1 Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (*Seriola lalandi*
2 Valenciennes 1883): selenium and temperature interaction
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Abstract

The aim of this study was to investigate the interactive effects of temperature and dietary selenium concentrations on antioxidant capacity, muscle histochemistry and the growth, of juvenile yellowtail kingfish (*Seriola lalandi*). The yellowtail kingfish were exposed to two temperatures (21°C or 26°C) and three selenium levels (0.0, 2.0 or 4.0 mg Se kg⁻¹ of feed) for 30 days. Final weight and specific growth rate (SGR) were significantly affected by water temperature ($p < 0.001$) and dietary Se ($p < 0.001$) supplementation, and there were significant differences in the interaction between these two factors. Juvenile yellowtail kingfish fed Se-supplemented diets, attained a higher final weight and SGR than those without Se supplementation at 21°C, but not at 26°C. Regardless of the temperature, the red blood cell (RBC) glutathione peroxidase (GPx) activity of yellowtail kingfish fed Se-supplemented diets was significantly higher ($p < 0.05$) than with the control diet. However, GPx activity of yellowtail kingfish when fed either 2.0 mg Se kg⁻¹ or 4.0 mg Se kg⁻¹ showed no significant difference ($p > 0.05$). Se concentration in the muscles of juvenile yellowtail kingfish fed Se-supplemented diets was higher than that of the yellowtail kingfish that were fed the control diet. A histopathological test confirmed that 20.3% of fish muscles exhibited lesions, which occurred in the absence of dietary Se. The outcome of the present study helps in understanding the interactive effects of dietary Se concentrations and the temperature in the farming of yellowtail kingfish.

Keywords: selenium, temperature, growth, antioxidant capacity, muscle histochemistry, yellowtail kingfish

Abbreviations: RBC, red blood cell; GPx, glutathione peroxidase; SGR, specific growth rate; DNA, deoxyribonucleic acid; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT,

catalase; GR, glutathione reductase; ACAAR, Australian Centre for Applied Aquaculture Research; DO, dissolved oxygen, RFI, relative feed intake; BW, body weight; NADPH, nicotinamide adenine dinucleotide phosphate; AES, atomic emission spectrometry; SE, standard error; ANOVA, analysis of variance; SPSS, Statistical Package for Social Sciences; mOsm, milliosmole; RMR, routine metabolic rate; US-EPA, United States Environmental Protection Agency; Se–Se–Se, triselenium linkage; S–Se–S, selenotrisulfide linkage; S–S, disulfide).

1. Introduction

Disease continues to be the major constraint in the development of sustainable aquaculture. Elevated stress levels are the root of most diseases in farmed fish (Turnbull, 2012; Wedemeyer, 1997). A number of studies have shown that increased disease susceptibility and reduced immune response were attributable to acute or chronic stress responses (Barton and Iwama, 1991; Dang et al., 2012; Davis et al., 2002; Dietrich et al., 2014; Fridell et al., 2007; Iguchi et al., 2003; Li et al., 2014; Nakano et al., 2014; Small and Bilodeau, 2005; Tacchi et al., 2015; Varsamos et al., 2006). Thus, in most cases diseases infect farmed fish following exposure to stress condition.

It is well recognised that fish previously exposed to a stressor may show oxidative stress response (Lushchak and Bagnyukova, 2006), which is a reflection of an imbalance between the levels of prooxidant and antioxidant properties (Sies, 1985). Prooxidant substances include those relating to reactive oxygen species (ROS), chemically reactive molecules containing oxygen, which are responsible for lipids, proteins and deoxyribonucleic acid (DNA) impairment, particularly when fish lack the capacity to deal with accumulated ROS production (Vinagre et al., 2012). Antioxidant properties are classically defined as any substance that provides

93 protection against oxidative damage (Pamplona and Costantini, 2011). The equilibrium between
94 ROS production and antioxidant stores that the fish maintain, therefore, regulate the extent to
95 which oxidative damage can occur in fish. Enzymatic antioxidants such as superoxide dismutase
96 (SOD), catalase (CAT) and glutathione peroxidase (GPx) establish a fundamental aspect of the
97 antioxidant response (Winston, 1991).

98 Selenium (Se), an essential micro-mineral required for maintaining the growth and metabolic
99 function of fish, serves as an integral structural element of the active core of GPx enzymes in red
100 blood cells (RBCs) (Rotruck et al., 1973). Although Se is needed only in trace amounts, Se
101 engrosses a distinctive attention among micro-minerals as the constraint in the antioxidant
102 enzyme biosynthesis. Se-containing GPx plays an important role in protecting cells and
103 membranes from oxidative stress by catalysing the reduction of hydrogen peroxide and lipid
104 peroxides using reduced glutathione (GR) (Watanabe et al., 1997). The activity of this enzyme is
105 modulated by sufficient Se intake (Dhur et al., 1990) and it is well known that, although fish can
106 accumulate Se via both surrounding water and food, dietary exposure to Se compounds
107 comprises the predominant source of Se for fish (Janz, 2011). Dietary supplementation of Se has
108 been reported to enhance the antioxidant enzyme capacity precursor in crayfish *Procambarus*
109 *clarkii* (Dörr et al., 2008), freshwater prawn *Macrobrachium rosenbergii* (Chiu et al., 2010),
110 whiteleg shrimp *Litopenaeus vannamei* (Parrilla-Taylor and Zenteno-Savín, 2011; Parrilla-
111 Taylor et al., 2013), common carp *Cyprinus carpio* (Elia et al., 2011), crucian carp *Carassius*
112 *auratus gibelio* (Zhou et al., 2009), rainbow trout *Oncorhynchus mykiss* (Kucukbay et al., 2009),
113 and cod *Gadus morhua* (Penglase et al., 2010). An additional benefit of using Se as a feed
114 supplement is that elevated dietary levels improve growth and feed utilisation in a variety of
115 aquatic species, including African catfish, *Clarias gariepinus* (Abdel-Tawwab et al., 2007), gibel

carp *Carassius auratus gibelio* (Han et al., 2011), and abalone, *Haliotis discus hannai* Ino (Wang et al., 2012). In most fish, Se deficiency leads to poor growth performance, lipid peroxidation and decreased GPx enzymatic activity (Bell et al., 1986; Hilton et al., 1980; Wang et al., 2007).

Se exists in two forms, namely inorganic Se (selenate and selenite) and organic Se (selenomethionine and selenocysteine) (Sunde, 2006). The amount of dietary Se demanded to enhance growth and antioxidant capacity is dependent on Se sources. For instance, using sodium selenite, Hilton et al. (1980) recommended that a concentration of $0.38 \text{ mg Se kg}^{-1}$ is suitable for rainbow trout *O. mykiss*. With the similar form of Se, Gatlin and Wilson (1984) demonstrated that the optimum dietary supplementation of Se for channel catfish *Ictalurus punctatus* is $0.25 \text{ mg Se kg}^{-1}$. However, in an investigation employing selenomethionine, the juvenile grouper *Epinephelus malabaricus* required $0.77 \text{ mg Se kg}^{-1}$ for their best growth performance (Lin and Shiau, 2005). Han et al. (2011) found that the dietary selenomethionine requirement for gibel carp *C. auratus gibelio* is $1.18 \text{ mg Se kg}^{-1}$. Another experiment on African catfish *C. gariepinus*, conducted by Abdel-Tawwab et al. (2007), showed that a selenomethionine concentration of $3.67 \text{ mg Se kg}^{-1}$ was proposed as the optimum level needed to improve fish growth and vitality against environmentally-induced stress.

Se nutrition of yellowtail kingfish (*Seriola lalandi*), an emerging species in Australia and New Zealand aquaculture, has been recently studied. Le and Fotadar (2014a) reported that selenomethionine and seleno-yeast were the most bioavailable sources of Se to yellowtail kingfish. Se from fishmeal-based diets was inadequate to maintain the growth performances of the species and therefore supplementation with organic Se was recommended. In addition, dietary Se significantly improved yellowtail kingfish survival, antibodies, and haematocrit, following exposure to bacterial challenge, and the role of Se as an antioxidant was established by

activities such as resistance of RBCs to peroxidation and GPx (Le and Fotedar, 2014b). However, very limited data and information is available on the effects of temperature on micro-minerals such as Se utilisation, which may in turn, influence the growth and health of the cultured species. In commercial aquaculture, yellowtail kingfish are exposed to fluctuating water temperatures, which can affect the level of basal metabolism (De Silva, 1995), and thus induce stress (Bowden et al., 2007; Nakano et al., 2014). The species are presently cultured in sea cages at temperatures ranging from 12°C to 28°C in Australia (Tanner and Fernandes, 2010) and 14°C to 22°C in New Zealand (Moran et al., 2009). Therefore, this study was designed to investigate the effect of organic Se supplementation on growth as well as health performance of juvenile yellowtail kingfish at two temperatures that represent ambient and elevated water temperatures. Adequate knowledge of mineral utilisation at relevant environmental temperatures is important for optimising dietary composition and feeding conditions (Kim et al., 2006), thus, improving fish performance under culture situations, particularly during the grow-out phase.

2. Materials and methods

2.1. Experimental fish and diets

Healthy yellowtail kingfish juveniles (66.85 g) were provided by the Australian Centre for Applied Aquaculture Research (ACAAR) Laboratory, at the Challenger Institute of Technology (Fremantle, Western Australia), where this study was carried out. Yellowtail kingfish juveniles were acclimated to laboratory conditions and fed the control diet twice daily at 4.75% body weight for one week prior to the commencement of experiment.

A basal mash of a commercially available yellowtail kingfish diet without any supplementation of Se was used to prepare the experimental diets. Three isonitrogenous and

isocalorific experimental diets were prepared and differed only by the supplementation level of organic Se (Sel-Plex[®], Alltech Inc., Lexington, Kentucky, USA) at 0.0 (control), 2.0, or 4.0 mg Se kg⁻¹ diet. As fishmeal-based diets contained around 3.40 mg Se kg⁻¹, the actual concentrations of Se were 3.35 mg Se kg⁻¹, 5.46 mg Se kg⁻¹ and 7.38 mg Se kg⁻¹ for three experimental diets. The experimental diets, with an approximate chemical composition of 46.42% of protein, 15.05% of lipids, 9.56% of ash, 91.58% of dry matter and 40 mg kg⁻¹ of vitamin E, were prepared at the Australasian Experimental Stockfeed Extrusion Centre (Roseworthy, Adelaide, Australia) as cooked, extruded, slow-sinking 3 mm pellets.

Fish handling procedures, care, and facilities complied with the guidelines of the Animal Ethics Committee of Curtin University and followed the Australian Code of Practice for the care and use of animals for scientific purposes.

2.2. Experimental design

Two similar flow-through systems were designed to raise fish at 21°C and 26°C water temperatures. Both systems were located indoors and were set up as two identical flow-through systems with a total volume per system of 1,800 L. Each system was equipped with nine cylindrical tanks (200 L each), a submersible water pump, and aeration. An air diffuser, central drain and sponge filter were installed in each tank to allow for oxygenation, waste removal and biological filtration, respectively. The water flow rate in each system was adjusted to 4.8 L min⁻¹, to maintain dissolved oxygen above 80% saturation. A 1,000-W titanium heater and digital controller (WEIPRO MX-1019) were set to maintain a stable water temperature. The water temperatures measured in the experimental tanks generally followed the two temperature regimes targeted in the study design as values from daily manual measurements in each tank were in agreement with records from the temperature digital controller.

Throughout the experiment, the water quality parameters of the rearing water were monitored daily. Dissolved oxygen was monitored by using a DO meter (CyberScan DO 300, Eutech Instruments, Singapore). Ammonia and pH were measured every two days using chemical test kits (Aquarium Pharmaceuticals Ltd, UK) and a pH meter (CyberScan pH 300, Eutech Instruments, Singapore). Water salinity was maintained every day between 34-35 ppt, and measured by a portable refractometer (RHS-10ATC).

Following acclimation, animals were transferred and reared in 21°C and 26°C treatment tanks, respectively, where yellowtail kingfish were stocked at ten fish per tank. Upon commencement of the experiment, the animals were physically inspected, and the initial weights of animals from each dietary treatment were recorded. Fish were fed to apparent satiation twice daily, at approximately 09:00 and 14:00 hrs. The feeding trial lasted for 30 days.

2.3. Sampling and analytical methods

The proximate composition, including crude protein, crude ash, gross energy and moisture content of basal diets, was determined following the protocol established by the Association of Official Analytical Chemists (1990). Crude protein was analysed by using the Kjeldahl method, with a Kjeltec Auto 1030 analyser; lipids, by extraction with petroleum ether using a Soxtec System; moisture, by drying at 105°C in an oven (Thermotec 2000, Contherm Scientific, Hutt, New Zealand) to a constant weight; and ash, by combustion at 550°C for 24 hours in an electric furnace (Carbolite, Sheffield, UK).

Throughout the trial, the growth performance indicators that were measured were the specific growth rate (SGR, % day⁻¹), relative feed intake (RFI, g day⁻¹), and survival (%), according to the following formulae:

$$\text{SGR} = (\ln W_2 - \ln W_1) t^{-1} * 100$$

where W_1 and W_2 are the initial and final weights, respectively; t is the number of days in the feeding trial (Sveier and Lied, 1998).

$$RFI=100*(\text{dry feed intake})*\left[\frac{BW_1+BW_2}{2}\right]^{-1}*(\text{days fed})^{-1}$$

where BW_1 and BW_2 are the initial and final body weights, respectively (Grisdale-Helland et al., 2008).

During the feeding course, the dead fish of the different treatments were recorded, to determine the final survival rate of the juvenile yellowtail kingfish. At the end of the feeding trial, following a 24-hour fast, six fish from each tank were selected and anaesthetised with tricaine methane sulphonate (MS222) (50 mg L^{-1}) for final weighing and blood collection.

2.4. Haematology and osmolality

Fish blood samples from three fish tank⁻¹ were drawn by caudal vein puncture, with a 1-mL plastic syringe. The extracted blood was divided into two sets of tubes. The first set contained K_2EDTA (BD Vacutainer® Plus Plastic), used as an anticoagulant, for haematology while the second set (Eppendorf tubes) was left without an anticoagulant, for osmolality. Hb kit (Randox Laboratories, Antrim, United Kingdom) was used to measure the haemoglobin (Hb). For separation of serum, blood samples were immediately transferred to a 1 mL vial and allowed to clot at 4°C , in a refrigerator. The blood was centrifuged for 30 minutes at 5,000 rpm (Eppendorf 5430 R, Eppendorf Ltd., Hamburg, Germany). The serum was finally transferred to a 0.5 mL vial for osmolality analysis in a cryoscopic osmometer–Osmomat 030-D (Genotec).

2.5. Histopathology assay

After blood sampling, left anterior dorsal muscle from three euthanised fish tank⁻¹ were quickly dissected and prepared for histological examination. Light microscopy samples were prepared using standard histological techniques (Luna, 1968). Tissue samples were fixed in 10%

buffered formalin, dehydrated in ethanol, before equilibration in xylene and embedment in paraffin wax. Sections of approximately 5 μm were cut and stained with haematoxylin and eosin for histological examination, under an Olympus BX40F4 light microscope.

2.6. Antioxidant glutathione peroxidase (GPx) assay

Erythrocytes' (red blood cells) GPx activities were quantitatively assayed by using the Randox Laboratories test combination (Ransel, Antrim, United Kingdom). According to the method of Paglia and Valentine (1967), GPx catalyses the oxidation of glutathione using cumene hydroperoxide in the presence of glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The oxidised glutathione (GSSG) was immediately converted to the reduced form, with a concomitant oxidation of NADPH to nicotinamide adenine dinucleotide phosphate (NADP⁺). The decrease in absorbance at 34 nm was measured at 37°C.

2.7. Selenium analysis

The Se concentration of muscle tissue and experimental diets were determined by the Marine and Freshwater Research Laboratory, Environmental Science, Murdoch University (Western Australia) based on the method of inductively coupled plasma atomic emission spectrometry (Agilent 720 ICP-AES). The detection limit for Se through this method is approximately 0.02 to 50 ppm. All Se levels for diet and tissue samples are recorded as dry weights.

2.8. Statistical analysis

All data were expressed as mean $\pm$ standard error (SE). The effects of dietary organic Se supplementation (3 levels: 0.0, 2.0 or 4.0 mg Se kg⁻¹ diet) and rearing temperature (2 levels: 21°C or 26°C) on growth performance, feed utilisation, survival, osmolality, plasma GPx, and Se concentration were analysed with the two-way ANOVA. If a significant interaction was detected between the main variables, then the variable was tested using a one-way ANOVA. The Duncan

method was applied to determine significant differences among treatment groups, and probability values $p < 0.05$ indicate a significant difference. All statistical analyses were performed using the IBM SPSS Statistics 22 (Australia).

3. Results

Final weight, specific growth rate (SGR), relative feed intake (RFI), survival and osmolality of juvenile yellowtail kingfish reared at two temperatures (21°C and 26°C) and fed the diets containing different levels of organic Se are presented in Table 1. Final weight and SGR were significantly affected by dietary Se level ($p < 0.001$) and water temperature ($p < 0.001$), and an interaction effect was present ($p < 0.001$). The significant interaction was reflected by a greater increase in final weight and SGR, in fish supplemented with 2.0 mg Se kg⁻¹ at 21°C than at 26°C ($p < 0.001$). There was no significant interaction between temperature and diet, with regards to RFI, and it was significantly affected by Se levels in the diet ($p = 0.024$). Fish that were fed diets supplemented with 2.0 and 4.0 mg Se kg⁻¹, attained lower RFI than those fed the control diet. The RFI of fish reared at 21°C and 26°C was similar ($p > 0.05$). The survival of the juvenile yellowtail kingfish ranging from 90% to 97% was not significantly different among any treatment groups over the course of the feeding experiment. No significant interaction was found between temperature and diet ($p > 0.05$) on fish osmolality. However, osmolality was significantly influenced by temperature, as higher osmolality (311 mOsm kg⁻¹) was observed in fish reared at 26°C ($p < 0.001$).

The erythrocyte GPx of juvenile yellowtail kingfish at 21°C was significantly different between Se unsupplemented and supplemented dietary treatments ($p < 0.05$), which were 83.73±2.87 U g⁻¹ Hb (control diet), 97.27±2.06 U g⁻¹ Hb (2.0 mg Se kg⁻¹), and 100.63±3.27 U

g^{-1} Hb ($4.0 \text{ mg Se kg}^{-1}$) (Fig. 1). A similar trend was observed in the erythrocyte GPx of fish reared at 26°C . However, the GPx activity of fish that were fed Se-supplemented diets was highest at 26°C ($p < 0.05$), ranging from 106.33 ± 1.87 to $109.60 \pm 3.16 \text{ U g}^{-1}$ Hb.

After one month of the feeding trial, muscle Se concentrations of fish that were fed different levels of Se supplementation at 21°C were significantly different. At 21°C , muscle Se content in fish generally increased, as the dietary Se supplementation increased. Muscle Se concentrations of fish that were fed the control, $2.0 \text{ mg Se kg}^{-1}$ and $4.0 \text{ mg Se kg}^{-1}$ supplementation diets were $1.47 \pm 0.10 \text{ mg kg}^{-1}$, $2.33 \pm 0.10 \text{ mg kg}^{-1}$ and $2.86 \pm 0.03 \text{ mg kg}^{-1}$ dry weight, respectively ($p < 0.001$). In contrast, when fish were reared at 26°C , the muscle Se concentration of those fed a diet supplemented with a $4.0 \text{ mg Se kg}^{-1}$ diet was significantly higher ($p < 0.05$), while there was no difference between the control and $2.0 \text{ mg Se kg}^{-1}$ dietary treatments (Fig. 2).

The histological analysis of the yellowtail kingfish showed necrotic fibres in the muscles of juvenile yellowtail kingfish fed with the control diet (Fig. 3). A histopathological test confirmed that 20.3% of fish exhibiting lesions in skeletal muscles occurred in the absence of Se supplementation in the fish diet, whereas such cases were not observed in fish fed diets supplemented with Se. However, the histological alteration in the muscles of yellowtail kingfish that were fed the control diet, from both ambient and elevated water temperatures, was similar. Additionally, an insignificant amount of fish (6 from 26°C) exhibited eye problems, which were detected during the final sampling.

4. Discussion

Both water temperature and Se level interactively modified the growth performance (final weight and SGR) of yellowtail kingfish, with the overall final weight and SGR were highest at

21°C (128.7 g and 2.2% body weight day⁻¹). The interaction between temperature and Se exposure observed in this study, suggested that elevated temperature was a strong inducer of low final weight and reduced growth in yellowtail kingfish. It appeared that, for Se, elevated temperature exacerbated toxicity. As water temperature increased, the fish fed diets supplemented with 2.0 mg kg⁻¹ decreased their final weight and SGR by about 30% and 54%, respectively. The imbalance between respiratory demands and respiratory capacity might have caused a physiological stress that resulted in lower final weight and SGR of fish in the elevated temperature. Moreover, at 26°C, the energetic demands of increased oxygen consumption and reduced respiratory capacity were not counteracted by an increase in feeding activity, indicating that Se could have placed further metabolic stress on these fish. However, of the limited information available on the synergistic effects of Se and temperature on aquatic animal growth and health indicators, poor reproduction has been detected when Se-deficient planktonic crustacean *Daphnia magna* were kept at 25°C, whilst such an effect was not detected at 20°C (Winner and Whitford, 1987). The so-called “winter stress syndrome” of Lemly (Lemly, 1993; Lemly, 1996), signalled by increased mortality, reduced activity and feeding as well as the reduced condition factor and decreased energy stores of bluegill sunfish *Lepomis macrochirus*, is the upshot of winter temperatures combined with elevated Se exposure. In addition, there is also a synergism between Se and raised temperatures causing mortalities and abnormal morphologies in white sturgeon *Acipenser transmontanus* and green sturgeon *A. medirostris* (Silvestre et al., 2010).

It appears from this study that, irrespective of the diet, rearing yellowtail kingfish at the elevated temperature of 26°C significantly resulted in reduced growth performance. The present finding contrasts with that of Abbink et al. (2012) which found that increasing the water

temperature from 21°C to 26.5°C resulted in a 54% increase in the final weight, after one month's exposure. Aside from the differences in culture conditions, this contradiction could be attributed to fish size. The larger initial weight (67 g) of yellowtail kingfish in our study than used by Abbink et al. (2012) could result in an ontogenetic shift in optimum temperature for growth as shown in majority of ectothermic animals (Angilletta and Dunham, 2003) such as cod *Gadus morhua* L. (Björnsson et al., 2001) and turbot *Scophthalmus maximus* L. (Imsland et al., 1996). It has been shown that the temperature required for optimum growth declines by 1–2°C when the larger fish (10 to 250 g) were used (Cuenco et al., 1985). Similar to our study, Pirozzi and Booth (2009) examined the influence of temperature exposure ranging from 10 to 35°C on the oxygen consumption or routine metabolic rate (RMR) of naturally occurring yellowtail kingfish and found that the thermo-sensitivity reaction of RMR appears as an indicator of the temperature profile and suggesting the preferred temperature ranges from 20 to 25°C. Martin and Huey (2008), through their “optimality model”, predicted that animals, if given a choice, will select temperatures that are somewhat lower than the temperature at which fitness is optimum. Therefore, the growth retardation at 26°C, as observed in our study, can be due to the increased metabolic rate at an elevated temperature. However, the increased demand for energy by yellowtail kingfish was not correspondingly denoted by an increase in relative feed intake (RFI), a circumstance that is normally observed when fish are exposed to temperature stress (Handeland et al., 2008).

In fish, thermal stress is accompanied by oxidative stress (Lushchak, 2011). Enzymatic antioxidant GPx plays a vital role in maintaining cellular defence against extreme ROS production and lipid peroxidation (Kohen and Nyska, 2002; Pedrero and Madrid, 2009). GPx metabolises hydroperoxides and is thus intimately engaged in cellular defence against oxidative

damage (Arthur et al., 2003). Therefore, Se is an essential micro-nutrient of major metabolic importance (Brown and Arthur, 2001; Hamilton, 2004). The results of the present study show a significant increase in the RBC GPx activity in elevated water temperatures, at least at 26°C and the experimental conditions employed in the present study. This indication reinforces the proposed hypothesis that the dietary requirement of Se is relatively higher in fish, in response to oxidative stress induced by an increased temperature level. Although there was no statistical difference in erythrocyte GPx activity between the Se supplementation levels of 2.0 and 4.0 mg kg⁻¹ diet at both 21°C and 26°C temperature regimes, enzymatic GPx activity levels were significantly higher in the erythrocytes of fish with Se supplementation than in those without Se supplementation. This would suggest that the dietary Se supplementation of 2.0 mg kg⁻¹ in the commercial fishmeal-based diets is needed to maintain maximum RBC GPx activity. Again, apparent enzymatic GPx activity levels, as observed in this study, confirm the earlier suggestion with similar species that Se supplementation has been reported to promote the enhanced physiological response of GPx activity during stress periods (Le and Fotedar, 2014b). The significance of Se to the antioxidant capacity in other species has also been documented (Chiu et al., 2010; Lin and Shiau, 2005; Liu et al., 2010; Rider et al., 2009; Wang et al., 2006; Zhou et al., 2009). Furthermore, Se-containing compound, selenoneine, was found to be the major form of organic Se in the muscle and other tissues of tuna, mackerel and tilapia (Yamashita et al., 2010). Se may exert an antioxidant effect by binding to oxygen-binding proteins such as hemoglobin and myoglobin, protecting them from autooxidation (Yamashita and Yamashita, 2015). The implication of Se for the growth performance of yellowtail kingfish might be associated with the antioxidant role of Se.

Se plays a vital role in amino acid metabolism and is also incorporated into some proteins. Organically bound Se, such as selenomethionine and selenocysteine, is efficiently absorbed throughout the gastrointestinal tract (Gropper et al., 2009). Although selenocysteine is a more available source of Se for selenoprotein synthesis, only selenomethionine is incorporated into body proteins (Schrauzer, 2000). Therefore, Se accumulation should occur in protein-rich tissues such as skeletal muscle (Dainty and Fox, 2005). In fish, it has been proved that muscle tissue is one of the primary sites of Se storage (Burk and Hill, 1993). In the present study, supplementing yellowtail kingfish diets with organic Se from a commercial product, in the form of selenomethionine (Sel-Plex[®]), led to a considerable increase in the concentration of Se in muscles. The overall increased accumulation of muscle Se in yellowtail kingfish that were fed Se-enriched diets, regardless of water temperature, was likely to have been caused by the non-specific incorporation of selenomethionine into proteins. Tissue accumulation of Se may be understood, with respect to bioavailability, as a sign of the superiority of organic sources over inorganic sources (Ornsrud and Lorentzen, 2002). In fact, at 21°C, there was a linear relationship between organic Se supplementation levels and Se accumulation in muscles. This finding parallels those reported for other fish. For example, a linear response was observed in channel catfish *I. punctatus* with respect to the dose in Se accumulation in muscles following 15 weeks of an organic Se (ranging from 0 to 15 mg Se kg⁻¹) feeding trial (Gatlin and Wilson, 1984). Similarly, during eight weeks of exposure, accumulation in the muscle Se of Atlantic salmon *Salmo salar* parr was directly related to dietary Se, when organic Se was included in the diet (Lorentzen et al., 1994). In contrast, at 26°C, Se concentration in the muscles of yellowtail kingfish supplemented with a 2.0 mg Se kg⁻¹ diet, was not significantly different from that supplemented with a 4.0 mg Se kg⁻¹ diet, and these concentrations were lower, compared to

those at 21°C. The reduction in muscle Se concentrations, as noticed in the present study, might be ascribed to the increased utilisation of Se against extreme thermal induced ROS production.

Elevated temperatures may also trigger osmotic imbalance, as shown in the present study. Serum osmolality was greater in fish kept at 26°C ($>305 \text{ mOsm kg}^{-1}$) than in those kept at 21°C. Although the osmotic pressure of the aqueous humour (watery fluid similar to plasma located in the anterior and posterior chambers of the fish eye) was not measured in the present study, there was a linearity between serum osmolality and aqueous humour osmolality, being indicative of cataract formation in salmonids (Iwata et al., 1987). Akiyama et al. (1986) studied the effects of temperature on the incidence of scoliosis and cataracts in chum salmon *Oncorhynchus keta* fry, and found that cataracts are apt to occur when metabolism is high, due to increased water temperatures. Similarly, Atlantic salmon *S. salar* parr kept at a constant higher temperature, developed more cataracts than parr raised at a constant, low water temperature (Bjerkås et al., 2001). Despite the insignificant number of fish that were found to exhibit a somewhat cloudy eye during the final sampling, it is not clear whether the signs of eye impairment are a direct or secondary effect of osmotic imbalance. Hence, further study should investigate whether acute or chronic Se exposure plays a part in the formation of the so-called “cloudy eye syndrome” or cataracts, one of the common disorders of the eye in finfish (1991).

In aquatic wildlife, Se is a widespread and naturally occurring element (US-EPA, 2004). Bioaccumulation of Se through food, leading aquatic animals to high levels of Se exposure, has been a significant concern. Thus, at levels exceeding those required, Se can induce harmful effects, which may be described principally by the failure of the protein metabolism to distinguish sulfur (S) amino acids and their seleno analogues. Because it is most similar to S through its chemical properties, Se is faultily substituted for S, when generated in excessive

amounts (Meyer et al., 1992). This may result in the formation of triselenium linkage (Se–Se–Se) or selenotrisulfide linkage (S–Se–S), either of which impede the formation of the functionally essential disulfide chemical bonds (S–S) (Lemly, 2002). The substitution of Se for S eventually results in weakened, dysfunctional enzymes and protein structures, which damages normal cellular biochemistry (Lemly, 2002).

Those Se-induced flaws in the protein biosynthesis bring about several metabolic consequences. Symptoms of Se toxicity in fish include elevated mortalities, lower feed intake, histopathological alterations in tissues, poor reproductive performance, and decreased growth response and haematocrit values (Gatlin and Wilson, 1984; Hamilton et al., 2002; Hilton et al., 1980; Jaramillo Jr et al., 2009; Lemly, 1997; Sorensen et al., 1984; Tashjian et al., 2006). However, the toxicity of Se appears to be impacted by the length of exposure and life stages of the affected animal (Lemly, 2002). The earliest life stages of fish are more Se-sensitive (Lemly, 1997), suggesting that those at embryo-alevin-fry stages may pose a higher risk of elevated Se levels than those at later stages, such as juvenile and adult fish. Whilst the diet containing 15.43 mg Se kg⁻¹ did not induce any toxic effects in 19.55 g yellowtail kingfish after 10 weeks of exposure (Le and Fotedar, 2014c), the diet with 13 mg Se kg⁻¹ caused reduced growth, poor feed intake, and high mortality in 1.3 g rainbow trout *O. mykiss*, after four weeks of feeding (Hilton et al., 1980). In spite of the proposed threshold level between 15.43 and 20.87 mg Se kg⁻¹ for yellowtail kingfish (Le and Fotedar, 2014c), the absence of detrimental effects on the growth and health parameters of juvenile yellowtail kingfish fed the highest Se supplementation level (4.0 mg kg⁻¹, providing the actual concentration of 7.38 mg kg⁻¹), employed in the present study, can therefore be established.

Conversely, Se deficiency in fish may manifest as the malfunction of various organs and tissues, including skeletal muscle, as described by Chariot and Bignani (2003). Muscle fibre degeneration necrosis of sea bass *Dicentrarchus labrax* has been associated with Se inadequacy in diets (López-Albors et al., 1995). Correspondingly, in the present study, in addition to the low antioxidant capacity of GPx, fish that were fed the control diet, exhibited muscle lesions, which is characterised by an alteration of skeletal muscle fibres, initiating contraction impairment, muscle atrophy and various degrees of limb or trunk severity (Lescure et al., 2009). Similar muscle conditions have been reported in various fishes, such as rainbow trout (*S. gairdneri*) and Atlantic salmon (*S. salar*) (Bell et al., 1986; Lorentzen et al., 1994). White muscle disease in domestic animals is also caused by a deficiency in Se, which negatively affected productive efficiency and animal health (Hefnawy and Tórtora-Pérez, 2010). In the present study, this “nutritional muscular dystrophy syndrome” occurs because the biological availability of Se from fishmeal appears to be low, possibly due to the binding of Se to mercury and other heavy metals (Webster and Lim, 2002). Therefore, Se supplementation may be required, to avoid deficiency syndromes, as well as maintain optimal growth and a functional immune system, in yellowtail kingfish.

5. Conclusion

In summary, the present study has demonstrated that there is an interactive relationship between Se level and temperature, in yellowtail kingfish. Se-supplemented diets significantly increased the final weight and SGR of fish reared at 21°C, but not at 26°C. Reduced growth, lower Se concentration in muscles and higher osmolality of fish reared at elevated water temperature might be linked to thermal-induced oxidative stress. However, the antioxidant

capacity of GPx, muscle Se level, and muscle histological performance were influenced by dietary Se intake. The present outcome may be relevant in portraying the impacts of temperature and Se level on antioxidant capacity, muscle histochemistry and growth for other marine finfish species.

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Table 1

Growth performance, relative feed intake (RFI), survival and osmolality of juvenile yellowtail kingfish fed with Se-supplemented and Se-unsupplemented diets at two different temperatures¹

Temperature	21°C			26°C			Analysis of variance (ANOVA) ²				
Se supplementation level (mg kg ⁻¹ diet)	0	2.0	4.0	0	2.0	4.0	Temp °C	Se supplementation level (mg kg ⁻¹)			Interaction
							21°C vs. 26°C	0	2.0	4.0	
Initial weight (g)	68.0±2.08	69.0±3.03	64.37±2.25	66.7±0.92	68.3±1.09	64.7±1.58					
Final weight (g)	101.1±2.75 ^a	128.7±4.05 ^c	113.3±1.5 ^b	86.1±0.64 ^a	90.7±1.48 ^a	101.7±1.6 ^b	>		*		*
SGR (% BWday ⁻¹)	1.37±0.04 ^b	2.20±0.06 ^c	1.99±0.11 ^c	0.85±0.02 ^a	0.99±0.01 ^a	1.51±0.05 ^b	>			*	*
RFI (% BWday ⁻¹)	1.04±0.12	1.32±0.42	1.22±0.26	1.02±0.18	1.12±0.21	1.05±0.37	NS	x	y	y	NS
Survival (%)	93±1.08	97±0.85	93±1.08	90±1.32	93±0.85	90±1.08	NS	NS			NS
Osmolality (mOsmkg ⁻¹)	293±1.52	298±0.57	299±4.35	307±1.52	310±0.57	315±1.15	<	NS			NS

^{x,y,z}For variables with a significant effect of diet and no significant interaction, values without a common letter are different (z indicated the highest value; $p < 0.05$).

^{a,b,c}For parameters with a significant interaction, differences in diets are compared within each temperature ($p < 0.05$), and values without a common superscript are different.

¹Means of three replicates ± SE.

²NS, non significant; *, $p < 0.05$. For variables with a significant effect of temperature ($p < 0.05$), and no interaction, < or > indicates whether the values measured at 21 °C were less than or greater than those measured at 26 °C.

Fig. 1. Red blood cell (RBC) glutathione peroxidase (GPx) activities of juvenile yellowtail kingfish fed diets containing different Se supplementation levels at 21°C and 26°C temperatures.

Fig. 2. Selenium concentration of muscles of juvenile yellowtail kingfish reared at 21°C (a) and 26°C (b) water temperatures.

Fig. 3. Histopathological analysis on juvenile yellowtail kingfish muscles showing the normal (A) and necrotic changes (B), scale bar = 50 μ m.