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EFFECT OF DDT ON TEMPERATURE SELECTION OF SOME SALMONIDS*

M. YAQUB JAVAID†

Department of Biology, Carleton University, Ottawa, Canada

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Abstract. Treatment for 24 hr with sublethal doses of DDT, ranging from 2 to 200 parts per billion (p.p.b.), resulted in shifts in temperature selection of three species of salmonids. In both the Atlantic salmon and brook trout, in general, low doses of DDT decreased the selected temperature, higher doses increased it. In rainbow trout exposure to DDT led to only an upward shift in selected temperature. A 'shock-response' to cold temperatures of the gradient was observed in both the Atlantic salmon and brook trout at higher DDT concentrations, while the rainbow-trout exhibited a 'shock-response' to warm temperatures. Exposure of Atlantic salmon to potassium cyanide doses, ranging from 1 to 200 p.p.b., resulted in a downwards shift in selected temperature which was dose-dependent. The ATP-uncoupler 2,4-dinitrophenol treatment was without any effect on temperature selection of Atlantic salmon. It seemed that DDT-induced changes in the behaviour of fish observed were due to interference by DDT with the thermal acclimation mechanism. A hypothesis of DDT action is discussed in which metabolic rate is the causitive factor in thermal acclimation mechanism.

In recent years a considerable body of literature has accumulated on the lethal effects of DDT on a variety of animals, including fish, but very little attention has been given to possible sublethal effects.

Allison *et al.*[1] studied the effects of chronic sublethal DDT treatment on the cutthroat trout (*Salmo clarki lewisi*). These authors observed that relatively high doses, which themselves did not appear to be the direct cause of death, resulted in marked delayed mortality, 'probably by reducing resistance to nonspecific stressors.' They did not investigate this hypothesis directly. However, DDT-exposure did result in higher-than-control mortality to the artificially induced stress.

Many authors have noted delayed mortalities in fish in areas of heavy DDT spray which could be attributed either directly to DDT by its progressive accumulation or indirectly to DDT by DDT-induced physiological changes which render the fish susceptible to one or more environmental conditions.[5,9,20,21,33]

It is also well established that DDT can exert its lethal effect, presumably directly in tissues, one generation removed from its initial absorption, by becoming passed on to, and greatly concentrated in, the yolk of eggs. The eggs may hatch but they succumb during the sac-fry stage.[1,2]

Behaviour is a special kind of physiological response. An interesting literature is developing on the effect of a variety of drugs on the behaviour of animals (Warner[32]) stimulated by the need for reliable bioassay tests. For insecticides, however, the documentation is poor. There are some reports indicating that sublethal doses of insecticides can produce behavioural changes in insects,[11,26] rodents,[3,31] birds[6] and man.[8] To our knowledge there are only two references to fish. Warner *et al.*[34] with the help of computer programming, measured several parameters of a conditioned avoidance response of goldfish before and after treatment with sublethal doses of toxaphene. It was shown that a 96-hr exposure to as little as 1.8 g/litre of toxaphene produced 'behavioural pathology' of considerable severity.

Ogilvie and Anderson[23] in a preliminary study showed that DDT in sublethal doses produced marked shifts in the selected temperature of Atlantic salmon, lower doses lowering it and higher doses raising it. The authors also described a curious 'cold-shock-response' in fish exposed to relatively high concentrations of DDT.

This paper presents the results of a more thorough investigation on the effects of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyle) ethane] on temperature selection of Atlantic salmon (*Salmo salar*) at various acclimation temperatures. The work was also extended to include brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). In addition, an attempt has been made to formulate a hypothesis of mechanism of DDT action.

Material and Methods

Animals

The three salmonid species used were in the fingerling stage and were 2–3 in in length. The sources and maintenance procedures of the fish have been described before.[14,15] As mentioned earlier,[14–16] the fish were always acclimated to any temperature for at least 4 weeks before use in experiments.

Procedure

(i) *DDT Treatment.* DDT (*p,p'*-isomer approximately 80%) treatment was carried out immediately prior to the determination of selected temperature, and consisted of exposing the fish, generally five or six at a time, to the desired concentration of DDT in 10 litre of water in a 11.5-litre glass container for 24 hr

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†Now at the Department of Zoology, University of the Punjab, New Campus, Lahore.

DDT was added to water in 0.5 ml of 95% ethanol. The concentration of DDT in the exposure containers in the experiments reported here ranged from 2 to 200 p.p.b. by weight. The water temperature during exposure was always the same as the acclimation temperature. The fish were not fed before use in an experiment once DDT exposure had begun. The fish in control experiments were treated as above except that the 0.5 ml of 95% ethanol contained no dissolved DDT.

The effect of DDT on the selected temperature was tested for acclimation temperatures 5, 10, 15, and 20°C for Atlantic salmon, 10 and 20°C for brook trout as well as rainbow trout.

(ii) *Potassium Cyanide Treatment.* The effect of KCN on selected temperature was investigated for 10°C acclimated Atlantic salmon. The KCN-treatments were for 24 hr at concentrations ranging from 2 to 200 p.p.b. The general procedure was the same as already described for DDT-treatment. KCN was added to the exposure jars in 0.5 ml distilled water. Fresh solutions of KCN were prepared before every treatment in view of the instability of KCN in aqueous solution.

(iii) 2,4-*Dinitrophenol Treatment.* The effect of 2,4-dinitrophenol (DNP) on selected temperature was investigated for 10°C acclimated Atlantic salmon. An aqueous solution of DNP was prepared and treatment of fish was carried out in the manner identical to that described for DDT and KCN administration. DNP concentrations ranged from 0.5 p.p.m. to 4 p.p.m.

The details of apparatus and experimental procedure have been described previously.[14] The selected temperature in all cases is defined as the temperature range which corresponded with the mode of the frequency distribution plot.

Results

The influence of various sublethal concentrations of DDT on selected temperature of the species under study is shown graphically (Figs. 1,2 and 3). Closed symbols denote results which differed significantly from controls. The method of statistical analysis has already been described.[16] The highest dose of DDT used in each case was determined by that dose which if exceeded would have resulted in unacceptable levels of mortality. Mortalities during the 24 hr exposure to maximum doses varied from 50% to 80% at the highest exposure dosage.

Atlantic Salmon

Figure 1 shows the effect of DDT concentrations, ranging from 2 to 100 p.p.b. on the selected temperature of Atlantic salmon for four acclimation temperatures 5, 10, 15, and 20°C. It is obvious that DDT affects the selected temperature but the sign of this effect is concentration-dependent, low doses lowering the selected temperature, high doses raising it. The DDT effect is also dependent upon acclimation temperature as evidenced by the dose causing the maximum lowering of the selected temperature tending to decrease with increasing acclimation temperature.

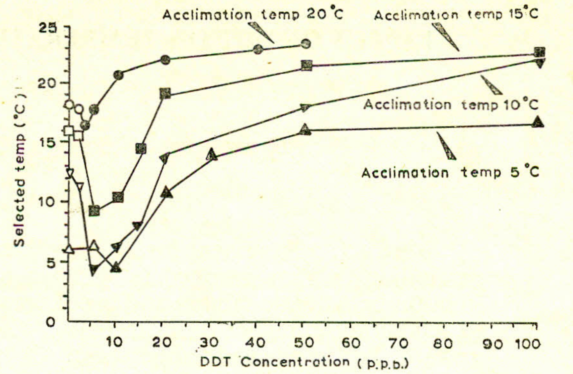

Fig. 1. The relation between selected temperature and DDT exposure in Atlantic salmon at four acclimation temperatures 5, 10, 15, and 20°C. Open symbols denote experiments in which selected temperature did not differ significantly from control values (on Y axis). Closed symbols are experiments differing significantly from controls.

The maximum lowering of the selected temperature occurs for 10°C acclimated fish and decreases at acclimation temperatures both above and below this temperature. There is a progressive decrease, however, in the raising (above control) of the selected temperature as the acclimation temperature is increased. The maximum total excursion of the selected temperature is remarkable, 18.0°C for the 10°C acclimated fish. The theoretical upper and lower limits of the DDT-induced shifts in selected temperature presumably would be set by the lower and upper lethal limits. For 20°C acclimation temperature the lowest recorded selected temperature of 16.4°C is clearly far removed from the lower lethal limit which is 5.5°C.[18] For 5 and 10°C acclimation temperature the gap, while still appreciable, is considerably narrowed since the selected temperature in both cases reaches a minimum of about 4.0°C and the lower lethal limit for 10°C acclimation temperature is 0.1°C, and presumably does not exist (except in theory) for the 5°C acclimated fish.[18] However, with regard to the maximum upwards shifts in selected temperature, the 10, 15, and 20°C acclimated fish are less than 2.0°C away from their upper lethal temperature (26.5°C for 20°C acclimation, 24.0°C for 10°C acclimation).[18]

The 'cold-shock-response' noted by Ogilvie and Anderson[23] was observed in the DDT-treated fish at all acclimation temperatures at relatively high doses. In 5°C acclimated fish it was noticeable only at the highest dose of 100 p.p.b. DDT, and even at this dose it was not very marked. At 10 and 15°C acclimation temperatures it was well pronounced at 50 and 100 p.p.b. DDT, and was present though much less apparent at 20 p.p.b. DDT. In the case of 20°C acclimated fish the cold-shock-response was very pronounced from doses of 20 p.p.b. DDT and greater.

No corresponding 'shock-response' to higher temperatures of the gradient was observed at any concentration of DDT.

Brook Trout

The effect of DDT concentrations, ranging from

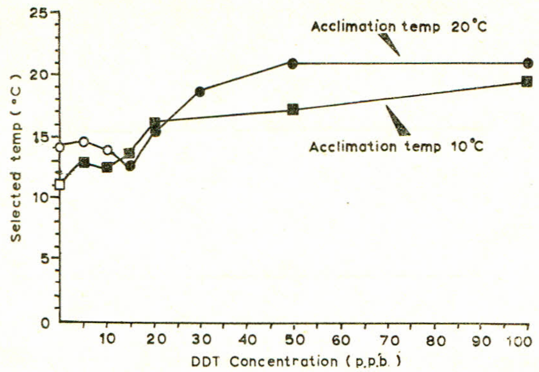

Fig. 2. The relation between selected temperature and DDT exposure in brook trout at two acclimation temperatures 10 and 20°C. Open symbols denote experiments in which selected temperature did not differ significantly from control values (on Y axis). Closed symbols represent experiments differing significantly from controls.

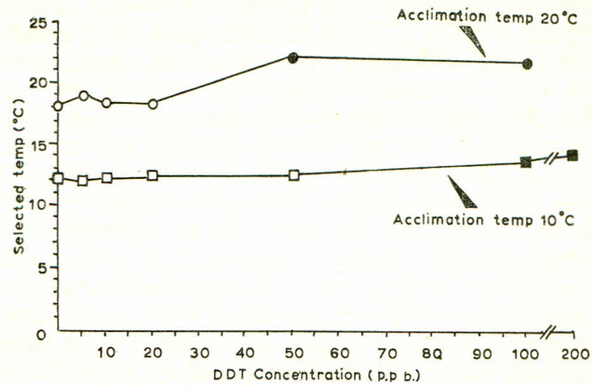

Fig. 3. The relation between selected temperature and DDT exposure in rainbow trout at two acclimation temperatures 10 and 20°C. Open symbols denote experiments in which selected temperature did not differ significantly from control values (on Y axis). Closed symbols represent experiments differing significantly from controls.

5 to 100 p.p.b. on the selected temperature of brook trout acclimated to 10 and 20°C is shown in Fig. 2. In general the results were the same as were seen in Atlantic salmon, except that there was no lowering of selected temperature for 10°C acclimated fish, and the temperature selection displacements were less in magnitude.

The cold-shock-response was apparent in brook trout also. It could be well recognised at the highest concentration of DDT used, that is, 100 p.p.b. was barely noticeable at 50 p.p.b. DDT, and was absent at lower DDT concentrations than these. Again no shock-response to warm temperatures of the gradient was noted.

Rainbow Trout

For rainbow trout there was no lowering of selected temperature at any of the DDT concentrations used, ranging from 5 to 200 p.p.b., for either 10 or 20°C acclimation temperature (Fig. 3). In addition, the selected temperature shifts were relatively small as compared to the other two species. Evidently, rainbow trout is much more resistant to DDT.

The cold-shock-response, so characteristic of Atlantic salmon and brook trout at higher doses of DDT, was found to be completely absent in this species. On exposure to higher concentrations of DDT, the fish tended, as a rule, to avoid the colder regions of the gardient but did not show any signs of distress upon the few occasions when they did venture into these regions. There was, however, a marked response in this species to warm water seen only at higher doses of DDT (100 and 200 p.p.b. DDT at 10°C acclimation; 50 and 100 p.p.b. DDT at 20°C acclimation). This response resembled in symptoms the cold-shock-response, and occurred when the fish encountered temperatures of 25°C or above. The fish showed signs of distress, became hyperactive, if not taken out of these temperatures immediately, swam upside down and died within a short time. If removed soon enough from the warm temperatures the fish usually recovered and soon exhibited behaviour indistinguishable from the other fish.

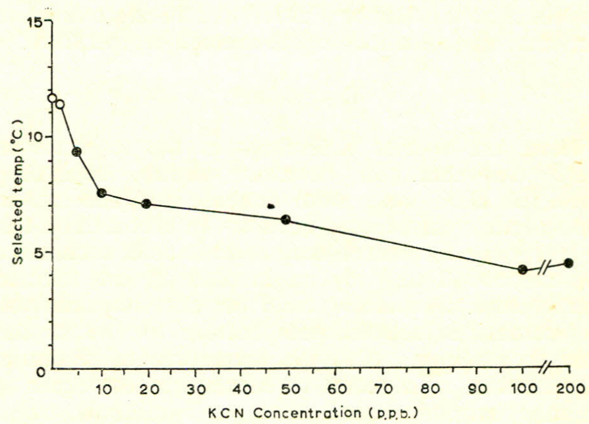

Fig. 4. The relation between selected temperature and potassium cyanide (KCN) exposure in 10°C acclimated Atlantic-salmon. Open circles denote experiments in which selected temperature did not differ significantly from control values (on Y axis). Closed circles represent experiments differing significantly from controls.

Effect of other Agents on Selected Temperature

Some preliminary work had suggested that in Atlantic salmon the metabolic rate of both the brain tissue[24] and the whole organism[18] is decreased and increased by low and high doses of DDT respectively. The thought occurred that the similarity of these results with the results obtained in this study, might be more than coincidental. Specifically, the suggestion arose that anything that lowers the metabolic rate brings about a lowering of selected temperature; anything that raises the metabolic rate, raises the selected temperature. It was, therefore, decided to try some agents which would lower and raise the metabolic rate of fish artificially, to see whether there was any effect on temperature selection.

(a) *Potassium Cyanide Treatment.* Potassium cyanide (KCN) is a well-known inhibitor of the cytochrome system, lowering the metabolic rate as measured by oxygen consumption. In Fig. 4 is shown the effect on temperature selection of 10°C acclimated

Atlantic salmon, of 24 hr exposure to various concentrations of KCN. The selected temperature decreases with increased KCN concentration, becoming asymptotic at, or perhaps before, 100 p.p.b. KCN. Of some interest, the asymptotic value for selected temperature is almost precisely the same as that recorded for the maximum DDT-induced lowering (Fig. 1).

(b) *2,4-Dinitrophenol Treatment.* Increasing the standard metabolic rate proved to be a greater challenge. Atlantic salmon, acclimated to 10°C, were exposed for 24 hr to various concentrations of 2,4-dinitrophenol (DNP). While DNP, acting as an ATP-uncoupler, is known to increase oxygen consumption when applied to tissues, there is no evidence that it can be absorbed from the water by fish and thereafter to stimulate metabolic rate. The possibility was not examined in this study. The results then, shown in Fig. 5, are somewhat equivocal. Up to 4 p.p.m. DNP (5 p.p.m. resulted in 100% mortality) there was no detectable change in selected temperature. It cannot be said with certainty if this was because DNP was with out effect on the fish, or if DNP had an effect which in turn had no influence on the selected temperature.

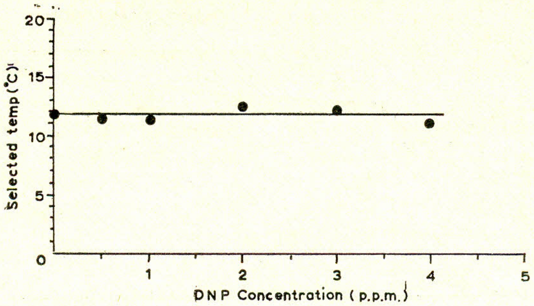

Fig. 5. The relation between selected temperature and 2,4-dinitrophenol (DNP) exposure in 10°C acclimated Atlantic salmon.

Discussion

There are species differences in the response to DDT treatment. In Atlantic salmon, depending upon the DDT dose, both increase and decrease in the selected temperature occurs. In brook trout this is true only for warm acclimation, cold acclimated fish responded only by an increase in the selected temperature. In rainbow trout the only response was an increase in selected temperature for warm and cold acclimation. Also the magnitude of response, regardless of sign, seems to decrease in the order of Atlantic salmon, brook trout and rainbow trout. And finally, rainbow trout exhibited no cold-shock-response but instead displayed a 'warm-shock-response' which was totally absent in the other two species. These differences cannot be due simply to variations between species of the toxicity of DDT to the tissues, or to variations in the rate of DDT-uptake (variations which certainly have been shown to exist between species[12]), because it will be recalled that in all cases the range of DDT doses used extended up to the lethal level. It would appear that independently of the toxicity of DDT, the species vary with respect to the sensitivity too, and even the kind of response induced by sublethal concentrations of DDT.

In all three species, the curve describing the relationship between selected temperature and DDT concentrations at various acclimation temperatures are more or less independent of each other, that is, the data for one acclimation temperature lies entirely above and below the data for the next lowest and highest acclimation temperature respectively. On the one hand this is to be expected since it is consistent with the effect of acclimation temperature on selected temperature. On the other hand it would not have been surprising to find considerable intersection of the curves on the basis of some of the known effects of DDT. It would be remembered that the temperature at which the fish were exposed to DDT was always the same as the acclimation temperature and in terms of toxicity at least, DDT is more effective at higher than at lower acclimation temperatures, test temperatures remaining constant.[22] Also at constant acclimation temperature DDT is known to be more toxic at higher than at lower test temperatures.[10,19] On both grounds then, it might be expected that the effect of DDT on selected temperature would have been greater at higher than at lower acclimation temperatures. The overall impression is that such is not the case. Of course the situation is complicated by the additional factor of the limit imposed by the upper and lower lethal temperatures, as referred to previously. The fact, however, that the lowest concentration of DDT, which just produces a change in selected temperature, tends to decrease with increasing acclimation temperature (brook trout being a notable exception) is consistent with the suggestion that DDT is more effective the higher the acclimation temperature.

The above results serve to focus attention on perhaps the most paramount question of all, viz., how does DDT act to bring about the striking behavioural changes associated with selected temperature. That there should have been DDT-induced changes seen in the selected temperature is by itself perhaps not too significant because behaviour is obviously a function of nervous activity and it is well known that DDT affects nervous structures, both peripheral and central. What, in my view, was highly significant was the fact that by varying the concentrations of DDT one could shift the selected temperature either upwards or downwards as if one had changed the thermal acclimation upwards or downwards respectively. Of course, no such alterations in acclimation temperature actually occurred, which encouraged the speculation that DDT might have been exerting its influence on behaviour by somehow interferring with the normal functioning of the thermal acclimation mechanism. This view gained additional support from the DDT-induced sensitivity to cold in which the cold-shock-response,[23] and the rise with DDT, of the lower lethal temperatures[18] resembled that which might normally be expected to be associated with increased thermal acclimation.

While peripheral thermal receptors (as yet unidentified) clearly participate in the temperature selection response,[30] all the evidence points to the central nervous system as the site for the changes in this response which are associated with thermal acclimation.[7]

Historically, it was the easily observable neurophysiological responses that DDT induced in sensory nerves that attracted the most attention.[28,35] Later impairment of central nervous system function was recognised as a common occurrence.[4,29] At the moment there is no general agreement as to which of the areas, peripheral or central, is the 'target' organ for DDT poisoning. There is, however, no question that DDT very quickly reaches the central nervous system of fish[26] and remains there.[13]

Regarding the nature of the thermal acclimation mechanism itself, there is no general agreement, although there is no lack of proposals.[17,25,27] To this list we now add yet another hypothesis. Specifically, it is proposed that metabolic rate is a causal agent in the thermal acclimatory mechanism. In the simple situation it would be the metabolic rate of the thermal acclimation mechanism itself which would determine the nature of the acclimation-associated changes, but there is no *a priori* reason why the metabolic rate of surrounding structures, or the animal as whole, could not indirectly affect the mechanism. To specify even further, it is proposed that increases in metabolic rate lead to response changes associated with warmwards acclimation, decreases in metabolic rate to response changes associated with coldwards acclimation. DDT, then, is visualized as inducing thermal acclimation-like responses by affecting metabolic rate, in the simplest case the metabolic rate of the acclimation mechanism itself. Our results on DDT and temperature selection, and some preliminary findings on DDT and metabolic rate mentioned earlier[18,24] are consistent with this hypothesis. The starvation[15] and cyanide-induced inhibition of standard metabolic rates in general led to decrease in selected temperature as predicted by our hypothesis. The results with DNP have been considered to be inconclusive rather than negative. Perhaps the most important information which is not consistent with our hypothesis is the well-known fact that following a change in acclimation temperature from one level to another the standard metabolic rate of the organism undergoes a change opposite in direction to the change occurring more or less simultaneously in the selected temperature, that is, following a cold-to-warm change in acclimation temperature, the standard metabolism gradually declines when as we have seen[16] the selected temperature gradually increases. In addition, the work indicating that the time course of change in selected temperature is temperature-independent[16] is not encouraging in the present context. When measured acutely, metabolism can hardly be temperature-independent and thus it would be expected that if metabolic rate was an integral part of the thermal acclimation mechanism, then acclimatory changes would take longer to occur going from warm to cold than cold to warm.

The fact remains, however, that in the main our hypothesis seems to be more consistent with the available evidence than any other we can think of.

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