

**EFFECTS OF SOCIAL STRESS IN RAINBOW TROUT
AT DIFFERENT STOCKING DENSITIES**

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Introduction

Conspecific social interactions are characteristic phenomena in nature and in laboratory conditions as well. In a confined space of aquaria without possibilities for flight or concealment, individuals are exposed to constant social stress (Noakes, Leatherland, 1977; Ejike, Schreck, 1980). Rainbow trout are widely used in various toxicological and physiological studies and are frequently maintained in relatively small aquaria and in small groups. When investigating social stress in such groups sometimes the death of subdominant fish was recorded (Yamagishi, 1962; Laidley, Laetherland, 1988; Pottinger, Pickering, 1992). However, according to our data mortality of subdominant fish in small groups of rainbow trout is rather frequently observed (Petrauskienė, 1991) as a result of exhaustion caused by social stress (Vosyliene, Petrauskienė, 1993)

The aim of the present study was to examine relation between group size and rate of mortality of subdominant fish and to investigate possible modifications of toxicant influent on rainbow trout kept at different stocking densities.

Materials and methods

The experiments have been carried out on underyearling rainbow trout, *Oncorhynchus mykiss* with body length 9-10 cm and weight 9-12 g. Mortality of subdominant fish was monitored within 1.5 month period in groups consisted of 2,3,5 and 10 individuals (20 replicates for every density). Groups of 2-5 individuals were placed into aquaria of 45 l volume, and groups of 10 individuals - into 100 l volume aquaria. Experiments were conducted in nonchlorinated and aerated still water that were renewed at 24 h intervals. Water specifications: temp. 9-10°, pH 7.2- 7.4; dissolved oxygen 8.1-8.5 mg/l, hardness 250-270 mg CaCO₃/l. Aggressive behavior was recorded visually in every group consisted of 3 individuals during 1 h observation sessions the first four days after fish were placed together. Later recordings were performed only in those groups of 3 individuals in which strong hierarchy developed and possibility of the death of subdominant fish (S) was expected. In this paper there is presented only number of attacks because this act may physically damage another partner. Attack: an approach towards another fish followed by bite.

Mortality of fish exposed to acutely toxic concentration of copper (0.5mg/l) for 96 h was recorded at the densities mentioned above. Changes in ventilation frequency of trout was compared among fish kept singly and in groups of five after 10 days treatment with copper of 0.1 mg/l concentration. Both acute and chronic tests were conducted in still water, solutions were renewed at 24 h intervals.

Results

a. Behavioristic patterns and mortality. A despotic hierarchy was observed in the majority of groups at all densities. One fish established itself as a dominant (D) within one hour in groups consisted of 2-5 individuals and within 1,5 h in groups of 10 individuals. All aggressive acts after that time were performed by the D only (with a few exception when two D occurred or linear hierarchy was formed).

With the purpose to ascertain if the subdominant fish that died the first (Sd) was attacked by D more often than other members of group and might be damaged heavier, than others, aggressive behavior in groups of 3 individuals was analysed. Although great variability in the course of time and in different groups were observed, in general, number of attacks Sd had got from D didn't differ significantly from those another subdominant fish had got (Table 1). It might mean that the main reason of death of Sd wasn't damages

Table 1. Number of attacks per h performed by dominant fish towards subdominant fish in groups consisted of 3 individuals. S-subdominant fish, Sd-subdominant fish that died the first in the group.

Fish attacked	Time				
	the first day	the second day	the third day	the fourth day	the day before daying
S	69.0±27.3	41.9±20.5	29.9±18.9	21.6±11.29	23.7±10.5
Sd	85.6±25.6	25.3±16.6	36.8±12.5	38.0±9.59	22.2±10.6

got during attacks and followed by bacterial diseases but rather by their inability to resist to social stress. This may be reflected in the fact as well that in 25% of all mortality cases (in groups at all densities) Sd individuals died suddenly, having only slight damages and not being ill with bacterial diseases. However, in majority of cases, some days before dying Sd lost equilibrium, their fins were heavily damaged, the caudal part of body was lacking scales, and very often they were ill with Saprolegnia.

Severe social stress that finally ended in the death of Sd developed earlier and mortality was higher in groups at low densities. In groups of 2,3,5 and 10 individuals the mean value of time interval in which Sd died was 6, 9, 14 and 20 days, respectively (Figure 1). The higher percents of number of groups in which Sd failed to survive in 1.5 month period was observed at low densities (Figure 2). The higher mortality was observed in groups with the great differences among D and Sd in body coloration, in feeding and swimming activity. In

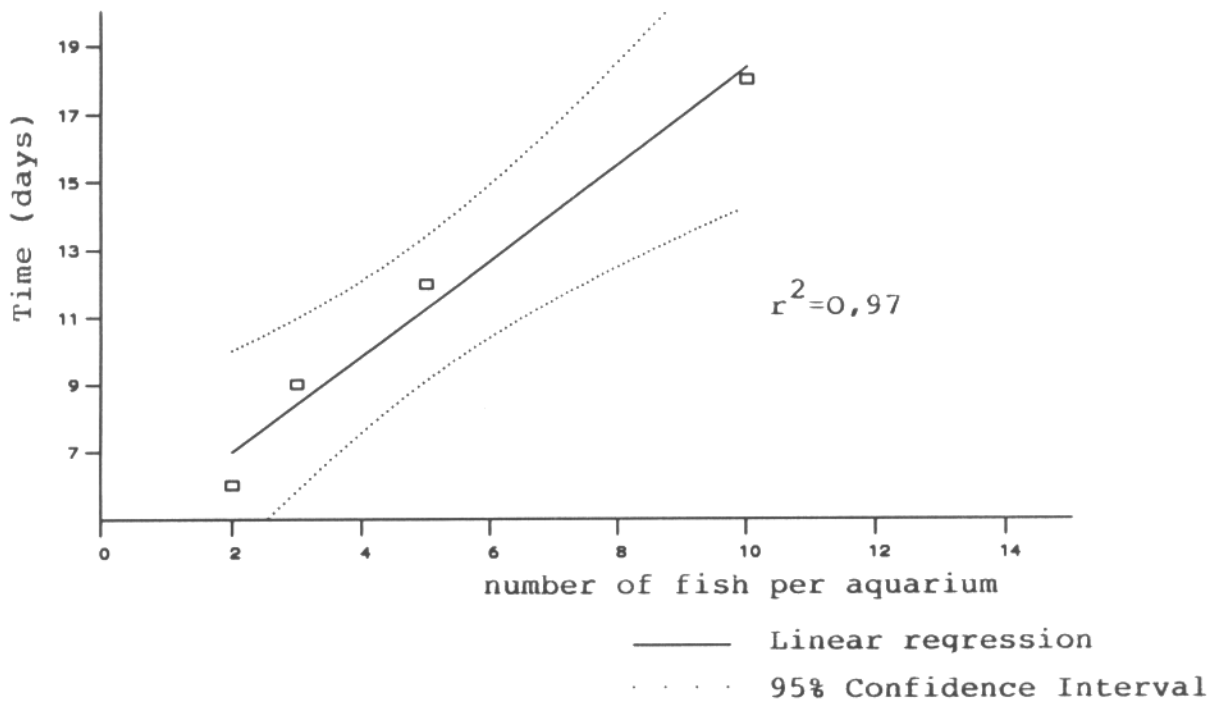


Figure 1. Changes of time interval in which the death of subdominant fish was recorded in groups at different densities

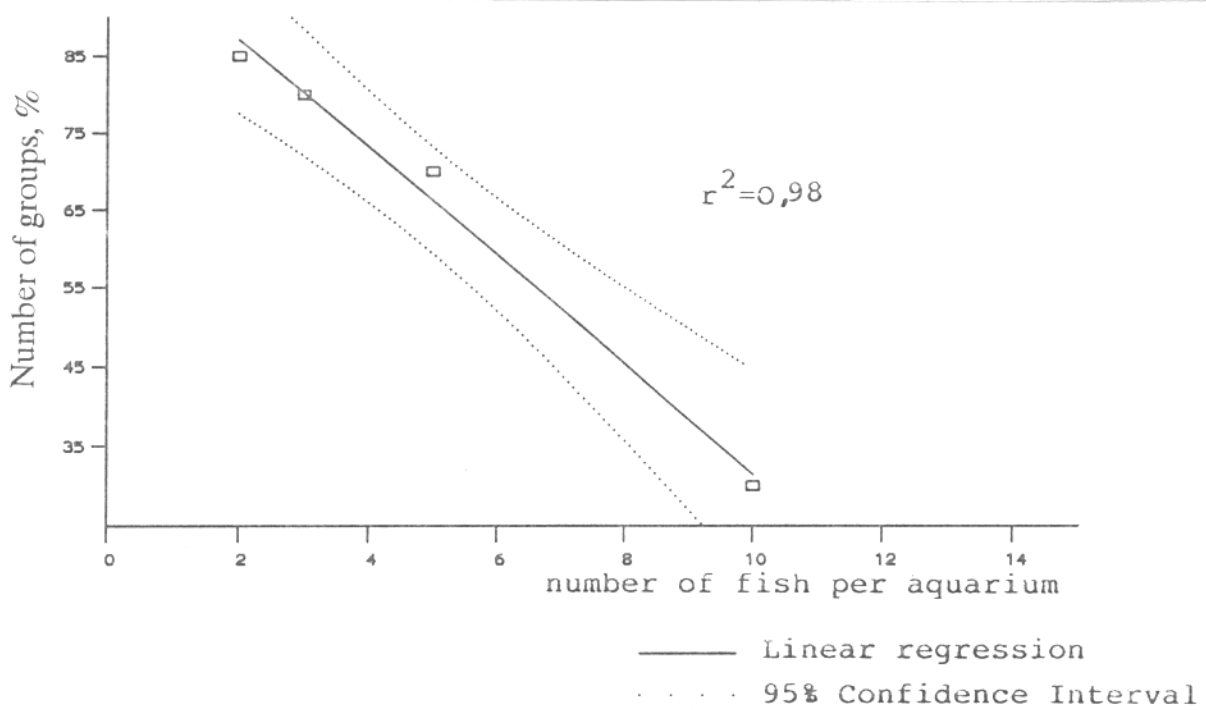


Figure 2. Changes of number of groups (in %) in which subdominant fish failed to survive the period of 1.5 months at different stocking densities.

those groups Sd were remarkable darker than D, Sd didn't feed and remained quiet for a long period holding position in the upper layer of water or on the bottom in the very corner of the aquarium. In groups with no mortality cases of Sd differences mentioned above were not so considerably expressed.

b. Modification of toxicant influence. Social interaction and social stress may modify influence of toxicants both increasing harmful effects and concealing them. Acute toxicity of copper might be increased in groups at low density (Table 2), most probably because of addition of chemical and social stress.

Table 2. The percent mortality of trout kept at different densities after 96 h exposure to 0.5 mg/l copper concentration. Number of fish at every density = 30.

Number of fish per aquarium	Mortality, %	
	Control	0.5 mg/l Cu
10	0	36.7
5	0	43.3
3	6.7	53.3
2	20	66.7

By recording physiological parameters of fish there is a possibility to overlooked changes of them due to great variability of parameters elicited by social stress. As seen in Table 3

Table 3. Changes in ventilation rate (n/min) after 10 days exposure to 0.1 mg/l copper concentration in groups of trout kept singly (a) and in groups of five individuals (b). *-value significantly different from control, $p=0.05$ (Student's t-test). a-10 replicates, b-2 replicates.

	Ventilation rate, n/min	
	a	b
control	96.4±0.65	110.3±6.7
0.1 mg/l Cu	93.5±0.99*	108.2±8.3

a significant decrease of ventilation rate was recorded in fish kept singly after treatment with copper, while in groups of five individuals no significant changes were observed. Usually ventilation rate in subdominant fish was higher, than in their dominant partner; difference might be 25% and more. Ventilation rate of D also can be rapidly increased to 20% and more after performing attacks towards other members of group. Thus, changes of ventilation rate occurred both in D and in S during social interactions may cover the influence of copper even at comparatively high concentration as 0,1 mg/l. In similar way may be confound results of any other toxicological, physiological and biochemical investigation.

Discussion

Final consequences of severe social stress that ended in the death of subdominant fish were rather frequently observed in the groups with low densities. Our previous investigations on physiological parameters of rainbow trout at various stages of social stress performed in groups with 2 and 3 individuals have shown that remarkable changes occurred in subdominant fish within 1-3 h after social interactions: darkening of body coloration, increasing of ventilation rate and blood glucose concentration, elevating of haematocrite level, decreasing of lymphocyte concentration, changes of catecholamines concentration in brain and head kidney (Vosyliënė, Petrauskienė, 1993; Vosyliënė et al., 1993). The stress reaction of subdominant fish was going on until exhaustion stage when sharply decreased glucose concentration and lymphocyte level were recorded before the death of fish.

Modifications of influence of toxicant on rainbow trout kept at different densities can be explained by increasing variability of physiological parameters by means of social interaction. Similar effects have been reported for bluegill: effects of Cd and Zn was found to be related to rank within the social hierarchy, while mortality of bluegills exposed to Zn was more rapid in subdominant fish, than in dominant one (Henry, Atchison, 1979). Pottinger and Pickering (1992) by examination the time-course of acclimation of rainbow trout to the stress of confinement found that acclimation of the fish confined in pairs was severely confounded by the development of hierarchical relationships between the fish; similar, though less severe evidence of social stress was observed among the fish confined as groups of five.

Possible effects of social stress on various parameters should be taken into account when arranging experimental design for aggressive, territorial fishes.

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