 15 and 30-day periods. The maintenance requirements were increased by

dietary methoxychlor.

At the end of experiments 2, 3, 4 and 5 all surviving fish were weighed and kept frozen for determination of dry weight, fat and methoxychlor content of whole fish. Residual methoxychlor increased with food consumption as greater fat was deposited in the tissues at higher rations.

Haemoglobin determinations after the 30-day exposure to dietary methoxychlor in experiments 4 and 5 revealed slightly higher values in methoxychlor-exposed fish at the lowest and intermediate feeding levels.

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# APPENDIX A

Computation of the calorific values of the diet used by Brown (1946b) and that used in the present study.

In order to determine the calorific values of the diet used by Brown in her studies, the percentage fat, carbohydrate and protein composition of liver and minced meat, obtained from Altman and Dittmer (eds. 1968) were as follows:

| | Fat | $CH_2O$ | Protein | Ash | Water |
|---|---|---|---|---|---|
| Liver | 2.82 | 3.94 | 14.78 | 0.96 | 77.5 |
| Minced Meat | 7.10 | - | 14.69 | 0.71 | 77.5 |
| Overall Mean | 5.67 | 1.31 | 14.72 | 0.79 | 77.5 |

To determine the caloric values of the diet, the digestibility of the food classes was taken into consideration. According to Phillips (1965), 3.9 kcal, 8.0 and 3.5 kcal of energy is available/g of protein, fat and carbohydrate respectively after deductions have been made for the indigestible portions.

Calorific values of Brown's diet:

Fat = 8000 cal X 0.0567 g = 453.60 cal.

Protein = 3900 cal X 0.1472 g = 574.08 cal.

$CH_2O$ = 3500 cal X 0.0131 g = 45.85 cal.

Approx. calorific value of Brown's diet =1073.53 cal/g wet weight.

From Brown's Figure 6, the amount of food consumed by

| | | |
|---|---|---|
| 20 g fish | = | 150 mg/g/week = 3 g/week for whole fish. |
| | = | 0.4236 g/day |
| if 1g | = | 1074 cal of energy |
| .4236 g | = | 460.31 cal. |
| 18 g fish | = | 152 mg/g/wk = 2.73 g/fish/week for whole fish |
| | = | 0.3709 g/day = 378.35 cals/day. |

The method for the determination of the calorie value of the diet used in the present study is similar to the one used for Brown's.

Diet composition:

| Protein | Fat | $CH_2O$ | Ash | Water |
|---|---|---|---|---|
| 24.12 | 3.76 | 5.13 | 2.81 | 64.18 |

Calorific values:

| | | | | |
|---|---|---|---|---|
| Protein | = | 3900 X .2412 g | = | 944.19 cal |
| Fat | = | 8000 X .0376 g | = | 300.80 cal |
| $CH_2O$ | = | 3500 X .0513 g | = | 179.55 cal |

Total calorific value of diet =

1424.54 cal/g wet weight

The calorific values of the food maintenance requirements for the entire duration of each experiment are as follows:

| Expt. No. | Control | Experimental |
|---|---|---|
| 2 | maintenance requirements = 1.08 g of diet/100 g wet wt. fish/day<br>1.08 g = 1538.80 cal<br>9.42 fish will consume $\frac{9.42}{100}$ X 1.08 = 144.93 cal/day | 212.02 cal/day |
| 3 | 230.99 cal/day | 325.90 cal/day |
| 4 | 161.39 cal/day | 203.23 cal/day |
| 5 | 209.92 cal/day | 241.41 cal/day |

# APPENDIX B

Calculation of the calorific energy requirement of yearling *Salmo clarki* as extrapolated from Brocksen's (1966) data, cited by Warren, E.W. and C.E. Davis in Gerking, S.D. 1967 (ed.). Biological Basis of Freshwater Fish Production (p. 198-199). John Wiley and Sons, Inc., New York. 495 p.

By extrapolation

Maintenance ration value for stream fish = 13.2 cal/kcal/day.
Fish on low ration have approximately 4.22 kcals/g dry weight.
21 g fish with about 21% dry weight = 4.41 g = 18.76 cals/day.

∴ 21 g fish requires 18.76 X 13.2 = 247.63 cals/day for maintenance.