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RESEARCH PAPER

Optimal Density of Asian Seabass (*Lates calcarifer*) in Combination with the Omani Abalone (*Haliotis mariae*), Brown Mussel (*Perna perna*) and Seaweed (*Ulva fasciata*) in a Land-based Recirculating Integrated Multi-trophic Aquaculture (IMTA) System

Balqees Al-Rashdi[1], Wenresti Gallardo[1,*], Gilha Yoon[1], Hussein Al-Masroori[1]

تأثيرالكثافة المثالية للقاروص الآسيوي (*Lates calcarifer*) بالاشتراك مع أذن البحر العماني (*Haliotis mariae*) وبلح البحر البني (*Perna perna*) والأعشاب البحرية (*Ulva fasciata*) في نظام متكامل للاستزراع المائي متعدد التغذية (IMTA) قائم على نظام تدوير أرضي

بلقيس الراشدي[١,٢] وونيريتسي جالاردو[١] وجلها يون[١] وحسين المسروري[١]

ABSTRACT. An experiment was conducted to develop a land-based recirculating integrated multi-trophic aquaculture (IMTA) system using a combination of the Omani abalone (Haliotis mariae) and Asian seabass (Lates calcarifer) as fed species, brown mussel (Perna perna) and seaweed (Ulva fasciata) as extractive species. Specifically, this study was carried out to determine the optimal seabass density (20, 40 and 60 individuals per 500-liter tank) on water quality, growth and survival of the cultured species in the system. Sampling of all species was done every two weeks to check their growth. Water samples were taken every two weeks for analysis of ammonia, nitrite, nitrate, phosphate, and silicate. Measurements of temperature, dissolved oxygen and salinity were done daily. Growth of abalone and mussels were higher in fish densities of 20/tank and 40/tank, respectively, while growth and survival of seabass were not significantly different between densities. Biomass of seaweeds decreased during the experiment period. Temperature, dissolved oxygen and salinity were within optimum levels. Ammonia levels decreased as nitrite increased but in some cases it remained high while nitrates did not increase, indicating that nitrites were not converted to nitrates most likely due to the lack of efficient bio-filtration in the mussel tanks.

KEYWORDS: Abalone; seabass; mussel; Ulva; IMTA

المستخلص: أجريت في هذه الدراسة تجربة لتطوير نظام متكامل للاستزراع المائي متعدد التغذية (IMTA) قائم على نظام تدوير أرضي باستخدام أذن البحر العماني (Haliotis mariae) والقاروص الآسيوي (Lates calcarifer) كأنواع تتغذى مع بلح البحر (Perna perna) والأعشاب البحرية (Ulva fasciata) كأنواع استخراجية، وكان الهدف الرئيسي لهذه التجربة هو تحديد كثافة القاروص المثلى (٢٠ أو ٤٠ أو ٦٠ سمكة لكل خزان ذو سعة ٥٠٠ لتر) على جودة المياه ونمو وبقاء الأنواع المستزرعة في النظام. تم أخذ عينات من جميع الأنواع كل أسبوعين للتحقق من نموها، كما تم أخذ عينات المياه كل أسبوعين لتحليل الأمونيا والنيتريت والنيترات والفوسفات والسيليكات، أما درجة الحرارة والأكسجين المذاب والملوحة فقد تم قياسها يومياً. وقد وجد أن نمو أذن البحر وبلح البحر كان أعلى في كثافة الأسماك من ٢٠/خزان و ٤٠/خزان على التوالي، بينما لم يكن نمو وبقاء القاروص مختلفين بشكل كبير بين الكثافات. وقد لوحظ أن الكتلة الحية من الأعشاب البحرية قد إنخفضت خلال فترة التجربة وكانت درجة الحرارة والأكسجين المذاب والملوحة ضمن المستويات المثلى، وقد لوحظ أيضا إنخفاض مستويات الأمونيا مع زيادة النيتريت، لكنها ظلت مرتفعة في بعض الحالات بينما لم تزد النيترات، الأمر الذي يشير إلى أن النيتريت لم يتم تحويلها إلى نيترات على الأرجح بسبب نقص الترشيح الحيوي الفعال في خزانات بلح البحر.

الكلمات المفتاحية : أذن البحر، القاروص الآسيوي، بلح البحر، أعشاب بحرية، النظام المتكامل للاستزراع المائي متعدد التغذية

Introduction

Aquaculture provides socio-economic benefits such as employment, food provision and income generation, but if not done properly, it can lead to adverse environmental impacts which usually come with the development of commercial aquaculture activities (FAO, 2018). By-product wastes in culturing species fed artificial diets in monoculture system are very high (Troell et al., 2003). Environment-friendly aquaculture techniques and systems, for example recirculating aquaculture systems (RAS), are necessary to ensure sustainable aquaculture development. One of the aquaculture systems that has high potential for environmental protection is the integrated multi-trophic aquaculture or IMTA (Neori et al., 2004; Chopin, 2006). IMTA can be sea-based culture system or land-based recirculating system. It involves the culture of a number of species belonging to different trophic levels, some of them are fed while others are extractive, in which the particulate

Wenresti Gallardo[1,*] (✉) gallardo@squ.edu.om, [1]Department of Marine Science and Fisheries, College of Agricultural and Marine Sciences, Sultan Qaboos University, Sultanate of Oman.

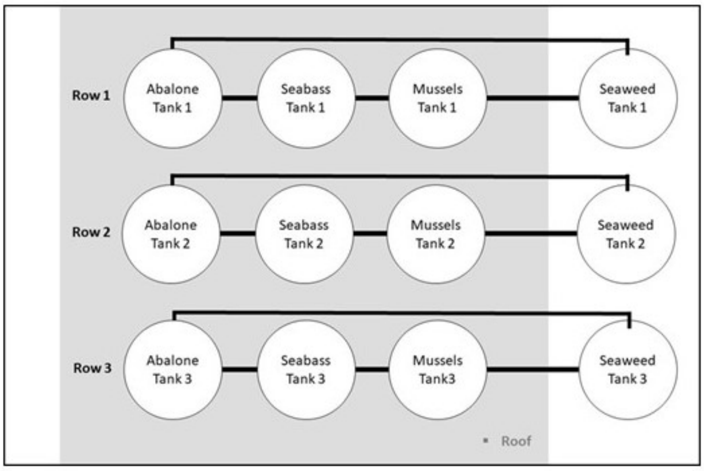

Figure 1. IMTA system of interconnected tanks with recirculating water system. Water from abalone to seaweed tanks flow by gravity while water from seaweed tanks are brought back to abalone tanks by submersible pumps

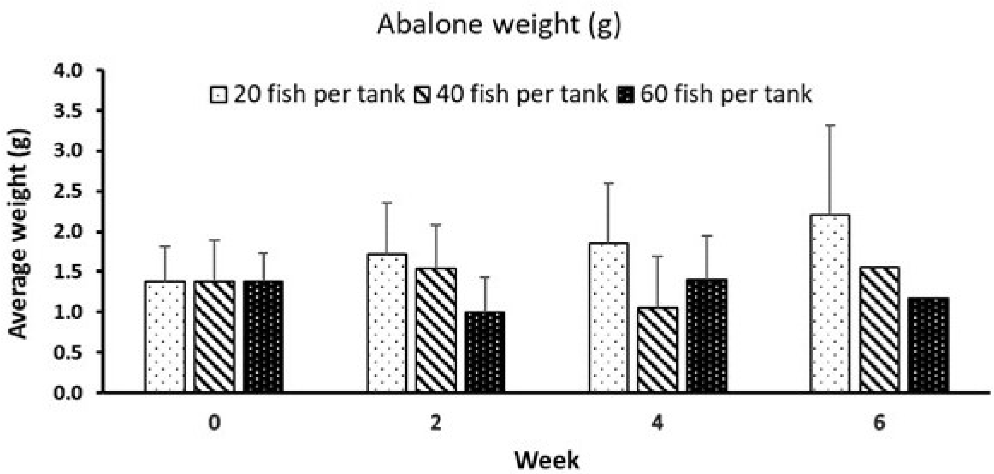

Figure 2. Average weight of abalone in combination with seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) density. Error bars are standard deviation

and dissolved wastes (uneaten feeds, feces, excretion) of other species are utilized by another species.

IMTA requires a careful selection of ecologically and economically important species, some of which can efficiently utilize the wastes from the production of the other species, thus, preventing pollution or eutrophication. In a conventional aquaculture system, wastes go to the environment causing over-enrichment and algal bloom which may eventually cause mass mortalities of cultured and wild species when there is algal die-off and oxygen depletion.

Among the extractive organisms that can be used in an IMTA system are bivalves and seaweeds. Bivalve species such as the mussel Perna perna can filter suspended particles and utilize organic matters in the water (Cheshuk et al., 2003; MacDonald et al., 2011) while seaweeds or macroalgae can take up nitrates which have been converted from ammonia and nitrite by the nitrifying bacteria. Among the seaweeds, Ulva fasciata which is an intertidal green macroalga with high nutrient absorption ability of up to 80% ammonia input (Neori et al., 2000), can be used as the bio-filtration component in an IMTA system (Chopin et al., 2001; Al-Hafedh et al., 2015). In this study, we used the commercially important Omani abalone Haliotis mariae (Al-Rashdi et al., 2008) and Asian seabass or "barramundi" Lates calcarifer as the fed species and the brown mussel Perna perna and seaweed Ulva fasciata as the extractive species.

The Omani abalone is naturally present in the coastal region of the southern part of Oman where it is heavily exploited due to its commercial value. To prevent its depletion, the Ministry of Agriculture and Fisheries Wealth of the Sultanate of Oman is producing juveniles in the hatchery for stock enhancement purposes; however, culturing them in a land-based IMTA system has not been tested yet. If it can be proven that the Omani

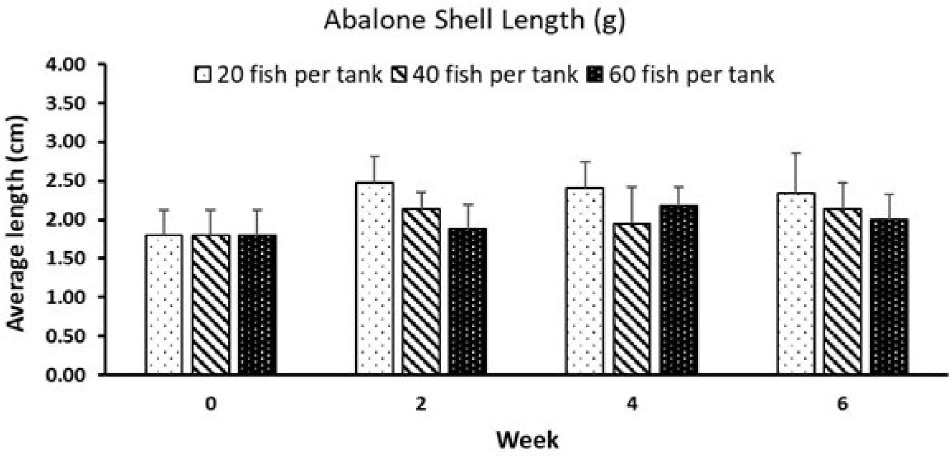

Figure 3. Average shell length of abalone in combination with seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density. Error bars are standard deviation

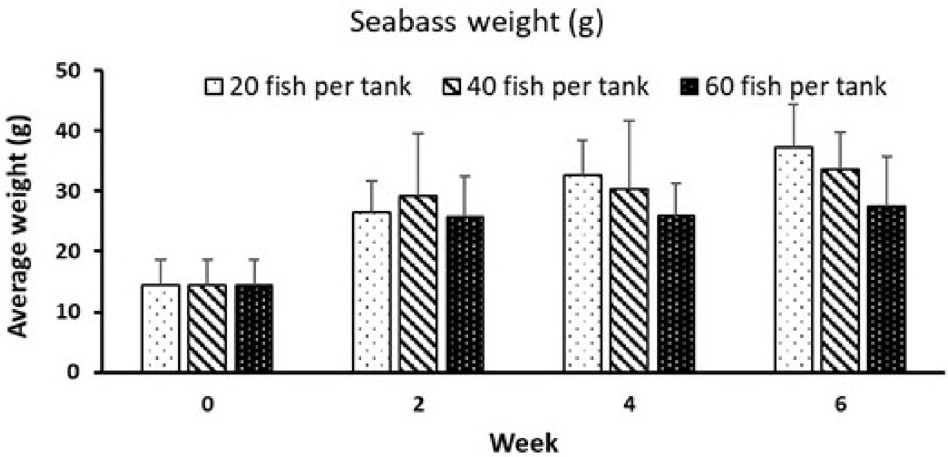

Figure 4. Average weight of seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) density. Error bars are standard deviation

abalone can survive and grow in a land-based recirculating IMTA system, then there is another option for their population to be maintained.

One of the critical factors in a culture system is the stocking density of the cultured species. The objective of the study was to determine the optimal density of the Asian seabass Lates calcarifer as one of the fed species in the land-based recirculating IMTA system, in relation to water quality, and the growth and survival of the cultured species.

Materials and Methods

System Design and Operation

The IMTA system (Figure 1) consists of interconnected 500-l tanks with recirculating water system. Tanks were organized in three rows, each row containing four tanks for the culture abalone (Haliotis mariae), seabass (Lates calcarifer), mussel (Perna perna), and seaweed (Ulva fasciata). Due to lack of tanks and space, replication was not possible.

Initially, 30 individuals of abalone, fish and mussel were selected randomly for weight and length measurements before distributing them to the tanks. The fishes were stocked at 20, 40 and 60 pieces per tank, hereafter designated as low, medium and high fish density. Seaweeds were distributed at 2 kg per tank. The abalone and mussels were distributed at 75 pieces per tank. The experiment was conducted for 6 weeks (42 days).

Abalone, sea bream and mussel tanks were placed under a roof to prevent direct exposure to sunlight and high temperatures, and to minimize water evaporation. The seaweed tanks were placed outside to allow some sunlight needed for photosynthesis but they were covered with a green mesh to minimize direct sunlight and

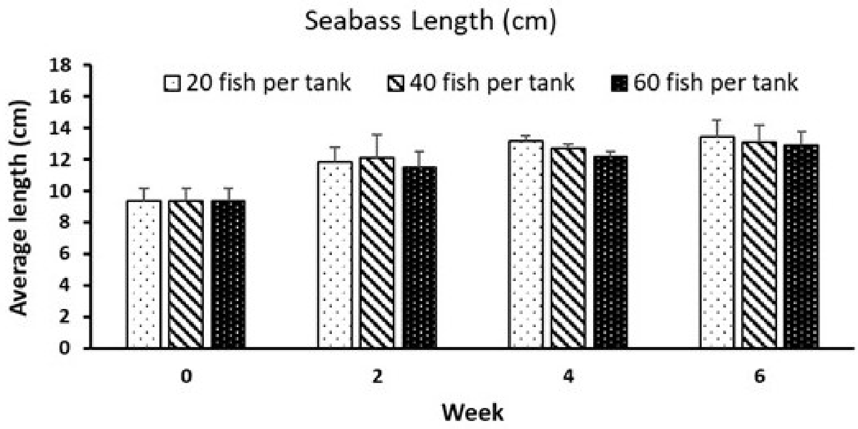

Figure 5. Average length of seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) density. Error bars are standard deviation

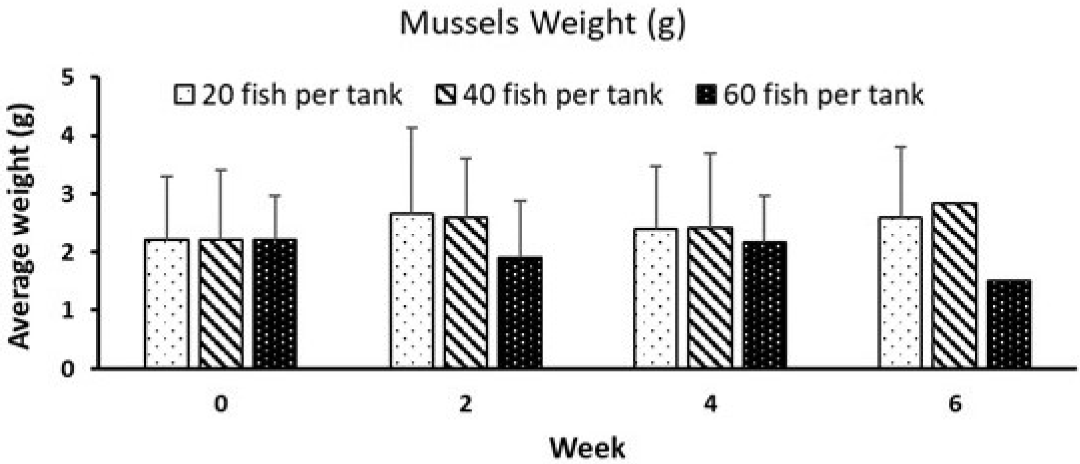

Figure 6. Average weight of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density. Error bars are standard deviation

excessive heat. Each seaweed tank was installed with a submersible pump that recirculates the water back to the abalone tank and the rest of the tanks at a rate of 1,200 liters per hour.

The initial feeding rate for seabass was 5% body weight and the amount of feed given to each fish tank was determined by the respective fish density. On the second, third and fourth week, the feeding rate was changed to 3% of body weight and the feed amount was adjusted according to fish density. The abalone were fed with Ulva from the seaweed tanks, approximately 10% of the abalone biomass.

Sampling

Sampling of all species was done every two weeks to check their growth. Water samples were taken every two weeks for measurement of water quality (ammonia, nitrite, nitrate, phosphate, and silicate). Measurements of temperature, dissolved oxygen and salinity were done daily at 8:30 AM and 4:30 PM.

Statistical Analysis

Repeated measures ANOVA was performed to determine any significant difference between treatments, followed by Tukey's test to identify which treatments were significantly different.

Results and Discussion

Growth of Abalone

The average initial weight of abalone was 1.4 g (Figure 2). At low fish density, abalone weight was increased until at the end of experiment while at medium fish density, abalone weight increased on week 2 but decreased on week 4 and increased again on week 6. At high fish den-

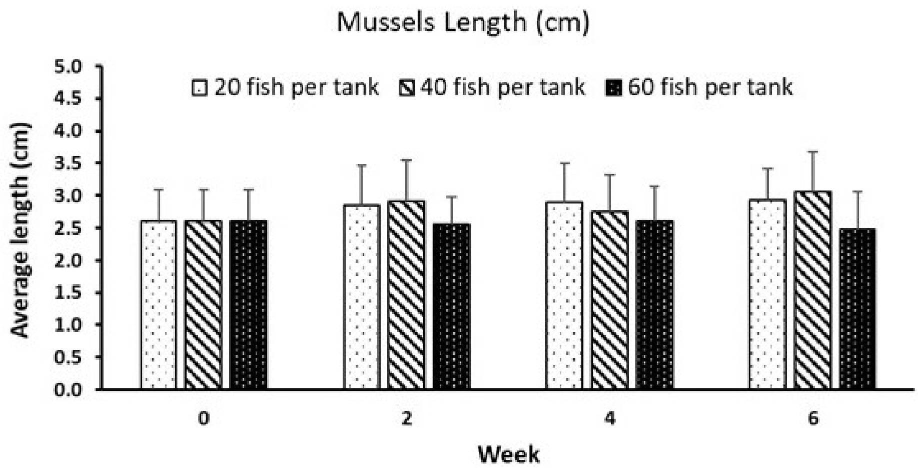

Figure 7. Average length of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density. Error bars are standard deviation.

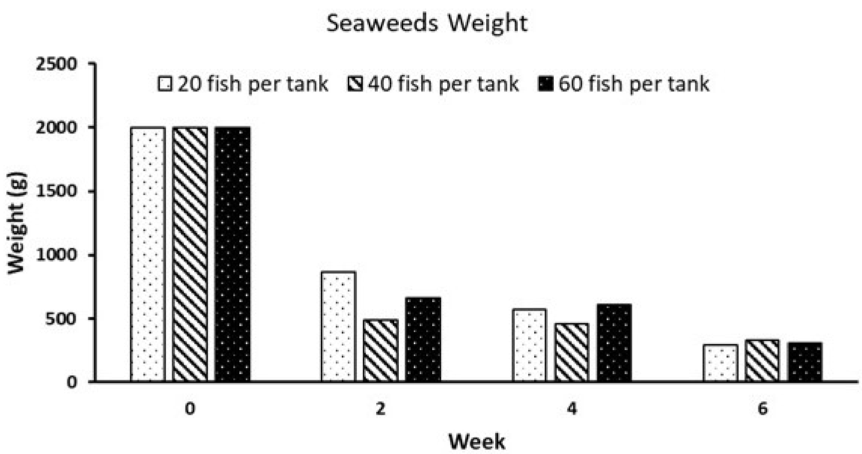

Figure 8. Weight of seaweeds at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density during the 6-week experiment period

sity, abalone weight decreased on week 2, increased on week 4 and decreased again on week 6. The average final weight was 2.2 g, 1.6 g and 1.2 g, at low, medium and high fish densities of 20, 40 and 60 individuals per tank, respectively. The P-value corresponding to the F-statistic of one-way ANOVA was less than 0.05 which indicated significant difference between treatments. Tukey's HSD test results showed no significant difference in abalone growth between Treatment 1 (low fish density) and Treatment 2 (medium fish density), and between Treatment 2 (medium fish density) and Treatment 3 (high fish density); however, there was significant difference between Treatment 1 (low fish density) and Treatment 3 (high fish density) as growth of abalone in Treatment 1 (low fish density) was higher. At high fish density, water quality was not as good (i.e. high ammonia and nitrite) as in low fish density, therefore, growth of abalone was better in the treatment with low fish density. As shown in the water quality data in section 3.5, ammonia, for example, was lower at low fish density.

The initial average length of abalone was 1.8 cm (Figure 3). At all three fish densities, abalone shell length increased although there were minor fluctuations among weeks. The average final length of abalone in relation to low, medium and high fish density was 2.2 cm, 2.1 cm, and 2.0 cm, respectively, without significant difference among them. Although there was significant difference in abalone weight between Treatment 1 (low fish density) and Treatment 3 (high fish density), there was no significant difference in terms of shell length indicating that body weight and shell length are not proportional or correlated and that weight is a better indicator of abalone growth.

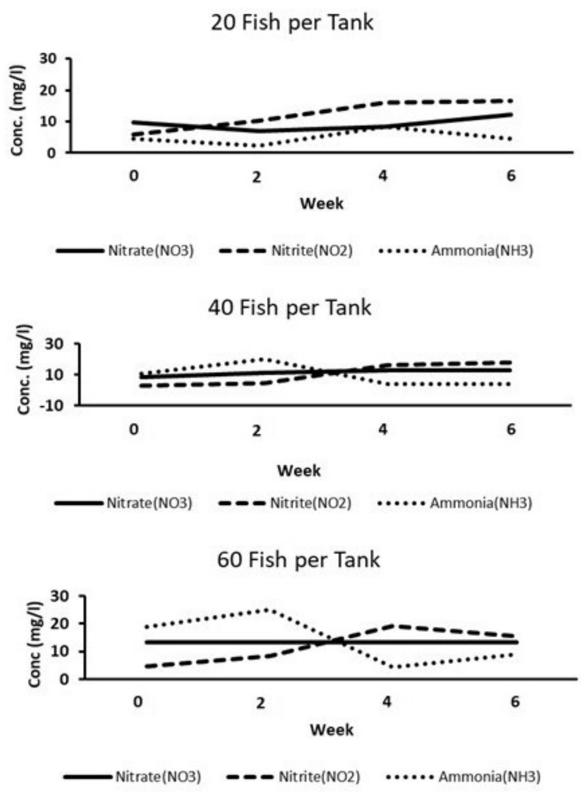

Figure 9. Concentration (mg/l) of ammonia, nitrite and nitrate at low, medium and high fish densities.

Growth and Survival of Seabass

The initial average weight of seabass was 14.4 g (Figure 4). Seabass weight increased from week 0 to week 6. The average final weights at low, medium and high density were 37.2 g, 33.6 g and 27.4 g, respectively. The P-value corresponding to the F-statistic of one-way ANOVA was higher than 0.05, suggesting that the treatments were not significantly different at different fish densities for that level of significance. The Tukey HSD test was also applied and showed the same result.

For commercial culture of sea bass in cages, stocking density of 15-20 fish/m^3 is recommended (Gaitan and Toledo, 2009). In the present experiment, the fish densities (20, 40 and 60/tank) in the 500-liter tanks are equivalent to 40, 80 and 120 fish/m^3 which are higher than the recommended stocking densities. In terms of biomass per cubic meter the initial densities tested are equivalent to 0.58, 1.15 and 1.73 kg/m^3. Ardiansyah and Fotedar (2016) reported that a stocking density of lower than 18.75 kg/m^3 is recommended for culturing in integrated recirculating aquaculture systems.

The initial average length of seabass was 9.4 cm. Its increase during the 6-week culture period is shown in Figure 5. The average final length of sea bass in Treatment 1 (20 fish/tank), Treatment 2 (40 fish/tank) and Treatment 3 (60 fish/tank) were 13.4 cm, 13.1 cm and 12.9 cm, respectively, and were not significantly different ($P>0.05$).

The fish survival rates were 100, 100 and 98% at low, medium and high fish density, respectively. The slightly higher mortality in the high density tank may be due to the relatively higher ammonia concentration observed in this tank. However, since the difference was not significant, this suggests that the fish densities used in the experiment can be also be used in commercial culture even if it is higher than the recommended density for commercial culture of seabass in non-IMTA system, at least for these relatively small fish.

Growth of Mussels

The initial average weight of mussels was 2.2 g. Its growth during the 6-week culture period is shown in Figure 6 with a decrease in mussel weight at high seabass density while at low and medium seabass density, mussel weight increased. The average final weights of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) fish densities were 2.6 g, 2.8 g and 1.5 g, respectively. The average final weight of mussels in combination with low and medium fish densities were significantly higher than with high seabass densities ($P = 0.026$), indicating that the high fish density did not result in good growth of the mussels. The number of mussels may have not been enough to filter the suspended particles coming from the tanks with high fish density.

The initial average length of mussels was 2.6 cm and its increase in length during the 6-week culture period is shown in Figure 7. The average final lengths of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) fish densities were 2.9 cm, 3.1 cm and 2.5 cm, respectively. Similar to the data on mussel weight, the growth in length of mussels was not high when combined with fish at high density. This suggests a need to increase the number of mussels in the next experiment.

Growth of Seaweeds

The initial average weight of seaweeds was 2,000 g. Figure 8 shows a significant decrease in biomass at week 2 and onwards. The final weights of seaweeds were 292.5 g, 327.3 g and 304.7 g, respectively, in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass densities.

Initially some seaweeds were taken and fed to the abalone but when their growth was not good, artificial feeds were instead given to the abalone. Overall there was a decrease in the final weight of seaweeds. This could be due to high temperature and the difference of environmental condition in the experiment area (Al-Hail)

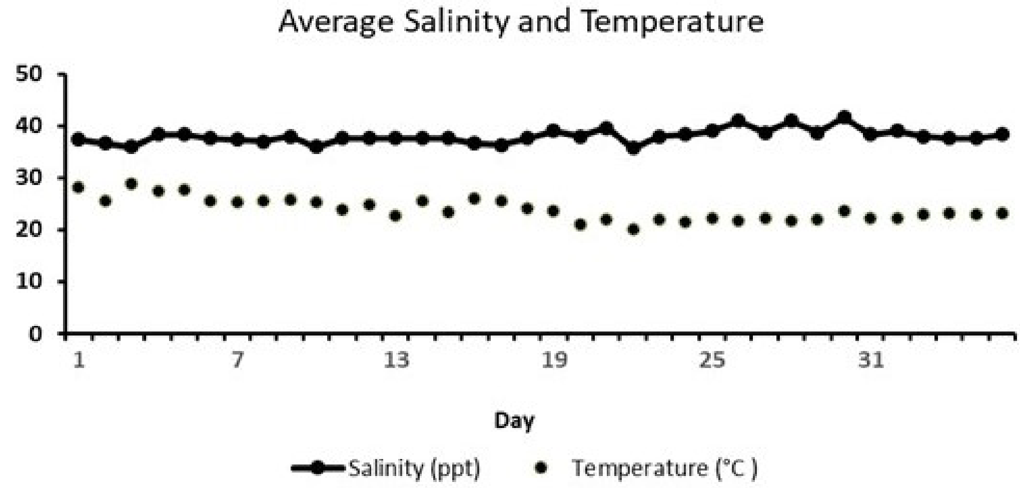

Figure 10. Average temperature and salinity in all tanks during the experiment period.

compared to the origin of seaweeds which were brought from Dhofar region which is usually cooler at temperatures ranging from 21 to 26 °C although the algae were acclimatized for one week prior to the experiment.

The low density of fish resulted in low waste production, thus, low production of nitrates needed for the seaweeds to grow. Yousef et al. (2014) suggested that increasing fish effluent flow in the seaweed culture tanks allows to duplicate the biomass yield. Also, they stated that the increase in water flow is adequate to maintain a high yield and that the stocking rate of 3 kg m^{-3} for Ulva seems to be the best one.

Water Quality

Concentrations of ammonia, nitrite and nitrate in the recirculating system are shown in Figure 9. At low fish density (20 per tank) the concentration of ammonia increased on week 4 and decreased on the week 6, while the nitrite increased on the week 4 and then levelled off and the nitrate increased on week 6. At medium fish density (40 per tank), ammonia increased on week 2 and decreased on weeks 4 and 6 while nitrite increased on week 4 and then levelled off and nitrate was gradually increasing. At high fish density (60 per tank), ammonia increased on week 2 and decreased on week 4 and increased on week 6 while nitrite increased on week 4 and decreased on the week 6 and nitrate was slightly increasing. These three cases indicate the conversion of ammonia to nitrite and then to nitrate but at medium and high fish densities, ammonia build up was earlier (week 2) than at low fish density (week 4).

Temperature and Salinity

Temperature in the culture tanks ranged from 20 to 29°C (Figure 10). At the beginning it was high during summer and then it decreased due to the start of winter season. In seabass culture, optimum temperature for growth and food conversion ranged between 26-32°C (Kungvankij et al., 1984). In our set up, salinity in abalone, seabass, mussels, and seaweed tanks ranged from 35.7 to 41.7 ppt. The reason why the salinity levels fluctuated could be due to addition of fresh water to lower the high salinity levels occurring in some tanks.

Dissolved Oxygen

Dissolved oxygen in seabass tanks ranged from 4.6 to 7.2 mg/l (Figure 11). At the beginning, the dissolved oxygen was high in all the tanks due to clean water used at the start of the experiment. Later on it started to decrease due to the increased production of waste which was acted upon by decomposing bacteria that consumed the oxygen along with the other species. This could also be due to the decrease in seaweeds biomass towards the later part of the culture period while dissolved oxygen level became constant as the waste utilization stabilized.

In the next experiments, we are considering to do the following: (i) increase the number of mussels in the biofilter tank in order to increase the filtration of suspended particles, (ii) add biofilter mat to increase the substrates (in addition to the mussels as substrates) for nitrifying bacteria, (iii) add sea cucumbers in the biofilter tank, for the utilization of detritus and pseudofeces of mussels, (iv) test other extractive species such as seaweeds particularly Gracilaria which have been found to be functioning as a natural filter for ammonia and nitrate (Largo et al., 2016), and (v) test the effect of partial recirculation (8-12 hours only) instead of 24 h recirculation, on water quality, growth and survival of cultured organisms and on the cost and benefit.

Conclusion

There was no significant difference in seabass growth and survival at densities of 20, 40, and 60 per 500-liter tank. However, the highest growth of abalone and mus-

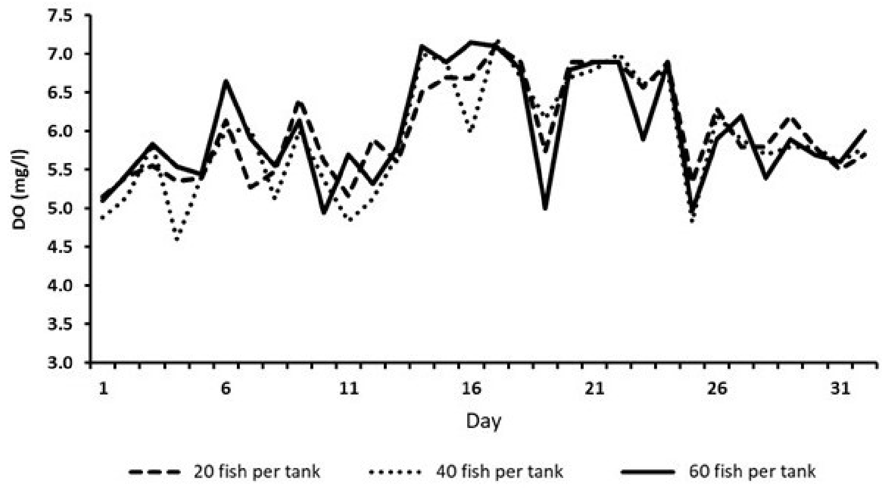

Figure 11. Dissolved oxygen (mg/l) at low, medium and high fish densities.

sels were in low and medium fish density (20 and 40 seabass per tank, respectively). Seaweeds showed a decrease in biomass during the experiment. Water quality parameters, such as temperature and dissolved oxygen were within optimum levels. Ammonia levels decreased as nitrite increased but in some cases it remained high while nitrates did not increase, indicating that nitrites were not converted to nitrates most likely due to the lack of efficient bio-filtration in the mussel tanks. This is the first report on the growth of the Omani abalone Haliotis mariae together with the Asian seabass Lates calcarifer, brown mussel Perna perna, and seaweed Ulva fasciata in a land-based recirculating integrated multi-trophic aquaculture (IMTA) system. Although results may not be highly conclusive due to lack of space and tanks for replication of treatments, the results are useful for further work to validate the findings that will lead to the development of land-based IMTA system especially for the Omani abalone.

Acknowledgements

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