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Ambient salinity and osmoregulation, energy metabolism and growth in juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes 1833) in a recirculating aquaculture system

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Abstract

The effects of salinity on plasma osmolality, branchial chloride cell density, feed consumption and conversion and growth performance of yellowtail kingfish (*Seriola lalandi*) were evaluated. Fish (11.6 ± 0.6 g) were kept for 29 days at 14, 18, 22, 26 (experimental) and 30 g L^{-1} (control) salinity in independent, pilot-scale recirculation aquaculture systems. No differences in plasma osmolality or chloride cell numbers in gills were observed, pointing to a strong osmoregulatory capacity in the juveniles. Fish at 14, 18 and 22 g L^{-1} (7.61 ± 0.19, 7.61 ± 0.01 and 7.61 ± 0.13% day^{-1}, respectively) had higher growth rates than fish at 26 and 30 g L^{-1} (7.10 ± 0.05 and 6.97 ± 0.06% day^{-1} respectively). The higher growth rate at lower salinity resulted from increased feed intake; feed conversion was not different. An evaluation of the impact of salinity on growth rate of on-growing stages (till market size) seems warranted to assess whether the profitable effects of low salinity persist in later stages of this important aquaculture species.

Keywords: physiological response, Seriola lalandi, salinity, growth performance

Introduction

Yellowtail kingfish (*Seriola lalandi* Valenciennes 1833) is a marine pelagic species widely distributed in warm, near-shore and off-shore waters of the southern hemisphere. Its fast growth and excellent flesh quality favour the culture of this species especially in Australia, New Zealand, Japan and Chile (Fowler, Ham & Jennings 2003; Moran 2007; Nakada 2008; Aguilera, Yany & Romero 2013). The grow-out phase in Australia and Japan typically focuses on sea cages (Nakada 2002; Miegel, Pain, van Wettere, Howarth & Stone 2010). In the Netherlands, commercial farming of yellowtail kingfish to market size occurs in recirculation aquaculture systems (RAS). RAS offer better biosecurity and waste management control than cages; in addition, strict control over water quality enables growth optimization for maximum economic return (Martins, Eding, Verdegem, Heinsbroek, Schneider, Blancheton, d'Orbcastel & Verreth 2010), a prerequisite for a lasting, economically viable intensive aquaculture (Tandler, Harel, Wilks, Levinson, Brickell, Christie, Avital & Barr 1989).

The optimum temperature and water pH for juvenile yellowtail kingfish in RAS have been established (Abbink, Blanco Garcia, Roques, Partridge,

Kloet & Schneider 2012). Ambient salinity strongly influences fish growth and physiology (Tandler *et al.* 1989; Morgan & Iwama 1991; Boeuf & Payan 2001; Partridge & Jenkins 2002; Faulk & Holt 2006). If a species adapts well and easily to less saline (more brackish) environment, the range of locations suitable for farming broadens.

To keep osmotic balance in full-strength seawater (30–34 g L^{-1}), marine fish spend significant energy on osmoregulation that could otherwise (in less saline waters) be allocated for growth. The body fluids of most marine teleosts are isosmotic with seawater of 11–14 g L^{-1}, i.e. brackish water (around 350 mOsm kg^{-1}; Gordon 1977). Consensus exists that growth performance improves at salinities where the least energy is required for osmoregulation (Brett 1979; Jobling 1994; O'Neill, De Raedemaecker, McGrath & Brophy 2011). However, optimum salinities for growth may vary considerably between species as well as within species at different life stages (Morgan & Iwama 1991; Morgan, Balfry, Vijayan & Iwama 1996).

There are only limited data available on optimum salinity for growth of *S. lalandi* or related species. The greater amberjack (*Seriola dumerilii* Risso 1810) is grown to commercial size at salinities between 14 and 33 g L^{-1} (Hu & Ji 2003).

We investigated the effects of salinities between 14 and 30 g L^{-1} on osmoregulation and growth performance in juvenile yellowtail kingfish over a 4-week period in RAS.

Material and methods

Fish

Yellowtail kingfish fingerlings (0.5 g) were flown in from the Challenger Institute of Technology in Western Australia to the IMARES facilities in the Netherlands. During a 6-week acclimatization period, fish were kept in a 5000-L recirculation tank, at 26.5°C, 30 g L^{-1} (the natural Oosterschelde seawater piped into the facility; salinity equivalent to 30 g L^{-1} sea salt or 885 mOsmol kg^{-1}) and a 16:8 light:dark photoperiod. Fish were fed eight times daily to satiation with commercial pellets containing 55% protein and 15% lipids (0.8–1.2 Gemma Diamond and R 2–3 Europa; Skretting, Boxmeer, The Netherlands). Salinity, dissolved oxygen, temperature and pH were monitored online; nitrogenous waste production was measured daily.

Experimental design

The experimental design of this experiment was described recently (Abbink *et al.* 2012). In short, growth performance and physiological response of the fish were measured after 29 days at salinities of 14, 18, 22, 26 and 30 g L^{-1} (413, 531, 649, 767 and 885 mOsmol kg^{-1} respectively). Salinities were realized by dilution of full-strength seawater with dechlorinated fresh water. For each salinity, an independent RAS consisting of three 800-L tanks was used as experimental unit. The RAS had a drum filter (Hydrotech HDF501; Vellinge, Sweden), a trickling biofilter and a combined ultraviolet (UV) sterilizing filter *plus* heat exchanger to guarantee a temperature of 25.5°C (Teco Seachill; Ravenna, Italy). Airstone aeration in each tank secured stable oxygen levels and removed carbon dioxide.

Each tank was stocked with 20 randomly selected fish that were individually weighed (body weight 8.38 ± 0.05 g, $n = 300$). Next, the fish were gradually acclimatized to the test salinities by dilution of the refreshment water with tap water to reduce salinity by 3 g L^{-1} day^{-1}. Once all groups reached their desired salinity, all fish were individually weighed again (body weight 11.6 ± 0.6 g, $n = 300$) and the experiment started. Fish were fed by hand to satiety eight times per day. Feed was added in small portions to avoid pellets to sink to the bottom of the tanks. In case of (odd) mortality, fish were removed from the system and weighed. Weights of dead fish were included in calculations where needed. The water refreshment rate in each RAS was set to 3 m^3 kg^{-1} feed day^{-1} to keep nitrogenous waste products at accepted low levels (Colt 2006).

Measurements and data analysis

During the trial, salinity, dissolved oxygen, temperature and pH were recorded with an IKS meter (IKS International, Rosmalen, The Netherlands). Total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) were measured daily using a spectrophotometer (Hach Lange DR 5000 UV/VIS; Hach Lange, Tiel, The Netherlands). Daily values were averaged for each replicate tank.

Growth performance was expressed as specific growth rate (SGR) according to the following formula: SGR (% day^{-1}) = $[\ln(W_f) - \ln(W_i)/t] * 100$; where W_f and W_i were the average final and average initial body weight of the fish in each tank

respectively and t (time in days) represents the duration of the experiment.

Feed intake was recorded daily for each tank as the feed consumed per 20 fish per day. Feed conversion rate (FCR) was calculated for each replicate according to: FCR = Feed Intake/(W_f * N_f) + W_d − (W_i * N_i); where W_f and W_i were the average final and average initial body weight of the fish, W_d is the weight of dead fish (when mortality occurred), and N_f and N_i are the final and initial fish number.

At the end of the experiment, all fish were anaesthetized (0.1% 2-phenoxyethanol; Sigma-Aldrich, St Louis, MO, USA) and individually weighed. Blood was taken by puncture of the caudal vessels from a subsample of five randomly selected fish from each tank using heparinized tuberculin syringes and 23-G needles. Blood was centrifuged at 14 171 ***g*** for 10 min at 4°C and the plasma thus obtained stored at −20°C till analyses.

Plasma levels of glucose, lactate and non-esterified fatty acids (NEFA) were assessed as readout for secondary stress response and energy status. Plasma osmolality, Na^+- and Cl^--concentrations reflect the osmoregulatory capacity of the fish. HCO_3^- and the pH serve to monitor acid–base status.

Plasma glucose, lactate, Na^+, HCO_3^- and pH were measured using a Stat Profile pHOx plus analyser (Nova Biomedical, Waltham, MA, USA). Plasma Cl^- and non-esterified fatty acids (NEFA) levels were determined using commercial test kits (Cl^-: Gentaur, Brussels, Belgium; NEFA: NEFA-HR (2) Instruchemie, Delfzijl, The Netherlands). Plasma osmolality was measured with a cryoscopic osmometer (Osmomat 030; Gonotec, Berlin, Germany). Deionized water (0 mOsm kg^{-1}) and a standard solution (300 mOsm kg^{-1}) were used as reference. Protocols were adapted for 96-well microplate application where possible.

The outer gill arches on both lateral sides from five fish were sampled. One gill arch was stored at −20°C in SEI buffer (150 mmol L^{-1} sucrose, 10 mmol L^{-1} EDTA, 50 mmol L^{-1} imidazole: pH 7.4) for later determination of Na^+/K^+-ATPase, the other gill arch was fixed in Bouin's fixative (15 volumes saturated picric acid: 5 volumes 37% formaldehyde: 1 volume glacial acetic acid) for immunohistochemical analysis.

Sodium- and potassium-dependent, ouabain-sensitive ATPase (Na^+/K^+-ATPase) activity is a measure of the sodium pump capacity of gills. Enzymic activity in whole gill homogenate was determined as the difference in ATP hydrolysis in the presence of Na^+ and K^+ (total activity) and in the absence of K^+ and presence of ouabain (ouabain-insensitive activity) as described in detail before (Metz, van den Burg, Wendelaar Bonga & Flik 2003). Branchial tissue was scraped off the arches with microscope slides on an ice-cooled Petri dish and homogenized in SEI buffer. Protein concentration was determined with a commercial kit (BioRad, Hercules, CA, USA) with bovine serum albumin as reference. The Na^+/K^+-ATPase specific activity was calculated by subtraction of ouabain-insensitive ATPase activity from total ATPase activity, and expressed as the amount of P*i* formed per hour and per mg protein.

For histology, the filaments were cross-sectioned (at 5–7 μm) to observe the chloride cells in the interlamellar epithelium and at the trailing edge. After dewaxing, blocking of endogenous peroxidase with 2% (v/v) H_2O_2 and blocking of non-specific sites with 10% (v/v) normal goat serum, the slides were incubated overnight with a monoclonal antibody against chicken Na^+/K^+-ATPase (IgGα5; Developmental Studies Hybridoma Bank, Department of Biological Sciences, University of Iowa, USA) at a final dilution of 1:500 (v/v). Goat anti-mouse (Nordic Immunology, Tilburg, The Netherlands) was used as secondary antibody at 1:150 (v/v) dilution. The slides were subsequently incubated with 1:150 (v/v) diluted mouse peroxidase anti-peroxidase (M-PAP; Nordic). Staining was performed in 0.025% (w/v) 3,3′-diaminobenzidine (DAB) and 0.0005% (v/v) H_2O_2 (Metz *et al.* 2003).

The number of Na^+/K^+-ATPase positive (chloride) cells in three filaments and spanning approximately 120 lamellae, were counted in duplicate by two independent (uninformed about experimental conditions) researchers.

Data are expressed as means ± SD. Levene's test was used to assess homogeneity of variance, and the Shapiro–Wilk test to confirm normal distribution of data. Significance of differences between data obtained from full-strength seawater (controls) and lower salinities (experimentals) was assessed by using one-way analysis of variance (ANOVA); Bonferroni corrections preceded Student's *t*-test to obtain the level of significance. Kruskal–Wallis (KW) was used for non-parametric data analysis, combined with Tukey's *post hoc* test. Significance was accepted when $P \leq 0.05$. SGR data required arcsine transformation prior to analysis.

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

Results

Measured daily salinities during the experimental period showed little variation and were 13.8 ± 0.85, 18.2 ± 0.47, 22.1 ± 0.9, 25.9 ± 1.51 and 30.4 ± 1.03 g L^{-1}, and all significantly differed from each other. TAN and nitrite never surpassed 0.4 and 0.6 mg L^{-1} respectively. Dissolved oxygen was above 7 mg L^{-1} in all tanks, well above the recommended minimum of 5.7 mg L^{-1} (Nakada 2000) for yellowtail culture. Water pH remained above 7.8 in all tanks, above the level of pH 6.58 shown to negatively impact growth of yellowtail (Abbink *et al.* 2012).

At salinities of 14, 18 and 22 g L^{-1}, survival was 100%, and at 26 and 30 g L^{-1}, one fish died in each group (out of 60 fish; Table 1). The differences in survival were not significant ($P = 0.58$). Growth of juvenile yellowtail kingfish was significantly affected by salinity (Table 1). Growth rates of the fish at the three lower salinities (14 g L^{-1}: SGR = 7.61 ± 0.19% day^{-1}, 18 g L^{-1}: SGR = 7.61 ± 0.01% day^{-1} and 22 g L^{-1}: SGR = 7.61 ± 0.13% day^{-1}) did not differ but were significantly higher than growth rates at 26 and 30 g L^{-1} (SGR = 7.10 ± 0.05% day^{-1} and 6.97 ± 0.06% day^{-1}, respectively); growth rates at the latter two salinities were similar.

Feed intake was also affected by salinity. At the three lower salinities of 14 g L^{-1} (2.49 ± 0.13 g $fish^{-1}$ day^{-1}), 18 g L^{-1} (2.38 ± 0.03 g $fish^{-1}$ day^{-1}) and 22 g L^{-1} (2.41 ± 0.02 g $fish^{-1}day^{-1}$) intake was significantly higher than at 30 g L^{-1} (2.07 ± 0.08 g $fish^{-1}$ day^{-1}). Feed intake at 26 g L^{-1} (2.16 ± 0.07 g $fish^{-1}$ day^{-1}) differed only from the feed intake at 14 and 22 g L^{-1}.

No differences were found for FCR between groups ($P = 0.09$), with values ranging between 0.76 ± 0.02 at 18 g L^{-1} and 0.82 ± 0.04 at 30 g L^{-1}.

Na^+/K^+-ATPase activity at 14 g L^{-1} (0.59 ± 0.55 μmol P_i^{-1} h^{-1} mg^{-1} protein) was significantly lower compared with the activity at 30 g L^{-1} (1.72 ± 0.90 μmol P_i^{-1} h^{-1} mg^{-1} protein). Analyses of the gills showed no differences in the number of chloride cells in the gill epithelium and no migration of cells from the epithelium to the lamella (an indicator of stress) was observed (Fig. 1). In addition, no change was found for plasma osmolality ($P = 0.38$), whereas mild effects were observed for chloride and HCO_3^- concentrations, indicating some acid–base imbalance.

Lactate levels at 30 g L^{-1} (8.05 ± 0.85 mmol L^{-1}) were significantly higher than at 14 g L^{-1} (6.93 ± 0.87 mmol L^{-1}), 18 g L^{-1} (7.20 ± 0.72 mmol L^{-1}) and 22 g L^{-1} (6.76 ± 0.72 mmol L^{-1}). No effects were found for plasma glucose ($P = 0.50$), NEFA ($P = 0.35$) and pH ($P = 0.5$; Table 1).

Discussion

This study shows that reduced salinities (compared with seawater of 30 g L^{-1}) positively affect growth of juvenile yellowtail kingfish in RAS, with enhanced growth performance at 14, 18 and 22 g L^{-1} compared with 26 and 30 g L^{-1}. It appeared that only feed intake was improved at the lower salinities; only slight and not statistically significant improvements in feed conversion efficiency were seen at these salinities and that therefore may have contributed to the improved growth performance at these salinities. The fish blood physiology was normal and they acclimated well to the reduced salinities.

Exposure for 28 days to reduced salinities sufficed to demonstrate effects on growth and physiology in this species with its phenomenal growth rate. The salinities chosen (14, 18, 22, 26, 30 g L^{-1}) were at least isosmotic, and thus osmotic water efflux and ion influxes may be predicted, fluxes that fish counteract by drinking and branchial excretion of sodium and chloride (Evans 2008). Branchial Na^+/K^+-ATPase activity decreased at the lower salinity of 14 g L^{-1} (the ambient salinity closest to the isosmotic point), whereas the number of chloride cells and their position on the epithelial membrane was unaffected (Fig. 1). This is indicative of reduced ion pump activity per chloride cell and most likely reflects an adaptation of the chloride cell population to decreased transport need for sodium and chloride. The turnover rate of branchial chloride cells is estimated to a matter of 3–4 days during salinity adaptation (Uchida & Kaneko 1996; Dang, Lock, Flik & Wendelaar Bonga 2000). The duration of the present study would have allowed for multiple turnover cycles to adjust

Table 1 Mean (±standard deviation) for growth performance and physiological parameters of fish kept at different salinities for 4 weeks. SGR, specific growth rate; FCR, feed conversion ratio; NEFA, non-esterified fatty acids; NKA, Na^+/K^+-ATPase activity. Shapiro–Wilk test was used to test homogeneity of data. AN, one-way ANOVA, KW, Kruskal–Wallis and *P* values are given. As *post hoc* test, Bonferroni was used for one-way ANOVA and Tukey was used for Kruskal–Wallis. Values followed by different lower case letters express significant differences ($P \leq 0.05$) between treatments

Group	14 g L^{-1}	18 g L^{-1}	22 g L^{-1}	26 g L^{-1}	30 g L^{-1}
Mortality (of 60 fish per treatment)	0	0	0	1	1
Body mass prior salinity acclimatization					
AN; $P = 0.06$	8.37 ± 0.43	8.31 ± 0.42	8.42 ± 0.43	8.44 ± 0.38	8.36 ± 0.45
Initial body mass (g)					
AN; $P < 0.05$	12.22 ± 2^{a}	11.79 ± 1.79^{ab}	11.90 ± 2.7^{a}	11.96 ± 2.01^{bc}	11.62 ± 1.96^{c}
Final body mass (g)					
AN; $P < 0.05$	103.2 ± 15.8^{a}	99.3 ± 11.0^{ab}	100.2 ± 11.5^{ab}	87.4 ± 13.5^{bc}	81.9 ± 11.6^{c}
SGR (% day^{-1})					
AN; $P < 0.05$	7.61 ± 0.19^{a}	7.61 ± 0.01^{a}	7.61 ± 0.13^{a}	7.10 ± 0.05^{b}	6.97 ± 0.06^{b}
FCR					
AN; $P = 0.09$	0.77 ± 0.03	0.76 ± 0.02	0.77 ± 0.02	0.81 ± 0.04	0.82 ± 0.04
Feed intake (g $fish^{-1}$ day^{-1})					
AN; $P < 0.05$	2.49 ± 0.13^{a}	2.38 ± 0.03^{ab}	2.41 ± 0.02^{a}	2.16 ± 0.07^{bc}	2.07 ± 0.08^{c}
Glucose (mmol L^{-1})					
KW; $P = 0.42$	6.55 ± 1.03	7.03 ± 0.98	7.17 ± 1.19	7.01 ± 0.92	7.07 ± 0.79
Lactate (mmol L^{-1})					
AN; $P < 0.05$	6.93 ± 0.87^{a}	7.20 ± 0.72^{ab}	6.76 ± 0.72^{a}	7.29 ± 0.86^{ab}	8.05 ± 0.85^{b}
NEFA (mmol L^{-1})					
KW; $P = 0.35$	0.13 ± 0.03	0.12 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.12 ± 0.03
Osmolality (mOsmol kg^{-1})					
KW; $P = 0.38$	388.8 ± 15.7	389.3 ± 9.9	384.3 ± 10.1	393.0 ± 17.2	394.7 ± 10.8
NKA (μmol P_i^{-1} h^{-1} mg $protein^{-1}$)					
KW; $P < 0.05$	0.59 ± 0.55^{a}	0.85 ± 0.56^{abc}	0.7 ± 0.33^{ab}	1.21 ± 0.76^{abc}	1.72 ± 0.90^{c}
Na^+ (mmol L^{-1})					
AN; $P = 0.25$	188.6 ± 3.1	186.5 ± 3.7	185.8 ± 2.7	189.0 ± 5.4	188.1 ± 5.8
Cl^- (mmol L^{-1})					
AN; $P < 0.05$	233.3 ± 19.3^{ab}	235.9 ± 17.1^{a}	221.1 ± 23.00^{ab}	213.9 ± 15.2^{b}	215.0 ± 17.2^{b}
HCO_3^- (mmol L^{-1})					
KW; $P < 0.05$	8.96 ± 0.92^{a}	9.62 ± 0.87^{ab}	11.16 ± 1.43^{c}	10.34 ± 0.76^{bc}	10.07 ± 0.99^{abc}
pH					
AN; $P = 0.5$	7.56 ± 0.05	7.53 ± 0.04	7.57 ± 0.04	7.54 ± 0.04	7.53 ± 0.06

osmoregulatory mechanisms in the gills and thus save energy at the lower salinities. Such rapid turnover of the chloride cell population is well illustrated in a study by Roques, Abbink, Geurds, van de Vis and Flik (2010), who showed that chloride cells had migrated from the filamental epithelium to lamellar regions within 6 h after a tailfin clip, a supposedly pain and stressful stimulus that evokes rapid adaptive endocrine responses and replacement of existing chloride cells in the gills.

In addition to the physiological adaptations, the strong growth performance at reduced salinities (SGR of 7–7.6% day^{-1} and a factor 8 in body mass increase) further substantiates the notion state that a period of 28 days suffices for successful acclimation and solid and lasting effects on growth and osmoregulation in this species.

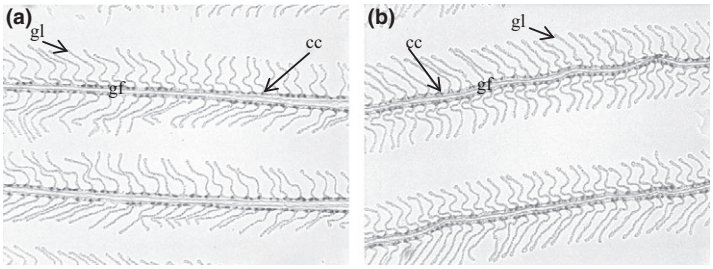

Figure 1 Details of two gill filaments (GF; horizontal structures) and the lamellae (gl, vertical structures) sampled from the outer gill arch of experimental fish reared at 14 L^{-1}(a) and 30 g L^{-1} (b). The samples were stained for chloride cells (CC), visible as dark cells in the filamental epithelium at the base of the lamellae. In case of chronic (osmoregulatory) stress, migration of cc from the filaments towards the lamella can occur. This was not observed in any of the sampled fish, indicating that the experimental conditions were not severely challenging for the fish.

Growth

The effects of salinity on growth of marine and estuarine fish vary widely among species. Some studies report better growth performance for gilthead sea bream (*Sparus aurata* Linnaeus 1758) at lower salinities (Laiz-Carrion, Sangiao-Alvarellos, Guzman, del Rio, Soengas & Mancera 2005) and for red porgy fry (*Pagrus pagrus* Linnaeus 1758) (Vargas-Chacoff, Calvo, Ruiz-Jarabo, Villarroel, Muñoz, Tinoco, Cardenas & Mancera 2011), others the opposite for dusky grouper (*Epinephelus marginatus* Lowe 1834) (Gracia López & Castelló-Orvay 2003) and for dusky kob (*Argyrosomus japonicas* Temminck & Schlegel 1843) juveniles (Bernatzeder, Cowley & Hecht 2010). Partridge and Jenkins (2002) found black bream (*Acanthopagrus butcheri* Munro 1949) juveniles to grow better at 24 g L^{-1} (also well above the isosmotic point) in a study where they tested a salinity range of 0–60 g L^{-1}.

Differences in salinity response with ontogeny seem to be species specific. Cardona (2000) observed higher growth rates in Mediterranean flathead grey mullet (*Mugil cephalus* Linnaeus 1758) in fresh water and low salinity waters (5–18 g L^{-1}); adults of this fish showed preference for higher salinities (18–30 g L^{-1}). Life stage dependence is species specific: studies of Imsland, Gustavsson, Gunnarsson, Foss, Arnason, Arnarson, Jonsson, Smaradottir and Thorarensen (2008) and Imsland, Gunnarsson, Asgeirsson, Kristjansson, Arnason, Jonsson, Smaradottir and Thorarensen (2010), showed Atlantic halibut (*Hippoglossus hippoglossus* Linnaeus 1758) grow best at intermediate/isosmotic salinities (15 g L^{-1}) independent of size and age. The ontogenetic differences in salinity tolerance of yellowtail kingfish juveniles indicate that generalization should be avoided and that such dependence needs to be assessed for every species studied.

Interrelationships between salinity, feed intake and FCR have been described for other marine and estuarine species. Lambert, Dutil and Munro (1994) and Imsland *et al.* (2010) found that in Atlantic cod (*Gadus morhua* Linnaeus 1758) and Atlantic halibut (*H. hippoglossus*), respectively, optimum growth performance at near isosmotic salinity resulted specifically from improved feed conversion, not from differences in feed intake. This is in contrast to our observations on *Seriola*; in this fish improved growth resulted from a higher feed intake. Both Partridge and Jenkins (2002) for black seabream and Klaoudatos and Conides (1996) for gilthead sea bream found optimal growth at 24–28 g L^{-1} as a result of a combination of improved feed conversion efficiency and feed intake.

Physiology

Our physiological analyses indicate that juvenile yellowtail kingfish possess a strong osmoregulatory capacity when exposed to reduced environmental salinity. No differences in plasma osmolality were observed in fish exposed for 28 days to various salinities. The smaller inward osmotic gradient at lower salinities requires less osmoregulatory actions of the fish (Morgan & Iwama 1991), and this we interpret to give room a redirection of energy flows to improve growth. This notion is corroborated by the strong decrease in the (energy consuming) branchial Na^+/K^+-ATPase activity at lower salinity.

The density of chloride cells in the gills was not affected by salinity. With a lower Na^+/K^+-ATPase

activity at lower salinity, it appears that new cells, with lower and less costly Na^+/K^+-ATPase expression levels, reflect the ambient salinity. Scaling down this activity seems an economical acclimation to lower environmental salinity. We did not observe migration of chloride cells to lamellar regions and this we take to indicate that these fish can healthily cope with the salinity challenges given in this study. In a variety of species exposed to stressful conditions, chloride cells will migrate to the lamellar epithelium (Roques *et al.* 2010). This phenomenon was not observed in the present study, underlining the robustness of this fish and its true euryhalinity.

Branchial Na^+/K^+-ATPase activity relates directly to Na^+ and Cl^- fluxes across gills (Flik, Kaneko, Greco, Li & Fenwick 1997). Several studies have shown how a decrease in Na^+/K^+-ATPase activity at reduced salinities is accompanied by a decrease in plasma Cl^- (Woo & Chung 1995; Foss, Evensen, Imsland & Oiestad 2001; Fielder, Allan, Pepperall & Pankhurst 2007; Partridge & Lymbery 2008) and an increase in Na^+ (Gaumet, Boeuf, Severe, Leroux & Mayergonstan 1995; Imsland *et al.* 2008). This relation was not found in the present study, where Cl^- concentrations were significantly higher at the lowest salinities and Na^+ values were unaffected. Future studies on branchial permeabilities and chloride transporter proteins should be undertaken to shed light on this apparent discrepancy.

The reduced salinities and subsequent increased feed intake did not result in higher values for glucose or NEFA. Glucose, NEFA, as well as lactate remained largely unaffected. The high basal levels for glucose and lactate reflect the highly active swimming behaviour of these fish. The stable levels also show that the fish were not stressed by the long-term exposure to reduced salinities; indeed, as reasoned above, the lower salinities do not seem to require increased energy availability, e.g. through hepatic glycogenolysis (Wendelaar Bonga 1997), once again supporting successful adaptation of the fish to the changed environment.

Conclusion and perspectives

Growth and physiological responses of juvenile yellowtail kingfish in RAS were studied in relation to ambient salinity. Enhanced growth performance (due to increased feed intake and SGR with a stable FCR) was seen at the reduced salinities (14, 18 and 22 g L^{-1} compared with 26 and 30 g L^{-1}) and this should assure and stimulate farmers to expand the range of salinities in which to culture this species. A robust osmoregulatory capacity is at the basis of low salinity tolerance and acclimation. Similar studies across a wider range of commercially relevant fish sizes seem warranted to examine whether shifts in salinity tolerance towards full-strength seawater occur in the more adult stages. It could be that energy-costly residence at sea in high salinity is a trade-off with ample food availability. Clearly in RAS such trade-off is not at stake.

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