Blood parameters and electrocardiogram in squeezed fish simulating the effect of net damage and recovery

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ABSTRACT: In the present study, rubber bands were tied around the girth of rainbow trout Oncorhynchus mykiss to simulate and assess the physiological damage and survival rates of fish that encounter gill nets. Physiological condition was assessed by analysis of blood lactate, pH, oxygen partial pressure and plasma potassium concentration before and after release from binding. Twenty-four hours after release, half of the test fish had died. Although the binding forces used to hamper fish did not differ significantly, blood lactate levels in the dead fish rose to 10-fold higher than those in the survivors. Consequently, the pH level fell in the group that died, whereas in the surviving group it fell only slightly and soon recovered. Potassium concentration increased after release from binding in fish that died, and the PO_2 levels in these fish continued to drop until just before death. It was concluded that when fish get entangled in fishing nets, blood lactate accumulates because of physical fatigue and the elevated lactate levels lead to metabolic acidosis. Finally, fatal metabolic conditions could cause higher mortality rates, even in escapees.

KEY WORDS: blood analysis, cannula, gill net, girth, lactate, net damage, rainbow trout, survival rate.

INTRODUCTION

Gill nets or the codends of trawl nets are designed to be selective; that is, if the body girth of a fish that encounters a net is smaller than the mesh size, the fish can pass through and escape. Generally, fisheries scientists have neglected the fact that escapees can be injured or even die after passing through the mesh or escaping. It has been pointed out that if escapees are injured or have lost scales, then significant numbers can die. Furthermore, the mortality rate of these fish depends on the relationship between body girth and mesh perimeter. It is likely that the pressure of the mesh string can induce fatal damage to the circulatory system and that the condition of fish affected in this way could deteriorate further after they escape.

It is also known that extreme exertion can cause metabolic acidosis in concert with a rise in blood lactate. The effect on fish of being captured by or escaping from hooks or gill nets has been studied by hematological analysis. However, it is difficult to withdraw blood from fish without handling them, especially if they are entangled in a gill net.

In the present study, the authors tried to reproduce the entrapment of fish in a gill net by binding fish around the girth with rubber bands in an experimental tank. Blood was taken without handling by means of a cannula inserted into the dorsal aorta. Blood samples were analyzed before binding, immediately after release from binding and following recovery. Hematological analyses were used to compare the physiological changes in fish that ultimately died with those of survivors to determine the cause of death in escaping fish.

MATERIALS AND METHODS

Animals

Rainbow trout Oncorhynchus mykiss were purchased from a local fish farm in Shizuoka Prefecture in central Japan and held at 15 ± 1°C in a rearing tank (100 cm × 80 cm × 200 cm). All fish were acclimated in the tank for more than 2 weeks before the experiment. Fifteen fish weighing between 730 and 1800 g were used for the following
experiment. For repetitive blood sampling without struggling, they were chronically cannulated in the dorsal aorta after anesthetization in water containing tricaine methane sulfonate (3-aminobenzoic acid ethyl; diluted 15 000 times). During the surgical operation, the gills were irrigated with the aerated anesthetic solution (diluted approximately 20 000 times). Electrocardiogram (ECG) was recorded through a electrode inserted into the pericardial cavity of the fish. The electrode was made of thin silver wire covered with a plastic tube peeled off at the tip, and connected to a small plate to attach it to the chest skin. Following surgery, the fish were allowed to recover for 48 h in a temperature-controlled experimental tank (45 cm × 45 cm × 120 cm: 15°C) filled with aerated water. Cannulae were flushed with heparin (0.3 g/10 mL) in saline before every sample.

**Experimental procedure**

Forty-eight hours after surgery, 0.7-mL blood samples were taken with a heparinized syringe (1 mL; Terumo Corp., Tokyo, Japan) through the cannula of each prebinding fish. The fish were then anesthetized again with a lower dose of tricaine methane sulfonate (diluted 20 000 times). While the fish were still in a state of slight torpor, the test fish were quickly bound around the girth just posterior to the opercula with several thin rubber bands. A small piece of U-shaped thin metal wire was placed on the back of the fish beneath the rubber bands for cutting the bands easily and quickly with scissors after the experiment. The binding force of the bands had been estimated beforehand by the stretch of rubber bands as ranging from 7.2 to 39.8 N, in accordance with the fish girth. After individual fish had been bound, each fish was immediately transferred to another experimental tank, which was the same size as that used above and which was filled with water containing over-saturated oxygen. Test fish recovered quickly from anesthesia in the tank. The rubber bands were kept in place for 85–250 min while in the recovery tank. When a binding duration of 120 min had elapsed, or if the fish appeared weak or tended to lie on the bottom of the tank, the binding was released. Blood samples were taken immediately after the bands had been cut and subsequently at 1- or 2-h intervals for 12 h after release. A final sample was taken 24 h after release, if the fish survived. If the fish rolled about or struggled after the release of the bands, however, blood samples were taken at 1-h intervals until the fish died. At every blood collection, the blood removed was replaced with an equivalent amount of saline. Control tests were also performed on a separate set of three fish that had been anesthetized but not bound. Blood samples were taken from the control fish just after anesthetization and every 2 h thereafter until 12 h had elapsed.

Blood samples were analyzed for lactate concentration, pH, oxygen partial pressure and plasma levels of potassium. Lactate was measured in 0.1-mL fractions of whole blood deproteinized in 0.2 mL of 8% perchloric acid and then centrifuged at 504 × g for 10 min. The supernatant was analyzed enzymatically by using a kit measuring blood lactic acid (Sigma, 826, NJ, USA). Blood pH and oxygen partial pressure were determined by injecting 0.1–0.2 mL of whole blood into a portable clinical analyzer, which corrected the value automatically at actual temperature (in this case 15°C; Analyzer 200; I-STAT, St Louis, MO, USA). The plasma level of potassium was also determined with the same clinical analyzer after centrifuging the remainder of the blood sample (504 × g, 10 min).

Each hematological parameter was compared between the surviving, dead and control fish groups. Survival or death after binding was defined by whether the fish were still alive 24 h after they had been released from their bindings. A Student's two-tailed *t*-test was used to compare hematological parameters at each elapsed time. Differences where *P* < 0.05 were accepted as significant.

**RESULTS**

Of 12 bound fish, six died and the remainder were still alive 24 h after release of the rubber bands. The mean binding forces, estimated by the relationship between tensile and expansion of the rubber bands, and the duration of binding were 17.1 N and 146 min for fish that survived and 18.7 N and 154 min for fish that died, respectively. However, neither of these differences were significant. Figure 1 shows the changes in blood lactate levels in both the groups of fish from the initial sampling at prebinding to 6 h after the application of binding. During binding, the blood lactate level, which was regarded as representative of the level of physical fatigue caused by struggling or insufficient oxygen supply, was slightly higher in the group that died later. The prebinding and post-binding levels in the group of survivors were lower and relatively stable, whereas the values in the dead group increased to a comparatively higher level post-binding. However, the differences in blood lactate concentrations between the surviving and dead groups post-binding were not significant. The time until death varied widely from 1 to 21 h after release of the bands in the
dead group, and the physiological parameters of these fish differed according to the survival periods. The time that had elapsed after release was not very important in a comparison of the physiological conditions between the group that survived and the group that died. Instead, the physiological values in the dead group were examined at two time points: 2 h before death and just before death. For the group of survivors, data 4 and 6 h after release from restriction with bands were used; these data were also considered in the group of fish that died. The mean survival time after release of the bands was 6 h. In the unbound control group, the time point ‘immediately after removal of binding’ corresponded to the time of recovery from anesthesia, and the time point ‘after 4 or 6 h’ represented the time elapsed after this recovery. Figure 2 compares blood lactate concentrations between the control, surviving and dead groups at similar times after release from binding. The mean blood lactate level in the group that died rose dramatically and peaked 2 h before or just before death, and was 10-fold higher than in the surviving group. In the surviving group, the mean blood lactate level increased slightly after binding and had returned to prebinding levels by 4 h after release. The values in the control group remained relatively low, both before and after anesthesia. There were apparent differences in the trends of blood lactate levels between the control, surviving and dead groups.

As a result of these changes in blood lactate level, the pH after binding fell in the group that died, whereas in the surviving group it dropped only slightly and soon recovered (Fig. 3). Even though the clinical analyzer (I-STAT) analyzed pH at the corrected temperature, the values were relatively high. Immediately after removal of binding pressure, the mean pH in the group that died lowered, but this was insignificant. After that, the pH became significantly lower until death. Although there was no significant difference in blood pH levels just after binding between the surviving and dead groups, levels 2 h prior to death (4 h after release from binding in the surviving group) and just before death (6 h after release from binding) were significantly lower in the group that died. In the group that survived, the pH had recovered to its prebinding level by 4 h after release from binding, whereas in the fish that eventually died, the pH continued to decrease toward a state of metabolic acidosis. Figure 4 shows the changes in plasma potassium concentration before and after binding. In resting fish, the concentrations of potassium in all three
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In both the control and surviving groups, potassium levels increased slightly immediately after removal of binding (or after recovery from anesthesia, in the case of the controls), but by 6 h after release, the values had recovered to the original prebinding levels. In the group that died, the potassium concentration increased after the removal of binding, and there were significant differences between non-surviving and surviving groups immediately after removal of the bands. Although the blood potassium concentration remained high in the group that died, and recovered in the surviving and control groups, the variance also increased in the group that died, such that there were no significant differences between it and either of the other two groups 1–2 h before death (4 h after release from binding) and just before death (6 h after release from binding). Although blood levels of lactate, pH and potassium among the control and dead groups were almost equal in prebinding conditions, the blood oxygen tension ($P_{O_2}$) of these groups varied at prebinding (Fig. 5). Furthermore, each prebinding value of $P_{O_2}$ was relatively high, because the water in the experimental tank was aerated continuously and oxygen was oversaturated during the experiment. Although the mean level of $P_{O_2}$ in the group that died was slightly (but not significantly) lower at prebinding than in the surviving group, the $P_{O_2}$ level in the former group 1–2 h before death and just before death continued to decline. The mean $P_{O_2}$ of the surviving and control groups was still high 6 h after release of the bands. There were significant differences in $P_{O_2}$ between the dead and the surviving groups 1–2 h before death (4 h after release of binding) and among the dead, surviving and control groups immediately before death (6 h after release of bindings or recovery from anesthesia).

DISCUSSION

Physiological changes during and after exhaustive exercise have been described in some species. In these studies, blood lactate concentrations were found to rise and pH to drop after the exercise, and these levels recovered following a period of rest. In the present study, fish fatigue after escaping from fishing gear was simulated. It was noted that the blood lactate concentration in the fish in the present test rose and the pH fell after...
the fish had been bound, even though little swimming activity or exercise was observed during the period of binding. After exhaustive exercise, typical recovery from lactate accumulation requires 12–24 h, and approximately 40% of fish can die over the 12 h following cessation of this exercise. In contrast, half of the bound fish in the present study died at an average of 6 h after the release of the binding bands, and the surviving fish showed no significant elevation of lactate levels. It is important to point out that the surviving fish were not exhausted, and only the fish that eventually died were fatigued after release from binding. However, it is quite difficult to explain fully how the physiological conditions changed after releasing from binding. The assumption was made that holding by means of binding around the girth sometimes causes fatal physiological stress. However, the reasons for lactate accumulation or fatal exhaustion during and post-binding in the present study are not entirely clear. It is presumed that during the binding process the fish suffered and resisted the binding force even though they hardly swam or exercised. Figure 6 shows the relationship between blood pH and lactate levels. As the individual differences were large and there were depressed pH levels even in surviving fish, high concentrations of lactate tended to cause low blood pH and acidic conditions. This suggests that the binding caused not only a metabolic or respiratory acidosis, but also had a profound effect on the circulatory system – one of many probable reasons for cardiac depression. Wood et al. found high potassium levels in dying fish after exhaustive swimming. In contrast, in the case of mammals, more than 5.5 mM of potassium is required to stop the heart. Thus, it is assumed that elevated potassium levels caused cardiac failure in the test fish in the present study, as the potassium levels in these fish 1–2 h before death and just before death approached 5 mM (Fig. 4). Furthermore, ECG obtained by means of attaching an electrode before binding to the chest skin of one of the test fish that died after binding showed changes in the duration of contraction of the ventricle (Fig. 7). The prolonged QRS complexes just before death indicated a reduction in the contractile action of the ventricle. However, it cannot be assumed that accumulation of potassium alone caused the decrease of the contraction of the heart muscle; more likely, there was a serious cycle of lack of oxygen intake, increase of blood lactate, depressed pH level, excessive blood potassium concentration, and cardiac failure in the group of fish that died.

A number of researchers have described fish mortality rates after encounters with fishing gear. It has also been pointed out that injuries to fish skin, loss of scales, and the force required to pass through the net mesh affect the escapees’ mortality rate. From the viewpoints described in those studies and in the current study, it is sug-
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ACKNOWLEDGMENTS

We thank Dr Tatsusuke Takeda of Kyushu University for his technical suggestion for implanting the cannula into the fish. We also express our gratitude to Dr E.P. Villoso, College of Fisheries and Ocean Sciences, University of the Philippines in the Visayas, for his critical readings and helpful suggestions. This study was supported in part by Grants-in-aid from the Research Fellowships of Japan Society for the Promotion of Science for Young Scientists (No. 10760121).

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