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The modification of schooling behaviour in larval sea bass, *Dicentrarchus labrax*, by sublethal concentrations of methyl parathion*

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Abstract: The species-typical schooling behavioural repertoire of larval sea bass, *Dicentrarchus labrax*, was examined and is described in this paper. Experiments were conducted to determine the effects of sublethal concentrations of methyl parathion, an organophosphate insecticide and anticholinesterase agent, on their schooling behaviour. The fish were exposed to 2.50, 5.00 and 10.00 ppm of the toxicant for periods of 2, 8, 24 and 48 hours. The results demonstrated distinct modifications in their schooling patterns; there was generally little or no correlation of dosage with period of exposure; deviations in schooling behaviour were maximal and deleterious in fish exposed to 10.00 ppm of methyl parathion. The most conspicuous modification was the deterioration of social attraction and interaction in the three groups of test fish, resulting in statistically significant declines in duration of parallel orientation and frequency of realignment in contrast to the controls. The test fish also exhibited changes in locomotor ability and movements, organization of schools and feeding. Behavioural modifications caused by subacute dosages of methyl parathion may be ecologically significant in the survival of schooling species of fish.

1. Introduction

The biological effects of pesticide contamination of the environment have been studied principally from physiological or developmental points of view. Another approach, which has received some attention, is concerned with the mechanism and patterns of behaviour of the organisms living in the altered environment (KLEEREKOPER, 1976; EISLER, 1979). Behaviour is the result of an animal's growth in its environment; when an animal lives in a species-typical environment, species-typical behaviour emerges. If, on the other hand, the animal's environment is atypical, subtle or conspicuous modifications occur and affect the expression of behaviour (WILLIAMS and SHAW, 1971; WILLIAMS, 1976). Since a pollutant alters the environment in some way, those organisms that are living in it will probably be affected behaviourally. If the behavioural modification is deleterious, there may be a gradual depletion of the species over a long period of time and eventual extinction.

Analyses of non-adaptive behavioural changes caused by pesticide pollution may be sensitive and meaningful indicators by which "safe" concentrations of pesticides may be determined (SPRAGUE, 1971; BULL and MCINERNEY, 1974; LIVINGSTON, 1977). In addition these analyses may be valuable in demonstrating that the behavioural modifications produced by the toxicant vary at different dosages, and may develop at concentrations which are considered too low to elicit other biological effects (HOLDEN, 1973). Exposure of fish to sublethal levels of pesticides may be more critical than individual deaths caused by lethal concentrations (KLEEREKOPER, 1974); if subtle or conspicuous changes occur in their behaviour, these may be of long-term ecological significance.

Schooling in fish was investigated in this research; it is a vital adaptative behaviour, ensuring species survival by facilitating life functions as protection from predators, reproduction, sociability, finding of food and energy conservation. Disruption of schooling may, indeed, jeopardize

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the animal's success in its environment. Schooling develops gradually with larval fish initially demonstrating patterns of approach and withdrawal which later decrease in frequency and are replaced by sustained parallel orientation and swimming in schools (WILLIAMS and SHAW, 1971, WILLIAMS, 1976).

The experiments were conducted in Concarneau, Brittany, France at the Laboratoire Biologie Marine, Collège de France, in order to determine the effects of a range of sublethal concentrations of methyl parathion on the species-typical schooling behavioural repertoire of sea bass, *D. labrax* (LINNAEUS, 1758), for varying perionds of exposure from 2 to 48 hours. These schooling fish are widely distributed in Breton coastal waters and estuaries and are exposed to fluctuating sublethal concentrations of the polyvalent organophosphate, methyl parathion; it is used as an agricultural insecticide and discharged into watersheds and the ocean where it undergoes dilution processes (ROUGER, 1988). Methyl parathion is an anticholinesterase agent which affects the nervous system of fish and may produce changes in their schooling behaviour. The subjects of the experiments were feeding larvae which are the most sensitive of the different life stages of fish to pesticides (BUTLER, 1964; MURTY, 1986). This study was designed to detect and quantify subtle or conspicuous behavioural modifications of the fish to the altered environment.

2. Materials and methods

Experimental fish and maintenance

Approximately 375 sea bass, *D. labrax*, 72 days of age, with a median length of 6.50 mm were obtained from the hatchery of the Centre National pour l'Exploration de Océans, Centre Océanologique de Bretagne (CNEXO) in Brest, France; they were a small part of a huge, genetically homogeneous population. The fish were maintained during the experiments in a tank (designated as home tank) which measured 91 cm in length and 50 cm in width; the water level was 26 cm and was natural, filtered sea water with a salinity of 24‰. It was continuously aerated and circulated by several air stones and was partially discarded and replenished on a daily basis. All tanks, containing the toxicant, were covered securely with plastic sheeting to prevent its volatization into the atmosphere. In addition, as with the home tank, regular and frequent replenishment of the medium was done to maintain constant concentrations of the insecticide. The lighting in the laboratory was natural with an ambient temperature of 21±1°C, similar to that of the hatchery in CNEXO; the fish were also fed the same diet of pellets of dried shrimp and fish, ten to twelve times daily. Rigid controls were applied to the handling of the fish and maintenance of stable environmental conditions for all parts of the experiment.

Methyl parathion is manufactured in France under the name of Ecadion methyl Sedagi, Ugine Kuhlmann (0,0-dimethyl 0-*p*-nitrophenyl phosphorothioate). The determination of sublethal dosages was made by conducting relative toxicity tests on 54 animals. Each test consisted of a group of 2 fish which was placed in a 1 litre container of thoroughly aerated sea water and a particular concentration of Ecadion methyl, ranging from 0.75 to 50.00 ppm. A total of 19 dosages plus 3 controls resulted in 27 tests; frequent observations on survival of the fish were made over a period of 48 hours. All of the fish died in the following concentrations: 12.50 ppm at 90 minutes, 25.00 ppm at 60 minutes and 50.00 ppm at 2 minutes. They survived in 16 different concentrations, ranging from 0.75 to 11.55 ppm for the exposure period of 48 hours. It was thus decided to expose and test the fish at 2.50, 5.00 and 10.00 ppm for 2, 8, 24 and 48 hours in order to comprehensively examine the effects of these subacute levels of methyl parathion on the species-typical schooling behavioural repertoire of larval *D. labrax*.

Procedures of grouping and exposure of fish to the pesticide

Table 1 summarizes the details of the experimental design. In order to acclimate the fish to the dimensions of the exposure tank, groups consisting of 8 fish were transferred from the home tank to small holding tanks for a period of 8 hours or overnight prior to exposure and testing. The holding and exposure tanks were of the same dimensions, measuring 28 cm×23 cm ×23 cm with a capacity of 3 litres of water. The tanks were covered and thoroughly aerated for several hours prior to reception of the fish.

Table 1. Experimental design — grouping and exposure of fish to pesticide.

Period of exposure (hours)	Group exposed to varying concentrations of methyl parathion in ppm					
	0.00 N of Group[a]	2.50 N of Group[a]	5.00 N of Group[a]	10.00 N of Group[a]	Total N of Tests	Total N of Fish
2	5	5	5	5	20	80
8	5	5	5	5	20	80
24	5	5	5	5	20	80
48	5	5	5	5	20	80
Totals					80	320[b]

N=number. [a] Four fish formed a group. [b] Fish were naive to testing and exposure to pesticide.

Procedures of testing and recording of observations

The methods of testing and recording of behaviour were basically similar to those done in previous studies (WILLIAMS and SHAW, 1971; WILLIAMS, 1976). The tank that was used for testing was circular, 70 cm in diameter with a water level of 5 cm and a capacity of 16 litres of water; it was illuminated directly by a 10-watt opaque light suspended 38 cm above it. The fresh sea water, which was introduced into the test tank for each test, contained the same concentration of methyl parathion to which the test fish were previously exposed. Prior to each test, the tank was covered with plastic sheeting, and the sea water was aerated for a minimum of 10 minutes.

The four test fish were individually and gently poured from a small cup into the test tank at specific times. The first fish remained alone for three minutes; the second was introduced at the end of that period; the third three minutes later and the fourth three minutes after that; the fish were introduced at the same location in the tank. During each three minute period, observations were made of the behaviour of the newly introduced fish. Table 2 summarizes the test procedure; a total of 23 minutes of recorded data was collected for each of the tests. All fish were naive to testing and exposure to methyl parathion.

Table 2. Procedures of testing of fish.

Time period	Length of time of observation	Procedure
1 through 3 min.	3 min.	Fish No. 1 introduced; no behaviour recorded
4 through 6 min.	3 min.	Fish No. 2 introduced; behaviour of fish No. 2 recorded
7 through 9 min.	3 min.	Fish No. 3 introduced; behaviour of fish No. 3 recorded
10 through 12 min.	3 min.	Fish No. 4 introduced; behaviour of fish No. 4 recorded
13 through 24 min.	12 min.	Behaviour of all fish recorded
30th min.	1 min.	,, ,, ,,
36th min.	1 min.	,, ,, ,,

The Rustrak Event Recorder, a miniature recorder and keyboard designed on the Esterline Angus multievent recorder (TOBACH *et al.*, 1962), was operated to simultaneously record sequence, frequency and duration of behaviour of the fish. Quantitative recordings were made of: (1) duration of approach-orientation: one fish approaches another to within 3 mm or less and both remain near each other; (2) duration of parallel orientation: two or more fish approach each other, orient into position parallel to each other and swim in a school; (3) frequency of realignment within the school: parallel reorientation of fish succeeded by change in direction of the school; this action is preceded by energetic and rapid swimming by the members, followed by a brief pause of 3 to 4 seconds, during which time they turn around simultaneously in the same direction to observe and wait for a solitary conspecific that is lagging behind, or one that is stationary and located near their swimming path; the fish joins the group; the parallel formation is subsequently compressed when the fish turn laterally in unison, reorient and swim in another direction.

Statistically significant differences among the means of the above parameters were determined by analysis of variance using IBM Scientific Subroutines in Fortran; all comparisons of means were on a pair basis.

Qualitative observations were made of the general organization of the schools, locomotor behaviour and unique behaviours demonstrated by the test fish. Feeding behaviour was also noted in both the home and exposure tanks as well as the general behaviour of the fish when they were transferred after testing to a community tank of fresh, aerated sea water.

3. Results

General results

The basic research that was conducted in these experiments involved the observation and analysis of the species-typical schooling behavioural repertoire of larval *D. labrax*; this would serve as the norm for comparison with the fish that were exposed to the pesticide or atypical environment. The repertoire consisted of the following patterns of behaviour: (1) limited duration of approach-orientation, non-polarized aggregating of fish when they approach each other and remain adjacent to within 3 mm; (2) the latter behaviour develops into the dominant activity, polarized parallel orientation, when the the fish approach each other and orient in sustained parallel formations or schools; the fish swim in the same direction with close intra-fish spacing of approximately 1 mm, augmented by synchronized swimming movements and speed, and (3) frequent realignment within the schools, as described above. Intrinsic to these three patterns of schooling behaviour are the mutual, positive biosocial attraction and interaction of the fish.

The results of the experiments generally indi-

Table 3. Intergroup comparisons of mean duration of approach-orientation.

Hours of exposure	Control group		Group exposed to 2.50 ppm methyl parathion		Group exposed to 5.00 ppm methyl parathion		Group exposed to 10.00 ppm methyl parathion	
	N	Mean[a]	N	Mean[a]	N	Mean[a]	N	Mean[a]
2	5	212.1	5	450.4	5	448.2	5	195.8
8	5	62.1	5	337.0	5	401.2	5	184.8
24	5	51.9	5	164.6	5	349.2[b]	5	131.0
48	5	46.4	5	48.9	5	28.9	5	36.0

N=number. [a] Means are expressed in seconds.
[b] This group is significantly different from the other three groups ($P<0.05$).

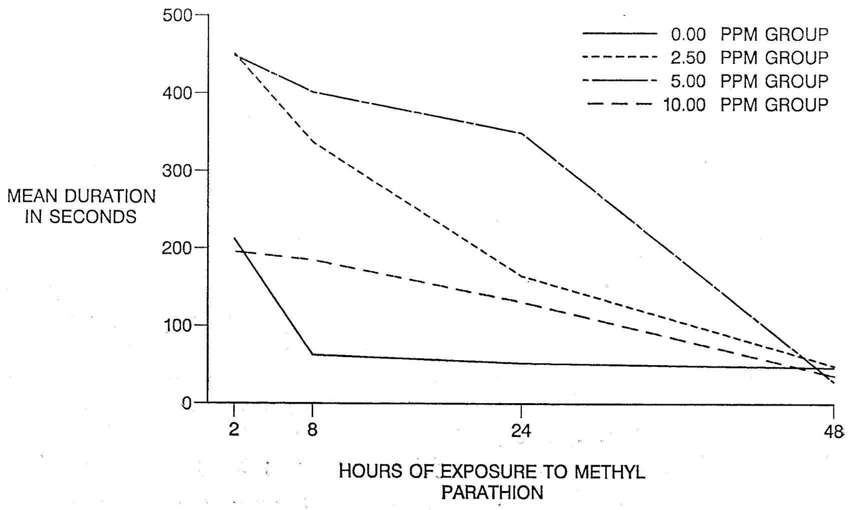

Fig. 1. Comparisons of duration of approach-orientation.

cated that schooling behaviour in larval *D. labrax* was considerably modified by exposure to subacute concentrations of methyl parathion. Duration of parallel orientation and frequency of realignment within the schools declined significantly in the three groups of test fish in contrast to the control group. The addition of the toxicant to the medium resulted in various new and deleterious behaviours in the three groups of fish. There was some diminution of locomotor ability. Dispersal of schools occurred with ongoing progression of the tests; schools when present were disorganized with wide intra-fish spacing. The fish were irritable and in some cases veered away from each other. Their tail regions were curved; they performed undulations, circumvolutions, serpentine movements and swam upright in the water as well as around the circumference of the tank. Social attraction and interaction were greatly deteriorated. The deviations in schooling patterns were maximal and conspicuous in the fish exposed to 10.00 ppm of methyl parathion for all periods of exposure.

Quantitative results

Intergroup and longitudinal comparisons of means of duration of approach-orientation, duration of parallel orientation and frequency of realignment within schools are presented, respectively, in Table 3, Fig. 1; Table 4, Fig. 2; Table 5, Fig. 3. Statistically significant differences ($P<0.05$) among the means are specified in the Tables.

Duration of approach-orientation was reduced in the control fish, while the test fish demonstrated slightly higher durations at 2, 8 and 24 hours with a subsequent decline at 48 hours, so

Table 4. Intergroup comparisons of mean duration of parallel orientation.

Hours of exposure	Control group		Group exposed to 2.50 ppm methyl parathion		Group exposed to 5.00 ppm methyl parathion		Group exposed to 10.00 ppm methyl parathion	
	N	Mean[a]	N	Mean[a]	N	Mean[a]	N	Mean[a]
2	5	727.2[b]	5	431.7	5	57.7	5	4.5
8	5	962.1[b]	5	650.6	5	118.0	5	6.3
24	5	1063.9[b]	5	874.2[b]	5	300.9	5	15.0
48	5	1087.2[b]	5	759.1	5	423.3	5	28.3

N=number. [a] Means are expressed in seconds. [b] This group is significantly different from the other two or three groups ($P<0.05$).

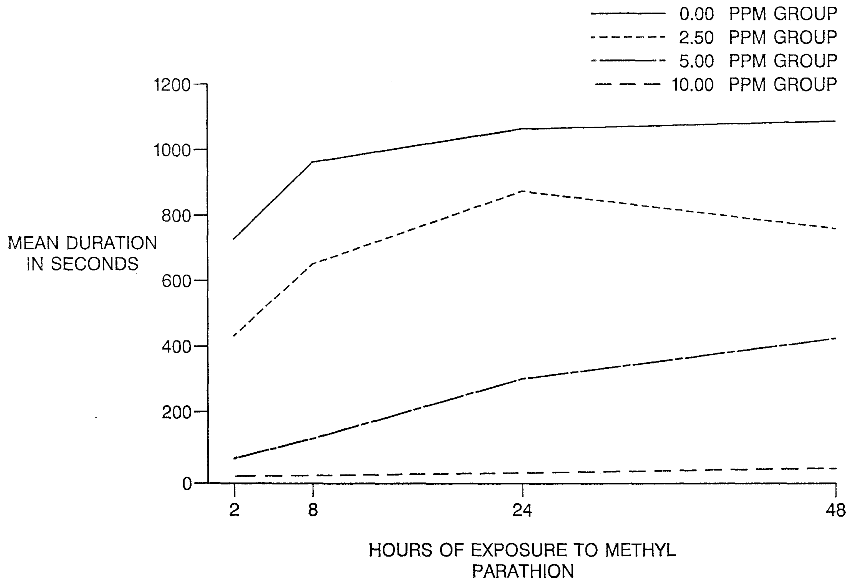

Fig. 2. Comparisons of duration of parallel orientation.

Table 5. Intergroup comparisons of mean frequencies of realignment.

Hours of exposure	Control group		Group exposed to 2.50 ppm methyl parathion		Group exposed to 5.00 ppm methyl parathion		Goup exposed to 10.00 ppm methyl parathion	
	N	Mean	N	Mean	N	Mean	N	Mean
2	5	66.2[a]	5	16.2	5	4.2	5	2.4
8	5	37.0[a]	5	9.4	5	4.4	5	6.4
24	5	40.8[a]	5	9.6	5	7.4	5	0.2
48	5	13.6[a]	5	2.6	5	7.8	5	0.2

N=number. [a] This group is significantly different from the other three groups ($P<0.05$).

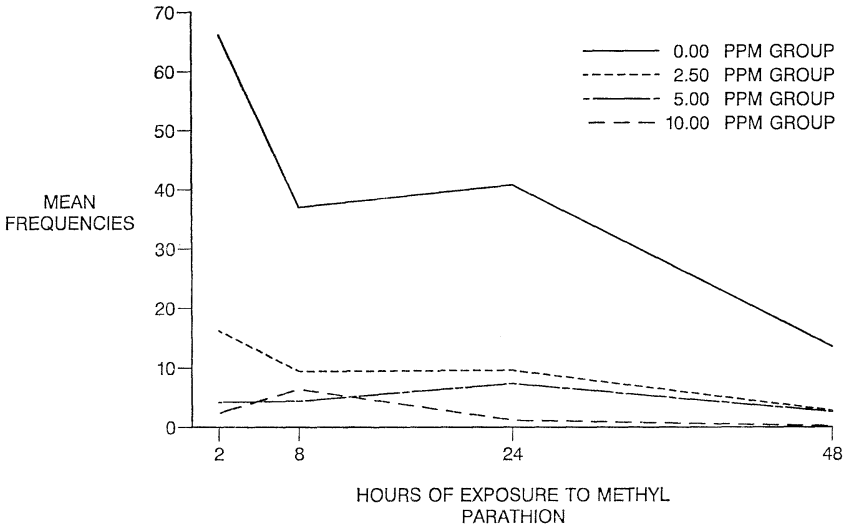

Fig. 3. Comparisons of frequencies of realignment.

that all four groups were the same for the maximal period of exposure (Fig. 1). The adverse effects of methyl parathion were demonstrated in duration of parallel orientation and frequency of realignment. The control fish displayed statistically significant higher duration and frequency, respectively, in these 2 parameters; there were no significant differences in the longitudinal comparisons (Table 4, Fig. 2; Table 5, Fig. 3).

Qualitative observations

Two hours

The control fish formed cohesive schools with the members closely spaced at 1 to 3 mm apart. They swam in short, darting movements, and there were some changes in the direction of the schools. When this occurred, the fish immediately oriented parallel to each other, subsequently synchronizing their swimming movements. This resulted in uniform schools usually composed of 4 fish and persisting for several seconds to a minute in duration. As the time progressed in the tests, the schools improved in cohesiveness and composition. There were many demonstrations of approach-orientation behaviour with fry grouping together at distances of 2 to 3 mm.

The fish that were exposed to 2.50 ppm methyl parathion for two hours exhibited a decline in locomotor ability in comparison to the controls; they swam at a slower pace; their approaches, followed by orientation, were slower. Schooling occurred, but most of the time was spent in orientation. Many of the fish appeared to be irritable and nipped at each other; their bodies assumed a curved posture especially in the tail region; other fish swam in circles. There was generally a decline in the duration of parallel orientation in contrast to the controls.

The behaviour of the fish exposed to 5.00

ppm of methyl parathion was characterized by a dramatic decline in parallel orientation; approach-orientation was the dominant activity. It was of long duration and frequent since the fish showed a definite interest in each other and in schooling together, but these attempts were thwarted by their inability to swim. They demonstrated their affinity for each other by huddling close together. Some of the fish had curved bodies, especially pronounced in the tail region. The fish were very much subdued in comparison to the fish exposed to 2.50 ppm. Aggressiveness was observed as fish nipped and occasionally chased each other which resulted in withdrawals.

The fish that were exposed to 10.00 ppm methyl parathion generally displayed some diminished locomotor ability, characterized mainly by undulating, quivering serpentine movements; their tails were curved. They swam past each other with no interaction or cognizance of each other. There were little or no approach-orientation behaviour and parallel swimming.

Eight hours

Schooling activity for the controls was generally cohesive. There was a decrease in duration of approach-orientation correlated with an increase in duration of parallel orientation in comparison to the fish tested after 2 hours. The fish to fish distance was close at 1 mm; swimming speed was fast and quick darting movements were conspicuous. Schooling improved with the progress of the test. Realignment occurred frequently with its characteristic social interaction among the fish. They were very much aware of each other as illustrated by members of a school which hesitated in their swimming for several seconds to turn around in order to observe a species mate that was following, or in some cases to observe a solitary, stationary fish. In each incident, the school waited for the conspecific to join it.

The fish that were exposed to 2.50 ppm for eight hours demonstrated haphazard schooling of short duration, but there was a definite improvement over the 2 hour exposure. The fish swam faster; some fish were irritable and moved in circles; their tails were curved. Their desire to school was indicated by aggregating closely, resulting in parallel swimming at 1 mm apart; some realignment was exhibited. The close communication among the fish was conspicuous. They huddled very tightly together and oriented to each other. Some fish exhibited irritability and sensitivity to environmental sounds and movements by darting in the water.

The fish that were exposed for eight hours to 5.00 ppm of the pesticide were inactive and lethargic. There was frequent approach-orientation; the fish grouped closely together and attempted to school, but this was frustrated because of diminished locomotor ability. The fish were extremely irritable. There were occasions in which the fish were located far from each other, moving in their individual spheres.

The fish exposed to 10.00 ppm of methyl parathion demonstrated similar behaviour as at 2 hours, but with the addition of circumvolutions produced mainly by the fish circling about their own axes or occasionally moving in small circles. They were totally unresponsive to each other even when swimming immediately adjacent to a species mate. There was the distinct absence of approach-orientation, parallel formations and realignment. They also appeared to be non-irritable and failed to respond to environmental sounds. The fish demonstrated a quivering, undulating movement as at 2 hours, but they also swam rapidly around the circumference of the tank.

Twenty-four hours

Schooling in the control group was cohesive and characterized by rapid swimming and close fish to fish distances of 1 mm. A frequent occurrence was realignment with its distinctive, positive social communication among the fish. The fish demonstrated a great affinity for each other in their realignment behaviour.

The fish that were exposed to 2.50 ppm exhibited an increase in parallel orientation over the 8 hour exposure to the pesticide. Schooling persisted, but it was haphazard, typified by frequent reversals in direction of swimming; this was initiated by one fish which was subsequently followed by the other members of the school. There were a few incidents in which the swimming movement was slow, and the fish appeared to be waiting for species mates to join up with them. Fish to fish distances were wide at 4 mm or more. The fish frequently circumrotated on their own axes. Improvement in schooling behaviour correlated with increase in progress of the test at 25 to 30 minutes.

Some schooling was exhibited by fish exposed to 5.00 ppm of insecticide. Swimming was characterized by slow, short, hesitant, undulating and meandering movements. There were sporadic bursts of rapid swimming in schools. Aggressiveness was expressed by frequent chasing of one fish after another with subsequent rapid withdrawals. The fish also demonstrated extreme irritability by a horizontal back and forth movement. Their bodies were curved and circumrotation occurred. They were acutely aware of each other as indicated when one fish was stationary; its conspecific approached and oriented for approximately a second and then swam away.

The schooling behaviour of the fish exposed to 10.00 ppm of methyl parathion for 24 hours was similar as at exposures of 2 and 8 hours. Locomotor ability, however, improved slightly, but interaction and awareness of the fish for each other, as well as schooling, were negligible. They exhibited occasional withdrawals from species mates which were located close by and were involved in circumrotating on their axes. Some fish sporadically demonstrated bouts of swimming around the circumference of the tank for as long as 60 seconds. They sometimes swam upright and close to the surface of the water; their bodies were curved. Movements were erratic, and each fish performed its own action, seemingly oblivious of species mates in the tank.

Forty-eight hours

The schooling of the controls at 48 hours was cohesive and well organized with close fish to fish distances. Again as at 2, 8 and 24 hours, the fish in a school hesitated in their locomotion for a slower-swimming species mate to catch up with them; there was a high duration of parallel orientation.

The fish exposed to 2.50 ppm of methyl parathion exhibited schools that were less cohesive than at 2, 8 and 24 hours. The distance between the fish was wide at 4 mm or more and swimming was rapid. There were frequent reversals of direction of swimming by members of the school. The duration of most schools was 60 seconds, and they dispersed with passage of time in the test. In addition there was a diminution of sensitivity and awareness of the fish of each other; they swam past each other without communication.

The fish exposed to 5.00 ppm of methyl parathion for 48 hours exhibited improved swimming ability compared to their species mates at 2, 8 and 24 hours at the same concentration. They swam closely past each other and did not appear to communicate. Their bodies quivered as they swam, and this action was more pronounced than at 24 hours; they swam quickly at times. Limited schooling occurred, but it was very brief in duration, highly incohesive and disorganized with wide fish to fish distances of 4 mm or more. There was dispersal of members with ongoing progression of the test.

The same behaviour prevailed for fish in 10.00 ppm insecticide for 48 hours as in the groups at 2, 8 and 24 hours. They were, however, livelier, swimming high in the water, occasionally exhibiting sudden bursts of energetic swimming; fish that passed each other in this manner appeared to be completely disinterested in each other. There was generally no interaction among the fish. Circumrotations occurred, but were much diminished in frequency in comparison to fish exposed to 10.00 ppm methyl parathion for 2, 8 and 24 hours.

General observations of fish in their living environments

The experimental fish did not feed in the holding tanks to which various concentrations of pesticide had been added. This was in dramatic contrast to the controls in the holding tanks and other fish living in the home tank, which fed avidly.

The fish that were exposed to methyl parathion were transferred after testing to tanks containing fresh, aerated, uncontaminated water. They exhibited all the effects of the pesticide after 24 hours. They did not school, but oriented close to each other; their swimming movements were slow and hesitant. Approximately 4 days later, all of the fish appeared to have recovered; they were swimming, schooling and feeding in the same way as were the control fish.

4. Discussion

Effects of sublethal concentrations of methyl parathion on schooling behaviour

The present experiments demonstrated the debilitating effect of the pesticide on locomotor ability of the experimental fish, *D. labrax*. Similar results have been reported in the literature. Sublethal concentrations of organophosphate pesticides decrease activity of fish brain acetylcholinesterase (WEISS, 1961). BONE (1978) concluded from his studies that acetylcholinesterase-mediated transmission of nerve impulses through the central and peripheral nervous systems is necessary for coordinated muscle movement. This suggests that swimming or locomotor activity may well be affected by exposure to these compounds. RAND (1977) demonstrated that the general locomotor behaviour of goldfish was adversely affected by subacute concentrations of methyl parathion. MURTY *et al.* (1984) also observed behavioural changes in the swimming performance of the freshwater catfish, *Mystus cavasius*, as a result of exposure to subacute levels of the toxicant. The affected fish swam rapidly and erratically, often performing somersaults. Significant inhibitory effects of subacute levels of two other organophosphate pesticides, EPN and guthion, on the swimming ability and brain acetylcholinesterase activity in the estuarine sheepshead minnow, *Cyprindon variegatus*, were reported by CRIPE *et al.* (1984).

The effect of subacute dosages of methyl parathion on the locomotor ability of *D. labrax* was primarily a retardation of swimming speed; at various times, the movements of fish were slow, hesitant, quivering, undulating or meandering, and sometimes there were rapid bursts of swimming. Nevertheless, all of the experimental fish retained the ability, although limited, to swim. Indeed, this slow locomotion may have contributed partially to the slightly higher duration of approach-orientation, a nonpolarized orientation, in the test fish, than that of the controls (Table 3, Fig. 1); the former fish remained together or aggregated for longer periods of time, whereas the latter showed a reduced duration of aggregation, and in most cases oriented in parallel swimming immediately after the approach. This behaviour was part of the species-typical schooling repertoire of larval *D. labrax*.

Locomotion is a component of schooling behaviour; sublethal concentrations of methyl parathion as high as 10.00 ppm for a period of exposure of 48 hours did not eliminate it completely. The fish in the 10.00 ppm groups were swimming, but parallel orientation was almost non-existent. Directly related to this phenomenon was a striking behavioural modification: the complete absence of social interaction. The fish swam past species mates which were situated immediately adjacent, without any communication or awareness. There was no affinity or attraction of the fish for each other. Methyl parathion appeared to have a stupefying effect. Similar behaviour, but of a lesser magnitude, was exhibited by the fish exposed to 2.50 and 5.00 ppm at 48 hours. The three test groups also showed statistically significant lower duration of parallel orientation in comparison with the control fish (Table 4, Fig. 2). The decline of social interaction in the test groups was also directly correlated with their decline in frequency of realignment behaviour, characterized by the unique and positive social communication of the fish (Table 5, Fig. 3).

Schooling behaviour is a social behaviour; it is initiated and based on biosocial mutual attraction of the fish which leads to sustained parallel orientation. Methyl parathion, in its role as an anticholinesterase agent, may have adversely affected the visual system of the fish which functions as the primary sensory modality in the attraction-approach mechanism and in parallel orientation. This system integrates with other sensory systems to provide information for sustained parallel orientation. When the fish swam past conspecies without any communication, this may have been caused by their inability to interact visually which resulted in the absence of a stimulus for the approach. When there was no approach, there was subsequently no parallel orientation.

During the dispersal of schools in the experimental groups, increases in the intra-fish spacing occurred in the schools at 4 mm and more. The compact control schools were composed of fish closely positioned at 1 mm apart. WEIS and WEIS (1974) also observed a spreading out of a school of Atlantic silversides, *Menidia menidia*, over a large area after a 24 hour exposure to Sevin, a carbamate compound, which acts similarly to an organophosphate by inhibition of acetylcholinesterase.

Parallel orientation is also facilitated by the lateral line system of fish which consists of nerves that function as receptors to detect vibratory stimuli of water movements made by adjacent fish. It may be possible that these pollutants had a detrimental effect on this system, so that the fish did not receive the critical information to properly orient in parallel formation and maintain positioning in the school.

It appeared that the adverse effects produced by sublethal concentrations of methyl parathion on larval *D. labrax* did not persist, since the fish were swimming without impairment, schooling and feeding after transfer to clean, uncontaminated water after approximately 4 days. It may be of value to study the fish more intensively with observations and testing in the pesticide-free environment for varying periods of residence, preceded by long periods of exposure to sublethal levels of the toxicant. This may provide information on the survival of the fish in the natural environment after chronic exposure to subacute concentrations of methyl parathion.

Effects of sublethal concentrations of methyl parathion on feeding bebaviour

Feeding declined significantly in fish that were exposed to sublethal concentrations of methyl parathion; in cntrast the control *D. labrax* fed avidly throughout the experiments. EISLER (1967, 1970) reported similar results in the northern puffer, *Sphaeroides maculatus*, and the mummichog, *Fundulus heteroclitus*, when they were exposed to methyl parathion. Reduced feeding was also observed by KLEEREKOPER (1974) in mullet when the food was contaminated with parathion. Similar decreases in feeding also occurred in coho salmon, *Oncorhynchus kisutch*, in a stream aquarium exposed to sublethal concetrations of the organophosphate, Sumithion (fenitrothion) (BULL and MCINERNEY, 1974). SPRAGUE (1971) reported that damage to peripheral sense organs by some pollutants negatively affected the feeding behaviour of fish.

The return to normal feeding patterns in addition to other behaviours occurred in the test *D. labrax* approximately three to four days after introduction to clean uncontaminated sea water. This is similar to the findings of WEIS and WEIS (1974) in their work on *M. mendia*. In addition HOLDEN (1973) observed that fish that had been exposed to a pesticide survived after transfer to fresh water, but concluded that they became more vulnerable to environmental variables.

Comparisons of schooling behaviour in larval D. labrax, M. menidia *and* Atherina mochon

The species-typical schooling behavioural repertoire was investigated in two species of developing atherinid fish: *M. menidia* from 1 to 55 days of age (WILLIAMS and SHAW, 1971) and *A. mochon* from 1 to 35 days of age (WILLIAMS, 1976). The present experiments involved *D. labrax* at 72 days of age. Data of the three studies are not comparable because of differences in ages of the fish; these age gaps encompass multitudinous changes that occur during the development of the fish. It may, nonetheless, be concluded from these researches that the three species exhibited similar behavioural development in their schooling patterns. They demonstrated, in general, reduced duration of approach-orientation with concommitant increased duration of parallel orientation, since the fish upon their mutual approaches immediately oriented into parallel formations.

There were, however, two striking differences in *D. labrax* as compared to the atherinid fish that merit attention. The schooling repertoire of the former fish showed the unique absence of approach-withdrawal behaviour which usually occurs initially in the development of schooling. It is characterized by mutual attraction and approach of the fish to within 3 mm and an immediate veering away from each other by one or both of the fish. Frequencies of approach-withdrawals in *M. menidia* tapered off steadily, starting at 25 days of age and declined to a few or none at 55 days of age. Their age-peers of *A. mochon* were, however, uniform in their frequencies throughout 1 to 35 days. It is possible that these fish may show diminution in approach-withdrawal behaviour after 35 days. Perhaps *D. labrax* experienced appraoch-withdrawals at an earlier period of development which then declined completely prior to 72 days of age.

Another provocative difference between *D. labrax* and the two atherinid fish is that the latter did not exhibit realignment behaviour with its unique social communication. Since they were studied respectively up to 55 and 35 days of age, and this behaviour was observed in 72 day old *D. labrax*, it may be that the sophisticated social interaction and parallel reorientation of the fish normally occur at a more mature age in the development of schooling repertoire. Further studies would have to be conducted on the three species at comparable ages in order to examine the above hypotheses.

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致死濃度以下のメチルパラチオンによるスズキ (*Dicentrarchus labrax*) 稚魚の集群習性の変化

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要旨: スズキ (*Dicentrarchus labrax*) 稚魚の集群習性に関する種特異性を研究した。研究においては，メチルパラチオンの致死濃度以下の濃度環境において集群習性を実験的に追及した。供試魚はメチルパラチオン濃度 2.50, 5.00, 10.00 ppm の実験環境において 2, 8, 24, 48 時間にわたって観察された。その結果，メチルパラチオンが明らかに集群習性を変化させる効果のあることが明らかになった。すなわち，メチルパラチオンの濃度効果と実験期間とには殆ど相関関係のないこと，10.00 ppm の濃度で集群習性における異常効果が最大となることなどである。最も顕著な影響は，3グループの供試魚群において誘引行動と相互作用に特徴的な効果が起り，対照実験に対して統計的に有意な並行遊泳行動や整列遊泳行動を行なう期間の短縮が認められたことである。供試魚群は，更に遊泳能力や群れの形成，摂餌行動においても変化をきたした。このようなメチルパラチオンが与える行動変化は，集群習性を持つ魚類の生存にとって生態学的に重要であろう。