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Fillet colour correlates with biochemical status in Australasian snapper (*Pagrus auratus*) during storage in refrigerated seawater

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ABSTRACT

We demonstrate that two commonly used chilled storage temperatures (4.15 and 0.3 °C) differ in their effects on whole rested harvested Australasian snapper (*Pagrus auratus*) stored in refrigerated seawater (RSW) with respect to white muscle biochemistry and skin and fillet colour. White muscle pH decreased from 7.64 to ca. 6.4 over 48 h at both temperatures, but remained significantly ($P<0.05$) elevated at 4.15 °C compared to 0.3 °C until 24 h post-mortem. This corresponded with significantly elevated potential energy and concentrations of ATP, ADP and glycogen. Depletion of these metabolites occurred by 24 h at both temperatures and was mirrored by rises in lactate and inosine monophosphate (IMP). At 24 h snapper stored at 4.15 °C remained in partial rigor, whereas animals at 0.3 °C were in firm rigor. After 72 h significantly higher inosine and hypoxanthine concentrations were present in the 4.15 °C group, demonstrating temperature related mass action. Tissue biochemistry significantly correlated with changes in fillet colour, particularly redness and yellowness, and we propose that fillet colour can be used as a non-destructive indicator of muscle biochemistry. We link these data with muscle ultrastructure and translucency through histology and with use of a novel laser penetration method.

1. Introduction

Rested harvesting of fish is the reduction or elimination of exhaustion and stress during harvesting. This can be achieved by introducing a narcotising agent such as a low irritant anaesthetic into the water, allowing the fish to enter late stage narcosis without disturbance, and then euthanising them in a rapid manner, such as by brain ablation (Jerrett et al., 1996). Using this procedure, it has been clearly established that energy reserves are preserved relative to harvesting regimens that produce stressed and/or exhausted fish. This is evidenced by the preservation of cellular pH, ATP and creatine phosphate concentrations in skeletal muscle immediately post-mortem that are close to concentrations reported in vivo (Forgan et al., 2010; Tuckey et al., 2010). Relative to traditional methods, rested harvesting has also been shown to produce post-mortem muscle that has increased metabolic rate (Forgan et al., 2010), increased time to the onset of rigor (Jerrett and Holland, 1998), lighter colour, firmer texture and decreased drip loss (Bosworth et al., 2007; Kiessling et al., 2004). Increased IMP concentrations are also generated during the post-harvest metabolic rundown and maintained longer during storage, which means fillets from rested fish have lower K-values during storage (Digre et al., 2011a; Tuckey et al., 2010). More recently, rested harvesting has been shown to improve animal welfare when compared with nitrogen and carbon dioxide sedation used for harvesting Atlantic salmon (*Salmo salar*) (Erikson, 2011). This is significant as animal welfare is becoming increasingly scrutinised in fish production systems, not just for public perception, marketing and product acceptance, but also in terms of production efficiency, quality and quantity (Ashley, 2007).

Flake ice, ice slurry, and to a lesser extent refrigerated seawater (RSW) systems have been shown to be effective for fish storage as they provide consistent temperature, and in the case of slurry and RSW, a somewhat protective physical medium (Digre et al., 2011b; Piñeiro et al., 2004). It is generally recommended that fresh fish is stored near or marginally below 0 °C as this slows autolytic biochemical processes in the tissue and inhibits microbial spoilage to the greatest extent possible, resulting in maximised shelf life (Digre et al., 2011b; Sigholt et al., 1997). However, if the tissue freezes, cellular structure is disrupted through the formation of ice crystals, causing irreversible damage that results in accelerated autolysis (Zhu et al., 2003). Also, given that the excessive or rapid chilling of live fish results in stress and ultimately death through disruption of important physiological and biochemical processes (Donaldson et al., 2008), it is also likely that very low post-mortem storage temperatures may significantly affect post-harvest metabolism and accelerate the initial degradative processes.

As such, the primary aim of the current study was to document the post-harvest metabolic rundown and fillet properties of whole rested harvested Australasian snapper (*Pagrus auratus*) during storage for 5 d in RSW at two commonly used chilled storage temperatures (i.e. 0.3

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and 4.15 °C). Previous work has identified a critical temperature transition for Australasian snapper tissues around 6 °C; however a lower limit of 2 °C was used (Jerrett et al., 2002). These temperatures, as well as being common chilled food storage temperatures, allow us to extend these observations close to 0 °C and also to examine the possibility of subtle differences in post-harvest tissue metabolism below this critical temperature transition. We also wished to determine if imaging technologies could be used to non-invasively monitor the post-harvest properties of the fillets, particularly the first 24 h post-mortem. To do this, we demonstrate the effects of these storage regimens on the colour profiles of the skin and fillets, and muscle translucency through the use of a novel laser penetration method and correlate these data with fillet biochemistry.

2. Materials and methods

2.1. Husbandry, harvesting and storage procedures

Australasian snapper (*P. auratus* Bloch and Schneider 1801, Perciformes, Sparidae) were hatchery-raised on site at Plant & Food Research (Nelson, New Zealand). They were housed in 4500 L circular tanks with aerated flow-through filtered seawater (55 L min^{-1}) and fed daily with a mixture of commercial fish pellets, mussels, alginate, squid and fish. Food was withheld for 24 h prior to harvest. They were acclimated to ambient seawater from Nelson Haven, which was 18.2 °C at the time of harvest. Harvesting took place in November 2010, which was late spring in New Zealand.

In total 102 fish were used in this study (mixed sex, weight 596.5 ± 10.2 g (±SEM); length 303 ± 1.9 mm, condition factor 2.2 ± 0.04, gonadosomatic index 2.9 ± 0.15, hepatosomatic index 1.1 ± 0.03). A rested harvest was conducted by introducing 20 ppm AQUI-S™ (active ingredient isoeugenol at 540 g L^{-1}; AQUI-S™ New Zealand Ltd, Lower Hutt, New Zealand) into the holding tank (18.2 °C) and rapidly mixed by vigorous aeration from a concentrated stock solution as per the manufacturer's instructions. Total exposure time was 30 min during which the dissolved oxygen did not fall below 84% air-saturation (Oxyguard Handy Polaris, Oxyguard International AS, Birkeroed, Denmark). Fish were regularly staged to determine the level of anaesthesia. All fish used for this study were adjudged to be in stage 3 anaesthesia (i.e. total loss of reactivity to handling and showing reduced ventilation) (Stoskopf and Posner, 2008). Following anaesthesia all fish were euthanised by pithing the brain with an *iki jime* tool. Six fish were sampled immediately with sampling procedures taking 50 min in total, and the remaining fish were randomly allocated to one of two treatment groups (4.15 °C or 0.3 °C, n = 48 per group). For the 4.15 °C treatment, fish were transferred in equal numbers to six plastic bins containing a total volume of approximately 200 L (i.e. 33 L each) UV sterilised and filtered refrigerated seawater (RSW) at 4.15 ± 0.54 °C. The plastic bins were stacked inside commercial freezer units (ELBA, Fisher and Paykel, Auckland, New Zealand) fitted with thermostats (ir33, Carell, Padova, Italy) and continuously aerated with air stones connected to a reticulated compressed air supply. For the 0.3 °C treatment, fish were placed in a 300 L insulated bin containing approximately 200 L filtered continuously aerated seawater maintained at 0.3 ± 0.54 °C with ice filled plastic bags. Temperatures in the RSW were continuously logged throughout the storage period using Hobo® pendant temperature loggers (Onset Computer Corporation, MA, USA).

These procedures conformed to all local laws and did not involve any manipulations as defined under the New Zealand Animal Welfare Act (1999).

2.2. Sampling procedures

Time measurements were calculated as the difference between sampling time and the time at which the anaesthetic was introduced into the tank. Six fish from each temperature treatment were removed for sampling following approximately 3, 6, 12, 24, 48, 72, 96 and 120 h storage. Fish were removed from the RSW for sampling individually and the time of removal recorded. Length, weight and temperature measurements from the body cavity and fillets were measured using 4600 Precision thermometers (YSI Inc., Ohio, USA) fitted with model 406 and 551 temperature probes respectively (Measurement Specialties, VA, USA). Rigor stage was assessed at this point as being pre-rigor, partial-rigor, firm-rigor and post-rigor, in a simplified version of the assessment presented in Sigholt et al. (1997). Digital external and skin-on fillet images were then taken using a custom-built imaging apparatus made up of the following components: a near white melamine background, a SPOT Flex digital camera (Diagnostic Instruments, Inc., MI, USA) fitted with a Sigma 20 mm F1.8 EX DG lens (Sigma Corporation of America, NY, USA) and four KFB RB 5004 HF lamps (Kaiser, Germany) fitted with Osram Dulux L fluorescent light tubes (Osram Ag, Munich, Germany). Sixteen megapixel digital images were captured using Image Pro version 7 software (Media Cybernetics, Inc., MD, USA) and colour calibration was carried out daily in the CIE L*a*b* colour space using a ColorChecker DC testchart and ProfileMaker Pro version 4.1.5 (X-Rite, MI, USA). First, external images were taken of the left, right and dorsal sides of the animal. Following this, skin-on fillets were removed and the flesh side imaged.

A second image of the fillets was then taken. In this image a 655 nm 10 mW laser (South Island Component Centre Limited, Christchurch, New Zealand) beam was aimed horizontally into the anterior surface of the D1 muscle block (the first block of muscle dorsal to the lateral line) and adjusted using a vernier mount to strike the tissue ca. 8 mm below the cut surface. A fresh slice from the left fillet was then removed and the cut surface pH measured from the D1 muscle block with a CyberScan pH300 handheld pH meter (Eutech Instruments Pte Ltd, Singapore) fitted with a Sensorex 450CD pH probe (Sensorex, CA, USA). A further fresh tissue sample from the left fillet was cut, also from the D1 tissue block, freeze clamped, wrapped in aluminium foil and stored at −80 °C for biochemical analysis. Following this, an approximately 40 mm square section, with a depth of 20 mm, was cut from the anterior end of the skin-on left fillet and fixed in isotonic 10% phosphate buffered formalin (50 mM, 154 mM NaCl, pH 7.5) (Sigma-Aldrich, Castle Hill, Australia) and stored for histology. Histology samples were collected at 24 h intervals. The gonads and liver were removed from the gut cavity and weighed. Combined with the length and weight data, these measurements were used to calculate condition factor (CF), gonadosomatic index (GSI) and hepatosomatic index (HSI) for each fish using the following formulae: $CF = L/BW^3 \times 100$; $GSI = G/BW \times 100$; $HSI = H/BW \times 100$, where G = total gonad weight (g), H = total liver weight (g), BW = total body weight (g), L = fork length (cm) (Love, 1980).

2.3. Sectioning and microscopy

Fixed skin/muscle samples were embedded in paraffin wax, sectioned at 2 μm and stained using haematoxylin and eosin (H&E). All sectioning and staining were carried out by Gribbles Veterinary Pathology (Christchurch, New Zealand). Sections were viewed using an Olympus BX50 microscope (Olympus Corporation, Tokyo, Japan) and images captured with a digital SPOT Flex camera and Image Pro 7 software.

2.4. Image analysis

Colour profiles were measured as the mean CIEL*a*b* (where L* represents lightness, a* represents redness and b* represents yellowness) values from the entire area of the left exterior and flesh surface of the left fillet excluding the belly region from the captured digital images using Image Pro 7 software. The left side was chosen as it was determined that no statistically significant differences existed between the left and right sides of the fish. Data from the laser penetration were gathered by isolating the laser spot using Image Pro 7 software. This was achieved