EVALUATION OF STRESS OF CARBON DIOXIDE ANAESTHESIA

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INTRODUCTION

The Salmonid Enhancement Program (SEP) has applied coded-wire tags to 7-12 million fish per year since the early 1980's, an operation that requires short-term (5-10 min) anaesthesia. Tricaine methane sulphonate (TMS) and 2-phenoxyethanol (TPE) were the chemicals most often used in the early years of the SEP. Several concerns, including the safety of hatchery workers using TPE, warnings about releasing fish into seawater soon after exposure to TMS and increasing restrictions on the availability of drugs for routine procedures has led to a switch to carbon dioxide gas as the preferred anaesthetic at SEP hatcheries. One of the marked differences between anaesthesia with carbon dioxide and other anaesthetics is that fish exhibit an extreme hyperactive response when first immersed in water containing a high concentration of carbon dioxide. This led to concerns that the fish were extraordinarily stressed by carbon dioxide anaesthesia, which led to this series of experiments to determine if the carbon dioxide caused a greater degree of measurable physiological stress than the other anaesthetics. Cortisol was chosen as the index of stress, in accordance with common practice (Donaldson, 1981).

METHODS

Experiments were carried out at Tenderfoot Creek Hatchery using juvenile coho salmon (*Oncorhynchus keta*) of about 18 g mean weight. Fish were netted from a holding tank where they had been resting in the shade for at least 2 d, exposed to the anaesthetics in 10 L plastic tubs and placed back into net enclosures in the holding tanks following treatment. Sample groups of fish were collected from each test group before, and at various times after treatment. The sampled fish were killed by a blow to the head, the caudal peduncle was severed and at least 100 μL of blood was drawn using heparinized capillary tubes. The blood samples were centrifuged, the plasma was pipetted off, placed in plastic vials, capped and frozen. The frozen plasma samples were later analyzed using a Baxter radioactive immunoassay (RIA) at the West Vancouver Laboratory.

Carbon dioxide was introduced into the anaesthetic baths in gas form using ceramic airstones. The concentration of carbon dioxide was measured titrimetrically (APHA, 1985). TMS and TPE were measured volummetrically from standard solutions.

A series of experiments was carried out:

1. Determine how fast cortisol rises following carbon dioxide anaesthesia, when it peaks and how fast it dissipates;
2. Compare the cortisol stress response of carbon dioxide anaesthesia with that of the

other two anaesthetics and a control that includes fish that have been handled the same amount without then being put into an anaesthetic bath;

3. Determine the cortisol stress response of different concentration of carbon dioxide in the anaesthetic bath and compare those to anaesthesia in buffered carbon dioxide (a common method of reducing apparent stress from exposure to acidic water) and pre-dosing the fish in a low concentration of carbon dioxide (a method that reduces the hyperactive response exhibited by the fish);
4. Compare the time that fish in a variety of anaesthetic protocols took to become fully anaesthetized.

RESULTS

The first experiment determined the cortisol stress response curve of coho juveniles following exposure to carbon dioxide anaesthesia at about 250 mg/L. A response curve was generated (Figure 1) indicating that the peak cortisol level occurred approximately 90 min following the treatment procedure, that dissipation should begin to be apparent at about 6 hrs after treatment and that by 24 hrs after treatment, the cortisol level would have dropped to a level that may not be distinguishable from background levels.

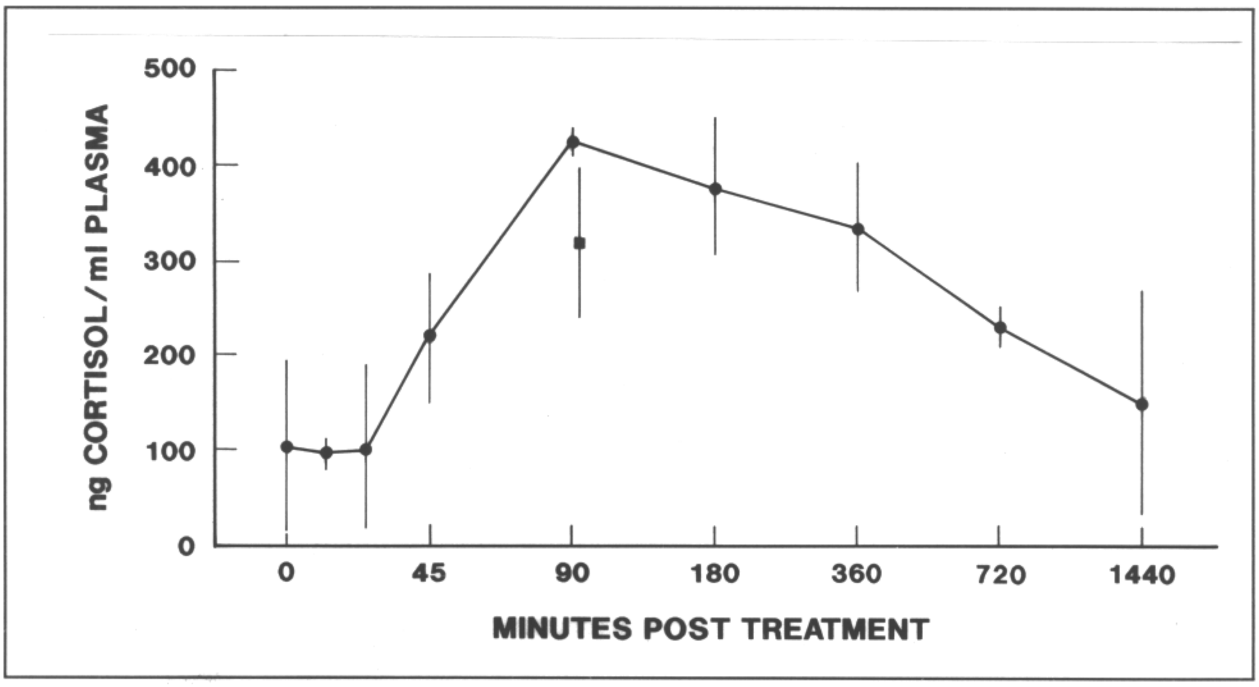


Figure 1. Cortisol concentration following carbon dioxide anaesthesia with handling (● = anaesthetic treatment; ■ = handled only [no anaesthetic])

The second experiment compared the cortisol stress responses of anaesthesia with carbon dioxide, TMS and TPE, and a group that were handled but not anaesthetized (Figure 2). While carbon dioxide had the highest cortisol at the 90 min post-treatment peak, it was not significantly higher and the carbon dioxide fish recovered more quickly than the other fish, including those that were not anaesthetized. It was concluded that the greatest stress was that of handling the fish, which masked any differences between the different anaesthetics. The more rapid clearance of cortisol following carbon dioxide anaesthesia may have been because the carbon dioxide was cleared from the fish more rapidly than the other chemicals, making for a quicker return to full respiration rate which would clear the cortisol quicker. This assumption is based on carbon dioxide being one of the main metabolites of fish that they have developed mechanisms to get rid of, whereas TMS and TPE are not natural metabolites.

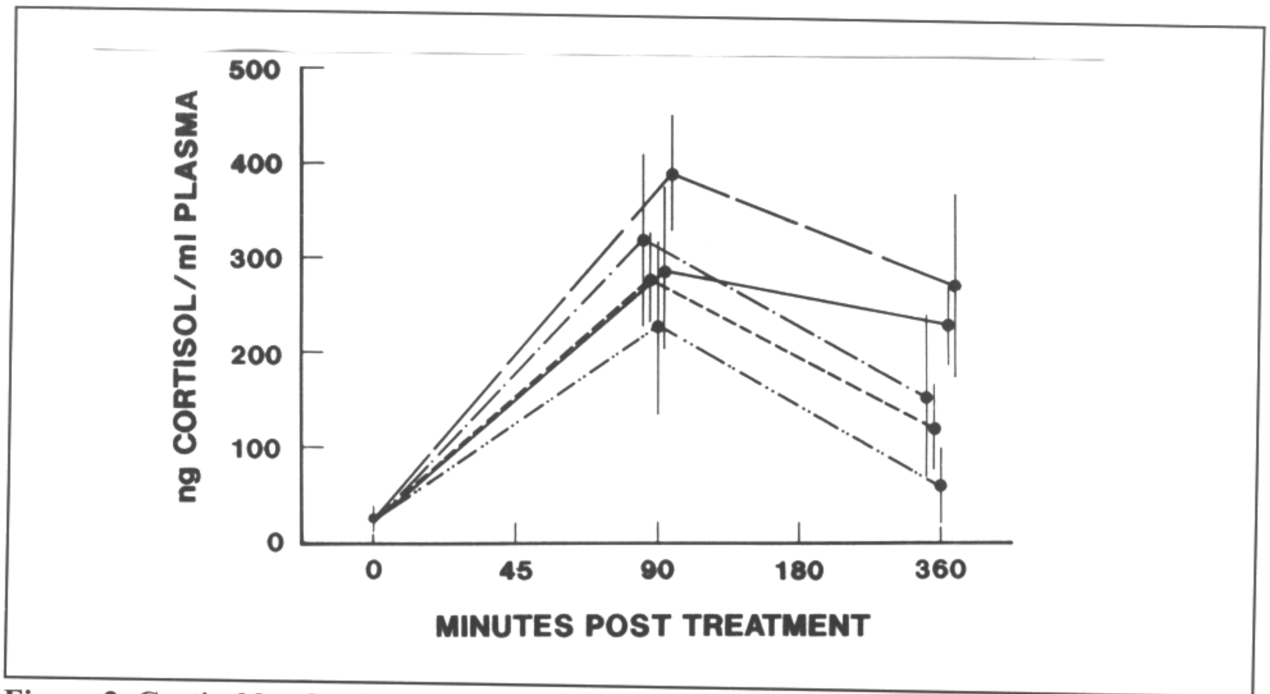


Figure 2. Cortisol levels after treatment with different levels of carbon dioxide anaesthesia. (400 mg/L = dot-dot-dash; 300 mg/L = short dash; 200 mg/L = dot-dash; pre-dosed = long dash; buffered = solid line)

The third experiment determined the cortisol stress response of different concentrations of carbon dioxide anaesthesia (Figure 3). The lowest serum cortisol level at both the 90 min post-treatment peak and at the 6 hr dissipation point was from the highest concentration of carbon dioxide (400 mg/L). Pre-dosing the fish with a low concentration of carbon dioxide (100 mg/L, then 300 mg/L) actually increased their stress level. Buffering the carbon dioxide solution (from a pH of about 5.4 to a pH of 6.5) also did not reduce the stress level and resulted in a slower dissipation of cortisol.

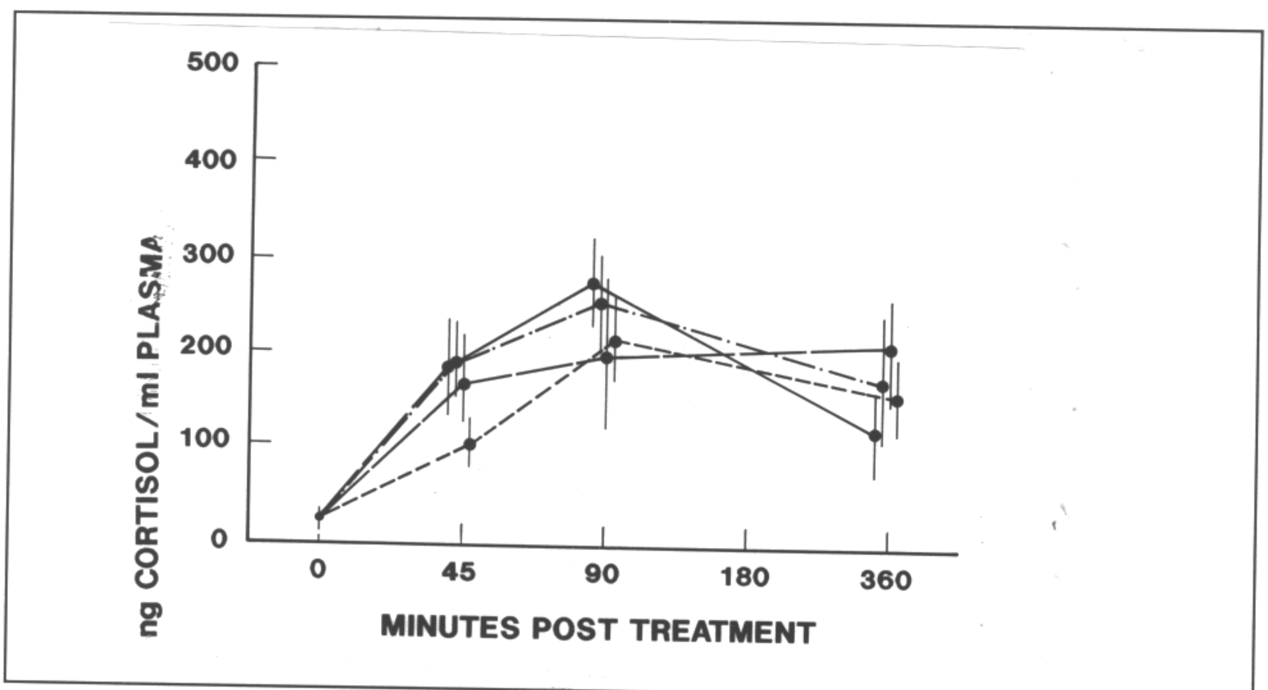
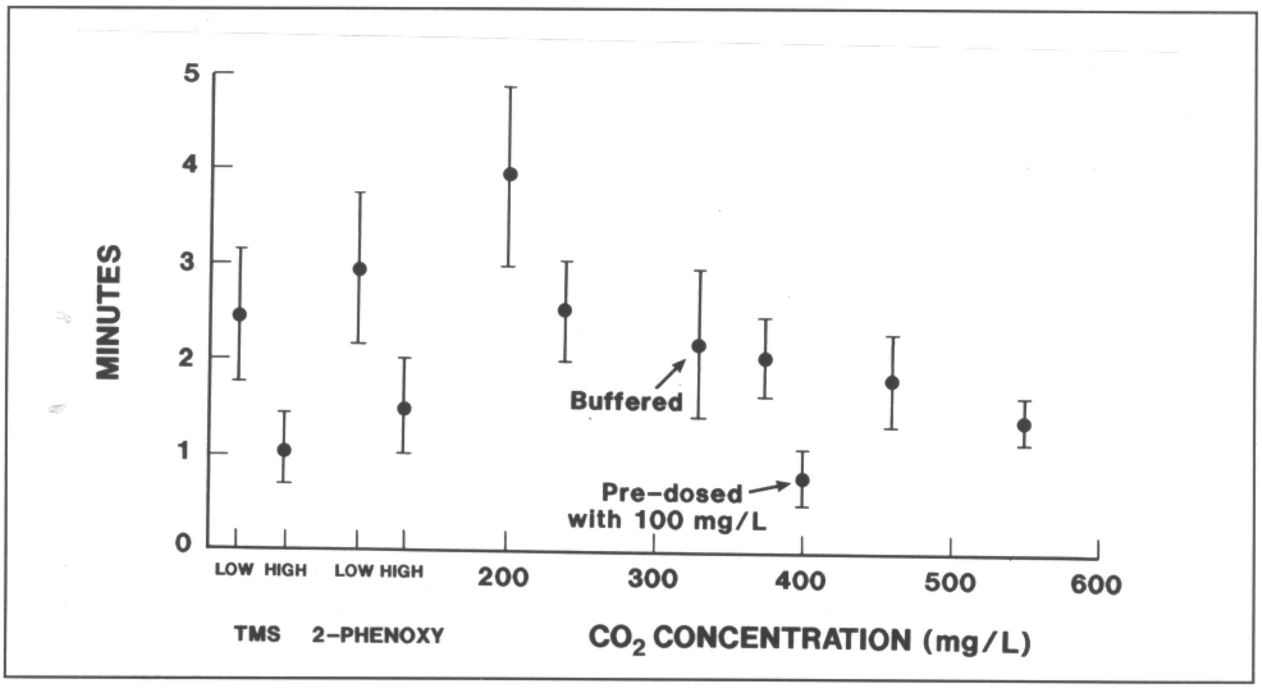


Figure 3. Comparative cortisol response of three anaesthetics and a handle-only control. (Carbon dioxide = solid line; TMS = dot-dash; TPE = long dash; handle-only = short dash)

The fourth experiment was carried out to determine the rapidity of onset of an anaesthetized state (cessation of movement, relaxation of muscles and no response to touch). As expected, higher levels of carbon dioxide resulted in more rapid inducement of anaesthesia (Figure 4). This explained why the higher levels of carbon dioxide showed a lower cortisol response — the fish were anaesthetized so quickly that they had less time for the sequence of cortisol-releasing events to take place before the stress response was shut down by complete anaesthesia.



CONCLUSIONS

Although we have not determined the cause of the hyperactive response of fish being anaesthetized with carbon dioxide, we conclude that such anaesthesia is no more stressful than with other chemicals, since the major component of the distress to the fish is the initial handling prior to being placed in the anaesthetic bath. Our major success in reducing the hyperactive response is by reducing the amount that fish are handled prior to being placed in the anaesthetic bath. This holds for adult salmon as well as juveniles.

Although it would appear from these experiments that the least stressful way to anaesthetize fish is to use high concentrations of carbon dioxide, other tests showed that exposure of fish to concentrations of carbon dioxide higher that 400 mg/L for periods as short as 10 min can cause significant mortalities. It would also appear, from a stress point of view, that buffering the carbon dioxide bath is of no benefit, however, we have observed that buffering does reduce mucous loss from fish, and is probably less damaging to the integument (including the gills) than an acid bath.

We highly recommend carbon dioxide as an effective and safe alternative to other anaesthetics.

REFERENCES

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