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ORIGINAL ARTICLE

Aquaculture

Salinization intensifies the effects of elevated temperatures on *Channa striata*, a common tropical freshwater aquaculture fish in the Mekong Delta, Vietnam

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Abstract
Increasing water temperatures and salinity intrusion due to climate change are serious challenges for freshwater aquaculture. In this study, we assessed the combined effects of salinization at 0, 6, and 9‰ and water temperatures of 28, 31, and 34 °C on the survival and growth rates, and feed utilization, of freshwater snakehead *Channa striata*. We also assessed feed digestibility in snakeheads to explore their physiological mechanisms. The lowest survival rate (66.7%) was found at 34 °C-9‰. The highest daily weight gain of 0.72 g/day was found at 34 °C-0‰, while the second highest (0.62 g/day) was at 31 °C-0‰. Chymotrypsin activity increased at higher temperatures; the activities of α-amylase and pepsin were lowest at 34 °C-9‰. The highest apparent digestibility coefficients (ADCs) for dry matter (80.3%), protein (95.3%), and lipid (97.9%) were observed at 31 °C-0‰ ($p < 0.05$). Elevated temperature (34 °C) combined with high salinity (9‰) was lethal for one-third of the fish. The lowest ADC and α-amylase activities led to the highest feed-conversion ratio. Snakehead growth rates were highest in the 31 °C-0‰ and 34 °C-0‰ treatments. The combined highest salinity and highest temperature treatment (34 °C-9‰) decreased snakehead growth, feed utilization efficiency, feed digestibility, and enzyme activities (except for chymotrypsin).

Keywords Snakehead · *Channa striata* · High temperature · Salinity levels · Digestibility · Feed utilization

Introduction

Global climate change is predicted to have an impact on biological diversity in certain regions of the world, e.g., in the Mekong Delta, Vietnam (Tuan et al. 2007). Current predictions of the effects of climate change include a rise in global temperatures (Stocker et al. 2013) and sea levels (Meehl et al. 2005), followed by an increase in the salinity of certain estuaries worldwide and salinity intrusion into freshwater ecosystems (Cloern and Jassby 2012). The impact of seawater intrusion on farmed freshwater species and on ecosystems is poorly understood, especially when it is combined with another stressor such as elevated temperature. Water temperature and salinity, either singly or combined, especially at elevated levels, are considered the major environmental factors that control food utilization at all stages of fish growth. The effects of combined salinity and temperature on fish growth performance and feed utilization have been well documented for various species. Fish performance and feed utilization efficiency increased with decreasing salinity at higher temperatures in Nile tilapia (Likongwe et al. 1996), juvenile turbot (Imsland et al. 2001, 2003), and genetically improved farmed tilapia (GIFT) (Qiang et al. 2013), whereas high salinity in combination with high temperature inhibited fish growth performance in Nile tilapia (Likongwe et al. 1996).

Snakehead (*Channa striata*) has become an important cultured freshwater fish species in recent years in the Mekong Delta. In the past decade, snakehead aquaculture has increased rapidly, e.g., in 2010, the total production of snakehead in the Mekong Delta was estimated at

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40,000 tons (Sinh and Chung 2011). The fish are reared in earthen ponds that are directly supplied with water from branches of the Mekong River. The adaptation of snakeheads to increased salinity and temperature has not been well studied. Huong and Trinh (2013) reported the effect of salinity on the osmotic regulation and growth performance of snakeheads. Snakeheads grew well at salinity levels below 10‰ despite stress induction and elevated energy consumption for branchial Na^+/K^+-ATPase activities (Nakkrasae et al. 2015; Amornsakun et al. 2017). Huong and Trinh (2013) also reported that snakehead growth rates were significantly lower at 9‰ salinity as compared to those at 0 and 3‰ salinity. Qin and Fast (1998) reported doubled growth rates in snakeheads cultured at 27 °C compared to those cultured at 21.7 °C.

In the Mekong Delta, the average temperature recorded from 2009 to 2013 was 27 °C (Gasparrini et al. 2017), and is predicted to increase to 33 °C in the following years of the twenty-first century [Intergovernmental Panel on Climate Change (IPCC) 2014]. With a salinity level of above 5‰, approximately 1.3 million ha of the delta is affected by saline water. Between March and April, saline water intrudes up to 40–50 km inland through estuaries of the main river systems (Nhan et al. 2012). In 2016, it was reported that mortality increased by up to 35% in stocked snakeheads in Tra Vinh province, a coastal province of the Mekong Delta where 8–10‰ salinity was recorded in combination with high temperatures in March and April of that year (Tra Vinh Province Report 2017). Quyen et al. (2016) reported that snakehead seed production in the Mekong Delta had to be suspended to deal with the increase in water salinity and high temperatures caused by climate change in the region. Study of the combined effects of water temperature and salinity on snakeheads should provide useful information on their adaption to climate change, and for their aquaculture in the Mekong Delta. Thus, in this study we assessed the combined effects of water temperature and salinity on feed digestibility, digestive enzyme activity, fish performance, and feed utilization in snakehead fingerlings.

Materials and methods

Specimen collection

We obtained 2000 snakehead fingerlings with an initial body weight of 5–6 g from a commercial hatchery in An Giang province, and transferred them to a wet laboratory at Can Tho University, where they were acclimated in two 4-m^3 tanks for 2 weeks. The fish were fed twice a day (at 0800 and 1600 hours) to satiation during the acclimatization period.

Growth experiment

This experiment was conducted to evaluate the growth and feed utilization of snakehead fingerlings reared in different water conditions, i.e., under combinations of three different water temperatures (an unadjusted water temperature of 27–28 °C, and adjusted water temperatures of 31 and 34 °C) and levels of salinity (0, 6, and 9‰). The experiment included nine treatments, and each treatment was carried out in triplicate. The snakehead fingerlings were fed with commercial pellets (AquaExcell; Cargill Vietnam) containing 45.6% crude protein, 6.9% crude lipid, 13.0% total ash, and 4340 kcal/kg from dried matter with a moisture content of 8.1%.

Fish (6.53 ± 0.09 g) were selected and randomly assigned to 27 composite tanks (250 l) with aeration; the stocking density was 30 fish/tank. The water temperature and salinity levels in the tanks were adjusted at rates of 2 °C/day and 3‰/day, respectively, to achieve the desired levels for the treatments. We recorded the amount of feed consumed and weighed and removed the excess feed and dead fish daily. Prepared water, at the same temperature and salinity as in the experimental tanks, was used to replace 30–50% of the water in the tanks every 3 days. The experiment lasted for 8 weeks. The pH (7.2–7.5) and dissolved oxygen (4.18–5.42 mg/l) of water were measured twice a day using a pH meter (SevenGo; Mettler Toledo, USA) and an oxygen meter (SevenGo pro; Mettler Toledo), respectively. The temperature was maintained at 27.7–28.9 °C for the treatment without temperature adjustment, at 30.7–31.6 °C for the treatments at 31 °C, and at 33.1–34.4 °C for the treatments at 34 °C, as recorded by the pH meter (SevenGo; Mettler Toledo). The nitrogen dioxide (nitrite ion) concentration ranged from 0.116 to 0.216 mg/l, and total ammonia nitrogen was below 1 mg/l, as measured using the Sera test kit (Germany). Salinity was measured using a Master Refractometer (Atago, Japan).

Initial mean weight (Wi) and final mean weight (Wf) of individual fish were determined before (initial fish) and after (final fish) the experiment. The survival rate (SR; %), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention were measured as follows (where t = time in days):

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

$$\text{DWG}\ (\text{g/day}) = \left(W_\text{f} - W_\text{i}\right)/t,$$

$$\text{FI}\left(\%\ \text{fish}^{-1}\ \text{day}^{-1}\right) = \text{consumed feed} / \left(\sqrt{W_\text{f} \times W_\text{i}} \times t\right),$$

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

$$\text{PER} = \left(W_\text{f} - W_\text{i}\right)/\text{protein intake},$$

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

Initial fish (six fish/tank) and final fish (six fish/tank) were collected from each tank, killed by placing in ice for 5 min, minced, and stored at − 20 °C until analysis of their chemical composition following the methods of Latimer (2016). The moisture content of the fish and feed was determined by drying the samples at 105 °C for 4–5 h to a constant weight. Crude protein was analyzed using the Kjeldahl method. Crude lipid was analyzed by petroleum ether extraction with a Soxhlet extractor. The ash content was determined after combusting samples in a furnace at 560–600 °C for 6 h.

Digestibility experiment

The experimental setup was similar to that of the growth trial, but with an external marker (chromic oxide) added to the feed. The feed was ground, mixed with 1% chromic oxide (Cr_2O_3), pelletted at 70 °C using a mini-extruder, and dried in an oven at 60 °C for ca. 10 h to reach a moisture content of approximately 10%. The experimental system for feces collection consisted of a series of 250-l composite tanks with aeration in which conical feces-collection chambers (5 l) were installed. The feces settled into the conical chamber and then into a 200-ml glass bottle (Duran Group, Germany), which was immersed into ice for the duration of the collection period (from 1800 to 0600 hours on the following day). Snakeheads (20.3 ± 0.5 g) were stocked in the settlement collection system at a density of 30 fish/tank and fed twice a day (at 0800 and 1600 hours) to satiation during the acclimatization period in which the desired water temperature and salinity were attained. Excess feed was removed daily. Feces collection began after 10 days of feeding and continued for 20 days, at which point the amount of feces (about 10 g dry matter) was sufficient for analysis. For feces collection, fish were fed once at 1400 hours, the uneaten feed removed after 2 h, the tanks cleaned, feces-collection bottles installed, and feces collected until the following morning. Feces were collected every day, washed using distilled water, dried at 60 °C, and stored at -20 °C until analysis. The pH (7.2–7.4) and dissolved oxygen (4.63–5.36 mg/l) of water were measured twice a day. The water temperature was 27.7–29.0 °C in tanks without temperature adjustment, 30.4–31.5 °C for the treatments at 31 °C, and 33.4–34.3 °C for the treatments at 34 °C.

Feces and experimental feed were analyzed for crude protein, crude lipid, crude ash, and moisture content following Latimer (2016). Gross energy was calculated based on the total energy obtained from the crude protein, crude lipid, and total carbohydrate in the samples. Chromic oxide in the fecal and feed samples was analyzed following the method of Furukawa and Tsukahara (1996). The following calculations were made:

$$\text{ADC}_\text{feed} = 1 - \frac{\%A}{\%B},$$

$$\text{ADC}_\text{Nu - feed} = 1 - \frac{\%A}{\%B} \times \frac{\%B'}{\%A'},$$

where the apparent digestibility coefficient (ADC) of feed = ADC_feed, and the ADC of nutrients in the feed = $\text{ADC}_\text{Nu-feed}$, where A is percent Cr_2O_3 in feed, B is percent Cr_2O_3 in feces, A' is percent nutrient in feed, and B' is percent nutrient in feces.

At the end of the experiment, three fish per tank were collected after 1 day of fasting for digestive enzyme analysis. The fish were killed by placing them in ice for 5 min. They were then dissected, and their stomach and intestines removed for analysis. Samples were then minced in a buffer solution (20 mM monopotassium phosphate and 6 mM sodium chloride) at pH 6.9. The supernatant was collected after centrifugation at 4200 r.p.m. for 30 min and stored at − 80 °C until analysis. The α-amylase contents in the intestine and stomach were analyzed following the method of Bernfeld (1951). Pepsin activity in the stomach and chymotrypsin activity in the intestine were analyzed following the methods of Worthington (1982). The enzyme analytical methods are described in Babaei et al. (2011).

Statistical analyses

Prior to statistical analyses, the data were checked for normal distribution and homogeneity of variance. To test for the effects of temperature, salinity, and their interaction with life history traits and biochemical compositions of the snakeheads, we ran two-way ANOVAs with temperature and salinity as fixed factors. Tukey's honest significant difference test was employed for specific comparisons, where appropriate. Differences were considered significant at $p < 0.05$. All statistical analyses were performed using SPSS software (version 16.0; IBM, USA).

Results

Growth experiment

Fish performance (i.e., growth, feed utilization efficiency, and nutrient retention ratio) was strongly affected ($p < 0.05$) by both salinity and water temperature. The interaction between water temperature and salinity was reflected in both the growth and survival rates of the experimental fish ($p < 0.05$). Survival rate was considerably reduced at 34 °C, especially at salinity levels of 6 and 9‰, generating the main effect of water temperature ($p < 0.001$) and its interaction with salinity ($p = 0.036$; Table 1). The highest growth rate (DWG) and feed consumption (FI) of snakeheads were at a salinity of 0‰ and a water temperature of 31 °C. There were no differences in FCR, PER, or protein retention values (Table 2) of fish treated with water at 0–6‰ salinity and temperatures of 28–31 °C. The lowest PER and the highest FCR values were recorded in the treatment at 34 °C and 9‰ salinity, and differed significantly from those obtained in the other treatments ($p < 0.05$); also, protein retention and FI values were lowest in this former treatment.

Interaction between salinity and water temperature ($p < 0.001$) was observed for fish body composition, with the exception of ash content. The crude protein and crude lipid contents in the fish body were significantly affected ($p < 0.001$) by salinity and temperature, but body moisture showed no significant difference ($p > 0.1$) among salinity levels (Table 3). Crude protein and crude lipid contents in the fish body were lowest at the highest salinity (9‰). Moisture and crude protein contents were lowest at the highest temperature. At 0 and 6‰ salinity, total fat content

Table 1 Daily weight gain (*DWG*) and survival rates (*SR*) of snakehead (*Channa striata*) reared under different combinations of water temperature (°C) and salinity (‰)

Treatments	Initial weight (g)	Final weight (g)	DWG (g/day)	SR (%)
28 °C—0‰	6.57 ± 0.12	35.0 ± 0.10^{d}	0.51 ± 0.01^{d}	91.1 ± 3.84^{c}
28 °C—6‰	6.53 ± 0.02	38.2 ± 0.51^{e}	0.57 ± 0.01^{e}	98.9 ± 1.92^{c}
28 °C—9‰	6.45 ± 0.04	31.4 ± 0.07^{c}	0.44 ± 0.01^{c}	93.3 ± 8.81^{c}
31 °C—0‰	6.56 ± 0.12	46.8 ± 0.28^{g}	0.72 ± 0.01^{g}	92.2 ± 1.92^{c}
31 °C—6‰	6.55 ± 0.08	35.1 ± 1.12^{d}	0.51 ± 0.02^{d}	87.8 ± 8.39b^{c}
31 °C—9‰	6.57 ± 0.13	31.1 ± 0.56^{c}	0.43 ± 0.01^{c}	98.4 ± 2.36^{c}
34 °C—0‰	6.52 ± 0.05	41.5 ± 1.88^{f}	0.62 ± 0.04^{f}	86.7 ± 8.39^{bc}
34 °C—6‰	6.56 ± 0.15	28.0 ± 0.13^{b}	0.38 ± 0.01^{b}	68.9 ± 10.2^{ab}
34 °C—9‰	6.50 ± 0.12	20.0 ± 1.10^{a}	0.24 ± 0.02^{a}	66.7 ± 5.77^{a}
P (temperature)	0.444	< 0.001	0.004	< 0.001
P (salinity)	0.597	< 0.001	< 0.001	0.565
P (temperature × salinity)	0.669	< 0.001	0.006	0.036

Data are presented as mean ± SD ($n = 3$). *Different lowercase letters* in the *same column* indicate statistically significant differences ($P < 0.05$)

Table 2 Feed intake (*FI*), feed conversion ratio (*FCR*), protein efficiency ratio (*PER*), and protein retention of snakeheads reared under different combinations of water temperature and salinity

Treatments	FI (%/fish/day)	FCR	PER (%)	Protein retention (%)
28 °C—0‰	3.39 ± 0.05^{bc}	0.88 ± 0.04^{a}	2.51 ± 0.10^{b}	41.9 ± 4.57^{bc}
28 °C—6‰	3.63 ± 0.05^{cd}	0.90 ± 0.10^{a}	2.46 ± 0.27^{b}	42.3 ± 4.53^{bc}
28 °C—9‰	3.05 ± 0.12^{ab}	0.91 ± 0.04^{a}	2.42 ± 0.09^{b}	40.9 ± 1.50^{bc}
31 °C—0‰	3.84 ± 0.08^{d}	0.82 ± 0.09^{a}	2.70 ± 0.31^{b}	46.2 ± 5.44^{c}
31 °C—6‰	3.30 ± 0.06^{bc}	0.87 ± 0.06^{a}	2.54 ± 0.15^{b}	42.6 ± 2.72^{bc}
31 °C—9‰	3.30 ± 0.07^{bc}	0.96 ± 0.03^{a}	2.29 ± 0.06^{b}	37.7 ± 1.41^{bc}
34 °C—0‰	3.60 ± 0.21^{cd}	0.84 ± 0.03^{a}	2.61 ± 0.08^{b}	43.2 ± 1.46^{bc}
34 °C—6‰	3.07 ± 0.22^{ab}	0.96 ± 0.05^{a}	2.29 ± 0.12^{b}	36.4 ± 1.93^{ab}
34 °C—9‰	2.83 ± 0.20^{a}	1.23 ± 0.03^{b}	1.79 ± 0.04^{a}	28.1 ± 0.80^{a}
P (temperature)	0.001	< 0.001	0.007	< 0.001
P (salinity)	< 0.001	< 0.001	< 0.001	< 0.001
P (temperature × salinity)	0.001	0.001	0.026	0.011

Data are presented as mean ± SD ($n = 3$). *Different lowercase letters* in the *same column* indicate statistically significant differences ($P < 0.05$)

Table 3 Chemical compositions of snakehead prior to (*Initial fish*) and after (*Final fish*) exposure to different combinations of water temperature and salinity

Treatments	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Initial fish	75.8 ± 0.01	15.5 ± 0.32	2.83 ± 0.15	4.88 ± 0.03
Final fish				
28 °C—0‰	71.3 ± 0.15^{bc}	17.4 ± 0.03^{cd}	5.24 ± 0.05^{b}	4.80 ± 0.08^{bc}
28 °C—6‰	71.9 ± 0.16^{e}	17.9 ± 0.08^{e}	5.28 ± 0.04^{b}	4.63 ± 0.10^{ab}
28 °C—9‰	71.8 ± 0.02^{e}	17.6 ± 0.19^{d}	5.25 ± 0.05^{b}	4.93 ± 0.17^{cd}
31 °C—0‰	71.7 ± 0.22^{de}	17.9 ± 0.08^{e}	5.50 ± 0.01^{d}	4.48 ± 0.05^{a}
31 °C—6‰	71.5 ± 0.32^{cd}	17.5 ± 0.27^{cd}	5.53 ± 0.05^{d}	4.86 ± 0.07^{bcd}
31 °C—9‰	71.4 ± 0.05^{c}	17.2 ± 0.15^{b}	5.36 ± 0.05^{c}	5.16 ± 0.21^{e}
34 °C—0‰	70.9 ± 0.11^{a}	17.4 ± 0.05^{c}	5.50 ± 0.01^{d}	4.78 ± 0.08^{bc}
34 °C—6‰	71.2 ± 0.10^{b}	16.8 ± 0.07^{a}	5.40 ± 0.03^{c}	5.06 ± 0.21^{de}
34 °C—9‰	71.3 ± 0.08^{bc}	16.7 ± 0.15^{a}	5.13 ± 0.05^{a}	5.53 ± 0.37^{f}
P (temperature)	< 0.001	< 0.001	< 0.001	< 0.001
P (salinity)	> 0.1	< 0.001	< 0.001	< 0.001
P (temperature × salinity)	< 0.001	< 0.001	< 0.001	> 0.1

Data are presented as mean ± SE ($n = 3$). *Different lowercase letters* in the *same column* indicate statistically significant differences ($P < 0.05$)

increased with an increase in temperature; however, at 9‰ salinity, total fat content was at its highest level at 31 °C, but dropped to its lowest level at 34 °C. The body ash content significantly increased with increasing salinity.

Digestibility experiment

The ADC values of dry matter, crude protein, crude fat, crude ash, and gross energy differed ($p < 0.05$) with different salinity and temperature levels (Table 4). An interaction was found between salinity and water temperature for the ADC values of all nutrients. The highest salinity (9‰) combined with the highest temperature (34 °C) decreased the ADC of nutrients radically, whereas the highest ADC values were observed at 0‰ salinity at 31 °C.

Similar to the ADC results, a strong negative impact of the combination of high salinity and high temperature was found for the activities of the measured digestive enzymes. Also, there was an interaction between water temperature and salinity for all digestive enzyme activities ($p < 0.05$). The activities of digestive enzymes in chyme (i.e., stomach and intestine) were lower at higher water salinity (Table 5). The activities of chymotrypsin and amylase in both the stomach and intestine were significantly affected by water temperature. However, water temperature did not affect pepsin activity in the intestine. At a water salinity of 9‰, pepsin activity was lowest at 31 and 34 °C. The chymotrypsin

Table 4 Apparent digestibility coefficient (*ADC*; %) of feed for dry matter, crude protein, crude lipid, crude ash, and gross energy of snakeheads under different combinations of water temperature and salinity

Treatments	ADC dry matter (%)	ADC protein (%)	ADC lipid (%)	ADC ash (%)	ADC energy (%)
28 °C—0‰	78.3 ± 0.70^{d}	93.8 ± 0.26^{b}	97.5 ± 0.14^{d}	36.4 ± 0.53^{de}	87.5 ± 0.41^{bc}
28 °C—6‰	75.9 ± 1.47^{c}	93.1 ± 0.73^{b}	97.2 ± 0.16^{cd}	26.1 ± 2.13^{cd}	86.5 ± 0.38^{ab}
28 °C—9‰	75.5 ± 0.23^{bc}	93.6 ± 0.34^{b}	96.7 ± 0.19^{b}	19.0 ± 0.60^{bc}	87.1 ± 0.03^{bc}
31 °C—0‰	80.3 ± 0.75^{e}	95.3 ± 0.38^{c}	97.9 ± 0.06^{e}	42.8 ± 1.69^{e}	88.7 ± 0.22^{c}
31 °C—6‰	76.1 ± 0.97^{c}	93.8 ± 0.59^{b}	97.1 ± 0.20^{c}	26.0 ± 0.78^{cd}	86.8 ± 0.09^{b}
31 °C—9‰	72.6 ± 1.14^{a}	92.9 ± 1.34^{b}	96.7 ± 0.15^{b}	7.88 ± 1.00^{a}	85.8 ± 0.07^{ab}
34 °C—0‰	76.6 ± 1.34^{cd}	93.8 ± 0.51^{b}	97.5 ± 0.15^{d}	34.1 ± 2.57^{de}	86.2 ± 0.41^{ab}
34 °C—6‰	73.9 ± 1.30^{ab}	92.7 ± 1.28^{b}	96.7 ± 0.17^{b}	14.5 ± 1.73^{ab}	86.1 ± 0.66^{ab}
34 °C—9‰	72.1 ± 2.20^{a}	90.5 ± 1.13^{a}	95.5 ± 0.38^{a}	7.96 ± 3.54^{a}	84.8 ± 0.60^{a}
P (temperature)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P (salinity)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P (temperature × salinity)	< 0.001	< 0.001	< 0.001	0.002	0.021

Data are presented as mean ± SE ($n = 3$). *Different lowercase letters* in the *same column* indicate statistically significant differences ($P < 0.05$)

Table 5 α-Amylase activity in the stomach and intestine, pepsin activity in the stomach, and chymotrypsin activity in the intestine of snakeheads under different combinations of water temperature and salinity

Treatments	α-Amylase in stomach	α-amylase in intestine	Pepsin	Chymotrypsin
28 °C—0‰	1.81 ± 0.05^{b}	1.45 ± 0.07^{ab}	1.17 ± 0.01^{ab}	262 ± 4.14^{a}
28 °C—6‰	1.86 ± 0.03^{b}	1.48 ± 0.05^{b}	1.18 ± 0.01^{bc}	268 ± 7.17^{a}
28 °C—9‰	1.87 ± 0.09^{b}	1.49 ± 0.06^{b}	1.19 ± 0.02^{bc}	261 ± 8.91^{a}
31 °C—0‰	1.88 ± 0.05^{b}	1.50 ± 0.11^{b}	1.19 ± 0.01^{bc}	347 ± 10.3^{c}
31 °C—6‰	1.90 ± 0.08^{b}	1.52 ± 0.07^{b}	1.20 ± 0.04^{c}	335 ± 7.48^{c}
31 °C—9‰	1.89 ± 0.03^{b}	1.48 ± 0.07^{b}	1.15 ± 0.01^{a}	311 ± 7.31^{b}
34 °C—0‰	1.87 ± 0.05^{b}	1.49 ± 0.05^{b}	1.18 ± 0.01^{bc}	365 ± 8.83^{d}
34 °C—6‰	1.80 ± 0.09^{b}	1.47 ± 0.06^{b}	1.19 ± 0.02^{bc}	362 ± 11.1^{d}
34 °C—9‰	1.61 ± 0.07^{a}	1.36 ± 0.07^{a}	1.15 ± 0.01^{a}	343 ± 9.82^{c}
P (temperature)	< 0.001	0.007	0.412	< 0.001
P (salinity)	0.001	0.068	< 0.001	< 0.001
P (temperature × salinity)	< 0.001	0.012	< 0.001	< 0.001

Enzyme activities are expressed as mU/min per mg protein. Data are presented as mean ± SE ($n = 3$). *Different lowercase letters* in the *same column* indicate statistically significant differences ($P < 0.05$)

activities in fish cultured at unadjusted water temperatures at a salinity of 0–9‰ were significantly lower than those in the other treatments ($p < 0.05$), i.e., increased water temperature significantly increased chymotrypsin activity regardless of the salinity of the water.

Discussion

It has been reported that salinization intensifies the effects of elevated temperatures on fish growth (Malloy and Targett 1991; Watanabe et al. 1993; Likongwe et al. 1996). In the current study, it was confirmed that water temperature and salinity combined significantly affected the performance, digestion, and feed utilization of snakeheads. Fish growth performance and feed utilization efficiency increased with decreasing salinity and higher temperatures. Our results are in agreement with those of previous studies on other fish species, e.g., Nile tilapia (Likongwe et al. 1996), juvenile turbot (Imsland et al. 2001), and GIFT (Qiang et al. 2013). The growth-inhibiting effect of high salinity at high temperature in this study agreed with the findings of Likongwe et al. (1996), and could be explained by a combination of lower feed consumption (Table 2) and lower feed digestibility (Table 4). In contrast, a positive effect (i.e., growth enhancement) of low salinity at high temperatures was found for fish species from brackish water (Sardella and Brauner 2008; Vargas-Chacoff et al. 2015).

In the present study, optimal fish growth occurred at 31 °C in freshwater, and was highly correlated to higher feed intake, nutrient utilization, and feed digestibility, including protein and lipid utilization. Higher temperatures may contribute to an increase in feed intake (or appetite) and feed utilization and, in turn, to more efficient digestion and absorption, as found by Musaka et al. (2009).

The survival and growth of the experimental snakeheads in this study started to exponentially decrease with an increase in water temperature at 6‰ water salinity and higher. At 0‰ salinity and 28 and 31 °C, fish growth increased with feed intake, which led to a decrease in the FCR. In contrast, the daily feed intake of snakeheads declined with increasing water salinity. This implies that the appetite of snakeheads reduces in saline water, which is in line with previous research (Handeland et al. 1998). This may also explain the decrease in ADCs followed by a decrease in the activity of digestive enzymes with increasing salinity. In addition, the FCR and the utilization of nutrients in the feed were adversely affected by the high salinity level of 9‰. The high metabolic cost of osmoregulation may explain the negative impact of salinity on fish growth and feed utilization of nutrients (Sardella and Brauner 2008; Vargas-Chacoff et al. 2015). Amornsakun et al. (2017) found that snakeheads grew well at a salinity of 0–10‰, but slower at salinity higher than 10‰, and even died after 8 days at 15‰ salinity. A recent study by Nakkrasae et al. (2015) concluded that it was possible for snakeheads to survive in saline water; however, the osmoregulatory mechanisms for this (despite the measured enhanced branchial Na^+/K^+-ATPase activity) were not clarified. That study also indicated an induced stress response (through elevated plasma cortisol and glucose levels) and muscle dehydration in snakeheads after exposure to 10‰ salinity. Nakkrasae et al.'s (2015) findings are in accordance with the low survival rate of snakeheads in the present study at the highest temperature (34 °C) combined with high salinity. Our results also agree with those of Wang et al. (1997) for common carp and those of Watanabe et al. (1993) for red tilapia. Lesions were found on Nile tilapia after they were reared in seawater (Likongwe

et al. 1996), and exposure to high salinity levels was thought to disrupt the epithelium and cause the edema of both gill filaments and secondary lamellae of the gill in Rohu fingerlings (Holliday and Jones 1967 cited in Islam et al. 2014).

In the present study, water salinity–temperature combinations strongly affected the digestibility of feed and the activity of digestive enzymes (i.e., α-amylase in the stomach and intestine, pepsin in the stomach, and chymotrypsin in the intestine) of the snakehead, with serious, negative impacts at the highest salinity (9‰). However, in a previous study on tilapia, protease activity was not significantly affected by exposure of the fish to different levels of salinity (Fang and Chiou 1989). Although a salinity level of 9‰ is lower than the iso-osmotic point of the snakehead (Huong and Trinh 2013; Nakkrasae et al. 2015), the ADC values of all nutrients, and the enzyme activities (excluding that of chymotrypsin) in the present study linearly decreased with an increase in culture temperature, and the values at this salinity were lower than those for the freshwater fish (initial fish). We suggest that the salt content of the feed due to the absorbance of saline water might have lead to a change in the salinity of the chyme fluid, and that the activities of the digestive enzymes may have been negatively affected by this. If this supposition is correct, the digestive environment would no longer have been optimal for the activities of the digestive enzymes, and the ADCs would have decreased as a consequence. In addition, the results of the present study revealed that digestion was most efficient in snakeheads at 31 °C in freshwater, which is in accordance with the results of Wee (1982).

The pattern of crude protein content in the snakehead body in response to temperature-salinity was similar to that of growth rate, and provides evidence for a relationship between fish-body protein content and growth. In freshwater, protein contents were highest in the snakeheads reared at 31 °C, but protein contents decreased with increasing culture water temperature when the fish were exposed to brackish water. These results are in line with those of Imsland et al. (2001), who found that the nutrient compositions of experimental fish were affected by water salinity.

Overall, and in agreement with existing literature on freshwater species, the present study revealed that growth performance, feed digestibility, and digestive enzyme activities of snakeheads increased with increasing water temperature at 0‰ salinity. However, when the experimental fish were exposed to higher salinity levels for a longer period of time, the effect of temperature on these parameters changed.

Combined temperature and salinity variations significantly affected snakehead growth and feed utilization rates. The highest growth rates of fish were observed in treatments at 31 °C and 0‰ salinity and 34 °C and 0‰ salinity, whereas significantly lower survival rates, growth performance, and feed utilization efficiencies of fish were observed in treatments at 34 °C and 6‰ salinity and 34 °C and 9‰ salinity as compared to the other treatments. Snakehead growth and feed utilization efficiency decreased with increasing salinity at higher temperatures.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval As regards the National Regulations for the Use of Animals in Research in Vietnam, the snakehead is not listed as either an endangered or critically endangered species (IB group) or a threatened or rare species (IIB group) (decree 32/2006/ND-CP, 2006), thus, this study did not require a permit or ethical approval.

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