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From: Mulyadi Idris (mulyadibrian26@yahoo.com)

To: ek.bioflux@gmail.com

Date: Sunday, May 17, 2020, 04:09 AM GMT+7

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Article Trachinotus blochii

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To: ek.bioflux@gmail.com

Date: Thursday, June 11, 2020, 03:51 AM GMT+7

This is my new revise article

Your sincerely

Mulyadi

Mulyadi article

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To: ek.bioflux@gmail.com

Date: Thursday, June 11, 2020, 04:04 AM GMT+7

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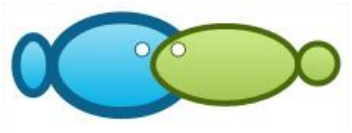

Osmotic performance rate, stress response~~,~~ and growth performance of silver pompano (*Trachinotus blochii*) reared in different salinities using recirculating culture system

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Abstract. Silver pompano (*Trachinotus blochii*) has received tremendous attention from the~~in~~ aquaculture sector, due to its favourable features, ~~i.e.~~such as a ~~-~~high~~ly~~ economic value, ~~high~~ its good adaptive response~~,~~ and its potential~~ity~~ to be cultured in various salinities. The aim of this study was to discover the effects of medium salinities on osmotic performance rate, blood cortisol, and growth performance (absolute growth weight and lenght, specific growth rate SGR, feed conversion FC, feed efficiency FE, survival rate SR) of silver pompano under recirculating system. The histological alterations (kidney and gill) and water quality (temperature, pH, DO, NH_3, NO_2 and NO_3) were also observed. The 56-day experiment was carried out in Balai Perikanan Budidaya Laut (BPBL) of Batam, Indonesia. A total of 225 fish especimens (11-13 cm in length, weighing 28-29 g) were raised in a 100 L-tank containing 80 L of water at a density of 1 fish/4 L (~~totally~~ 20 fish in total). They were fed with commercial pellet (46% protein) at 3% of fish biomass and 3 times a day. The experiment was conducted according to a Completely Randomized Design with 5 levels of salinity: P1=25‰, P2=20‰, P3=15‰, P4=10 ‰ and P5=5‰, by ~~performed~~ performing triplicates measurements for each treatment. ~~As~~ The treatment with~~the results,~~ 15 ‰ salinity showed the best effects, yielding an osmotic performance rate of 3 mOsm~~/L~~ L^{-1} H2O, a blood cortisol level of 50,923 nmol~~/L~~ L^{-1}, an absolute growth weight and length of 17~~,~~73±1~~,~~25 g and 2~~,~~32±0~~,~~21 cm, respectively, an SGR of 0~~,~~87 ± 0~~,~~05%, an FC of 1.24±0~~,~~00, an FE of 80.79±0~~,~~58 and an SR of 88~~,~~33±2~~,~~88%. Histologically, there were no anomalies in the structure of gill and kidney of the fish cultured in 5‰ ~~-~~25‰ salinities. Water quality was acceptable for ~~growth~~ growing ~~of~~ silver pompano.

Key Words: osmotic performance rate, cortisol hormone, growth, histology, and *Trachinotus blochii*.

Introduction. The farming of silver pompano (*Trachinotus blochii*) has currently gained great popularity in Indonesia. The market demand for the species has continuously increased in international trade, due to its high~~ly~~ economic value, its ~~high~~ good adaptive response~~,~~ and its potential~~ity~~ to be cultured in various water conditions. The price of the fish reaches aproximately ~~Rp.~~ Rp. 60~~,~~000 kg^{-1} in local market, but it may reach Rp. 200~~,~~000 kg^{-1} in export market (Mo 2017). Since 2015, silver pompano is ~~touted~~ considered as a promising commodity in marine fisheries sector with total production of 1~~,~~900 tons in 2015, demonstrating the annual rise of 31~~,~~5% (Prahadi 2015).

T. blochii was reported to exert a high adaptive response towards salinity changes. Survival rate of the species at various salinity levels 32‰, 24‰, 14‰, and 4‰ ~~for~~ during the 28-day trial showed no significant difference, indicating that the fish could adapt at lower salinity over seawater, as well as ~~showed~~ confirming the possibility of fish ~~culture~~ farming diversification ~~in~~ through ~~low salinity~~ culture systems ~~regarding to diversification of fish culture~~ in brackish water (Arrokhman ~~et al.~~et al~~,~~ 2012).

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Salinity tolerance in fish closely relates to the osmotic pressure balance between inside and outside the fish body. Osmotic pressure inside the fish is lower than ~~the~~ outside. The imbalance may cause disturbance of the physiological functions, which in turn disrupt~~ing~~ the fish growth ~~of the fish~~. However, under a normal osmotic gradient, the metabolic activity could reach an optimum rate, as indicated by a good appetite and an enhanced feed intake, allowing ~~to allocate~~allocating more energy for the growth (Carrion ~~et al.~~et al~~,~~ 2005). Fujaya (2004) suggested that the osmotic balance was achieved through regulation of body fluid transportation, ~~which is well~~ known as osmoregulation~~.~~. ~~It is noteworthy to notice that t~~ The adjustment activities needed for balancing internal and external osmotic pressures ~~between inside and outside the fish always~~ require a high energy consumption, leading to ~~generation of~~ stress generation, as indicated by the production of blood cortisol.

Important osmoregulatory organs including gills and kidney play a crucial role in the process ~~during the osmoregulation~~. Defective tissues in these organs offer a sign of failure in osmoregulation. Consequently, the defect would adversely affect physiological functions, causing the ~~loss~~ decrease of feed consumption and fish growth (Putri ~~et al.~~et al~~,~~ 2014). In this matter, gills seem to be the most susceptible osmoregulatory organ towards environmental changes~~,~~ such as physicochemical properties of water and presence of toxic compounds. The gill lamellae become~~s~~ the weakest part, in which presence of stressors directly induces ionic homeostasis that remarkably imparts osmoregulation. Indeed, the chronic stressors lead to destructive effects on the gill. Macroscopic and microscopic defects in the gill can serve as biomarker of fish health status (Camargo ~~and~~ & Martinez 2007). Besides, Thophon ~~et al.~~et al (2003) also argued that kidney ~~showed~~ is a susceptible organ to ~~wards~~ the external stressors exposure, ~~since it shows~~due to its essential role~~s~~ in maintaining homeostasis. Based on the above elaboration, ~~there is~~it can be concluded that there is a need for investigating the osmotic response, stress level~~,~~ and growth performance of silver pompano reared in a recirculation system~~,~~ at different salinity levels. In this work, the histological alterations ~~defect~~ in gill and kidney ~~was~~ were also observed.

Material and Method

Study site. The experiment was carried out in the Agency for Marine Fisheries Culture (Balai Perikanan Budidaya Laut~~—~~-BPPL), Batam, Indonesia, for 56 days.
~~Animals and Feed.~~ A total of 225 *T. blochii* ~~silver pompano~~ seeds (average length of 11-13 cm, weight of 28-29 g) were reared in an experimental container (capacity 100 L) filled with 80 L of water. The density referred to Indonesia National Standard ~~2013~~ (~~abbreviated~~ SNI 2013), ~~i.e.~~which recommends~~—~~ 1 individual~~/~~ 4 L^{-1} (equal to 20 individuals ~~/~~80 L^{-1}). Commercial pellet (Megami GR 2) was used, containing ~~protein~~ 46% ~~,~~ protein, ~~fat~~ 9%~~,~~ fat, ~~crude fiber~~ 1~~,~~.9% crude fiber and ~~moisture~~ 8%~~.~~ moisture. The ~~fish~~ specimens were fed at 3% of ~~fish~~ their weight, three times a day.

Preparation of the culture container. The close recirculating system was prepared. The container was filled with seawater at different salinity levels, then connected to PVC gutter (50 cm x 14 cm x 14 cm) at the upside of the chamber. The ~~water from~~ filtration unit water was transported into the culture container through a PVC pipe (2~~,~~.5 cm diameter). ~~Filtration~~ The filtration unit was filled with 50 bioballs (each gutter), as previously prescribed by Nelvia ~~et al.~~et al (2015). The water was subsequently pumped to the filtration unit with the aid of a 50 W~~-~~ water pump.

Experimental design. ~~Completely~~ The completely randomized design was arranged, consisting of 1 factor and 5 levels (with triplicates) as follows: P1~~-~~=~~-~~25~~-~~‰, P2~~-~~=~~-~~20~~-~~‰, P3~~-~~=~~-~~15~~-~~‰, P4~~-~~=~~-~~10~~-~~‰ and P5~~-~~=~~-~~5~~-~~‰. The effect of salinity was studied, focusing on the osmotic response, the content of blood cortisol, the tissue histology (gills and kidney), the absolute growth weight (Wm)~~,~~ ~~and~~ the growth length (Lm), the specific growth rate (SGR), the survival rate (SR)~~,~~ and the water quality (temperature, pH, DO, NH_3, NO_2 and NO_3).

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Determination of the osmotic response. Osmotic response was evaluated at the end of experiment and determined using micro-osmometer, as explained by Cambell et al.et al (2012). The fish sample was acclimatized for 7 days, and, for the first 5 days, the fish was fed. A total of 10 fish specimens were used for each salinity level, in which they were exposed to the salinity levels, according to treatments arranged. After 7 days of exposure, the blood of living fish (1 mL) was collected from the heart, then centrifuged at 3000 rpm for 3 minutes to obtain blood plasma. The plasma oOsmotic pressure of the plasma was measured using a micro-osmometer. The oOsmoregulatory capacity refers to the difference between osmotic pressure of the medium and internal osmotic pressure of the fish blood plasma. The calculation was described as follows (Anggoro and & Nakamura, 1996):

$$TKO = [POsmoBlood - POsmoMedium]$$

Where:
TKO is osmotic response (mOsm/L L^{-1} H2O);
POsmoBlood is biota osmolarity (mOsm/L L^{-1} H2O);
POsmoMedium is medium osmolarity, and the bracket [ ] means absolute value.

Blood cortisol analysis. The analysis was performed at 3 periods: day 1, day 28, and day 56 of the experiment, using enzyme-linked immunosorbent assay (ELISA). Before the blood withdrawal, the fish was anaesthetsized using phenoxyethanol at dose of 0,3 mL/L L^{-1} water (Rigal et al.et al, 2008). Briefly, blood was collected via the vena caudalis, using a heparinized syringe (1 mL), then centrifuged to collect plasma. Plasma cortisol was quantified using RIA (radio immuno-assay) Cortisol (125I) RIA KIT IZOTOP (Ramsay et al.et al, 2006). In this regard, blood plasma (0,3 mL) was frozen at -20°C. To maintain the hormone in the plasma, the sample was packed within a cool-box containing dry ice exactly at day 57 of experiment, then immediately transported into laboratory for analysis.

Histological analysis. Gill and kidney tissue of the sample was collected at the first and last days of the experiment, fromcarried out in the fish specimens exposed to a salinity of 30 ppt (natural habitat condition). Gill cover (overculum) was lifted and the base was cut off to release the gill. Afterwards, the kidney was obtained by opening the abdominal cavity. The phosphate-buffered formalin (NBF) at 10% was used for organ fixation, carried out for 24-48 h (Raškoviæ et al.et al, 2011). After fixation, the tissues were dehydrated in graded series of alcohol and xylol, then embedded in paraffin. All these stages were conducted using tissue processor. The sections obtained were cut at thickness of 3-5 µm, then incubated in a water bath at 40°C. Object glass was used to attract the section in water bath, then immediately air-dried for 1 h. Afterwards, it was stained using haematoxylin-eosin (HE), and observed under light microscope at magnification of 400×.

Growth performance. The weight and length of fish were recorded each 14 days. Growth performance included following parameters:
a. Weight gain (g) = final weight – initial weight;
b. Absolute growth length (cm) = average final length (cm) – average initial length (cm);
c. Specific growth rate (SGR) (%) = (Ln mean final fish weight – Ln mean initial fish weight)/ culture period (day) × 100 %;
d. Feed efficiency (FE) (%) = increased fish mass/total feed consumed;
e. Survival rate (%) = (final number of fish/initial number of fish) × 100 %.

Water quality. Temperature was daily observed using a thermometer, while chemical indicators were checked each 14 days, including pH (using pH meter), DO (using DO meter), NH_3, NO_2 and NO_3 (using a spectrophotometer).

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Results and Discussion

Osmotic performance rate. Osmotic response of fish to thewards variation of salinity was shown in Figure 1, indicating that salinity caused a remarkable effect on medium osmolarity, blood plasma, and osmotic performance rate of silver pompano ($p<0.05$). In this case, the lowest rate (3 mOsm/L L^{-1} H_2O) was found at P3 (15‰), while the highest one (87 mOsm/L L^{-1} H_2O) was attributed to P5 (5‰). This clearly imparts the effect of salinity on medium and plasma osmolarity, leading to noticeable changes in osmotic performance rate of silver pompano seeds. The extreme difference between medium osmolarity and internal fish osmolarity could cause significant changes in fish behaviors and physiological conditions, and as a consequentlycc, altering the feed consumption rate and fish growth.

In water of salinity (15‰) near the isoosmoticisosmotic condition, the fish allocated more energy for enhancing their growth. Energy metabolism required for osmoregulation in fish is fundamentally associated with osmotic performance rate as a rapid response towards changes in medium osmolarity. In this case, osmotic performance rate shows linear correlation to energy consumption for osmoregulatory activities. On the contrary, hypoosmotic (salinity of 30‰, 25‰ and 20‰) and hyperosmotic (10‰ and 5‰) condition leads to increment of osmotic response, which needs higher energy requirement for osmoregulation (Carrion et al.et al 2005). Arjona et al.et al (2008) reported that there was clear evidence that higher osmotic response led to higher energy use for osmoregulation. Our data demonstrated a variety of osmotic response with progression of salinity. As mentioned by Putri et al.et al (2014), medium that possesses higher salinity (far away from isoosmoticisosmotic condition) would increase osmotic performance rate of the fish. At the condition in which the medium condition is tolerable, osmotic performance rate tended to attenuate, thus allowing energy use for fish growth.

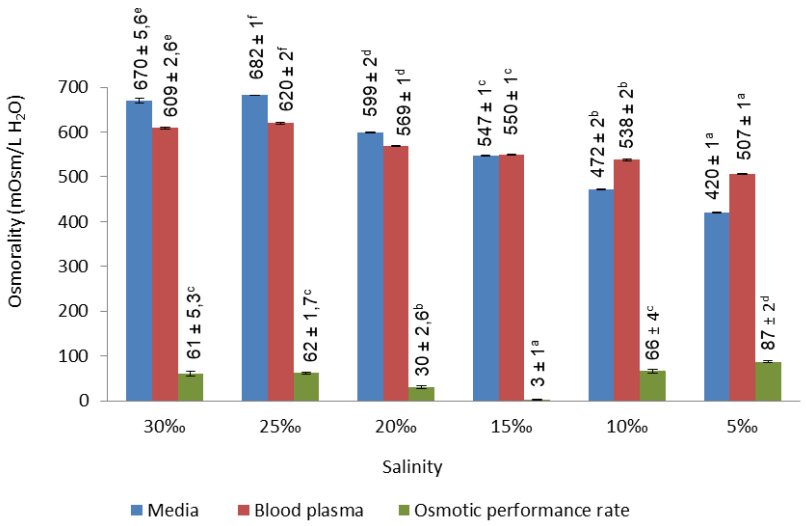

Figure 1. Average osmolarity of rearing medium and blood plasma, and osmotic performance rate of silver pompano cultured in various salinity levels. Different superscripts above the bar showed significant difference at $p<0.05$.

This present work reveals that silver pompano is able to adapt to the medium salinity of medium at 15 ppt, suggesting that the juvenile (proper size and age) can tolerate the condition. Additionally, Retnani and & Abdulgani (2013) argued that such adaptive capability of fish relied on size and growth stage, in which osmoregulatory activity of fish

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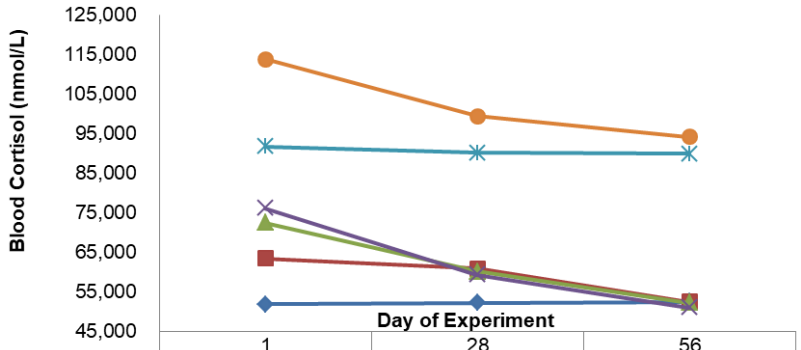
may differ depending on age. Lantu (2010) stated that osmoregulation of euryhaline fish strongly related to osmo-sensitivity of chloride cells. They serve as receptor, mainly responding to the salinity level of the medium. When ~~entered~~ immersed in water media with differ~~r~~ent salinity, chloride cells in euryhaline fish transmit signals to central nervous system, primarily to the pituitary gland~~s~~ responsible for controlling secretion of growth hormone. Subsequently, the hormone regulates the development of chloride cells in osmoregulatory organs such as gills, kidney and digestive tract~~; and~~. T~~th~~~~erefore~~us, the ~~quantity~~ amount of chloride cells is adjusted, ~~as well as~~ causing changes in the physiological mechanisms ~~for~~ of secretion or absorbtion of ions by chloride cells. At ~~the~~ a higher salinity, chloride cells would ~~be more~~have a higher rate of proliferation~~produced~~, and vice~~-~~versa. Over the~~In~~ long term, this controlling mechanism may also cause genetic expression.

Syakirin ~~et al.~~et al (2018) stated that a higher concentration of ions in water would rise salinity level and osmolar density. ~~The "abstruse"~~For instance, the hybrid grouper is a kind of marine fish that has blood osmolarity (internal osmotic fluid pressure) lower than the environment~~ of~~al osmotic pressure. Therefore, the water will pass from the body of the fish to the environment by the osmotic process through the kidney, gill, as well as in the body. Salinity demonstrates a relationship with the osmoregulation of aquatic animals. ~~If there is a~~A sudden fluctuation of salinity~~, it will provo~~ make~~s difficulty to have~~ the body osmoregulation difficult ~~in their body~~ and induces the animal mortality ~~of the animals~~. ~~Osmoregulation is stated with t~~The osmoregulation capacity ~~which~~ is is determined by the difference between the blood osmotic pressure (fish) ~~with~~ and the media osmotic pressure ~~media~~. Osmoregulation relates to the difference of ~~osmotic pressure in~~ fish blood osmolarity and media osmolarity ~~in the environment~~, ~~which is then~~ known as the osmotic work level: the response to the salinity change increases with this difference. ~~In short, it will be higher as response to the greater difference of the two osmolarities.~~

Stress level in ~~T.~~ *T. blochii*~~silver pompano~~. The increment of blood cortisol in fish indicates stressful condition. Our data suggested that blood cortisol in all treated samples declined at different extent ~~as~~ during the experiment pogression ~~of experiment periods~~, indicating that the fish exhibited adaptive capacities to lower salinity (Figure 2).



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30‰	51,828	52,135	52,471
25‰	63,463	60,872	52,450
20‰	72,466	60,116	52,174
15‰	76,118	59,291	50,923
10‰	91,737	90,148	89,960
5‰	113,838	99,465	94,160

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Day if experiment

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Figure 2. Concentration of blood cortisol in ~~silver pompano (~~*Trachinotus blochii* ~~Lacepede)~~ cultured in various levels of salinity.

As depicted in Figure 2, the concentration of blood cortisol on day 1 increased as ~~lower~~ the level of salinity was lower compared to the control (30‰). This clearly showed a stress response to the new environment. Setiyoningsih (2014) argued that stress in fish existed as rapid response towards enviromental pressure, thus they secreted glucocorticoid (cortisol) and catecholamine hormone to cope with the stress condition.

Furthermore, cortisol level declined consistently from day 28 to day 56 in all treatments, indicating adaptive capacities. On day 56, the lowest cortisol level (50~~.~~,923 nmol ~~/~~L^{-1}) was found at 15‰, while the highest one (94~~.~~,160 nmol~~/L~~ L^{-1}) was found at 5‰. In salinity 15‰, the fish showed the best osmoregulatory activity through balancing osmotic pressures. Pamungkas (2012) argued that osmoregulation in fish was modulated by two hormones, ~~i.e~~namely~~.~~ prolactin and cortisol. Cortisol is a crucial hormone in euryhaline fish since it modulates excretion of ions via gills able to stimulate chloride cells; thus, when migrating, the concentration of plasma cortisol increases. In addition, Scabra (2018) found the depletion of blood volume, leukocyte, and liver glycogen in stressed fish, but the concentration of cortisol increased. Stress condition results mainly from external changes such as salinity, and during this stressful period, ~~the~~ fish activate homeostatic ~~procedure~~ processes by accelerating their metabolic activities, leading to the rise of oxygen intake.

At the end of experiment, the concentration of cortisol in ~~salinity~~ 15‰, 20‰ and 25‰ salinity reached ~~was~~ a level close to the value measured for ~~that in~~the control salinity (30‰). This represents the successful attempt of the fish to reach stability. Hastuti ~~*et al.*~~et al (2004) reported that concentrations of cortisol in plasma of normal fish ranged between 20~~.~~65~~--~~53~~.~~22 µg ~~/~~dL^{-1}~~L~~.

Water quality. Table 1 presents the parameters of water quality, including temperature, dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). The results showed that these parameters tended to be similar in all salinity levels, within the following ranges: temperature 27-29~~—~~°C, pH 5~~.~~9-7~~.~~9, DO 5~~.~~9-10~~.~~9 mg~~/L~~ L^{-1}, NH_3 0~~.~~01~~--~~0~~.~~131 mg~~/~~ L^{-1}, NO_2 0~~.~~050~~--~~0~~.~~090, and NO_3 0~~.~~190~~--~~0~~.~~890. It is noticeable that these values correspond to~~are~~ a set of good conditions for growth and survival rate of silver pompano. As discussed by Sitta ~~and~~ & Hermawan (2011), the optimum condition for silver pompano included temperature 28-32~~-~~°C, pH 6~~.~~8-8~~.~~4, while DO ranged 4~~.~~8 – 5 mg~~/L~~ L^{-1} in aquarium and ±7~~.~~3 mg~~/L~~ L^{-1} in floating net cage. Boyd (2015) found that ammonia at level of 0~~.~~2~~--~~2~~.~~0 mg~~/L~~ L^{-1} could be detrimental to fish~~, .~~ ~~Mean~~while~~,~~ nitrite was acceptable for fish at a concentration ~~of~~ < 1mg/L, unsafe at 1-5 mg~~/L~~ L^{-1}, and poisonous at 16 mg~~/L~~ L^{-1} (Siikavuopio ~~and~~ & Saether 2006).

Table 1

~~Table 1.~~ Mean water quality parameters during the research period

Parameters	*Unit*	*Salinity*				
		25‰	*20‰*	*15‰*	*10‰*	*5‰*
Temperature	°C	27.3-28.8	27.4-28.6	27.8-29.1	27.3-28.6	27.5-28.6
pH	-	7.4-7.9	7.0-7.9	6.9-7.9	6.1-7.9	5.9-7.8
DO	mg/L	5.9-6.6	8.8-10.9	5.9-6.8	5.9-7.03	6-7.1
NH_3	mg/L	<0.01-0.105	<0.01-0.101	<0.01-0.099	0.010-0.109	0.060-0.131
NO_2	mg/L	0.050-0.070	0.050-0.059	0.050-0.059	0.060-0.071	0.060-0.090
NO_3	mg/L	0.340-0.750	0.360-0.780	0.360-0.890	0.190-0.570	0.206-0.345

During the experiment, water quality was maintained at ~~particular~~ a large extent to ensure ~~those~~ acceptable parameters ~~acceptable~~ for fish growth. Water filtration was

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installed in the culture system, comprising of physical (synthetic cotton), chemical (zeolite and active carbon), and biological (bioball) filter. Cotton filter ~~serves~~ served to capture uneaten feed and feces, while zeolite and active carbon enabled ~~to~~ the ~~absorb~~ absorption of toxic compounds such as ammonia and nitrite (Supriyono ~~*et al.*~~et al~~,~~ 2007). Bioball is important as attachment site for nitrifying bacteria capable of converting nitrogen into unharmful form, i.e. nitrate (Dewi ~~and~~ & Masithoh~~,~~ 2013). Nurhidayat ~~*et al.*~~et al (2012) also augmented that the combination of zeolite, ative carbon, and bioball showed satisfying results of maintaining water quality through oxidation of ammonia and enrichment of non-pathogenic nitrifying bacteria.

Growth performance and survival rate. Salinity demonstrated significant impacts to absolute growth weight, absolute growth length, SGR, feed conversion, FE, and SR of silver pompano (~~*p*<0,05~~p<0.05). Statistical test of Newman-~~-~~Kkeuls revealed that two salinity levels, i.e. 5~~-~~‰ and 10~~-~~‰, did not result in any significant difference in some parameters including absolute growth weight and growth length, SGR, feed conversion, and FE. Meanwhile, SR tended to be similar between treatments (Table 2).

Table 2

~~Table 2.~~ Growth performance of silver pompano reared in different salinity levels

Parameters	*Salinity*				
	25‰	*20‰*	*15‰*	*10‰*	*5‰*
Absolute growth weight (g)	14.03±1.18^{ab}	15.87±1.05^{bc}	17.73±1.25^{c}	12.73±1.70^{a}	11.93±1.66^{a}
Absolute growth length (cm)	1.93±0.34	1.82±0.14	2.32±0.21	2.09±0.23	1.84±0.07
Specific growth rate (%)	0.75±0.08^{ab}	0.87±0.04^{b}	0.87±0.05^{b}	0.67±0.09^{a}	0.62±0.07^{a}
Feed conversion	1.31±0.01^{a}	1.26±0.01^{a}	1.24±0.00^{b}	1.33±0.01^{c}	1.34±0.01^{d}
Feed efficiency (%)	76.62±0.8^{a}	79.07±1.03^{a}	80.79±0.58^{b}	75.22±0.76^{c}	74.37±0.28^{a}
Survival rate (%)	81.67±2.88^{ab}	83.33±2.88^{ab}	88.33±2.88^{b}	83.33±2.88^{ab}	76.67±2.88^{a}

Different superscripts following means in the same line showed significant difference at p<0.05.

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As presented in Table 2, 15‰ salinity demonstrated the most satisfying effects on growth of silver pompano, meaning that the fish could perform proper feed utilization and osmoregulation. Wulandari (2006) reported that optimum energy use could be achieved at osmotic condition; thus, more energy was used for their growth instead of osmoregulatory activities.

Retnani ~~and&~~ Abdulgani (2013) reported that growth of silver pompano cultured in 4-24‰ salinity was better than that in 32-34‰ salinity. The salinity modification ~~of to salinity~~ less than the seawater salinity level provoked a decline of the used energy owing to the attenuation of ion exchanges by gill's chloride cells. Such a condition minimized the energy demand for osmoregulation, thereby enhancing the fish growth.

Considering that size, age, stock density~~,~~ and feed are similar, the difference of the fish growth is undoubtedly affected by the enviromental salinities. We also noted that water quality ~~was found at proper ranges~~ ranged within value intervals appropriate for the culture of silver pompano. The importance of salinity ~~towards~~ for the fish growth is associated with changes in physiolo~~g~~fical functions. Fish cultured in high salinity (control) performed a more active transport in order to release excessive ions of Na from gill,

which is a highly energy-consuming activity. Gill chloride cells are responsible for fish osmoregulation. Proliferated on the lamellae, they are extremely sensitive to external salinity. When moving to the new medium with different salinity, euryhaline fish activated chloride cells~~,~~ and delivered signals to central nervous system. ~~At~~ In a culture medium with higher salinity, the proliferation of chloride cells was more intensive; conversely, they were less produced under lower salinity conditions (Bone ~~&and~~ Moore~~,~~ 2008; Fujaya~~,~~ 2004).

The specific growth rate (SGR) was the highest in fish cultured in 15‰ and 20‰ salinity (0~~,~~.87%), and the lowest in 25‰ (0.75%), 10‰ (0,67%) and 5‰ (0.62%) salinity, respectively. Retnani ~~and~~ & Abdulgani (2013) reported a higher SGR in silver pompano cultured in 24‰ salinity (10~~,~~.594%) than ~~that~~ in 32-34‰ salinity (10~~,~~.359%). Noticeably, the SGR of silver pompano farmed in brackish salinity ranged from 14‰ to 24‰.

Feed efficiency (FE) reached the highest level in fish cultured ~~in~~ at 15‰ salinity, indicating that isosmotic condition was achieved. Therefore, energy expenditure is devoted to fish growth instead of osmoregulation. Febrianti ~~*et al.*~~et al (2007) argued the efficient utilization of feed must be greater than 50%. We further noted that FE of silver pompano exceeded 50% across the treatments, meaning that the fish could utilize~~d~~ the feed efficiently in various salinities.

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In respect of the survival rate (SR), the ~~high~~ percentage ~~was~~ recorded across the treatments~~,~~ ~~ranging~~ ranged within 76~~,~~.67-88~~,~~.33%, while fish cultured ~~in~~ at 15‰ salinity reached the highest level. It is noticeable that silver pompano can properly adapt ~~at~~ to a wide range of ~~salinity~~salinities, i.e. 30‰~~—~~-5‰. Additionally, Arrokhman et al (2012) reported a SR of 99~~,~~.03~~–~~–100% in silver pompano reared in 4~~—~~-34‰ salinity. This suggests that the fish ~~is~~ has the potential to be~~y~~ farmed in brackish water.

Histological alterations. Histological observation on the fish gills did not find anomalies in all treatments studied, as indicated by the clear appearance of the secondary lamellae, epithelium, thrombocytes and pillar cells. No abnormalities were observed in gill chloride cells, including the absence of hypertrophy and edema, despite a moderate hyperplasia ~~found in small intensity~~ (Figure 3). In this case, hyperplasia was linked to parasites in the medium. As reported by Wahyuni ~~*et al.*~~et al (2017), normal tissue of fish gill was characterized by obvious appearance of secondary lamellae, pillar cells, lacunae, and thrombocytes.

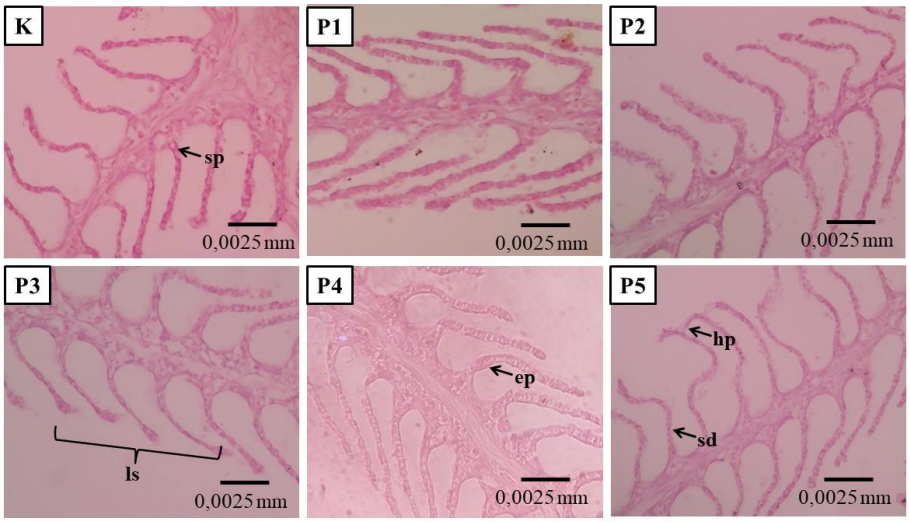

Figure 3. Structure of gill tissue of silver pompano cultured in different levels of salinity. K-=-~~Control~~control, P_{1}-=-25‰, P_{2}-=-20‰, P_{3}-=-15‰, P_{4}-=-10‰ and P_{5}-=-5‰, sp-= pillar cells, ls-=-~~ceondary~~ secondary lamellae, ep-=-epithelium, sd-=-thrombocyte, hp-= hyperplasia.

The histological analysis on fish kidney ~~also~~ did not found any~~no~~ significant abnormalities ~~in all treatments~~, as indicated by condition of Bowman's capsule and glomerulus. The cells were intact and not infected, but a bleeding part was observed (Figure 4). Mc Gavin ~~and~~ & Zachary (2007) offered a description of the~~about~~ kidney histology comprised of main parts such as glomerulus, tubulus and blood vessels. In addition, Takashima ~~and~~ & Hibiya (1995) reported that Bowman's capsule surrounds glomerulus as an indicator of healthy kidney. These organs perform an essential role, i.e. filtering metabolites in bloods. The excretory fluids enter the tubule, while minerals, glucose, and other fluids are re-absorbed. The number and size of glomerulus in freshwater fish were greater than those in seawater fish, considering their importance in retaining salt in body and releasing urine.

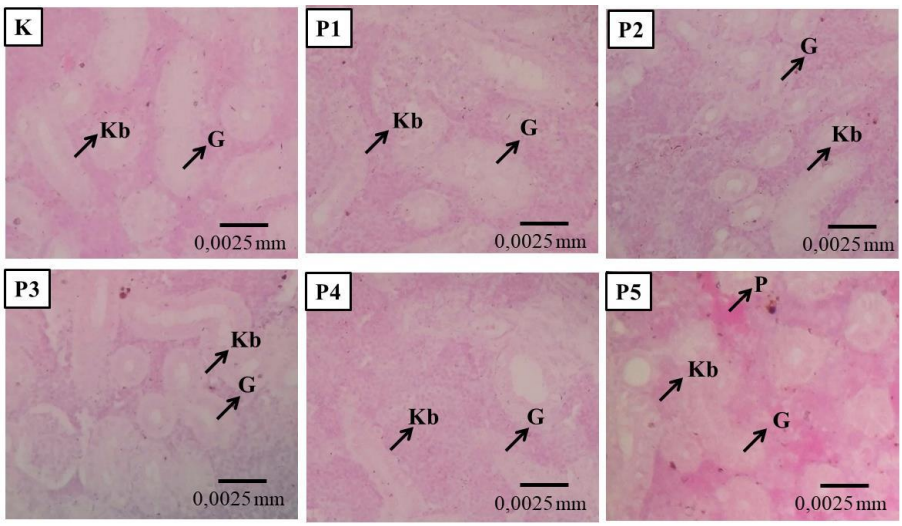

Figure 4. Structure of ~~gill~~ kidney tissue of silver pompano cultured in different levels of salinity. K-=-control, P_{1}-=-25‰, P_{2}-=-20‰, P_{3}-=-15‰, P_{4}-=-10‰ and P_{5}-= 5‰, Kb-=-Bowman's capsule, G-=-glomerulus, P-=-bleeding.

Conclusions.The experimental data revealed the significant effects of salinity on the osmoregulatory activities, blood cortisol level, and growth performance of silver pompano ($p<0.05$). The variance of salinities did not cause any difference in the structure of gill and kidney~~,~~ and did not show~~ed~~ any~~no~~ ~~abnormalities~~ abnormality in these organs. The treatment ~~of~~ at 15‰ salinity exhibited the best outcome, resulting in: osmotic pressure of 3 mOsm~~/L~~ L^{-1} H_2O (closer to the ~~isoosmotic~~isosmotic condition), blood cortisol of 50,923 nmol/L, absolute growth weight of 17~~,~~73±1~~,~~25 g, absolute growth leng~~h~~th of 2~~,~~32±0~~,~~21 cm, specific growth rate of 0~~,~~87 ± 0~~,~~05%, feed conversion of 1.24±0~~,~~00, feed efficiency of 80.79±0~~,~~58 and survival rate of 88~~,~~33±2~~,~~88%. Parameters of water quality were at ~~proper~~ the appropriate level for ~~growth~~ silver pompano growing~~of silver pompano~~. Furthermore, the fish could exert adaptive capacities to medium salinities below the seawater salinity, ~~in which~~ the most satisfying level ~~was~~ being achieved at 15‰ salinity, when cultured in a recirculation system.

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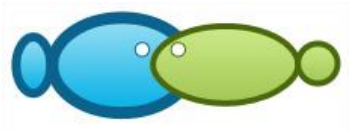

Osmotic performance rate, stress response and growth performance of silver pompano (*Trachinotus blochii*) reared in different salinities using recirculating culture system

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Abstract. Silver pompano (*Trachinotus blochii*) has received tremendous attention from the aquaculture sector, due to its favourable features, such as a high economic value, its good adaptive response, and its potential to be cultured in various salinities. The aim of this study was to discover the effects of medium salinities on osmotic performance rate, blood cortisol, and growth performance (absolute growth weight and lenght, specific growth rate SGR, feed conversion FC, feed efficiency FE, survival rate SR) of silver pompano under recirculating system. The histological alterations (kidney and gill) and water quality (temperature, pH, DO, NH_3, NO_2 and NO_3) were also observed. The 56-day experiment was carried out in Balai Perikanan Budidaya Laut (BPBL) of Batam, Indonesia. A total of 225 fish specimens (11-13 cm in length, weighing 28-29 g) were raised in a 100 L-tank containing 80 L of water at a density of 1 fish/4 L (20 fish in total). They were fed with commercial pellet (46% protein) at 3% of fish biomass and 3 times a day. The experiment was conducted according to a Completely Randomized Design with 5 levels of salinity: P1=25‰, P2=20‰, P3=15‰, P4=10 ‰ and P5=5‰, by performing triplicate measurements for each treatment. The treatment with, 15 ‰ salinity showed the best effects, yielding an osmotic performance rate of 3 mOsm L^{-1} H_2O, a blood cortisol level of 50,923 nmol L^{-1}, an absolute growth weight and length of 17.73±1.25 g and 2.32±0.21 cm, respectively, an SGR of 0.87 ± 0.05%, an FC of 1.24±0.00, an FE of 80.79±0.58 and an SR of 88.33±2.88%. Histologically, there were no anomalies in the structure of gill and kidney of the fish cultured in 5‰ - 25‰ salinities. Water quality was acceptable for growing silver pompano.

Key Words: gill, kidney, blood cortisol, histology, and *Trachinotus blochii*.

Introduction. The farming of silver pompano (*Trachinotus blochii*) has currently gained great popularity in Indonesia. The market demand for the species has continuously increased in international trade, due to its high economic value, its good adaptive response and its potential to be cultured in various water conditions. The price of the fish reaches aproximately USD. 4.25 kg^{-1} in local market, but it may reach USD. 14.144 kg^{-1} in export market (Mo 2017). Since 2015, silver pompano is considered as a promising commodity in marine fisheries sector with total production of 1,900 tons in 2015, demonstrating the annual rise of 31.5% (Prahadi 2015).

T. blochii was reported to exert a high adaptive response towards salinity changes. Survival rate of the species at various salinity levels 32‰, 24‰, 14‰, and 4‰ during the 28-day trial showed no significant difference, indicating that the fish could adapt at lower salinity over seawater, as well as confirming the possibility of fish farming diversification through culture systems in brackish water (Arrokhman et al 2012).

Salinity tolerance in fish closely relates to the osmotic pressure balance between inside and outside the fish body. Osmotic pressure inside the fish is lower than outside.

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The imbalance may cause disturbance of the physiological functions, which in turn disrupt the fish growth. However, under a normal osmotic gradient, the metabolic activity could reach an optimum rate, as indicated by a good appetite and an enhanced feed intake, allowing allocating more energy for the growth (Carrion et al 2005). Fujaya (2004) suggested that the osmotic balance was achieved through regulation of body fluid transportation, known as osmoregulation. The adjustment activities needed for balancing internal and external osmotic pressures require a high energy consumption, leading to stress generation, as indicated by the production of blood cortisol.

Important osmoregulatory organs including gills and kidney play a crucial role in the process. Defective tissues in these organs offer a sign of failure in osmoregulation. Consequently, the defect would adversely affect physiological functions, causing the decrease of feed consumption and fish growth (Putri et al 2014). In this matter, gills seem to be the most susceptible osmoregulatory organ towards environmental changes, such as physicochemical properties of water and presence of toxic compounds. The gill lamellae become the weakest part, in which presence of stressors directly induces ionic homeostasis that remarkably imparts osmoregulation. Indeed, the chronic stressors lead to destructive effects on the gill. Macroscopic and microscopic defects in the gill can serve as biomarker of fish health status (Camargo & Martinez 2007). Besides, Thophon et al (2003) also argued that kidney is a susceptible organ to the external stressors exposure, due to its essential role in maintaining homeostasis. Based on the above elaboration, it can be concluded that there is a need for investigating the osmotic response, stress level and growth performance of silver pompano reared in a recirculation system, at different salinity levels. In this work, the histological alterations in gill and kidney were also observed.

Material and Method

Study site. The experiment was carried out in the Agency for Marine Fisheries Culture (Balai Perikanan Budidaya Laut-BPPL), Batam, Indonesia, for 56 days.

A total of 225 *T. blochii* seeds (average length of 11.90-12.55 cm, weight of 24.30-28.90 g) were reared in an experimental container (capacity 100 L) filled with 80 L of water. The density referred to Indonesia National Standard (SNI 2013), which recommends 1 individual 4 L^{-1} (equal to 20 individuals 80 L^{-1}). Commercial pellet (Megami GR 2) was used, containing 46% protein, 9% fat, 1.9% crude fiber and 8% moisture. The specimens were fed at 3% of their weight, three times a day.

Preparation of the culture container. The close recirculating system was prepared. The container was filled with seawater at different salinity levels, then connected to PVC gutter (50 cm x 14 cm x 14 cm) at the upside of the chamber. The filtration unit water was transported into the culture container through a PVC pipe (2.5 cm diameter). The filtration unit was filled with 50 bioballs (each gutter), as previously prescribed by Nelvia et al (2015). The water was subsequently pumped to the filtration unit with the aid of a 50 W water pump.

Experimental design. The completely randomized design was arranged, consisting of 1 factor and 5 levels (with triplicates) as follows: P1=25‰, P2=20‰, P3=15‰, P4=10‰ and P5=5‰. The effect of salinity was studied, focusing on the osmotic response, the content of blood cortisol, the tissue histology (gills and kidney), the absolute growth weight (Wm), the growth length (Lm), the specific growth rate (SGR), the survival rate (SR) and the water quality (temperature, pH, DO, NH_3, NO_2 and NO_3).

Determination of the osmotic response. Osmotic response was evaluated at the end of experiment and determined using micro-osmometer, as explained by Cambell et al (2012). The fish sample was acclimatized for 7 days and, for the first 5 days, the fish was fed. A total of 10 fish specimens were used for each salinity level, in which they were exposed to the salinity treatments. After 7 days of exposure, the blood of living fish (1 mL) was collected from the heart, then centrifuged at 3000 rpm for 3 minutes to obtain blood plasma. The plasma osmotic pressure was measured using a micro-osmometer.

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The osmoregulatory capacity refers to the difference between osmotic pressure of the medium and internal osmotic pressure of the fish blood plasma. The calculation was described as follows (Anggoro & Nakamura 1996):

$$TKO = [POsmoBlood - POsmoMedium]$$

Where:
TKO - osmotic response (mOsm L^{-1} H2O);
POsmoBlood - biota osmolarity (mOsm L^{-1} H2O);
POsmoMedium - medium osmolarity, and the bracket [ ] means absolute value.

Blood cortisol analysis. The analysis was performed at 3 periods: day 1, day 28 and day 56 of the experiment, using enzyme-linked immunosorbent assay (ELISA). Before the blood withdrawal, the fish was anaesthetized using phenoxyethanol at dose of 0.3 mL L^{-1} water (Rigal et al 2008). Briefly, blood was collected via the vena caudalis, using a heparinized syringe (1 mL), then centrifuged to collect plasma. Plasma cortisol was quantified using RIA (radio immuno–assay) Cortisol (125I) RIA KIT IZOTOP (Ramsay et al 2006). In this regard, blood plasma (0.3 mL) was frozen at -20–°C. To maintain the hormone in the plasma, the sample was packed within a cool-box containing dry ice exactly at day 57 of experiment, then immediately transported into laboratory for analysis.

Histological analysis. Gill and kidney tissue of the sample was collected at the first and last days of the experiment, from the fish specimens exposed to a salinity of 30 ppt (natural habitat condition). Gill cover (overculum) was lifted and the base was cut off to release the gill. Afterwards, the kidney was obtained by opening the abdominal cavity. The phosphate-buffered formalin (NBF) at 10% was used for organ fixation, carried out for 24-48 h (Raškoviæ et al 2011). After fixation, the tissues were dehydrated in graded series of alcohol and xylol, then embedded in paraffin. All these stages were conducted using tissue processor. The sections obtained were cut at thickness of 3-5 µm, then incubated in a water bath at 40 °C. Object glass was placed in the water bath, then immediately air-dried for 1 h. Afterwards, it was stained using haematoxylin-eosin (HE), and observed under light microscope at magnification of 400×.

Growth performance. The weight and length of fish were recorded each 14 days. Growth performance included following parameters:
a. Weight gain (g) = final weight – initial weight;
b. Absolute growth length (cm) = average final length (cm) – average initial length (cm);
c. Specific growth rate (SGR) (%) = (Ln mean final fish weight – Ln mean initial fish weight)/ culture period (day) × 100 %;
d. Feed efficiency (FE) (%) = increased fish mass/total feed consumed;
e. Survival rate (%) = (final number of fish/initial number of fish) × 100 %.

Water quality. Temperature was daily observed– using a thermometer, while chemical indicators were checked each 14 days, including pH (using pH meter), DO (using DO meter), NH_3, NO_2 and NO_3 (using a spectrophotometer).

Results and Discussion

Osmotic performance rate. Osmotic response of fish to the variation of salinity was shown in Figure 1, indicating that salinity caused a remarkable effect on medium osmolarity, blood plasma, and osmotic performance rate of silver pompano ($p<0.05$). In this case, the lowest rate (3 mOsm L^{-1} H_2O) was found at P3 (15–‰), while the highest one (87 mOsm L^{-1} H_2O) was attributed to P5 (5‰). This clearly imparts the effect of salinity on medium and plasma osmolarity, leading to noticeable changes in osmotic performance rate of silver pompano seeds. The extreme difference between medium

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osmolarity and internal fish osmolarity could cause significant changes in fish behaviors and physiological conditions, consequently altering the feed consumption rate and fish growth.

In water of salinity (15–‰) near the isosmotic condition, the fish allocated more energy for enhancing their growth. Energy metabolism required for osmoregulation in fish is fundamentally associated with osmotic performance rate as a rapid response towards changes in medium osmolarity. In this case, osmotic performance rate shows linear correlation to energy consumption for osmoregulatory activities. On the contrary, hypoosmotic (salinity of 30‰, 25‰ and 20‰) and hyperosmotic (10‰ and 5‰) condition leads to increment of osmotic response, which needs higher energy requirement for osmoregulation (Carrion et al 2005). Arjona et al (2009) reported that there was clear evidence that higher osmotic response led to higher energy use for osmoregulation. Our data demonstrated a variety of osmotic response with progression of salinity. As mentioned by Putri et al (2014), medium that possesses higher salinity (far away from isosmotic condition) would increase osmotic performance rate of the fish. At the condition in which the medium condition is tolerable, osmotic performance rate tended to attenuate, thus allowing energy use for fish growth.

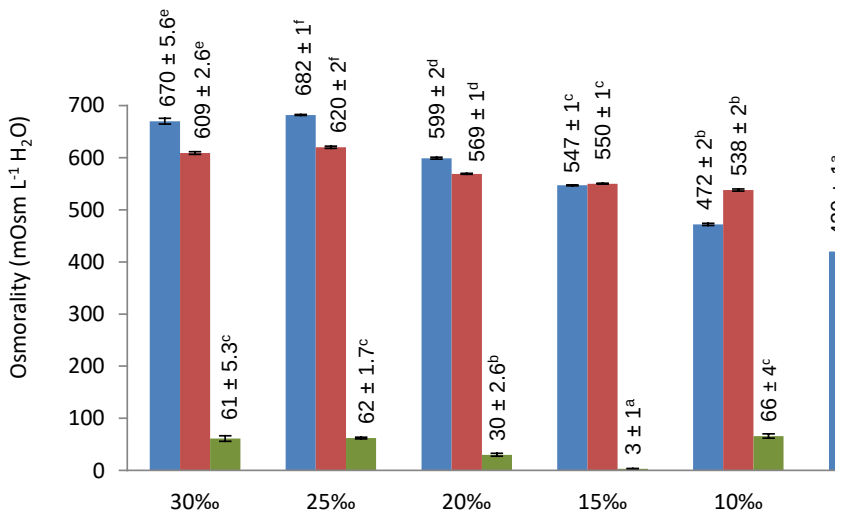

Figure 1. Average osmolarity of rearing medium and blood plasma, and osmotic performance rate of silver pompano cultured in various salinity levels. Different superscripts above the bar showed significant difference at $p<0.05$.

This present work reveals that *T. blochii* is able to adapt to the medium salinity at 15 ppt, suggesting that the juvenile (average length of 12.43-12.48 cm, weight 28.00-28.70 g and age 2 months) can tolerate the condition. Additionally, Retnani & Abdulgani (2013) argued that such adaptive capability of fish relied on size and growth stage, in which osmoregulatory activity of fish may differ depending on age. Lantu (2010) stated that osmoregulation of euryhaline fish strongly related to osmo-sensitivity of chloride cells. They serve as receptor, mainly responding to the salinity level of the medium. When immersed in water media with different salinity, chloride cells in euryhaline fish transmit signals to central nervous system, primarily to the pituitary gland responsible for controlling secretion of growth hormone. Subsequently, the hormone regulates the development of chloride cells in osmoregulatory organs such as gills, kidney and digestive tract. Thus, the amount of chloride cells is adjusted, causing changes in the physiological mechanisms of secretion or absorbtion of ions by chloride cells. At a higher

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salinity, chloride cells would have a higher rate of proliferation, and vice-versa. Over the long term, this controlling mechanism may also cause genetic expression.

Syakirin et al (2018) stated that a higher concentration of ions in water would rise salinity level and osmolar density. For instance, the hybrid grouper is a kind of marine fish that has blood osmolarity (internal osmotic fluid pressure) lower than the environmental osmotic pressure. Therefore, the water will pass from the body of the fish to the environment by the osmotic process through the kidney, gill, as well as in the body. Salinity demonstrates a relationship with the osmoregulation of aquatic animals. A sudden fluctuation of salinity makes the body osmoregulation difficult and induces the animal mortality. The osmoregulation capacity is is determined by the difference between the blood osmotic pressure (fish) and the media osmotic pressure. Osmoregulation relates to the difference of fish blood osmolarity and media osmolarity, known as the osmotic work level: the response to the salinity change increases with this difference.

Stress level in *T. blochii*. The increment of blood cortisol in fish indicates stressful condition. Our data suggested that blood cortisol in all treated samples declined at different extent during the experiment pogression, indicating that the fish exhibited adaptive capacities to lower salinity (Figure 2).

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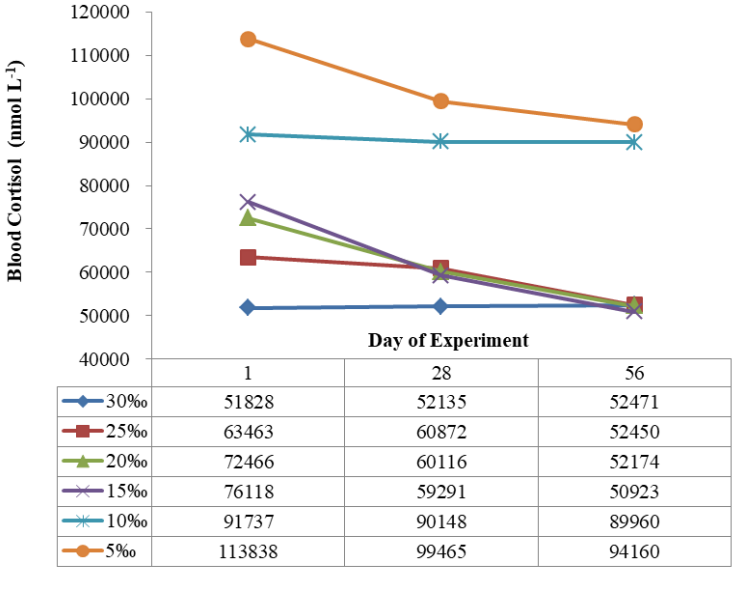

	1	28	56
30‰	51828	52135	52471
25‰	63463	60872	52450
20‰	72466	60116	52174
15‰	76118	59291	50923
10‰	91737	90148	89960
5‰	113838	99465	94160

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Day if experiment

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Figure 2. Concentration of blood cortisol in *Trachinotus blochii* cultured in various levels of salinity.

As depicted in Figure 2, the concentration of blood cortisol on day 1 increased as the level of salinity was lower compared to the control (30‰). This clearly showed a stress response to the new environment. Setiyoningsih (2014) argued that stress in fish existed as rapid response towards enviromental pressure, thus they secreted glucocorticoid (cortisol) and catecholamine hormone to cope with the stress condition.

Furthermore, cortisol level declined consistently from day 28 to day 56 in all treatments, indicating adaptive capacities. On day 56, the lowest cortisol level (50,923 nmol L^{-1}) was found at 15‰, while the highest one (94,160 nmol L^{-1}) was found at 5‰. In salinity 15‰, the fish showed the best osmoregulatory activity through balancing

osmotic pressures. Pamungkas (2012) argued that osmoregulation in fish was modulated by two hormones, namely prolactin and cortisol. Cortisol is a crucial hormone in euryhaline fish since it modulates excretion of ions via gills able to stimulate chloride cells; thus, when migrating, the concentration of plasma cortisol increases. In addition, Scabra (2018) found the depletion of blood volume, leukocyte, and liver glycogen in stressed fish, but the concentration of cortisol increased. Stress condition results mainly from external changes such as salinity, and during this stressful period, fish activate homeostatic processes by accelerating their metabolic activities, leading to the rise of oxygen intake.

At the end of experiment, the concentration of cortisol in 15‰, 20‰ and 25‰ salinity reached a level close to the value measured for the control salinity (30‰). This represents the successful attempt of the fish to reach stability. Hastuti et al (2004) reported that concentrations of cortisol in plasma of normal fish ranged between 569.73–1,468.34 nmol L^{-1}.

Water quality. Table 1 presents the parameters of water quality, including temperature, dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). The results showed that these parameters tended to be similar in all salinity levels, within the following ranges: temperature 27-29°C, pH 5.9-7.9, DO 5.9-10.9 mg L^{-1}, NH_3 0.01–0.131 mg L^{-1}, NO_2 0.050–0.090, and NO_3 0.190–0.890. It is noticeable that these values correspond to a set of good conditions for growth and survival rate of silver pompano. As discussed by Sitta & Hermawan (2011), the optimum condition for silver pompano included temperature 28-32°C, pH 6.8-8.4, while DO ranged 4.8 – 5 mg L^{-1} in aquarium and ±7.3 mg L^{-1} in floating net cage. Boyd (2015) found that ammonia at level of 0.2–-2.0 mg L^{-1} could be detrimental to fish, while nitrite was acceptable for fish at a concentration < 1mg/L, unsafe at 1-5 mg L^{-1}, and poisonous at 16 mg L^{-1} (Siikavuopio & Saether 2006).

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Table 1

Mean water quality parameters during the research period

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Parameters	*Unit*	*Salinity*				
		25‰	*20‰*	*15‰*	*10‰*	*5‰*
Temperature	°C	27.3-28.8	27.4-28.6	27.8-29.1	27.3-28.6	27.5-28.6
pH	-	7.4-7.9	7.0-7.9	6.9-7.9	6.1-7.9	5.9-7.8
DO	mg/L	5.9-6.6	8.8-10.9	5.9-6.8	5.9-7.03	6-7.1
NH_3	mg/L	<0.01-0.105	<0.01-0.101	<0.01-0.099	0.010-0.109	0.060-0.131
NO_2	mg/L	0.050-0.070	0.050-0.059	0.050-0.059	0.060-0.071	0.060-0.090
NO_3	mg/L	0.340-0.750	0.360-0.780	0.360-0.890	0.190-0.570	0.206-0.345

During the experiment, water quality was maintained at a large extent to ensure acceptable parameters for fish growth. Water filtration was installed in the culture system, comprising of physical (synthetic cotton), chemical (zeolite and active carbon), and biological (bioball) filter. Cotton filter served to capture uneaten feed and feces, while zeolite and active carbon enabled the absorption of toxic compounds such as ammonia and nitrite (Supriyono et al 2007). Bioball is important as attachment site for nitrifying bacteria capable of converting nitrogen into unharmful form, i.e. nitrate (Dewi & Masithoh 2013). Nurhidayat et al (2012) also augmented that the combination of zeolite, ative carbon, and bioball showed satisfying results of maintaining water quality through oxidation of ammonia and enrichment of non-pathogenic nitrifying bacteria.

Growth performance and survival rate. Salinity demonstrated significant impacts to absolute growth weight, absolute growth length, SGR, feed conversion, FE, and SR of silver pompano ($p<0.05$). Statistical test of Newman-Keuls revealed that two salinity levels, i.e. 5‰ and 10‰, did not result in any significant difference in some parameters including absolute growth weight and growth length, SGR, feed conversion, and FE. Meanwhile, SR tended to be similar between treatments (Table 2).

Table 2

Growth performance of silver pompano reared in different salinity levels

Parameters	*Salinity*				
	25‰	*20‰*	*15‰*	*10‰*	*5‰*
Absolute growth weight (g)	14.03±1.18^{ab}	15.87±1.05^{bc}	17.73±1.25^{c}	12.73±1.70^{a}	11.93±1.66^{a}
Absolute growth length (cm)	1.93±0.34	1.82±0.14	2.32±0.21	2.09±0.23	1.84±0.07
Specific growth rate (%)	0.75±0.08^{ab}	0.87±0.04^{b}	0.87±0.05^{b}	0.67±0.09^{a}	0.62±0.07^{a}
Feed conversion	1.31±0.01^{a}	1.26±0.01^{a}	1.24±0.00^{b}	1.33±0.01^{c}	1.34±0.01^{d}
Feed efficiency (%)	76.62±0.8^{a}	79.07±1.03^{a}	80.79±0.58^{b}	75.22±0.76^{c}	74.37±0.28^{a}
Survival rate (%)	81.67±2.88^{ab}	83.33±2.88^{ab}	88.33±2.88^{b}	83.33±2.88^{ab}	76.67±2.88^{a}

Means with different superscripts (a, b, c and d) were significantly different while ab and bc were not significantly different ($p < 0.05$).

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As presented in Table 2, 15‰ salinity demonstrated the most satisfying effects on growth of silver pompano, meaning that the fish could perform proper feed utilization and osmoregulation. Wulandari (2006) reported that optimum energy use could be achieved at osmotic condition; thus, more energy was used for their growth instead of osmoregulatory activities.

Retnani & Abdulgani (2013) reported that growth of silver pompano cultured in 4-24‰ salinity was better than that in 32-34‰ salinity. The salinity modification to less than the seawater salinity level provoked a decline of the used energy owing to the attenuation of ion exchanges by gill's chloride cells. Such a condition minimized the energy demand for osmoregulation, thereby enhancing the fish growth.

Considering that size, age, stock density and feed are similar, the difference of the fish growth is undoubtedly affected by the enviromental salinities. We also noted that water quality ranged within value intervals appropriate for the culture of silver pompano. The importance of salinity for the fish growth is associated with changes in physiological functions. Fish cultured in high salinity (control) performed a more active transport in order to release excessive ions of Na from gill, which is a highly energy-consuming activity. Gill chloride cells are responsible for fish osmoregulation. Proliferated on the lamellae, they are extremely sensitive to external salinity. When moving to the new medium with different salinity, euryhaline fish activated chloride cells, and delivered signals to central nervous system. In a culture medium with higher salinity, the proliferation of chloride cells was more intensive; conversely, they were less produced under lower salinity conditions (Bone & Moore 2008; Fujaya 2004).

The specific growth rate (SGR) was the highest in fish cultured in 15‰ and 20‰ salinity (0.87%), and the lowest in 25‰ (0.75%), 10‰ (0,67%) and 5‰ (0.62%)

salinity, respectively. Retnani & Abdulgani (2013) reported a higher SGR in silver pompano cultured in 24‰ salinity (10.594%) than in 32-34‰ salinity (10.359%). Noticeably, the SGR of silver pompano farmed in brackish salinity ranged from 14‰ to 24‰.

Feed efficiency (FE) reached the highest level in fish cultured at 15‰ salinity, indicating that isosmotic condition was achieved. Therefore, energy expenditure is devoted to fish growth instead of osmoregulation. Febrianti et al (2016) argued the efficient utilization of feed must be greater than 50%. We further noted that FE of silver pompano exceeded 50% across the treatments, meaning that the fish could utilized the feed efficiently in various salinities.

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In respect of the survival rate (SR), the percentage recorded across the treatments ranged within 76.67-88.33%, while fish cultured at 15‰ salinity reached the highest level. It is noticeable that silver pompano can properly adapt to a wide range of salinities, i.e. 30‰-5‰. Additionally, Arrokhman et al (2012) reported a SR of 99.03–100% in silver pompano reared in 4-34‰ salinity. This suggests that the fish has the potential to be farmed in brackish water.

Histological alterations. Histological observation on the fish gills did not find anomalies in all treatments studied, as indicated by the clear appearance of the secondary lamellae, epithelium, thrombocytes and pillar cells. No abnormalities were observed in gill chloride cells, including the absence of hypertrophy and edema, despite a moderate hyperplasia (Figure 3). In this case, hyperplasia was linked to parasites in the medium. As reported by Wahyuni et al (2017), normal tissue of fish gill was characterized by obvious appearance of secondary lamellae, pillar cells, lacunae, and thrombocytes.

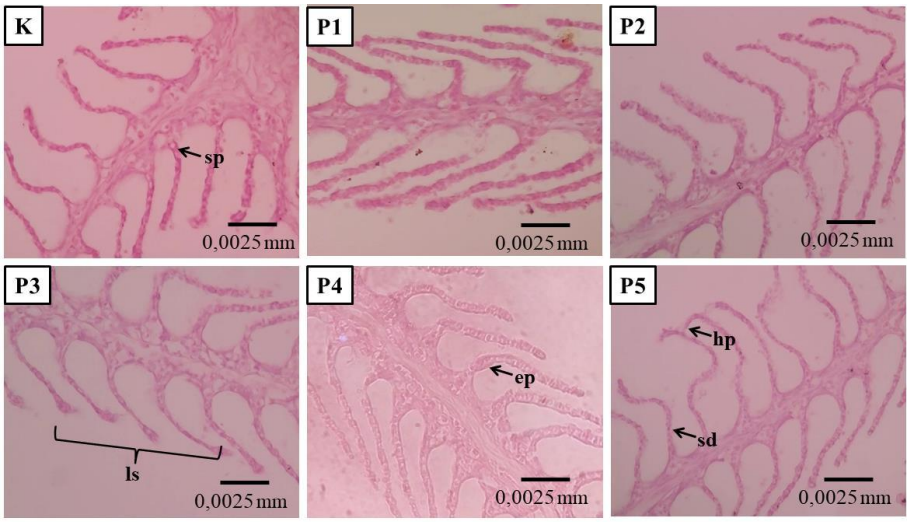

Figure 3. Structure of gill tissue of silver pompano cultured in different levels of salinity. K=control, P_1=25‰, P_2=20‰, P_3=15‰, P_4=10‰ and P_5=5‰, sp=pillar cells, ls= secondary lamellae, ep=epithelium, sd=thrombocyte, hp=hyperplasia.

The histological analysis on fish kidney did not found any significant abnormalities, as indicated by condition of Bowman's capsule and glomerulus. The cells were intact and not infected, but a bleeding part was observed (Figure 4). Mc Gavin & Zachary (2007) offered a description of the kidney histology comprised of main parts such as glomerulus, tubulus and blood vessels. In addition, Takashima & Hibiya (1995) reported that Bowman's capsule surrounds glomerulus as an indicator of healthy kidney. These organs perform an essential role, i.e. filtering metabolites in bloods. The excretory fluids enter the tubule,

while minerals, glucose, and other fluids are re-absorbed. The number and size of glomerulus in freshwater fish were greater than those in seawater fish, considering their importance in retaining salt in body and releasing urine.

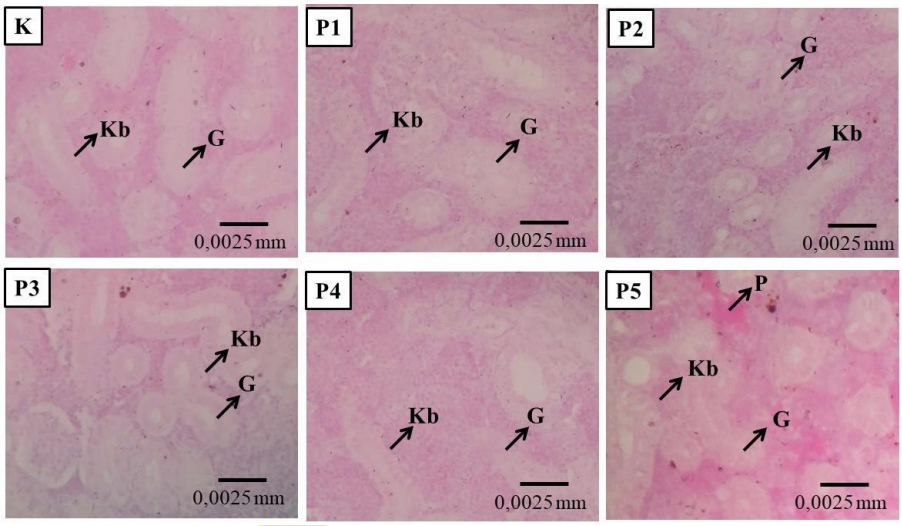

Figure 4. Structure of kidney tissue of silver pompano cultured in different levels of salinity. K=control, P_1=25‰, P_2=20‰, P_3=15‰, P_4=10‰ and P_5=5‰, Kb=Bowman's capsule, G=glomerulus, P=bleeding.

Conclusions.The experimental data revealed the significant effects of salinity on the osmoregulatory activities, blood cortisol level, and growth performance of silver pompano ($p<0.05$). The variance of salinities did not cause any difference in the structure of gill and kidney and did not show any abnormality in these organs. The treatment at 15‰ salinity exhibited the best outcome, resulting in: osmotic pressure of 3 mOsm L^{-1} H2O (closer to the isosmotic condition), blood cortisol of 50,923 nmol L^{-1}, absolute growth weight of 17.73±1.25 g, absolute growth length of 2.32±0.21 cm, specific growth rate of 0.87 ± 0.05%, feed conversion of 1.24±0.00, feed efficiency of 80.79±0.58 and survival rate of 88.33±2.88%. Parameters of water quality were at the appropriate level for silver pompano growing. Furthermore, the fish could exert adaptive capacities to medium salinities below the seawater salinity, the most satisfying level being achieved at 15‰ salinity, when cultured in a recirculation system.

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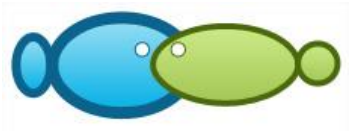

Osmotic performance rate, stress response and growth performance of silver pompano (*Trachinotus blochii*) reared in different salinities using recirculating culture system

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Abstract. Silver pompano (*Trachinotus blochii*) has received tremendous attention from the aquaculture sector, due to its favourable features, such as a high economic value, its good adaptive response and its potential to be cultured in various salinities. The aim of this study was to discover the effects of medium salinities on osmotic performance rate, blood cortisol, and growth performance (absolute growth weight and lenght, specific growth rate (SGR), feed conversion (FC), feed efficiency (FE), survival rate (SR)) of *T. blochii* under recirculating system. The histological alterations (kidney and gill) and water quality (temperature, pH, DO, NH_3, NO_2 and NO_3) were also observed. The 56-day experiment was carried out in Balai Perikanan Budidaya Laut (BPBL) of Batam, Indonesia. A total of 225 fish specimens (11-13 cm in length, weighing 28-29 g) were raised in a 100 L tank containing 80 L of water at a density of 1 fish 4 L^{-1} (20 fish in total). They were fed with commercial pellet (46% protein) at 3% of fish biomass and 3 times a day. The experiment was conducted according to a completely randomized design with 5 levels of salinity: P1=25‰, P2=20‰, P3=15‰, P4=10 ‰ and P5=5‰, by performing triplicate measurements for each treatment. The treatment with 15 ‰ salinity showed the best effects, yielding an osmotic performance rate of 3 mOsm L^{-1} H_2O, a blood cortisol level of 50,923 nmol L^{-1}, an absolute growth weight and length of 17.73±1.25 g and 2.32±0.21 cm, respectively, an SGR of 0.87 ± 0.05%, an FC of 1.24±0.00, an FE of 80.79±0.58 and an SR of 88.33±2.88%. Histologically, there were no anomalies in the structure of gill and kidney of the fish cultured in 5‰-25‰ salinities. Water quality was acceptable for growing *T. blochii*.
Key Words: gill, kidney, blood cortisol, histology, water quality.

Introduction. The farming of silver pompano (*Trachinotus blochii*) has currently gained great popularity in Indonesia. The market demand for the species has continuously increased in international trade, due to its high economic value, its good adaptive response and its potential to be cultured in various water conditions. The price of the fish reaches aproximately USD. 4.25 kg^{-1} in local market, but it may reach USD. 14.144 kg^{-1} in export market (Mo 2017). Since 2015, *T. blochii* is considered as a promising commodity in marine fisheries sector with total production of 1,900 tons in 2015, demonstrating the annual rise of 31.5% (Prahadi 2015).

T. blochii was reported to exert a high adaptive response towards salinity changes. Survival rate of the species at various salinity levels 32‰, 24‰, 14‰, and 4‰ during the 28-day trial showed no significant difference, indicating that the fish could adapt at lower salinity over seawater, as well as confirming the possibility of fish farming diversification through culture systems in brackish water (Arrokhman et al 2012).

Salinity tolerance in fish closely relates to the osmotic pressure balance between inside and outside the fish body. Osmotic pressure inside the fish is lower than outside.

The imbalance may cause disturbance of the physiological functions, which in turn disrupt the fish growth. However, under a normal osmotic gradient, the metabolic activity could reach an optimum rate, as indicated by a good appetite and an enhanced feed intake, allowing allocating more energy for the growth (Carrion et al 2005). Fujaya (2004) suggested that the osmotic balance was achieved through regulation of body fluid transportation, known as osmoregulation. The adjustment activities needed for balancing internal and external osmotic pressures require a high energy consumption, leading to stress generation, as indicated by the production of blood cortisol.

Important osmoregulatory organs including gills and kidney play a crucial role in the process. Defective tissues in these organs offer a sign of failure in osmoregulation. Consequently, the defect would adversely affect physiological functions, causing the decrease of feed consumption and fish growth (Putri et al 2014). In this matter, gills seem to be the most susceptible osmoregulatory organ towards environmental changes, such as physicochemical properties of water and presence of toxic compounds. The gill lamellae become the weakest part, in which presence of stressors directly induces ionic homeostasis that remarkably imparts osmoregulation. Indeed, the chronic stressors lead to destructive effects on the gill. Macroscopic and microscopic defects in the gill can serve as biomarker of fish health status (Camargo & Martinez 2007). Besides, Thophon et al (2003) also argued that kidney is a susceptible organ to the external stressors exposure, due to its essential role in maintaining homeostasis. Based on the above elaboration, it can be concluded that there is a need for investigating the osmotic response, stress level and growth performance of *T. blochii* reared in a recirculation system, at different salinity levels. In this work, the histological alterations in gill and kidney were also observed.

Material and Method

Study site. The experiment was carried out in the Agency for Marine Fisheries Culture (Balai Perikanan Budidaya Laut-BPPL), Batam, Indonesia, for 56 days. A total of 225 *T. blochii* seeds (average length of 11-13 cm, weight of 28-29 g) were reared in an experimental container (capacity 100 L) filled with 80 L of water. The density referred to Indonesia National Standard (SNI 2013), which recommends 1 individual 4 L^{-1} (equal to 20 individuals 80 L^{-1}). Commercial pellet (Megami GR 2) was used, containing 46% protein, 9% fat, 1.9% crude fiber and 8% moisture. The specimens were fed at 3% of their weight, three times a day.

Preparation of the culture container. The close recirculating system was prepared. The container was filled with seawater at different salinity levels, then connected to PVC gutter (50 cm x 14 cm x 14 cm) at the upside of the chamber. The filtration unit water was transported into the culture container through a PVC pipe (2.5 cm diameter). The filtration unit was filled with 50 bioballs (each gutter), as previously prescribed by Nelvia et al (2015). The water was subsequently pumped to the filtration unit with the aid of a 50 W water pump.

Experimental design. The completely randomized design was arranged, consisting of 1 factor and 5 levels (with triplicates), as follows: P1=25‰, P2=20‰, P3=15‰, P4=10‰ and P5=5‰. The effect of salinity was studied, focusing on the osmotic response, the content of blood cortisol, the tissue histology (gills and kidney), the absolute growth weight (Wm), the growth length (Lm), the specific growth rate (SGR), the survival rate (SR) and the water quality (temperature, pH, DO, NH_3, NO_2 and NO_3).

Determination of the osmotic response. Osmotic response was evaluated at the end of experiment and determined using micro-osmometer, as explained by Cambell et al (2012). The fish sample was acclimatized for 7 days and, for the first 5 days, the fish was fed. A total of 10 fish specimens were used for each salinity level, in which they were exposed to the salinity treatments. After 7 days of exposure, the blood of living fish (1 mL) was collected from the heart, and then centrifuged at 3000 rpm for 3 minutes to obtain blood plasma. The plasma osmotic pressure was measured using a micro-osmometer. The osmoregulatory capacity refers to the difference between osmotic

pressure of the medium and internal osmotic pressure of the fish blood plasma. The calculation was described as follows (Anggoro & Nakamura 1996):

$$TKO = [POsmoBlood - POsmoMedium]$$

Where:
TKO - osmotic response (mOsm L^{-1} H_2O);
POsmoBlood - biota osmolarity (mOsm L^{-1} H_2O);
POsmoMedium - medium osmolarity, and the bracket [ ] means absolute value.

Blood cortisol analysis. The analysis was performed at 3 periods: day 1, day 28 and day 56 of the experiment, using enzyme-linked immunosorbent assay (ELISA). Before the blood withdrawal, the fish was anaesthetized using phenoxyethanol at dose of 0.3 mL L^{-1} water (Rigal et al 2008). Briefly, blood was collected via the vena caudalis, using a heparinized syringe (1 mL), then centrifuged to collect plasma. Plasma cortisol was quantified using RIA (radio immunoassay) Cortisol (125I) RIA KIT IZOTOP (Ramsay et al 2006). In this regard, blood plasma (0.3 mL) was frozen at -20°C. To maintain the hormone in the plasma, the sample was packed within a cool-box containing dry ice exactly at day 57 of experiment, then immediately transported into laboratory for analysis.

Histological analysis. Gill and kidney tissue of the sample was collected at the first and last days of the experiment, from the fish specimens exposed to a salinity of 30 ppt (natural habitat condition). Gill cover (overculum) was lifted and the base was cut off to release the gill. Afterwards, the kidney was obtained by opening the abdominal cavity. The phosphate-buffered formalin (NBF) at 10% was used for organ fixation, carried out for 24-48 h (Raškoviæ et al 2011). After fixation, the tissues were dehydrated in graded series of alcohol and xylol, and then embedded in paraffin. All these stages were conducted using tissue processor. The sections obtained were cut at thickness of 3-5 µm, then incubated in a water bath at 40°C and then immediately air-dried for 1 h. Afterwards, it was stained using haematoxylin-eosin (HE), and observed under light microscope at magnification of 400×.

Growth performance. The weight and length of fish were recorded each 14 days. Growth performance included following parameters:
a. Weight gain (g) = final weight – initial weight;
b. Absolute growth length (cm) = average final length (cm) – average initial length (cm);
c. Specific growth rate (SGR) (%) = (Ln mean final fish weight – Ln mean initial fish weight) / culture period (day) × 100 %;
d. Feed efficiency (FE) (%) = increased fish mass/total feed consumed;
e. Survival rate (%) = (final number of fish/initial number of fish) × 100 %.

Water quality. Temperature was observed daily using a thermometer, while chemical indicators were checked each 14 days, including pH (using pH meter), DO (using DO meter), NH_3, NO_2 and NO_3 (using a spectrophotometer).

Results and Discussion

Osmotic performance rate. Osmotic response of fish to the variation of salinity was shown in Figure 1, indicating that salinity caused a remarkable effect on medium osmolarity, blood plasma, and osmotic performance rate of *T. blochii* ($p<0.05$). In this case, the lowest rate (3 mOsm L^{-1} H_2O) was found at P3 (15‰), while the highest one (87 mOsm L^{-1} H_2O) was attributed to P5 (5‰). This clearly imparts the effect of salinity on medium and plasma osmolarity, leading to noticeable changes in osmotic performance rate of *T. blochii* seeds. The extreme difference between medium osmolarity and internal

fish osmolarity could cause significant changes in fish behaviors and physiological conditions, consequently altering the feed consumption rate and fish growth.

In water of salinity near the isosmotic condition (15‰), the fish allocated more energy for enhancing their growth. Energy metabolism required for osmoregulation in fish is fundamentally associated with osmotic performance rate as a rapid response towards changes in medium osmolarity. In this case, osmotic performance rate shows linear correlation to energy consumption for osmoregulatory activities. On the contrary, hypoosmotic (salinity of 30‰, 25‰ and 20‰) and hyperosmotic (10‰ and 5‰) condition leads to increment of osmotic response, which needs higher energy requirement for osmoregulation (Carrion et al 2005). Arjona et al (2009) reported that there was clear evidence that higher osmotic response led to higher energy use for osmoregulation. Our data demonstrated a variety of osmotic response with progression of salinity. As mentioned by Putri et al (2014), medium that possesses higher salinity (far away from isosmotic condition) would increase osmotic performance rate of the fish. At the condition in which the medium condition is tolerable, osmotic performance rate tended to attenuate, thus allowing energy use for fish growth.

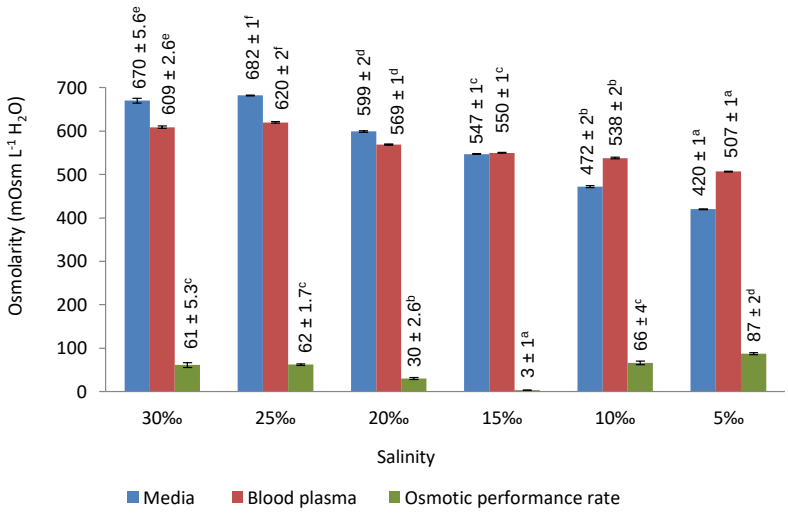


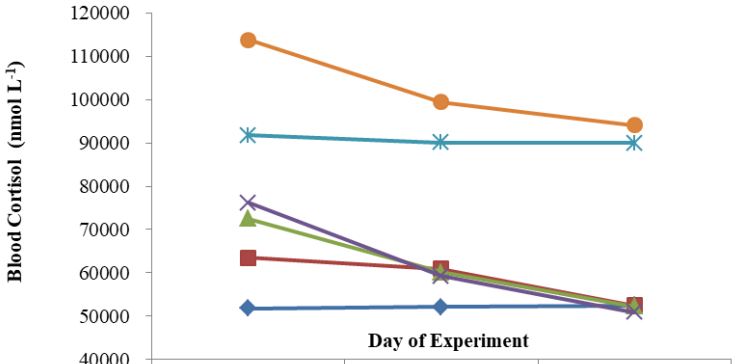
Figure 1. Average osmolarity of rearing medium and blood plasma, and osmotic performance rate of *Trachinotus blochii* cultured in various salinity levels. Different superscripts above the bar showed significant difference at $p<0.05$.

This present work reveals that *T. blochii* is able to adapt to the medium salinity at 15 ppt, suggesting that the juvenile (average length of 12.43-12.48 cm, weight 28.00-28.70 g and age of 2 months) can tolerate the condition. Additionally, Retnani & Abdulgani (2013) argued that such adaptive capability of fish relied on size and growth stage, in which osmoregulatory activity of fish may differ depending on age. Lantu (2010) stated that osmoregulation of euryhaline fish strongly related to osmo-sensitivity of chloride cells. They serve as receptor, mainly responding to the salinity level of the medium. When immersed in water media with different salinity, chloride cells in euryhaline fish transmit signals to central nervous system, primarily to the pituitary gland responsible for controlling secretion of growth hormone. Subsequently, the hormone regulates the development of chloride cells in osmoregulatory organs such as gills, kidney and digestive tract. Thus, the amount of chloride cells was adjusted, causing changes in the physiological mechanisms of secretion or absorbtion of ions by chloride cells. At a higher

salinity, chloride cells would have a higher rate of proliferation, and vice-versa. Over the long term, this controlling mechanism may also cause genetic expression.

Syakirin et al (2018) stated that a higher concentration of ions in water would rise salinity level and osmolar density. For instance, the hybrid grouper is a kind of marine fish that has blood osmolarity (internal osmotic fluid pressure) lower than the environmental osmotic pressure. Therefore, the water will pass from the body of the fish to the environment by the osmotic process through the kidney, gill, as well as in the body. Salinity demonstrates a relationship with the osmoregulation of aquatic animals. A sudden fluctuation of salinity makesthe body osmoregulation difficult and induces the animal mortality. The osmoregulation capacity is is determined by the difference between the blood osmotic pressure (fish) and the media osmotic pressure. Osmoregulation relates to the difference of fish blood osmolarity and media osmolarity, known as the osmotic work level: the response to the salinity change increases with this difference.

***Stress level in T. blochii*.** The increment of blood cortisol in fish indicates stressful condition. Our data suggested that blood cortisol in all treated samples declined at different extent during the experiment pogression, indicating that the fish exhibited adaptive capacities to lower salinity (Figure 2).



	1	28	56
30‰	51828	52135	52471
25‰	63463	60872	52450
20‰	72466	60116	52174
15‰	76118	59291	50923
10‰	91737	90148	89960
5‰	113838	99465	94160

Figure 2. Concentration of blood cortisol in *Trachinotus blochii* cultured in various levels of salinity.

As depicted in Figure 2, the concentration of blood cortisol on day 1 increased as the level of salinity was lower compared to the control (30‰). This clearly showed a stress response to the new environment. Setiyoningsih (2014) argued that stress in fish existed as rapid response towards enviromental pressure, thus they secreted glucocorticoid (cortisol) and catecholamine hormone to cope with the stress condition.

Furthermore, cortisol level declined consistently from day 28 to day 56 in all treatments, indicating adaptive capacities. On day 56, the lowest cortisol level (50,923 nmol L^{-1}) was found at 15‰, while the highest one (94,160 nmol L^{-1}) was found at 5‰. In salinity 15‰, the fish showed the best osmoregulatory activity through balancing osmotic pressures. Pamungkas (2012) argued that osmoregulation in fish was modulated by two hormones, namely prolactin and cortisol. Cortisol is a crucial hormone in

euryhaline fish since it modulates excretion of ions via gills able to stimulate chloride cells, thus, when migrating, the concentration of plasma cortisol increases. In addition, Scabra (2018) found the depletion of blood volume, leukocyte, and liver glycogen in stressed fish, but the concentration of cortisol increased. Stress condition results mainly from external changes such as salinity, and during this stressful period, fish activate homeostatic processes by accelerating their metabolic activities, leading to the rise of oxygen intake.

At the end of experiment, the concentration of cortisol in 15‰, 20‰ and 25‰ salinity reached a level close to the value measured for the control salinity (30‰). This represents the successful attempt of the fish to reach stability. Hastuti et al (2004) reported that concentrations of cortisol in plasma of normal fish ranged between 20.65–53.22 µg dL^{-1}.

Water quality. Table 1 presents the parameters of water quality, including temperature, dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). The results showed that these parameters tended to be similar in all salinity levels, within the following ranges: temperature 27-29°C, pH 5.9-7.9, DO 5.9-10.9 mg L^{-1}, NH_3 0.01–0.131 mg L^{-1}, NO_2 0.050–0.090, and NO_3 0.190–0.890. It is noticeable that these values correspond to a set of good conditions for growth and survival rate of *T. blochii*. As discussed by Sitta & Hermawan (2011), the optimum condition for *T. blochii* included temperature 28-32°C, pH 6.8-8.4, while DO ranged 4.8 – 5 mg L^{-1} in aquarium and ±7.3 mg L^{-1} in floating net cage. Boyd (2015) found that ammonia at level of 0.2–2.0 mg L^{-1} could be detrimental to fish, while nitrite was acceptable for fish at a concentration <1mg/L, unsafe at 1-5 mg L^{-1}, and poisonous at 16 mg L^{-1} (Siikavuopio & Saether 2006).

Table 1

Mean water quality parameters during the research period

Parameters	*Unit*	*Salinity*				
		25‰	*20‰*	*15‰*	*10‰*	*5‰*
Temperature	°C	27.3-28.8	27.4-28.6	27.8-29.1	27.3-28.6	27.5-28.6
pH	-	7.4-7.9	7.0-7.9	6.9-7.9	6.1-7.9	5.9-7.8
DO	mg/L	5.9-6.6	8.8-10.9	5.9-6.8	5.9-7.03	6-7.1
NH_3	mg/L	<0.01-0.105	<0.01-0.101	<0.01-0.099	0.010-0.109	0.060-0.131
NO_2	mg/L	0.050-0.070	0.050-0.059	0.050-0.059	0.060-0.071	0.060-0.090
NO_3	mg/L	0.340-0.750	0.360-0.780	0.360-0.890	0.190-0.570	0.206-0.345

During the experiment, water quality was maintained at a large extent to ensure acceptable parameters for fish growth. Water filtration was installed in the culture system, comprising of physical (synthetic cotton), chemical (zeolite and active carbon), and biological (bioball) filter. Cotton filter served to capture uneaten feed and feces, while zeolite and active carbon enabled the absorption of toxic compounds such as ammonia and nitrite (Supriyono et al 2007). Bioball is important as attachment site for nitrifying bacteria capable of converting nitrogen into unharmful form, i.e. nitrate (Dewi & Masithoh 2013). Nurhidayat et al (2012) also augmented that the combination of zeolite, ative carbon, and bioball showed satisfying results of maintaining water quality through oxidation of ammonia and enrichment of non-pathogenic nitrifying bacteria.

Growth performance and survival rate. Salinity demonstrated significant impacts to absolute growth weight, absolute growth length, SGR, feed conversion, FE, and SR of *T. blochii* ($p<0.05$). Statistical test of Newman-Keuls revealed that two salinity levels, i.e. 5‰ and 10‰, did not result in any significant difference in some parameters including absolute growth weight and growth length, SGR, feed conversion, and FE. Meanwhile, SR tended to be similar between treatments (Table 2).

Table 2

Growth performance of *Trachinotus blochii* reared in different salinity levels

Parameters	*Salinity*				
	25‰	*20‰*	*15‰*	*10‰*	*5‰*
Absolute growth weight (g)	14.03±1.18^{ab}	15.87±1.05^{bc}	17.73±1.25^{c}	12.73±1.70^{a}	11.93±1.66^{a}
Absolute growth length (cm)	1.93±0.34	1.82±0.14	2.32±0.21	2.09±0.23	1.84±0.07
Specific growth rate (%)	0.75±0.08^{ab}	0.87±0.04^{b}	0.87±0.05^{b}	0.67±0.09^{a}	0.62±0.07^{a}
Feed conversion	1.31±0.01^{a}	1.26±0.01^{a}	1.24±0.00^{b}	1.33±0.01^{c}	1.34±0.01^{d}
Feed efficiency (%)	76.62±0.8^{a}	79.07±1.03^{a}	80.79±0.58^{b}	75.22±0.76^{c}	74.37±0.28^{a}
Survival rate (%)	81.67±2.88^{ab}	83.33±2.88^{ab}	88.33±2.88^{b}	83.33±2.88^{ab}	76.67±2.88^{a}

a, b, c and d were significantly different; ab and bc were not significantly different ($p<0.05$).

As presented in Table 2, 15‰ salinity demonstrated the most satisfying effects on growth of *T. blochii*, meaning that the fish could perform proper feed utilization and osmoregulation. Wulandari (2006) reported that optimum energy use could be achieved at osmotic condition; thus, more energy was used for their growth instead of osmoregulatory activities.

Retnani & Abdulgani (2013) reported that growth of *T. blochii* cultured in 4-24‰ salinity was better than that in 32-34‰ salinity. The salinity modification to less than the seawater salinity level provoked a decline of the used energy owing to the attenuation of ion exchanges by gill's chloride cells. Such a condition minimized the energy demand for osmoregulation, thereby enhancing the fish growth.

Considering that size, age, stock density and feed are similar, the difference of the fish growth is undoubtedly affected by the enviromental salinities. We also noted that water quality ranged within value intervals appropriate for the culture of *T. blochii*. The importance of salinity for the fish growth is associated with changes in physiological functions. Fish cultured in high salinity (control) performed a more active transport in order to release excessive ions of Na from gill, which is a highly energy-consuming activity. Gill chloride cells are responsible for fish osmoregulation. Proliferated on the lamellae, they are extremely sensitive to external salinity. When moving to the new medium with different salinity, euryhaline fish activated chloride cells and delivered signals to central nervous system. In a culture medium with higher salinity, the proliferation of chloride cells was more intensive; conversely, they were less produced under lower salinity conditions (Bone & Moore 2008; Fujaya 2004).

The specific growth rate (SGR) was the highest in fish cultured in 15‰ and 20‰ salinity (0.87%), and the lowest in 25‰ (0.75%), 10‰ (0,67%) and 5‰ (0.62%) salinity, respectively. Retnani & Abdulgani (2013) reported a higher SGR in *T. blochii* cultured in 24‰ salinity (10.594%) than in 32-34‰ salinity (10.359%). Noticeably, the SGR of *T. blochii* farmed in brackish salinity ranged from 14‰ to 24‰.

Feed efficiency (FE) reached the highest level in fish cultured at 15‰ salinity, indicating that isosmotic condition was achieved. Therefore, energy expenditure is devoted to fish growth instead of osmoregulation. Febrianti et al (2016) argued the efficient utilization of feed must be greater than 50%. We further noted that FE of *T.*

blochii exceeded 50% across the treatments, meaning that the fish could utilize the feed efficiently in various salinities.

In respect of the survival rate (SR), the percentage recorded across the treatments ranged within 76.67-88.33%, while fish cultured at 15‰ salinity reached the highest level. It is noticeable that *T. blochii* can properly adapt to a wide range of salinities, i.e. 30‰-5‰. Additionally, Arrokhman et al (2012) reported a SR of 99.03–100% in *T. blochii* reared in 4-34‰ salinity. This suggests that the fish has the potential to be farmed in brackish water.

Histological alterations. Histological observation on the fish gills did not find anomalies in all treatments studied, as indicated by the clear appearance of the secondary lamellae, epithelium, thrombocytes and pillar cells. No abnormalities were observed in gill chloride cells, including the absence of hypertrophy and edema, despite a moderate hyperplasia (Figure 3). In this case, hyperplasia was linked to parasites in the medium. As reported by Wahyuni et al (2017), normal tissue of fish gill was characterized by obvious apprearance of secondary lamellae, pillar cells, lacunae, and thrombocytes.

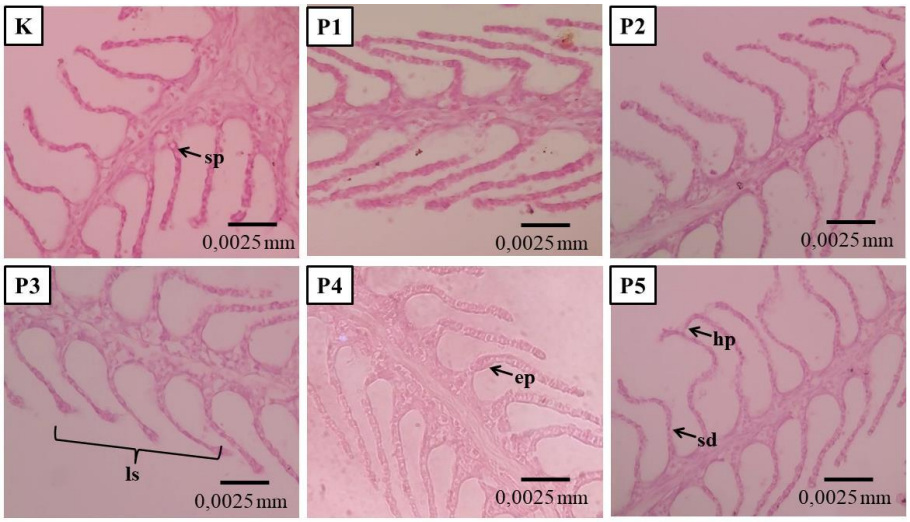


Figure 3. Structure of gill tissue of *Trachinotus blochii* cultured in different levels of salinity. K-control, P_1=25‰, P_2=20‰, P_3=15‰, P_4=10‰ and P_5=5‰, sp-pillar cells, ls-secondary lamellae, ep-epithelium, sd-thrombocyte, hp-hyperplasia.

The histological analysis on fish kidney did not found any significant abnormalities, as indicated by condition of Bowman's capsule and glomerulus. The cells were intact and not infected, but a bleeding part was observed (Figure 4). Mc Gavin & Zachary (2007) offered a description of the kidney histology comprised of main parts such as glomerulus, tubulus and blood vessels. In addition, Takashima & Hibiya (1995) reported that Bowman's capsule surrounds glomerulus as an indicator of healthy kidney. These organs perform an essential role, i.e. filtering metabolites in bloods. The excretory fluids enter the tubule, while minerals, glucose, and other fluids are re-absorbed. The number and size of glomerulus in freshwater fish were greater than those in seawater fish, considering their importance in retaining salt in body and releasing urine.

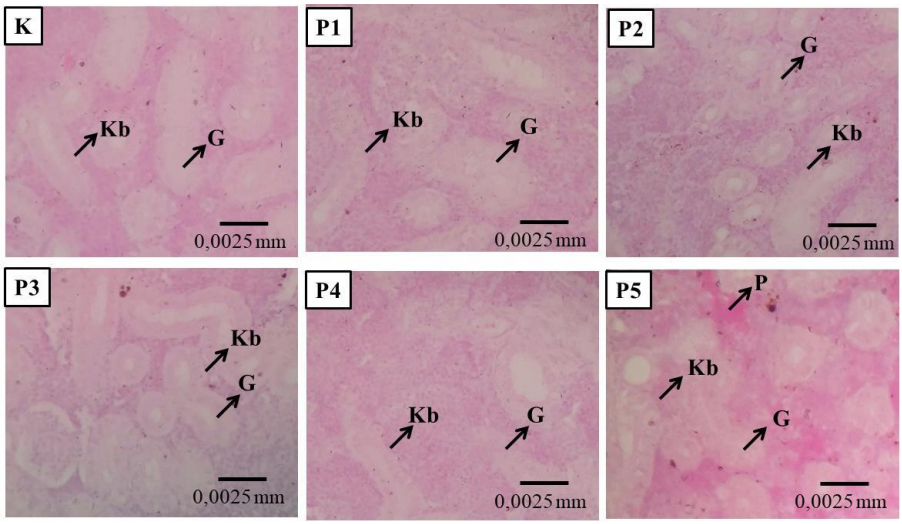


Figure 4. Structure of kidney tissue of *Trachinotus blochii* cultured in different levels of salinity. K-control, P_1=25‰, P_2=20‰, P_3=15‰, P_4=10‰ and P_5=5‰, Kb-Bowman's capsule, G-glomerulus, P-bleeding.

Conclusions.The experimental data revealed the significant effects of salinity on the osmoregulatory activities, blood cortisol level, and growth performance of *T. blochii* ($p<0.05$). The variance of salinities did not cause any difference in the structure of gill and kidney and did not show any abnormality in these organs. The treatment at 15‰ salinity exhibited the best outcome, resulting in: osmotic pressure of 3 mOsm L^{-1} H2O (closer to the isosmotic condition), blood cortisol of 50,923 nmol L^{-1}, absolute growth weight of 17.73±1.25 g, absolute growth length of 2.32±0.21 cm, specific growth rate of 0.87 ± 0.05%, feed conversion of 1.24±0.00, feed efficiency of 80.79±0.58 and survival rate of 88.33±2.88%. Parameters of water quality were at the appropriate level for *T. blochii* growing. Furthermore, the fish could exert adaptive capacities to medium salinities below the seawater salinity, the most satisfying level being achieved at 15‰ salinity, when cultured in a recirculation system.

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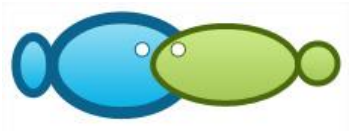

Osmotic performance rate, stress response and growth performance of silver pompano (*Trachinotus blochii*) reared in different salinities using recirculating culture system

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Abstract. Silver pompano (*Trachinotus blochii*) has received tremendous attention from the aquaculture sector, due to its favourable features, such as a high economic value, its good adaptive response, and its potential to be cultured in various salinities. The aim of this study was to discover the effects of medium salinities on osmotic performance rate, blood cortisol, and growth performance (absolute growth weight and lenght, specific growth rate SGR, feed conversion FC, feed efficiency FE, survival rate SR) of silver pompano under recirculating system. The histological alterations (kidney and gill) and water quality (temperature, pH, DO, NH_3, NO_2 and NO_3) were also observed. The 56-day experiment was carried out in Balai Perikanan Budidaya Laut (BPBL) of Batam, Indonesia. A total of 300 fish specimens (11-13 cm in length, weighing 28-29 g) were raised in a 100 L-tank containing 80 L of water at a density of 1 fish/4 L (20 fish in total). They were fed with commercial pellet (46% protein) at 3% of fish biomass and 3 times a day. The experiment was conducted according to a Completely Randomized Design with 5 levels of salinity: P1=25‰, P2=20‰, P3=15‰, P4=10 ‰ and P5=5‰, by performing triplicate measurements for each treatment. The treatment with, 15 ‰ salinity showed the best effects, yielding an osmotic performance rate of 3 mOsm L^{-1} H_2O, a blood cortisol level of 50,923 nmol L^{-1}, an absolute growth weight and length of 17.73±1.25 g and 2.32±0.21 cm, respectively, an SGR of 0.87 ± 0.05%, an FC of 1.24±0.00, an FE of 80.79±0.58 and an SR of 88.33±2.88%. Histologically, there were no anomalies in the structure of gill and kidney of the fish cultured in 5‰ - 25‰ salinities. Water quality was acceptable for growing silver pompano.

Key Words: gill, kidney, blood cortisol, histology, and *Trachinotus blochii*.

Introduction. The farming of silver pompano (*Trachinotus blochii*) has currently gained great popularity in Indonesia. The market demand for the species has continuously increased in international trade, due to its high economic value, its good adaptive response and its potential to be cultured in various water conditions. The price of the fish reaches aproximately USD. 4.25 kg^{-1} in local market, but it may reach USD. 14.144 kg^{-1} in export market (Mo 2017). Since 2015, silver pompano is considered as a promising commodity in marine fisheries sector with total production of 1,900 tons in 2015, demonstrating the annual rise of 31.5% (Prahadi 2015).

T. blochii was reported to exert a high adaptive response towards salinity changes. Survival rate of the species at various salinity levels 32‰, 24‰, 14‰, and 4‰ during the 28-day trial showed no significant difference, indicating that the fish could adapt at lower salinity over seawater, as well as confirming the possibility of fish farming diversification through culture systems in brackish water (Arrokhman et al 2012).

Salinity tolerance in fish closely relates to the osmotic pressure balance between inside and outside the fish body. Osmotic pressure inside the fish is lower than outside.

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The imbalance may cause disturbance of the physiological functions, which in turn disrupt the fish growth. However, under a normal osmotic gradient, the metabolic activity could reach an optimum rate, as indicated by a good appetite and an enhanced feed intake, allowing allocating more energy for the growth (Carrion et al 2005). Fujaya (2004) suggested that the osmotic balance was achieved through regulation of body fluid transportation, known as osmoregulation. The adjustment activities needed for balancing internal and external osmotic pressures require a high energy consumption, leading to stress generation, as indicated by the production of blood cortisol.

Important osmoregulatory organs including gills and kidney play a crucial role in the process. Defective tissues in these organs offer a sign of failure in osmoregulation. Consequently, the defect would adversely affect physiological functions, causing the decrease of feed consumption and fish growth (Putri et al 2014). In this matter, gills seem to be the most susceptible osmoregulatory organ towards environmental changes, such as physicochemical properties of water and presence of toxic compounds. The gill lamellae become the weakest part, in which presence of stressors directly induces ionic homeostasis that remarkably imparts osmoregulation. Indeed, the chronic stressors lead to destructive effects on the gill. Macroscopic and microscopic defects in the gill can serve as biomarker of fish health status (Camargo & Martinez 2007). Besides, Thophon et al (2003) also argued that kidney is a susceptible organ to the external stressors exposure, due to its essential role in maintaining homeostasis. Based on the above elaboration, it can be concluded that there is a need for investigating the osmotic response, stress level and growth performance of silver pompano reared in a recirculation system, at different salinity levels. In this work, the histological alterations in gill and kidney were also observed.

Material and Method

Study site. The experiment was carried out in the Agency for Marine Fisheries Culture (Balai Perikanan Budidaya Laut-BPPL), Batam, Indonesia, for 56 days.

A total of 225 *T. blochii* seeds (average length of 11.90-12.55 cm, weight of 24.30-28.90 g) were reared in an experimental container (capacity 100 L) filled with 80 L of water. The density referred to Indonesia National Standard (SNI 2013), which recommends 1 individual 4 L^{-1} (equal to 20 individuals 80 L^{-1}). Commercial pellet (Megami GR 2) was used, containing 46% protein, 9% fat, 1.9% crude fiber and 8% moisture. The specimens were fed at 3% of their weight, three times a day.

Preparation of the culture container. The close recirculating system was prepared. The container was filled with seawater at different salinity levels, then connected to PVC gutter (50 cm x 14 cm x 14 cm) at the upside of the chamber. The filtration unit water was transported into the culture container through a PVC pipe (2.5 cm diameter). The filtration unit was filled with 50 bioballs (each gutter), as previously prescribed by Nelvia et al (2015). The water was subsequently pumped to the filtration unit with the aid of a 50 W water pump.

Experimental design. The completely randomized design was arranged, consisting of 1 factor and 5 levels (with triplicates) as follows: P1=25‰, P2=20‰, P3=15‰, P4=10‰ and P5=5‰. The effect of salinity was studied, focusing on the osmotic response, the content of blood cortisol, the tissue histology (gills and kidney), the absolute growth weight (Wm), the growth length (Lm), the specific growth rate (SGR), the survival rate (SR) and the water quality (temperature, pH, DO, NH_3, NO_2 and NO_3).

Determination of the osmotic response. Osmotic response was evaluated at the end of experiment and determined using micro-osmometer, as explained by Cambell et al (2012). The fish sample was acclimatized for 7 days and, for the first 5 days, the fish was fed. A total of 10 fish specimens were used for each salinity level, in which they were exposed to the salinity treatments. After 7 days of exposure, the blood of living fish (1 mL) was collected from the heart, then centrifuged at 3000 rpm for 3 minutes to obtain blood plasma. The plasma osmotic pressure was measured using a micro-osmometer.

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The osmoregulatory capacity refers to the difference between osmotic pressure of the medium and internal osmotic pressure of the fish blood plasma. The calculation was described as follows (Anggoro & Nakamura 1996):

$$TKO = [POsmoBlood - POsmoMedium]$$

Where:
TKO - osmotic response (mOsm L^{-1} H2O);
POsmoBlood - biota osmolarity (mOsm L^{-1} H2O);
POsmoMedium - medium osmolarity, and the bracket [ ] means absolute value.

Blood cortisol analysis. The analysis was performed at 3 periods: day 1, day 28 and day 56 of the experiment, using enzyme-linked immunosorbent assay (ELISA). Before the blood withdrawal, the fish was anaesthetized using phenoxyethanol at dose of 0.3 mL L^{-1} water (Rigal et al 2008). Briefly, blood was collected via the vena caudalis, using a heparinized syringe (1 mL), then centrifuged to collect plasma. Plasma cortisol was quantified using RIA (radio immuno–assay) Cortisol (125I) RIA KIT IZOTOP (Ramsay et al 2006). In this regard, blood plasma (0.3 mL) was frozen at -20–°C. To maintain the hormone in the plasma, the sample was packed within a cool-box containing dry ice exactly at day 57 of experiment, then immediately transported into laboratory for analysis.

Histological analysis. Gill and kidney tissue of the sample was collected at the first and last days of the experiment, from the fish specimens exposed to a salinity of 30 ppt (natural habitat condition). Gill cover (overculum) was lifted and the base was cut off to release the gill. Afterwards, the kidney was obtained by opening the abdominal cavity. The phosphate-buffered formalin (NBF) at 10% was used for organ fixation, carried out for 24-48 h (Raškoviæ et al 2011). After fixation, the tissues were dehydrated in graded series of alcohol and xylol, then embedded in paraffin. All these stages were conducted using tissue processor. The sections obtained were cut at thickness of 3-5 µm, then incubated in a water bath at 40 °C. Object glass was placed in the water bath, then immediately air-dried for 1 h. Afterwards, it was stained using haematoxylin-eosin (HE), and observed under light microscope at magnification of 400×.

Growth performance. The weight and length of fish were recorded each 14 days. Growth performance included following parameters:
a. Weight gain (g) = final weight – initial weight;
b. Absolute growth length (cm) = average final length (cm) – average initial length (cm);
c. Specific growth rate (SGR) (%) = (Ln mean final fish weight – Ln mean initial fish weight)/ culture period (day) × 100 %;
d. Feed efficiency (FE) (%) = increased fish mass/total feed consumed;
e. Survival rate (%) = (final number of fish/initial number of fish) × 100 %.

Water quality. Temperature was daily observed– using a thermometer, while chemical indicators were checked each 14 days, including pH (using pH meter), DO (using DO meter), NH_3, NO_2 and NO_3 (using a spectrophotometer).

Results and Discussion

Osmotic performance rate. Osmotic response of fish to the variation of salinity was shown in Figure 1, indicating that salinity caused a remarkable effect on medium osmolarity, blood plasma, and osmotic performance rate of silver pompano ($p<0.05$). In this case, the lowest rate (3 mOsm L^{-1} H_2O) was found at P3 (15–‰), while the highest one (87 mOsm L^{-1} H_2O) was attributed to P5 (5‰). This clearly imparts the effect of salinity on medium and plasma osmolarity, leading to noticeable changes in osmotic performance rate of silver pompano seeds. The extreme difference between medium

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osmolarity and internal fish osmolarity could cause significant changes in fish behaviors and physiological conditions, consequently altering the feed consumption rate and fish growth.

In water of salinity (15–‰) near the isosmotic condition, the fish allocated more energy for enhancing their growth. Energy metabolism required for osmoregulation in fish is fundamentally associated with osmotic performance rate as a rapid response towards changes in medium osmolarity. In this case, osmotic performance rate shows linear correlation to energy consumption for osmoregulatory activities. On the contrary, hypoosmotic (salinity of 30‰, 25‰ and 20‰) and hyperosmotic (10‰ and 5‰) condition leads to increment of osmotic response, which needs higher energy requirement for osmoregulation (Carrion et al 2005). Arjona et al (2009) reported that there was clear evidence that higher osmotic response led to higher energy use for osmoregulation. Our data demonstrated a variety of osmotic response with progression of salinity. As mentioned by Putri et al (2014), medium that possesses higher salinity (far away from isosmotic condition) would increase osmotic performance rate of the fish. At the condition in which the medium condition is tolerable, osmotic performance rate tended to attenuate, thus allowing energy use for fish growth.

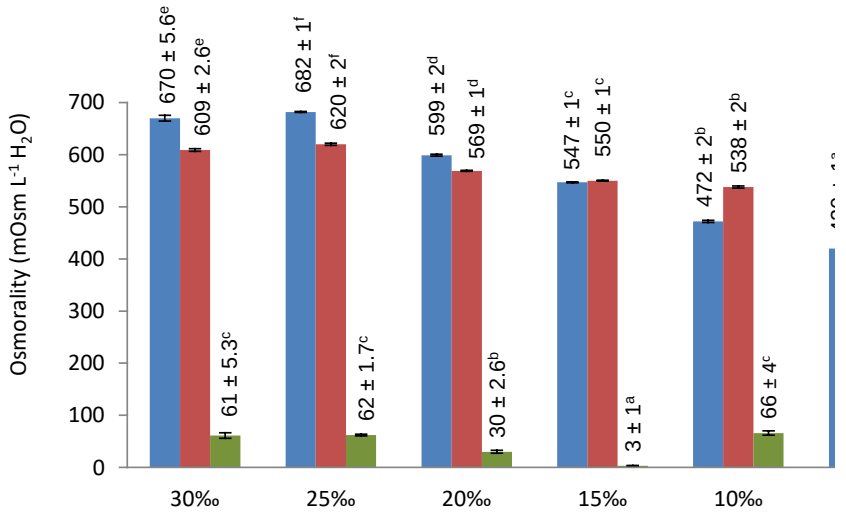

Figure 1. Average osmolarity of rearing medium and blood plasma, and osmotic performance rate of silver pompano cultured in various salinity levels. Different superscripts above the bar showed significant difference at $p<0.05$.

This present work reveals that *T. blochii* is able to adapt to the medium salinity at 15 ppt, suggesting that the juvenile (average length of 12.43-12.48 cm, weight 28.00-28.70 g and age 2 months) can tolerate the condition. Additionally, Retnani & Abdulgani (2013) argued that such adaptive capability of fish relied on size and growth stage, in which osmoregulatory activity of fish may differ depending on age. Lantu (2010) stated that osmoregulation of euryhaline fish strongly related to osmo-sensitivity of chloride cells. They serve as receptor, mainly responding to the salinity level of the medium. When immersed in water media with different salinity, chloride cells in euryhaline fish transmit signals to central nervous system, primarily to the pituitary gland responsible for controlling secretion of growth hormone. Subsequently, the hormone regulates the development of chloride cells in osmoregulatory organs such as gills, kidney and digestive tract. Thus, the amount of chloride cells is adjusted, causing changes in the physiological mechanisms of secretion or absorbtion of ions by chloride cells. At a higher

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salinity, chloride cells would have a higher rate of proliferation, and vice-versa. Over the long term, this controlling mechanism may also cause genetic expression.

Syakirin et al (2018) stated that a higher concentration of ions in water would rise salinity level and osmolar density. For instance, the hybrid grouper is a kind of marine fish that has blood osmolarity (internal osmotic fluid pressure) lower than the environmental osmotic pressure. Therefore, the water will pass from the body of the fish to the environment by the osmotic process through the kidney, gill, as well as in the body. Salinity demonstrates a relationship with the osmoregulation of aquatic animals. A sudden fluctuation of salinity makes the body osmoregulation difficult and induces the animal mortality. The osmoregulation capacity is is determined by the difference between the blood osmotic pressure (fish) and the media osmotic pressure. Osmoregulation relates to the difference of fish blood osmolarity and media osmolarity, known as the osmotic work level: the response to the salinity change increases with this difference.

Stress level in *T. blochii*. The increment of blood cortisol in fish indicates stressful condition. Our data suggested that blood cortisol in all treated samples declined at different extent during the experiment pogression, indicating that the fish exhibited adaptive capacities to lower salinity (Figure 2).

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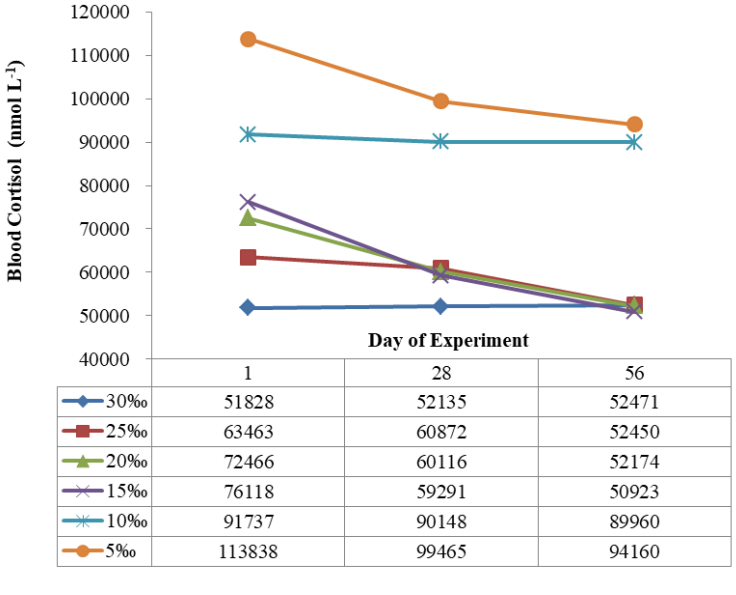

	1	28	56
30‰	51828	52135	52471
25‰	63463	60872	52450
20‰	72466	60116	52174
15‰	76118	59291	50923
10‰	91737	90148	89960
5‰	113838	99465	94160

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Day if experiment

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Figure 2. Concentration of blood cortisol in *Trachinotus blochii* cultured in various levels of salinity.

As depicted in Figure 2, the concentration of blood cortisol on day 1 increased as the level of salinity was lower compared to the control (30‰). This clearly showed a stress response to the new environment. Setiyoningsih (2014) argued that stress in fish existed as rapid response towards enviromental pressure, thus they secreted glucocorticoid (cortisol) and catecholamine hormone to cope with the stress condition.

Furthermore, cortisol level declined consistently from day 28 to day 56 in all treatments, indicating adaptive capacities. On day 56, the lowest cortisol level (50,923 nmol L^{-1}) was found at 15‰, while the highest one (94,160 nmol L^{-1}) was found at 5‰. In salinity 15‰, the fish showed the best osmoregulatory activity through balancing

osmotic pressures. Pamungkas (2012) argued that osmoregulation in fish was modulated by two hormones, namely prolactin and cortisol. Cortisol is a crucial hormone in euryhaline fish since it modulates excretion of ions via gills able to stimulate chloride cells; thus, when migrating, the concentration of plasma cortisol increases. In addition, Scabra (2018) found the depletion of blood volume, leukocyte, and liver glycogen in stressed fish, but the concentration of cortisol increased. Stress condition results mainly from external changes such as salinity, and during this stressful period, fish activate homeostatic processes by accelerating their metabolic activities, leading to the rise of oxygen intake.

At the end of experiment, the concentration of cortisol in 15‰, 20‰ and 25‰ salinity reached a level close to the value measured for the control salinity (30‰). This represents the successful attempt of the fish to reach stability. Hastuti et al (2004) reported that concentrations of cortisol in plasma of normal fish ranged between 569.73–1,468.34 nmol L^{-1}.

Water quality. Table 1 presents the parameters of water quality, including temperature, dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). The results showed that these parameters tended to be similar in all salinity levels, within the following ranges: temperature 27-29°C, pH 5.9-7.9, DO 5.9-10.9 mg L^{-1}, NH_3 0.01–0.131 mg L^{-1}, NO_2 0.050–0.090, and NO_3 0.190–0.890. It is noticeable that these values correspond to a set of good conditions for growth and survival rate of silver pompano. As discussed by Sitta & Hermawan (2011), the optimum condition for silver pompano included temperature 28-32°C, pH 6.8-8.4, while DO ranged 4.8 – 5 mg L^{-1} in aquarium and ±7.3 mg L^{-1} in floating net cage. Boyd (2015) found that ammonia at level of 0.2–-2.0 mg L^{-1} could be detrimental to fish, while nitrite was acceptable for fish at a concentration < 1mg/L, unsafe at 1-5 mg L^{-1}, and poisonous at 16 mg L^{-1} (Siikavuopio & Saether 2006).

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Table 1

Mean water quality parameters during the research period

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Parameters	*Unit*	*Salinity*				
		25‰	*20‰*	*15‰*	*10‰*	*5‰*
Temperature	°C	27.3-28.8	27.4-28.6	27.8-29.1	27.3-28.6	27.5-28.6
pH	-	7.4-7.9	7.0-7.9	6.9-7.9	6.1-7.9	5.9-7.8
DO	mg/L	5.9-6.6	8.8-10.9	5.9-6.8	5.9-7.03	6-7.1
NH_3	mg/L	<0.01-0.105	<0.01-0.101	<0.01-0.099	0.010-0.109	0.060-0.131
NO_2	mg/L	0.050-0.070	0.050-0.059	0.050-0.059	0.060-0.071	0.060-0.090
NO_3	mg/L	0.340-0.750	0.360-0.780	0.360-0.890	0.190-0.570	0.206-0.345

During the experiment, water quality was maintained at a large extent to ensure acceptable parameters for fish growth. Water filtration was installed in the culture system, comprising of physical (synthetic cotton), chemical (zeolite and active carbon), and biological (bioball) filter. Cotton filter served to capture uneaten feed and feces, while zeolite and active carbon enabled the absorption of toxic compounds such as ammonia and nitrite (Supriyono et al 2007). Bioball is important as attachment site for nitrifying bacteria capable of converting nitrogen into unharmful form, i.e. nitrate (Dewi & Masithoh 2013). Nurhidayat et al (2012) also augmented that the combination of zeolite, ative carbon, and bioball showed satisfying results of maintaining water quality through oxidation of ammonia and enrichment of non-pathogenic nitrifying bacteria.

Growth performance and survival rate. Salinity demonstrated significant impacts to absolute growth weight, absolute growth length, SGR, feed conversion, FE, and SR of silver pompano ($p<0.05$). Statistical test of Newman-Keuls revealed that two salinity levels, i.e. 5‰ and 10‰, did not result in any significant difference in some parameters including absolute growth weight and growth length, SGR, feed conversion, and FE. Meanwhile, SR tended to be similar between treatments (Table 2).

Table 2

Growth performance of silver pompano reared in different salinity levels

Parameters	*Salinity*				
	25‰	*20‰*	*15‰*	*10‰*	*5‰*
Absolute growth weight (g)	14.03±1.18^{ab}	15.87±1.05^{bc}	17.73±1.25^{c}	12.73±1.70^{a}	11.93±1.66^{a}
Absolute growth length (cm)	1.93±0.34	1.82±0.14	2.32±0.21	2.09±0.23	1.84±0.07
Specific growth rate (%)	0.75±0.08^{ab}	0.87±0.04^{b}	0.87±0.05^{b}	0.67±0.09^{a}	0.62±0.07^{a}
Feed conversion	1.31±0.01^{a}	1.26±0.01^{a}	1.24±0.00^{b}	1.33±0.01^{c}	1.34±0.01^{d}
Feed efficiency (%)	76.62±0.8^{a}	79.07±1.03^{a}	80.79±0.58^{b}	75.22±0.76^{c}	74.37±0.28^{a}
Survival rate (%)	81.67±2.88^{ab}	83.33±2.88^{ab}	88.33±2.88^{b}	83.33±2.88^{ab}	76.67±2.88^{a}

Means with different superscripts (a, b, c and d) were significantly different while ab and bc were not significantly different ($p < 0.05$).

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As presented in Table 2, 15‰ salinity demonstrated the most satisfying effects on growth of silver pompano, meaning that the fish could perform proper feed utilization and osmoregulation. Wulandari (2006) reported that optimum energy use could be achieved at osmotic condition; thus, more energy was used for their growth instead of osmoregulatory activities.

Retnani & Abdulgani (2013) reported that growth of silver pompano cultured in 4-24‰ salinity was better than that in 32-34‰ salinity. The salinity modification to less than the seawater salinity level provoked a decline of the used energy owing to the attenuation of ion exchanges by gill's chloride cells. Such a condition minimized the energy demand for osmoregulation, thereby enhancing the fish growth.

Considering that size, age, stock density and feed are similar, the difference of the fish growth is undoubtedly affected by the enviromental salinities. We also noted that water quality ranged within value intervals appropriate for the culture of silver pompano. The importance of salinity for the fish growth is associated with changes in physiological functions. Fish cultured in high salinity (control) performed a more active transport in order to release excessive ions of Na from gill, which is a highly energy-consuming activity. Gill chloride cells are responsible for fish osmoregulation. Proliferated on the lamellae, they are extremely sensitive to external salinity. When moving to the new medium with different salinity, euryhaline fish activated chloride cells, and delivered signals to central nervous system. In a culture medium with higher salinity, the proliferation of chloride cells was more intensive; conversely, they were less produced under lower salinity conditions (Bone & Moore 2008; Fujaya 2004).

The specific growth rate (SGR) was the highest in fish cultured in 15‰ and 20‰ salinity (0.87%), and the lowest in 25‰ (0.75%), 10‰ (0,67%) and 5‰ (0.62%)

salinity, respectively. Retnani & Abdulgani (2013) reported a higher SGR in silver pompano cultured in 24‰ salinity (10.594%) than in 32-34‰ salinity (10.359%). Noticeably, the SGR of silver pompano farmed in brackish salinity ranged from 14‰ to 24‰.

Feed efficiency (FE) reached the highest level in fish cultured at 15‰ salinity, indicating that isosmotic condition was achieved. Therefore, energy expenditure is devoted to fish growth instead of osmoregulation. Febrianti et al (2016) argued the efficient utilization of feed must be greater than 50%. We further noted that FE of silver pompano exceeded 50% across the treatments, meaning that the fish could utilized the feed efficiently in various salinities.

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In respect of the survival rate (SR), the percentage recorded across the treatments ranged within 76.67-88.33%, while fish cultured at 15‰ salinity reached the highest level. It is noticeable that silver pompano can properly adapt to a wide range of salinities, i.e. 30‰-5‰. Additionally, Arrokhman et al (2012) reported a SR of 99.03–100% in silver pompano reared in 4-34‰ salinity. This suggests that the fish has the potential to be farmed in brackish water.

Histological alterations. Histological observation on the fish gills did not find anomalies in all treatments studied, as indicated by the clear appearance of the secondary lamellae, epithelium, thrombocytes and pillar cells. No abnormalities were observed in gill chloride cells, including the absence of hypertrophy and edema, despite a moderate hyperplasia (Figure 3). In this case, hyperplasia was linked to parasites in the medium. As reported by Wahyuni et al (2017), normal tissue of fish gill was characterized by obvious appearance of secondary lamellae, pillar cells, lacunae, and thrombocytes.

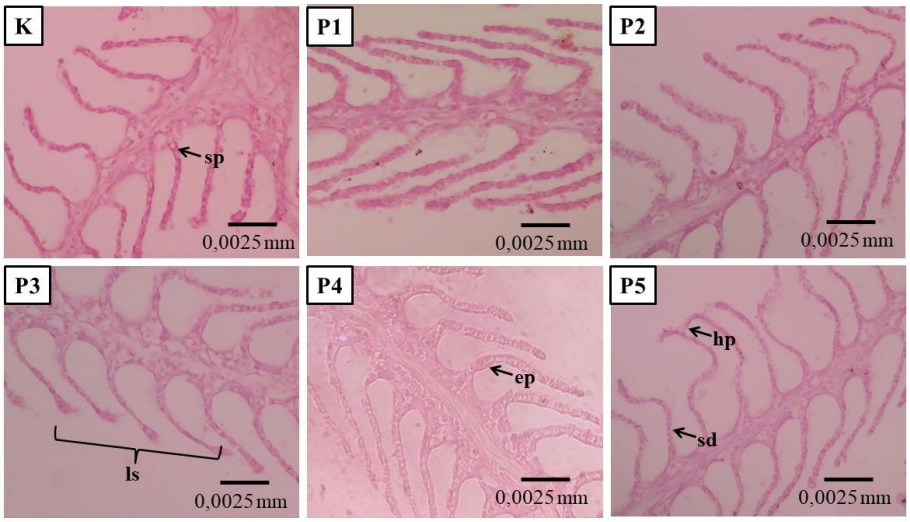

Figure 3. Structure of gill tissue of silver pompano cultured in different levels of salinity. K=control, P_1=25‰, P_2=20‰, P_3=15‰, P_4=10‰ and P_5=5‰, sp=pillar cells, ls= secondary lamellae, ep=epithelium, sd=thrombocyte, hp=hyperplasia.

The histological analysis on fish kidney did not found any significant abnormalities, as indicated by condition of Bowman's capsule and glomerulus. The cells were intact and not infected, but a bleeding part was observed (Figure 4). Mc Gavin & Zachary (2007) offered a description of the kidney histology comprised of main parts such as glomerulus, tubulus and blood vessels. In addition, Takashima & Hibiya (1995) reported that Bowman's capsule surrounds glomerulus as an indicator of healthy kidney. These organs perform an essential role, i.e. filtering metabolites in bloods. The excretory fluids enter the tubule,

while minerals, glucose, and other fluids are re-absorbed. The number and size of glomerulus in freshwater fish were greater than those in seawater fish, considering their importance in retaining salt in body and releasing urine.

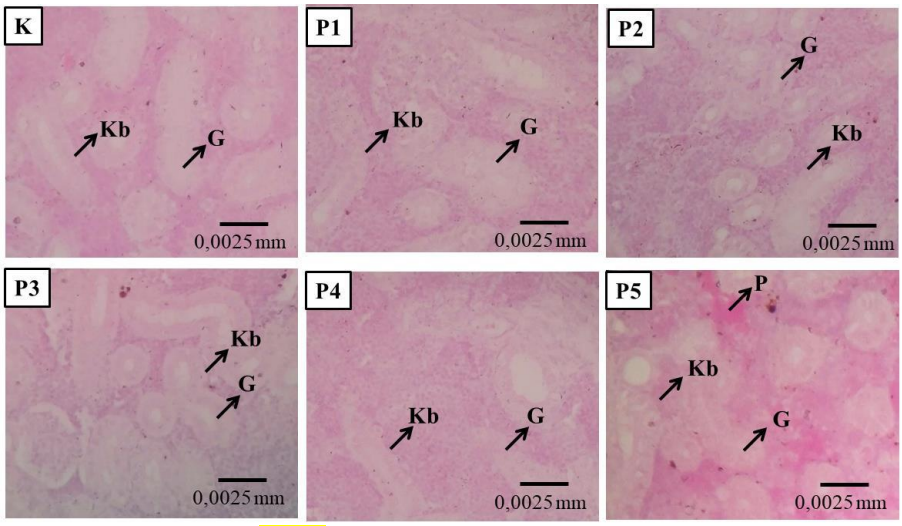

Figure 4. Structure of kidney tissue of silver pompano cultured in different levels of salinity. K=control, P_1=25‰, P_2=20‰, P_3=15‰, P_4=10‰ and P_5=5‰, Kb=Bowman's capsule, G=glomerulus, P=bleeding.

Conclusions.The experimental data revealed the significant effects of salinity on the osmoregulatory activities, blood cortisol level, and growth performance of silver pompano ($p<0.05$). The variance of salinities did not cause any difference in the structure of gill and kidney and did not show any abnormality in these organs. The treatment at 15‰ salinity exhibited the best outcome, resulting in: osmotic pressure of 3 mOsm L^{-1} H2O (closer to the isosmotic condition), blood cortisol of 50,923 nmol L^{-1}, absolute growth weight of 17.73±1.25 g, absolute growth length of 2.32±0.21 cm, specific growth rate of 0.87 ± 0.05%, feed conversion of 1.24±0.00, feed efficiency of 80.79±0.58 and survival rate of 88.33±2.88%. Parameters of water quality were at the appropriate level for silver pompano growing. Furthermore, the fish could exert adaptive capacities to medium salinities below the seawater salinity, the most satisfying level being achieved at 15‰ salinity, when cultured in a recirculation system.

Acknowledgements. Authors would like to acknowledge Balai Perikanan Budidaya Laut (BPBL) of Batam for providing research facilities. We also would like to thank Lembaga Penelitian dan Pengabdian Masyarakat (LPPM), University of Riau for research funding, as well as Razuli, Eva Selvia, Hidayati and Fitriulan for technical assistance.

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