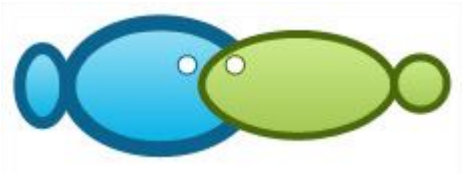

Changes of glucose, cortisol, and ions in spotted scat *Scatophagus argus* under different ambient salinities

[1]Huy V. Nguyen, [2]Quynh B. Le, [1]Vinh H. Nguyen, [1]Tue M. Le, [1]Huu T. Le, [1]Chat T. Ton

[1] Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 530000 Hue city, Vietnam; [2] Tuan Kiet TS Company Limited, 530000 Hue city, Vietnam.
Corresponding author: H. V. Nguyen, huy.nguyen@hueuni.edu.vn

Abstract. Spotted scat, *Scatophagus argus* is a euryhaline fish and is widely distributed in brackish and marine waters. Salinity influences the standard metabolic rate of fish, fish ingestion rate, feed conversion efficiency, metabolism, and hormone secretion, thus affecting the body growth and the accumulation of nutrients in the fish. This study aims to determine the optimal salinity for growth performance, and physiological characteristics of *S. argus*. The experiment was conducted in triplicates with 12 composite tanks at 4 different salinity treatments: 10 ‰; 15 ‰ (control); 20 ‰, and 25 ‰, with a completely randomized design method. The weight and total length of fish at different salinities were measured before and after the experiment. The results show that the specific growth rate (SGR), survival of fish, and feed conversion ratio (FCR) varied significantly between treatments of 15 ‰ and 25 ‰ ($p < 0.05$). For fish, all treatments had a survival rate as high as 100%. The FCR in the 15 ‰ group was lower than that of the 25 ‰ group ($p < 0.05$), however no significant difference in FCR was found among the 10‰, 15 ‰, and 20 ‰ groups ($p > 0.05$). There was a significant difference in plasma cortisol levels between the 10% and 15% treatments when compared to 20% and 25% ($p < 0.05$). There was no significant difference in glucose concentrations among salinity treatments ($p > 0.05$). Changes in ionic concentrations of Na^+ and Cl^- were at the highest value at the treatment of 25 ‰ and all treatments reached the peak on d-1 and then declined until the end of the experiment. Changes in the concentrations of glucose, Ca^{++}, and K^+ during the time course of the experiment were not significantly different under different salinities. As a result, we propose using a salinity of 15 ‰ for *S. argus* farming in order to achieve a higher growth rate and lower the FCR, and thus better production.
Key Words: growth rates, physiological changes, *S. argus,* salinity, stress.

Introduction. The Tam Giang-Cau Hai Lagoon in Vietnam is the largest brackish lagoon in Southeast Asia and has a surface area of 22,000 hectares. It is home to a wide range of aquatic species, including many highly valuable fish. The spotted scat (*Scatophagus argus*) is a typical species of this ecosystem and one of the most economically important aquaculture species. *S. argus* is a euryhaline fish and widely distributed in brackish and marine waters of the Indo-Pacific and Southeast Asia (Mookkan et al 2014; Su et al 2019). Furthermore, *S. argus* is considered a typical species of this ecosystem and is one of Southeast Asia's most economically important brackish water fish species, both for human consumption and as ornamental fish (Mandal et al 2020). *S. argus* has become a popular species and important food fish due to its good taste, nutrient quality with high protein content. In Vietnam, *S. argus* is considered the main target species for aquaculture because of its high adaptive capacity to environmental stress (Su et al 2016) and its suitability to mono- and polyculture systems (Gupta 2016).

Salinity affects the standard metabolic rate of fish, fish ingestion rate, feed conversion efficiency, metabolism, and hormone secretion, thus affecting the growth of the body and the accumulation of nutrients in the fish (Boeuf & Payan 2001; Ern et al 2014; Xu et al 2020). An increase in water salinity exposes fish to acute extracellular osmolarity and shrinks their tissues (Jensen et al 2002). Cortisol, glucose, and lactate levels are general stress indicators in fish (Santos & Pacheco 1996; Pacheco & Santos

2001). Changing in serum cortisol and glucose levels of tilapia *Oreochromis mossambicus* under salinity stress has been reported by Cataldi et al (2005). Similarly, grouper *Epinephelus malabaricus*, a marine fish, also encountered sudden stress during the salinity experiment (Tsui et al 2012).

Environmental factors such as salinity strongly influence fish growth (Barton & Zitzow 1995; Bœuf & Payan 2001). Even though studies on teleosts like groupers (*E. malabaricus*), tilapia (*O. mossambicus*), catfish (*Pangasianodon hypophthalmus*), and *Mugil cephalus* have been done for osmoregulatory processes, only a few studies documented the effects of salinity stress on growth performance and the change of plasma cortisol, glucose, ions of *S. argus*. The present study aims to determine optimal salinity for growth performance and physiological characteristics of *S. argus* under different salinities.

Material and Method

Experimental design. This study was conducted from 15 May 2022 to 24 June 2022 in the farm of Tuan Kiet TS Company Limited in Central Vietnam. The initial body weight and total length of *S. argus* were 34.37±0.30 g and 11.49±0.02 cm, respectively. All experimental fish were kept in 4 m^3 composite tanks with brackish water at a salinity of 15 ‰ for three days prior to the experiment (similar to salinity in farm). The experiment was conducted in triplicates with 12 composite tanks at 4 different salinity treatments: 10 ‰ ; 15 ‰ (control); 20 ‰ , and 25 ‰ , with a completely randomized design method. Each tank has a volume of 500 liters and was stocked with 30 fish, aerated constantly with an air stone (90 fish per treatment). Fish were fed twice a day until satiation (7 a.m. and 6 p.m.) with commercial finfish-floating feed containing 40% protein (Sea Masster, SHENG LONG BIO-TECH INTERNATIONAL CO., LTD.). During the experimental period, waste from the tank bottom was siphoned in combination with 20% water exchange every day. The water quality of the tanks was meticulously recorded twice daily. Dissolved oxygen (DO) was measured in mg L^{-1} using a digital DO meter (Model: CE 225908), water temperature was measured by using digital thermometer (model: CE 225908) in ^oC, and pH was recorded by digital pH meter (Model: CE 224469). Water temperature, DO and pH were kept at 28.3±1.2^oC, 5.8±0.36 mg L^{-1}, and 7.8± 0.42, respectively.

Fish growth measurement. Weight and length of fish were measured before and after the experiment to reduce stress and disturbance for the fish. Electronic scale and ruler calculated the weight and length of fish. The specific growth rate (SGR) in length (SGR_L) and weight (SGR_W) were calculated using the formula:

$$SGR_L or\ SGR_W(\%) = \left[\frac{\ln(X_2)-\ln(X_1)}{T_2-T_1}\right] \times 100$$

where: X_1 = the weight or length of fish at the beginning of the experiment (T_1);
X_2 = the weight or length of fish at the end of experiment (T_2);
T2–T1 = experimental duration (days).

Feed conversion ratio (FCR) was calculated using the formula:

$$FCR = \frac{Feed\ given\ \ (g)}{Fish\ weight\ gain\ \ (g)}$$

Survival rate (SR) (%) was calculated using the formula:

$$SR\ (\%) = \frac{Numbers\ of\ fish\ harvested}{Number\ of\ fish\ stocked} \times 100$$

Blood sampling and analysis. Day-0 (d-0), day-1 (d-1, 24 h), day-5 (d-5), day-10 (d-10), day-20 (d-20), and day-40 were the exposure times (d-40). Before sampling, fish were anesthetized with AQUI-S® 10 mL m^{-3}. Individual fish were supported with a foam rubber cradle upside down in a water-filled bowl immediately after captured. To reduce stress, the fish's head was covered with a moist towel. A 1 mL sterile pre-heparinized syringe was used to draw blood from the caudal vein. Blood samples were divided into

1.5 mL Eppendorf tubes before centrifugation at 4°C, 6,000 rpm for 5 minutes, and stored at -80°C for later analysis. Cortisol level was measured using an enzyme-linked immunosorbent assay (ELISA) method with Cobas e 601 machines and Elecsys test kit reagent. Glucose level was measured by HITACHI COBAS C 311, with COBAS C Test kit reagent. The concentrations of [Na^+], [K^+], [Ca^{++}], [Cl^-] ions were measured by using the COBAS pro-parameter analyzer using the ISE test kit reagent, following the ISE run procedure. Blood plasma analyses were performed in triplicates.

Statistical analysis. The effects of the salinity on plasma glucose, cortisol, and ions [Na^+], [K^+], [Ca^{++}], [Cl^-] concentrations were evaluated using a one-way analysis of variance (ANOVA) by using the SPSS version 20.0 to determine the sample variance. Statistical significance for all tests was found at a level of ($p < 0.05$) with Turkey's test.

Results. Table 1 summarizes the experiment's fish growth parameters after 40 days. Salinity influenced significantly the growth performance of *S. argus* ($p < 0.05$). Fish biomass production was higher (60.35 g $fish^{-1}$) at salinity treatment of 15 ‰, followed by 10 ‰ (58.71 g $fish^{-1}$) and 20 ‰ (57.82 g $fish^{-1}$), and then 25 ‰ (55.08 g $fish^{-1}$). The SGR, survival of fish, and FCR varied significantly between treatments of 15 ‰ and 25 ‰ ($p < 0.05$). However, these parameters were not significantly different among treatments of 10 ‰ or 20 ‰, compared to other treatments ($p > 0.05$) (Table 1). Specific growth rate in weight (SGR_W) was 1.32%, 1.44%, 1.30%, and 1.19% day^{-1} in salinities of 10 ‰, 15 ‰, 20 ‰, and 25 ‰, respectively. All treatments demonstrated a high as 100% fish survival rate, indicating that this species has a high adaptability to changes in ambient salinity. There was a significant change in FCR in relation to the experimental salinities. The FCR in the 15 ‰ group was lower than that of the 25 ‰ group ($p < 0.05$). No significant difference in FCR was found among the 10 ‰, 15 ‰ and 20 ‰ groups ($p > 0.05$). The highest FCR occurred in the treatment of 25 ‰, while the lowest one occurred in the 15 ‰ treatment.

Table 1

Growth performance, survival rate, and FCR of *S. argus* during experiment

Fish growth parameters	*Treatments*			
	10 ‰	*15 ‰*	*20 ‰*	*25 ‰*
Ls (cm)	11.52 ± 0.08^{a}	11.49 ± 0.09^{a}	11.46 ± 0.17^{a}	11.50 ± 0.15^{a}
Le (cm)	13.34 ± 0.43^{ab}	13.55 ± 0.04^{b}	13.23 ± 0.23^{ab}	13.21 ± 0.14^{ab}
SGR_L (% day^{-1})	0.37 ± 0.01^{ab}	0.41 ± 0.02^{b}	0.36 ± 0.01^{ab}	0.35 ± 0.03^{a}
Ws (g)	34.69 ± 1.31^{a}	33.97 ± 1.42^{a}	34.34 ± 2.00^{a}	34.28 ± 1.36^{a}
We (g)	58.71 ± 1.72^{ab}	60.35 ± 0.74^{b}	57.82 ± 1.05^{ab}	55.08 ± 0.98^{a}
SGR_W (% day^{-1})	1.32 ± 0.04^{ab}	1.44 ± 0.05^{b}	1.30 ± 0.13^{ab}	1.19 ± 0.04^{a}
SR (%)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
FCR	1.35 ± 0.05^{ab}	1.28 ± 0.09^{b}	1.39 ± 0.08^{ab}	1.54 ± 0.11^{a}

Data with the different letter $^{a, b, c}$ on the same row shows the significantly different at level of $p < 0.05$. Ls and Ws are the length and weight of fish at the start of experiment; Le and We are the length and weight of fish at the end of experiment.

Figure 1 shows the physiological changes of *S. argus* under different salinities. All parameters of glucose, cortisol, and ions (Na^+, K^+, Ca^{++}, Cl^-) increased suddenly from d-0 to d-1, before decreasing gradually until the end of the experiment (d-40). After being transferred from holding to experimental tanks, fish were stressed on the first day. Fish gradually adapted to the stress environment by controlling their physiology.

Plasma cortisol levels in all treatments followed a similar pattern, peaking at d-1 (after 24 hours) and then gradually decreasing to as low as the beginning at d-40. Except at d-40, there was a significant difference in cortisol concentrations between treatments of 10 ‰ or 15 ‰ and compared to 20 ‰ and 25 ‰ over the sampling points ($p < 0.05$), indicating that fish seems to be adapted to the change in ambient salinity. However, no significant differences in plasma cortisol levels were found between treatments 10 ‰ and 15 ‰ or 20 ‰ and 25 ‰ ($p > 0.05$). The plasma cortisol concentrations of fish at d-1 and d-40 in treatments of 10, 15, 20, and 25 ‰ were 129.27, 126.56, 172.90, and

195.53 nmol L^{-1}; and 20.70, 21.67, 22.97, and 26.93 nmol L^{-1}, respectively. In any of the treatments, there was no significant difference in challenge salinity on glucose concentrations ($p > 0.05$). From day 5 onwards, glucose concentration in all treatments tended to decrease gradually. Similarly, changes in Na+ and Cl- ionic concentrations were highest at treatment 25, and all treatments reached a peak on d-1 and then declined until the end of the experiment. There was a significant difference in plasma Na+ concentrations between salinities of 10 ‰ to 15 ‰ and 20 ‰ to 25 ‰ ($p < 0.05$), but no difference was found between 10 ‰ and 15 ‰ or 20 ‰ and 25 ‰ ($p > 0.05$).

On the other hand, there were a few changes in the concentrations of glucose, Ca^{++}, and K^{+} during the time-course of the experiment. These parameters increased abruptly at d-1 before gradually decreasing thereafter, but no significant differences were found between treatments over the sampling period ($p > 0.05$).

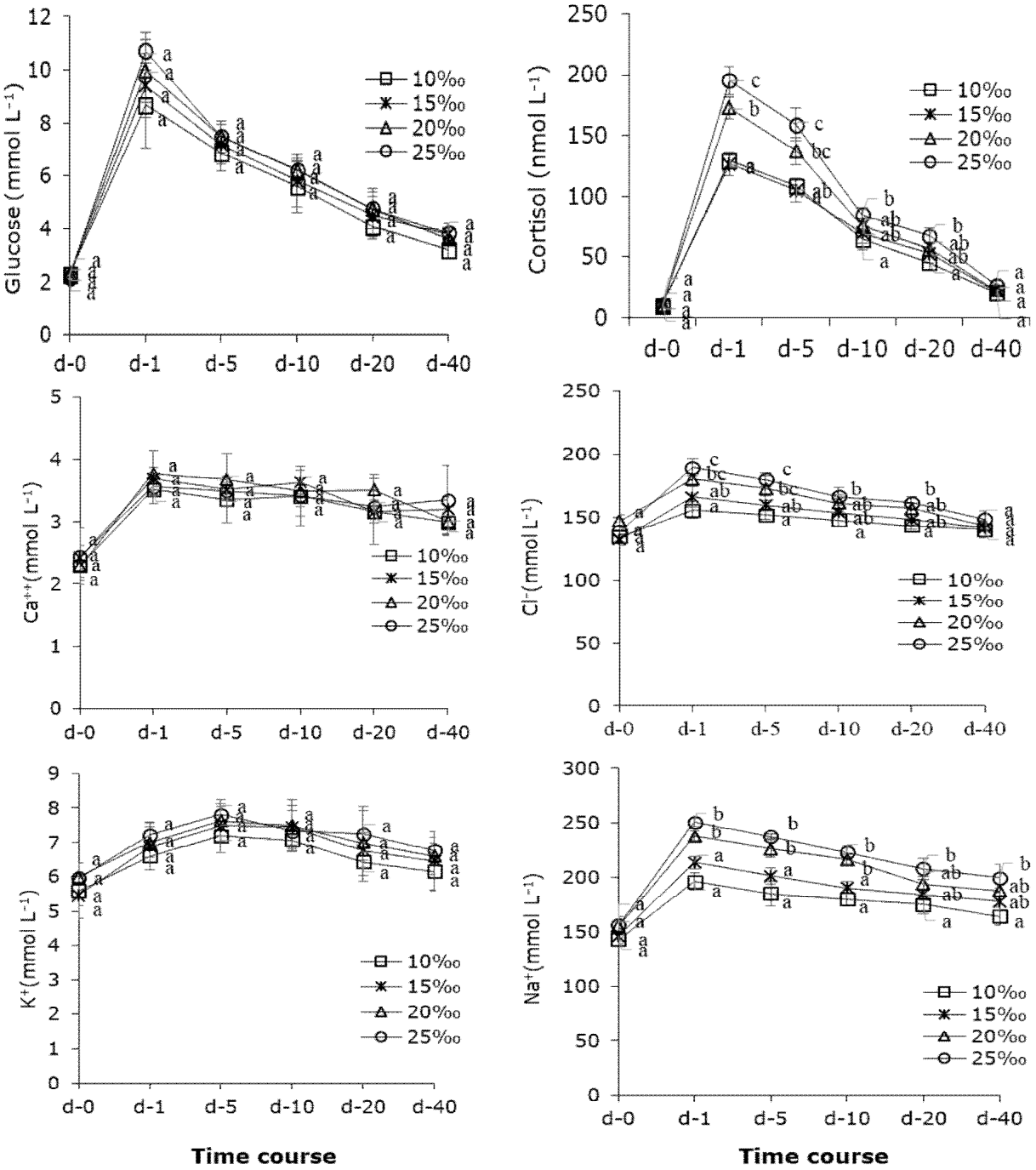

Figure 1. Time-course changes in cortisol, glucose, and ionic concentrations of *S. argus* under different salinity stresses.

Discussion. The current study aims to evaluate the growth, survival rates, and FCR of *S. argus* at different salinities comparable to those they might experience in the coastal lagoon for 40 days experiment. Furthermore, we measured the physiological parameters in blood plasma to elucidate the stressful status of fish under the impact of ambient salinities. During the experiment, each group's body weight increased significantly, as did the SGR, SR, and FCR. It appears that rearing *S. argus* at a salinity of 25 ‰ resulted in lower growth and higher FCR than other treatments. These results are in line with the results obtained by Saoud et al (2007). The higher energy cost for osmoregulation derived from growth can explain why high salinity inhibits fish growth (Prunet & Bornancin 1989).

The physiological stress response is generally independent of the type of the stressor. For example, acclimation temperature of striped bass, *Morone saxatilis*, at 10 and 16^{o}C had lower physiological change and faster recovery in comparison to 5 and 30^{o}C (Davis & Parker 1990). Similarly, serum cortisol and ion changes of Nile tilapia was affected in salinity stressed groups (10 and 15 ‰), compared to the control group (0 ‰) (Mohamed et al 2021). Several authors have also reported that certain fish species can grow faster in low salinity than in high salinity. Fish can adapt to different salinities by adjusting their osmotic pressure to balance the concentration of ions in their internal and external bodies. A change in ambient salinity from 10 to 25 ‰ is a challenge that *S. argus* in Vietnam's Tam Giang - Cau Hai lagoon would face naturally. The current study shows that rearing *S. argus* at salinity 15 ‰ is the best for growth because the fish expends less energy regulating osmotic pressure and other metabolic processes. The change induced a transient rise in plasma cortisol, glucose, and ion concentrations (Figure 1). Cortisol and glucose are important indicators to assess the stress levels of fish. The elevation of plasma cortisol levels is the result of hepatic glucose production and processes involved in osmotic regulation in fish (Mommsen et al 1999). Cortisol level in teleost fish is considered normal at the values between 20.549 and 102.747 nmol L^{-1} (McDonald & Milligan 1997). A study on juvenile groupers *Epinephelus fuscoguttatus* concluded that cortisol levels were initially higher and then gradually decreased to resting cortisol levels (Tahir et al 2018).

The current study found that cortisol concentrations in fish in all treatments were slightly higher than this threshold from d-0 to d-5, particularly from d-0 to d-1. Most fish species tested show the greatest plasma cortisol increase within 0.5-1 hr. of a stressful disturbance (Barton & Iwama 1991). Previous research found that serum cortisol and glucose levels in groupers *E. malabaricus* increased dramatically after being transferred to 29 ‰ and 34 ‰ salinities, respectively, as an early stress response (Tsui et al 2012). Barton & Zitzow (1995) investigated the physiological stress responses of juvenile walleyes (*Stizostedion vitreum*) to net handling by measuring plasma cortisol levels, which increased rapidly within 15 minutes of handling. Nguyen et al (2013) also reported that cortisol levels of Tra striped catfish (*P. hypophthalmus*) increased significantly in higher salinity treatment compared to lower cases after 24 h before decreasing gradually over time. The role of cortisol in regulating seawater tolerance of the Atlantic sturgeon (*Acipenser oxyrinchus*) has been documented by McCormick et al (2020) who suggested that plasma cortisol and glucose were elevated after 1 day and reached peak levels after 2 days in seawater (25 ‰) and started to decrease from the peak but remained slightly high until day 14. Our findings support these arguments and the fact that cortisol levels do not decrease for hours after stress (Redding et al 1984; Barton et al 1986). This was explained by the fact that plasma glucose is required in fish to provide metabolic energy that is self-sufficient in glycogen sources (Pérez-Robles et al 2012). In other words, glucose levels in the blood rise to provide energy for animals in response to stress (Bonga 1997). Additionally, according to Fazio et al (2013), the stresses of *M. cephalus* caused by changes in water salinity can alter metabolic energy production or consumption. The change of glucose from the present study was consistent with research from Laiz-Carrión et al (2005), who reported that there was no significant variation between plasma glucose levels in gilt-head bream *Sparus aurata* for various salinity treatments. Similarly, Huong et al (2021) demonstrated that the glucose level was not

significantly changed after 90 days of rearing snakehead fish *Channa striata* in different salinities.

Our findings show that Na^+ and Cl^- concentrations in blood of *S. argus* were higher at the first stage of the experiment and then slightly decreased over the time course. There were significant differences in concentrations of Na^+ and Cl^- of fish between group salinity of 10 to 15 ‰ and group of 20 to 25 ‰. This was in agreement with Nguyen et al (2013) who demonstrated that Na^+ level is significantly higher in fish blood at high salinity (14 and 18 ‰) than those at low salinity treatments (2, 6, and 10 ‰). In contrast, the concentration of Na^+ ions in blood plasma of tilapia decreases after 2 months of culture but the difference is not significant when salinity increases from 0 to 10 ‰ (Sun et al 1994). In the same way, Na^+ ion concentration in the blood plasma of perch (*Anabas testudineus*) was stable when cultured at low salinity from 0 to 9 ‰ (Huong et al 2021). Kammerer et al (2010) also reported that tilapia blood plasma Na^+ and Cl- increased as early as 6-8 h and all solute concentrations reached a peak at 24h of seawater of 25 ‰ salinity. The increase in plasma Na^+ and Cl^- resulted from rising osmolality in fish. There were no significant changes in *S. argus* blood plasma Ca^{++} and K^+ under different salinities and time course of the experiment.

Conclusions. Our findings demonstrate that *S. argus* is a highly tolerant species to different salinities. Thus, we conclude that 15 ‰ is the optimal salinity for the grow-out of *S. argus*. The results indicate that growth performance of *S. argus* was significantly greater at 15 ‰ than at 25 ‰, but there was no significant difference in growth performance, FCR, among treatments of 10, 15, and 20 ‰ salinity. Changes in plasma glucose, cortisol, and ions reflected the growth performance, SR, and FCR at different salinities treatments. The concentrations of plasma glucose, cortisol, and ions sharply increased on the first day after the fish was transferred to the experimental tanks and then decreased gradually over the course of the experiment. There was a significant difference in plasma cortisol level between either treatment of 10 ‰ or 15 ‰ when compared to 20 ‰ and 25 ‰ ($p < 0.05$). There was no significant difference in challenge salinity on glucose concentrations in any treatments ($p > 0.05$). Changes in ionic concentrations of Na^+ and Cl^- were at the highest value at the treatment of 25 ‰ and all treatments reached the peak on d-1 and then declined until the end of the experiment. Changes in the concentrations of glucose, Ca^{++}, and K^+ during the time course of the experiment were not significantly different under different salinities. Therefore, we propose applying a salinity of 15 ‰ for *S. argus* farming to achieve a higher growth rate and lower the FCR, thus increasing subsequent production.

Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Huy Van Nguyen, Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 530000 Hue city, Vietnam, e-mail: huy.nguyen@hueuni.edu.vn
Quynh Ba Le, Tuan Kiet TS Company Limited, 530000 Hue city, Vietnam, e-mail: quynh3579@yahoo.com
Vinh Huu Nguyen, Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 530000 Hue city, Vietnam, e-mail: vinhntts@gmail.com
Huu Tien Le, Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 530000 Hue city, Vietnam, E-mail: letienhuu@hueuni.edu.vn
Tue Minh Le, Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 530000 Hue city, Vietnam, e-mail: leminhtue@huaf.edu.vn
Chat That Ton, Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 530000 Hue city, Vietnam, e-mail: tonthatchat@huaf.edu.vn
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