

Female Tilapia Strategising Energy Mobilisation Differently For Growth Or Reproduction Depend On Living Environments

Ros Suhaida Razali

Universiti Malaysia Terengganu

Sharifah Rahmah

Universiti Malaysia Terengganu

Mazlan Abd Ghaffar

Universiti Malaysia Terengganu

Leong-Seng Lim

Universiti Malaysia Sabah

Thumronk Amomsakun

Prince of Songkla University

Hua Thai Nhan

Can Tho University

Yu Mei Chang

Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences

Li Qun Liang

Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences

Young-Mao Chen

National Taiwan Ocean University

HON JUNG LIEW (✉ honjung@umt.edu.my)

Universiti Malaysia Terengganu <https://orcid.org/0000-0002-7002-8338>

Research Article

Keywords: energy, growth, feeding, ionoregulation, physiology, tilapia

Posted Date: August 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-746351/v1>

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Abstract

This study was conducted to investigate the energy mobilization and ionoregulation pattern of tilapia living recirculating aquaculture system (RAS) and cage culture environments. Three different groups of tilapia were compared as tilapia cultured in RAS (Group I - RAS), tilapia cultured in open water cage (Group II - Cage) and tilapia transferred from cage to RAS (Group III - Compensation) as physiology compromising model. Results revealed that Group II tilapia mobilized glycogen as primarily energy for daily exercise activity and promoted growth, whilst tilapia from Group I and III mobilised lipid to support gonadogenesis and protein reserved for somatogenesis. The gills and kidney NKA activities remained relative stable to maintain balance homeostasis with a electrolytes level. As a remark, this study revealed that tilapia re-strategized their energy mobilization pattern in accessing glycogen as easy energy to support exercise metabolism and mobilized lipid and protein for growth and gonadal development.

Highlights

- i. Tilapia mobilised muscle glycogen to support exercise metabolism, prioritized lipid for gonadogenesis and protein for somatogenesis.
- ii. Tilapia mobilized energy pattern differently to accommodate changing environments.
- iii. NKA activities in both gills and kidney important to maintain balance homeostasis.

Introduction

Tilapia from the genus *Oreochromis* is the second most farmed species after cyprinid that accounted for more than 6.93 million tonnes in 2020 and expected to continue rising with the increasing global population (FAO, 2020; Zeng et al., 2021). In 2021, the market of tilapia had continuously survive even under Covid-19 pandemic and expected to continue gains steady to reach 7.3 million (FAO, 2021a). Popularity of tilapia farming crossing more than 140 countries with global production market values recorded at USD12 billion in 2018 and expected to reach USD25 billion by 2028 (FAO, 2018).

Tilapia as a hardy fish which can be farmed from extensive to super intensive production systems from conventional pond to cage culture systems or either partial or complete recirculating tank system (Kabir et al., 2019). However, high density farming leads to stress in competition for growth which may induce stress related immune inhibition and increase disease susceptibility (Munangandu et al., 2016). However, production lost that worth about USD10 billion that accounted 40% in tilapia farming is predicted by year 2028, if production line continuous expose to environment stresses, diseases and quality stock (Owens, 2012; Joshi et al., 2021). Some reports highlighted that open water cage culture of tilapia exhibited slow growth and mortality reaching 60–70% occurred yearly especially during the dry season (Siti-Zahrah et al., 2008). The causes are unclear and require convincing evidence to elucidate the issue thoroughly. In Malaysia, Como River – Kenyir Lake located at Terengganu state of Malaysia is categorised for open cage farming. However, many farmers experience production lost and slow growth, which resulted in abandonment of their farm operation (Siti-Zahrah et al., 2008). By putting disease infection apart, the

losses of the production could be related to physiological stress affected by living environment. One of the possibility may be due to extra energy expenses to cope with the living condition and maintain the homeostasis of active ion uptake. Active swimming is known require extra energy expenses to support exercising metabolism (Liew et al., 2012; Liew et al., 2013). In addition, maintaining active ionoregulation is crucial to retain and remain ionic stability in the body for all biology processes. Performing active ionoregulation is an energetically expensive process that require about 1–20% of the total ATP demand (Evan and Claiborne 2008; Moyson et al 2015). Thereby, prioritizing energy metabolism is believe reserve for exercise and ionoregulation that be promote somatogenesis in tilapia cultured in cage system (Waldrop et al., 2018; Inoue et al., 2019).

However, due to Covid-19 pandemic with restriction in operation under Movement Control Order (MCO) in many countries (Waiho et al., 2020), domestic food supply and chain become important to ensure nation food security during pandemic situation. As tilapia is one of the most popular choice in Malaysia markets. Thus, micro tilapia farming operation have restarted by using abandoned backyard earth ponds to support local community needs and contribute to household income during this pandemic period. Nevertheless, a lot of these micro tilapia farmers complaining that the growth rate of tilapia cultured in pond was not comparable to cage culture tilapia. With this question raised up by local farmers, we speculated that tilapia that cultured in pond system tend to spend energy for secondary maturation and reproduction. As tilapia is a mouthbrooder species that reached sexually maturation at size of about 100 g body weight (Specker and Kishida, 2000). The male tilapia started establish territory site digging, cleaning and guarding his territory to attract female tilapia. Once spawning process take place, female tilapia immediately collect fertilized eggs and incubate in her mouth for two weeks (FAO, 2021b). Thereby, our first hypothesis assume that tilapia tend to priority their energy for reproduction especially female tilapia which slow growth is expected. Whereas, tilapia that cultured in cage system spent their energy for exercise and growth, because of cage condition not suitable for reproduction. Secondly, maintaining homeostasis balancing is important in order to order to perform all basal metabolism needs. As compared tilapia that cultured in open cage with recirculating aquaculture system (RAS), tilapia in cage culture would have to maintain high level of ionoregulatory activity such as sodium pump (NKA activity) to maintain essential ion. While, high NKA activity also expected in RAS cultured tilapia which believed in associate with ammonia excretion. Increase ammonia excretion efficiency concurrently induces an increase NKA activity also been reported previously on other species such as common carp (Liew et al., 2012), rainbow trout (Sinha et al., 2013), climbing perch (Chew et al., 2014).

With this hypothesis as background, the objective of this study aimed to investigate the energy mobilisation pattern and ionoregulation of tilapia cultured in different living conditions. The status of energy expenses and ionoregulation of freshwater teleost in the open water cage culture still remains to be investigated. Therefore, with special interest on the metabolic adjustments that include energy mobilization and ionoregulation pattern were pursued on the tilapia farmed at open water cage culture at Como River, Kenyir Lake (Group II - Cage) in compared with tilapia cultured in recirculating aquaculture system (Group I - RAS). In terms of energy mobilisation, we hypothesized that lipid was the priority energy being mobilized followed by glycogen and protein with greater ionoregulatory activities under this

circumstances. In addition, we also examined their physiological remodelling strategy of tilapia by transferring the tilapia from the open water cage of Kenyir Lake back to indoor recirculating aquaculture system (RAS) and cultured for 4 weeks (Group III - Compensation) to compare the energy and ionoregulation patterns.

Materials And Methods

Source of specimens and management

Hybrid red tilapia *Oreochromis* sp. was used in this study, the experimental tilapia were divided into three different groups. Group I - RAS referred to the hybrid red tilapia that were cultured in enclosed recirculating aquaculture system (RAS) for two month at the hatchery facility in AKUATROP, UMT. Group II - Cage referred to the hybrid red tilapia that were cultured in the crystal clear open water floating cages (5°02'22.1"N 102°50'41.1"E) at Como River, Kenyir Lake, Terengganu. Fish from Group II were harvested after two months period of cultivation. Group III – Compensation referred to the hybrid red tilapia that were cultured at the crystal clear open water floating cages, which were then transferred back to the hatchery AKUATROP hatchery and cultured in RAS facility for two months. Fish were fed 3% of body weight (BW) with commercial tilapia pellet (TP-2 Star-Feed®) (28% protein and 3% crude fat) twice daily at 8:30h and 16:30h. During cultivation period for all groups, water pH was maintained at 6.5–7.5, temperature at 26.5–28.5°C and dissolved oxygen at 5.2–6.8 mg/L. A volume of 50 ml water samples were collected from all groups for ionic measurement.

Experiment series I – physiological compensatory strategy comparison of hybrid red tilapia living in Group I - RAS and Group II - Cage

In order to compare the physiological responses of hybrid red tilapia living in different conditions, both groups of tilapia and water samples were analysed. For Group I (RAS) – 20 hybrid red tilapia that have been cultured for 2 months were sampled directly from RAS at a body weight (BW) of 167.69 ± 4.35 g and body length (BL) of 21.12 ± 0.20 cm. For Group I, 20 tilapia were introduced randomly into a rounded fiberglass tanks at a capacity of 2000 L equipped with an external filtration system at a capacity of 1 tonne in volume. Filter system consisted of 5 compartments with particle settlement as the first unit followed by sponges and bio-rings units to maintain good water quality level. Throughout culturing period, only settlement and sponges units were cleaned weekly to remove excessive wastes. Water in the system were refreshed weekly at 40% and monitored by using YSI multiple meter (YSI-556 MPS).

For Group II Cage from Como River cage culture - a total of 20 hybrid red tilapia were sampled from crystal clear open water cage culture at an average BW of 180.11 ± 8.79 g and BL of 20.52 ± 0.31 cm. Fish were sampled randomly from different cages and dissected immediately to sample gills, muscle tissue, liver, kidney and gonad (if available). During sampling, *in-situ* physical water parameters both inside and outside the cages were measured using YSI multiple meter (YSI-556 MPS).

Experiment series II – physiological compensation strategy of hybrid red tilapia

For Group III – Compensation, a total of 80 hybrid red tilapia from crystal clear open water cages culture system were transferred back to the hatchery and cultured under RAS facility. All fishes were distributed randomly into 4 RAS units (similar system design as Hatchery RAS group) with stocking density of 20 fishes per tank and cultured for 4 weeks (compensation recovery strategy). Weekly, 10 fishes were sampled randomly from the system to examine their physiological strategy to compare their physiological parameters. During cultivation, similar feeding schedule and feed were applied for compensation recovery strategy group. Water in the system were refreshed weekly at 40% and monitored by using YSI multiple meter (YSI-556 MPS).

Sampling procedures

At every sampling time point intervals, a total of 10 female fishes were collected randomly for biometric characteristics measurement and tissue collection for biochemistry analysis. During sampling process, all selected fishes were anesthetized with clove oil at 10 mg/L (Thalid et al., 2021). The clove oil was first mixed with ethanol to make a stock solution at a ratio of 1:10 (clove oil:ethanol) before use in order to assist emulsification. After fish showed passive operculum movement and loss of equilibrium, they were immediately removed and blotted for biometric measurement followed by blood sampling. Blood were drawn via caudal peduncle using 1 ml heparinized syringe and carefully expelled into heparinized 1.5 ml bullet tube. Samples were immediately centrifuged at 5,000 g under 4°C for 30 sec. Thereafter, plasma samples were transferred into another 1.5 ml bullet tube and immediately frozen in liquid nitrogen (N₂). In order to collect other tissues, fish were euthanized with a sharp blow to the head. Thereafter, gills, liver, kidney, muscle tissues and gonad (if available) were excised quickly. Wet liver mass was measured and all other tissues were wrapped in aluminium foil individually. All samples were frozen in liquid nitrogen immediately and stored in -80°C freezer until analysis. Both liver and muscle tissues were used for bioenergy analysis, while gills and kidney samples were used for enzymatic electrolytes ATPase transporters analysis. Biometric measurement were used to calculate condition factor, whereas liver mass and gonad volume were used to calculate hepatosomatic index (HSI) and gonadosomatic index (GSI). Condition factor was calculated as $K\text{-factor} = \frac{1}{4} 100 \times (W/L^3)$, where, W is the body weight of fish (g) and L³ is the total length (cm). Whereas, $HSI = 100 \times (Lw/BW)$, where Lw is weight of the liver mass (g) and BW is body weight (g). GSI was calculated as $GSI = 100 \times (Gw/BW)$, where Gw is the weight of ovary (g) and BW is the body weight (g). In this study, GSI was measured only for female tilapia, as female is the parent performing mouthbrooding incubation with no food intake during this period.

Tissue metabolites

For bioenergy analysis, both liver and muscle tissues weighed about 2g was homogenized using handheld homogenizer under ice chilled condition (Liew et al., 2015). Homogenization was performed at 5x folds dilution factor with ultrapure water (Milli-Q grade). Thereafter, total bioenergy of the liver and

muscle tissue were analysed for lipid, protein and glycogen contents. Lipid was extracted by methanol-chloroform and measured with a tripalmitin standard reference (Bligh and Dyer, 1959). Protein measurement was performed by following Bradford method (Bradford, 1976) and using bovine serum albumin as standard reference. While, glycogen content was measured using Anthron method with glycogen standard as reference (Roe and Dailey, 1966).

Plasma osmolarity and electrolytes

Plasma osmolality levels were measured using Osmometer (Advanced Instrument Inc. – Model 3320) with unit expressed as mOsm/l. Plasma electrolytes such as Na^+ , K^+ , Cl^- , Ca^{2+} and Mg^{2+} were measured using Ion Chromatography Analyzer (Metrohm 81 Compact IC Plus - Model 883) with unit expressed as mmol/L.

Gills and kidney enzymatic Na^+/K^+ ATPase activity

Gills and kidney Na^+/K^+ ATPase (NKA) activity was measured according to the method described by McCormick (1993) and Liew et al. (2015). A total of 8 samples from each gills and kidney samples were randomly selected for electrolytes enzymatic ATPase activity analysis. Selected samples were homogenized with the mixture of ice cooled neutralized SEI/SEID buffer solution (SEI – 150 mM sucrose; 10 mM EDTA; 50 mM imidazole solution / SEI with 0.1% sodium deoxycholate solution) with buffer solution pH 7.5 at ratio of 4:1. Thereafter, samples were centrifuged for 1 min at 5,000 g under 4°C to obtain enzyme supernatant. During enzymatic measurement, duplication of 10 μl supernatant samples were pipetted and carefully transferred into 96-wells microplate in two series. A freshly made 200 μl mixture cocktail assay solution A (400 U lactate dehydrogenase; 500 U pyruvate kinase; 2.8 mM phosphoenolpyruvate; 0.7 mM ATP; 0.22 mM NADH; 50 mM imidazole) were added into the first series supernatant and 200 μl mixture cocktail assay solution B (mixture assay A with 0.4 mM ouabain) were added into the second series supernatant on the microplate. The NKA activity were measured kinetically by using spectrophotometer (MultiskanTM FC microplate photometer, ThermoFisher ScientificTM) read at 340 nm for 10 min with 15 s intervals. Adenosine diphosphate (ADP) was used as standard reference. NKA activity were calculated by subtracting the oxidation rate of NADH in the presence of ouabain from the oxidation rate to the NAD in the absence of ouabain. The crude homogenate protein was determined by using bovine serum albumin (US Biochemical, Cleveland, OH, USA) as standard reference and read at 430 nm according to Bradford (1976). The NKA activity unit was expressed as $\mu\text{mol ATP/h/mg protein}$ (Liew et al., 2020).

Statistical analysis

The results of growth indication, plasma osmolality, electrolytes, bioenergy and NKA ATPase activities were presented as mean \pm standard error mean (SEM) ($n = 10$). Prior to significance analysis, all data were checked for normality distribution by using Shapiro-Wilk test and homogeneity of variance by using Levene test. In case of failure to fulfil normality and homogeneity requirement, data were either log or arcsine square root transformed prior further analysis. Data collected from experiment series I and II (Group I-RAS, Group II-Cage and Group III-Compensation) on weekly progress were compared by using

one-way analysis of variance (ANOVA). Tukey HSD post-hoc test was performed to identify significant differences among experimental series groups set at 95% confident limit at $p < 0.05$.

Results

Biometric indication

In term of growth performance in term of BW and BL, no obvious differences was noticed (Table 2). Highest BW was recorded in Group II at $212.11 \pm 3.79\text{g}$, which was significantly highest compared to Group I at $167.69 \pm 2.35\text{g}$ and Group III at week-1 with BW recorded only at $162.31 \pm 2.49\text{g}$. Whereas, BL have no significant different at all groups. As shown in Table 2, the average hepatosomatic index (HSI) of tilapia from Group I was recorded at 1.45 ± 0.04 , which was significantly higher compared to tilapia from Group II at 1.02 ± 0.05 ($P < 0.01$). For Group III, the HSI increased significantly from week-1 until week-3 of culture period. The HSI noted at week-1 was 1.23 ± 0.18 , had shown a significant increasing pattern with 1.51 ± 0.10 at week-2 and reached the highest at week-3 with HSI of 1.95 ± 0.21 . However, a slightly decreasing trend was noticed at week-4 with HSI at 1.57 ± 0.11 . Whereas, GSI for Group I was recorded at 4.48 ± 0.15 and Group II at 3.15 ± 0.51 , respectively. When in compared with Group III, GSI for week-1 was 3.36 ± 0.24 , week-2 at 3.45 ± 0.24 and week-3 at 4.09 ± 0.59 , which were insignificant compared to Group II. However at week-4, GSI was recorded at 4.61 ± 0.56 that similar at Group I, but significantly higher compared to Group II and those from Group III at week-1, -2 and -3, respectively (Table 2). Condition factor (K-factor) varied considerably from 1.54 ± 0.01 and 2.07 ± 0.04 (Table 2). Lowest K-factor was recorded in Group I at 1.54 ± 0.01 and the highest K-factor was recorded in Group II at 2.07 ± 0.04 (Table 2). K-factor for Group III tilapia showed a decreasing trend from 2.02 ± 0.02 at week-1 and decreased gradually to 1.99 ± 0.02 at week-2. This value was further decreased to 1.95 ± 0.02 at week - 3 and reached to the lowest point at week-4 with K-factor of 1.74 ± 0.01 ($P < 0.05$; Table 2).

Tissue bioenergy

Overall muscle glycogen in all groups of tilapia were considerably low. As for tilapia from Group I and Group II, muscle glycogen levels were low but not significantly different compared to Group III ($P > 0.05$; Fig. 1.A). Muscle glycogen for Group I was recorded at 1.41 ± 0.20 mg/g, which was significantly higher compared to Group II at 0.90 ± 0.14 mg/g. Nevertheless in Group III, tilapia that adapted to RAS for the week-1 of recovery had the lowest muscle glycogen level at 0.85 ± 0.12 mg/g. However, muscle glycogen levels showed a significant increment at week-3 with 1.56 ± 0.20 mg/g and week-4 with 1.61 ± 0.33 mg/g as compared to week-1 at 0.86 ± 0.12 mg/g ($P < 0.05$; Fig. 1.A). While for liver glycogen, the lowest value was recorded in Group II at 40.44 ± 2.89 mg/g and the highest liver glycogen level was recorded in Group III at week-2 at 62.17 ± 3.59 mg/g ($P < 0.05$; Fig. 1.B).

Both muscle and liver protein from Group I were recorded at 18.45 ± 0.39 mg/g and 30.31 ± 2.14 mg/g (Fig. 1) respectively, which were significantly lower compared to Group II with muscle protein recorded at 23.71 ± 0.87 mg/g and liver protein at 53.80 ± 5.39 mg/g ($P < 0.05$; Fig. 1.C). Liver protein level found in

Group II was recorded at 53.80 ± 5.38 mg/g considerably higher as compared to Group I and Group III from week-2 onwards ($P < 0.05$; Fig. 1.D).

Muscle lipid levels remained relatively stable among all groups of tilapia from Group I, Group II and Group III ($P > 0.05$; Fig. 1.E). Differently, lowest liver lipid was recorded in Group I and Group III at week-4 with 40.59 ± 3.44 mg/g and 39.54 ± 2.44 mg/g, respectively ($P < 0.05$; Fig. 2.B). Surprisingly, liver lipid level from Group II was not significantly different compared to Group III at week-1,-2 and -3 ($P > 0.05$; Fig. 1.F).

Plasma osmolarity and electrolytes

Plasma osmolarity and electrolytes concentration was presented in Fig. 4. Plasma osmolarity for all groups were remained relatively stable with 295.54 ± 1.44 mOsm/L recorded for Group I and 291.52 ± 1.65 mOsm/L ($P > 0.05$; Fig. 4.A). Meanwhile, plasma osmolarity level in Group III were recorded at a range of 300.05 ± 1.78 mOsm/L to 306.28 ± 2.81 mOsm/L, which were relatively stable within 4 weeks ($P > 0.05$; Fig. 4.A). The lowest plasma sodium (Na^+) was found from Group II with only 135.39 ± 1.60 mmol/L compared to all other groups ($P < 0.05$; Fig. 4.B). Plasma Na^+ level for Group I and Group III for all weeks were maintained relatively stable at range of 146.95 ± 4.23 mmol/L to 156.24 ± 7.69 mmol/L ($P > 0.05$; Fig. 4.B). Potassium (K^+) is the second important cation for biological processes in organism. In this study found that plasma K^+ levels in all groups were not significantly differences ranging from 3.71 ± 0.78 to 4.60 ± 0.89 mmol/L ($P > 0.05$; Fig. 4.C). Differently for plasma chloride (Cl^-) levels in all groups, Group I exhibited lowest plasma Cl^- level for Group I was recorded at 128.67 ± 2.78 mmol/L and the lowest plasma Cl^- was noted in Group II at 122.58 ± 1.82 mmol/L compared to Group III at week-1 and week-2, which were 143.85 ± 1.48 mmol/L and 145.05 ± 4.56 mmol/L ($P < 0.05$; Fig. 4.D). Similarly trend was also noticed for plasma calcium (Ca^{2+}) with the lowest plasma Ca^{2+} observed at Group II at only 6.57 ± 0.29 mmol/L compared to other groups ($P < 0.05$; Fig. 4.E). Plasma Ca^{2+} for Group I and III were remained insignificantly different ranging from 7.58 ± 0.26 mmol/L to 8.43 ± 0.59 mmol/L ($P > 0.05$; Fig. 4.E). Whereas for plasma magnesium (Mg^{2+}), lowest value was noticed in Group I with only 0.69 ± 0.04 mmol/L ($P < 0.05$; Fig. 4.F). Overall, highest plasma Mg^{2+} was noticed in Group II at 1.05 ± 0.09 mmol/L, but was not significantly different compared to Group III ranging from 0.82 ± 0.12 mmol/L to 0.97 ± 0.07 mmol/L ($P > 0.05$; Fig. 4.F).

Gills and kidney enzymatic Na^+/K^+ ATPase activity

Sodium pump Na^+/K^+ ATPase activity (NKA) for the gills and kidney of tilapia cultured under different environment were presented in Fig. 5. In general, both gills and kidney NKA activities were presented in similar pattern (Fig. 5.A and 5.B). As for gills NKA no significant differences noticed for all groups. Although, Group I expressed lowest NKA activities with only 3.61 ± 0.49 $\mu\text{mol Pi/mg protein/h}$ compared to all other groups ($P > 0.05$; Fig. 5.A). High gill NKA activities was recorded in tilapia from Group II with NKA activities at 4.80 ± 0.67 $\mu\text{mol Pi/mg protein/h}$. Whereas NKA activities for Group III were recorded ranging from 3.85 ± 0.55 $\mu\text{mol Pi/mg protein/h}$ to 4.54 ± 0.35 $\mu\text{mol Pi/mg protein/h}$, respectively ($P > 0.05$; Fig. 5.A).

Similar trend was also noticed in kidney NKA activity with no significant difference observed for all groups ($P > 0.05$; Fig. 5.B). Nevertheless, highest kidney NKA activities noticed in Group II at 2.62 ± 0.22 $\mu\text{mol Pi/mg protein/h}$, followed by Group III on week-1 and week-2 at 2.25 ± 0.31 $\mu\text{mol Pi/mg protein/h}$ and 2.21 ± 0.36 $\mu\text{mol Pi/mg protein/h}$ ($P > 0.05$; Fig. 5.B). Again similar as gills, lowest kidney NKA activities were recorded from Group III on week-4 at 1.83 ± 0.22 $\mu\text{mol Pi/mg protein/h}$, which was comparable with Group I at 1.99 ± 0.61 $\mu\text{mol Pi/mg protein/h}$, respectively.

Discussion

Growth and energy mobilisation pattern of tilapia living in different environments

The K-factor of a fish reflects physical and biological quality of a species or individual of fishes (Binner et al. 2008). Through its variations, information on the physiological state of the fish in relation to its welfare can be recognized (Lizama & Ambrosio 2002). K-factor is also an indicator of the general fish condition because condition factor reflecting interactions between biotic and abiotic factors in the physiological condition of fishes. Moreover, body condition provides an alternative to the expensive in vitro proximate analyses of tissues (Sutton et al. 2000). Therefore, information of K-factor can be vital to culture system management because they provide the producer with information of the specific condition under which organisms are developing (Araneda et al. 2008). The values of K-factor recorded in the present study were 1.54 ± 0.01 for Group I, 2.07 ± 0.04 for Group II and range from 1.74 ± 0.01 to 2.02 ± 0.02 for Group III respectively. Condition factor of greater than one showed the well-being of fishes (Datta et al. 2013). Although all fishes were in a good condition, low K-factor in Group I and Group III at week-4 could be related to reproduction pheromone when more energy were channelled to reproduction. Whereas, fish in Group II were cultured in cage culture system. This may head to unsuitability for reproduction. Thereby energy intake was speculated to be used for growth. Similarly for tilapia in Group III at week-1 and -3. The K-factor decreases at the start of the spawning period due to high metabolic rates as noticed with increased of GSI. There is normally a gradual increase in the condition factor during the reproductive period and normalization occurs immediately afterwards (Lizama et al. 2002; Gupta et al. 2011). Energy that is surplus to the essential standard metabolic requirements (i.e., maintenance, locomotion, predation avoidance, and feeding activity) is allocated to somatic growth, energy storage, or reproduction after the fish reaches sexual maturation. The priority with which this surplus energy is allocated to each of the above biological functions differs among fish species (Nunes et. al. 2011). Fish is under the indeterminate growth as must consider the survival costs and the available energy for reproduction and must make an allocation decision between current and future reproduction as an adaptation to the fluctuating environmental conditions.

Hepatosomatic Index (HSI) is defined as the ratio of liver mass to body weight, where HSI is commonly use as reference to define status of feeding and nutrition intake (Facey et al. 1999) of an organism with energy storage for growth and reproduction (Nunes et al., 2011; Sadekarpawar & Parikh, 2013). As data

obtained in this study, lower HSI was observed in Group II which could be attributed to the important role of the liver in nutrient metabolism, which was associated with high activity and metabolic rates. As tilapia from Group II were actively swimming in circle that led to high energy expenditure for exercise while maintained efficient aerobic metabolism. Thereby, accelerates energy mobilization thus reduced HSI value. Differently for tilapia in Group I and Group III, HSI values relatively higher compared to tilapia from Group II. This is because tilapia from Group I and III were cultured in the RAS system with low exercise capacity, thus reserves energy for secondary maturation and reproduction. Evidently, this was noticed in Group III tilapia with significant HSI increment till week-3 and started reduces on week-4, which was believed in associated with energy mobilisation for reproduction. HSI is used as a good indicator of total energy reserves on Atlantic cod *Gadus morhua*, which also correlated positively with fish K-condition (Lambert and Dutil, 1997).

The HSI reflected total energy reserved in the liver of tilapia, this was obvious based on bioenergy mobilisation pattern of tilapia in Group I where both liver protein and lipid were highly mobilized to support reproduction process, while reserving glycogen for routine metabolism needs. This was supported with spawning process and mouth incubation occurred during the study period. Similar energy mobilisation pattern was noticed in Group III, where tilapia from cage culture system transferred back to RAS showed significant energy reserved for the first two weeks acclimation. Thereafter, week-3 onwards liver protein and lipid were mobilized significantly which was associated with reproduction process. At week-4, liver protein and lipid reached to the similar level as tilapia from Group I with mouthbrooding behaviour noticed.

Together HSI indication and energy mobilisation pattern showing a contribution to support GSI development as an indication of readiness for spawning process especially tilapia from Group III with an increasing GSI noticed. This also in agreement with our hypothesis that tilapia cultured in RAS condition (Group I and Group III) more suitable for spawning as bottom base of the tank provided spawning ground as territory site and allow female tilapia to collect fertilized eggs after spawning. As compared to tilapia that culture in cage system (Group II) without bottom base which is difficult to perform fertilization and eggs collection processes, therefore lower GSI was obtained in Group II.

Glycogen is one of the important energy sources to maintain basal metabolism (Javed & Usmani 2015) and serves as readily energy supply to fuel metabolic needs under unfavourable environment challenges (Mehjbeen & Nazura 2015). This corresponded to the tilapia from Group II having the lowest liver glycogen, which was believed to be related to their exercise capacity in cage. On the other hand, muscle glycogen reached to the lowest level on week-2 and restored to higher level on week-3 onwards in Group III. Possibility first two weeks tilapia from Group III could be related with acclimation process from cage to RAS conditions. Meanwhile, liver glycogen reached to peak level was recorded at week-2 and returned to level similar as Group I and Group III at week-1, -3 and -4, although the liver glycogen levels were not significantly different. This shows that tilapia reserved liver glycogen as readily energy to support exercise activity when required and this indicated that all nutrients intake were sufficient and able to deposit in the liver.

In fish, protein is known to be more efficiently catabolized into energy sources to support aerobic exercise performance as compared to lipids and glycogen (Ballantyne, 2001; Liew et al., 2012; Rahmah et al., 2020). As Group II tilapia cultured in floating cage was not suitable for spawning, therefore protein was reserved for somatogenesis as higher muscle and liver protein were recorded. Whereas, protein level for tilapia from Group I and III were displayed relatively similar level. Protein was mobilized to support reproduction process, where mating and spawning process were noticed (FAO 2021b). This was in agreement with previous studies reported that at mature age, fish mobilized protein to prioritise gonadosomatic development (Van Dijk et al. 2005; Encina & Granado-Lorencio 1997; Santos et al. 2010). Protein is known as a central role in production that allows the fish to reallocate energy used for growth to other metabolic needs at different life stage based on priority (Ferrari et al., 2011; Moyson et al. 2015) as well as to improve adaptability performance in different environment changes (Cara et al. 2007).

On the other hand, lipid mobilisation were not notified in muscle lipid, but liver lipid mobilisation were distinguished in tilapia from Group I and Group II week-4. Significantly liver lipid mobilisation is believed not only to support basal metabolism and somatogenesis, but also to support secondary maturation for reproduction (Kolditz et al. 2008). Energy requirements for gonad maturation appeared to come from liver reserves and it is noted that 1g of lipid contains 2 times higher energy than 1g of carbohydrates or 1g of protein (Jobling et al. 1998; Robb et al. 2002; Wood et al. 2003).

Ionoregulation of tilapia living in different environments

Higher plasma osmolality was recorded in Group II tilapia indicated that tilapia increased their osmolality to facilitate active ion uptake as well as enhance metabolites such as glucose and/or glycogen for routine and active metabolic activities. Facilitating active ion uptake was in parallel with high NKA activity found in both gill and kidney. According to Morgan et al. (1997), maintaining or increasing plasma osmolality is important to conserve stable ionic concentration in body fluid with support of active osmoregulation. High NKA activities in Group II tilapia was also believed to relate with swimming activity as highlighted in goldfish and common carp when forced to swim actively, significantly accelerated the gill NKA activity (Liew et al., 2013). Contradictory, lower plasma Na^+ was noticed in Group II tilapia, while plasma Na^+ in tilapia from Group I and III were relatively stable. Although higher NKA activities in gills and kidney were noticed in Group II, this does not retain Na^+ level efficiently. Loss of Na^+ might occur in Group II tilapia could be associated with the living condition in the lake water which is relatively clean and low ionic levels compared to tilapia that lives in the RAS water. In Group III, gills and kidney NKA activities were noticed relatively stable as for tilapia in Group I.

On the other hand, plasma K^+ levels were relatively stable, except Group I tilapia that had slightly higher K^+ level. High plasma K^+ could be released from the tissue into the blood stream in cooperate NKA activity not only to facilitate Na^+ uptake but also helped in ammonia excretion (Liew et al., 2013). Another possible explanation could be correlated with defensive behaviour of Group I tilapia due to active swimming and defending territory for mating and spawning. Active exercise resulted in tissue K^+ leaked into body fluid was reported in common carp (Knudsen and Jensen, 1997). Na^+ , K^+ and Cl^- are important

ions which provide the sustainability of the osmotic pressure of body fluids as well as acid-base balance (Keleştemur 2012; Karnaky 1998). As one of the essential ions, plasma Cl⁻ level was maintained consistently in Group III, but lightly decreased in Group I and II tilapia. Stability of plasma Cl⁻ is probably correlated with an increase of Na⁺ uptake via the Na⁺/Cl⁻ exchanger via dietary intake (Liew et al., 2020). Feeding is known to provide excessive base which consequently led to the uptake of Cl⁻ via branchial Cl⁻/HCO₃⁻ exchanger as reported in rainbow trout (Bucking and Wood, 2006; 2008). As important ions, the association of unidirectional influx and efflux of Na⁺ and Cl⁻ (Perry and Fryer, 1997) to be adjusted to a net flux via Cl⁻ uptake at gills Cl⁻/HCO₃⁻ exchanger was noted during alkalosis metabolism (Perry and Goss, 1994).

As all fishes in all groups were fed twice daily, dietary Ca²⁺ uptake seemed to sufficiently support basal metabolic needs. Higher plasma Ca²⁺ levels in Group II and Group III seemed to be a strategy to retain Ca²⁺ in the body active uptake through Ca²⁺ transporter and Ca²⁺ channel. Ca²⁺ is known to play important role in bone and scale formation. Therefore, it is highly essential for tilapia from Group II and III to maintain sufficient level of Ca²⁺ to support basal metabolic needs. Differently from Group I that lived in enclosed system at all time with consistent water Ca²⁺ level as well as received sufficient Ca²⁺ from dietary supply, which allowed tilapia from this group to maintain Ca²⁺ for basal metabolism. Mg²⁺ is the second most abundant cation that exists in intracellular fluid that acts as a functional co-factor for enzymes as well as play an important role in neurochemical impulse transmission and muscle excitability (Keleştemur 2012). Changes in plasma Mg²⁺ level is always associated with stress or environmental changes (Iversen et al. 2009; Iversen & Eliassen 2009). This phenomenon was noticed in this study where plasma Mg²⁺ levels were inconsistent where higher Mg²⁺ level found in Group II tilapia, lower in Group I and fluctuate in Group III. It was believed that the different levels of ions was influenced by the living conditions which could be associated with territory competition for mating and water ionic status especially in Group II condition.

Conclusion

The present study revealed that tilapia prioritize energy mobilization differently for growth and reproduction according to living conditions. As tilapia living in open cage mobilized glycogen to fuel swimming activity and prioritized energy for growth performance. Whilst, tilapia living under enclosed condition mobilized lipid and protein to prioritise reproduction purpose. This remark supported our hypothesis highlighted that energy mobilization was confirmed for tilapia living in enclosed condition. Whereas, our second hypothesis on the gills and kidney NKA activities was rejected. The gills and kidney NKA activities in all groups of tilapia remained steady to support balance homeostasis for basal metabolism, without influenced by living conditions.

Declarations

ACKNOWLEDGEMENT

Thanks to Terengganu Department of Fisheries, Central Terengganu Development Authority (KETENGAH), Research Laboratory and Hatchery staffs from Institute Tropical of Aquaculture and Fisheries (AKUATROP) and Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu for their assistance.

FUNDING

This research was supported by Fundamental Research Grant Scheme from Ministry of Higher Education Malaysia (MOHE) with register ID: FRGS/1/2014/STWN03/UMT/01/1 and UMT ID: FRGS – Vote No. 59333 with research title “Modelling of nutrient budget and water quality fluctuation in relation to tilapia (*Oreochromis* sp.) production at Como River cage culture, Kenyir Lake, Terengganu”. This research grant was awarded by MOHE to Hon Jung Liew as principal investigator.

CONFLICT OF INTEREST

The authors declared that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this manuscript.

STATEMENT OF ETHIC ANIMAL MANAGEMENT

Fish handling practice during experimentation was followed and approved by Universiti Malaysia Terengganu Animal Care Committee according to the guidelines of the Laboratory Animal Ethic Regulation (UMT/JKEPHT/2019/38).

DATA AVAILABILITY

All the data presented in this study are provided within the main manuscript. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

DECLARATION OF COMPETING INTEREST

The authors would like to declare there is no conflict of interest either financial, personal interest or authorship arrangement among authors listed in this manuscript.

AUTHOR CONTRIBUTION

All authors contributed to the study conception, design and planning. System setup was performed by Razali, R.S., Rahmah, S. and Lim, L.S. Experimentation, data collection and tissue analytical were performed by Razali, R.S., Rahmah, S., Lim, L.S. and Liew, H.J. Enzyme activity analysis was facilitated and financially supported by Chang, Y.M. and Liang, L.Q. Data analysis performed by Razali, R.S. guided by Amornsakum, T. Draft preparation, writing and editing were performed by Rahmah, S., Ghaffar, M.A., Nhan, H.T., Chen, Y.M. and Liew, H.J.

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Tables

Table 1: Water ions concentration from Group I - hatchery recirculating aquaculture system (RAS), Group II - Como River cage culture (Cage) and Group III - RAS (Compensation).

	Group I (RAS)	Group II (Cage)	Group III (Compensation)
Na ⁺	0.0950 ± 0.0005 ^a	0.0776 ± 0.0002 ^b	0.0921 ± 0.0014 ^a
K ⁺	0.0580 ± 0.0002 ^a	0.0289 ± 0.0005 ^b	0.0523 ± 0.0008 ^a
Cl ⁻	0.0901 ± 0.0006 ^a	0.0547 ± 0.0003 ^b	0.0937 ± 0.0003 ^a
Ca ²⁺	0.1630 ± 0.0008 ^a	0.0742 ± 0.0003 ^b	0.1618 ± 0.0009 ^a
Mg ²⁺	0.0611 ± 0.0003 ^a	0.0294 ± 0.0003 ^b	0.0625 ± 0.0011 ^a

The ionic concentrations of sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), calcium (Ca²⁺) and magnesium (Mg²⁺) in water sources from Group I - (RAS) hatchery recirculating aquaculture system tilapia culture system, Group II - (Cage) water sample from Como River cage culture water and Group III – (Compensation) RAS system from compensation. Different superscript letters in the same row indicate significant differences of ionic concentration amongst different types of water (P < 0.05). Results are presented as mean ± standard error mean (mean ± SEM).

Table 2: Biometric data of hybrid red tilapia *Oreochromis* sp. from Group I (RAS), Group II (Cage) and Group III (Compensation).

Biometric	Group I	Group II	Group III			
	RAS	Cage	Compensation			
			Week-1	Week-2	Week-3	Week-4
Final body weight (g)	167.69 ± 2.35 ^a	212.11 ± 3.79 ^b	162.31 ± 2.49 ^a	175.59 ± 10.15 ^{ab}	184.92 ± 7.06 ^{ab}	182.44 ± 10.12 ^{ab}
Final body length (cm)	21.12 ± 0.20	20.52 ± 0.31	19.87 ± 0.86	20.26 ± 0.69	20.02 ± 0.37	21.78 ± 0.65
Hepatosomatic Index (HSI)	1.45 ± 0.04 ^b	1.02 ± 0.05 ^a	1.23 ± 0.18 ^{ab}	1.51 ± 0.10 ^b	1.95 ± 0.21 ^c	1.57 ± 0.11 ^{bc}
Gonadosomatic Index (GSI)	4.48 ± 0.15 ^a	3.35 ± 0.51 ^b	3.36 ± 0.24 ^b	3.45 ± 0.24 ^b	4.09 ± 0.59 ^{ab}	4.61 ± 0.56 ^a
Condition factor (K-factor)	1.54 ± 0.01 ^a	2.07 ± 0.04 ^b	2.02 ± 0.02 ^b	1.99 ± 0.02 ^b	1.95 ± 0.02 ^b	1.74 ± 0.01 ^c

The biometric data of hybrid red tilapia *Oreochromis* sp. from Group I (RAS), Group II (Cage) and Group III (RAS-Compensation) which include final body weight, final body length, HIS, GSI and K-Factor. Different lowercase letters indicate significant differences among groups (RAS, Cage and RAS-compensation). Results are presented as mean ± standard error mean (mean±SEM, P<0.05, n = 10).

Figures

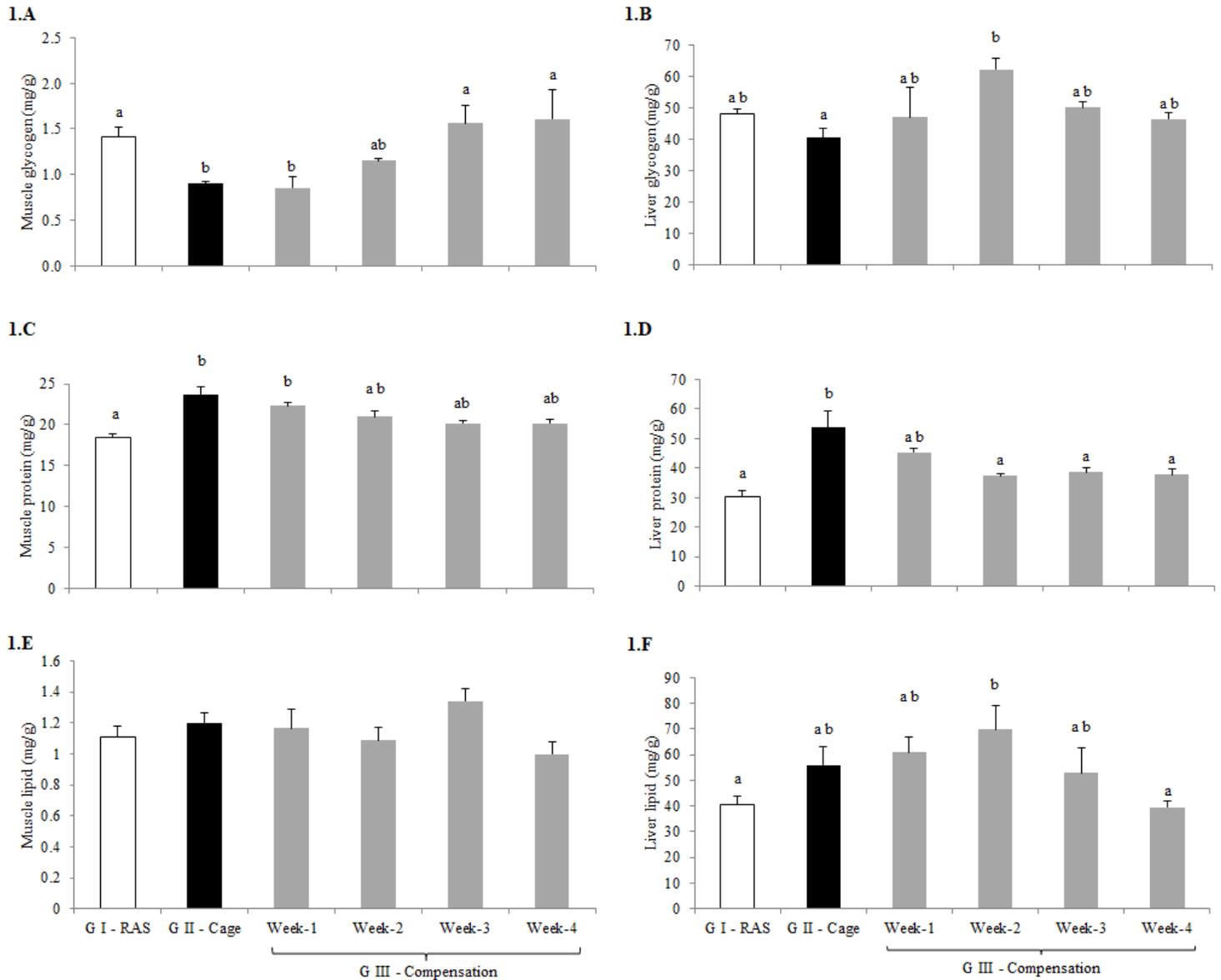


Figure 1

Total energy of (A) muscle glycogen (B) liver glycogen (C) muscle protein (D) liver protein (E) muscle lipid and (F) liver lipid levels of hybrid red tilapia *Oreochromis sp.* from Group I – RAS (white bar), Group II – Cage (black bar) and Group III – Compensation (grey bars) for week-1, week-2, week-3 and week-4. All values are means \pm standard error of the mean (SEM) (n=10). Superscript small letters indicates significant differences amongst cultured in different groups (P<0.05).

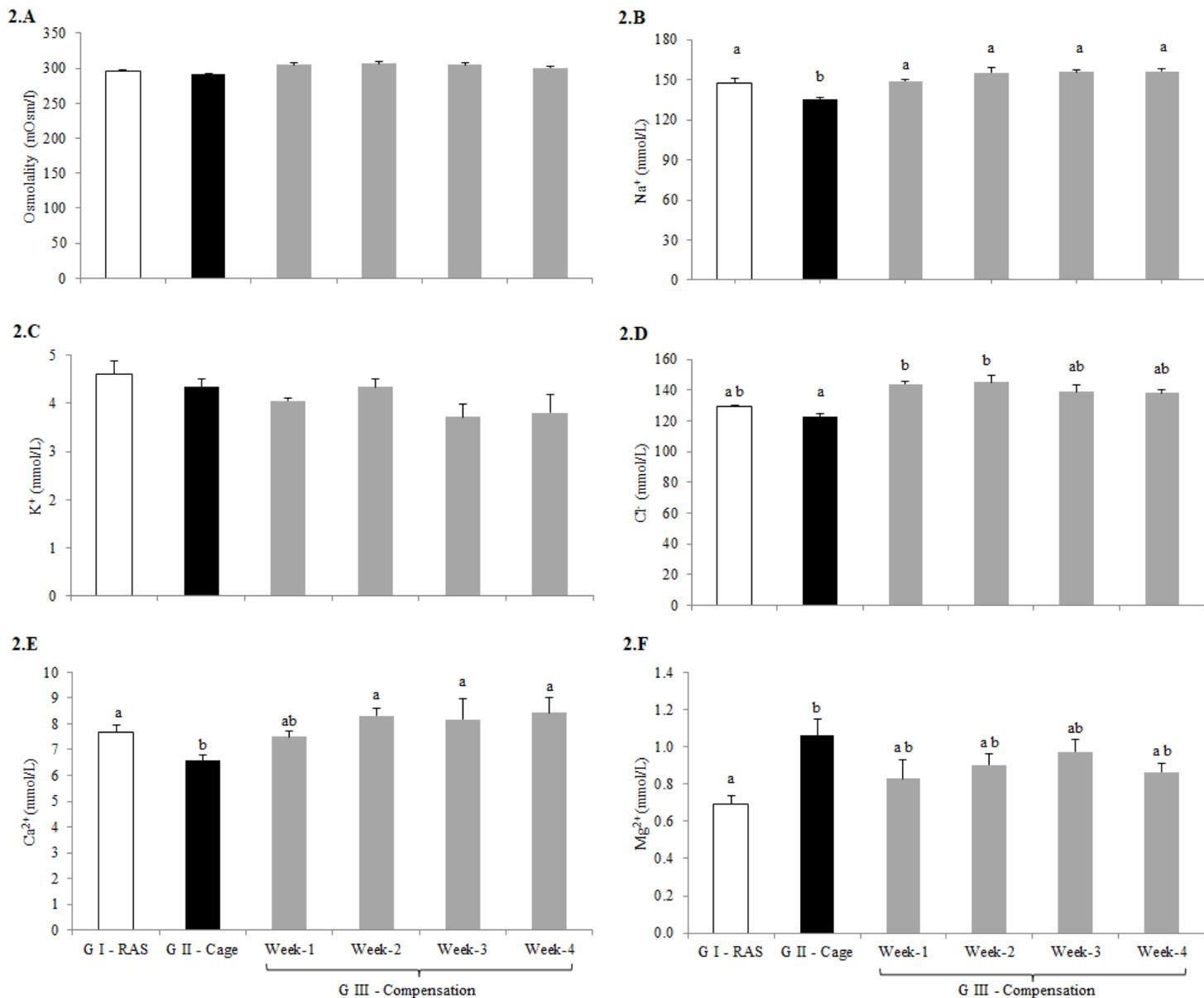
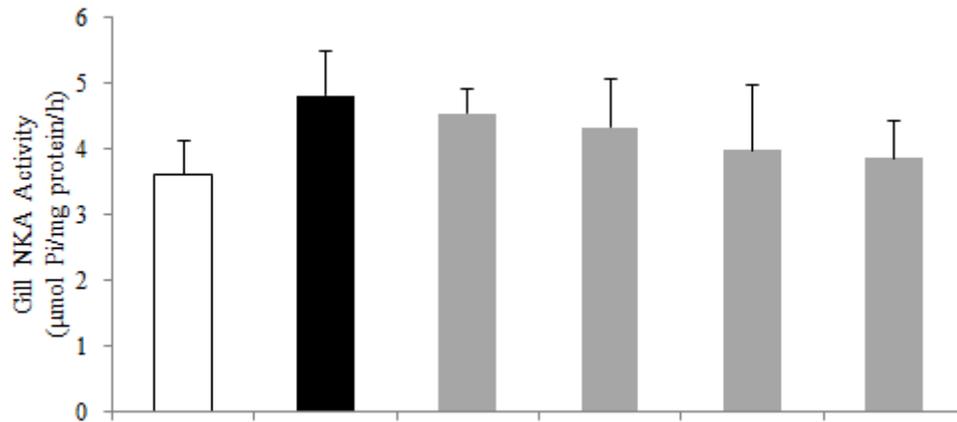


Figure 2

Plasma composition (A) osmolarity (B) Na⁺ (C) K⁺ (D) Cl⁻ (E) Ca²⁺ and (F) Mg²⁺ levels of hybrid red tilapia *Oreochromis sp.* from Group I – RAS (white bar), Group II – Cage (black bar) and Group III – Compensation (grey bars) for week-1, week-2, week-3 and week-4. All values are means ± standard error of the mean (SEM) (n=10). Superscript small letters indicates significant differences amongst cultured in different groups (P<0.05).

3.A



3.B

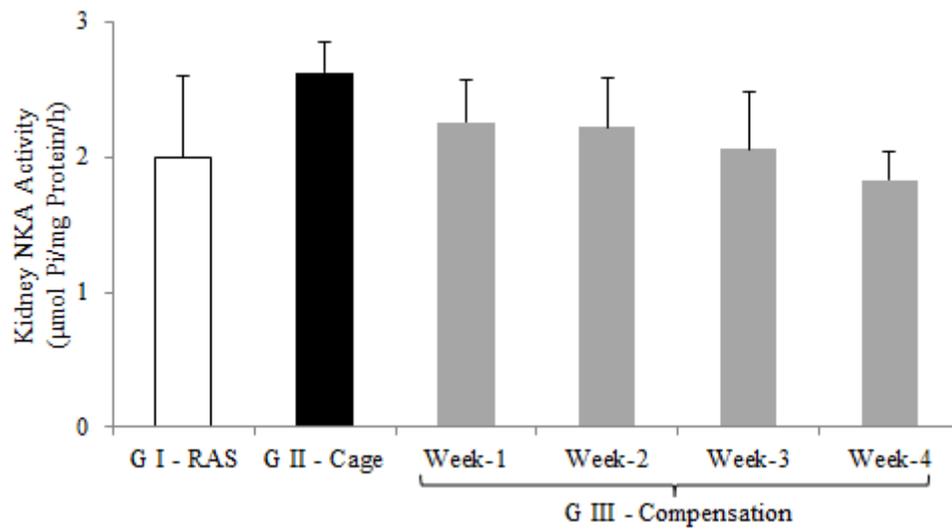


Figure 3

(A) Gills and (B) kidney Na^+/K^+ ATPase activities levels of hybrid red tilapia *Oreochromis sp.* from Group I – RAS (white bar), Group II – Cage (black bar) and Group III – Compensation (grey bars) for week-1, week-2, week-3 and week-4. All values are means \pm standard error of the mean (SEM) (n=10). Superscript small letters indicates significant differences amongst cultured in different groups ($P < 0.05$).