

Original Research Article

Dynamics of Functional Diversity of Bacterioplankton in Fishponds and Potential Link to Fish Productivity

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Abstract

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This study investigated the community-level physiological profiling (CLPP) of bacterioplankton in yellow catfish (*Pelteobagrus fulvidraco*) intensive culture ponds during the growing season employing Biolog EcoPlates technology to study the dynamics of functional diversity of bacterioplankton communities and potential link to fish yield. It was found that bacterioplankton communities had significantly different patterns of carbon source utilization during different culture periods and that several indices of carbon source metabolic diversity of bacterioplankton communities tended to decrease significantly as the culture progressed. The McIntosh index of bacterioplankton communities during the mid and later period of the culture exhibited a significantly positive correlation with fish yield, suggesting that pond water with higher microbial metabolic diversity was inclined to have higher fish yield. The bacterioplankton communities with relatively higher rates of utilization for specific carbon sources during the specific month appeared to have a significant correlation with the culture yield under our experimental conditions. Therefore, the present study contributed to understand the physiological function dynamics of bacterioplankton communities and the interactions with fish yield in real-scale intensive yellow catfish culture pond. This finding will help us to integrate the knowledge into routine bacterial community management and culture practices to optimize fish yield.

Keywords: Pond aquaculture, Biolog Ecoplates, Microbial diversity, Culture yield

INTRODUCTION

In developing countries, aquaculture is considered to be a relatively easy entry point for practicing fishery productions particularly for small-scale producers, where pond culture forms the largest proportion of freshwater fisheries (Mischke, 2013). Freshwater fishpond area accounted for 44.71% of the freshwater aquaculture area in China and provided 44.46% of the total freshwater aquaculture production (The Fishery Bureau in Ministry of Agriculture of the People's Republic of China, 2017). As the most important aquaculture mode in China, fishpond production guarantees the aquatic product supply of modern society and provides the most efficient, high quality and inexpensive protein for humans. It is now well-established that the stabilization of fishpond production is much needed for the continued

development of sustainable aquaculture in China (Zhang et al., 2011).

The optimum fish production is dependent on the physical, chemical and biological qualities of water to most of the extent (Bhatnagar and Devi, 2013). Managing water quality within the tolerance limits to optimize growth, survival, production, product quality and feed conversion is challenging as the tendency of increasing intensification in static ponds (Iwama et al., 2000). Microorganisms have major roles in pond culture, particularly with respect to productivity, nutrient cycling, and the nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent (Moriarty, 1997; Fan et al., 2016). More recently, assessment has considered microorganisms as biological

indicators of water health (Murray et al., 2001). The metabolic dynamics of bacterioplankton communities reflect the microbial decomposition and mineralization capacity of culture water which could reveal the health status of environment (Yuan et al., 2010; Li et al., 2014). Microbial communities of aquaculture ponds play pivotal roles in pond productivity and fish production success, practicing fish culturists need to consider this variable in pond management decision-making (Kurten and Barkoh, 2014).

Biofloc technology is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or elevated carbon content of the feed. This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006). Thousands of hectares of commercial shrimp and tilapia production ponds, located in many countries, currently use the Biofloc technology (BFT approach). These systems are considered to be environmentally and a low-cost sustainable constituent to future aquaculture development (Bosma and Verdegem, 2011; Wang et al., 2015; Wang et al., 2016; Brito et al., 2016; Rego et al., 2017). The nutritional properties of the flocs are influenced by the type of carbon source used to produce the flocs (Crab, 2010; Crab et al., 2010a) and the choice of carbon source used for growing bioflocs is of prime importance (Wei et al., 2016). However, the indigenous microbiota present in the pond water will put forth a characteristic effect that needs to be considered (Crab et al., 2012). The questions about which substrates category were most utilized by the indigenous microbial communities and how they shift with the culture course has not been identified. We should find out the carbon utilization properties of the indigenous microbial communities first and then add the corresponding carbon source to use the biofloc technology used in aquaculture.

BIOLOG Eco-plates provide one method for determination of functional diversity indices and community-level physiological profiling (CLPP) of microbial populations based on carbon substrate utilization (Garland and Mills, 1991). Biolog Eco method has advantages over both classic cell culturing techniques and molecular level RNA amplification as these techniques are time consuming and require specialized expertise (Garland, 1997). By dealing with a large number of interdependently correlated variables associated with efficient and meaningful statistical methods the community-level physiological profiling of microbial populations can be obtained (Grove et al., 2004; Tiquia, 2008; Lyons and Dobbs, 2012) and can be used as a tool to assess ecosystem health (Dickerson and Williams, 2014). The CLPP method appears to be robust and enables assessment of heterotrophic microbial community functional characteristics such as relative diversity, similarity, and

community functional activity (Kurten and Barkoh, 2014). In recent years, applying of the technology in the research of functional diversity of microbial communities has been performed in aquaculture environment. The effects of application of *Bacillus* in tilapia pond on water metabolic functions of microbial communities were studied using Biolog Ecoplates (Yuan et al., 2010). Water microbial community function of different grass carp polyculture system and Hybrid Snakehead (*Channamaculata*♀×*Channa argus*♂) and largemouth bass (*Micropterus salmoides*) culture ponds in Southern China were compared (Li et al., 2014; Tian et al., 2012). The Biolog Ecoplates were also taken to gain perspective on the spatial and temporal variation of microbial communities in aquaculture systems of varying design (Kteily et al., 2014). Determination of the relation of functional diversity of microbial communities and fish productivity would present basic knowledge for evaluation of the importance of bacterioplankton on fish productivity and may provide a novel strategy for maintaining proper microbial diversity when applying the artificial improving practice especially the Biofloc technology. However, relatively little has been known about the correlations between water microbial properties and fish productivity in such studies.

For the study described herein, metabolic profiling methods were used (1) to provide profiles of the variation of metabolic capabilities of bacterioplankton communities during the whole culture course. (2) to determine any relationships between the function of the bacterioplankton community and the fish productivity, and the primary carbon sources that have effect on the fish yield.

MATERIAL AND METHODS

Site description

The study site was located in Jingzhou city (30°16' N, 112°18' E), Hubei Province (part of central China with a typical subtropical climate). Five earthen fishponds were assigned for the experiment. Each of the pond's surface area was 0.06 ha with an average depth of about 1.5 m. Before stocking the fish, all of the ponds were drained, and the bottom of the ponds was disinfected with lime. Water pumped from a nearby reservoir was then used to fill the culture pond to a depth of approximately 1.5 m; thereafter, the replacement of the water lost due to evaporation was mainly achieved from the groundwater and rainfall. In this study, a polyculture strategy for the stocking of the fish was adopted. Yellow catfish (*Pelteobagrus fulvidraco*) as the main culture specie (10000 ind/667 m², 7.8g/ind) were raised, mixed with a minor quantity of filter-feeding fish, Silver carp (*Hypophthalmichthys molitrix*, 200 ind/667m², 15.1g/ind). During the study, the fish in every pond was fed using

same feeding strategy. All the ponds were configured to bottom micropore aerator, timely start oxygen according to the weather and water dissolved oxygen condition.

Samples and analysis

Measurement of water quality

Water samples were collected respectively on June (the initial stage of culture), August (the middle stage of culture), and October (the latter stage of culture), 2012 which almost covered the consecutive production cycle. The mixed samples, consisting of central and surrounding water in a pond were collected at a depth of 10–15 cm from the surface and then stored in a sterile glass bottle (500 ml) at low temperature. Finally, samples were brought to a laboratory for measurements. Water quality parameters including electrical conductivity (EC), oxidation-reduction potential (ORP), pH and dissolved oxygen (DO), were measured in situ with a YSI6600 V2 multiparametric sonde (Yellow Spring Instruments, USA). The ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrite nitrogen ($\text{NO}_2^-\text{-N}$), total nitrogen (TN), total phosphorus (TP), COD and algal chlorophyll (Chl-a) were analyzed as related references (State EPA of China, 2002).

Measurement of total bacterial counts

The method of Liao et al. (2012) was used for the enumeration of total bacterial counts by epifluorescence microscopy with the mud procedure eliminated. 80 μL water diluents were collected to a centrifuge tube where 10 μL ($10\times$) SYBR Green I fluorochrome was added. After shady staining for 1 min, 10 μL anti fading agent was added to make staining samples. Finally, enumeration data can be observed with a fluorescence microscope.

Community-level metabolic fingerprinting of bacterioplankton

Biolog Ecoplates (BIOLOG Inc., Hayward CA., USA) were used to determine the community-level physiological profiling of bacterioplankton populations in the fishpond. Biolog-Ecoplates are 96-well microtiter plates containing 31 carbon sources and a blank in triplicate. Each well contains the redox dye tetrazolium violet in a dry film together with the carbon source. As the carbon source is oxidized, formazan is formed which can be quantified spectrophotometrically. Taking microbial metabolic pathway of three major nutrients as the basic delineation standards, the 31 carbon sources in Biolog Ecoplates were divided into four types. They were carbohydrates and their derivatives (12 kinds), amino acid and their derivatives (6 kinds), fat acid and lipid (5 kinds),

metabolic mediators and secondary metabolites (8 kinds) (Zhang et al., 2009).

Each well of the plate was inoculated with 150 μL of water sample and then the plates were incubated in the dark at 25°C. Color development in the plates was measured every 24h at 590nm till the end of incubation of 168 h by using a Biolog reader. Using absorbance of wells in the plates to characterize functional diversity of microbial community, the analysis was performed as follows: a raw absorbance value for each well was blanked against the control well, and negative absorbance values were set to zero according to Garland (1997). The diversity indices including Shannon index (H'), Simpson index (D), Shannon evenness index (E_h), McIntosh index (U), and McIntosh evenness index (E_u) were used to assess water microbial functional diversity (Gomez et al., 2006). The Simpson index is weighted toward the abundances of the most common species, and the Shannon index indicates the richness of water microorganisms (Magurran, 1988), whereas the McIntosh index indicates the evenness or homogeneity of water microorganisms (Atlas, 1984). All the parameters were determined by calculating the mean of every well's absorbance value after 72h of incubation, which corresponded to the time of maximal microbial growth in the plates.

Shannon index:

$$H' = -\sum P_i \ln P_i$$

Shannon evenness:

$$E_h = H' / \ln S$$

Simpson index:

$$D = \sum n_i(n_i - 1) / [N(N - 1)]$$

McIntosh index :

$$U = \sqrt{\sum (n_i^2)}$$

McIntosh evenness :

$$E_u = (N - U) / (N - N / \sqrt{S})$$

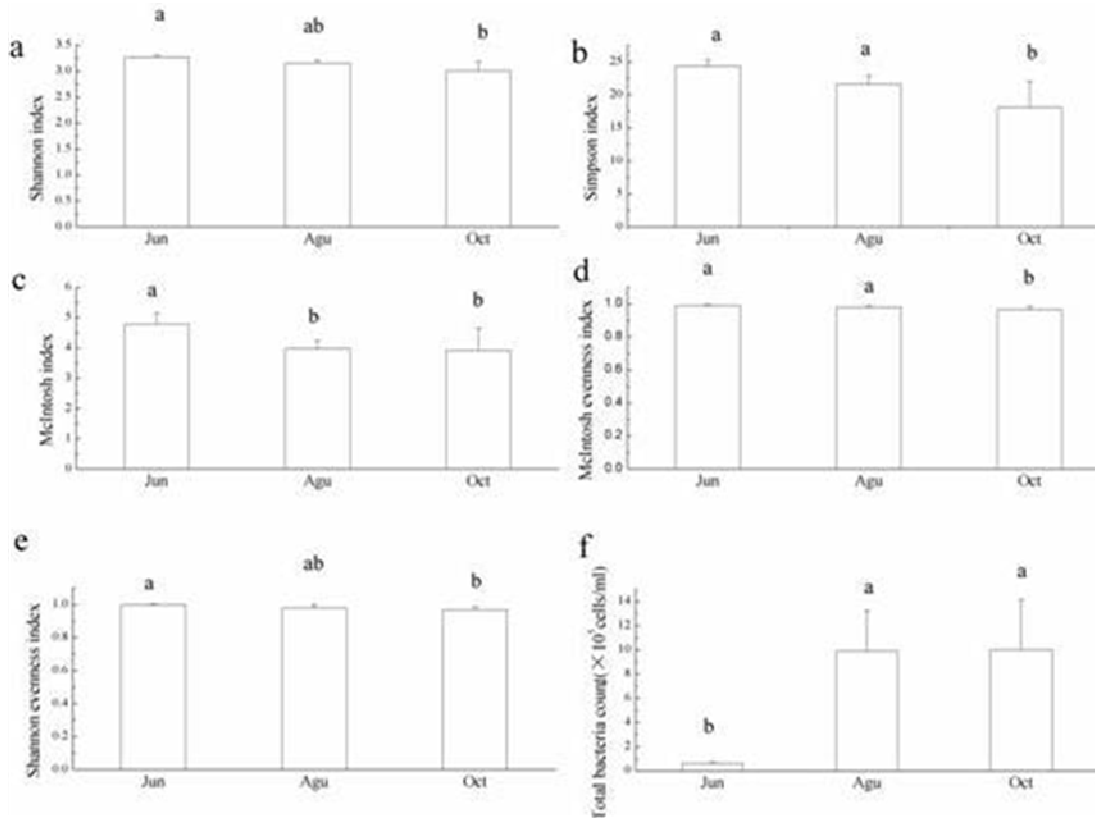
Where P_i is the proportion of the absorbance of each well to the sum of absorbance of all wells; N is the total absorbance values of all wells, and S is the number of wells with the substrate being used ($\text{OD}_{590} > 0.15$) by the community (Zhang *et al.*, 2013). The Simpson index is expressed as the reciprocal ($1/D$).

Data analysis

Statistical analyses were performed using the SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Differences between means for diversity indices in different months were compared using a one-way analysis of variance (ANOVA). The microbial utilization on carbon sources was analyzed with principle components analysis (PCA). Pearson Correlation analysis was used to analyze the relationship between the diversity of bacterioplankton communities and fish yield and the relationship between relative utilization of sole carbon source and fish yield.

Table 1. The physical-chemical parameters of water in the fishpond

Parameter	EC, μ S/cm	DO,mg/L	pH	ORP,mV	NO ₂ ⁻ -N,mg/L	NH ₄ ⁺ -N,mg/L	COD,mg/L	TN,mg/L	TP,mg/L	Chl-a, μ g/L
Value	444 \pm 8	3.04 \pm 0.10	7.39 \pm 0.02	94 \pm 1	0.086 \pm 0.044	1.64 \pm 0.13	10.5 \pm 0.5	3.75 \pm 0.05	0.36 \pm 0.06	79.2 \pm 18.3

**Figure 1.** Dynamics of metabolic diversities of bacterioplankton during the culture period

^a Columns followed by the same letter are not significantly different at $P < 0.05$

The values were considered to be significantly different at a 95% confidence level. The values in the figures and tables correspond to the average data \pm standard deviations (SD).

RESULTS

The physical-chemical characteristics of the water quality

The mean variations recorded for water physico-chemical parameters of the fishponds during the experimental period from 8th June to 15th October, 2012 were shown in table 1. It was observed that the DO, conductivity, ORP and pH in the pond water was averaged as 3.04 mg/L, 444 μ S/cm, 94 mV and 7.39 in the whole farming period respectively. The TN, TP, NH₄⁺-N, NO₂⁻-N, COD and Chl-a content were averaged as 3.75 mg/L, 0.36 mg/L,

1.64 mg/L, 0.086 mg/L, 10.5 mg/L and 79.2 μ g/L on the sampling days respectively.

Dynamics of community-level physiological profiling of bacterioplankton in the fishpond

As shown in figure 1, the values of the McIntosh evenness index and Simpson index of bacterioplankton communities in the fishpond were continuously decreased along the culture course and significant difference was observed in October (later period of culture course) ($P < 0.05$), whereas the McIntosh index decreased significantly in August (middle period of culture course) and continuously declined in October although the difference was not significant. The Shannon index and Shannon evenness index in October were significantly lower than that in June but not significant lower than that in August ($P < 0.05$). The results showed

Table 2. Correlation coefficients of different carbon sources with the first two principal components ($|r| > 0.8$)

Carbon source type	Carbon source	Jun		Aug		Oct	
		PC1	PC2	PC1	PC2	PC1	PC2
Carbohydrates and their derivants	D-Xylose		0.929		-0.819		
	I-Erythritol	0.907					
	D-Galacturonic Acid		0.942	0.953			0.877
	D-Galactonic Acid γ -Lactone			0.953			-0.997
	β -Methyl-D-Glucoside						-0.862
	D-Mannitol						-0.994
	N-Acetyl-D-Glucosamine						-0.945
	D-Cellobiose						-0.995
	D-Glucosaminic Acid						0.960
	α -Cyclodextrin						0.908
Metabolic mediates and secondary metabolites	Glucose-1-Phosphate	-0.868					
	D,L- α -Glycerol Phosphate	-0.954					
	4-Hydroxy Benzoic Acid	-0.916					
	α -Ketobutyric Acid			0.870			
	D-Malic Acid			-0.980			
	2-Hydroxy Benzoic Acid			0.928			
Amino acid substrates and their derivants	L-Threonine	-0.819					
	L-Arginine						0.836
	Glycyl-L-Glutamic Acid			0.952			
	L-Asparagine						0.859
	L-Serine						0.916
	Itaconic Acid	0.915			0.966		0.943
Fat acid and lipid	Pyruvic Acid Methyl Ester				-0.935		
	γ -Hydroxybutyric Acid			0.948			-0.865

coefficient) in June. And the 8 kinds of carbon sources included 3 kinds of carbohydrates and their derivants (I-Erythritol, D-Xylose and D-Galacturonic Acid), 1 kind of amino acid substrates and their derivants (L-Threonine), 1 kind of fat acid and lipid (Itaconic Acid) and 3 kinds of metabolic mediates and secondary metabolites (Glucose-1-Phosphate, D,L- α -Glycerol Phosphate and 4-Hydroxy Benzoic Acid). There were mainly 10 kinds of carbon sources which had effects on the first two principal components in August. And the 10 kinds of carbon sources included 3 kinds of carbohydrates and their derivants (D-Xylose, D-Galacturonic Acid and D-Galactonic Acid γ -Lactone), 1 kind of amino acid substrates and their derivants (Glycyl-L-Glutamic Acid), 3 kinds of fat acid and lipid (Itaconic Acid, Pyruvic Acid Methyl Ester and γ -Hydroxybutyric Acid), 3 kinds of metabolic mediates and secondary metabolites (α -Ketobutyric Acid, D-Malic Acid and 2-Hydroxy Benzoic Acid). In the same way, there were mainly 13 kinds of carbon sources which had impacts on the first two principal components in October. And the 13 kinds of

carbon sources included 8 kinds of carbohydrates and their derivants (D-Galacturonic Acid, D-Galactonic Acid γ -Lactone, β -Methyl-D-Glucoside, D-Mannitol, N-Acetyl-D-Glucosamine, D-Cellobiose, D-Glucosaminic Acid and α -Cyclodextrin), 3 kinds of amino acid substrates and their derivants (L-Arginine, L-Asparagine and L-Serine), 2 kinds of fat acid and lipid (γ -Hydroxybutyric Acid and Pyruvic Acid Methyl Ester). The results above indicated that the carbon source playing the primary role of distinguishing the bacterioplankton communities varied markedly along the culture course and the influential carbon source that categorized into amino acid substrates and their derivants and carbohydrates and their derivants were increased while the carbon belong to metabolic mediates and secondary metabolites decreased.

Correlations between yellow catfish yield and functional diversity of bacterioplankton

The relationships between bacterioplankton diversity par-

Table 3. Pearson correlations between functional diversity indices of bacterioplankton and fish yield

Diversity indices	Month	Yield
Shannon index	Jun	-0.43
	Aug	0.27
	Oct	0.73
Simpson index	Jun	-0.03
	Aug	0.31
	Oct	0.77
Shannon evenness index	Jun	-0.23
	Aug	0.71
	Oct	0.44
McIntosh index	Jun	0.57
	Aug	0.938 ^a
	Oct	0.942 ^a
McIntosh evenness index	Jun	0.86
	Aug	0.78
	Oct	0.66
Total bacterial count	Jun	0.24
	Aug	0.18
	Oct	0.73

^a * $P < 0.05$

Table 4. Pearson correlations between relative utilization of sole carbon source and fish yield

Month	Carbon source type	Carbon Source	Yield
Jun	Carbohydrates and their derivants	D-Galactonic Acid γ -Lactone	0.891 [*]
		Itaconic Acid	0.930 [*]
Aug	Carbohydrates and their derivants	α -D-Lactose	-0.881 [*]
		D-Xylose	0.931 [*]
Oct	Carbohydrates and their derivants	D-Galacturonic Acid	0.909 [*]
		D-Glucosaminic Acid	0.926 [*]
	Amino acid substrates and their derivants	L-Asparagine	0.912 [*]
		Metabolic mediates and secondary metabolites	D,L- α -Glycerol Phosphate

^a * $P < 0.05$, the correction coefficient not significant is not presented.

ameters in fishpond and yellow catfish culture yield were depicted by Pearson Correlation Analysis (table 3). The results showed that the culture yield of yellow catfish was significantly correlated with the McIntosh index in both August and October ($r=0.938$, $P < 0.05$; $r=0.942$, $P < 0.05$). Furthermore, it is worth noticing that the culture yield was positively correlated with almost all diversity indices and total bacterial count in October with high correlation coefficients although the correlations were too weak to be significant. Such results illustrated that the yellow catfish culture yield was positively correlated with the metabolism diversity of bacterioplankton communities in fishpond during the mid and later period of culture course.

Table 4 showed the specific correlation between the sole carbon source relative utilization rate of the bacterioplankton communities in fishpond and fish yield. Among the 31 types of carbon sources in Biolog Eco plates, the yellow catfish culture yield was positively correlated with the utilization rates of D-Galactonic Acid γ -Lactone (carbohydrates and their derivants) and Itaconic Acid (carbohydrates and their derivants) in June, α -D-Lactose (carbohydrates and their derivants) in August and D-Xylose (carbohydrates and their derivants), D-Galacturonic Acid (carbohydrates and their derivants), L-Asparagine (amino acid substrates and their derivants), D-Glucosaminic Acid (metabolic mediates and secondary metabolites) and D,L- α -Glycerol (metabolic mediates and

secondary metabolites) in October respectively. The results demonstrated that the culture yield could be influenced by the carbon substrate utilization patterns of bacterioplankton in the fishpond and the correlated carbon substrates were highly variable along the culture course with the most correlated carbon substrates and carbon categories occurred in the later period of culture.

DISCUSSION

The average pH values of 7.4 recorded in this study was within pH values of 6.5 -9.0 which is regarded as ideal values in an aquaculture pond (Wurts and Durborow, 1992; Bhatnagar and Devi, 2013). Mean DO value of 3.04 mg/L recorded was unproductive as it should be above 5.0 mg/L for good production (Banerjee, 1967), but it was in desirable range. The concentration of total NH_4^+ -N and NO_2^- -N was in desirable range of 0-2 mg/L and 0-1 mg/L (Stone and Thomforde, 2006). However, the TN, TP and COD content were in high level and demonstrated that the ponds were under eutrophic status (Prapaiwong and Boyd, 2012). Thus, the fluctuations of water physico-chemical parameters recorded were within the acceptable range for the freshwater fish culture throughout farming phase to guarantee an acceptable fish production.

Microbial population diversity index is a comprehensive index for revealing species richness and evenness, and its change could accurately reveal the total dynamic change in metabolism diversity of microbial population (Zhang *et al.*, 2013). A collapse in bacterial species diversity might indicate impending environmental stress and disease. Therefore, a better understanding of bacterial community function could lead to improved fish husbandry practices (Arias *et al.*, 2006). The functional diversity of water microbes inferred from the CLPP data is useful in evaluating capacity of bacterial communities to utilize a set of sole carbon and monitoring carbon sources utilization changes in microbial diversity (Derry *et al.*, 1998). A larger diversity index indicated that the microbial community functional diversity was higher; on the contrary, the smaller the diversity index was, the lower the microbial community functional diversity was. The larger the evenness index (E) was, the more evenly the individuals distributed (Zhang *et al.*, 2013). It was found that carbon source metabolism indices of the bacterioplankton communities in yellow catfish culture water, namely Shannon index, Simpson index, Shannon evenness index, McIntosh index, and McIntosh evenness index was inclined to decrease significantly as the culture continued with an increasing trend of total bacteria count on the contrary. Such result was comparable with the results of Yan *et al.* (2014) who observed that carbon metabolism diversity indices including Shannon and Simpson index of aquatic microorganism in *Stichopus japonicus* pond were lower in summer and autumn and

and was partially consistent with the result that bacterioplankton communities experience a dramatic drop in richness in late summer (Kent *et al.*, 2004). The overall water qualities in the ponds were mainly excellent during the early period and deteriorated in the mid to late period (Gao *et al.*, 2009). It might be speculated that the similar trends of the decrease of microbial diversity could possibly relate to the deteriorated water quality during the later period of culture course when harmful substances accumulated and may reflect on some microbial functions in the water.

Sample distribution in the PC axis was relevant to carbon source substrates utilization capacity of microbes. From the PCA results above, it could be seen that bacterioplankton communities distributed intently and had their unique models of carbon utilization during the different culture period, with the strongest capacity of carbon utilization in October. It turned out that bacterioplankton communities in October dispersed separately and the bacterioplankton communities of the five fishponds had different features of carbon utilization in October. The carbon sources playing the primary role of distinguishing the bacterioplankton communities were highly variable in different culture period. Furthermore, the influential carbon source that categorized into amino acid substrates and their derivants and carbohydrates and their derivants were increased while the carbon categorized into metabolic mediates and secondary metabolites decreased in this study. Apart from the temperature difference, the reason was mainly associated with the changes of carbon source quantity and quality which was provided to bacterioplankton communities. Nowadays, most ponds are fed, either by home-made or industrial aquafeeds (Tacon, 2007). However, not all nutrients from artificial feed are assimilated into fish tissue and a large fraction of the ration administered to the pond is instead released into the pond environment. Uneaten feed continuously accumulated in pond water that can lead to high concentration of carbohydrates, lipid and amino acids in the pond water (Cao *et al.*, 2014), especially for the high protein feed (38% crude protein, 3.5% fat, 5% cellulose and 1.4% TP) fed for the yellow catfish during the culture course. The changes in the carbon source substrate utilization observed in the current study were likely the result of an adaptation to the specific conditions in the ponds.

The result that carbon source utilization McIntosh index of bacterioplankton communities during the mid and later period of culture course were positively correlated to pond culture yield implied a different pattern in carbon utilization for bacterioplankton communities in fishpond with different production level. The yellow catfish is a bottom omnivore fishes which can possibly directly graze on organic detritus in water and epibacteria, i.e. bacteria attached to the suspended organic particles may be a carbon source to the cultured yellow catfish which

may ultimately cause the changes in bacterioplankton community's structure and the high bacterioplankton diversity that corresponded to superior water quality may help the fish growth. As a result, it could be speculated that the fishpond with higher bacterioplankton diversity can decompose and turnover more residual feed demonstrating a preference for higher fish productivity. The Pearson Correlation analysis identified the community level physiological profiling of bacterioplankton communities that correlated with culture yield. Through the association of the carbon source utilization pattern of bacterioplankton communities with fish production helped to elucidate that the bacterioplankton with relatively higher utilization rates of these carbon sources in the specific month appeared to have positively/negatively influence on the culture yield under our experimental condition to some extent. There is certain difference in metabolism diversity of aquatic microorganism under different culture modes, which may possibly have direct relation with the difference in pond culture management modes (such as feedstuff adding etc.) (Li *et al.*, 2014). Based on the above findings, management practice such as supplementary of the positively related carbon source to increase the diversity and metabolism ability of probiotics in the pond could be taken as a water quality improvement practice to enhance the fish productivity from a production standpoint.

Biolog technology can quickly and simply acquire the information related to the structure and function diversity of microbial population (Toolan *et al.*, 1991). However, limitations of Biolog Ecoplates should also be taken into consideration. The method can only focus on easily cultivable microorganisms and mainly fast growing microorganisms are involved (Cycoń *et al.*, 2013; Zak *et al.*, 1994). The biological functions of microorganism can be disclosed more effectively and comprehensively by a further and long-term monitoring analysis in future by using molecular biology and other means. This paper discussed merely the correlation between community level metabolism diversity characteristics of bacterioplankton communities and culture yield on the statistical level in the case of yellow catfish culture. Further analysis is needed to determine if observed correlations are causal. The environmental factors affecting culture yield of farmed fishes needed to be analyzed by comprehensive analysis including water quality and various growth demands of culture varieties in this regard. Therefore, further research is required before a firm conclusion can be obtained.

CONCLUSIONS

In the present study, microbial functional diversity in yellow catfish ponds was assessed by Biolog EcoPlates. Several indices of carbon source metabolic diversity of bacterioplankton communities in yellow catfish culture

ponds, including the Shannon index, Shannon evenness index, McIntosh index, and McIntosh evenness index, tended to decrease significantly as the culture progressed. The McIntosh index of bacterioplankton communities during the mid and later period of the cultures exhibited a significant positive correlation with fish yield, suggesting that fishponds with higher bacterioplankton metabolic diversity were inclined to have higher productivity. The Principal component analysis (PCA) demonstrated that the carbon sources playing the primary role of distinguishing microbial communities were highly variable throughout the course of the culture, with the highest number of correlated carbon substrates and types being found later in the culture. The bacterioplankton communities that have relatively high rates of utilization for specific carbon sources in each specific month appeared to have a significant influence on the culture yield under our experimental conditions. This result indicates that community-level physiological profiling (CLPP) of bacterioplankton can be applied to evaluate fishpond productivity potential. This research has contributed to the understanding of interactions between the physiological dynamics of bacterioplankton communities and fish yield in real-scale intensive commercial yellow catfish culture systems. Further investigations are required to determine if the observed correlations between bacterioplankton communities and production yield are causative

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REFERENCES

- Arias, C.R., Abernathy J.W., Liu Z., 2006. Combined use of 16S ribosomal DNA and automated ribosomal intergenic space analysis to study the bacterial community in catfish ponds. *Lett. Appl. Microbiol.* 43, 287-292.
- Atlas, R.M., 1984. Diversity of microbial communities. *Adv. Microb. Ecol.* 7, 1-47.
- Banerjee, S.M., 1967. Water quality and soil condition of fishponds in some states of India in relation to fish production. *Indian J. Fish.* 14, 115-144.
- Bhatnagar, D., Devi, P., 2013. Water quality guidelines for the management of pond fish culture. *Int. J. Environ. Sci.* 3, 1980-1996.
- Bosma, R.H., Verdegem, M.C.J., 2011. Sustainable aquaculture in ponds: principles, practices and limits. *Livest. Sci.* 139, 58-68.
- Brito, C., Valle, B., Interaminense, J., Peixoto, S., Lima-Filho, J.V., Roberta, S., 2016. Microbiological quality of *Litopenaeus vannamei* culture using conventional and biofloc systems. *Aquacult. Res.* 47, 3098-3108.
- Cao, Y. C., Li, Z. J., Wen, G. L., Yuan, C. L., Yang, Y. Y., Hu, X. J., Lin, X. T., 2014. Dynamics of microbial community and its metabolisms of different carbon sources in tilapia ponds. *J. Agro-Environ. Sci.* 33, 172-177.

- Crab R., Defoirdt T., Bossier P., Verstraete W., 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture* 356-357, 351-356.
- Crab, R., 2010. Bioflocs technology: an integrated system for the removal of nutrients and simultaneous production of feed in aquaculture. PhD thesis, Ghent University. pp. 178.
- Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W., 2010a. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* post larvae. *Aquacult. Res.* 41, 559–567.
- Cycoń, M., Markowicz, A., Borymski, S., Wójcik, M., Piotrowska-Seget, Z., 2013. Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. *J. Environ. Manage.* 131, 55-65.
- Derry, A.M., Staddon, W.J., Trevors, J.T., 1998. Functional diversity and community structure of microorganisms in uncontaminated and creosote-contaminated soils as determined by sole-carbon-source-utilization. *World J. Microbiol. Biotechnol.* 14, 571-578.
- Dickerson, T.L., Williams, H.N., 2014. Functional Diversity of Bacterioplankton in Three North Florida Freshwater Lakes over an Annual Cycle. *Microb. Ecol.* 67, 34-44.
- Fan, L.M., Barry, K., Hu, G.D., 2016. Bacterioplankton community analysis in tilapia ponds by Illumina high-throughput sequencing. *World J. Microbiol. Biotechnol.* 32, 1-11.
- Gao, P., Jiang, M., Zhao, Y.J., Wu, F., Liu, W., Leng, X.J., Wen, H., 2009. Variation rules of water quality and budget of nitrogen and phosphorus in ponds with grass carp as the dominant cultured species. *J. Yunnan Agricultural University*, 24, 71-77.
- Garland, J. L., 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol. Ecol.* 24, 289-300.
- Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.* 57, 2351-2359.
- Gomez, E., Ferreras, L., Toresani, S., 2006. Soil bacterial functional diversity as influenced by organic amendment application. *Bioresour. Technol.* 97, 1484-1489.
- Grove, J.A., Kautola, H., Javadpour, S., Moo-Young, M., Anderson, W.A., 2004. Assessment of changes in the microorganism community in a biofilter. *Biochem. Eng. J.* 18, 111-114.
- Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. *Aquacult. Eng.* 34, 344-363.
- Iwama, G.K., Vijayan, M.M., Morgan, J.D., 2000. The stress response in fish. *Ichthyology, recent research advances.* Oxford and IBH Publishing Co, Pvt. Ltd, New Delhi
- Kent, A.D., Jones, S.E., Yannarell, A. C., Graham, J.M., Lauster, G.H., Kratz, T.K., Triplett, E.W., 2004. Annual patterns in bacterioplankton community variability in a humic lake. *Microb. Ecol.* 48, 550-560.
- Kteily, N.S., 2014. Microbial community characterization and pathogen profiling of land-based aquaculture systems using culture-based and molecular-based fingerprinting techniques. Theses and Dissertations (Comprehensive). 1691. Wilfrid Laurier University.
- Kurten, G.L., Barkoh, A., 2016. Evaluation of community-level physiological profiling for monitoring microbial community function in aquaculture ponds. *N. Am. J. Aquacult.* 78, 33-44.
- Li, Z.F., Xie, J., Yu, E.M., Wang, G.J., 2014. Carbon metabolic diversity of microbial communities in intensive ponds for hybrid snakehead and largemouth bass based on Biolog-ECO Plates. *J. Agro-Environ. Sci.* 33, 185-192.
- Liao, M.J., He, X.G., Xie, C.X. A fluorescence microscopic counting detection method of bacteria in soil and sediment. *Huazhong Agricultural University, CN201110187705.5.* 2013
- Lyons, M.M., Dobbs, F.C., 2012. Differential utilization of carbon substrates by aggregate-associated and water-associated heterotrophic bacterial communities. *Hydrobiologia* 686, 181-193.
- Magurran, A. E., 1988. *Ecological diversity and its measurement.* Princeton University Press, New Jersey, USA.
- Mischke, C.C., 2012. Aquaculture pond fertilization: impacts of nutrient input on production. *Aquaculture Pond Fertilization.* pp. 65-72.
- Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture* 151, 333-349.
- Murray, K.S., Fisher, L.E., Therrien, J., George, B., Gillespie, J., 2001. Assessment and use of indicator bacteria to determine sources of pollution to an urban river. *J. Great Lakes Res.* 27, 220-229.
- Prapaiwong, N., Boyd C.E., 2012. Effects of major water quality variables on shrimp production in inland, low-salinity ponds in Alabama. *J. World Aquacult. Soc.* 43, 349-361.
- Rego, M.A.S., Sabbag, O.J., Soares, R., Peixoto, S., 2017. Risk analysis of the insertion of biofloc technology in a marine shrimp *Litopenaeus vannamei*, production in a farm in Pernambuco, Brazil: A case study. *Aquaculture* 469, 67-71.
- State EPA of China, 2002. *Monitoring and Determination Methods for Water and Wastewater.* 4th ed., China Environmental Science Press, Beijing, China.
- Stone, N. M., Thomforde, H.K., 2006. *Understanding your fish pond.* Water analysis report. Cooperative Aquaculture/Fisheries Extension Program, University of Arkansas . Pine Bluff .US Department of Agriculture and County Governments cooperating.
- Tacon, A.G.J., 2007. Global aquaculture production highlights and estimated compound aquafeed use in 2005. *Int. Aquafeed.* 10, 40-44.
- The Fishery Bureau in Ministry of Agriculture of the People's Republic of China, 2017. *Fishery Yearbook*, Beijing, China.
- Tian, X.L., Zheng, Y.Y., Liu, B.J., 2012. Abundance dynamics and community functional diversity of bacteria in grass carp polyculture systems. *Periodi. Ocean Uni. Chi.* 42, 19-27.
- Tiquia, S.M., 2010. Metabolic diversity of the heterotrophic microorganisms and potential link to pollution of the Rouge River. *Environ. Pollut.* 158, 1435-1443.
- Toolan, T., Wehr, J. D., Findlay, S., 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. *Appl. Environ. Microb.* 57, 2074-2078.
- Wang, C., Pan, L., Zhang, K.Q., Xu, W.J., Zhao, D., Lin M., 2016. Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. *Aquacult. Res.* 47, 3307-3318.
- Wang, G.J., Yu, E.M., Xie, J., Yu, D.G., Li, Z.F., Luo, W., Qiu, L.J., Zheng, Z.L., 2015. Effect of C/N ratio on water quality in zero-waste exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus* . *Aquaculture* 443, 98-104.
- Wei, Y.F., Liao, S.A., Wang, A.L., 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. *Aquaculture* 465, 88-93.
- Wurts, W.A., Durborow, R.M., 1992. Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. *SRAC publication.* 464.
- Yan, F.J., Tian, X.L., Dong, S.L., Yang, G., 2014. Seasonal variation of functional diversity of aquatic microbial community in *Apostichopus japonicus* cultural pond. *Chin. J. Appl. Ecol.* 25, 1499-1505.
- Yuan, C.L., Li, Z.J., Yang, Y.Y., Lin, X.T., 2010. Effects of *Bacillus* preparation on metabolic function of microbial communities in tilapia ponds at early stock stage. *Chi. J. Ecol.* 29, 2464-2470.
- Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.* 26, 1101-1108.
- Zhang, H.F., Li, G., Song, X.L., Yang, D.L., Li, Y.J., Qiao, J., Zhang, J.N., Zhao, S.L., 2013. Changes in soil microbial functional diversity under different vegetation restoration patterns for Hulunbeier Sandy Land. *Acta Ecol. Sin.* 33, 38-44.
- Zhang, S.Y., Li, G., Wu, H. B., Liu, X.G., Yao, Y.H., Tao, L., Liu, H., 2011. An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *Aquacult. Eng.* 45, 93-102.
- Zhang, Y.Y., Qu, L.Y., Chen, L.D., 2009. An amendment on information extraction of Biolog EcoPlate™. *Microbiology* 36, 1083-1091.