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Research article

Effects of ammonia, temperature and their interaction on oxygen consumption rate of Asian seabass (*Lates calcarifer*) juveniles

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Abstract

Importance of the work: Global warming presents a major challenge to intensive aquaculture and live fish transportation, due to its effects on oxygen consumption (OC) and total ammonia nitrogen; therefore, investigation is necessary of the interaction between these parameters.
Objectives: To evaluate the interaction effect of temperature and total ammonia nitrogen (TAN) on the oxygen consumption rate (OCR) of Asian seabass (*Lates calcarifer*) juveniles.
Materials & Methods: The OCR of Asian seabass juveniles (mean weight ± SD of 8.09 ± 0.30 g and mean length ± SD of 7.25 ± 0.45 cm) were measured at three temperatures (29 °C, 32 °C and 36 °C) and three TAN levels (0 mg/L, 0.5 mg/L and 1.0 mg/L). The OC levels of fish in a respiratory chamber were measured, from which their OCRs were calculated.
Results: This study was the first to show the interaction of temperature and TAN on the OCR of Asian seabass, which was highly significant (F = 7.449, df = 4, $p = 0.001$). The presence of TAN (0.5–1.0 mg/L) enhanced the adverse effect of rising temperature (≥ 32 °C), with the OCR affected. The developed polynomial equation was OCR = (0.0021 × Temperature × TAN) + 0.018, with a coefficient of determination of 0.935 that indicated a good fit to the data.
Main finding: The interaction of temperature and TAN influenced the OCR of Asian seabass juveniles. The developed equation could be applied to forecast the OCR, which would benefit managing the stocking density, oxygen demand and transportation.

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Introduction

Dissolved oxygen is one of the most important water quality parameters in aquaculture because it is indispensable for aquatic animals to maintain their routine activities and growth (Post and Lee, 1996; Helfman et al., 2009; Mazumder et al., 2019). Therefore, hypoxia conditions would deter growth (Shi et al., 2011) and the survival rate of aquatic animals (Boyd, 1998).

The oxygen consumption rate (OCR) of fish varies according to species, sizes, movement conditions and nutritional behaviors, and environmental factors, such as the total ammonia nitrogen (TAN; Barbieri and Doi, 2012) and temperature (Urbina and Glover, 2013). In particular, small fish have higher OCRs than larger ones (Fidhiany and Winckler, 1998; Helfman et al., 2009; Is-haak et al., 2019), while the OCR increases with incremental TAN content in the water (Lawson, 1995; Jensen et al., 2013; Campos et al., 2017). In addition, the water temperature affects the OCR of aquatic animals, with an increase in the water temperature directly increasing the OCR (Lawson, 1995; Cooper et al., 2019; Das et al., 2021). The impacts of environmental factors on the OCR have been studied in many aquatic species; however, they have focused mainly on only a single factor, such as the effect of weight or size (Fidhiany and Winckler, 1998), water temperature (Nerici, et al., 2012; Longwu et al., 2017; Cooper et al., 2019), ammonia concentrations (Harris et al., 1998; Jensen et al., 2013; Campos et al., 2017) or salinity (Via et al., 1998).

Despite limited studies, there are indications that each factor does not independently affect the OCR but that rather there are interactions among factors. For example, the interaction of increasing fish size and lowering water velocity reduced the OCR of red tilapia (Is-haak et al., 2019); decreasing temperature in combination with a reduction in photoperiod led to a low OCR for juvenile dark-banded rockfish (Oh and Noh, 2006); low temperature and reduced fish weight caused a reduction in the OCR of Asian seabass (Glencross and Felsing, 2006).

Currently, the rising water temperature due to global warming has become a major challenge for aquaculture because it affects both the OCR of fish and the TAN in fishponds, especially in intensive aquaculture systems (Pörtner and Knust, 2007) and fish transportation (Grottum et al., 2008; Hong et al., 2019). Therefore, the combined effects of temperature and TAN on the OCR merit investigation. The current study used Asian seabass because of the economic importance of this species, especially for Thailand, which is a top producer of fingerlings as well as marketable fish for both domestic and international sale (Senanan et al., 2015). Therefore, the current study was conducted to evaluate the effects of combinations of the two main factors, water temperature and the TAN in the water, on the OCR of Asian seabass. The knowledge gained from this study should benefit water quality management of Asian seabass and related species, particularly in coping with rising temperatures due to global warming.

Materials and Methods

Experimental fish and set-up

A sample of 45 Asian seabass fingerlings (mean weight ± SD of 8.09 ± 0.30 g and mean length ± SD of 7.25 ± 0.45 cm) were obtained from Phumipat Farm, Chachoengsao province, Thailand, where they were acclimated to freshwater for 10 d before being transported to the Fisheries Science Department, Rajamangala University of Technology Suvannabhumi, Ayuthaya province, Thailand.

The experimental fish were stocked in 3 aquariums (30 cm × 60 cm × 40 cm, volume = 60 L) with aeration (Resun air pump, Ap-30) at three temperatures, 29 °C (Hailea chiller, HS-28a), 32 °C (ambient temperature) and 36°C (Sobo heater, HQ-300W), respectively. They were fed with 45% crude protein commercial feed (CP, Higrade, 9006T) twice daily at 5% of fish weight. Fish were acclimated under the experimental conditions for 14 d before the trial commenced.

Experimental design

To measure the oxygen consumption rate (OCR) of Asian seabass fingerlings at three temperatures (29 °C, 32 °C and 36 °C) and three levels of TAN (0 mg/L, 0.5 mg/L and 1.0 mg/L) were arranged in a 3×3 factorial experiment in a randomized complete block design. Days were considered as blocks and the experiment was conducted for 5 d, with nine experimental units and five replications. A single fish in each respiratory chamber represented a replication.

Respiratory chamber design and set-up

The experimental unit consisted of a water storage chamber (WSC) and a respiratory chamber (RC). The WSC and RC were made of acrylic vessels (diameter 25 cm, height 35 cm, with a volume of 17.17 L) with cover lids. The WSC and RC lids were mounted with a dissolved oxygen meter (YSI, model ProSolo; model Pro 20i). The WSC lid was installed with aeration (Resun air pump, Ap-30) with three air stones and a water outlet. The water was pumped from the WSC outlet to the RC inlet (peristaltic pump, Longer BT100-2J) and discharged water through the RC outlet (Fig. 1). To maintain the designated temperatures, the experimental units were immersed in water basins with a chiller (29 °C) or a heater (36 °C), while the control unit (32 °C) was placed at ambient temperature.

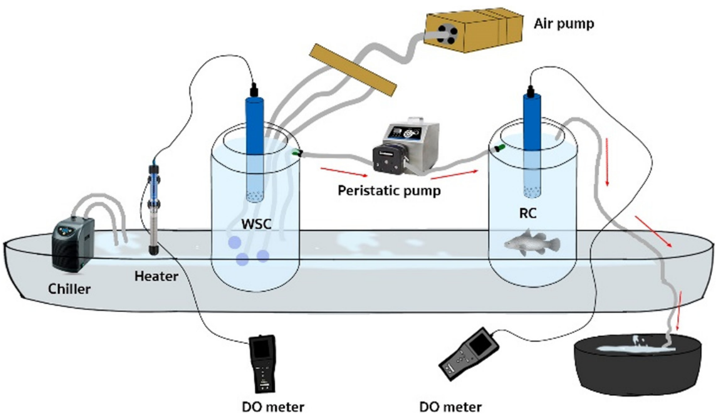

Fig. 1 Diagram of the experimental unit, where WSC = water storage chamber, RC = respiratory chamber and DO = dissolved oxygen

Stocking water was prepared with varying TAN levels according to treatments. First, the well-aerated water was adjusted to the designated temperature. Then, 1 mg/mL ammonium stock solution (99.5% NH_4Cl, Salmiac) was added to the stocking water to final concentrations of 0.5 mg/L or 1.0 mg/L before running the trial. After that, the experimental units (WSC and RC) were filled with stocking water. The mean water flow rate ± SD from the WSC to RC was set at 250 ± 3.15 mL/h; finally, the excess water from the RC overflowed into the temperature-controlled basin. A fish from the designated temperature stocking tank was starved for 12 h and then transferred and acclimated in each RC unit for 10 min before recording the dissolved oxygen (DO) data every minute for 15 min, during which time, the swimming behavior and gill operculum movement speed of all experimental fish was observed before weighing each fish.

Calculation of oxygen consumption rate

The OCR measured in milligrams per liter per hour per gram weight of fish, was calculated using Equation 1 (Cao and Wang, 2015):

$$OCR = [(DO_{in} - DO_{out}) \times V\,] / W \qquad (1)$$

where DO_{in} is the dissolved oxygen in the WSC, DO_{out} is the dissolved oxygen in the RC (both measured in milligrams per liter), V is the water rate (in liters per hour) and W is the weight of the experimental fish (in grams).

Statistical analysis

The effects of temperature, TAN and their interaction were analyzed using two-way analysis of variance in randomized complete block design, where the day was considered as blocks. Prior to the analysis, data were tested for homogeneity. Tukey's honestly significant difference tests were used to determine significant differences among means at $p < 0.01$. The correlations for OCR with temperature and TAN were analyzed using polynomial regression. Results were presented as mean value ± SD.

Ethics statements

Three of the researchers had licenses for keeping animals for scientific purposes: Supavadee Koydon (no. U1-O2667-2559), Jesada Is-haak (no. U1-O2666-2559) and Chansawang Ngamphongsai (no. U1-O1734-2558), which were issued by the Institute of Animals for Scientific Purposes Development, National Research Council of Thailand.

Results

The swimming behavior and gill operculum movement speed of all experimental fish were relatively normal except for the fish reared at 36 °C with 1.0 mg/L TAN that had a somewhat higher rate of operculum beat compared to the other groups who showed regular swimming and operculum movement. However, the fish exposed to 36 °C and 1.0 mg/L TAN did not lose their balance and no mortality was observed.

Effects of temperature and total ammonia nitrogen on oxygen consumption rate

The results of the analysis of variance showed that the water temperature, TAN, and the interaction of both factors had a highly significant influence on the OCR of Asian seabass fingerlings, as shown in Figs. 2–4 ($F = 1193.714$, $df = 2$, $p = 0.000$ for temperature; $F = 48.110$, $df = 2$, $p = 0.000$ for TAN and $F = 7.449$, $df = 4$, $p = 0.001$ for their interaction).

The effect of temperature is shown in Fig. 2 and that of TAN in Fig. 3. It was evident that when the temperature increased, the OCR increased accordingly (from 0.045 ± 0.002 mg/L/h/g at 29 °C to 0.059 ± 0.002 mg/L/h/g at 36 °C). Likewise, when the TAN increased, the OCR of fish increased accordingly (from 0.014 ± 0.009 mg/L/h/g at 0 mg/L TAN to 0.083 ± 0.007 mg/L/h/g at 1 mg/L TAN).

The effect of the interaction between temperature and TAN is displayed in Fig. 4. It was apparent that at 29 °C, the OCR significantly increased as the TAN increased. In contrast, an increase in the temperature alone (TAN = 0 mg/L) also elevated the OCR. Interestingly, with the presence of TAN, the change in temperature from 29 °C to 32 °C significantly increased the OCR (for example, at 0.5 mg/L TAN, OCR = 0.054 ± 0.0029 mg/L/h/g at 29 °C, whereas this increased to 0.061 ± 0.0020 mg/L/h/g at 32 °C). The same enhanced effect was apparent for 1 mg/L TAN with the same temperature increment (OCR = 0.075 ± 0.0066 mg/L/h/g at 29 °C that increased to 0.086 ± 0.0009 mg/L/h/g at 32 °C). However, this effect was not detected when the temperature increased from 32 °C to 36 °C (Fig. 4). It should be noted that when TAN was not considered (OCR at each temperature was averaged across TAN), increasing the temperature from 29 °C to 32 °C significantly enhanced the OCR (Fig. 2).

Simulated equations of oxygen consumption

Polynomial equations were developed to model the interaction between temperature and TAN. The best equation for OCR estimation was the polynomial equation: OCR = (0.0021 × Temp × TAN) + 0.018, with an R^2 value of 0.935, which indicated a good fit.

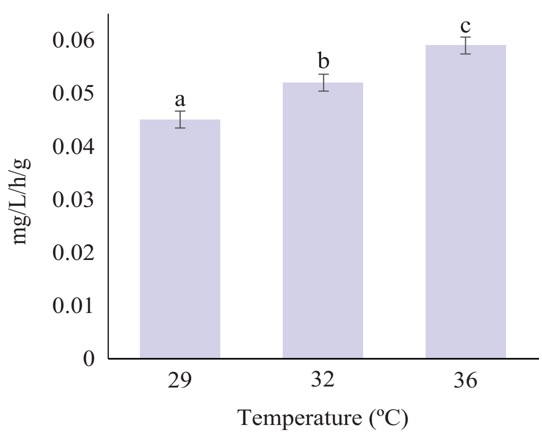

Fig. 2 Average oxygen consumption rate (OCR) of Asian seabass juveniles at three different water temperatures: 29 °C, 32 °C and 36 °C averaged across total ammonia nitrogen concentrations, where different lowercase letters above bars indicate highly significant ($p < 0.01$) differences among treatments and error bars represent ± SD.

Discussion

The current study clearly demonstrated that either temperature or TAN could not be considered alone because their interaction affected the OCR. Notably, when temperature

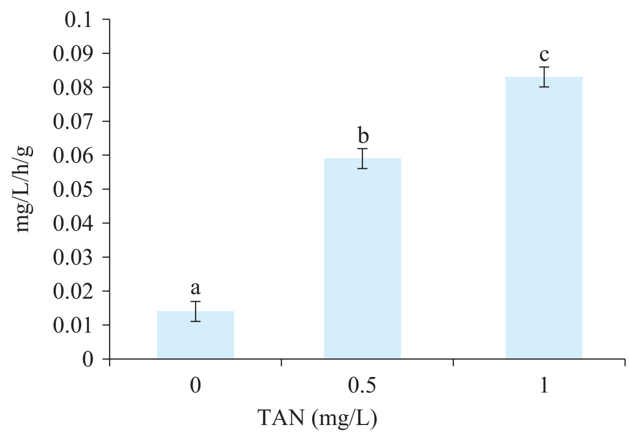

Fig. 3 Average oxygen consumption rate (OCR) of Asian seabass juveniles at three different levels of total ammonia nitrogen (TAN) at 0 mg/L, 0.5 mg/L and 1 mg/L averaged across temperatures, where different lowercase letters above bars indicate highly significant ($p < 0.01$) differences among treatments and error bars represent ± SD.

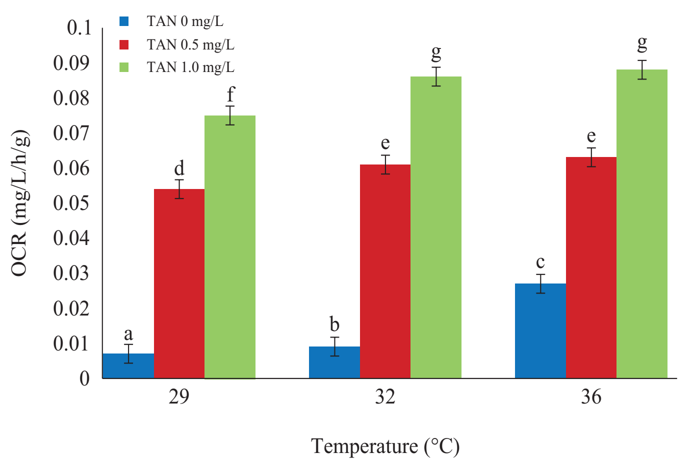

Fig. 4 Histogram showing interaction of three temperatures and of three concentrations of total ammonia nitrogen (TAN) on mean oxygen consumption rate (OCR) of Asian seabass juveniles, where different lowercase letters above bars indicate highly significant ($p < 0.01$) differences among treatments and error bar represent ± SD.

was considered alone, raising the temperature from 29°C to 36 °C increased the OCR. However, when the interaction was considered (Fig. 4), the same increment in temperature for TAN = 0 mg/L did not affect the OCR, whereas, in the presence of TAN, increasing the temperature from 29 °C to 32 °C enhanced the OCR. Katersky and Carter (2005) mentioned that Asian seabass (*Lates calcarifer*) were tolerant to a maximum of 39 °C. Therefore, no mortality was observed in the current research (temperature range of 29–36 °C). Above 36 °C, the increase in metabolism approaching the maximum tolerance temperature resulted in decreases feed intake and a subsequent reduction in both body protein and body fat (Jobling, 1997; Katersky and Carter, 2005).

The Asian seabass lives in a wide range of temperatures (15–40 °C) based on other studies (Tucker et al., 2002; Eme and Bennett, 2009; Das et al., 2021). The current results agreed with reports that the suitable temperature for Asian seabass was in the range 22–35 °C (Tian and Qin, 2003; Anil et al., 2010; Campos et al., 2017). However, some studies reported a wider suitable temperature range for this fish of 27–36 °C (Katersky and Carter, 2005). Such differences might have been because the experimental fish in these studies lived in higher ambient temperatures and had adapted to that environment. Similar to temperature, the effects of ammonia have been well studied. The safe concentration of un-ionized ammonia-nitrogen (UIA-N) for Asian seabass was 0.001–0.05 mg/L (Ignatius, 2009). An increase in the value of UIA-N from 0.200 mg/L to 0.500 mg/L led to an increased mortality rate of Asian seabass (Venkatachalam et al., 2018). The 96 h LC_{50} value for seabass fingerlings was 1.7 mg/L UIA-N or 40 mg/L TAN (Person-Le Ruyet et al., 1995). The concentrations of TAN used in the current study converted into UIA-N were in the range 0.019–0.037 mg/L (1.0 mg/L TAN at 26–36 °C), which was less than the lethal concentration; hence no mortality occurred, but the fish suffered from stress, as seen by the increase in the OCR. The current results showed that raising the TAN concentration elevated the OCR, which was the same outcome for cobia (Barbieri and Doi, 2012).

Ammonia is the end product of protein catabolism, produced in the fish liver and eliminated via the gills. Accumulation of ammonia is toxic; defense mechanisms convert ammonia to less toxic compounds, such as glutamine and urea as well excretion. (Randall and Wright, 1987; Ip et al., 2001). Ammonia excretion mainly involves NH_4^+ active transport, NH_3 passive diffusion and NH_4^+/Na^+ exchange (Randall and Wright, 1987; Wilkie, 1997; Ip et la., 2001). It has been confirmed that ammonia excretion in fish involved rhesus glycoproteins in the branchial and cutaneous epithelia (Ip et al., 2001). However, the branchial ammonia excretion mechanisms vary between fish species and environments (Randall and Wright, 1987).

At a sublethal concentration, ammonia causes damage to the gills, liver, kidneys and spleen, reduced growth rate and an increased OCR (Lin and Chen, 2003; Barbieri and Bondioli, 2015; Shokr, 2019). Histopathological studies of ammonia-exposed common carp (Chezhian et al., 2012) and silver catfish (Miron et al., 2008) showed necrosis, hyperplasia, edema and lamellar fusion of gills. Furthermore, ammonia might damage the hematopoietic tissue of the liver, kidneys and spleen, resulting in decreasing numbers and changing shapes of red blood cells (Das, 1998; Das et al., 2004; Shokr, 2019). Notably, sensitivity to ammonia is species-dependent; for example, juvenile European seabass (*Dicentrarchus labrax*) were more sensitive than seabream (*Sparus aurata*) and turbot (*Scophthalmus maximus*), according to Person-Le Ruyet et al. (1995).

Besides the sole effect of temperature or TAN, the current study was the first to show the interaction between temperature and TAN on the OCR of Asian seabass. The study showed that the presence of TAN enhanced the adverse effect of rising temperature. Likewise, increasing the temperature from 29 °C to 32 °C enhanced the adverse effects of TAN at both concentrations. However, such enhanced impact on temperature was not produced with a temperature change from 32 °C to 36 °C, possibly because at 32 °C, the fish were already under stress (US Environmental Protection Agency, 1998; Randall and Tsui, 2002), and thus, TAN had accumulated in the blood. However, in the absence of ambient TAN, the fish could maintain osmoregulation, and hence regular OCR was observed. In the presence of ambient ammonia (such as 0.5 mg/L, Ignatius, 2009), the elevated ammonia uptake from the environment caused decreased ammonia excretion, resulting in the accumulation of ammonia in the blood. The ammonia detoxification of fish would require increase OCR for the conversion of ammonia into less toxic forms, such as urea and glutamine, reduction of ammonia production, as well as active ammonia excretion (Ip et al., 2001; Randall and Tsui, 2002; Eddy, 2005) for these reasons.

The global warming effect has elevated global temperatures (Franklin and Edward, 2019). Because of this, TAN in ponds must be strictly controlled because a safe level of TAN may restrict its detrimental effects on increasing ambient temperature and adversely affecting the optimal feeding rate, aeration and stocking density. Pond management, such as by using water exchange, waste removal or the introduction of nitrifying bacteria can be used as standard procedures to reduce TAN toxicity (Peng et al., 2013; Refaey and Li, 2018; Vanderzwalmen et al., 2019).

During transport, water quality deteriorates, increasing the TAN and CO_2 levels, decreasing the dissolved oxygen and pH and leading to stress on fish (Gomes et al., 1999; Grottum et al., 2008; Hong et al., 2019). Live fish transportation has involved pre-transportation starving (Shrivastava et al., 2017), adding sodium chloride or using an anesthetic (Zhang et al., 2018; Hong et al., 2019), controlling the temperature (Hong et al., 2019), optimizing the stocking density (Gomes et al., 2003, Hong et al., 2019) and oxygenation (Hahn and Pérez, 2015; Zhang et al., 2017; Hong et al., 2019).

The OCR equation could be applied to forecast the oxygen consumption of fish, which would be useful information for Asian seabass farmers to manage the fish stocking density, aeration and transportation based on the analysis of the water temperature and TAN concentration. For further study, multiple correlations of other parameters should be investigated, such as size, stage or age, strain and water quality, to understand the interactions among such parameters and to subsequently apply this information for aquaculture purposes.

The current study provided baseline information for the culture of Asian seabass fingerlings, specifically that a temperature range of 29–32 °C (in water free of TAN) was optimal, as evidenced by the unchanged OCRs at the upper and lower temperatures, with the normal range in the OCR of Asian seabass fingerlings (8 g body weight) being 0.007–0.009 mg/L/h/g (at 29–32 °C). Notably, the OCR increased with size; for example, it was 0.081 mg/L/h/g for Asian seabass fingerlings with 56.4 g body weight, despite a slightly lower rearing temperature (28–29 °C), according to Khongthon et al. (2021). Changing the water temperature so that it exceeds the optimum range would increase the metabolic rate and hence increase the OCR as a result of stress (Jobling, 1997; Katersky and Carter, 2005).

The interaction of the two factors of water temperature and ammonia concentration influenced the OCR of the fish. At temperatures ≥ 32 °C, the OCR was affected by the presence of TAN (0.5–1.0 mg/L), as represented by the equation OCR = (0.0021 × Temperature × TAN) + 0.018 (R^2 = 0.935). The Asian seabass farmers could apply this OCR equation to forecast oxygen consumption, which would benefit the management of stocking density, oxygen demand and transportation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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