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## Accepted Manuscript

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**The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems**

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**Abstract**

A search for a viable new fish species for culture in recirculating aquaculture systems (RAS) in the Netherlands identified yellowtail kingfish *Seriola lalandi* as having excellent potential. To assist in determining the most appropriate water quality conditions for this species in RAS, the effect of water temperature (21, 23.5, 25, 26.5 and 29 °C) and pH (6.58, 7.16 and 7.85) were tested in two separate experiments. Growth performance, feed conversion, stress-physiological and metabolic parameters were assessed in juvenile yellowtail kingfish grown in pilot-scale RAS. Growth was optimised at a water temperature of 26.5 °C, in combination with maximum food intake and optimum food conversion ratio (FCR). Increasing temperature from 21 °C to 26.5 °C resulted in a 54 % increase in the fish's final weight after 30 days. A water pH of 6.58 resulted in mortality and inhibited both growth and FCR due to physiological disruptions to which the fish could not adapt.

Key words: growth performance, physiology, dissolved carbon dioxide, hypercapnia

## 1. Introduction

The development of new species for Dutch recirculating aquaculture systems (RAS) is a high priority, since existing industries are under environmental, societal and economic pressure. The annual production of European eel *Anguilla anguilla* and African catfish *Clarias gariepinus* in RAS in The Netherlands is approximately 4000 metric tonnes each (Van Duijn et al., 2010). The sustainability of the European eel industry is in question due to its reliance on wild seed and the effects such capture is seen to be having on wild eel populations (Van Duijn et al., 2010). Catfish farming, on the other hand, is under economic pressures imposed through the importation of competing Asian catfish (Van Duijn et al., 2010). A classification scheme based on a recent desktop study (Le-Francois et al., 2002) assessed a range of new candidate species for culture in RAS and based on factors including their closed life cycle, rapid growth rate to market size (ca. 3 kg in 1 year) and marketability, yellowtail kingfish (*Seriola lalandi*) was identified as a species with great potential.

Yellowtail kingfish is a pelagic marine species found globally in temperate and sub-tropical coastal waters. This and other closely related species such as *Seriola quinqueradiata* and *Seriola dumerili* are being cultured (or being investigated for culture) in countries including Japan, Australia, New Zealand, South Africa and Chile (Nakada, 2000; Hutson et al., 2007; Moran et al., 2009). Japan is the largest producer of all three species however culture in this country is reliant on the collection of wild-caught juveniles (Watanabe and Vassallo-Agius 2003, Nakada, 2000).

In those countries other than Japan in which yellowtail kingfish is being commercially cultured, juveniles are produced in hatcheries from closed life cycle spawning (Poortenaar et

al., 2001; Chen et al., 2006) and aquaculture production does not, therefore, depend on the capture of wild juveniles. Growout production in all countries is currently focused on net pen culture, however some production has occurred in RAS, primarily on a research scale (Partridge et al., 2003). Such systems have better bio-security and waste management capabilities than net pens. In addition, their ability to control water quality enables growth to be optimised for maximising economic returns (Mazzola et al., 2000; Kohbara et al., 2003, Miegel et al., 2010; Martins et al., 2010).

This study investigated the effects of two key environmental parameters - temperature and pH, on the growth and physiological response of juvenile yellowtail kingfish in RAS operating under these same specific conditions. There appear to be no published data on the optimum temperature for growth of yellowtail kingfish. Yet given the ability to precisely control water temperature in RAS, this data is of critical importance for optimising fish performance and subsequently economic return. Although the pH of seawater typically ranges between 7.85 and 8.5, factors such as nitrification and carbon dioxide excretion cause pH in RAS to decline (Spotte, 1992). Although this decline is compensated for by the addition of a base to maintain both pH and alkalinity, pH values are usually maintained below typical seawater values for reasons including better biofilter performance and lower costs of buffering.

## **2. Materials and Methods**

### **2.1 Fish**

Juvenile yellowtail kingfish were transported from Challenger Institute of Technology, Western Australia to IMARES, The Netherlands at a size of 0.3 g. Prior to experiments, fish were acclimatized for four weeks in a 5000 L recirculating tank at 25 °C, 30 ‰ salinity and a photoperiod of 16:8 h light:dark. The fish were fed daily to satiety (ca. 5-7 % BW/day) with commercial pellets containing 57% protein and 15% lipids (R-0.5-3 Europa, Skretting, Boxmeer, The Netherlands). Dissolved oxygen, temperature and pH were monitored continuously and the concentration of nitrogenous waste products was measured daily. The tank received a water refreshment of 1 m<sup>3</sup>/kg feed/day in order to maintain nitrate at less than 10 mg/L NO<sub>3</sub>-N.

## 2.2 Experimental Design and System

In Experiment 1, we measured growth performance and physiological responses of juvenile yellowtail kingfish kept at 21 °C, 23.5 °C, 25 °C, 26.5 °C and 29 °C for 32 days. Five independent RAS were used, each consisting of three replicate 800 L tanks. Tanks contained 20 randomly selected fish (initial body weight of the stock fish 4.11 ± 0.03 g, n = 300, Table 2).

Each RAS comprised a drum filter (Hydrotech HDF501; Vellinge, Sweden) for the removal of particulate waste, a trickling biofilter for the conversion of ammonia to nitrate and a combined ultraviolet (UV) sterilising filter and heat exchanger (Teco Seachill; Ravenna, Italy) to reduce bacteria and maintain temperature, respectively. Aeration within each tank provided oxygen and removed carbon dioxide. The water refreshment rate in each RAS was set to 3 m<sup>3</sup>/kg feed/day to maintain NO<sub>3</sub>-N levels at less than 10 mg/L NO<sub>3</sub>-N.

On completion of Experiment 1, three replicate tanks within three of the aforementioned RAS were used for Experiment 2. Each tank was stocked with 20 fish (initial weight  $32.7 \pm 0.6$  g,  $n = 180$ ; Table 2). The fish used for Experiment 2 were different to those used in Experiment 1. This second experiment investigated the effect of target pH values of 8.0, 7.0 and 6.5 on the growth and physiological response of juvenile yellowtail kingfish for 27 days. Based on the results of Experiment 1, this trial was conducted at a temperature of  $26.5$  °C. The target pH values of 7.0 and 6.5 were achieved using pH controllers (Electronic Metering Equipment and Control Ltd (EMEC); LPH 230; Italy). A pH probe (IKS; Rosmalen, The Netherlands) in the sump of each RAS constantly monitored pH and a 5 % HCl solution automatically dosed in response to this measurement via a dosing pump (Fluid and Water Technologies; FX CS/DL; Italy). The treatment of pH 8.0 received no dosing.

In both trials, fish were fed eight times daily to satiety on the aforementioned diet and the quantity of food ingested by fish in each tank was recorded. Fish were fed slowly to ensure there was no waste of feed pellets in the tanks.

### 2.3 Measurements and Statistical Analysis

Growth performance in each experiment was assessed as specific growth rate (SGR (%)/day) according to the following formula:

$$\text{SGR} = (\ln(W_f) - \ln(W_i)) \times 100/t$$

Where:  $W_f$  = final mean body weight (g),  
 $W_i$  = initial mean body weight (g)  
 $t$  = trial duration (days).



Feed intake was recorded daily for each tank. Food conversion ratio (FCR) was calculated for each tank as the ratio of biomass gain to food intake. Lower survival occurred in the pH 6.5 group. FCR data for this treatment were corrected for mortality by adding the weight gained by these mortalities prior to death to the total weight gain.

At the end of each experiment, all fish were anaesthetised (0.005% 2-phenoxyethanol, Sigma-Aldrich, St. Louis, MO, USA) and individually weighed. Blood was taken from the caudal vein from six fish (Experiment 1) or five fish (Experiment 2) from each tank (randomly selected) using heparinised syringes and needles. Blood was centrifuged at 13000 rpm for 10 minutes at 4 °C and the plasma then stored at -20 °C prior to analysis. Plasma osmolality was measured with a cryoscopic osmometer (Osmomat 030, Gonotec, Germany). De-ionized water (0 mOsm/kg) and a standard solution (300 mOsm/kg) were used as calibrators. Plasma glucose, lactate,  $\text{Na}^+$  (mmol/L),  $\text{HCO}_3^-$  (mmol/L) and pH were measured using a Stat Profile pHox plus analyser (Nova Biomedical, Waltham, MA, USA) and were recalculated to mg/dL for glucose and lactate. Plasma  $\text{Cl}^-$  and non-esterified fatty acids (NEFA) levels were determined using commercial test kits ( $\text{Cl}^-$ : Gentaur, Brussels, Belgium; NEFA: NEFA-HR (2) Instruchemie, Delfzijl, The Netherlands). The protocols were adapted for 96-well micro plate application and NEFA was recalculated to mg/dL.

Two gill arches per fish from the aforementioned six and five fish from Experiment 1 and 2, respectively were sampled and stored at -20 °C in SEI buffer (150 mmol/L sucrose, 10 mmol/L EDTA, 50 mmol/L imidazole: pH 7.4) prior to the determination of branchial  $\text{Na}^+/\text{K}^+$ -ATPase (NKA) activity according to Metz et al. (2003).

During each trial, dissolved oxygen, temperature and pH were measured with a multimeter (Hach Lange HQ40d, Tiel, The Netherlands), salinity was measured with a conductivity meter (Hach Lange HQ14d), total ammonia nitrogen (TAN), nitrite nitrogen (NO<sub>2</sub>-N) and nitrate nitrogen (NO<sub>3</sub>-N) were measured using a spectrophotometer (Hach Lange DR 5000 UV/VIS). All parameters were measured daily and these daily values averaged for each replicate tank. Values for free ammonia nitrogen (FAN) were calculated from TAN, temperature and pH values.

All data are expressed as mean  $\pm$  S.E. Levene's test was used to test for homogeneity of variance, Shapiro-Wilk to test for normal distribution of data. Significance of difference between treatments was calculated by one-way analysis of variance (ANOVA; further referred to as AN in the text) when the conditions of validity passed, differences between groups were calculated by Bonferroni post-hoc test. Kruskal Wallis (KW) was used when no normal distribution of data was found, followed by Tukey post hoc test. Significance was accepted when  $P \leq 0.05$ . The SGR data were arcsine transformed prior to analysis.

The study was approved by the ethical committee for animal research, Livestock Research, Lelystad, The Netherlands and is in accordance with the code of ethics of the world medical association for animal experiments (Declaration of Helsinki).

### **3. Results**

#### **3.1 Experiment 1**

Temperature had a significant effect on the growth of juvenile yellowtail kingfish (AN;  $P < 0.001$ ; Table 2). Those cultured at 26.5 °C had the fastest rate of growth (SGR =  $7.75 \pm 0.14$  %/day), significantly faster than those at all other temperatures ( $P < 0.001$  with 21 and 23.5 °C,  $P = 0.002$  with 29 °C and  $P = 0.01$  with 25 °C). The growth rate of fish at 25 °C ( $7.24 \pm 0.03$  %/day) and 29 °C ( $7.12 \pm 0.02$  %/day) did not differ ( $P = 1.000$ ), and were significantly faster than those at 21 °C ( $6.33 \pm 0.02$  %/day;  $P < 0.001$  with 25 °C and 29 °C) and 23.5 °C ( $6.63 \pm 0.02$  %/day;  $P = 0.003$  with 25 °C and  $P = 0.015$  with 29 °C), which did not differ from each other ( $P = 0.204$ ).

Feed intake (AN;  $P < 0.001$ ) at 26.5 °C ( $1.23 \pm 0.03$  g/fish/day) was significantly higher compared to 21 °C ( $0.98 \pm 0.03$  g/fish/day;  $P < 0.0001$ ) and 23.5 °C ( $1.05 \pm 0.02$  g/fish/day  $P = 0.006$ ) Table 2). Those fish reared at 26.5 °C had the lowest (best) FCR ( $0.88 \pm 0.05$ ) (AN;  $P = 0.002$ ), significantly lower than at 21 °C ( $1.13 \pm 0.04$ ;  $P = 0.001$ ) and 23.5 °C ( $1.11 \pm 0.01$ ;  $P = 0.001$ ), but not different to that achieved at 25 °C ( $0.98 \pm 0.03$ ;  $P = 0.366$ ) and 29 °C ( $1.00 \pm 0.01$ ;  $P = 0.185$ ; Table 2).

Temperature had a significant effect on most of the measured physiological parameters (Table 2). Glucose, lactate, osmolality,  $\text{Cl}^-$  and NKA showed a general increase with increasing temperature. Glucose (KW;  $P < 0.001$ ) was significantly lower at 21 °C ( $112 \pm 3.1$  mg/dL) than all other temperatures ( $P = 0.04$  for 23.5 °C,  $P = 0.003$  for 25 °C,  $P < 0.0001$  for 26.5 °C,  $P < 0.0001$  for 29 °C). Lactate (AN;  $P = 0.002$ ) at 21 °C ( $62 \pm 3.2$  mg/dL) was significantly lower than at both 25 °C ( $77 \pm 3.0$  mg/dL,  $P = 0.008$ ) and 26.5 °C ( $78 \pm 3.2$  mg/dL,  $P = 0.002$ ). At the highest temperature, NEFA (KW;  $P = 0.002$ ) was significantly lower ( $6.2 \pm 0.4$  mg/dL) than all other temperatures, except 23.5 °C ( $7.2 \pm 0.2$  mg/dL,  $P = 0.232$ ). Osmolality (AN;  $P = 0.001$ ) at 21 °C ( $385 \pm 2.6$  mOsm/kg) was significantly lower

than at 25 °C ( $394 \pm 2.5$  mOsm/kg,  $P = 0.04$ ) and 26.5 °C ( $398 \pm 1.5$  mOsm/kg,  $P = 0.001$ ) and coincided with a significantly lower  $\text{Cl}^-$  concentration (AN;  $P = 0.032$ ) at this lowest temperature ( $149 \pm 3.7$  mmol/L) compared with at 26.5 °C ( $163 \pm 3.7$  mmol/L,  $P = 0.04$ ). Levels of  $\text{Na}^+$  (AN;  $P = 0.613$ ), plasma pH (AN;  $P = 0.45$ ) and  $\text{HCO}_3^-$  (KW;  $P = 0.091$ ) were unaffected by temperature.

All water quality parameters (Table 1) remained well within widely accepted ranges for fish production (Colt 2006). Survival of fish in all treatments was 100%.

### 3.2 Experiment 2

Average daily pH values for the target pH values of 8, 7 and 6.5 were  $7.85 \pm 0.01$ ,  $7.16 \pm 0.02$  and  $6.58 \pm 0.02$ , respectively (AN;  $P < 0.001$ ; Table 1)

pH had an effect on both the survival and growth of juvenile yellowtail kingfish. Survival at a pH of 6.58 was 92 % and this was considerably lower than at pH 7.16 and pH 7.85, which both experienced 100 % survival. The growth (AN;  $P < 0.001$ ) of fish at a pH of 6.58 ( $4.52 \pm 0.11\%$ /day) was significantly slower than at both other pH values (pH 7.16;  $5.77 \pm 0.18\%$ /day,  $P = 0.001$ ; pH 7.85;  $6.05 \pm 0.05\%$ /day,  $P < 0.001$ ).

Feed intake was also significantly affected by pH (AN;  $P < 0.001$ ). Those fish reared in water with a pH of 6.58 ate significantly less food ( $3.3 \pm 0.1$  g/fish/day) than those in waters with pH 7.16 ( $4.4 \pm 0.1$  g/fish/day;  $P = 0.001$ ) and 7.85 ( $4.8 \pm 0.1$  g/fish/day;  $P < 0.001$ ), which were not different from each other ( $P = 0.052$ ; Table 2). The food conversion ratio (FCR; AN;  $P < 0.001$ ) of fish grown at pH 6.58 ( $1.26 \pm 0.03$ ) was significantly higher (worse) than

those cultured at 7.16 ( $0.98 \pm 0.02$ ,  $P = 0.002$ ) and 7.85 ( $0.98 \pm 0.004$ ,  $P = 0.002$ ), which did not differ from each other.

Glucose (KW;  $P = 0.019$ ) in the pH 6.58 group ( $153 \pm 2.2$  mg/dL) was significantly higher compared to the pH 7.85 group ( $131 \pm 6.3$  mg/dL;  $P = 0.007$ ). The lactate concentration (KW;  $P = 0.004$ ) in the pH 6.58 group ( $76 \pm 5.9$  mg/dL) was significantly lower than in the pH 7.16 group ( $97 \pm 3.15$  mg/dL;  $P = 0.003$ ). NEFA (AN;  $P = 0.041$ ) in the pH 6.58 ( $2.1 \pm 0.2$  mg/dL) group was significantly lower than both other groups ( $P = 0.04$  for pH 7.16 and  $P = 0.03$  for pH 7.85), which did not differ from each other.  $\text{Na}^+/\text{K}^+$ -ATPase activity (KW;  $P = 0.001$ ) in the pH 6.58 group ( $1.13 \pm 0.14$   $\mu\text{mol P}_i/\text{h}/\text{mg}$  protein) was more than twice that in the pH 7.16 ( $0.49 \pm 0.07$   $\mu\text{mol P}_i/\text{h}/\text{mg}$  protein;  $P < 0.001$ ) and pH 7.85 groups ( $0.51 \pm 0.05$   $\mu\text{mol P}_i/\text{h}/\text{mg}$  protein;  $P < 0.001$ ); a difference that was highly significant. Osmolality (KW;  $P = 0.001$ ) was significantly higher in the pH 6.58 group compared with both other groups ( $P = 0.037$  for pH 7.16 and  $P < 0.001$  for pH 7.85). Plasma pH (KW;  $P = 0.021$ ) in the pH 6.58 group (7.40) was significantly lower than both other groups (7.48 for both pH 7.16 and 7.85 groups;  $P = 0.002$ ). There was no effect of pH on plasma  $\text{Na}^+$  (AN;  $P = 0.215$ ) and  $\text{Cl}^-$  (KW;  $P = 0.348$ ).

Water quality data for Experiment 2 are presented in Table 1. All water quality parameters remained well within widely accepted ranges for fish production (Colt 2006).

#### 4. Discussion

This study presents the first reported data on optimum temperature and pH conditions for maximising growth of early juvenile yellowtail kingfish within recirculating aquaculture

systems. Although replicate tanks were nested within RAS (due to constraints to providing an independent RAS on each replicate tank), all water quality parameters (other than those under direct investigation) were well within widely accepted ranges for fish production (Colt, 2006). For example, although significant differences were found in dissolved oxygen between treatments the absolute magnitude of these differences were small and dissolved oxygen in all treatments always remained above the level of 5.7 mg/L described by Nakada (2000) as necessary for optimum growth of the congeneric yellowtail, *Seriola quinqueradiata*. Likewise, small but significant differences in nitrite and nitrate concentrations were found between treatments, however the highest values recorded for each parameter were 0.75 mg/L and 6.3 mg/L, respectively. These values are low compared to those recommended in intensive aquaculture systems. Siikavuopio and Sæther (2006) recommended maintaining nitrite values below 1.0 mg NO<sub>2</sub>-N for Atlantic cod *Gadus morhua*, and Frakes and Hoff (1982) recommended maintaining nitrate values below 20 mg/L for clownfish *Amphirion ocelluris*. Due to the very strong effect that pH has on FAN, significant differences in FAN were observed between treatments in Experiment 2, however the highest FAN was less than one part per billion; significantly lower than the recommended level for RAS of 50 – 100 parts per billion (Timmons et al. 2002). Furthermore, it was typically in the best performing treatments that these highest values occurred, providing further evidence that they had little impact on the fish's growth. These results effectively demonstrate that our significant findings were the results of only the parameter under investigation.

Temperature is a key (abiotic) factor affecting fish growth (Brett, 1979) and our data clearly demonstrate this relationship for juvenile yellowtail kingfish. Growth was optimised at a water temperature of 26.5 °C; a temperature higher than which the species is currently

cultured in seacages in South Australia (10 °C to 24 °C; Miegel et al., 2010) and New Zealand (14 °C to 22 °C; Moran et al., 2009).

Previous reports on the optimum temperature for growth of this species are limited. Pirozzi and Booth (2009) measured the routine metabolic rate of 206 g yellowtail kingfish at six temperatures ranging from 10 °C to 32.5 °C. The optimum temperature was considered that at which  $Q_{10}$  was minimised and was calculated as the asymptotic value of the polynomial regression of  $Q_{10}$  vs temperature. That value was calculated to be 22.8 °C; somewhat lower than the optimum found in the current study. Although the  $R^2$  value of that regression was fairly low (0.65), we believe the major contributing factor to the difference in temperature optima between the two studies was the difference in fish sizes. A decreasing ontogenetic shift in optimum temperature is common for most ectotherms (Angilletta and Dunham 2003) and has been described for a range of other marine fish including Atlantic cod (*Gadus morhua*; Lafrance et al., 2005; Bjornsson et al., 2001), turbot (*Scophthalmus maximus*; Imsland et al., 1996), Atlantic halibut (*Hippoglossus hippoglossus*; Hallaråker et al., 1995) and plaice (*Pleuronectes platessa*; Fonds et al., 1992). Further studies investigating temperature optima for various sizes of yellowtail kingfish will be of benefit for optimising fish performance in RAS where temperature can be easily manipulated to ensure fish always grow at their optimum rate.

Although there appear to be no data on growth of juvenile yellowtail kingfish at the high temperatures tested in the current study, the rates of growth we achieved at the lower temperatures are comparable with similar sized conspecifics. Masumoto (2002), for example, reported that Japanese yellowtail grew from 3.7 to 35.9 g over 35 days in water that increased in temperature from 20 °C to 24 °C during the study. This specific growth rate of 6.5%

BW/day is very similar to those rates achieved at 21 °C ( $6.33 \pm 0.02$  %/day) and 23.5 °C ( $6.63 \pm 0.10$  %/day) in this study; providing further support that the water quality in all RAS was appropriate and non-limiting.

The growth of juvenile yellowtail kingfish was significantly reduced at the extreme temperature of 29 °C compared with the optimal temperature of 26.5 °C. This parabolic temperature response is typical of all species for which upper thermal tolerances have been studied (Bermudes et al., 2010; Bjornsson et al., 2001; Imsland et al., 2006). The significant reduction in food intake coupled with an increased demand for energy at such high temperatures are the likely major factors contributing to the poor performance of yellowtail kingfish at 29 °C. Data on metabolic rates of Japanese yellowtail by Watanabe et al. (1998), for example, show that maintenance energy requirements increase significantly from ca. 90 kJ kg BW<sup>-0.8</sup>day<sup>-1</sup> at temperatures between 21 and 25 °C to 138 kJ kg BW<sup>-0.8</sup>day<sup>-1</sup> at 27 °C. Pirozzi and Booth (2009) reported similar values for yellowtail kingfish. Despite this increase in energy demand, we observed appetite suppression at the most extreme temperature; a feature that has been described for a number of species and which has been attributed to stress (Bermudes et al., 2010; Imsland et al., 2006).

Although the 12% reduction in FCR at 29 °C compared with 26.5 °C was not significant, it may however be of biological importance. A reduction in feed utilisation efficiency at extreme water temperatures has been reported for barramundi *Lates calcarifer* (Bermudes et al., 2010), wolf fish *Anarhichas lupus* (Imsland et al., 2006) and European seabass *Dicentrarchus labrax* (Person-Le Reyut et al., 2004) and has been attributed to factors including increased protein turnover (McCarthy et al., 1999). Optimum FCR in the current study was achieved at the same temperature as optimum growth. This is of clear benefit for



the culture of yellowtail kingfish in RAS and is not the case for all species (Bjornsson et al., 2001; Berumudes et al., 2010; Person-Le Ruyet et al., 2004). The significantly higher FCR achieved at the lower two temperatures may be attributed to a lower protease activity and subsequent poorer protein digestibility as described for Japanese yellowtail reared in cool water by Kofuji et al. (2005). This is also consistent with another study on yellowtail kingfish in which FCR was significantly better in warm water (17 °C to 22 °C) compared with cool water (14 °C to 17 °C; Moran et al., 2009).

Changes in water temperature have been demonstrated to affect osmoregulatory capacities of fish (Staurnes et al., 1994; Handeland et al., 1998). In this study, those fish grown at 21 °C had significantly lower osmolality than those grown at the optimum temperature of 26.5 °C, which was due primarily to a significant reduction in plasma Cl<sup>-</sup>. Although no significant differences were measured in branchial Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, the enzyme showed typical increase in activity with temperature. This is the expected response to the increased drinking and ion uptake rates associated with the increase in metabolism seen with increasing temperature (Ando et al., 2003)

Plasma glucose concentration increased with temperature to 26.5 °C and then plateaued. This suggests that more glucose is being made available for energy via liver glycogenolysis to meet the concomitant increasing metabolic demand (Wendelaar Bonga, 1997) and is supported by the same temperature related response of lactate, which increases with the increased energy usage with higher temperatures. At the highest temperature tested, glucose and lactate did not increase further. This is despite the assumption that metabolism is likely to have increased significantly at this temperature as previously described. Our data showing a significant decrease in NEFA at this highest temperature suggest that lipid catabolism may be

used preferentially over glucose to fuel metabolism at this extreme temperature. Alternatively, the lower NEFA at the highest temperature may reflect the reduced food intake at this temperature, as Arjona et al. (2009) suggested that plasma NEFA levels are influenced by feed intake rather than mobilisation from body stores

Data from Experiment 2 demonstrate the negative consequences of growing juvenile yellowtail kingfish in water with pH 6.58. With the acidification of seawater resulting in the conversion of carbonates to carbon dioxide (Stumm and Morgan, 1996), the resulting hypercapnia may have been a contributing factor to the poor performance of fish at these reduced pH values. Following Experiment 2, a pH-CO<sub>2</sub> standard curve was prepared by titrating hydrochloric acid into seawater (alkalinity 2.5 mEq/L) whilst measuring dissolved carbon dioxide (Oxyguard, G02C2P Portable CO<sub>2</sub> analyzer). This titration indicated that acidification to pH 7.16 increased dissolved CO<sub>2</sub> to 10 mg/L; whereas acidification to pH 6.58 increased dissolved CO<sub>2</sub> to 23 mg/L.

Although there appear to be no data on the effects of pH on yellowtail kingfish, data exist on the effects of acutely elevated CO<sub>2</sub> concentrations. In a simulated transport trial, Moran et al. (2008) exposed juvenile yellowtail kingfish to dissolved CO<sub>2</sub> concentrations as high as 75 mg/L for five hours and reported only limited physiological disturbance and little mortality. In the related Japanese yellowtail, however, exposure to the same concentration for 72 hours resulted in 100% mortality, whilst exposure to 14 mg/L resulted in mild physiological adaptations including decreased plasma pH and elevated blood HCO<sub>3</sub><sup>-</sup> (Lee et al., 2003).

Based on the data provided from the aforementioned pH-CO<sub>2</sub> standard curve, the current study provides some indication on the effects of a more chronic, low level exposure of dissolved CO<sub>2</sub>. At the water pH of 7.16, physiological homeostasis in juvenile yellowtail kingfish was maintained and the negative impact of this pH on growth was caused only by appetite suppression and not reduced food conversion efficiency. A reduction in appetite has been described for juvenile European seabass exposed to elevated concentrations of dissolved CO<sub>2</sub> (Cecchini et al., 2001).

The effects seen at pH 6.58 were more severe than at pH 7.16, with a reduction in survival, growth, appetite and food conversion efficiency and a disruption to physiological homeostasis. At this water pH, blood acidosis was occurring, as seen by the significant reduction in blood plasma pH. This is typical of hypercapnia and the doubling of branchial Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in these fish is the anticipated response to blood acidosis, as it enables increased HCO<sub>3</sub><sup>-</sup> uptake via the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> carrier mechanism in the apical membranes of the Cl<sup>-</sup> cells in the gill epithelium (Perry and Gilmour, 2006). Wendelaar Bonga and co-workers (1990) also showed that long term plasma acidification results in increased number and turn-over rate of Cl<sup>-</sup> cells. That plasma HCO<sub>3</sub><sup>-</sup> was depleted in the pH 6.58 group compared to the pH 7.16 and pH 7.85 groups, demonstrates that despite a doubling of the activity of this enzyme, homeostasis had not been restored. These findings are consistent with those of Lee et al. (2003) who acutely exposed Japanese yellowtail to 75 mg/L CO<sub>2</sub> for 72 hours and found that plasma pH was not completely restored despite evidence of increased bicarbonate uptake. As carbonic acid is a weak acid, direct addition of carbon dioxide gas into seawater to achieve the same concentration of dissolved carbon dioxide as by acidification with HCl results in a smaller decline in pH. As such, we cannot

attribute all of the negative effects in the current trial only to dissolved CO<sub>2</sub> and the low pH per se is likely to also have contributed.

The significant decrease in plasma NEFA in the pH 6.58 group is likely the result of the increased energy demand to fuel the aforementioned physiological mechanisms required to overcome the disruption of homeostasis. The increased blood glucose concentration seen in this group suggests that glucose was not being used as an energy substrate, however, elevated glucose is a common secondary stress response in fish (Wells and Pankhurst, 1999) and this is the likely reason for its elevated concentration at this low pH.

## **5. Conclusion**

This study investigated two key water quality parameters for juvenile yellowtail kingfish in RAS. Juvenile yellowtail kingfish showed the best performance at a water temperature of 26.5 °C. Increasing temperature from 21 °C to 26.5 °C resulted in a 54% increase in the fish's final weight after 30 days. The optimum temperature for growth coincided with the optimum temperature for food intake and conversion efficiency. Such improvements will significantly decrease the time to reach the market size of 3-4 kg and cohort turnover rate; factors that are critical for the economic optimisation of RAS. Growth and FCR are inhibited at both lower and warmer temperatures, however, mechanisms to maintain homeostasis only become clearly evident at lower temperatures of 21 °C. Further studies investigating how temperature optima change with age are necessary to ensure fish performance in RAS is maintained at the highest rate possible.

A water pH level of 6.58 resulted in mortality, inhibited growth and FCR as a result of physiological disruptions that the fish were not able to adapt to. Given that performance was better in fish reared at pH 7.85 compared to pH 7.16 demonstrates that the water within RAS should be maintained at greater than pH 7.16 to prevent acidity related consequences in juvenile yellowtail kingfish.

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