Effects of anesthesia and surgery on U crit performance and MO2 in chum salmon, *Oncorhynchus keta*

Running title: Effects of surgery on recovery of chum salmon

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Abstract

Increasing threats posed by overfishing and dams to wild migratory fish make understanding their migration patterns essential. Telemetry is a useful technique for elucidating salmon behaviour, but the recovery periods before fish can be safely released after the attachment of telemetry devices have not yet been established. Reported recovery times vary widely, from 2 h to 13 d. We examined how anaesthesia and surgery to attach external electromyogram (EMG) transmitters affected chum salmon (*Oncorhynchus keta*) recovery based on three physiological parameters. Fish subjected to anaesthesia plus EMG transmitter attachment (EMG group), anaesthesia only (AO group), and no handling (control) were placed in a swim tunnel. Critical swimming speed (U_{crit}), oxygen consumption (MO_2), and muscle activity (EMG values) were assessed 0, 1, 6, 12, 24, and 30 h after treatment. The MO_2 in the EMG and AO groups was higher than in the control group 1 h after treatment, but the U_{crit} and EMG values were not significantly different from the control group at any other sampling time. We concluded that chum salmon had fully recovered their swimming ability by 1 h after treatment and could be safely released into the natural environment.

Keywords: critical swimming speeds (U_{crit}); EMG transmitter; oxygen consumption; telemetry

Introduction

Understanding the migratory patterns of fish is critically important because of increasing threats posed by human activities, such as overfishing and dam construction. Telemetry is a useful technique for elucidating fish behaviour in the wild (McKinley and Power 1992; Økland et al. 1997; Hinch and Rand 1998; Cooke et al. 2004). Telemetry research on fish involves anaesthesia, surgery, and recovery, followed by either release into the field for behavioural tracking or laboratory experiments (Weatherley et al. 1982; Økland et al. 1997; Hinch and Bratty 2000). Following anaesthesia and surgery, all adult Pacific salmon, including sockeye (*Oncorhynchus nerka* (Walbaum 1792)), masu (*O. masou* (Brevoort 1856)), pink (*O. gorbuscha* (Walbaum 1792)), and chum (*O. keta* (Walbaum 1792)), initially exhibit abnormal behaviour (i.e., wide gill flapping) and require more than ten minutes to regain normal orientation in the water (i.e., dorsal fins positioned vertically) after regaining consciousness. However, longer holding periods stress fish and result in both higher mortality rates (Donaldson et al. 2011) and a greater risk of damage to or detachment of telemetry equipment (Bridger and Booth 2003). Therefore, pre-spawning fish should be released as soon as possible after telemetry equipment attachment.

Reported recovery periods after transmitter attachment range from 2 h to 13 d before release into the field (Beddow and McKinley 1999; Akita et al. 2006; Enders et al. 2007; Scruton et al. 2007; Makiguchi et al. 2008; Pon et al. 2009; Clark et al. 2010; Cocherell et al. 2011), although some studies relied only on visual observations of fish behaviour. Although there have been many reports on the physiological effects of anaesthesia (Keene et al. 1998; Woody et al. 2002; Perdikaris et al. 2010), the time required for fish recovery following the attachment of telemetry devices remains unresolved.

Transmitters can be attached externally, inserted intragastrically, or implanted into the abdominal cavity of fish (Bridger and Booth 2003). External attachment causes the most hindrance to swimming (McCleave and Stred 1975; Adams et al. 1998; Makiguchi and Ueda 2009), impairs swimming stability (Bridger and Booth 2003), and increases oxygen consumption (Steinhausen et al. 2006). Moreover, externally-attached transmitters may cause serious damage to the muscles and scales of fish (Mellas and Haynes 1985; Bridger and Booth 2003). Therefore, recovery following surgery to attach an external transmitter would be expected to take longer than surgery to implant other types of transmitter and can establish an upper limit on safe recovery times.

We assumed that full recovery of fish after the attachment of telemetry devices is indicated by physiologically-normal swimming activity. Therefore, this study evaluated the time required for chum salmon to recover swimming ability after anaesthesia and EMG transmitter attachment; chum salmon are the most popular target for fish telemetry studies in Japan (Kitahashi et al. 2000; Tanaka et al. 2005; Akita et al. 2006; Makiguchi et al. 2011). The fish were physiologically assessed based on critical swimming speed (U_{crit}), oxygen consumption (MO_2), and muscle activity in a swim tunnel. Our methods provide baseline data on physiological recovery time in salmon after anaesthesia/surgery.

Materials and methods

Fish capture, handling, and experimental conditions

Twenty-six adult chum salmon (mean ± SE; fork length: 62.3 ± 4.1 cm; body weight: 2.66 ± 0.61 kg) of both sexes were captured using a waterwheel located about 70 km from the mouth of the Chitose River of western Hokkaido, Japan, during their upstream spawning migration. Experiments were conducted at the Chitose Salmon Aquarium in September and December 2010. Fish were individually transferred to compact fish cages (L × W × H = 1.8 × 0.9 × 0.6 m) in an artificially-flowing stream. Fresh Chitose River water was used in all experiments.

Fish were subjected to one of three treatments, each with an equal number of males and females: control (n = 12; fork length: 61.6 ± 4.8 cm; body weight: 2.55 ± 0.64 kg), anaesthesia only (AO group; total n = 6; fork length: 64.2 ± 4.3 cm; body weight: 3.08 ± 0.74 kg), and anaesthesia with EMG transmitter attachment (EMG group; n = 8; fork length: 62.0 ± 2.5 cm; body weight: 2.50 ± 0.30 kg). Control fish were exposed to air for a few seconds during transfer to the swim tunnel. The EMG group was anaesthetized with 0.5 ml L^{-1} FA100 (eugenol; Tanabe Seiyaku, Osaka, Japan) for about 8 min, then EMG transmitters were attached externally using a standard procedure developed by Makiguchi et al. (2011). Briefly, EMG transmitters (CEMG-R11, Lotek Engineering, Newmarket, Ontario, Canada: 18.0 g, 16.0 mm diam., 53.0 mm long) were pushed through the dorsal muscle using nylon ties, and Teflon-coated electrodes with brass muscle-anchoring tips (dimension 5 × 1 mm) were inserted subcutaneously using a hypodermic needle at approximately 0.7×

the body length on the left side of the fish. Paired electrode tips were positioned approximately 10 mm apart and secured in the lateral red muscle toward the rear of the fish. The surgery took about 7 min, during which the fish were exposed to air and their gills were irrigated. The AO group was anaesthetized as described above then held in air with gill irrigation for 7 min to control for the exposure time of surgery. The anaesthetic fluid was rinsed off with water, and fish were evaluated immediately after anaesthesia/surgery.

Determination of critical swimming speeds (U_{crit})

A swim tunnel (West Japan Fluid Engineering Laboratory Co. Ltd, Nagasaki, Japan) was used to measure U_{crit}, MO_2, and muscle activity (Fig. 1). The swim tunnel was sealed with an acrylic board to prevent gas exchange, and fresh river water was pumped into it before each trial. The water temperature during all experiments ranged from 12.1 to 14.7ºC. Within any one experiment, water temperature varied by ≤ 1ºC.

The U_{crit} quantifies the sub-maximum and largely aerobic swimming ability of fish and is approximately the speed at which fish become fatigued during incremental velocity trials (Brett 1964, 1967; Hammer 1995). Experimental fish were individually assessed for U_{crit} as a gauge of recovery. In each U_{crit} trial, the initial flow velocity (V) of 0.350 body lengths (BL) s^{-1} was increased by 0.175 BL s^{-1} every 15 min until the fish were fatigued and became lodged at the end of the swimming section of the tunnel. Flow velocity and the point of fatigue within the terminal 15-min period were used to calculate U_{crit}, normalized for BL, as described by Brett (1964):

$$U_{\mathrm{crit}} = U + [(T\,Ti^{-1})\,Ui] \qquad (1)$$

where U is the flow velocity, corrected to account for the solid blocking effects (Gehrke et al. 1990) described by Bell and Terhune (1970), at which the fish last swam for the full 15-min period; Ui is the velocity increment (0.175 BL s^{-1}); T is the length of time in minutes that fish were able to swim at the terminal flow velocity that produced fatigue, and Ti is the time between velocity increments (900 s).

In total, six trials were conducted, at 0, 1, 6, 12, 24, and 30 h after anaesthesia/surgery. Because each U_{crit} measurement took more than an hour, the fish used in the first trial were not used again. The same individuals were used in each of the second to sixth trials. In the first trial, the fish were immediately measured for U_{crit}, with no acclimatization period. Before the second trial, the fish were allowed to acclimate to a current velocity of V = 0.175 m s^{-1} for 1 h before the trial began. Fish were allowed to rest for ~2–3 h between trials. Wagner et al. (2005) reported that fish that rested for 45 min between U_{crit} trials had similar oxygen consumption values in both trials. Thus, we assumed that a resting period of 2–3 h between trials was sufficient for independent measurements of U_{crit} and MO_2.

Measurement of oxygen consumption (MO_2)

To measure MO_2 of fish during the trials, oxygen concentration in the swim tunnel was measured at 1-min intervals using a U-50 Multiparameter Water Quality Meter (Horiba Ltd., Kyoto, Japan) housed in a flow-through outside the swim tunnel (Fig. 1d, e). Before the fish were introduced, the swim tunnel was operated to remove air bubbles, and oxygen levels in the tunnel were replenished with fresh river water between trials. Oxygen consumption per 15-min period for each fish was calculated as the difference in oxygen concentration between the start and end of the period. The MO_2 (mg O_2 kg^{-1} h^{-1}) for individual fish during a velocity increment was calculated as $MO_2 = [O_2]\ v\ m^{-1}$, where the change in oxygen concentration $[O_2]$ is measured in mg·O_2 per l^{-1} h^{-1}, v is the water volume of the swim tunnel (L), and m is the body mass of the fish (kg).

Measurement of EMG values

Muscle activity in the EMG group was monitored with EMG transmitters. The EMG voltage was calibrated and sampled at 2-s intervals. At the end of each 2-s interval, the average value was assigned a unitless activity level (EMG signal) ranging from 0 to 50 and then transmitted to a radio receiver (model SRX_600, Lotek Engineering Inc., Newmarket, Ontario, Canada). The mean EMG value was calculated for each swimming velocity and mean and coefficient of variation (CV) were calculated for each trial and for the acclimatization period.

Data analysis and statistics

Data are presented as the mean ± the standard error (SE). One-factor ANOVAs were performed to assess differences in U_{crit}, MO_2, and EMG value among trials (using flow velocity as the factor) and among treatments (using treatment as the factor). Control fish did not have EMG transmitters, so EMG values were lacking for this group. The MO_2 data for three treatments in trials 2–6 were subsequently analysed by the Tukey-Kramer test. The EMG CV was analysed using one-factor ANOVA with trial as the single factor. Statistical significance was set at $P < 0.05$. Statistical analysis was performed using Excel 2007 (Microsoft, Redmond, WA, USA) with the add-in Statcal3 (Yanai 2011).

Results

There were no significant differences (U_{crit}: $P > 0.05$) between the sexes in any experiment, so male and female datasets were combined for each treatment.

Critical swimming speed (U_{crit})

The U_{crit} values for each trial are shown in Fig. 2. In the first trial, the fish in the AO and EMG groups were not able to wake and swim forward for several minutes (fish remained upside down or slanted, AO group: 5.13 min ± 4.20; EMG group: 10.39 min ± 7.08). To recover normal orientation, the fish required a further 20 min after being placed in the swim tunnel. Therefore, U_{crit} could not be measured in these fish in trial 1. For the control group, there were no significant differences in average U_{crit} between the first and subsequent trials ($P > 0.34$ in all comparisons). No significant differences in average U_{crit} were found among treatment groups in any of the subsequent trials ($P > 0.37$ in all comparisons). Thus, after anaesthesia/surgery, fish regained normal swimming ability within 1 h.

Oxygen consumption (MO_2)

For the control group, there were no significant differences in average MO_2 between the first and subsequent trials ($P > 0.17$ in all comparisons). Significant differences were found in MO_2 among all treatments in trial 2 (Fig. 3; $P < 0.01$ or 0.05), but no significant differences were found in MO_2 among any treatment groups in the other trials ($P > 0.09$ in all comparisons). For both AO and EMG groups, MO_2 in the first trial (Fig. 3b) differed from subsequent trials (Fig. 3c–f), in which MO_2 increased with swimming speed, although there were minor variations. In the AO and EMG groups, MO_2 levels were higher immediately after acclimatization (at V = 0.175 BL s^{-1}) than at U_{crit} (Fig. 3b). Oxygen consumption in the first trial of the EMG group declined over the first 1.25 h of the trial (until V = 1.05 BL s^{-1}), but stabilized thereafter. In the AO group, MO_2 decreased over the first 30 min of the first trial (until V = 0.525 BL s^{-1}), then began to slowly increase, as in the control. In all post-anaesthesia/surgery trials, maximum MO_2 at U_{crit} was approximately 6-7 mg O_2 kg^{-1} h^{-1}.

EMG values

Muscle activity (EMG values) in the EMG group increased with flow velocity in all trials (Fig. 4), and there were no significant differences among trials (*P* > 0.99 in all comparisons). The CV of the EMG values varied during the acclimatization phase more than in other phases, but no significant differences were observed (*P* > 0.77 in all comparisons) in the subsequent trials (Fig. 5).

Discussion

We evaluated the time needed for chum salmon to regain full physiological swimming ability (as measured by U_{crit}, MO_2, and EMG values) after anaesthesia and surgery for EMG transmitter attachment. Mean U_{crit} values were approximately 1.5 BL s^{-1}, comparable to the 1.6 BL s^{-1} reported for adult chum salmon by Makiguchi et al. (2008) and for coho salmon (*O. kisutch* (Walbaum 1792)) by Lee et al. (2003). We found no significant differences in mean U_{crit} values between the EMG group and either the control or AO groups in any of the five trials conducted between 1–30 h after anaesthesia/surgery. We conducted similar research using adult rainbow trout (*O. mykiss* (Walbaum 1792), total *n* = 28, 14 males, 14 females; mean ± SE; fork length: 52.0 ± 4.1 cm; body weight: 1.53 ± 0.36 kg) and found that swimming ability was also regained within 1 h after anaesthesia/surgery (unpublished data). Our fish required 5–10 min to recover normal orientation after anaesthesia/surgery. In comparison, Lacroix et al. (2004) reported that juvenile Atlantic salmon began to recover from anaesthesia about 2–3 min after being returned to fresh water and fully regained equilibrium and darting behaviour within 60 min. Meka et al. (2003) reported that adult rainbow trout could be released ~20–30 min after the start of anaesthesia/surgery, which took ~5–6 min. Obviously, the recovery period must be determined for each species and life stage prior to release.

The MO_2 of the EMG and AO groups were substantially higher than the control 1 h after anaesthesia/surgery. The fact that both groups had elevated MO_2 levels indicated that the 7 min of exposure to air affected the fish. Because the decline in MO_2 stopped 1.5 h into the trial (when V = 1.05 BL s^{-1}; MO_2: 6.0), we can assume that the effects of surgery had receded by this time. The MO_2 values were no longer significantly different from the control at V=0.700 BL s^{-1} ($P > 0.09$). As fish swim faster, their active metabolic rate increases (Brett 1964; Wagner et al. 2006), and MO_2 should increase as well. In all subsequent trials, MO_2 tended to increase with flow velocity and did not differ significantly among the control, AO, and

EMG treatments, indicating that the fish had fully recovered from anaesthesia/surgery.

Maximum oxygen uptake is generally accepted to occur at U_{crit} (Farrell and Steffensen 1987), when maximum aerobic capacity can be estimated (Hammer 1995). In none of our trials did the MO_2 values at U_{crit} differ among treatments. Moreover, the increase in MO_2 appeared to slow or even reverse immediately before U_{crit} was reached, similar to findings in chinook salmon (Geist et al. 2003). In all cases, the EMG group consumed substantially more oxygen 1 h after anaesthesia/surgery than in subsequent trials, but because neither U_{crit} nor MO_2 at U_{crit} differed from the control in the first trial, we concluded that the elevated MO_2 value did not affect swimming activity.

In all post-surgery trials, EMG values in the EMG group increased with flow velocity, in agreement with the report of Makiguchi et al. (2011) demonstrating that EMG values in chum salmon increased with swimming speed. There were no significant differences in average EMG values among trials. These results indicate that muscular activity in fish attached with EMG transmitters had recovered to normal levels within 1 h of anaesthesia/surgery. In addition, no significant differences in the EMG value CV were found among trials. During the acclimatization period (0–1 h) when the fish were waking, there was substantial variation in EMG values.

This study provided clear evidence that chum salmon that migrated to the Chitose River to spawn recovered within 1 h from both anaesthesia and surgery to attach external EMG transmitters, as indicated by three physiological measures, normal swimming behaviour, U_{crit}, MO_2, and EMG values. Their swimming ability remained stable thereafter. Thus, we concluded that chum salmon can be used for telemetry experiments 1 h after the attachment of an external transmitter without significant physiological disability. Our findings are likely to apply to intragastric and abdominally-implanted transmitters as well, because external transmitters are more likely to affect swimming ability (McCleave and Stred 1975; Adams et al. 1998; Makiguchi and Ueda 2009). Thorstad et al. (2000) reported no differences in swimming endurance of adult Atlantic salmon among control fish, those with small or large external dummy transmitters, or fish with surgical implants.

The importance of telemetry in understanding fish migration ensures that the number of telemetry studies will continue to increase as the devices become more compact and affordable. A variety of anaesthetics and equipment will be used on different species in different conditions, including water temperature, and fish age class (e.g., young, adult, spawning), and behavioural phase (e.g., downstream versus upstream migration), that might affect recovery time. Pike, for example, recovered quickly when anesthetized at 12°C, but required several hours to fully recover when anaesthetized at temperatures of <2°C (Jepsen et al. 2001). Our method should prove practical in evaluating a range of species under many different conditions. We are convinced that proper use of telemetry, including reasonable recovery and release times, will yield high quality data that will help to resolve various problems for migrating salmon, including fishways (Roscoe et al. 2011), dams (Cocherell et al. 2011), and global climate change (Hasler et al. 2012).

In summary, the current research showed that chum salmon had fully recovered from surgery to attach external telemetry equipment within 1 h. This study was the first to attempt to understand the physiological effects of anaesthesia/surgery on the recovery of chum salmon. The results provided baseline information on appropriate release times for chum salmon after the attachment of telemetry devices. Furthermore, our methods should be widely applicable to other species, types of telemetry device, and environmental conditions.

Acknowledgements

The authors thank A. Hirayama for comments on the study design; M. Kubo for advice about statistical techniques; M. Kikuchi for input on the selection of the experimental site; Chitose Salmon Aquarium and Chitosegawa River Office for access to the experimental site; and the Sea of Japan Salmon Propagative Association for providing fish for the experiments.

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Figure Legends

Fig. 1 Swim tunnel used in the swimming trials (length: 1.5 m; diam.: 0.3 m). Water flow was generated using a voltage-controlled motor and propeller, with the voltage calibrated against flow velocity. (a) Anticlockwise water flow with a water volume of 450 L. (b) Swimming area (L = 1.5 m). (c) Water quality sensor. (d) Water quality indicator/data logger. (e) Flow velocity controller. (f) Voltage-controlled motor and propeller. (g) Cooler. The water temperature was set at 12°C

Fig. 2. Relationship between the trials after anaesthesia/surgery and U_{crit} in chum salmon (N = 6–12 per treatment). Immediately after anaesthesia/surgery, fish in the anaesthesia only (AO) and EMG transmitter attachment (EMG) groups could not swim, so their U_{crit} could not be measured in the first trial at 0 h. Subsequent trials were begun 1, 6, 12, 24, and 30 h after anaesthesia/surgery. None of the measured U_{crit} values were significantly different from any other ($P > 0.05$).

Fig. 3. Relationship between flow velocity and oxygen consumption (N = 6–12 per treatment). Immediately after anaesthesia/surgery, fish in the anaesthesia only (AO) and EMG transmitter attachment (EMG) groups could not swim, so their MO_2 could not be measured in the first trial at 0 h. Subsequent trials were begun 1, 6, 12, 24, and 30 h after anaesthesia/surgery. Except in trial 2 (begun 1 h after anaesthesia/surgery), oxygen consumption increased with flow velocity. For trial 2, significant differences were found until V = 0.525 BL s^{-1} (*$P < 0.05$, **$P < 0.01$ by one-factor ANOVA followed by the Tukey-Kramer test).

Fig. 4. Relationship between flow velocity and muscle activity (EMG value) in fish with externally-attached EMG transmitters (N = 8). Trials were begun 1, 6, 12, 24, and 30 h after anaesthesia/surgery. For each flow velocity in each trial, five EMG values were averaged. Muscle activity increased with flow velocity in all trials, and no significant differences were observed among trials at each flow velocity ($P > 0.05$).

Fig. 5. Relationship between the trials after anaesthesia/surgery and the mean coefficient of variation (CV) of

the EMG value. Although substantial variation in the EMG CV occurred during the acclimatization period (~0–1 h) after anaesthesia/surgery, no significant differences were observed in EMG CV ($P > 0.05$).

Fig. 1

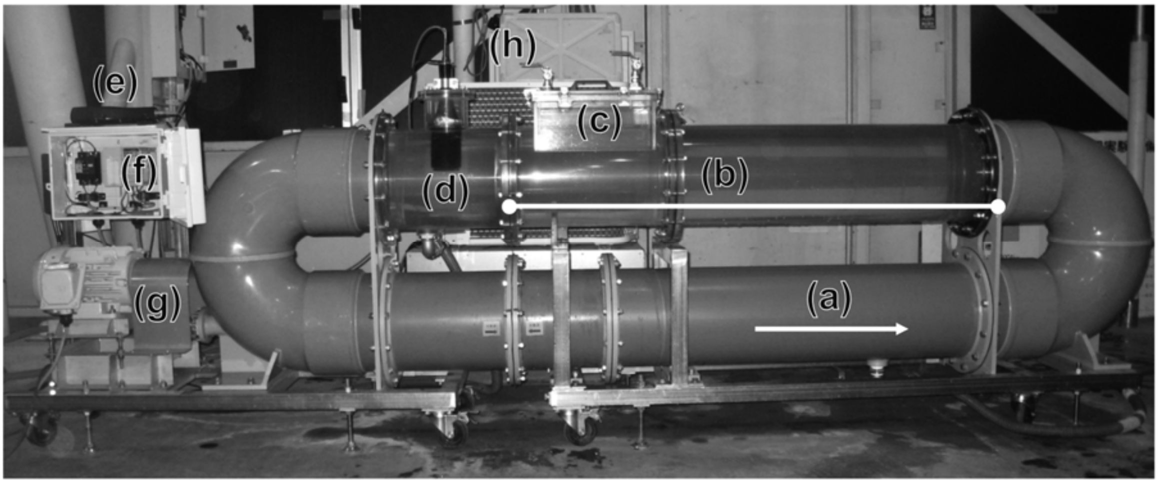


Fig. 2

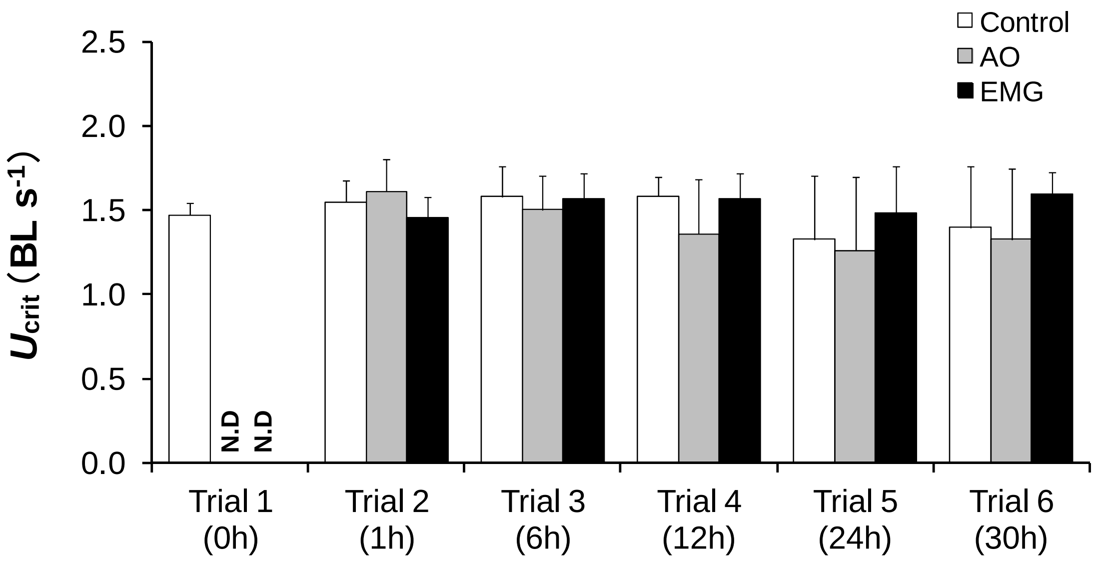

Control
AO
EMG
2.5
2.0
1.5
1.0
0.5
0.0
Ucrit（BL s-1）
N.D
N.D
Trial 1 (0h)
Trial 2 (1h)
Trial 3 (6h)
Trial 4 (12h)
Trial 5 (24h)
Trial 6 (30h)

Fig. 3

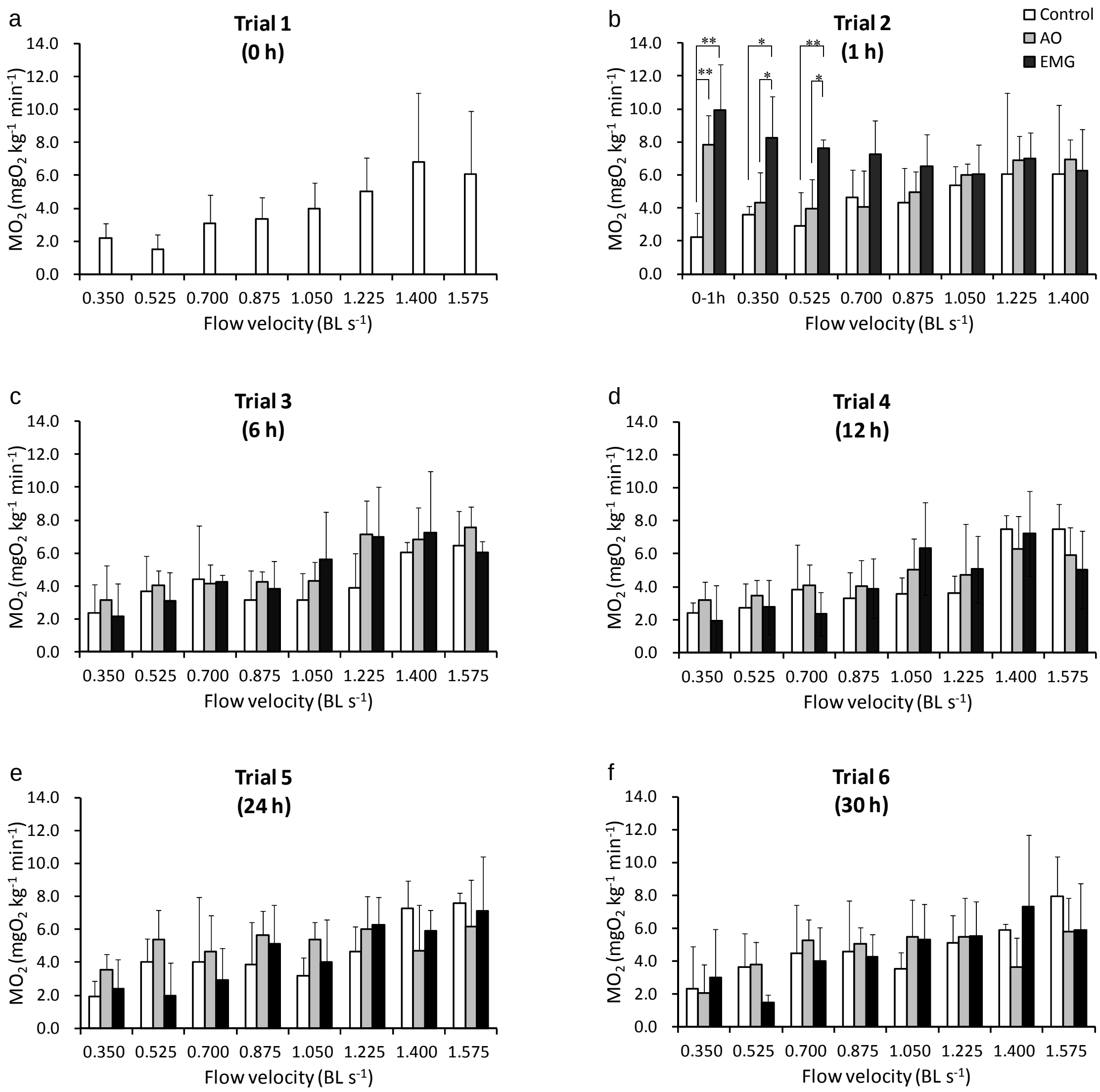
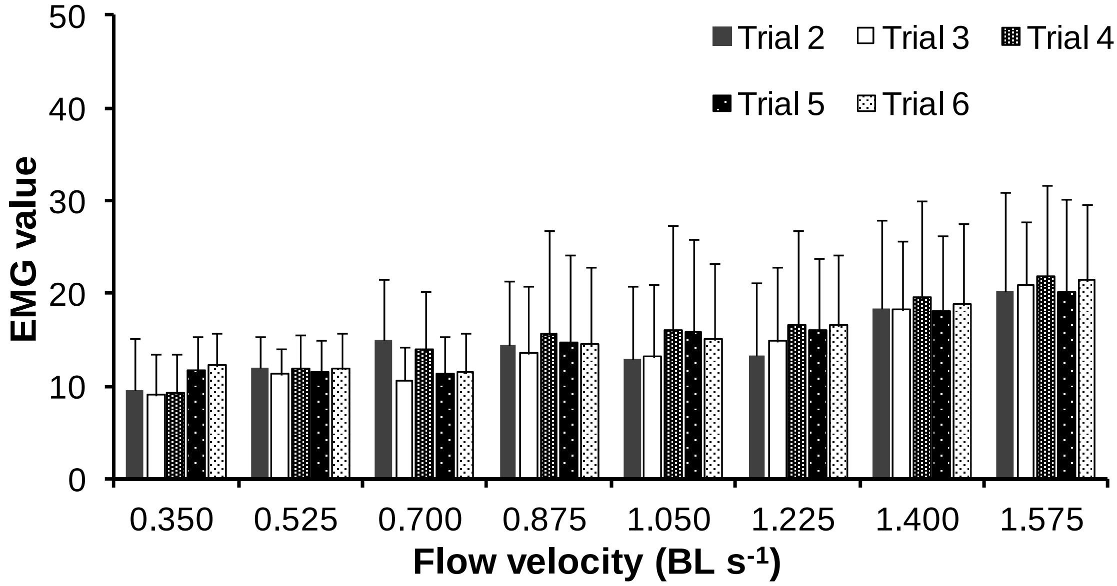

a
Trial 1
(0 h)
b
Trial 2
(1 h)
Control
AO
EMG
0-1h
c
Trial 3
(6 h)
d
Trial 4
(12 h)
e
Trial 5
(24 h)
f
Trial 6
(30 h)
MO2 (mgO2 kg-1 min-1)
Flow velocity (BL s-1)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
0.350
0.525
0.700
0.875
1.050
1.225
1.400
1.575
**
*

Fig. 4



Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
EMG value
50
40
30
20
10
0
0.350
0.525
0.700
0.875
1.050
1.225
1.400
1.575
Flow velocity (BL s-1)

Fig. 5