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Research Article

Growth performance and energy budget of the yellowfin seabream, *Acanthopagrus arabicus* (Iwatsuki, 2013) affected by the combined effects of water temperature and salinity

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Abstract

A 56-day research was carried out to determine the interactive effects of water temperature (WT) and salinity (WS) on physiological responses of yellowfin seabream, *Acanthopagrus arabicus* (Iwatsuki, 2013) (10.0±0.2 g). Three salinities (S; 12, 35 and 50 ‰) and four temperatures (T; 16, 23, 30 and 35°C) were selected to design 12 experimental treatments. The husbandry system consisted of thirty-six 300-L cylindrical polyethylene tanks that were filled with 250 L of water. Each treatment had three replicates (tanks) containing 20 fish in each tank. Fish were handfed on a commercial feed (440 g kg^{-1} crude protein and 180 g kg^{-1} crude fat) twice daily at visual satiation. Five fish from each tank were transferred into an experimental chamber to evaluate their oxygen consumption and ammonia excretion. There was a positive correlation between fish oxygen consumption and WT (r=0.594; p=0.001). In addition, there was a negative relationship between WS and ammonia excretion (AE) (r = -0.865; p=0.029), meanwhile the relationship between WT and AE was positive (r=0.422; P=0.01). Growth parameters were significantly affected by WT, WS and their interactions so that the highest final body was recorded in the highest temperature and salinity treatment. On the other hand, the lowest body weight was observed in the lowest WT (i.e., 16℃) irresponsive to WS. The moisture level in the whole body was affected by WT (p=0.012). The crude lipid in the whole body was influenced by only WS (p=0.04). The ash content in the whole body was profoundly affected by WT (p=0.0001), WS (p=0.0001) and their interaction effect (p=0.04). The energy content of the whole body was only affected by WT (p=0.025). The energy budget of fish was influenced by WT (p=0.0001) and its interaction with WS (p=0.015). In summary, the present findings indicated that the preferred WT for *A. arabicus* is between 30 to 35°C in brackish water.

Keywords: *Acanthopagrus arabicus*, Bioenergetics, Euryhaline fish, Feed utilization, Thermal requirements

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Introduction

During the recent years, different native marine fish species were considered as candidates for aquaculture diversification in Iran. Among different marine fish species, yellowfin seabream is considered as an aquaculture candidate because of its ability to spawn in captivity, high tolerance to culture conditions, reasonable growth rate and preferable feed conversion ratio (Tamadoni *et al.,* 2020). The yellowfin seabream complex species are euryhaline carnivorous fish and have a wide distribution range from the coasts of Japan, southern Korea, Taiwan, China, northern Vietnam and the Indo-West Pacific Ocean and the Persian Gulf (Iwatsuki, 2013). The genus *Acanthopagrus* includes five different subspecies, including *Acanthopagrus latus* (Houttuyn, 1782), *Acanthopagrus longispinnis* (Valenciennes, 1830), *Acanthopagrus morrisoni* (Iwatsuki 2013), *Acanthopagrus arabicus* (Iwatsuki 2013), and *Acanthopagrus sheim* (Iwatsuki 2013). There is a lot of information on the ecophysiology of these species (Zakeri *et al.,* 2010 ; Nasari *et al.,* 2014 ; Surinejad *et al.,* 2015; Vahabnejad *et al.,* 2017; Namjou *et al.,* 2019; Wang *et al.,* 2018). However, the information regarding the interactive effects of WT and WS in this species is scarce. Thus, the present study it was aimed to evaluate the interactive influence of WT and AS on survival, growth, the biochemical composition of the whole body, and energy budget of *Acanthopagrus arabicus*.

According to the findings of above mentioned studies, non-optimal WT and WS abate growth performance and feed utilization, increase oxygen consumption (OC) and ammonia excretion (AE) as well as metabolic rate, compromise fish immunocompetence against pathogens and adversely affect reproduction in different fish species (Burel *et al.*, 1996; Wedemeyer *et al.,* 1999; Boeuf and Payan, 2001; Ruyet *et al.,* 2004; Luo and Xie 2008; Zheng *et al.,* 2008; Fang *et al.,* 2010; Pérez-Robles *et al.*, 2012). In this sense, the interactive impact of WT and WS on the physiological responses of fish becomes more complex. Thus, a precise understanding of these interactions is required to manage climate change risks in aquatic ecosystems. In this context, previous studies demonstrated significant interactions between WT and WS on growth, energy budget, metabolic rate, OC and AE in different marine fish species, such as turbot (*Scophthalmus maximus*, Imsland *et al.,* 2001), spotted wolffish (*Anarhichas minor,* Magnussen *et al.,* 2008), *Harpagifer antarcticus* (Navarro *et al.,* 2019) and miiuy croaker (*Miichthys miiuy*, Zheng *et al.,* 2008). The above-mentioned studies indicated that the optimal combination of WT and WS not only improves the survival rate but also enhances growth and feed utilization and channels most of the feed energy to somatic growth. However, beyond the optimal ranges, the interactive impacts of WT and WS result in a lower survival rate, disturb normal OC and AE, as well as reduce growth rate in different fish species (Imsland *et al.*, 2001; Zheng *et al.*, 2008; Navarro *et al.*, 2019). A study of the optimal ranges of WT and WS is also required for the successful production of a candidate fish species in the aquaculture industry. Thus, the present study is aimed to evaluate the interactive influence of WT and WS on survival, growth, the biochemical composition of the whole body, and energy budget of *A. arabicus*.

Materials and methods

Experimental design

The present study was carried out in a marine fish hatchery (Sarbandar, Iran). For evaluating the interactive effects of WT and WS, three salinities (12, 35 and 50‰) and four temperatures (16, 23, 30 and 35°C) were selected for designing 12 experimental treatments, including $S_{12}T_{16}$, $S_{12}T_{23}$, $S_{12}T_{30}$, $S_{12}T_{35}$, $S_{35}T_{16}$, $S_{35}T_{23}$, $S_{12}T_{30}$, $S_{12}T_{35}$, $S_{50}T_{16}$, $S_{50}T_{23}$, $S_{50}T_{30}$ and $S_{50}T_{35}$ (Fig. 1). The salinity values were chosen according to the salinity ranges of brackish water bodies (*Ca.* 12‰), marine water (*Ca.* 35‰), and hypersaline bays (*Ca.* 50‰) are existing in the local water bodies of Khuzestan province. The WT ranges were also selected according to the local WT during cold seasons (fall and winter, *Ca.* 16°C), spring (*Ca.* 23°C), and summer (30°C). The highest WT (*Ca.* 35°C) was selected according to the thermal tolerance of *A. latus* reported by Jian *et al.* (2003).

Experimental setup

The present study was carried out in a private marine fish hatchery (Sarbandar, Iran). For evaluating the interactive effects of WT and WS, three salinities (12, 35 and 50‰) and four temperatures (16, 23, 30 and 35°C) were selected for designing 12 experimental treatments including $S_{12}T_{16}$, $S_{12}T_{23}$, $S_{12}T_{30}$, $S_{12}T_{35}$, $S_{35}T_{16}$, $S_{35}T_{23}$, $S_{12}T_{30}$, $S_{12}T_{35}$, $S_{50}T_{16}$, $S_{50}T_{23}$, $S_{50}T_{30}$ and $S_{50}T_{35}$ (Fig. 1).

Figure 1: Schematic overview of the experimental design.

Salinity (S)	**12**				**35**				**50**			
Temperature (T)	**16**	**23**	**30**	**35**	**16**	**23**	**30**	**35**	**16**	**23**	**30**	**35**
Treatments	$S_{12}T_{16}$	$S_{12}T_{23}$	$S_{12}T_{30}$	$S_{12}T_{35}$	$S_{35}T_{16}$	$S_{35}T_{23}$	$S_{12}T_{30}$	$S_{12}T_{35}$	$S_{50}T_{16}$	$S_{50}T_{23}$	$S_{50}T_{30}$	$S_{50}T_{35}$

The husbandry system consisted of thirty-six 300-L cylindrical polyethylene tanks that were filled with 250 L water of the selected salinities. At the beginning of the experiment, ten fish specimens were taken from the fish stock and euthanized by an overdose of 2-phenoxyethanol (1000 ppm; the optimum dosage is about 100 ppm) and kept at -80ºC for biochemical analyses. Seven hundred and twenty juvenile *A. arabicus* (initial weight=10±0.2, mean±standard error) were stocked into the husbandry system (n=20 fish per each tank). Fish were weighed individually prior to the start of the trial to obtain information about the biomass within each tank. Each treatment was tested with three replicates (tanks). The different salinities were prepared in 10-tonne concrete tanks and then transferred into the husbandry system. In this regard, seawater with 50‰ salinity was supplied from Khor Musa Bay, then for preparing lower salinities including 35 and 12‰ it was diluted with disinfected (*i.e.* chlorinated (10 ppm)) fresh water in 10-tonne tanks. The experimental trial was carried out during the winter and except for the lowest WT (16°C), for stabilizing WT at 23 and 30°C, each tank was equipped with a 300-watt aquarium thermostat heater equipped with precise temperature control (Mahiran, Tehran, Iran). For increasing WT in the 35 °C treatments, each tank was equipped with a 500-watt aquarium heater. Water temperature and WS were monitored twice a day at 0800 and 1800 h. The fluctuations of WT and WS were 0.4°C and 0.1‰ during the day, respectively. The average water quality values (mean±standard deviation) for pH and dissolved oxygen ranged from 7.8±0.4 and 6.5±0.6 mg L^{-1} in different treatments, respectively, and the photoperiod was 12L: 12D (Light: Darkness) during the fish husbandry period. About 50% of the water in the experimental tanks was exchanged with new water daily. Fish were handfed on a commercial feed (Partian Gostar Shayna, Iran; particle size: 2 mm, 440 g kg^{-1} crude protein, 180 g kg^{-1} crude fat, 85 g kg^{-1} ash, 100 g kg^{-1} moisture, 22g kg^{-1} fiber and 20 MJ kg^{-1}) twice daily (0900 and 1400 h) at visual satiation for eight weeks. Uneaten feed was siphoned one hour after feeding and weighed after drying (60°C, 24 h) to estimate the feed efficiency. In the morning, feces in each tank were collected by siphoning into a mesh screen (40 µm), then rinsed with distilled water and kept at −20 °C until their analysis (Yaghoubi *et al.*, 2016). The feces collected from a single tank pooled over time to give a single sample for each tank at the end of the trial.

Biometry and sampling

At the end of the feeding trial, fish were fasted for a day, and then their weight and length were individually measured. Three fish from each tank were sacrificed with an overdose of anesthetic for determining the liver weight; then their bodies were kept in a freezer for further biochemical analyses. For determining growth performance and feed utilization, the following standard equations were used:

Specific growth rate (% initial weight day^{-1}, SGR) = $((\ln BW_f - \ln BW_i) / t) \times 100$, where t is experimental period = 56 days.

Weight gain (%, WG)=$((BW_f - BW_i)/BW_i) \times 100$.

Survival (%) = number of fish in each group remaining on day 56/initial number of fish) × 100.

Feed intake (FI, g) = total feed intake per tank (g) / number of fish.

Feed conversion ratio (FCR)=(feed intake (g) / weight gain (g)).

Hepatosomatic index (%, HSI)= (liver weight (g) / BW_f (g)) × 100.

Fulton’s condition factor (%, K)=(BW_f (g)/ standard length (cm)^3) × 100.

The initial and final body weight was denoted by BW_i and BW_f, respectively.

Biochemical analyses

Proximate analyses of the whole body and feces were determined according to the standard methods (AOAC, 2005). Samples were dried at 105°C to a constant weight to determine their moisture content in an oven (D-63450; Heraeus, Hanau, Germany). Crude protein content was determined using an Auto Kjeldahl System (N×6.25; Kjeltec Auto Analyzer; FOSS, Hillerød, Denmark) and estimated by multiplying nitrogen by 6.25. Crude lipid was extracted by a mixture of chloroform/methanol (2:1) solvent using the Soxhlet method (Barnstead/Electrothermal, UK). Ash content was determined gravimetrically following the loss of mass after combustion of a sample in a muffle furnace (Muffle furnace, Isuzu, Tokyo, Japan) at 550°C for 8 h. Dietary crud fiber was measured using an automatic fiber analyzer (Fibertec System M, Tecator, Sweden). The gross energy contents of the whole body and feces were determined by an isoperibol oxygen bomb calorimeter (C6000, IKA, Germany).

The evaluation of fish OC, and AE were determined at the end of the husbandry trial according to Zheng *et al.* (2008). Water with the prescribed salinities was boiled two times to remove possible bacterial contamination; then their salinities were calibrated with distilled water again. After that, water was aerated for at least 12 h and WT calibrated according to the corresponding temperature by an aquarium heater and finally transferred into a 10-L plastic columnar chamber. As recommended by Sun and Chen (2014) to avoid the pollution of the experimental chambers, a day before the experiment the fish was fasted to empty their guts. Five fish from each tank were transferred into an experimental chamber, which was placed in a water bath with the respective temperature, and the lid was sealed, and then incubated for one hour. Each treatment was replicated in triplicate, and three blank chambers without fish were used as the control. To decrease the impact of body weight on OC and AE, fish within narrow weight ranges was chosen (mean weight: 13.5±0.2 g) from each tank. To reduce the impacts of the stress response on the OC and AE of fish, they were acclimated in the chambers until their gill covers opened and closed normally. The dissolved oxygen (DO) was measured at the beginning and the end of the incubation period with a DO meter (HI 9142, Hanna). In addition, water samples were collected (10 mL) at the start and the end of the experiment and then transferred into a freezer (-20°C) for further determination of the ammonia. Water NH_3 was quantified by spectrophotometric method at 640 nm, according to Grasshoff *et al.* (1983) by a spectrophotometer (DR/2000 Spectrophotometer, Hach, Loveland, CO, USA).

Oxygen consumption and AE were calculated as (Zheng *et al.,* 2008):

$$\frac{[A2\ (mg) - A1\ (mg)] - [B2\ (mg) - B1\ (mg)] /\ V\ (L)}{BW\ (Kg) \times T\ (h)}$$

A_1 and A_2 are the oxygen or ammonium contents in the experimental chamber at the beginning and the end of the test.

B_1 and B_2 are the oxygen or the ammonia contents in the blank chamber at the beginning and the end of the test.

V is the volume of the chamber, which was 10 L.

BW is the biomass of fish in the experimental chamber.

T is the incubation time, which was 1 hour.

Calculation of energy budget

Determination of energy budget of *A. arabicus* under interactive influence of WT, and WS were estimated as described by Carfoot (1987):

$C=G+F+U+R$

In which C is energy consumed, G is energy deposited for growth in the whole body, F is the loss of energy through feces, U is the loss of energy by ammonia excretion, and R is loss of energy by respiration. The G value was calculated according to the following formula:

$G=[BW_f\,(g)\times E_2\,(J\ g^{-1})]–[BW_i\,(g)\times E_1\,(J^{-1})]$

in which BW_f, and BW_i are the final and initial weights of fish, respectively, and E_2 and E_1 are the final and initial energy contents in the whole body of the fish, respectively.

To evaluate the energy value of U, Ammonia-N concentration in water was multiplied by the coefficient of 24.83 kJ g^{-1} N (Elliott, 1976). Furthermore, the value of oxygen consumption was multiplied by 13.84 J mg^{-1} O2 for determining energy value for respiration (R) (Guinea and Fernandez, 1997). For determination of assimilation efficiency (K, %), the following equation was used (Ye *et al.*, 2009):

$K=[(G+R)/(G+R+U)]\times 100$

Statistics

Data were analyzed using the SPSS ver. 16.0 (Chicago, IL, USA). After confirmation of the normality, and homogeneity of data by Shapiro-Wilk and Leven tests, respectively, the effects of salinity, and WT and their interaction effect were determined using a two-way ANOVA. If the effects of independent factors were significant, then these effects were evaluated separately by a one-way ANOVA. Turkey's post hoc analysis was performed following significant differences between groups. The Pearson product-moment correlation test was used to determine any correlation among parameters. The $p<0.05$ was considered significant for all statistical tests.

Results

Growth performance and feed utilization

The results of the present study demonstrated that the survival and growth performance of *A. arabicus* juveniles were remarkably affected by either WT or WS, and their interaction effect (Table 1). Fish in the $S_{50}T_{35}$ group had a lower survival rate (90%) compared to other treatments, and survival rates were profoundly affected by salinity (*p*=0.019), WT (*p*=0.0001) and their interactive effect (*p*=0.0001). The survival rate inversely correlated with WT (*r*=-0.362; *p*=0.03). Also, growth parameters, including WG, and SGR, were strikingly influenced by the main effect of WS, WT, and their interaction. The Pearson product-moment correlation method also revealed that WT was positively correlated to the BW_f (*r*=0.837; *p*=0.0001), WG (*r*=0.674; *p*=0.0001) and SGR (*r*=0.815; *p*=0.0001). Fish in the $S_{50}T_{35}$ group had the highest final body weight, and fish reared at 16ºC, irrespective of the WS, had the lowest final body weights. In addition, FI was pronouncedly affected by WT (*p*=0.0001) and the interactive effects of WS and WT (*p*=0.0001); however, this factor did not affect WS.

The feed conversion ratio was noticeably influenced by WT (*p*=0.0002), but not by salinity and their interactions. Moreover, FI positively correlated with WT (*r*=0.750; *p*=0.001), whereas there was a negative correlation between FCR, and WT (*r*=-0.347; *p*=0.038). Hepatosomatic index and K were not influenced by salinity, WT, or their interaction effect. There was no correlation between water salinity and growth parameters.

Whole body and feces biochemical composition

The whole body and feces biochemical composition were pronouncedly affected by treatments (Table 2). The moisture content in the whole body was significantly affected by WT (*p*=0.012), and a negative correlation was found between these two parameters, but it was not significant (*r*=-0.307; *p*=0.069). The protein content of the whole body was not affected by the main factors or their interaction. The crude lipid in the whole body was pronouncedly influenced by WS (*p*=0.04), but it was not affected by WT or their interactive effects. The ash content in the whole body was profoundly affected by WT (*p*=0.0001), salinity (*p*=0.0001), and their interaction effect (*p*=0.04). There were positive correlations among whole body ash content and WT (*r*= 0.620; *p*=0.0001) along with salinity (*r*=0.320; *p*=0.019). The energy content of the whole body only was affected by WT (*p*=0.025). The biochemical components of the feces were profoundly affected by different treatments (Table 3).

Except for the insignificant effects of WT on the moisture content of the feces, all of the biochemical parameters in the feces were significantly influenced by salinity, WT, and their interactive effect (*p*<0.05). The correlation between salinity and feces ash content was positive (*r*=0.536; *p*=0.001). Meanwhile, there was an inverse correlation between feces protein content, and salinity (*r*=-0.392; *p*=0.018). In addition, there were inverse relationships among WT and feces protein (*r*=-0.783; *p*=0.0001), lipid (*r*=-0.760; *p*=0.001) and energy contents (*r*=-0.824; *p*=0.0001), whereas a positive correlation was found between WT and feces ash level (*r*=0.582; *p*=0.001).

Table 1: Growth performance and feed utilization of *A. arabicus* juveniles under different salinity and temperature treatments (means±SE, n=3). A different superscript in the same row denotes statistically significant differences (*p*<0.05).

	Treatments													**Two-way ANOVA**		
	S_{12} T_{16}	**S_{12} T_{23}**	**S_{12} T_{30}**	**S_{12} T_{35}**	**S_{35} T_{16}**	**S_{35} T_{23}**	**S_{35} T_{30}**	**S_{35} T_{35}**	**S_{50} T_{16}**	**S_{50} T_{23}**	**S_{50} T_{30}**	**S_{50} T_{35}**	Pooled SEM	**Salinity**	**Temperature**	**Interactions**
BW_i (g)	10.4	10.1	10.0	9.9	9.9	10.2	10.1	10.3	10.0	10.1	10	10.2	0.2	1.000	1.000	1.000
BW_f (g)	13.4^{f}	15.2^{cd}	16.5^{b}	16.5^{b}	13.8^{ef}	14.5^{de}	16.7^{b}	15.8^{c}	13.8^{ef}	15.3^{cd}	16.1^{bc}	18.9^{a}	0.3	0.006	0.0001	0.0001
WG (%)	28.9^{g}	50.3^{de}	65^{bc}	67.8^{b}	40.2^{ef}	41.7^{ef}	65^{bc}	54.1^{cd}	38.9^{fg}	51.7^{de}	60.9^{cd}	84.6^{a}	2.7	0.011	0.0001	0.002
SGR (% BW_i day^{-1})	0.45^{g}	0.72^{de}	0.89^{bc}	0.92^{b}	0.58^{ef}	0.6^{ef}	0.89^{bc}	0.77^{cd}	0.58^{fg}	0.74^{de}	0.84^{cd}	1.1^{a}	0.1	0.021	0.0001	0.005
Survival (%)	95^{a}	97.5^{a}	97.5^{a}	95^{a}	97.5^{a}	100^{a}	97.5^{a}	97.5^{a}	97.5^{a}	97.5^{a}	100^{a}	90^{b}	1.0	0.019	0.0001	0.0001
FI (g $fish^{-1}$)	5.2^{f}	7.0^{cd}	7.5^{bc}	7.8^{bc}	6.2^{de}	6.4^{de}	7.0^{cd}	8.2^{b}	6.1^{e}	5.6^{e}	6.3^{e}	10.0^{a}	1.3	0.782	0.0001	0.0001
FCR	2.2^{a}	1.4^{bc}	1.2^{bc}	1.3^{bc}	1.7^{ab}	1.9^{ab}	1.1^{c}	1.7^{ab}	1.7^{ab}	1.1^{c}	1.0^{c}	1.8^{ab}	0.1	0.492	0.002	0.091
HSI (%)	1.7	1.2	1.6	1.2	1.3	1.5	1.2	1.2	1.3	1.6	1.2	1.4	0.1	0.249	0.275	0.542
K (%)	2.1	2.1	2.0	2.0	2.2	2.1	2.1	2.2	2.1	2.1	2.2	2.3	0.1	0.09	0.092	0.48

Abbreviations: BW_i: initial body weight; BW_f: final body weight; WG: weight gain; SGR: specific growth rate; FI: feed intake; FCR: feed conversion ratio; HSI: hepatosomatic index; K: Fulton's condition factor, SEM: standard error mean.

Table 2: Whole body and feces chemical composition (%) and energy content (KJ g^{-1}) in *A. arabicus* juveniles under different salinity and temperature treatments (means±SE, n=3). A different superscript in the same row denotes statistically significant differences (*p*<0.05). *SEM: standard error mean.

	Treatments														Two-way ANOVA		
	Initial	**S_{12} T_{16}**	**S_{12} T_{23}**	**S_{12} T_{30}**	**S_{12} T_{35}**	**S_{35} T_{16}**	**S_{35} T_{23}**	**S_{35} T_{30}**	**S_{35} T_{35}**	**S_{50} T_{16}**	**S_{50} T_{23}**	**S_{50} T_{30}**	**S_{50} T_{35}**	Pooled SEM	**Salinity**	**Temperature**	**Interactions**
Whole body																	
Moisture	71.2	69.6^{a}	68.3^{ab}	65.8^{bc}	66.7^{bc}	66.2^{bc}	67.0^{bc}	66.1^{bc}	67.0^{bc}	66.8^{bc}	68.2^{ab}	65.4^{c}	66.5^{bc}	0.3	0.206	0.012	0.213
Crude protein	17.8	18.3	19.9	19.2	19.5	20.3	18.5	19.0	19.1	20.3	18.9	21.1	20.1	0.2	0.141	0.681	0.178
Crude lipid	8.2	8.4^{c}	8.1^{c}	10.7^{a}	9.3^{bc}	9.4^{bc}	10.2^{ab}	10.2^{ab}	9.7^{ab}	9.0^{bc}	8.5^{c}	8.8^{bc}	8.8^{bc}	0.2	0.014	0.073	0.100
Ash	2.8	3.7^{c}	3.7^{c}	4.3^{ab}	4.5^{ab}	4.1^{bc}	4.3^{ab}	4.7^{a}	4.2^{ab}	3.9^{bc}	4.4^{ab}	4.7^{a}	4.6^{a}	0.1	0.0001	0.0001	0.04
Energy	7.4	7.7^{c}	7.9^{bc}	8.7^{a}	8.3^{bc}	8.5^{ab}	8.4^{ab}	8.5^{ab}	8.3^{bc}	8.4^{ab}	7.8^{bc}	8.4^{ab}	8.2^{bc}	0.1	0.164	0.025	0.125
Faeces																	
Moisture	-	80.1^{c}	82.1^{ab}	81.1^{bc}	79.9^{c}	83.0^{ab}	83.2^{ab}	81.9^{ab}	83.6^{a}	79.9^{c}	79.8^{c}	82.5^{ab}	82.5^{ab}	0.3	0.0001	0.064	0.0001
Crude protein	-	6.1^{a}	5.2^{bc}	5.5^{b}	4.9^{cd}	5.5^{b}	4.8^{d}	4.4^{e}	3.9^{f}	5.9^{a}	4.9^{cd}	4.3^{e}	4.3^{e}	0.1	0.0001	0.0001	0.004
Crude lipid	-	1.3^{a}	0.8^{d}	0.7^{e}	0.6^{f}	1.1^{b}	0.7^{e}	0.4^{g}	0.4^{g}	1.4^{a}	0.9^{c}	0.9^{c}	0.9^{c}	0.3	0.0001	0.0001	0.0001
Ash	-	5.9^{fg}	5.6^{g}	6.4^{f}	8.1^{b}	6.1^{f}	6.1^{f}	6.8^{de}	7.2^{cd}	7.6^{bc}	8.8^{a}	8.1^{b}	8.1^{b}	0.2	0.0001	0.0001	0.0001
Energy	-	2.0^{a}	1.5^{c}	1.6^{c}	1.4^{d}	1.7^{c}	1.4^{d}	1.1^{e}	1.05^{f}	1.9^{b}	1.5^{c}	1.4^{d}	1.4^{d}	0.05	0.0001	0.0001	0.009

Table 3: Energy budget components in *A. arabicus* juveniles under different salinity and temperature treatments (means±SE, n=3). A different superscript in the same row denotes statistically significant differences ($p<0.05$).

	Treatments													Two-way ANOVA		
	S_{12} T_{16}	S_{12} T_{23}	S_{12} T_{30}	S_{12} T_{35}	S_{35} T_{16}	S_{35} T_{23}	S_{35} T_{30}	S_{35} T_{35}	S_{50} T_{16}	S_{50} T_{23}	S_{50} T_{30}	S_{50} T_{35}	Pooled SEM	**Salinity**	**Temperature**	**Interactions**
C (J g^{-1} h^{-1})	31.3^{e}	53.3^{cd}	77.6^{ab}	75.3^{ab}	49.8^{cd}	56.0^{cd}	66.2^{bc}	81.7^{ab}	49.3^{d}	54.5^{cd}	71.2^{b}	90.3^{a}	3.0	0.150	0.0001	0.015
G (% of C)	50.0^{c}	53.9^{b}	57.3^{a}	49.3^{c}	59.1^{a}	50.0^{c}	51.1^{bc}	53.9^{b}	55.1^{ab}	52.3^{bc}	56.1^{ab}	58.0^{a}	0.8	0.537	0.034	0.192
R (% of C)	44.8^{ab}	41.7^{b}	39.0^{c}	47.8^{a}	37.5	45.6^{a}	46.7^{a}	42.2^{b}	41.1^{b}	43.9^{ab}	40.0^{bc}	39.2^{c}	0.8	0.026	0.001	0.013
U (% of C)	1.5^{b}	1.6^{b}	1.9^{a}	1.5^{b}	0.7^{d}	1.5^{b}	0.7^{d}	0.9^{c}	0.9^{c}	0.9^{c}	1.2^{bc}	1.0^{c}	0.1	0.001	0.032	0.107
F (% of C)	3.7^{a}	2.8^{a}	1.8^{b}	1.4^{c}	2.7^{ab}	2.9^{a}	1.5^{c}	3.0^{a}	2.9^{a}	2.9^{a}	2.7^{ab}	1.8^{b}	0.1	0.735	0.0001	0.005
K (%)	98.4^{c}	98.5^{c}	98.8^{ab}	98.9^{ab}	99.2^{a}	98.6^{bc}	98.8^{bc}	99.3^{a}	98.7^{bc}	98.6^{bc}	98.9^{ab}	99.0^{a}	0.1	0.059	0.021	0.148

Abbreviations: C: energy budget; G: the energy accumulated for growth; R: the energy loss through respiration; U: the energy loss as ammonia excretion; F: the energy loss in feces; K: assimilation efficiency, SEM: standard error mean.

Oxygen consumption and ammonia excretion

The oxygen consumption of fish was significantly affected by WT (p=0.0001), and the interactive effect of WT and WS (P=0.006). In addition, a positive correlation was found between fish OC, and WT (r=0.594; p=0.001). Ammonia excretion was affected by the WS (p=0.043), and WT (p=0.001), but there was no interactive effect of WT, and WS on AE (p=0.142) (Fig. 2b). There was a negative relationship between WS, and AE (r=-0.865; p=0.029), meanwhile the correlation between WT, and AE was positive (r=0.422; p=0.01).

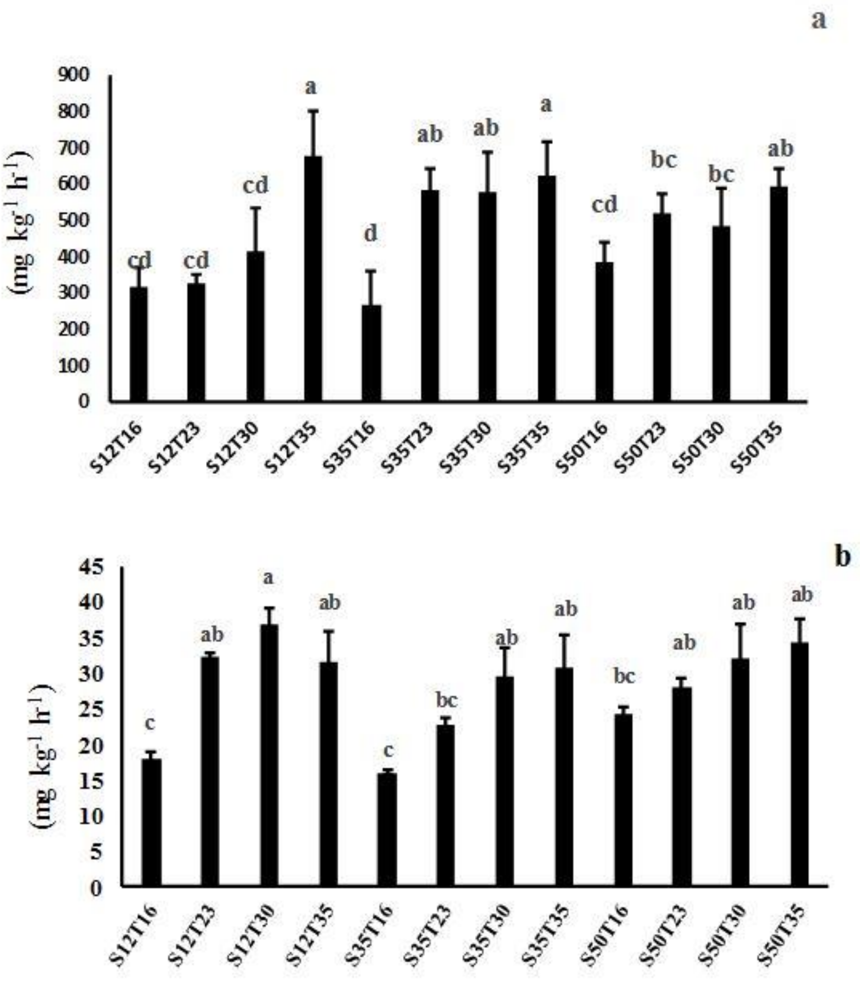

Figure 2: Oxygen consumption (a) and ammonia excretion (b) in *A. arabicus* juveniles under different salinity and temperature treatments (means±SE, n=3). A different superscript in the same row denotes statistically significant differences (*p*<0.05).

Energy budget

The energy budget of fish was influenced by WT (p=0.0001), and its interactive effect with WS (p=0.015). In addition, a positive correlation was found between energy budget and WT (r=0.798; P=0.0001). Regarding energy allocations, the main part of energy budget was spread to growth (G) in all treatments (between 49.3% and 58.0%), and respiration (R; between 37.5 and 46.7%). The excretion through feces (F; between 1.5% and 3.7%) and ammonia (U; between 0.7% and 1.9%) comprised the small fraction of the energy budget in *A. arabicus*. The following energy budget equations were determined for different treatments, including:

100C= 50G+44.8R+1.5U+3.7F $(S_{12}T_{16})$,

100C= 53.9G+41.7R+1.6U+2.8F $(S_{12}T_{23})$,

100C= 57.3G+39.0R+1.9U+1.8F $(S_{12}T_{30})$,

100C= 49.3G+47.8R+1.5U+1.4F $(S_{12}T_{35})$,

100C= 59.1G+37.5R+0.7U+2.7F $(S_{35}T_{16})$,

$100C= 50G+45.6R+1.5U+2.9F$ ($S_{35}T_{23}$), $100C= 53.9G+43.2R+0.9U+3.0F$ ($S_{35}T_{35}$), $100C= 55.1G+41.1R+0.9U+1.9F$ ($S_{50}T_{16}$), $100C= 52.3G+43.9R+0.95U+2.9F$ ($S_{50}T_{23}$), $100C= 56.1G+40.0R+1.2U+2.7F$ ($S_{50}T_{30}$), $100C= 58.0G+39.2R+1.0U+1.8F$ ($S_{50}T_{35}$). The assimilation efficiency (K) affected by WT (p=0.021), but it did not affect by WS or interaction between WS and WT. In addition, fish reared at $S_{50}T_{35}$ treatment showed higher assimilation efficiency but fish reared at $S_{12}T_{16}$ and $S_{12}T_{23}$ demonstrated lower assimilated efficiency values.

Discussion

The previous study by Jian *et al.* (2003) demonstrated that the upper incipient lethal WT in *A. latus* juveniles ranged between 32.8-36.4°C. Furthermore, critical maximum salinity in *A. latus* juveniles was reported to range between 54-69 ‰. In the current study, fish reared in 35 °C groups experienced the upper incipient lethal WT that was reported for *A. latus*; however, except for fish in $S_{50}T_{35}$, the survival rate of fish in $S_{12}T_{35}$ and $S_{35}T_{35}$ treatments were the same as the other experimental groups. Despite the lower survival rate, fish in the $S_{50}T_{35}$ group showed the highest growth rate, indicating that increasing WT and WS were unlikely to be direct causes of mortality in this treatment. It should be mentioned that the upper and lower incipient lethal WT, and WS for *A. arabicus* may be higher than those reported in *A. latus* (Jian *et al.*, 2003) because *A. arabicus* is found in the Persian Gulf with tropical weather compared to *A. latus* that is found in Japan coasts with temperate weather. Similar to our results, Hong Phuc *et al.* (2015) reported that the interaction of salinity (0‰), and WT (35°C) reduced the survival rate in striped catfish (*Pangasianodon hypophthalmus*).

Generally, the growth rate of fish enhances with increasing WT up to the upper extreme temperature range tolerated by the fish species (Jobling 1997; Ham *et al.*, 2003). In the current study, fish in the $S_{50}T_{35}$ group had a better growth rate compared to other treatments, and growth performance positively correlated with WT, indicating the positive thermal-growth coefficient effect (Schulte, 2011). However, the worst FCR in $S_{50}T_{35}$ was mainly attributed to lower survival rate in this group. Increasing WT induces the greater specific dynamic action that was associated with the acceleration of metabolic rate, digestion and nutrients assimilation, which consequently improved growth (Xie and Sun 1993; Sun *et al.*, 2014). Also, at higher WT, the feed consumption in fish was enhanced, mainly due to increments of appetite (Jobling, 1997). Similarly, increasing WT significantly enhanced the growth performance that coincided with an increase in FI, and FCR in different fish species, such as Asian seabass (*Lates calcarifer*, Bermudes *et* *al.*, 2010), tongue sole (*Cynoglossus semilaevis*, Fang *et al.*, 2010), turbot (Ham *et al.*, 2003), juvenile lumpfish (*Cyclopterus lumpus*; Nytro *et al.*, 2014), European seabass (*Dicentrachus labrax*; Ruyet *et al.*, 2004) and cobia (*Rachycentron canadum*, Sun and Chen, 2009). Also, the findings of the present study showed a significant influence of WS on growth performance; however, there was no positive correlation between these parameters. In this sense, it has been proved that iso-osmotic conditions improve growth as a consequence of lower energetic expenditure for osmoregulation that reduces maintenance metabolic rate and thus, more energy is channelled into somatic growth (Boeuf and Payan, 2001).

In this study, FI was significantly affected by WT, and they were positively correlated, which might be due to increased metabolic rate as also reported in juvenile spotted wolffish, *Anarhichas minor* (Magnussen *et al*., 2008), cobia (Sun and Chen 2009) and black bream (*Acanthopagrus butcheri*, Partridge and Jenkins 2002). Furthermore, FCR gradually reduced with increasing WT from 16 to 30°C, suggesting an increase in nutrient digestibility and assimilation. However, FCR was markedly increased in fish reared at 35°C, indicating nutrient assimilation may be disturbed at this temperature. In the current study, condition factor and HSI was not influenced by treatments. In contrast, different research reported that HSI was significantly influenced by WT, such as minnow (*Phoxinus phoxinus*; Cui and Wootton 1988), *Cirrhinus mrigala* (Singh *et al.*, 2008), Asian catfish, (*Clarias batrachus*; Singh *et al.*, 2009), which might be due to the drastic alternation of biochemical composition, especially lipid content (Cui and Wootton, 1988).

The findings of the present research demonstrated that the biochemical composition of the whole body and feces of fish was markedly affected by WT and salinity. Similarly, it has been reported that WT noticeably affects body composition in different fish species, and its effect was species-specific (Singh *et al.*, 2009; Fang *et al.*, 2010; Fatma and Ahmed, 2020). Temperature can affect the biochemical composition of the whole body by altering FI (Jobling, 1997); however, due to the restricted number of research with inconsistent findings, no generalizations can yet be made regarding the influence of WT on the biochemical composition of fish. In the present study, the moisture of the whole body was reduced by increasing WT which coincided with an increment of whole body ash content, as previously reported in juvenile Nile tilapia (Xie *et al.*, 2011) and white seabream (*Diplodus sargus*, Anacleto *et al.*, 2018). In addition, whole-body lipid content gradually increased from the 16 to 30°C groups associated with the enhancement of FI and improvement of FCR. However, whole-body lipid content was decreased in 35°C treatments, maybe as a consequence of enhanced metabolic expenditure and disorder of nutrients' assimilation at high temperatures (El-Sayed *et al.,* 1996; García-Guerrero *et al.,* 2003). In accordance with the results of the current study, Sun *et al.* (2015) reported that the protein and lipid contents in the whole body of the leopard coral grouper (*Plectropomus leopardus*) increased when WT was enhanced from 15 to 30°C, but their values remarkably decreased at 35°C. In the current study, inverse relationships were found among WT and feces protein, lipid, and energy contents, which might be due to the improvement of nutrients' assimilation as a consequence of increased digestive enzymes activities at higher WT. Furthermore, the feces energy content in fish reared at 16°C was greater than in other treatments, suggesting that lower digestibility of nutrients at low WT may be due to the reduction of digestive enzymes activities, and less efficient assimilation of nutrients (Caulton, 1978).

Previous research has reported that various factors, such as WT, salinity, dissolved oxygen, SDA, and escape behavior, significantly affect C and AE in marine teleosts (Gracia-Lopez *et al.,* 2006; Zheng *et al.,* 2008). Increased WT normally results in an increment of OC because at higher WT locomotion, SDA, digestion, and the cost of protein synthesis are running at their maximum rate inducing aerobic metabolism, and enhancing OC (Lyndon *et al.,* 1992; Luo and Xie 2008). In this study, OC enhanced with increasing WT in *A. arabicus* was consistent with the results of previous studies in different fish species (Requena *et al.,* 1997; Das *et al.,* 2005; Zheng *et al.,* 2008; Xing *et al.,* 2019). On the other hand, the findings of the current study demonstrated that OC was profoundly affected by WT rather than salinity, as *A. arabicus* is a euryhaline species, and can tolerate a wide range of salinity (Movahedinia *et al.,* 2009; Farshadian *et al.*, 2019). Similarly, previous studies in different marine fish species demonstrated that alterations in the metabolic rate, and OC as a result of changes in salinity might be very small because of the euryhaline characteristics of these species (da Silva Rocha *et al.,* 2005; Wuenschel *et al.,* 2005; Zheng *et al.,* 2008; Pérez-Robles *et al.,* 2012; Xu *et al.,* 2019).

In the present study, alternation in AE was changed by salinity, and there was an inverse relationship between AE and salinity, as previously reported in other euryhaline marine fish species, such as mudskipper (*Boleophthalmus pectinirostris*, Cao and Wang 2015), *Centropomus parallelus* (da Silva *et al.,* 2005), the inanga (*Galaxias maculatus,* Urbina and Glover 2015), tide pool sculpin (*Oligocottus maculosus,* Wraght *et al.,* 1995), Spotted Scat (*Scatophagus argus,* Xu *et al.,* 2019) and Mozambique tilapia *Oreochromis mossambicus* (Zikos *et al.,* 2015) in which AE increased in lower salinities but decreased in higher salinities. It has been confirmed that under an increase in salinity, a shift from ammonotelism to ureotelism in fish occurred, or nitrogenous wastes converted into free amino acids (Gershanovich and

Pototskij, 1995; Frick and Wright, 2002). In addition, AE was linearly enhanced by increasing WT, as also reported in other marine fish species, such as juvenile *Dipludus sargus* (Anacleto *et al.*, 2018) and areolated group (*Epinephelus areolatus*) and mangrove snapper (*Lutjanus argentimaculatus*) (Leung *et al.*, 1999).

In the current study, the higher proportion of food energy was allocated to growth (49.3–59.1%) and metabolism (39.0–47.8%), whereas in many previous studies, the energy costs for metabolism and growth comprised 50 and 30% of the total energy budget, respectively (Sandersfeld *et al.*, 2015; Sun *et al.*, 2006, 2009, 2014). These discrepancies between the findings of the present study and the others are possibly due to the differences in species, acclimation duration, experimental design, and measurement methodology (Jobling, 1997). It should be mentioned that the fish used in this study were in the juvenile stage, and more portion of feed energy may be used for somatic growth, as previously described in *D. sargus* (Anacleto *et al.*, 2018). According to Sun *et al.* (2006, 2009), feces (F) and nitrogenous excretion (U) only account for a small portion of energy allocations in carnivorous fish species and do not have a pronounced effect on the proportion of feed energy allocated to growth. On the other hand, despite some statistical differences among components of energy budget in *A. arabicus*, the proportion of dietary energy to each component of energy budget (C) were not varied between 16 to 35°C, as previously reported in white seabream (Anacleto *et al.,* 2018), and cobia (Sun *et al.*, 2006, 2009, 2014). According to the findings of the current study, the assimilated energy (K) was significantly affected by WT, and it seems the assimilated energy enhanced by augmentation of WT might be attributed to the higher SDA of fish at high WT.

In summary, the findings of the current investigation indicated that *A. arabicus* has high thermal adaptation owing to its specific ecological niche. Furthermore, as *A. arabicus* is a euryhaline species, the effects of WT were more pronounced than salinity on its growth performance and energy budget. Also, there was a positive correlation between WT and growth parameters of *A. arabicus*, attributed to higher FI in this fish species. The whole body and feces biochemical composition profoundly were affected by WT and salinity, suggesting the significant effects of these parameters on energy retention and waste of this species. Oxygen consumption was dramatically affected by WT, and a large proportion of feed energy was also allocated to respiration (R); meanwhile, a small portion of the energy budget was allocated to waste excretions (F+U). Moreover, AE positively correlated with WT, but its correlation with salinity was negative. According to the results mentioned above, the preferred WT for this species is between 30 to 35°C under intermediate salinity.

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