Effects of heat pollution and organo-chlorinated pesticides on fish reproduction

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Project Title : Effects of heat pollution and organo-chlorinated pesticides on fish reproduction.

I - EFFECTS OF TEMPERATURE ON TELEOST FISH REPRODUCTION

A - Effects on gametogenesis of goldfish (Carassius auratus) reproduction

Goldfish of both sexes were raised at 4 constant temperatures : 10, 17, 24 and 30°C, or submitted to two types of circadian heat fluctuations varying by 4°C around 17 and 24°C. A control put into natural environmental conditions was submitted to regular increase of temperature between February and August (10 to 27°C). The criteria used for evaluation were : plasmatic and pituitary levels of c-GTH gonadotropic hormone, evolution of R.G.S., quantitative analysis of gametogenesis.

The results show that at breeding temperatures equal to or higher than 17°C, with circadian variations or not, plasma c-GTH levels are always significantly ($P < 0.001$) higher than those of animals kept at 10°C for the first 4 months of treatment (February to May). In June and July, the situation is reversed and the fish raised in a high temperature present a lower level ($P < 0.05$) than subjects raised at 10°C. The pituitary c-GTH level in April is significantly higher at a high temperature (> 17°C) than at 10°C ($P < 0.05$), while in June the reverse is seen, indicating a negative balance between synthesis and release at a high temperature.

Analysis of R.G.S. and gametogenesis shows that at a breeding temperature of 30°C, spermatogenesis and ovogenesis are very rapidly inhibited; in April only spermatogonia A are found. In groups raised at lower temperatures, most of the cell types are conserved and spermagenic efficiency is maximum in April at 24°C and in June at 10°C. Such a study leads to very original conclusions : gonad and pituitary heat requirements are different. High breeding temperatures (30°C) inhibit gametogenesis by direct action on the gonads and enhance GTH secretion. It may be that this increase in gonadotropic secretion is due

to the suppression of a negative feedback usually exercised by the functional glands on the hypothalamo-pituitary system. This raises questions as to the physiological significance of these differential heat requirements, especially if it is remembered that the final heat preferendum of this species is about 30°C. It should be noted that this inhibition is reversible, and that resumption of gametogenesis has been observed after the fish have remained at 30°C for 6 months.

B - Effect on gamete survival and fertilization in the rainbow trout (Salmo gairdneri)

The experimental procedure was the following :
- effects on fertilization by submitting eggs and sperm to experimental temperatures only during insemination. Immediately after their collection, the gametes were progressively adapted over a 20-min period to experimental temperatures between 1 and 30 °C. Insemination was then practised with an insemination diluent (D.I.) previously brought to the same temperatures. The eggs were then left at room temperature for 10 min before transfer into fresh water incubators.
- effects on gamete survival. After dilution the gametes were independently exposed to experimental temperatures for 20 and 40 mins. Insemination was then practised using sperm or eggs left at 10°C. The effects were evaluated by the percentage of embryonic eggs observed after 10 days of incubation at 10°C.

The results show that the optimal fertilization temperature ranges between 5 and 15°C. At 20°C or more, fertilization rate diminishes, especially if dilution rate is high. When spermatozoa are put into movement after dilution in D.I., they retain their fertilizing ability longer at 0 and 5°C than at 10 and 15°C. If spermatozoa are immobilized by enriching the D.I. with K^+, their fertilizing ability significantly decreases at 20°C and in some cases at 15°C. After 40 min of exposure, ovule fertilizability is only conserved at 1 and 5°C. It thus seems important to control the temperature during Salmonid insemination in fish-farming, and in natural conditions it is probable that temperatures higher than 15°C affect fertilization yield.

II - EFFECT OF LINDANE ON REPRODUCTION IN THE TROUT.

A.- Establishment of lethal doses

The objective of one of the proposed experiments was to administer non-lethal

amounts of lindane to trout during gametogenesis. These doses were determined in a preliminary experiment in which the dose of lindane was varied between 0 and 50 mg/kg of live weight per day. Mortality was found on day 3 of treatment for 50 and 25 mg doses and on day 9 for the 10 mg dose. Maximum mortality occurred on day 9 with the 50 mg dose and on day 20 with the 25 mg dose. After 3 weeks, mortality was negligeable with the 10 mg dose; it was 20 % with the 25 mg dose and 50 % with that of 50 mg. The doses used in further experiments were 0.5 and 5 mg of lindane per kg of live weight per day, or 50 and 500 mg of lindane per kg of feed with a daily feed rate of 1 % of live weight.

B - Effect on gametogenesis

The breeding fish were fed during the period comprising the end of vitellogenesis and spermiogenesis (September, October) with feeds containing lindane (see II-A); the control was a commercial feed. Gametogenesis occurred normally, and all treated males and females spermiated and ovulated normally. The sperm fertilization rate of treated males did not differ from that of the controls. Although the ovules treated with the strongest lindane dose presented the highest relative lindane levels (3.3 ppm in relation to fat content), they presented a normal rate of fertilization and embryonic development comparable to the controls.

C - Effects on gamete survival and reproduction

The lindane being incorporated into the D.I., the experimental procedure is analogous to that described previously for testing the effects of temperature. Fertilization percentage decreased when the dose of lindane reached 25 ppm in the D.I. The effect was more marked when dilution rate was higher. It seems that fertilization rate was affected when the lindane was present in high amounts in the medium used for insemination.

III - EFFECTS OF SOME METALS ON GAMETE SURVIVAL AND ON FERTILIZATION. A PROPOSED TEST FOR TOXICITY.

With the methodology used in the experiments reported in paragraphs I-B and II-C, we carried out an experiment to test the toxicity of some metals introduced into the D.I. Hg, Fe, Cn, Cr are the most toxic for gametes.

It thus appeared that the methodology employed might constitute a toxicity test which could profitably complete already-existing tests and be used to evaluate the effects of various pollutions on a particularly sensitive period in the life of fish.

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Contract number : 045-74-1 ENV. F, project 2

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Title of the project : Fish, pollution indicator : utilization of rainbow trout reactions to sub-lethal concentrations of toxic substances in water

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General description of studies and results

In the field of this contract, we tried to work out a detection test of the toxic effect of substances in solution in water at sub-lethal concentrations for a fish species : rainbow trout Salmo gairdneri. This test is simple, unexpensive and easy to carry out. Three apparatuses were designed :

- an electrical tester : research in the variation of the threshold of fish sensibility to electric current ;
- a rectilinear water current tester ;
- a circular water current tester in a funnel.

1. ELECTRICAL TESTER

1.1. Principle

The study was limited to the action of a detergent on fish sensibility to electric current. The hypothesis was that the threshold of fish sensibility to electric current is influenced by a chemical pollutant at sub-lethal concentrations. We tried to show up this fact by putting young trout for 24 h in solutions of various concentrations. The fish, placed in a trough between two electrodes, is submitted to increa-

sing electrical impulses, until it shows a reaction. Measures are made, the fish facing the cathode and the electrodes exactly fitting the fish length.

1.2. Working out

This apparatus was composed by an impulse generator, an electrode polarity reverser, a transparent plastic trough equipped with a fixed electrode and a mobile one giving the possibility to exactly adjust the trough useful length to that of the fish.

The toxicant used is an anionic detergent (Linear Alkylbenzene Sodium Sulfonate, activity 46.2% LAS), LC 50-24 h : 1.90 mg/l of active substance. Tests were made at 17° C.

The tested fish were rainbow trout kept in a rearing closed circuit.

1.3. Results and conclusions

With the four tested concentrations, it is noted that the averages of the threshold of sensibility are too close for their difference to be significant. The results obtained cannot therefore be utilized and the experimentation with that kind of apparatus was interrupted.

2. RECTILINEAR WATER CURRENT TESTER

2.1. Principle

This tester has been very much used to estimate the quality of fish intended for restocking. In our study, the fish introduced in a rectilinear pipe is submitted to a water current, the speed of which is increased by steps. The stamina of the tested fish is measured.

2.2. Working out

This apparatus was composed by a rectilinear glass pipe in which a constant water current is running (30 cm/s). This water current is created by a pump.recovering, in a tank, the water coming out of the

pipe. A mechanical grid above the pipe prevents the swimming up of fish beyond the trial area ; an electric fence, down the pipe, stimulates the swimming of fish until exhaustion. Various systems (water gates, electronic control) enable to steady the water current inside the pipe.

The toxic substance used was the same as in the precedent case, the fish tested were coming from the same batch as in the electrical test.

2.3. Conclusions

Under a constant speed of 30 cm/s young trout offered resistance during many days. In these conditions, it was difficult to determine when the test was finished, without designing an apparatus much more sophisticated and therefore considerably more expensive. This apparatus was then eliminated.

3. CIRCULAR WATER CURRENT TESTER IN A FUNNEL

3.1. Principle

The fish is put inside a funnel in which the solution is propelled by a rotary motion round a vortex which caps the outlet. Thus the fish struggles against the water current (rheotactism) and against the vortex suction. When it reaches its stamina limits, the exhausted fish stops the funnel mouth ; the stamina duration of the fish is then measured.

3.2. Working out

3.2.1. Apparatus (fig. 1)

3.2.2. Tested substances

- an anionic detergent : Linear Alkylbenzene Sodium Sulfonate, activity 46.2% LAS, CL 50-24 h : 1.90 mg/1 of active substance. The tests were made at 17° C ;

- a cationic detergent : Quaternary Ammonium Salt (Dimethyl Ditallow Ammonium Chloride), activity 75%, CL 50-24 h : 40 mg/1 of

active substance. Tests were made at 17° C ;

- a non ionic detergent : Ethoxylated Fatty Alcohol, activity 99.9%. Tests were made at 20 and 24° C. CL 50-24 h at 20° C was 2.75 mg/l of active substance.

The swimming tests and CL 50 determinations were made with natural mineral waters of commercial distribution (Evian, Source Cachat) as reference water and dilution.

3.2.3. Tested fish : rainbow trout, length class 7/10 cm, kept in a rearing closed circuit.

3.3. Results (fig. 2)

It appears that the relation dose-effect is quite clear below a CL 50-24 h conventionnally established. The low concentrations of a toxic agent can be underlined by the test of fish swimming endurance. Tests show that it is possible to study in fish both effects of temperature and toxicity of substances in solution.

3.4. Conclusions. Future of the apparatus

The utilization of an active method leads to the increase of fish sensibility to the tested toxicants and allows to estimate the influence of low concentrations on it. A patent application for this apparatus has been presented. A further test for the toxicity estimation of an industrial rough effluent was carried out. It has been possible to show a qualitative alteration of water for fish, alteration which is a linear function of the toxic concentration. But the appreciation of a permanent pollution through a "fish-test" can be representative as far as the fish are all the time kept in the water to control and thus undergo all the fluctuations of environment characteristics. These observations led to the working out of a pollution biodetector, made on an industrial scale (Biodetector ERMAT) and which is the practical issue of the studies carried out within this contract.

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Fig. 1

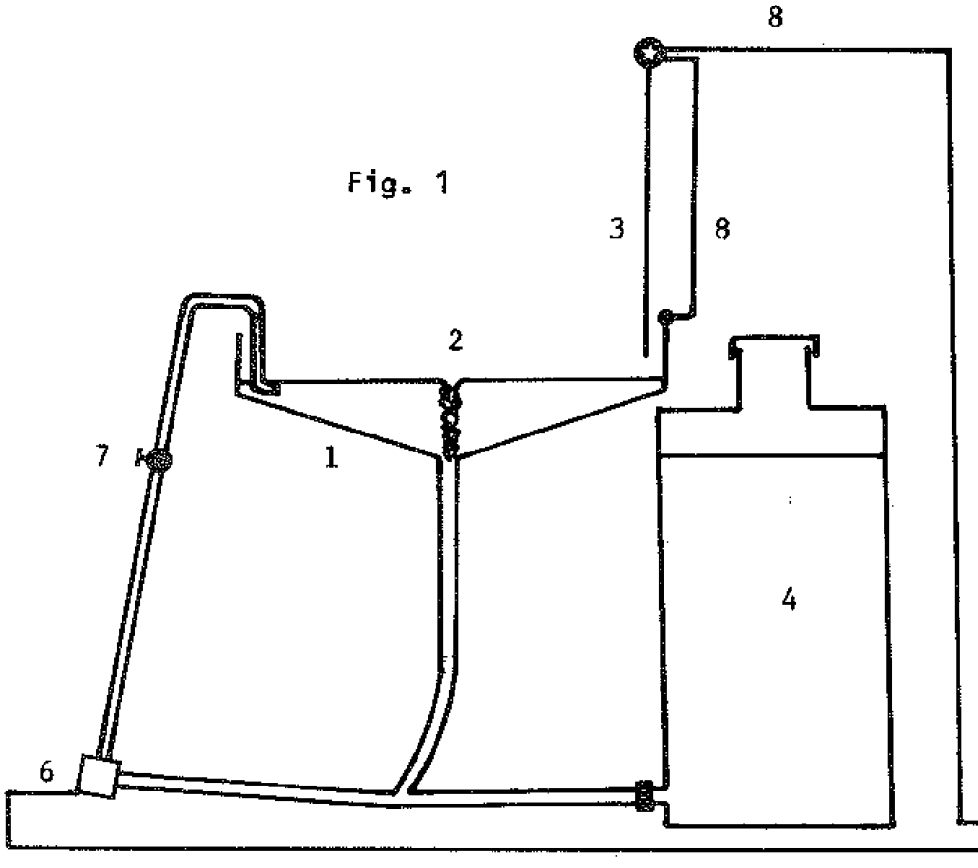

Diagram of the circular water current tester

1 - Funnel
2 - Vortex
3 - Level indicator
4 - Buffer tank
5 - Electric clock
6 - Pump
7 - Control gate
8 - Electrical circuit

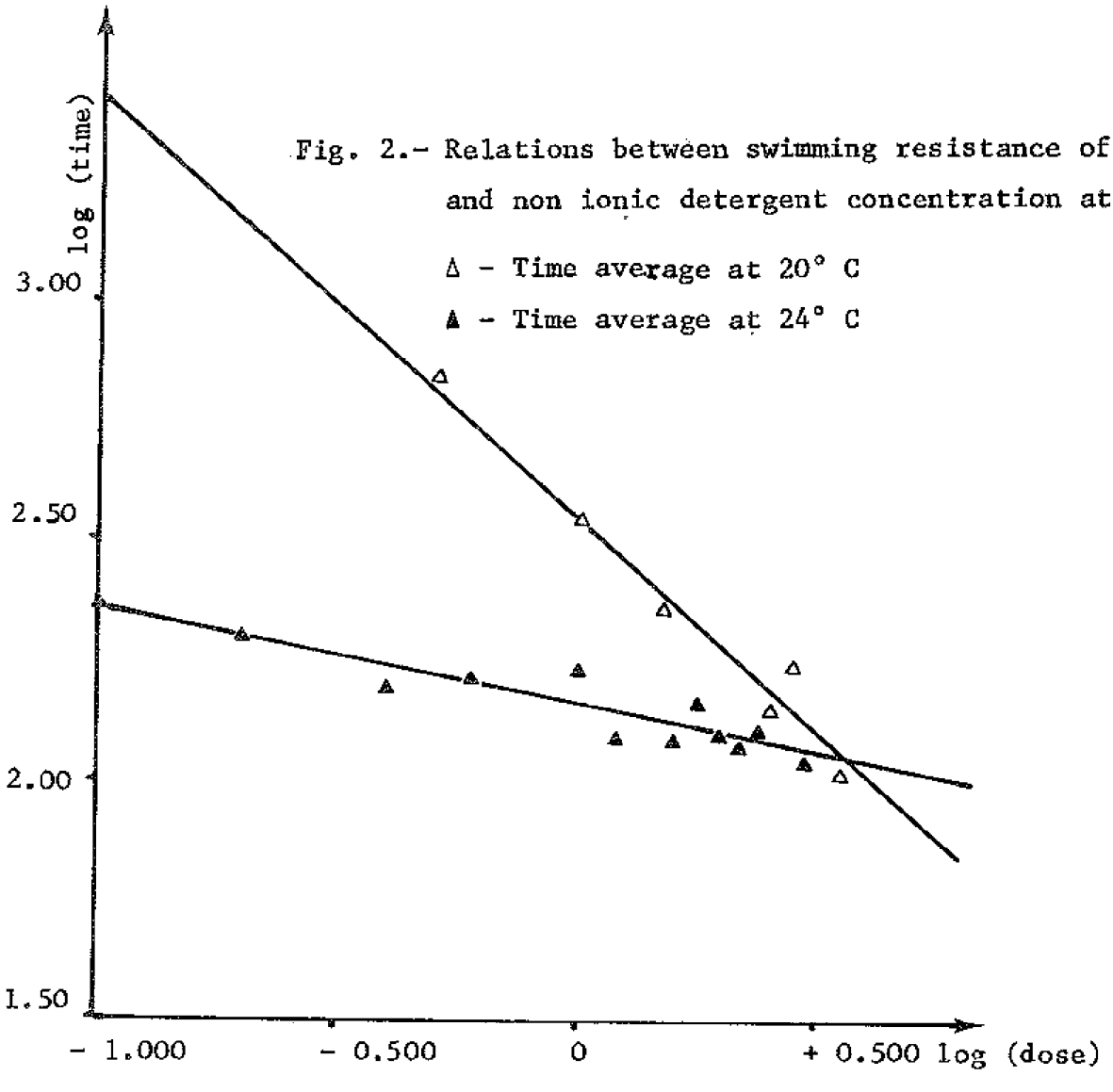

Fig. 2.- Relations between swimming resistance of young trout and non ionic detergent concentration at 20 and 24°C

Δ - Time average at 20° C

▲ - Time average at 24° C