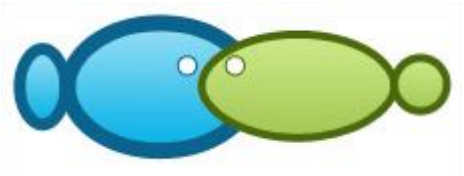

Combined effects of elevated salinity and temperature on growth performance and feed utilization in hybrid red tilapia fingerlings (*Oreochromis mossambicus* x *O. niloticus*)

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Abstract. The effects of elevated salinity (0, 6, 9 and 12 mg L^{-1}) and temperature (27-28°C, ambient; 31°C; and 34°C) on growth performance and feed utilization were investigated in hybrid red tilapia fingerlings (*Oreochromis mossambicus* x *O. niloticus*). The hybrid red tilapia fingerlings had the highest growth rate when reared in freshwater (0 mg L^{-1}) at ambient, 31°C, or 34°C or when reared in 6 mg L^{-1} at 31°C and at 34°C, 9 mg L^{-1} at 34°C, or 12 mg L^{-1} at 34°C. Rearing fingerlings in freshwater versus higher salinities (9 and 12 mg L^{-1}) or at higher temperatures did not impact survival rate; however, both feed intake and protein retention were significantly reduced at higher salinities compared to freshwater. Rearing fingerlings at 31°C and 34°C instead of at ambient temperature also increased feed intake significantly. Apparent digestibility coefficient (ADC) of feed in dry matter and proteins was significantly reduced in fingerlings reared in 12 mg L^{-1} compared to freshwater while neither ADC metric was affected by temperature. All other assessed indices were unaffected by elevated salinity, elevated temperature, or their interaction. We recommend that hybrid red tilapia fingerlings can be reared at a high temperature of 34°C with elevated salinities of 6-12 mg L^{-1}.
Key Words: digestibility, digestive enzymes, salinity, temperature, tilapia.

Introduction. Current climate change predictions include temperature rise (IPCC 2018) and salinity intrusion into freshwater ecosystems (Cloern & Jassby 2012). Such impacts of climate change on biological diversification have already been observed in the Mekong Delta in Vietnam (Tuan et al 2007). Elevated water temperature and salinity, alone and together, are considered to be the major environmental factors that influence fish growth and feed utilization (Imsland et al 2001). For example, fish growth performance and feed utilization both increased under reduced salinity and elevated temperature conditions for Nile tilapia (*Oreochromis niloticus*) (Likongwe et al 1996), juvenile turbot (*Scophthalmus maximus*) (Imsland et al 2001), and GIFT tilapia (*O. niloticus*) (Qiang et al 2013) whereas concurrent elevation of salinity and temperature inhibited growth performance in Nile tilapia (Likongwe et al 1996).

Hybrid red tilapia has been become a popular aquaculture species around the world, especially in Southeast Asia (Jayaprasad et al 2011). Red tilapia is a common hybrid between *O. mossambicus* and *O. niloticus* or *O. aureus* that exhibits rapid growth rate, elevated salinity tolerance, and high adaptability to most aquaculture systems (Pradeep et al 2014); however, the various hybrid red tilapia strains do not exhibit consistent responses to salinity and temperature conditions (Watanabe et al 1993; Pongthana et al 2010; Hassanen et al 2014; Barreto-Curiel et al 2015). For example, hybrid red tilapia (*O. mossambicus* × *O. aureus*) fingerlings reared in 34 mg L^{-1} seawater and freshwater for 90 days exhibited no significant differences in growth performance or biological indices (Barreto-Curiel et al 2015) while hybrid red tilapia (*O. niloticus* x *O. aureus*) fingerlings were reported to performed best at salinities up to 52 mg L^{-1} at 24 to 28°C (Hassanen et al 2014). Moreover, growth performance of hybrid red tilapia

(*Oreochromis* spp.) was observed to be significantly lower in saline compared to freshwater environments (Pongthana et al 2010) while Florida hybrid red tilapia (*O. urolepis hornorum* x *O. mossambicus*) were reported to exhibit an increase in feed consumption and growth rate at 18 mg L^{-1} compared to 0 or 36 mg L^{-1} salinity (Watanabe et al 1993). Taken together, the influence of temperature and salinity on feed utilization and growth performance appears to be linked to the specific genetic makeup of each hybrid red tilapia strain.

In the Mekong Delta, Vietnam, the average temperature is 27°C (Gasparrini et al 2017) but this is predicted to increase to 33°C in the 21st century (IPCC 2018). Between March and April approx. 1.3 million hectares are affected by saline water (> 5 mg L^{-1}) that intrudes 40-50 km inland from estuaries through the main river systems (Nhan et al 2012). The predominant red tilapia hybrid found in the Mekong Delta in Vietnam is a novel hybrid produced from the natural crossbreeding of *O. mossambicus* x *O. niloticus* hybrid red tilapia (Mather et al 2001). Given the potential for hybrid red tilapia to exhibit unique adaptability to both elevated temperature and salinity conditions (i.e., aquaculture amenability and climate change tolerance) we assessed the combined effects of elevated salinity and temperature on: (1) daily weight gain, (2) feed conversion ratio, (3) survival rate, (4) feed intake, (5) protein efficiency ratio, (6) protein retention, (7) biochemical composition indices (moisture, total ash, crude proteins, crude lipids), (8) feed digestibility (apparent digestibility coefficients), and (9) digestive enzyme abundance (intestinal α-amylase and trypsin).

Material and Method

Growth experiment. All fingerlings (10-11 g initial weight; *n* = 2000 total) were transferred to the wet laboratory at Can Tho University to acclimate in 0.5 m^3 tanks for 2 weeks at ambient temperature (27-28°C). Obtained fingerlings were the progeny of wild crossbred *Oreochromis mossambicus* x *O. niloticus* hybrid red tilapia; therefore, given their unknown parentage, the hybrid red tilapias used in this study are referred to as hybrid red tilapia. The hybrid red tilapia fingerlings were fed a tilapia commercial pellet to satiation twice daily (8:00 and 16:00) during 2-week acclimation period.

To ensure that the growth experiment utilized the correct salinity and isosmotic conditions both salinity tolerance and isosmotic point were determined for the hybrid red tilapia fingerlings using 0, 4, 8, 12, 16, 20, and 24 mg L^{-1} at ambient temperature (27-28°C). The hybrid red tilapia exhibited no mortality in salinity up to 26 mg L^{-1} under ambient temperature; however, mortality reached 50% at 29 mg L^{-1} under ambient temperature. Regression analysis of serum osmolality at 0, 4, 8, 12, 16, 20, and 24 mg L^{-1} revealed an isosmotic point of 12 mg L^{-1}. Salinities of 0, 6, 9 and 12 mg L^{-1} were used in the growth experiment as these equated to 0, 50, 75 and 100% of isosmotic point values, respectively.

Following acclimation, fingerlings (10.9±0.57 g) were randomly selected and assigned to one of the following aerated triplicate 150L treatment tanks to a final stocking density of 30 fingerlings per tank (*n* = 1080 total fingerings across 36 treatment tanks): (1) 0 mg L^{-1} at ambient temperature (27-28°C), (2) 6 mg L^{-1} at ambient temperature, (3) 9 mg L^{-1} at ambient temperature, (4) 12 mg L^{-1} at ambient temperature, (5) 0 mg L^{-1} at 31°C, (6) 6 mg L^{-1} at 31°C, (7) 9 mg L^{-1} at 31°C, (8) 12 mg L^{-1} at 31°C, (9) 0 mg L^{-1} at 34°C, (10) 6 mg L^{-1} at 34°C, (11) 9 mg L^{-1} at 34°C, and (12) 12 mg L^{-1} at 34°C. For each treatment tank water temperature and salinity were adjusted at the rate of 1°C and 2 mg L^{-1} per day, respectively, until the desired water conditions were obtained (Selong et al 2001). Water (30-50%) in each treatment tank was exchanged with water prepared at the same temperature and salinity every three days. The fingerlings were fed tilapia commercial pellet to satiation twice daily (8:00 and 16:00). The dry commercial pellets fed to fingerlings contained 36% crude protein, 5.6% crude lipid, 10.9% total ash, 19.1 KJ g^{-1}, and 1.2 mm in size. The experiment lasted for 59 days.

The amount of feed provided, excess feed recovered, and weight of removed dead fingerlings were recorded daily throughout the experiment. Salinity was measured once

daily using a Master Refractometer (Atago, Japan) and nitrite (NO_2^-) and total ammonia nitrogen (TAN) were measured once daily using a Sera test kit (Heinsberg, Germany). Dissolved oxygen (DO), temperature and pH were measured twice daily (at 9 am and 5 pm) by Hanna pH/DO/temperature tester. Heaters in the 31 and 34°C treatment tanks were only run from 8 am to 6 pm daily to mimic the natural daily increase in daytime temperature.

Initial mean weight (W_i) and final mean weight (W_f) were determined for each fingerling before and after the experiment, respectively. Survival rate (SR, %), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR) were calculated using the following formulas (where t = time in days):

$$SR\ (\%) = (\text{number of fish at the end of experiment})/(\text{number of initial fish}) \times 100$$

$$DWG\ (g\ day^{-1}) = (W_f - W_i)/t$$

$$FI\ (\%\ fish^{-1}\ day^{-1}) = 100 \times \text{consumed feed}\ /[(Wi + Wf)/2 \times t]$$

$$FCR = \text{amount of consumed dry feed/weight gain}$$

$$PER = (W_f - W_i)/\text{protein intake}$$

$$PR = (\text{protein of final fish} - \text{protein of initial fish})/\text{protein intake}$$

Initial fish (10 fish) and final fish (10 fish $tank^{-1}$) were collected from each treatment tank, euthanized by ice slurry (5 min), minced, and stored at -20°C until chemical composition analyses (e.g., moisture, crude protein, crude lipid, and total ash; AOAC 2016). Experimental diets (100 g) were also subjected to the same chemical composition analyses.

Digestibility experiment. The same stocking density, feeding regime, water quality monitoring, and experimental design (triplicate tanks for each of the 12 discrete water condition treatments) were used as described above for the growth experiment except that larger (40-45 g) hybrid red tilapia and modified experimental feed were used.

Experimental feed was modified by grinding, supplementing with 1% chromic oxide (Cr_2O_3), pelleting by using mini pelleting machine (Can Tho University, Vietnam), and then drying in oven (60°C, ≈10 hours) until moisture content reached ≈10%. Feces collection began after 7 days of feeding the modified experimental feed, when feces amount was sufficient for analysis (≈10 g dry matter), and continued for 21 days. More specifically, fish were fed once at 8:00 then uneaten feed was removed at 9:00, tank cleaning was done onward and feces was collected at 15:00 using small hand net (10 cm x 10 cm; 1 mm mesh size). Collected feces were washed with distilled water, dried at 60°C, and stored at -20°C until contents analyses were performed.

Feces and experimental feed were analyzed for crude protein, crude lipid, crude ash and moisture following AOAC (2016). The gross energy was calculated based on the total energy from crude protein, crude lipid, and total carbohydrate in the samples. Chromic oxide (Cr_2O_3) was analyzed following the method of Furukawa & Tsukahara (1966). Apparent digestibility coefficient of diet (ADC_{diet}) and coefficient of nutrients in diet ($ADC_{Nu\text{-}diet}$) were determined using the following formulas:

$$ADC\ of\ diet\ (ADCdiet, \%) = 100 - \{(\%Cr2O3\ in\ diet)/\%\ Cr2O3\ in\ feces)\}$$

$$ADC\ of\ nutrient\ in\ diet\ (ADCNu\text{-}Diet, \%) = 100 - \{(\%Cr2O3\ in\ feed)/\%\ Cr2O3\ in\ feces) \times (\%\ nutrient\ in\ feces)/\%\ nutrient\ in\ diet)\}$$

At the end of the experiment, three fish per tank were collected after one day without feeding for digestive enzyme analyses on intestinal tissue samples. Intestinal tissue samples were excised from each fish after being killed by putting them into ice water for 30 minutes. All visible fat was removed from each intestinal tissue sample using

a scalpel before homogenization in buffer (20 mM KH_2PO_4 and 6 mM NaCl; pH 6.9), centrifugation (4200 rpm for 30 min), supernatant retention, and storage at -80°C. Analysis of intestinal α-amylase followed Bernfeld (1955) while analysis of intestinal trypsin activity followed Worthington (1982) as described in Babaei et al (2011).

Data analysis. All data were checked for normal distribution using the One-Sample Kolmogorov-Smirnov test and on the homogeneity of variances using Levene's test. Two-way ANOVA analyses were used to determine the effect of temperature, salinity, and their interaction (temperature and salinity as fixed factors). Relevant pairwise comparisons were assessed for statistical significance using Tukey HSD post-hoc. All statistical analyses were performed in SPSS (version 16.0, IBM, USA) using an alpha value of 0.05.

Ethic statement. Based on the National Regulations for the Use of Animals in Research in Vietnam, the hybrid red tilapia is not listed in either of the two groups, IB (endangered and critically endangered species) and IIB (threatened and rare species) (Decree 32/2006/ND-CP 2006). Therefore, this study did not require a permit or ethical approval.

Results

Growth experiment. Across the 59 days experiment salinity reached the desirable values by adjudgment daily while NO_2^- and TAN levels ranged between 0.72-0.84 mg L^{-1} and 1.3-2.1 mg L^{-1}, respectively. DO and pH ranged between 4.53-5.11 mg L^{-1} and 6.5-7.1, respectively. The ambient temperature treatment tanks ranged between 26.8-27.1°C in the morning and 28.2-28.6°C in the afternoon, respectively, while the 31°C and 34°C temperature treatment tanks ranged between 27.2-27.5°C and 27.8-28.1°C in the morning and between 30.8-31.6°C and 33.3-34.0°C in the afternoon, respectively.

There was no significant difference in initial body weight across all 12 treatments ($p > 0.05$); however, both daily weight gain (DWG) and feed conversion ratio (FCR) were significantly affected by the interaction effect between salinity and temperature ($p < 0.05$) (Table 1). The 0 mg L^{-1} treatments exhibited no differences in DWG or FCR. The 6 mg L^{-1} at ambient treatment exhibited significantly lower DWG than the 6 mg L^{-1} at 31°C and 34°C treatments whereas there was no significant difference in FCR between 6 mg L^{-1} treatments. The 9 mg L^{-1} at ambient and 31°C treatments exhibited significantly lower DWG and higher FCR than the 9 mg L^{-1} at 34°C treatment; however, the 9 mg L^{-1} at ambient and 9 mg L^{-1} at 31°C treatments did not differ significantly from one another. The 12 mg L^{-1} at ambient and 31°C treatments exhibited significantly lower DWG than the 12 mg L^{-1} at 34°C treatment whereas there was no significant difference in FCR between 12 mg L^{-1} treatments.

The hybrid red tilapia exhibited the highest growth rates (final weight and daily weight gain) in the 0 mg L^{-1} salinity at ambient, 31°C, and 34°C thermal conditions followed by the 6 mg L^{-1} at 31°C, 6 mg L^{-1} in 34°C, 9 mg L^{-1} at 34°C, and 12 mg L^{-1} at 34°C treatments. Fish growth performance was significantly reduced in the following high salinity and low temperature treatments: 6 mg L^{-1} at ambient, 9 mg L^{-1} at ambient, 9 mg L^{-1} at 31°C, 12 mg L^{-1} at ambient, and 12 mg L^{-1} at 31°C ($p < 0.05$). The FCR observed in the 9 mg L^{-1} at 31°C and 9 mg L^{-1} at ambient temperature treatments was significantly higher than FCR values observed in all other treatments ($p < 0.05$); however, FCR values did not differ significantly between the 9 mg L^{-1} at 31°C and 9 mg L^{-1} at ambient temperature treatments ($p > 0.05$).

Table 1

Daily weight gain (DWG) and feed conversion ratio (FCR) of hybrid red tilapia fingerlings reared under different salinity (mg L^{-1}) and temperature combinations

Treatment	*Initial weight (g)*	*Final weight (g)*	*DWG (g day^{-1})*	*FCR*
0 mg L^{-1} at ambient	10.7±0.2	61.3±2.69^{c}	0.86±0.05^{b}	1.16±0.05^{ab}
0 mg L^{-1} at 31°C	10.8±0.1	62.3±4.86^{c}	0.87±0.08^{b}	1.16±0.08^{ab}
0 mg L^{-1} at 34°C	10.8±0.1	62.8±5.83^{c}	0.88±0.10^{b}	1.13±0.05^{a}
6 mg L^{-1} at ambient	10.9±0.1	47.4±2.0^{ab}	0.62±0.04^{a}	1.28±0.04^{abc}
6 mg L^{-1} at 31°C	10.8±0.1	54.4±5.61^{bc}	0.74±0.10^{b}	1.34±0.14^{c}
6 mg L^{-1} at 34°C	10.9±0.1	58.9±5.01^{c}	0.81±0.08^{b}	1.32±0.1^{bc}
9 mg L^{-1} at ambient	10.6±0.1	42.0±0.22^{a}	0.53±0^{a}	1.59±0.16^{d}
9 mg L^{-1} at 31°C	10.6±0.1	40.9±2.81^{a}	0.52±0.05^{a}	1.53±0.10^{d}
9 mg L^{-1} at 34°C	10.7±0.2	59.9±3.49^{c}	0.84±0.06^{b}	1.24±0.06^{abc}
12 mg L^{-1} at ambient	10.8±0.2	44.7±1.22^{a}	0.58±0.03^{a}	1.29±0.01^{abc}
12 mg L^{-1} at 31°C	10.7±0.1	49.2±7.13^{ab}	0.65±0.12^{a}	1.32±0.17^{bc}
12 mg L^{-1} at 34°C	10.7±0.2	54.2±5.32^{bc}	0.74±0.09^{b}	1.26±0.05^{abc}
P (temperature x salinity)	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Data are presented as mean±standard deviation. Different letters in the same column represent significant difference ($p < 0.05$).

Survival rate (SR), feed intake (FI), protein efficiency ratio (PER), and protein retention (PR) indices were not significantly affected by the interaction between salinity and temperature in the hybrid red tilapia ($p > 0.05$) (Table 2). When all three temperatures were considered collectively for each salinity treatment there was no significant difference in PER ($p > 0.05$) while significant differences were observed for SR, FI, and PR ($p < 0.05$). More specifically, SR was significantly higher in the 12 mg L^{-1} treatment than the 9 mg L^{-1} treatment ($p < 0.05$) but not significantly different between the 0, 6, and 9 mg L^{-1} treatments or the 0, 6, and 12 mg L^{-1} treatments ($p > 0.05$). FI was significantly higher in the 0mg L^{-1} treatment than in the 9 and 12 mg L^{-1} treatments ($p < 0.05$) but not the 6 mg L^{-1} treatment ($p > 0.05$) while there was no significant difference in FI between the 6, 9, and 12 mg L^{-1} treatments ($p > 0.05$). PR was significantly higher in the 0 mg L^{-1} treatment than in the 6, 9, and 12 mg L^{-1} treatments ($p < 0.05$) while the 6 mg L^{-1} treatment also exhibited a significantly higher PR than the 9 and 12 mg L^{-1} treatments, which did not differ from each other ($p > 0.05$). When all four salinities were considered collectively for each temperature treatment there was no significant difference in SR, PER, or PR indices ($p > 0.05$); however, there was a significant increase in FI between each temperature treatment ($p < 0.05$).

Table 2

Survival rate (SR), feed intake (FI), protein efficiency ratio (PER), and protein retention (PR) of hybrid red tilapia fingerlings reared under different salinity and temperature combinations

Salinity (mg L^{-1})	*SR (%)*	*FI (g $fish^{-1}$ day^{-1})*	*PER*	*PR*
0	89.6±9.00^{ab}	0.98±0.05^{b}	2.34±0.10	86.0±4.72^{c}
6	88.5±9.00^{ab}	0.92±0.13^{ab}	2.03±0.18	75.2±10.3^{b}
9	86.7±7.00^{a}	0.82±0.16^{a}	1.87±0.29	68.3±13.9^{a}
12	93.3±9.00^{b}	0.86±0.13^{a}	2.09±0.16	66.0±5.92^{a}
P (salinity)	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$
Temperature (°C)				
Ambient	88.2±6.56	0.79±0.11^{a}	2.05±0.27	73.8±11.9
31	90.9±4.96	0.89±0.12^{b}	2.06±0.29	71.9±12.8
34	90.0±5.13	1.00±0.10^{c}	2.17±0.18	76.4±11.5
P (temperature)	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
P (temperature x salinity)	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

Data are presented as mean±standard deviation. Different letters in the same column denote a significant difference ($p < 0.05$).

None of the assessed biochemical composition indices (moisture, total ash, crude lipids, and crude proteins) were affected by salinity, temperature, or their interaction ($p > 0.05$) (Table 3).

Table 3

Biochemical composition indices of hybrid red tilapia fingerlings reared under different salinity and temperature combinations

Salinity (mg L^{-1})	*Moisture (%)*	*Total ash (%)*	*Crude lipids (%)*	*Crude proteins (%)*
0	74.7±1.01	4.0±0.25	7.8±0.49	13.0±0.69
6	74.4±2.07	4.1±0.47	7.6±0.73	13.3±1.12
9	74.1±2.53	4.0±0.42	7.9±1.26	14.0±1.36
12	73.6±1.42	4.0±0.39	8.4±0.62	12.6±1.04
P (salinity)	p > 0.05	p > 0.05	p > 0.05	p > 0.05
Temperature (°C)				
Ambient	74.4±2.15	4.0±0.29	7.6±1.12	13.3±1.27
31	74.6±1.77	3.9±0.41	8.0±0.8	12.9±0.91
34	73.7±1.53	4.1±0.43	8.2±0.44	13.3±1.26
P (temperature)	p > 0.05	p > 0.05	p > 0.05	p > 0.05
P (temperature x salinity)	p > 0.05	p > 0.05	p > 0.05	p > 0.05

Data are presented as mean±standard deviation.

Digestibility experiment. Across the 30-day experiment, DO and pH ranged between 4.62-5.39 mg L^{-1} and 7.1-7.6, respectively. The ambient temperature treatment tanks ranged between 27.0 and 27.2°C in the morning and 28.0-28.2°C in the afternoon, respectively, while the 31°C and 34°C temperature treatment tanks ranged between 27.1-27.6°C and 27.0-27.6°C in the morning and between 31.0-31.4°C and 34.0-34.4°C in the afternoon, respectively.

Apparent digestibility coefficient (ADC) of feed in dry matter, crude protein, and crude lipids were not significantly affected by the interaction between salinity and temperature in the hybrid red tilapia ($p > 0.05$) (Table 4). When all three temperatures were considered collectively for each salinity treatment there was no significant difference in ADC of crude lipids ($p > 0.05$) while significant differences were observed for ADC of dry matter and crude protein ($p < 0.05$). More specifically, ADC of dry matter and protein were significantly higher in the 0 mg L^{-1} treatment than the 12 mg L^{-1} treatment ($p < 0.05$) but not significantly different between the 0, 6, and 9 mg L^{-1} treatments or the 6, 9, and 12 mg L^{-1} treatments ($p > 0.05$). When all four salinities were considered collectively for each temperature treatment there was no significant difference in ADC of dry matter, crude protein, or crude lipids ($p > 0.05$).

Table 4

Apparent digestibility coefficient (ADC; %) of feed in dry mater, crude proteins, and crude lipids of hybrid red tilapia fingerlings reared under different salinity and temperature combinations

Salinity (mg L^{-1})	*ADC dry matter (%)*	*ADC proteins (%)*	*ADC lipids (%)*
0	$76.3±1.46^{b}$	$86.0±1.12^{b}$	90.3±1.20
6	$75.2±1.76^{ab}$	$84.9±1.44^{ab}$	90.9±2.37
9	$74.4±1.74^{ab}$	$84.8±1.68^{ab}$	90.1±4.62
12	$74.0±2.26^{a}$	$83.7±1.82^{a}$	91.9±3.01
P (salinity)	p < 0.05	p < 0.05	p > 0.05
Temperature (°C)			
Ambient	75.0±1.87	85.1±2.19	90.8±2.01
31	75.7±1.4	85.2±1.13	90.9±2.75
34	74.5±2.4	84.3±1.60	90.7±4.29
P (temperature)	p > 0.05	p > 0.05	p > 0.05
P (temperature x salinity)	p > 0.05	p > 0.05	p > 0.05

Data are presented as mean±standard deviation. Different letters in the same column in each salinity or temperature represent significant difference ($p < 0.05$).

Intestinal α-amylase (mU mg^{-1} protein) and trypsin (mU mg^{-1} protein) were not affected by salinity, temperature, or their interaction ($p > 0.05$) (Table 5). While not statistically significant, both α-amylase and trypsin levels were observed to be highest at 31°C regardless of salinity.

Table 5

Intestinal α-amylase (mU mg^{-1} protein) and intestinal trypsin (mU mg^{-1} protein) of hybrid red tilapia fingerlings reared under different salinity and temperature combinations

Salinity (mg L^{-1})	*Intestinal α-amylase (mU mg^{-1} protein)*	*Intestinal trypsin (mU mg^{-1} protein)*
0	51.1±13.2	32.1±12.0
6	60.2±14.6	34.9±9.26
9	63.7±20.5	26.2±4.64
12	71.3±23.8	27.6±4.92
P (salinity)	p > 0.05	p > 0.05
Temperature (°C)		
Ambient	60.0±20.5	29.7±6.78
31	71.4±20.3	33.8±10.1
34	53.3±12.6	27.0±8.09
P (temperature)	p > 0.05	p > 0.05
P (temperature x salinity)	p > 0.05	p > 0.05

Data are presented as mean±standard deviation.

Discussion. The novel hybrid red tilapia strain used in this study exhibited an isosmotic point of 12 mg L^{-1} and is, thus, a freshwater species. Fingerlings reared in 12 mg L^{-1} at 34°C had significantly higher daily weight gain (0.74±0.09) compared to fingerlings reared at ambient and 31°C (0.58±0.03 and 0.65±0.12), respectively. This could be due to the reduction in required energy when fingerlings are reared at their species-specific isosmotic point. For example, Kang'ombe & Brown (2008) revealed that less energy was required to maintain ion balance in redbreast tilapia (*T. rendalli*) when reared in an isosmotic environment due to the minimal ionic gradient between extracellular fluid and water. This is further supported by more recent studies that demonstrated the optimal performance of Nile tilapia (*O. niloticus*) and a Sub-Antarctic Notothenioid fish (*Eleginops maclovinus*) when reared under isosmotic conditions (Hassan et al 2013; Vargas-Chacoff et al 2015). The significantly higher daily weight gain observed for fingerlings reared in freshwater and 34°C treatments correspond with the significantly higher feed intake values observed for these treatments (Table 2), which could be due to more efficient digestion and nutrient absorption under ideal conditions (Musuka et al 2009). Overall, these findings agreed with previous observations that rearing tilapia in freshwater at elevated temperatures can maximize growth (Wanatabe et al 1993; dos Santos et al 2013).

Rearing the hybrid red tilapia fingerlings in non-freshwater conditions significantly reduced daily weight gain compared to fingerlings reared in freshwater except when reared at 31°C (6 mg L^{-1}) or 34°C (6 mg L^{-1}, 9 mg L^{-1}, and 12 mg L^{-1}) (Table 1). This suggests that daily weight gain (i.e., growth performance) of the hybrid red tilapia fingerlings is inversely corelated with salinity, which is consistent with the findings of previous studies on Nile tilapia (Likongwe et al 1996), juvenile turbot (Imsland et al 2001), hybrid red tilapia (Pongthana et al 2010), and GIFT tilapia (Qiang et al 2013). The high metabolic cost of osmoregulation (up to 10% of total energy utilization) could underlie the negative impact of elevated salinity on growth performance (Bœuf & Payan 2001; Sardella & Brauner 2008; Vargas-Chacoff et al 2015). This is further supported by the observation that Malaysian red tilapia reared in low salinity at low temperature (9.5°C) significantly up-regulated T-complex protein (TCP-1) mRNA expression while Malaysian red tilapia reared in mild and moderate salinity (5-15 mg L^{-1}) at low temperature (9.5°C) significantly down-regulated TCP-1 gene expression (He et al 2017).

Survival rate (SR) of the hybrid red tilapia fingerings was significantly highest for fingerlings reared in 12 mg L^{-1}; however, there was no difference in SR between fingerlings reared in 12 mg L^{-1} and freshwater (Table 2). A similar result (Mian & Siddiqui 2020) was also found in hybrid tilapia (*O. mossambicus x O. niloticus*), the survival rate tended to increase with the increase of water salinity but decreased at high salinity (35 mg L^{-1}). Conversely, feed intake (FI) and protein retention (PR) of fingerlings reared in 9 mg L^{-1} and 12 mg L^{-1} were significantly reduced compared to fingerlings reared in freshwater (Table 2). SR, FI, and PR all increased with fingerling rearing temperature but only FI was significantly different between temperature treatments (Table 2). Concurrent reduction in FI and PR with increasing salinity has been previously observed in Atlantic halibut (Imsland et al 2001) and snakehead (Lan et al 2020). The feed appetite is influenced by salty taste leading to less feed intake could be a reason. The decrease of feed intake with the rise of water salinity was found in many studies, freshwater catfish *Clarias batrachus* (Sahoo et al 2003), and common carp *Cyprinus carpid* (Wang et al 1997). However, the opposite effect of salinity on the feed intake happened in marine species. The high feed intake was reported in high water salinity, e.g., sole (Vinagre et al 2007), hybrid grouper *Epinephelus fuscoguttatus*× *E. lanceolatus* (Noor et al 2018). There is a relationship between feed intake and fish osmoregulation. Vinagre et al (2007) explained that the fish would drink more water in high water salinity, leading to increased chyme fluid movement, increasing the evacuation rate, and increasing the feed intake.

Neither salinity nor temperature had a significant effect on any of the measured biochemical composition indices, i.e., moisture, total ash, crude lipids, and crude proteins (Table 3). The effect of water temperature and salinity on the fish body compositions is depending on fish species. In this study, the moisture content tended to reduce with the increase of water salinity. It was consistent with the study on spotted catfish (Xu et al 2020), silver pompano *Trachinotus blochii* (Hamed et al 2016). Hamed et al (2016) explained that marine species in low water salinity drank more water to maintain the osmotic leading to the increase of moisture content of the fish body, and freshwater species would converse. The other chemical compositions (protein, lipid, and ash) did not show any effect of salinity. Mandal et al (2020) reported that striped catfish cultured in water salinity from 0 to 14 mg L^{-1} did not express any difference in body chemical compositions. In hybrid tilapia *Oreochromis mossambicus* x *Oreochromis niloticus* cultured in water salinities from 0 to 35 mg L^{-1} did not show any difference in fish muscle protein and lipid (Mian & Siddiqui 2020). Related to the effect of water temperature or the combined effect of water temperature and salinity on the fish body compositions, GIFT tilapia cultured in different water temperatures showed no effect on the fish body protein and ash (Zeng et al 2020).

Apparent digestibility coefficient (ADC) of feed in dry matter and proteins was significantly reduced in fingerlings reared in 12 mg L^{-1} compared to freshwater whereas no effect was observed for salinity on ADC of crude lipids nor temperature on all three ADC metrics (Table 4). The activity of measured digestive enzymes (intestinal α-amylase and trypsin) was not affected by salinity, temperature, or their interaction (Table 5). The effect of salinity on intestinal digestive enzymes of fish depends on species. Consistent with this observation, Fang & Chiou (1989) reported a lack of protease activities in tilapia exposed to various salinities. Lan et al (2020) and Huong et al (2020) reported the activity of intestinal α-amylase and chymotrypsin of snakehead was not different in different water salinities. However, many studies revealed that the activity of intestinal digestive enzymes decreased with the increase of water salinity, e.g., in sparid *Sparus aurata* (Moutou et al 2004; Woo & Kelly 1995). The reduced digestive enzyme activity could lead to reducing in digestibility, e.g., common carp *Cyprinus carpio* (Wang et al 1997), milkfish *Chanos chanos* (Ferraris et al 1986). However, the decrease of ADC of feed when the water salinity increases could be affected by various factors, e.g., osmoregulation, drinking water, chyme fluid movement.

Conclusions. This is the first study conducted on the hybrid red tilapia on the effects of high water temperature and salinity on growth performance and digestibility. We observed that the hybrid red tilapia fingerlings had the highest growth rate (daily weight gain) when reared in freshwater at ambient, 31°C, or 34°C or when reared in water salinity of 6 mg L^{-1} at 31°C, 6 mg L^{-1} at 34°C, 9 mg L^{-1} at 34°C, or 12 mg L^{-1} at 34°C. Rearing fingerlings in freshwater versus higher salinities (9 mg L^{-1} and 12 mg L^{-1}) did not significant alter survival rate; however, both feed intake and protein retention were significantly reduced. Rearing fingerlings at 31°C and 34°C instead of at ambient temperature significantly increased feed intake. Apparent digestibility coefficient of feed in dry matter and proteins was significantly reduced in fingerlings reared in 12 mg L^{-1} compared to freshwater.

Protein efficiency ratio, biochemical composition indices (moisture, total ash, crude proteins, crude lipids), ADC of lipids, and activity of digestive enzymes (intestinal α-amylase and trypsin) were all unaffected by salinity, temperature, or their interaction. Future studies should aim to elucidate the effects of elevated salinity and temperature as well as their combined effect on hybrid red tilapia reproduction capabilities given the importance of this wild hybrid for aquaculture.

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