Rigor Contractions in "Rested" and "Partially Exercised" Chinook Salmon White Muscle as Affected by Temperature

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ABSTRACT

Time lapse video was used to determine the effects of temperature and fatigue on the development of rigor contractions in chinook salmon (*Oncorhynchus tshawytscha*) white muscle. Minimal handling and chemical anaesthesia (AQUI-S™) produced rested fish. After removal of one fillet from the rested fish, post-mortem electrical stimulation of the carcass was used to partially exercise the remaining muscle. Specific strips of rested and partially exercised muscle were held in physiological saline at 0, 4, 6, 8 and 12°C. Contraction onset and end were delayed by decreasing temperature in the rested treatment, but were unaffected in the partially exercised treatment. The final contracted length was affected by fatigue state and temperature.

Key Words: salmon, fatigue effects, rigor contractions, partial exercise, texture

INTRODUCTION

AN EXPERIMENTAL PROTOCOL WAS DEVELOPED THAT ENABLED the examination of the post-harvest characteristics of chinook salmon muscle in a near rested state. Rested "white" muscle retained its native tensile strength post-harvest significantly longer than fatigued muscle (Jerrett et al., 1996). In addition, substantial delays in the onset of rigor contractions and a reduction in the magnitude of isometric rigor contraction stress were reported (Jerrett and Holland, 1997). These beneficial attributes of rested muscle were attributable to the extended viability of the tissue during storage as indicated by the prolonged electrical excitability of the muscle (Jerrett et al., 1996). This viability, in turn, was attributed to the sparing effect of the low exercise harvesting regime on the phosphagen stores of the tissue (Bendall, 1960; Dobson and Hochachka, 1987).

Excessive chilling of fish muscle has been reported to elicit an acceleration of rigor mortis (or other "cold shortening" effects) in several temperate and tropical fish species (Ushio et al., 1991; Johansen et al., 1996). Irrespective of the underlying mechanism, unnecessary muscle contractions or an increase in energy expended for homeostasis would result in an undesirable reduction in the duration of the pre-rigor state. Depression of the post-mortem (PM) metabolic rate by chilling must, therefore, be balanced against any increased energy expenditure by the muscle in response to hypothermic stress.

In a previous study, it was noted that the "white" muscle of chinook salmon could retain electrical excitability when the fish carcass as a whole showed substantial stiffening (Jerrett et al., 1996). A similar discrepancy was reported by Curran et al. (1986) in iced tilapia (*Oreochromis aureus/niloticus*). In our current study the objective was to determine if any acceleration of rigor contraction onset or other "cold shortening" effects were detectable specifically in "rested" and moderately fatigued chinook salmon "white" muscle.

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MATERIALS & METHODS

Experimental animals

Twenty three ♀ chinook salmon (*Oncorhynchus tshawytscha*) were used. These animals were sampled from a mixed sex, tank population of 260 fish which had been reared indoors in a 28 m^3 oval tank from smoltification (35 to 116g). The rearing tank was supplied with filtered seawater on a flow-through basis with a typical turnover of one tank volume per 3.5h. Auxiliary aeration maintained the dissolved oxygen concentration at 90% of saturation.

The mean fish weight was 732g (SEM=42) and fork length was 373 mm (SEM=7). The mean condition factor (Love, 1980) of these animals was 1.38 (SEM=0.03). The mean liver weight was 8.52g (SEM=0.66) and the mean gonad weight was 2.41g (SEM=0.30).

Conditioning for capture and fish sampling

The conditioning and sampling protocol used by Jerrett et al. (1996) was used with the exception that in this study only one animal was held in the 500L holding tank at any time. Experimental timing was measured from the time that the fish brain was destroyed by insertion of a Japanese fish pick or *iki jime* tool through the cranium. The fish were sampled in August 1993 during which time the rearing tank water temperature ranged from 10.0 to 12.5±0.2°C (mean=11.2°C; SEM=0.1).

Electrical stimulation

One fillet was removed from each fish immediately after pithing and was assigned to the "rested" treatment. The second fillet was retained on the frame until completion of the PM exercise protocol. A Hewlett-Packard 7804A pacemaker unit was used to deliver 180, 15 Vdc, 2 ms duration square wave pulses at a rate of 1 pulse per 1.8s to this fillet. These fillets were assigned to the "partially exercised" treatment (Jerrett and Holland, 1997). The positive electrode lead terminated in a 16 gauge hypodermic needle. This needle was inserted into the dorsal musculature immediately posterior to the skull until contact was made with the vertebrae. Care was taken to ensure that the electrode was placed symmetrically on the dorsa-ventral axis of each fish. The negative electrode lead terminated in an "alligator"-style clip. This clip was attached near the termination of the spine in the fork of the tail.

Muscle sample preparation and storage

Fillets from both treatments were skinned when the electrical stimulation of the fillet assigned to the partially exercised treatment had been completed. Once skinned, a 100 mm by 15 mm strip was dissected from the anterior of each fillet. This was the same location as used in experiments on isometric rigorometry (Fig. 1; Jerrett and Holland, 1997). All visible "red" muscle was then removed leaving a strip almost rectangular in cross-section. After the red muscle was removed, each strip was cut to 80 mm±0.5 mm length. This yielded a mean muscle strip weight of 7.84g (SEM=0.34); thickness of 7.4 mm (SEM=1.6) and mean strip width of 10.3 mm (SEM=0.3). The muscle was handled as little as possible during these procedures.

Rigorometry

In the apparatus used to measure changes in length (Fig. 1), the muscle strips were immersed in a stirred bath of marine Ringers solu-

tion (Nilssen and Fange, 1969) pre-cooled to 0, 4, 6, 8 or 12°C. Changes in length were recorded on videotape using a CCD camera (Ikegami Model ICD-42E, Japan) fitted with a 6 mm focal length, F1.4 auto iris lens (Computer Model HG0614AFCS, Japan), and connected to a time lapse video recorder (Panasonic Model AG-6720A, Japan). One video frame was captured every 3.2 sec by the recorder. A scale ruler immersed in the bath, in the same focal plane as the muscle strips, allowed changes in strip length to be taken directly from a 35 cm monitor on review of the videotape.

Temperature control

A Peltier effect refrigeration unit (Tropicool TC2000, Christchurch, NZ) was modified to accept an insulated aluminum bath. Temperature control of the Ringers solution within ±0.2°C was achieved by using the built-in proportional control system of the Peltier unit to control one thermoelectric element while controlling the second element with a thermostat (SAE Model PMP33, Maron di Brugnera, PN, Italy). The accuracy of the temperature control was confirmed using a type T thermocouple logged at 1 min intervals by a data logger (Model LI-1000, LI-COR Inc., Lincoln, NE) fitted with a 1000-10 Thermocouple Terminal Block. The logger and thermocouples had been calibrated in a stirred water bath at the freezing point of water and against a reference thermometer at higher temperatures.

Cut-surface pH measurements

A surface pH measurement was made on the surface freshly exposed by a transverse section of the muscle at the anterior end of each strip used for rigor contraction measurements (A-A; Fig. 1; Jerrett and Holland, 1997).

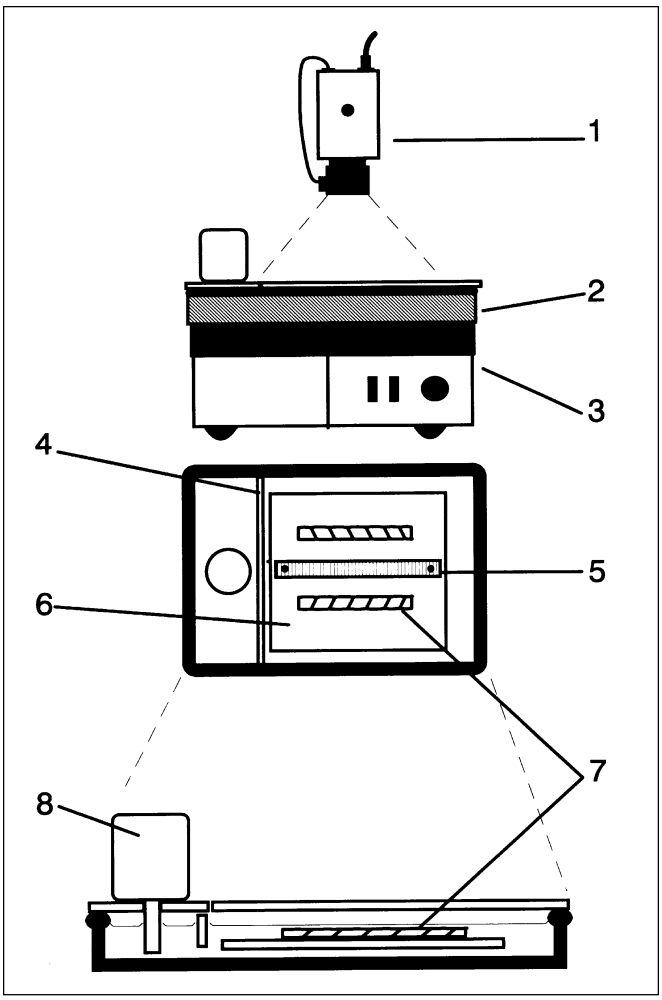

Fig. 1—Time lapse video rigorometry apparatus. (1) CCD camera; (2) insulated water bath with glass cover; (3) thermoelectric refrigeration unit; (4) baffle; (5) scale ruler; (6) raised perspex sample platform; (7) muscle strip; (8) stirrer.

Statistical analysis

Data from the rested and partially exercised fish were analyzed separately using analysis of variance techniques with the temperature effect partitioned into linear, quadratic and cubic contrasts. Initial pH, date of measurement, condition factor, gonad weight, liver weight and temperature of acclimation were tested as potential covariates. The fit of time to the onset of rigor contraction on temperature was subjected to nonlinear modelling and a 4-factor logistic curve was fitted. The analyses were carried out using Genstat^{TM} Version 5.0 (Rothamsted Experimental Station, UK, 1987).

RESULTS & DISCUSSION

Methodology

The time-lapse video rigorometry method we used was developed to allow the simultaneous recording of unrestricted changes in muscle strip dimensions while ensuring that the strips experienced the same environmental conditions. We assumed that support of aerobic metabolism via O_2 diffusion would be similar in each of the treatments and that this would be primarily a surface phenomenon. We also assumed that the response to immersion in the marine Ringers would be similar in both treatments and that this would simulate the conditions experienced by the muscle tissue *in situ*. The Ringers solution provided the necessary lubrication, support and control of desiccation required for the method.

An earlier study using conventional isometric rigorometry produced very similar rigor contraction onset and end times as this method using the same protocols to produce the rested and partially exercised muscle. In the isometric study, rigor contraction onset (at 15°C occurred in the rested fish after about 6.2h and in the partially exercised treatment after about 2.7h (Jerrett and Holland, 1997). In this study the mean rigor onset time was 5.72h (SEM=0.07) in the rested treatment and 2.69h (SEM=0.54) in the partially exercised treatment. The video method detected the onset of rigor contraction earlier than the isometric method although the animals had been acclimated to a lower temperature (12°C vs 15°C). This may be attributed to biological variation or perhaps to the higher sensitivity of the video method.

In this current study, the results (Fig. 2, 3 and 4) were obtained from muscle strips dissected from pairs of rested and partially exercised fillets taken from the same fish. In the 12°C treatment, the image of one exercised muscle strip was obscured by foam and discarded. In the 0°C treatment, two of the exercised samples were obscured by condensation and similarly discarded. In those cases, the "rested" treatment observations were unaffected and were retained.

Characterization of the fatigue states of the treatments

The initial mean cut-surface pH of the rested muscle strips was 7.33 (SEM=0.12) and the immediate post-stimulation pH of the partially exercised treatment strips was 6.88 (SEM=0.16). These values were similar to those reported (Jerrett et al., 1996; Jerrett and Holland, 1997) using the same protocol while the mean rested value was comparable with values reported by van den Thillart et al. (1989) from rested, anaesthetized carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*). As in previous work, muscle in the 180 contraction, partially exercised treatment was judged to be in a similar state to muscle taken from moderately fatigued fish that had been dip-netted as they surfaced to feed, restrained and pithed within 15 to 30 sec of capture (Jerrett et al., 1996). In chinook, a muscle cut-surface pH of 7.0 to 7.1 would indicate the beginning of lactic acid accumulation (Jerrett et al., 1996).

Temperature effects on time to contraction onset and end

None of the covariates affected the time to onset of rigor contraction in either rested or partially exercised muscle. Temperature did not

alter the time to rigor contraction onset for the partially exercised white muscle ($F_{4,15}$=0.91; p=0.43). However, with the rested muscle there was a linear decline ($F_{1,18}$=66.49; p<0.001) with a cubic component ($F_{1,18}$=6.95; p=0.02) in the time to rigor contraction as temperature increased (Fig. 2). The sigmoidal trend in time to onset of rigor in the rested treatment warranted further analysis. The response variance was stabilized across the temperature scale by taking the logarithms of the time to rigor contraction onset and the transformed values fitted to temperature using a 4-factor logistic function:

$$y = d+(a-d)/(1+b*e^{(-c*x)})$$

where y=ln time to contraction onset and x=storage temperature (°C). The coefficients and standard errors were: a=2.94, se=0.09; b=0.0047, se=0.0087; c=-0.81, se=0.28; d=1.73, se=0.12. The fit appeared to represent the data well at all temperatures ($F_{3,19}$=35.8; p<0.001) with the model explaining almost 85% of the variation in the transformed response.

The end of contraction was determined as the point of minimum sample length and zero change in length on review of the video tape. As was the case for the onset of contraction, temperature had little impact on time duration to end of rigor contraction in the 180 pulse partially exercised treatment (Fig. 3). Decreasing temperature prolonged contraction and delayed contraction end in the rested treatment. However, the sigmoidal relationship observed in the time to rigor contraction onset was not repeated.

The partial exercise treatment apparently took the muscle to the early stages of lactic acid accumulation, as indicated by the mean post-stimulation pH of 6.88 (Jerrett et al., 1996). Thus these data suggested that before the onset of glycolysis the metabolic rate was temperature-sensitive, but after onset of anaerobic glycolysis the factors governing rigor contraction were insensitive to temperature. We did not observe any acceleration of time to onset or end of contraction in either exercise treatment with decreasing temperature. This suggested that the onset and end of contraction were dictated by relative rates of ATP depletion rather than cold-elicited contraction. However, significant differences (p=0.05; t-test) in the final contracted length of the muscle strips were observed between strips incubated at 12°C and those at 0°C for both exercise treatments (Fig. 4). If we assume that the white muscle metabolic rate was either slowed by or insensitive to hypothermia, the reasons for this apparent increase in contraction "intensity" are not obvious.

In our previous study, using isometric rigorometry, rested chinook white muscle, acclimated to and stored PM at 15°C, produced a slow developing PM "relaxation stress" (perhaps a swelling) prior to onset of contraction (Jerrett and Holland, 1997). The relaxation stress, if allowed time to develop, was of similar magnitude to the rigor contraction stress, but decreased with increasing muscle fatigue and the more rapid onset of rigor contractions. This observation provided the basis for a simple model that would explain the commonly observed increases in apparent rigor intensity with muscle fatigue. If we consider that a relaxation stress produced a resistance to rigor contraction, then this model would explain the difference in final lengths of strips due to fatigue (Fig. 4), but would not entirely explain the temperature effects.

In the previous report we suggested that the relaxation stress could be attributable to tonicity changes in the muscle cells (Bonnet et al., 1992). Fish erythrocytes are known to swell as a response for increasing intra-erythrocytic pH during hypoxic stress (Soivio and Nikinmaa, 1981). This may be a generalized response of fish tissue to low intracellular pH. The relative circulatory isolation of fish "white" muscle make it likely that such mechanisms would exist. Soivio and Nikinmaa (1981) had reported that swelling of rainbow trout (*Salmo gairdnerii*) erythrocytes was temperature-dependent with the effect being eliminated at lower temperatures. If this temperature-dependent swelling also occurs in chinook white muscle then the reduction in the final contraction length with decreasing temperature (Fig. 4) could be explained by the elimination of swelling and, therefore, the resistance to rigor contraction. This mechanism could also explain the reduction in rigor or cold shock stiffness in the tropical species bighead (*Aristychthys nobilis*) produced by delaying PM icing (Parry et al., 1987). The parallels cannot be compared closely, however, as the temperature dependency of erythrocyte swelling was observed at different acclimated temperatures whereas we applied hypothermia to our treatments.

We have demonstrated that the time to rigor contraction onset and end in chinook salmon "white" muscle was extended or unaffected by PM hypothermia (down to 0°C). The response was primarily dependent on the peri-mortem fatigue state of the tissue. Since rigor contraction was associated with ATP depletion, our results indicated that hypothermia did not accelerate the metabolic rate of the PM muscle, nonetheless, hypothermia affected the extent of muscle contraction. The apparent "intensity" of the rigor process could, therefore, be affected by hypothermia without "cold shock" contractions (Curran et al., 1986) or hypothermic stress reactions (Ushio et al., 1991) being present.

CONCLUSIONS

THE CONTRAST IN RESPONSE OF OUR "PARTIALLY EXERCISED" AND "rested" treatments to PM temperature indicates the need for strict control over peri-mortem fatigue in commercial and experimental animals. Chilling chinook salmon to 0°C would not adversely affect the

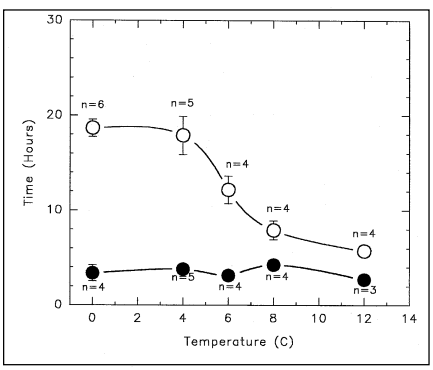

Fig. 2—Time to rigor contraction onset as related to temperature. "Rested" muscle (○),180 pulse "partially" exercised muscle (●) (means ± SEM: some error bars are obscured by symbol size in the exercised treatment). Curves fitted by cubic spline interpolation.

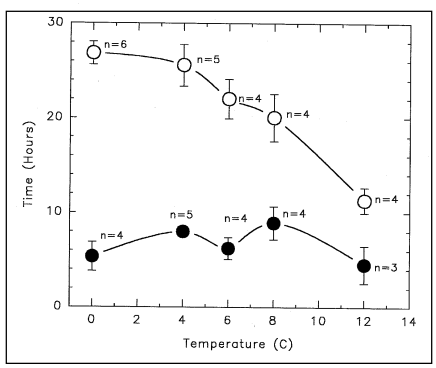

Fig. 3—Time to the end of rigor contraction as related to temperature. "Rested" muscle (○), 180 pulse "partially" exercised muscle (●) (means ± SEM). Curves fitted by cubic spline interpolation.

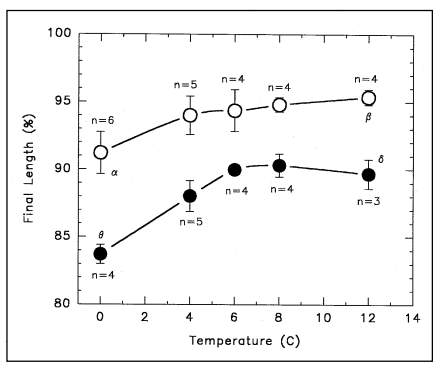

Fig. 4—Final length of the muscle strips after rigor contraction (percentage of the length measured 5 min after excision) as related to temperature. "Rested"muscle (○), 180 pulse "partially" exercised muscle (●)(means ± SEM). Curves fitted by cubic spline interpolation. Greek letters denote significantly different mean values (p=0.05).

substantial benefits associated with rested harvesting. The impact of subzero temperatures on rested muscle requires further investigation.

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