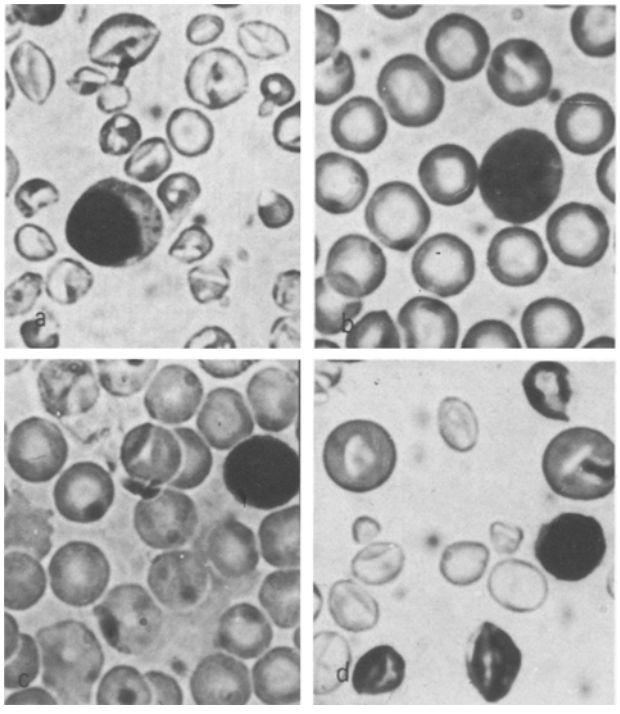

Red blood cells of b/b rat before, during and after parabiotic union with an normal partner. *A* Before parabiosis; *B* 4 weeks in parabiosis; *C* 5 weeks following separation; *D* 14 weeks following separation. × 2200.

shrivelled red blood cells to recover, and acquire a practically normal appearance, suggests that perhaps the structure of these cells is not irreparably damaged. The presence of red blood cells belonging to the anemic partner in the blood of the normal rat has been observed. The presence of red blood cells from the normal partner in the anemic animal cannot be easily established, as some normal looking red cells are always to be found in the peripheral blood of the anemic rat. From each pair of experimental rats closely comparable results were obtained. After separating the partners many of the peripheral red blood cells retained their normal appearance for 4–5 weeks (figure, C). With the further passage of time the circulating blood continued to undergo gradual but steady deterioration. 14 weeks following separation the peripheral blood returned to its original defective state (figure, D).

The effect which the parabiotic union had on the general health of the anemic rat was equally impressive. It is not without interest that in anemic rats very often the number of white blood cells may be considerably greater than in their normal controls (table), a fact totally overlooked in our previous studies.

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Effects of loading density on catfish blood[1]

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Summary. Results from this study indicate that a fish density stress syndrome exists for red blood cell morphology. Smaller (by 3.5%) and rounder (by 0.6%) red blood cells were consistently found in intensive fish cultures.

Some effects of overstocking fish ponds include smaller size fish, behavioral changes in the fish, and even marked fish losses[2,3]. These effects may result in excessive cost to fisheries managers at fish hatcheries and farms, and also make assessments of bioassay data in the laboratory more difficult. Since fish are frequently used to test water quality, it is important to isolate the toxicant effect (at sublethal concentrations) from the effect of culturing practices.

There have been few studies on physiological effects of crowding stress. Hematological changes have been identified with crowding stress for salmon and goldfish[4–7]. This paper reports additional effects of intensive crowding on catfish hematology.

Materials and methods. Channel catfish (*Ictalurus punctatus* Rafinesque) were cultured at 4 different loading densities, ranging from 0.18 kg/m^3 for the controls to 224 kg/m^3, 337 kg/m^3, and 433 kg/m^3 for the intensive cultures. Fish ranged in age from 1 to 4 years. Dissolved oxygen was at saturation levels, and water temperatures were similar for all cultures.

Control fish were collected by angling from a commercial fishout pond in northwestern Alabama. The intensive cultures were kept in concrete raceways (15.2 × 1.2 × 1.1 m) at Gallatin, Tennessee. Water replacement time of the 20,000 liter raceways was about 25 min. Raceway fish were caught with a dip-net.

Fish used in this study were free of epizootics and ranged in size from 0.22 to 0.45 kg. Samples were collected from February to August 1973. Blood was collected by cardiac puncture, and blood smear slides were made. Wright's stain was used to differentiate cell types. Length and width measurements of red blood cells were taken from camera lucida drawings. Cell roundness was determined from the width/length ratios. Cell area was calculated by multiplying the width-length product by $\pi/4$.

10 fish from each of the 4 cultures, with 50 cell measurements per fish, were used to test for density differences. A nested 1-way analysis of variance test was used for the determination. The 95% least significant difference test was used to locate differences between the means[5]. In addition measurements from 20 control fish were used to determine differences in sample size. A t-test was used to compare the 2 groups: 50 measurements vs 250 measurements.

Results. The effects of intensive culture practices of catfish on red blood cell morphology are summarized in table 1. These results indicate 3 distinct groupings of the data for cell length, width, and area, viz the control density (0.18 kg/m^3), medium density (224 kg/m^3), and high densi-

Table 1. Effects of loading density on cell morphology of channel catfish

Loading density	Length (μm)	Width (μm)	Roundness	Area (μm²)
0.18 kg/m³	10.17±0.04	7.79±0.03	0.783±0.004	62.04±0.33
224 kg/m³	9.38±0.04	7.30±0.03	0.786±0.004	53.94±0.33
337 kg/m³	8.70±0.04	7.08±0.03	0.821±0.004	48.52±0.33
433 kg/m³	8.68±0.04	7.15±0.03	0.830±0.004	48.92±0.33
Between groups	*	*	*	*
95% LSD	0.11	0.09	0.012	0.91
Among fish/group	*	*	*	*

* f>0.0001. Means±SE are indicated.

Table 2. Comparison of cell morphology for group 1 (50 cell measurements per fish) and group 2 (250 cell measurements per fish)

	Length (μm)	Width (μm)	Roundness	Area (μm²)
Group 1	10.12±0.04	7.89±0.03	0.792±0.004	62.70±0.35
Group 2	9.77±0.02	7.61±0.01	0.787±0.002	58.49±0.14
CV (%)				
1	13.16	12.06	15.64	17.69
2	11.74	9.95	13.56	16.39
t	*	*	NS	*

* t>0.0001; NS, not significant. Means±SE are indicated.

ty (337 kg/m³ and 433 kg/m³). Results for cell roundness indicate 2 distinct groups where data from control and medium densities comprised 1 group, and the data from both high densities comprised the other group. Medium and high loading densities tended to decrease cell length by 7.8% and 14.6%, cell width by 6.3% and 9.1%, cell area by 13.1% and 21.8%, and high density tended to increase cell roundness by about 5%.

While these changes were highly significant, it was desirable to see if better statistical resolution could be obtained with more than 50 cellular measurements. A comparison of 50 with 250 cellular measurements was made. These results are shown in table 2. The mean values were significantly reduced by 250 measurements as compared to 50 measurements, 3.5% for cell length and width, and 6.7% for cell area. The mean values for cell roundness were similar for both groups. As expected, the coefficients of variation for the 250 measurements group were consistently less than those for the 50 measurement group, indicating better resolution of the mean.

Discussion. The biological significance of red blood cell morphology is at least 2-fold: a) cell size determines the available volume for oxygen transport, gaseous exchange, and transport of nutrients and biochemical products, whereas b) cell shape, which influences the surface to volume ratios, determines, in part, the rates of cellular exchanges. The cell morphology associated with fish cultured under normal density conditions is assumed to be optimum for red blood cell functions. Since cell morphology and physiology are closely allied, deviations from the normal morphology may indicate physiological perturbations. If a density syndrome were revealed by red blood cell morphology, then it should be possible to identify and isolate the effects of density stress from a physiological stress resulting from a toxicant.

Since only 1 study has reported the effects of density on red blood cell morphology, the comparable data base is meager. Nevertheless, the results of this study reinforce those observed for goldfish[3]. In both studies red blood cells from fish in the control group had longer, wider cells, with more area than did those cells from fish cultured at higher loading densities. The only apparent difference between the 2 studies is in the cell shape (roundness). In the goldfish study there was no significant difference between the control group and the high density group. In the present study, cell roundness increased significantly at the high densities. Results in table 1 indicate no difference, however, between the control group and the medium density group. Since the medium density group for the present study had a higher density than the high density in the goldfish study, the conclusion is similar for both studies, viz cell shape changes only under prolonged extreme stress conditions.

When this study began, the proper number of cellular measurements was not known. This number was determined empirically in the goldfish study to be approximately 200 cell measurements for best statistical characterization of the cell features. Results from this study indicate that fewer measurements can be used with good results provided that the statistical model used is sensitive enough, such as hierarchical designs.

Results from this study support those observed in a previous study. Smaller and rounder red blood cells were consistently found in fish loaded at high densities. Evidently, in fish a density stress syndrome exists for red blood cell morphology[7].

1 Acknowledgments. This work was conducted as part of the Federal Interagency Energy/Environmental Research and Development Program with funds administered through the Environmental Protection Agency (EPA Contract No. EPA-IAG-D8-E721-DR, TVA Contract No. TV-41967A). Dr Ralph H. Brooks and Billy G. Isom are acknowledged for making this study possible. The data were kindly donated by Dr R.W. Williams and W.E. Barksdale, both formerly with the Department of Biology, University of North Alabama, Florence, Alabama.

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