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WATER QUALITY AND STOCKING DENSITY AS STRESSORS OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS* RAF.)

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ABSTRACT

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The influences of heated recirculated water and well water on three stocking densities of channel catfish (*Ictalurus punctatus* Raf.) were compared, based on several physiological and hematological parameters. Controls were kept in well water only. The leucocyte count of fish kept in well water generally decreased, and their thrombocyte, lymphocyte and granulocyte counts were lower at higher stocking densities. In addition, a slight hemoconcentration was observed. The fish kept in recirculated water showed a decrease only in thrombocyte count. Plasma cortisol and blood glucose did not change and in all groups were at a level characteristic of resting fish. In contrast, the blood lactate levels were always high. This may have been caused by the anesthetization. The alterations found are regarded as symptoms of the "general adaptation syndrome" induced by the stocking rates. High lymphocyte and granulocyte counts among the fish in reused water are signs of local adaptations to reduced water quality.

INTRODUCTION

The economic importance of intensive fish culture is well known. However, the high stocking densities in artificial rearing systems are far higher than in natural environments. Meade (1978) and Wedemeyer (1980) confirmed that this could produce severe stress in the fish, and if corrective action is not taken, heavy loss will occur.

Stressors elicit a broad, but nevertheless distinct, display of reactions in an organism, which are called the "general adaptation syndrome" (GAS) by Selye (1956). There is evidence that this syndrome occurs in teleosts (Gronow, 1974; Mazeaud et al., 1977; Peters et al., 1980). It permits the organism to adapt to environmental change, but too much stress reduces

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resistance against pathogenic agents, and finally leads to exhaustion and death. To quantify stress, the symptoms must be evaluated as deleterious or within the normal range of adaptation by the animal (Sprague, 1971).

Intensive fish culture in recirculating systems is an example of a sophisticated rearing technology. Little is known about possible effects of processed water on the well-being of fish. Schulze-Wiehenbrauck (1978) stated that in batch cultures of *Tilapia zillii*, the water exchange rates affect growth rate, food conversion and mortality.

We carried out an experiment to compare the influence of recirculated water and well water on channel catfish, *Ictalurus punctatus* Raf. at three stocking densities. The condition of the fish was monitored by measuring metabolic and hematological parameters which are known to change under the influence of stress.

MATERIAL AND METHODS

Channel catfish reared in a closed warm water system (Hilge, 1980) were used for the experiment. The fish were stocked in circular tanks, 1 m in diameter, at three stocking densities, 10, 50 and 120 individuals per tank, and maintained for 7 weeks. The average body weight of the different groups varied from 565 to 740 g. The stocking densities and the fish to water ratios are listed in Table I. The tanks were supplied with well water heated to

TABLE I

Parameters of stocking density of channel catfish in fresh and recirculated water

Fish per tank	Stocking density (kg/m^3)	Fish : water ratio
10	18	1 : 55
50	80	1 : 12
120	215	1 : 5

about 22.5°C in an open system or with recirculated water at the same temperature from a recycling system with a total volume of 6.5 m^3. Nitrification took place in a plastic-medium trickling filter, and suspended solids were removed from the water in a sedimentation unit directly behind the fish tanks. A special denitrification step in a by-pass was used to maintain nitrate and water pH at acceptable levels.

Duplicate trials were made at each stocking density. The water volume of the green plastic tanks was 350 l, and the water flow rate was adjusted to 9 l/min to guarantee an inlet oxygen concentration of 6—8.5 ppm and of 4.5—6 ppm in the tanks. Seven control animals of similar body weight were kept individually in rectangular, glass 40 l aquaria with a 2 l/min water supply. The aquaria walls were covered with a black plastic foil in order to

TABLE II

Water parameters (mean, standard deviation and range) at the inlet of the rearing tanks during the experiment. No ranges are given for the well water parameters due to insignificant variations (n.d., not detectable)

		Recycled water	Well water
Temperature (°C)		22.1 ± 0.5 (21.6 – 22.9)	22.8 ± 0.3
BOD_5	(mg/l)	6.7 ± 2.9 (4.3 – 10.0)	n.d.
pH		7.2 ± 0.6 (6.2 – 7.7)	8.0 ± 0.1
Ca^{2+}	(mg/l)	79.2 ± 6.4 (73.5 – 90.2)	43.8
Mg^{2+}	(mg/l)	17.9 ± 4.0 (12.1 – 22.3)	n.d.
PO_4^{3-}	(mg/l)	25.9 ± 2.3 (23.4 – 29.6)	0.3
SO_4^{2-}	(mg/l)	130.4 ± 35.5 (89.2 – 173.8)	35.5
Cl^-	(mg/l)	62.5 ± 20.2 (43.7 – 92.0)	22.5 ± 1.4
NH_4^+	(mg/l)	0.8 ± 1.3 (0.19– 3.2)	0.01
NO_2^-	(mg/l)	1.1 ± 0.4 (0.69– 1.70)	0.01
NO_3^-	(mg/l)	234 ± 35 (202 – 285)	7.2 ± 3.7

exclude outside disturbances. All fish were fed twice a day (08.00 h and 15.00 h) with commercial trout pellets totalling 1% of their body weight.

Water temperature and dissolved oxygen in the tanks were measured daily about 30 min after the morning feeding. Samples of the recirculated water were taken from the tank inlets three times a week and the well water was sampled twice a week before first feeding. The following parameters were measured using German Standard Methods (Anon., 1960—1979): BOD_5, Ca^{2+}, Mg^{2+}, PO_4^{3-}, SO_4^{2-}, Cl^-, NH_4^+, NO_2^-, and NO_3^- (Table II).

The experiments at the first two stocking densities and the control were performed at the same time. The third group was tested subsequently. Blood was collected from six catfish taken simultaneously from a tank and anesthetized in 60—75 s with neutralized MS 222 (0.28 mg/l). A 1—2 ml sample of freely running blood was taken by cardiac puncture through a No. 19 G needle into heparin-coated dry plastic tubes (Greiner Ltd., Germany).

All determinations were carried out by standard micro and semi-micro methods using test kits (Merck, Boehringer Co.). The leucocrit was measured according to McLeay and Gordon (1977). Total cell counts were performed using a Neubauer counting chamber, and differential cell counts by means of

blood smears stained by the Pappenheim method (Peters et al., 1980). Basophilic and eosinophilic granulocytes were too few to take into account during the blood smear analysis. All cell types were classified according to Williams and Warner (1976). Cortisol was measured by means of a radioimmunoassay (Peters et al., 1980). The results were checked statistically using the *F*-test (Sachs, 1978). The controls were not taken into consideration because not enough were available.

RESULTS

No mortality occurred during the experiments. The results for the fish kept in well water and the controls are given in Table III, and those for the fish from recirculated water in Table IV. The values for blood glucose and lactate concentrations show neither significant differences nor any relationships to other factors. The lactate levels were very high compared with undisturbed fish (Caillouet, 1968; Scott and Rogers, 1981). The plasma cortisol level was low in all groups. The leucocrit corresponds fairly well to the leucocyte count and seems to depend on the proportion of large leucocytes, such as the granulocytes. The hematocrit and hemoglobin content increased directly with the stocking density, which indicates a hemoconcentration.

The following changes occurred with increasing stocking density.

(1) The leucocyte counts decreased to the level of the control.

(2) In the blood smears from both groups, the thrombocyte counts decreased, as did the proportions of lymphocytes and granulocytes in smears from the well water group.

TABLE III

Blood parameters of catfish kept at three stocking densities in well water and controls kept single. All values $\bar{x} \pm S_D$

Parameter		Stocking density			
		18 kg/m³	80 kg/m³	215 kg/m³	Control
Hematocrit	(Vol%)	38.5 ± 3.2	40.5 ± 3.8	43.0 ± 4.1	35.1 ± 9.1
Hemoglobin concentration	(g%)	9.4 ± 0.8	10.3 ± 1.0	10.3 ± 0.6	8.5 ± 1.5
Leucocrit	(Vol%)	1.3 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2
Leucocyte count	(×10³/mm³)	121 ± 10	101 ± 12	84 ± 18	85 ± 16
Thrombocytes	(×10³/mm³)	73 ± 14	61 ± 8	50 ± 10	44 ± 14
Lymphocytes	(×10³/mm³)	39 ± 16	33 ± 6	29 ± 10	35 ± 7
Granulocytes	(×10³/mm³)	8 ± 5	4 ± 3	4 ± 2	2 ± 2
Monocytes	(×10³/mm³)	2 ± 1	3 ± 2	1 ± 1	3 ± 2
Cortisol	(*nmol/l*)	19 ± 16	27 ± 23	10.8*	21 ± 14
Blood glucose	(mmol/l)	1.9 ± 0.4	2.0 ± 0.5	2.4 ± 0.6	1.9 ± 0.4
Blood lactate	(mmol/l)	3.9 ± 0.8	4.2 ± 0.8	2.7 ± 1.1	3.1 ± 0.7
N		12	12	12	7

N, numbers of samples. *Pooled sample

TABLE IV

Blood parameters of catfish kept at three stocking densities in recirculated water. All values $\bar{x} \pm S_D$

Parameter		Stocking density 18 kg/m³	80 kg/m³	215 kg/m³
Hematocrit	(Vol%)	38.4 ± 4.3	43.1 ± 3.3	42.2 ± 3.6
Hemoglobin concentration	(g%)	9.6 ± 1.0	10.2 ± 0.9	9.9 ± 1.2
Leucocrit	(Vol%)	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.3
Leucocyte count	($\times 10^3$/mm³)	121 ± 26	114 ± 17	103 ± 13
Thrombocytes	($\times 10^3$/mm³)	74 ± 12	68 ± 15	60 ± 10
Lymphocytes	($\times 10^3$/mm³)	34 ± 7	37 ± 9	35 ± 7
Granulocytes	($\times 10^3$/mm³)	8 ± 10	8 ± 4	8 ± 6
Monocytes	($\times 10^3$/mm³)	4 ± 3	3 ± 1	2 ± 1
Cortisol	(nmol/l)	27 ± 20	11 ± 8	16.2*
Blood glucose	(mmol/l)	1.8 ± 0.5	1.5 ± 0.4	1.9 ± 0.6
Blood lactate	(mmol/l)	4.1 ± 1.1	3.8 ± 0.8	3.9 ± 2.2
N		12	12	12

N, numbers of samples. *Pooled sample.

TABLE V

Significant differences between all experimental groups (*F*-test)

Stocking density (kg/m³)	Differences
Well water group	
20— 80	Leucocyte count, leucocrit, hemoglobin concentration, thrombo- and granulocyte count $P \leq 0.05$
80—220	Leucocyte count $P \leq 0.05$; thrombocyte count $P \leq 0.01$
20—220	Hematocrit, hemoglobin concentration, granulocyte count $P \leq 0.01$; leucocyte count, thrombocyte count $P \leq 0.001$
Recirculated water group	
20— 80	Hematocrit $P \leq 0.01$
80—220	n.s.
20—220	Leucocyte count, hematocrit $P \leq 0.05$; thrombocyte count $P \leq 0.01$
Well water group—recirculated water group	
20	n.s.
80	Leucocyte count, granulocyte count $P \leq 0.05$
220	Thrombo- and granulocyte count $P \leq 0.05$; leucocyte count, leucocrit $P \leq 0.01$

n.s., not significant.

(3) A slight, insignificant hemoconcentration was observed.

(4) Fewer fights to establish a rank order occurred, evidenced by fewer injuries from bites at higher stocking densities.

When recirculated water was used instead of well water to maintain the fish at high densities, the following were observed.

(1) The decrease in the leucocyte count for a given stocking density was diminished.

(2) The granulocyte counts remained high.

(3) The number of lymphocytes remained unchanged.

To evaluate the results, we compared them with the known stress phenomena rather than relying on the statistics in Table V.

DISCUSSION

Stress is defined as a general and nonspecific reaction by an organism to exogenous and endogenous stimulations, called stressors (Selye, 1956). The effects of the stressors, the "general adaptation syndrome" (GAS), appear in response to the activation of distinct physiological pathways which originate in the hypothalamus. Two equivalent pathways can be distinguished that serve for transmitting the reaction:

(1) A nervous pathway via the sympathetic nerve and the suprarenal organ, releasing its hormones, the catecholamines.

(2) A hormonal pathway via the pituitary and the interrenal organ, in which glucocorticoids are synthesized (Mazeaud et al., 1977).

A great variety of physiological and morphological changes result, some interfering with one another.

The main glucocorticoid in fish is normally cortisol. In our experiment, all groups contained less than 10 ng/ml. Boehlke et al. (1966) defined the ratio of the glucocorticoids in catfish to one another as follows: cortisone: corticosterone: cortisol = 15:7:2, but the fluorimetric and colorimetric methods they used provide less specifity and sensitivity than the radio-immunoassays which are in common use now. Simco and Davies (personal communication, 1980) claimed that cortisol is the main glucocorticoid in catfish. Strange (1980) found an increase of cortisol in *Ictalurus* from 50 to approximately 100 ng/ml within 30 min after confinement; concomittantly, a high mortality occurred. It is therefore clear that the second pathway had not been activated in our experiment at the time of testing.

Blood glucose, which is influenced directly by catecholamines and indirectly by glucocorticoids, was found to be normal. The values we found are comparable to those Strange (1980) reported for undisturbed control catfish.

We found much higher blood lactate levels in all groups than those reported by Caillouet (1968) for control fish. This may be due to the intense activity during anesthetization because comparable values were found in

catfish shortly after anoxia was induced (Caillouet, 1968). A direct increase in lactate caused by the use of MS 222 is not necessarily to be expected (Houston et al., 1971; Oikari and Soivio, 1975; Smit et al., 1979). However, our experimental fish showed no significant differences between the groups or within a single group that could be related to anesthetization.

Although the stressors, stocking density and water quality did not activate the GAS at the basis level as far as we could determine, there are some significant alterations in the hematological parameters, which are known to be typical stress effects. These are related mainly to the leucocytes and only concern the red blood parameters to a lesser degree.

The hemoconcentration at higher stocking densities is typical for the alarm phase of the GAS (Selye, 1976). This may have several reasons: alteration in the ion regulation which changes the water content of the blood, swelling of the erythrocytes through increased CO_2 tension and lactate concentration, and mobilization of the blood reserved (Stevens 1968; Soivio et al., 1974; Soivio and Oikari, 1976; Yamamoto et al., 1980). Wedemeyer (1980) described a severe disturbance to the ion balance in salmon which were kept in biologically processed recirculating systems at high CO_2 tension. It is also possible that the relatively high nitrite content of the processed water impairs the function of the gills. Thus a hemoconcentration can result from stress caused by both social interactions and physiological malfunctions produced by poor water quality.

Leucopenia, and especially lymphopenia and a simultaneous granulocytosis, is well known as a stress reaction (McLeay, 1975; Johannson-Sjöbeck et al., 1978; Peters et al., 1980). Lymphopenia is directly related to the immunosuppressive effect of corticosteroids (Selye, 1976). In our experiment, the well-known effect of a high cortisol level was still detectable, but the base level of the hormone was normal. The decrease in granulocyte count with increasing stocking rates can be explained by the less intensive fighting for rank at higher stocking densities. An indication of this was that fewer injuries from bites occurred. Granulocytes occur in wounds during the inflammation response (Ellis, 1977). Catfish bathed in malachite green (Grizzle, 1977) and trout injected with turpentine (Weinreb, 1958) also display neutrophilia. Johannson-Sjöbeck et al. (1978) injected eels with cortisol and then detected lymphopenia and neutrophilia, but they could not deny the possibility that these conditions indicate the beginning of inflammation due to the injection.

The granulocyte counts of the group kept in recirculated water remained high, and the lymphocyte counts did not decrease. Apparently, the immunosuppressive effect of stress, as demonstrated by the group kept in well water, is counteracted by an activation of the immune system. A noxious or irritating effect of the processed water on the integument, gills or gut cannot be excluded, considering the granulocyte counts and the function of these cells. It seems pointless to seek an explanation for this by considering the values for the parameters measured in the processed water, because they re-

present only a small proportion of the entire chemical and biological picture.

The alteration in the thrombocyte counts are difficult to evaluate. A direct influence of cortisol on this cell type is not likely in the eel (Johannson-Sjöbeck et al., 1978), but it was demonstrated in *Oncorhynchus kisutch* after treatment with dexamethasone (McLeay, 1973). McLeay claimed that small lymphocytes are the precursors of thrombocytes and that both react in the same manner to ACTH or corticosteroids. After a short period of stress, the thrombocyte counts increased, resulting in a shorter clotting time (Casillas and Smith, 1977). An activation of the immune system and fast clotting seem to be appropriate reactions to injuries from bites or cell damage.

It is likely that the experimental fishes had passed the alarm phase, elicited by the stocking density stressor. The basic value for cortisol in this case should be normal and its after effects weakened. Thus, the animals are in the adaptation phase. The local symptoms, which are due to water quality and biting, should then be regarded as part of the "local adaptation syndrome" (LAS), as described by Selye (1956). This syndrome can cause specific effects. Both syndromes, GAS and LAS, are inseparable and superimpose on each other. In this case, the predominant LAS may actuate the GAS, so that the fish cannot maintain the adaptation phase and shift to the exhaustion phase. This undoubtedly brings about severe losses.

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