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Teratology

**OXYGEN DEFICIENCY AND NEGATIVE TEMPERATURE  
AS TERATOGENIC FACTORS IN RAINBOW TROUT  
(*SALMO GAIRDNERI* RICH.)**

**NIEDOBÓR TLEŃOWY I UJEMNA TEMPERATURA  
JAKO CZYNNIKI POWODUJĄCE ANOMALIE ROZWOJOWE  
PSTRĄGA TĘCZOWEGO (*SALMO GAIRDNERI* RICH.)**

Research on effects of low temperature and oxygen deficiency affecting, separately and jointly, developing embryos of rainbow trout (*Salmo gairdneri* Rich) demonstrated the occurrence of various malformations (spine distortions, twin forms) as well as vast damages of blood vessels leading to death of the embryos and larvae affected to be brought about. The number of teratologic changes and mortality rate of the embryos and larvae were found to be much higher at a joint action of the two factors than the same effects obtained with each adverse factor acting separately.

**INTRODUCTION**

The occurrence of teratologic changes and – in general – anatomical anomalies in embryos, larvae, and adult fish has been extensively treated in the literature (Gemill, 1912; Freund, 1923; Dawson, 1964, 1966, 1971). Opinions as to mechanisms underlying the changes and their causative agents tend to vary.

The changes may be pre-determined genetically (Schäperclaus, 1954; Rosenthal et al., 1958; Orska, 1962; Mattheis, 1964; Aulstand and Kittelsen, 1971). They may be brought

about by various deleterious external (or intrinsic) effects early in the ontogenesis. In this latter case, many authors relate the malformations to under- or overripened gametes (Mršič, 1923; Dieterich, 1938; Schnakenbeck, 1956); others hold parasites (e.g., certain protozoans) or bacteria responsible (Hoffman et al., 1962; Reichenbach-Klinke, 1966); still others maintain water temperature (Hein, 1911; Persov, 1950; Orska, 1956; Vasiliev, 1957; Hubbs, 1959; Tatarko, 1968; Kokurewicz et al., 1978), the absence of certain chemicals in the water (Matlak, 1969), oxygen deficiency (Willer, 1928, 1933; Lieder, 1955; Alderdice et al. 1958; Gottwald, 1965; Jurovickij and Resnicenko, 1961; Gottwald and Kaniewski, 1967; Gulidov, 1969; Matlak, 1969) or excess of oxygen (Gulidov, 1969) and also water pollution (Rucker et al., 1970; Matlak, 1972; Matsusato, 1973; Bucke, 1974), wrong diet, lack of vitamin D (Wunder, 1934; Otterstrom, 1939; Rucker et al., 1970) to be the causative agents.

Those authors dealing with effects of environmental factors on anatomical malformations in fish tend to restrict their considerations to a single selected factor, other factors, possibly affecting the embryo at the same time, being disregarded. The present paper is an attempt to explain a possible synergic action of two adverse factors capable of causing anatomical malformations.

## MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri* Rich.) eggs obtained from the Bukowo Morskie Hatchery were studied in spring 1979. Fertilised eggs were incubated in Petri dishes placed in a  $9^{\circ}\text{C} \pm 1\text{C}$  water bath. The water used for incubation, control, and experiment was found to contain  $9.8\text{ mg O}_2/1$  and  $11.8\text{ mg CO}_2/1$ .

The experiment was run in 3 variants.

Variant 1 covered low temperature effects. Three h after fertilisation the eggs were transferred to empty vials and placed in a refrigerator at  $-2^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ ; over the 5 subsequent days 100 refrigerated eggs were transferred every 24 h back to the control water and kept there until hatching. In another sample eggs developing normally were subject to refrigeration over 24 h during eyeing.

Variant 2 involved testing the effects of oxygen deficiency; 3 h after fertilisation the eggs were transferred from the control to dishes containing filtered water ( $2.8\text{ mg O}_2/1$ ;  $19.8\text{ mg CO}_2(1)$ ) and incubated until the eye pigment appeared, after which the eggs were transferred back to dishes containing the control water. In another series the eggs incubated in the control water until the blood system formation were transferred into the filtered water and incubated.

Variant 3 was a combination of the previous two.

All the experimental samples and the control were thoroughly observed, malformations being photographed live or after an intravital benzidine staining, preliminary alcohol fixation, and glycerin mounting and the changes were analysed.

## RESULTS

The observations demonstrated malformations and teratologic forms to appear among the regularly developing embryos in all the three experimental variants (low temperature, oxygen deficiency, and the two factors combined). The anomalies varied from the simplest single distortions of the body via more complex skeleton malformations and changes within the blood system to twin terases.

Low temperature effects on embryos from fertilisation until cleavage completion were reflected by malformations present in 1.84% of the individuals, the specimens affected showing various spine distortions in the horizontal (Fig. 1 a) or vertical planes: from simple lordoses, kyphoses, or scolioses to more complex malformations (Fig. 1 b), mostly in the caudal part.

Negative temperatures administered at later stages of egg development ("eyeing") induced no anomalies.

Adverse breathing conditions (low O<sub>2</sub> and high CO<sub>2</sub> contents) persistent from fertilisation to eyeing gave rise to malformations occurring in 3.2% of the embryos observed. The malformations, similarly to the previous experimental variant, involved various skeletal distortions, twins, and lesions in the course of blood vessels. Typical of the latter were congestions occurring at the terminal stage of the embryonal development, followed by effusions in some parts of the body, particularly in the caudal fin. A high (about 40%) percentage of normally developing eyeing embryos, when transferred into the filtered water, were found to die just before hatching. Those embryos commonly showed damaged blood vessels and ecchymoses.

Combined effects of the two factors on fertilised eggs during cleavage were manifest in variously malformed larvae, their number amounting to 7.3% of all the individuals observed, 1.8% of them being twins. The latter included different forms, from symmetrical twins (*Autosita*, according to Tur, 1927) to forms in which one of the pair had degenerated, i.e., asymmetrical twins (*Parasita*). The *Autosita* included both the *Anacatydimi*, i.e., individuals accreting through the yolk sac with heads and posterior parts of the bodies separated, in other words two independent individuals interconnected through the surface of the yolk sac ("omphalophagus" type), and the *Catadidymi*, i.e., individuals accreted through the yolk sac and posterior part of the body (Fig. 1 c).

Among the *Parasita*, various forms of the reduced individual's degeneration were recorded. Disregarding the size variability, malformations in this groups of embryos included distortion of the caudal part (Fig. 1 d), a poorer development of the head (Fig. 1 e), or an advanced reduction of the eyes (Fig. 1 f).

The hatched twin larvae very seldom moved from place to place, their movements being clumsy and depending on the adhesion width and extent of the twin individual's degeneration. When in an aquarium, they cannot move above 2–3 cm from the bottom and die after resorption of the yolk sac. Those larvae with distorted spines behave in a similar manner, their chances of survival being negligible as well.

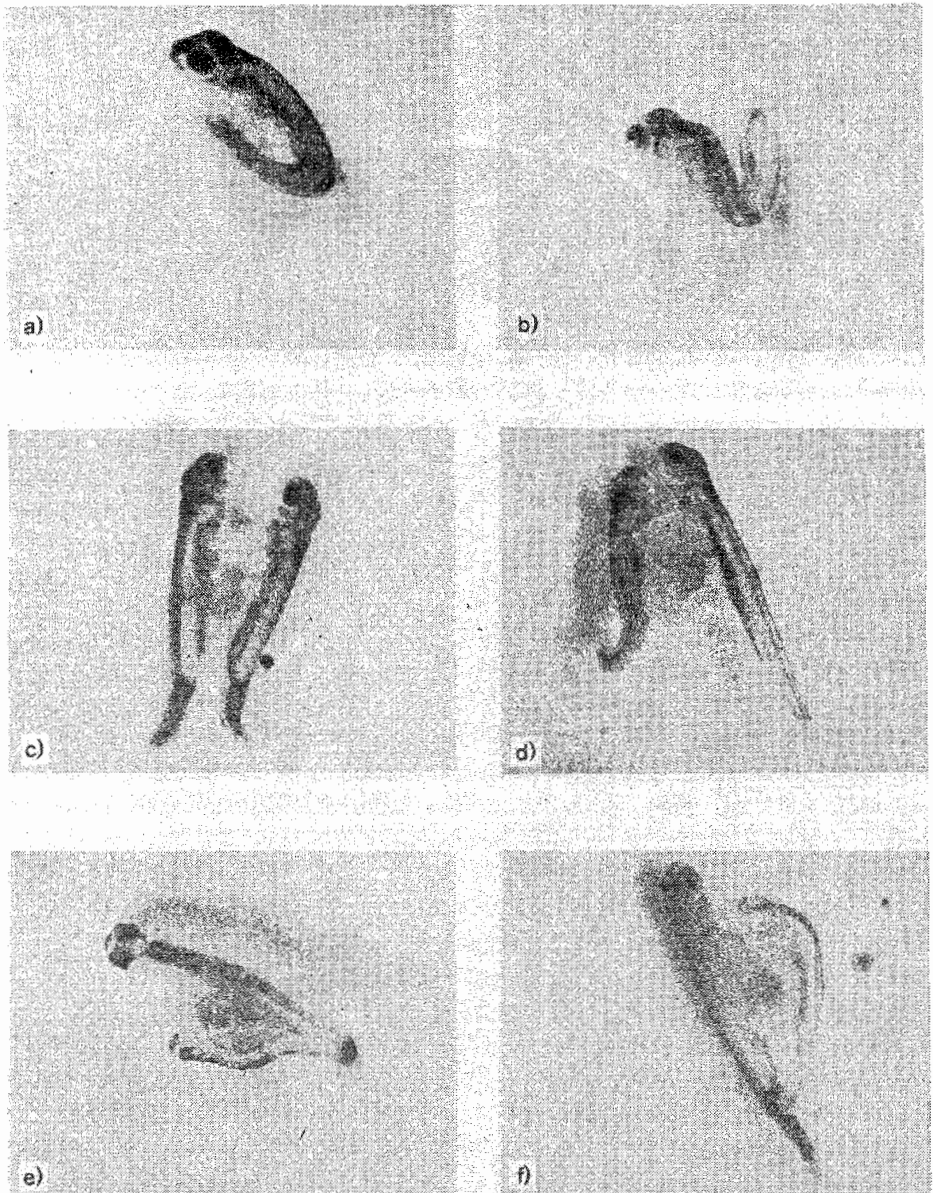


Fig. 1. Anatomical anomalies in embryos induced by oxygen deficiency and negative temperature. a. spine distortion in the horizontal plane; b. spiral multifarious distortion of the body; c. twin forms – omphalophagus type; d. caudal part distortion in one of twins; e. underdevelopment of the head in one of twins; f. underdevelopment of the eyes.

## CONCLUDING REMARKS

The results obtained confirm known facts of negative temperatures (Wawrzyniak, 1975) and oxygen deficiency (Gottwald, 1965) being teratogenic factors for salmonids. The below-zero decrease in temperature results basically in changes of the skeleton structure only, with all consequences in the external form and structure of the fish body, while adverse breathing conditions at early developmental stages give additionally rise to more far-reaching changes, including the formation of accreted twins.

Both the amount of various malformations and, particularly significant, the amount of twins increase when the two factors act jointly.

The percentage monstrosity rates found in the present study tend to be very high. It is possible that not only these two selected factors and their combinations are responsible. The quality of eggs might have played a role, too, although no developmental anomaly was found in the control. It is beyond doubt, however, that detrimental factors acting during cleavage bring about, as a rule, general changes in symmetry and structure of the body and formation of twins; the same factors acting during late embryogenesis result in local changes only, usually uniform in their nature, manifest as an altered structure of the system. It is also a fact beyond doubt that the amount of teratologic changes resulting from the combined action of the two adverse factors is never an arithmetic sum total of the malformation cases brought about by the factors acting separately being much higher than such a sum.

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(*SALMO GAIRDNERI* RICH.)

STRESZCZENIE

Badania nad wpływem obniżonej temperatury i niedoboru tlenu oddziaływujących oddzielnie i łącznie na rozwijające się zarodki pstrąga tęczowego (*Salmo gairdneri* Rich.) wykazały powstawanie różnych form potworkowatych (skrzywienia kręgosłupa, formy bliźniacze), jak również rozległe pęknięcie naczyń krwionośnych prowadzące do śmierci zarodków i larw. W przypadku wspólnego oddziaływania obu tych czynników ilość powstałych zmian teratologicznych oraz odsetek śmiertelności zarodków i larw jest znacznie większy niż suma tychże efektów uzyskanych w trakcie oddzielnego działania obu niekorzystnych czynników.

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НЕДОСТАТОЧНОСТЬ КИСЛОРОДА И ПОНИЖЕННАЯ ТЕМПЕРАТУРА В КАЧЕСТВЕ  
ФАКТОРОВ ВЫЗЫВАЮЩИХ АНАТОМИЧЕСКИЕ АНОМАЛИИ В РАЗВИТИИ  
РАДУЖНОЙ ФОРЕЛИ (*SALMO GAIRDNERI* RICH.)

Р е з ю м е

Исследования над влиянием пониженной температуры и недостатка кислорода действующих отдельно и совместно на развивающиеся зародыши радужной форели (*Salmo gairdneri* Rich.). выявили образование различных форм анатомических аномалий (искривление позвоночника, близнецовые формы) а также

наличие обширных трещин кровеносных сосудов, которые приводят к смертельному исходу зародышей и личинок. В случае совместного воздействия обоих этих факторов, количество тератологических изменений, а также процент смертности зародышей и личинок является значительно большим, чем сумма тех же эффектов полученных путем отдельного воздействия обоих неблагоприятных факторов.

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Received: 30 Sept. 1982