Bull. Environ. Contam. Toxicol. (1996) 57:842-848

Environmental Contamination and Toxicology

Carp Reproduction in Highly Eutrophic Pond Conditions

K. Bieniarz, P. Epler, M. Sokolowska-Mikolajczyk, J. Chyb, W. Popek

Department of Ichthyobiology and Fisheries, Agricultural Academy, ul. Prof. T. Spiczakowa 6, Kraków 30-149, Poland

Received: 12 December 1995/Accepted: 10 May 1996

Increasing water eutrophication is the reason for initial increase and later diminution of fish populations leading in consequence to a total decline of fish in a reservoire (Colby et al. 1972). It is not yet clear if the diminution of fish populations is caused by the direct toxic effect of the nitrogen-containing compounds, especially ammonia, on fish (Ball 1967; Colt and Tchobonoglous 1976; Bath and Eddy 1980; Scarno and Saroglia 1984; Williams and Eddy 1988 a,b), or on one of the reproductive stages, which lowers the number of offspring. Penaz (1965) showed that a short exposure of fertilized brown trout (*Salmo trutta*) eggs during later stages of embryonal development to concentrations of 50 mg/l of unionized ammonia at 10° C reduced hatching success. Concentrations of 0.3-0.4 mg/l of unionized ammonia inhibited trout spawning (Wuhrmann and Woker 1948) and chronic exposure to 0.017 mg/l delayed maturation, decreased oocyte diameter and lowered gonadosomatic index (GSI) in *Channa punctatus* (Dey and Bhattacharya 1989).

Besides ammonia, high concentrations of nitrates and nitrites are present in eutrophic waters (Piotrowska-Opuszynska and Szymacha 1992). However, according to our knowledge, there are no data on the negative effects of these compounds on fish reproduction. For this reason the current investigations (*in vivo* and *in vitro*) were undertaken. The aim of this study was to find an effect of nitrates and nitrites, (which may be responsible for diminution of fish populations in eutrophic waters) on the course of reproductive processes in carp.

MATERIALS AND METHODS

Experiments were conducted on 5-year old carp spawners held for four seasons (from fingerlings to spawners - males and females together) in 8 conventional carp ponds (4 of them highly eutrophic and 4 control ponds - area of 1000 m^2 each), having a natural productivity of 350 kg/ha. Stocking density in the first year

Correspondence to: P. Epler

of the experiment was 2400 and in the fourth year - 560 fish/ha. Fish were fed only with oat grain (2800 kg/ha) in the first year of experiment and in the consecutive three years fish utilized only natural feed present in the pond. Eutrophication of ponds was obtained by fertilization with urea ($CO(NH_2)_2$) and superphosphate. As a consequence of this fertilization 0.26 to 0.50 kg/m^3 of total nitrogen and 0.13 to 0.50 kg/m^3 of phosphorus were introduced into the ponds during the year. After four years of culture, with the use of routine methods, the following parameters were investigated: individual body weight gains, survival rate, GSI, relative fecundity (thousands of oocytes which have completed vitellogenesis per 1 kg of body weight), volume of sperm and effectiveness of artificial spawning (percentage of spawned females after hypophysation). The state of health was determined on the basis of a fish survey and calculation of the percentage of the specimens showing external pathological symptoms: ulcers, pathological changes on the skin and gills. The state of ovarian maturity was determined in the samples taken by biopsy (Bieniarz and Epler 1976). After clearing of the ovarian sample in the turpentine oil, the percentage of mature oocytes (after germinal vesicle break down - GVBD) was determined.

In the *in vitro* experiments ovulated eggs from 16 control females were fertilized in Petri dishes (on average 150 eggs in one dish) with milt sampled from 3 males. Fertilized eggs were incubated in 0, 1.0, 10.0 and 20.0 mg/l of NO^-_2 or in 0, 15.0, 150.0 and 500.0 mg/l of NO^-_3. The percentage of hatching was calculated for each concentration of NO^-_2 and NO^-_3 in 10 replicates.

Simultaneously with experiments on fish, hydrobiological (Witeska 1995) and hydrochemical (Piotrowska-Opuszynska and Szymacha 1992) investigations were conducted in the control and experimental ponds. At the time of the experiments the average content of the inorganic nitrogen changed from 1.38 to 6.77 mg/l in the eutrophic ponds and from 0.82 to 1.79 mg/l in the control ponds. The content of the phosphorus changed from 0.72 to 1.02 mg/l in the eutrophic ponds and from 0.09 to 0.36 mg/l in the control ponds.

The average plankton biomass in the eutrophic ponds was 2.205 mg/l and in the control ponds 1.356 mg/l. In the same time the average benthos biomass in the eutrophic ponds was 4048 mg/l and in the control ponds 1917 mg/l. Comparison of the eutrophic and the control ponds showed that the eutrophic ponds contained more of the chlorophyll "a" (20.6 vs 13.5 µg/l), the pheopigment (11.2 vs 4.8 µg/l), the molecular, organic carbon (6.17 vs 4.83 µg/l) and the organic seston (2.1 vs 1.61 µg/l).

RESULTS AND DISCUSSION

The average individual body weight gains changed from 225 to 1286 g in the eutrophic ponds and from 211 to 1051 g in the control ponds. The differences

were not significant (Fig.1). Survival rate in the course of the experiment in the eutrophic ponds (63%) was significantly lower ($p<0.05$) than in the control ponds (87%). Over 30% of fish from the experimental ponds, but not from the control ponds, showed symptoms of erythrodermatitis.

There was no significant differences in GSI of males cultured in the eutrophic (4.3 - 12%) and the control ponds (8.3 - 14.1%). GSI of the females from the eutrophic ponds (1.8 - 16.4%) was significantly lower ($p< 0.05$) than of fish kept in the control ponds (2.2 - 21.1%) (Fig. 2).

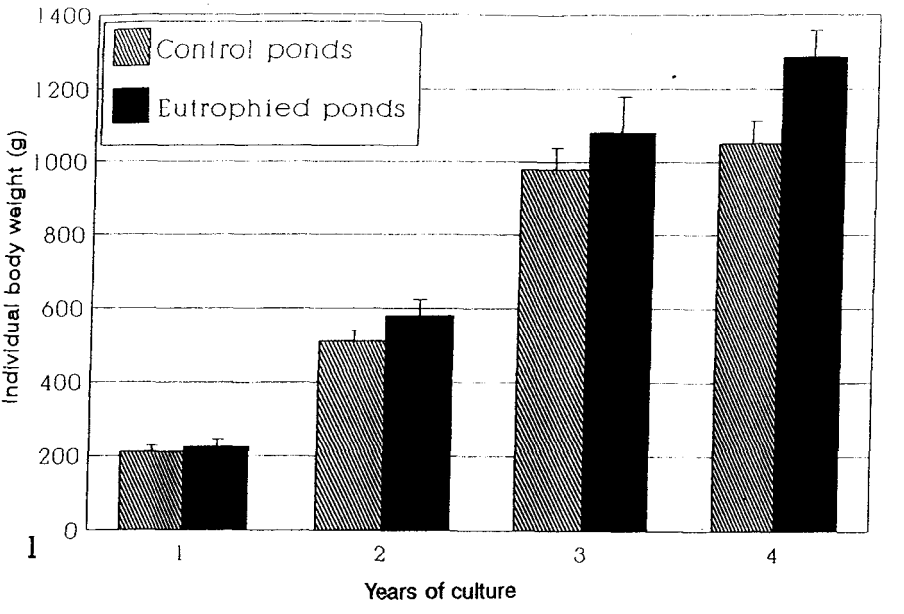
The percentage of the mature oocytes (after GVBD) in females from the eutrophic ponds (26.2 - 32.5%) was significantly lower ($p< 0.05$) than in fish held in the control ponds (41.7 - 56.9%) (Fig. 3).

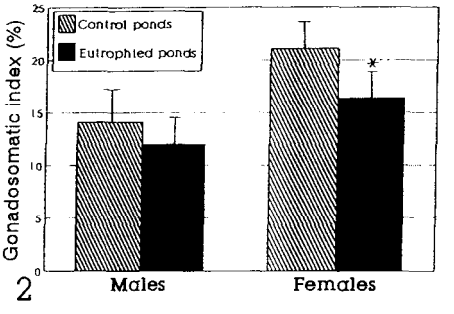
The fecundity of spawners after four years of culture in the eutrophic ponds (average of 58286 oocytes after completed vitellogenesis per kg body weight) was significantly lower ($p<0.05$) than of fish from the control ponds (217166 oocytes) (Fig. 4).

The volume of sperm in males from the fertilized ponds (2.13 - 6.67 ml) was significantly lower ($p<0.05$) than in fish from the control ponds (4.52 - 8.37 ml) (Fig. 5).

The percentage of artificially spawned females, derived from the eutrophic ponds (4.4%) and from the control ponds (11%) was not significantly different. The mean percentage of hatching in the control incubations (48%) was significantly higher ($p<0.05$) in comparison with the mean percentage of hatching in NO^-_2 concentrations of 1 mg/l (38%) 10 mg/l (36%) and 20 mg/l (33%). Also the mean percentage of hatching in the control incubations was significantly higher ($p<0.05$) in comparison with mean percentage of hatching in NO^-_3 concentrations of 15 mg/l (28%) 150 mg/l(23%) and 500 mg/l(l5%) (Fig. 6).

Intensive use of the mineral nitrogen fertilizers in agricultural production causes an increase of the nitrogen-containing compounds in soil and in water. It was demonstrated (Kaminski and Wróbel 1991) 8 fold increase of the nitrogen compounds (NH^+_4-N to 8 mg/l and NO^-_3-N from 0.14 to 2.20 mg/l) in Vistula waters in the last 60 years. Analysis of daily average flow of NH^+_4-N and NO^-_3-N showed that the maximum concentration of these compounds is observed from February to March, confirming the agricultural origin of these pollutants. The high level of the nitrogen-containing compounds is accompanied by the high level of the phosphates, whose daily concentration increased from 1800 kg in 1970 to 2750 kg in 1983 (Kaminski and Wrróbel 1991).
Hydrochemical and hydrobiological investigations (Piotrowska-Opuszynska and Szymacha 1992; Witeska 1995) showed that in the eutrophic ponds, in comparison with the control ponds, there was a higher level of the phosphorus-





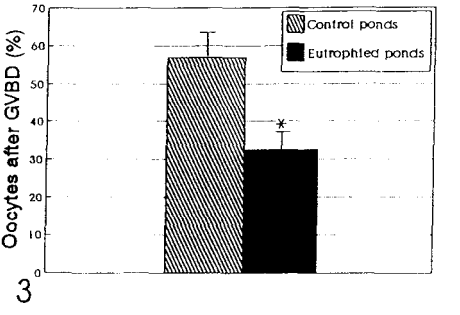
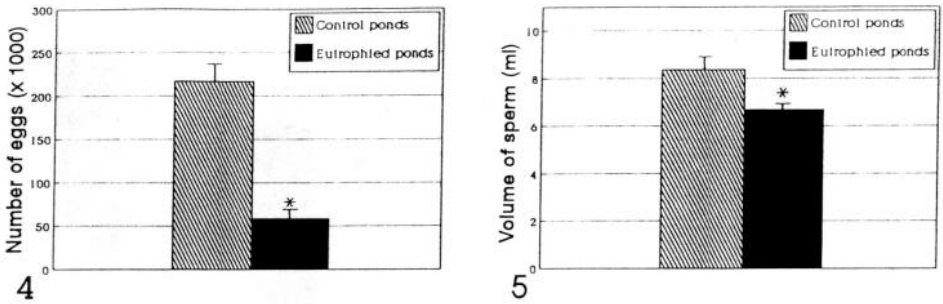


Figure 1. Average individual body weight gains of carp cultured for four years in control and fertilized ponds. Bars represent mean ±SE.

Figure 2. Gonadosomatic index (GSI) of male and female carp cultured for four years in conrol and fertilized ponds. Bars represent mean±SE (asterisk (*) - $p<0.05$ vs control).

Figure 3. Percentage of mature oocytes in female carp cultured for four years in control and fertilized ponds. Bars represent mean±SE (asterisk (*) - $p<0.05$ vs control).



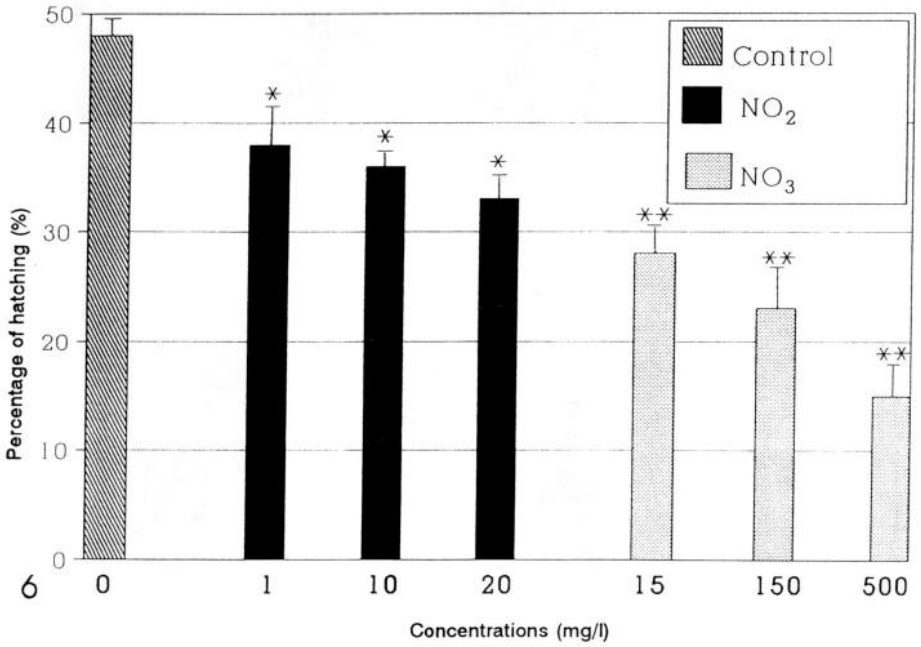


Figure 4. Relative fecundity of female carp cultured for four years in control and fertilized ponds. Bars represent mean±SE (asterisk (*) - $p<0.05$ vs control).

Figure 5. Volume of sperm collected from male carp cultured for four years in control and fertilized ponds. Bars represent mean±SE (asterisk (*) - $p<0.05$ vs control).

Figure 6. Percentage of hatching in different concentrations of nitrites and nitrates. Bars represent mean±SE (asterisk (*) - $p<0.05$ vs control, asterisks (**) - $p<0.01$ vs control).

containing and nitrogen-containing compounds as well as higher biomass of the zooplankton, phytoplankton and benthos. The content of the phosphorus and the nitrogen (NO^-_2-N and NO^-_3-N) during the whole experimental period was several fold higher in the eutrophic ponds than in the control ponds, which proves that fish were held in a eutrophic environment. The carp from such eutrophic ponds in comparison with fish from the control ponds was characterized by the higher body weight gains, lower survival rate, lower gonadosomatic index, lower volume of semen and lower fecundity. The *in vitro* experiments proved that the nitrites at the concentrations from 1 mg/l and the nitrates from 15 mg/l caused an increase of embryos mortality rate and significantly decreased the hatchability rate.

Among many eutrophication factors only the nitrogen compounds, mainly the free ammonia and the nitrites, were found to significantly affect the fish. The free ammonia affects the gills, liver, kidney and blood vessels (Smart 1976; Flis 1968) and the nitrites have an adverse influence on fish, causing, among others, the creation of methemoglobin (Smith and Williams 1974; Smith and Russo 1975; Williams and Eddy 1988 b). Our results suggest that high levels of the nitrogen compounds can lower reproductive effort in fish. They suggest also, that the unfavorable effects of both nitrogen-containing compounds on fish reproduction is exerted at the time of gonadal development (fecundity, quantity and quality of eggs and sperm) and at the time of the embryonal development. However, there was no effects on the ovulation and spawning, because the percentage of artificially spawned females (hypophysation) in the fertilized and in the control ponds were similar. The high levels of the nitrogen-containing and phosphorus-containing compounds in the eutrophic ponds increases the amount of natural food for carp. The abundance of food stimulate fish growth and their resistance against diseases, and in this way it softens detrimental effects of sublethal concentrations of the nitrogen.

The results obtained in this work suggest that, as observed by Colby et al. (1972), the decreased population in highly eutrophic waters may be caused by the deterioration of fish health, decreased survival rate of adult fish and embryos, decreased gonadosomatic index, fecundity and volume of sperm.

REFERENCES

Ball JR (1967) The relative susceptibility of some species of fresh water fish to poisons. J Ammonia Water Res 1: 767-775

Bath RN, Eddy FB (1980) Transport of nitrite across fish gills. J Exp Zoo1 214: 119-121

Bieniarz K, Epler P (1976) Preliminary results on the in vivo studies on the ovarian resorption in carp. J Fish Biol 8: 449-451

Colby PJ, Spangler GR, Hyrley DA, McCombie AM (1972) Effects of eutrophication on salmonid communities in oligotrophic lakes. J Fish Res Board Can 29: 975-983

Colt J, Tchobanoglous G (1976) Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish *Ictalurus punctatus.* Aquaculture 8: 209-224

Dey S, Bhattacharya S (1989) Ovarian damage to *Channa punctatus* after chronic exposure to low concentrations of elsan, mercury and ammonia. Ecotoxicol Environ Safety 17: 247-257

Flis J (1968) Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L) by ammonia water. Part 2. Effects of subtoxic concentration. Acta Hydrobiol 10: 225-238

Kaminski B, Wróbel S (1991) Water pollution. In: The basin of upper part of Vistula river. II. PWN, Warszawa-Krakow, 27-42 (in Polish)

Penaz M (1965) The effects of ammonia on eggs and hatch of trout *(Salmo trutta m. fario.* Zool Listy 14: 47-54 (in Chech)

Piotrowska-Opuszynska W, Szymacha J (1992) The effect of fish on the eutrophication of the ecosystem - model investigation in fish ponds. Part I. Zesz Nauk AR Wroclaw - Zootechnika XXXVII 218: 131-142

Scarno G, Saroglia MG (1984) Recovery of fish from functional and haemolytic anaemia after brief exposure to a lethal concentration of nitrites. Aquaculture 43: 421-426

Smart G (1976) The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J Fish Biol 8: 471-475

Smith CE, Williams WG (1974) Experimental nitrite toxicity in trout and in chinook salmon. Transactions of the American Fisheries Society 103 : 389-390

Smith CE, Russo RC (1975) Nitrite-induced methemoglobinemia in rainbow trout. Progressive Fish Culturist 37: 3

Williams EM, Eddy FB (1988a) Regulation of blood haemoglobin and electrolytes in rainbow *trout Salmo gairdneri* (Richardson) exposed to nitrite. Aquat Toxicol 13: 13-28

Williams EM, Eddy FB (1988b) Anion transport, chloride cell number and nitrite induced methaemoglobinaemia in rainbow trout (*Salmo gairdneri*) and carp (*Cyprirms carpio*). Aquat Toxicol 13 : 29-42

Witeska M (1995) Changes in plankton communities of ponds under conditions of fish stock and nutrient supply. Acta Hydrobiol (in press)

Wuhrmann K, Woker H (1948) Experimentelle Untersuchungen über die Ammoniak- und Blausärevergiftung. Schweiz Z Hydrol 11: 210-244