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Application of photosynthetic microalgae as efficient pH bio-stabilizers and bio-purifiers in sustainable aquaculture of *Clarias gariepinus* (African catfish) fry

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Abstract

***Chlorella lewinii* LC172265 and *Scenedesmus dimorphus* NIES-93 were grown with BG-11 medium and then transferred to fish seedlings' farm and the effects on changes in the pH, nitrite, total ammonia nitrogen and toxic ammonia were studied. Inoculation of the ponds with *C. lewinii, S. dimorphus* and the combined culture of *C. lewinii* and *S. dimorphus* significantly reduced the rise in the pH of the ponds ($p<0.05$). With these three cultures, the pond pH increased from 6.3±0.03 to only 6.5±0.2, 6.7±0.6 and 6.4±0.1 respectively within a period of 240 hours, as against control pond's pH which increased from 6.3±0.03 to 9.0±0.1 within the same period. Furthermore, inoculation with *S. dimorphus* reduced the nitrite concentration in the ponds to zero on the 144th hour and the concentration remained zero throughout the experiment. This was closely followed by the combined culture of *C. lewinii* and *S. dimorphus* which reduced the nitrite concentration to zero at 240th hour while *C. lewinii* was the least in nitrite removal. Toxic ammonia was also zeroed by the combined culture of *C. lewinii* and *S. dimorphus* at 144th hour of experiment which was followed by *C. lewinii* (at 192nd hour). Aquaculture ponds co-cultured with microalgae witnessed a maximum fry death rate of 40% which was much lower than 80% death rate observed in the control ponds. These results show that *C. lewinii* and *S. dimorphus* are very efficient in sustaining the quality of aquaculture water, and thus prolonging the length of time water can be used before changing.**

Keywords: nitrogen removal, ammonia, nitrite, fishery, fish pond

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Introduction

Fisheries and aquaculture play a critical role in food and nutrition security and in providing for the livelihood of several millions of people. Aquaculture supplies more than half of all the fish for human consumption (FAO, 2016) and also generates employment opportunities which have grown faster than employment in traditional agriculture with about 56 million people directly engaged in the fisheries sector (FAO, 2014; FAO Committee on Fisheries, 2014; FAO, 2016; Umaru et al., 2016). Fish consumption provides protein and a range of other nutrients, particularly essential fatty acids (such as the long-chain polyunsaturated fatty acids (LCPUFA)), minerals and vitamins (Beveridge et al., 2013; Kareem and Olanrewaju, 2015; FAO, 2016). Long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are the main building blocks of the neural system (Nelson, 2006) and are very important for optimal brain and neurodevelopment in young children (Thilsted et al., 2014; USAID, 2016).

Despite the breakthrough with the use of hormone in induced spawning of *Clarias gariepinus*, fry survival is still beset with a number of challenges (Adewumi, 2015). A major abiotic challenge stems from depletion in water quality partly resulting from the accumulation of poisonous nitrogenous wastes such as ammonia and nitrite and the consequent increase in water pH in aquaculture ponds (Kroupova et al., 2005).

Ammonia is the basic end product in the breakdown of feed proteins in fish (Ebeling et al., 2006; Lazur 2007; Chen et al., 2012). It is excreted either through their gills or feces (Durborow et al., 1997; YSI Environmental, 2010; Hii et al., 2011). Ammonia is present in two forms in biological fluids or water, namely the nontoxic ionized or dissociated form (NH_4^+) and the toxic un-ionized or molecular (non-dissociated) form (NH_3) (Lazur, 2007; Ogbonna and Chinomso, 2010; Somerville, et al., 2014). Both of them are always in equilibrium and the relative concentrations depend on the pH and temperature of the water (Crab et al., 2007; Lazur, 2007; Somerville et al. 2014). Nitrite (NO_2^-) is the most ephemeral and intermediate nitrogenous waste substance in aquaculture (Lazur, 2007; Bhatnagar and Devi, 2013; Taziki et al., 2015). It is produced when autotrophic *Nitrosomonas* bacteria oxidize ammonia (Lazur, 2007; Bhatnagar and Devi, 2013) or from the reduction of nitrates by denitrifying bacteria (Svobodova et al., 1993; Kroupova et al., 2005). Both of them are very toxic to fish (Lazur, 2007; YSI Environmental, 2010; Heidari et al., 2011; Somerville et al., 2014) and should be kept close to zero especially in fish seedling aquaculture (Bhatnagar and Devi, 2013). This is usually done by changing the pond water as soon as the concentrations of these nitrogen species increase above their critical values, a process that is expensive and limit aquaculture to areas with readily available sources of water.

Microalgae are microscopic unicellular organisms (Priyadarshani and Rath, 2012; Kiepper, 2013; Bleeke et al., 2014) or pluricellular organisms (Mimouni et al., 2012) with a simple structure, ranging from three to 80 µm in size (Scharff, 2015). Microalgae can efficiently absorb nutrients and other contaminants from aquaculture effluent (Heidari et al., 2011). They are responsible for approximately 70% of global nitrogen assimilation with about 65% consumed as reduced nitrogen (ammonia and organic nitrogen), approximately 10% through nitrogen fixation and the remaining as nitrate (Taziki et al., 2015). Microalgae have been widely used for nutrient removal in waste water both as free and immobilized cells (Ogbonna et al., 2000; Banerjee et al., 2015; Kaparapu and Geddada, 2016). Therefore algae growth in fish ponds can aid in reducing the concentrations of ammonia (Durborow et al., 1997) and other nitrogenous waste (Taziki et al., 2015) and at the same time produce microalgae biomass which have various applications (Chen et al., 2012).

The aim of this study was therefore to develop a method of prolonging the life span of water, and thus reduce the frequency of water changes in fish ponds by regulating and/or stabilizing pond water pH, and maintaining low concentrations of poisonous nitrogenous wastes.

Materials and methods

Preparation of media and culture collection

BG-11 medium was prepared according to the modified methods described by Rippka et al. (1979), Bittencourt-Oliveira et al. (2011) and Bortolia et al. (2014). It was composed of the following major nutrients; KNO_3, 1.5g/L; Na_2HPO_4, 0.04g/L; $MgSO_4.7H_2O$, 0.075g/L; $CaCl_2.2H_2O$, 0.036g/L; Citric acid, 0.006g/L; Ferric ammonium salt, 0.006g/L; Disodium magnesium EDTA, 0.001g/L; and Na_2CO_3, 0.02g/L. The trace nutrients were; H_3BO_3, 2.86g/L; $MnCl_2.4H_2O$, 1.8g/L; $ZnSO_4.7H_2O$, 0.222g/L; $NaMoO_4.2H_2O$, 0.39g/L; $CuSO_4.5H_2O$, 0.079g/L; and $Co(NO_3)_2.6H_2O$, 49.4mg/L. Typed cultures of *Chlorella lewinii* LC172265 and *Scenedesmus dimorphus* NIES-93 (10 mL each) were inoculated into two different 250 mL conical flasks containing 100 mL of BG-11 medium. The conical flasks were incubated at room temperature under fluorescent lamps of 1,000 lux intensity with occasional shaking for 21 days. The microalgae were sub-cultured into 20 conical flasks containing BG 11 medium (10 for *C. lewinii* and 10 for *S. dimorphus*) and were incubated at room temperature under fluorescent lamps of 1,000 lux intensity with

Standardization of microalgae cells

C. lewinii and *S. dimorphus* were serially diluted in test tubes with distilled water and the serially diluted cells were counted with Neubauer haemocytometer and microscope. The absorbance of the various serially diluted cell solutions was determined at 680 nm with a spectrophotometer. Calibration curves were established by plotting the cell numbers against the optical densities of *C. lewinii* and *S. dimorphus.*

Hatching of experimental fish and pond setup

Healthy male and female brood stocks of *C. gariepinus* were procured from Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. Artificial spawning and hatching of the experimental fry were done according to the methods of Potangham and Miller, (2006); and Bosworth et al. (2011). Hatched fry were maintained for 13 days in the fish seedling's facility by initially feeding them with artemia and then with 0.20 mm Coppens floating feed. Transparent plastic ponds with dimensions of 50cm x 30cm x 35cm were filled with water to 20 cm depth and setup as nursery for fry at the fish farm of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria. The experimental ponds were partially covered to accommodate both the photophobic fry and photophilic photosynthetic microalgae. Ten (10) fourteen-day old fry were introduced into each set of the partially covered experimental ponds. Initial inoculum of $1x10^9$ cells of *C. lewinii* and $1x10^9$ cells of *S. dimorphus* were inoculated into separate sets of the experimental ponds while combined inoculum of 5 $x10^8$ cells of *C. lewinii* and 5 $x10^8$ cells of *S. dimorphus* were inoculated into another set of the experimental ponds. The control ponds also contained 10 fry each, were partially shaded under sunlight but without photosynthetic microalgae. All the aquaculture ponds were covered with transparent plastic roofing to allow only sunlight and not rain into the systems. The fry were feed with 0.20 to 0.50 mm Coppens floating feed with daily feeding ration of 6% body weight as modified from Nasir et al. (2015).

Analysis of water parameters

The pond water temperature, pH, ammonia, and nitrite were assayed every 48 hours using Hach's® water testing kit Model FF-1A (Cat No. 2430-02) at the laboratory of the Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

Water pH

Aquaculture water pH was assayed using Hach®'s pH testing kit. Two viewing tubes were thoroughly rinsed with water sample and filled to the 5 mL marks to which six drops of Wide Range 4 pH Indicator Solution were added to one of the tubes and swirled to mix. The tube containing the indicator was then inserted into the right-hand opening of the Colour Comparator and the tube of untreated sample was also inserted into the left-hand opening of the Comparator. The Comparator was held up to light and viewed through the two openings in the front while rotating the colour disc until a colour match was obtained and the pH was read through the scale window and recorded.

Ammonia-nitrogen

The concentration of ammonia-nitrogen was determined with Hach®'s water ammonia-nitrogen testing kit according to the manufacturer's manual. One viewing tube was filled to the 5 mL mark with deionized water which served as the reagent blank while the second viewing tube was also filled to the 5 mL mark with the sample from the pond. A drop of Rochelle Salt Solution was added to each viewing tube and swirled to mix after which three drops of Nessler Reagent was added to each viewing tube, stoppered and swirled to mix. The viewing tubes were allowed to stand for 10 minutes for colour development**.** Thereafter, the prepared sample was inserted into the right hand opening of the Colour Comparator while the reagent blank was inserted into the left hand opening. The Comparator was held up to a light source and the disc rotated until the colours in the left and right windows match. The concentration of ammonia nitrogen in mg/L (N) was read through the scale window. The Toxic ammonia (NH_3) concentration (mg/L) was calculated as:

$$\frac{\text{mg/L } NH_3 \text{ as N x value } from \text{ Appendix 1}}{100} \times 1.2$$

Nitrite-nitrogen

The concentration of nitrite-nitrogen was determined with Hach®'s water nitrite-nitrogen testing kit. One viewing tube was rinsed several times with the water sample and then filled to the 5 mL mark. The content of one NitriVer® 3 Powder Pillow was then added to the 5 mL sample after which the viewing tube was stoppered and shook vigorously for exactly one minute. The prepared sample was allowed to sit undisturbed for 10 to 15 minutes and the tube was placed into the right opening of the Colour Comparator. A control tube was filled to the 5 mL mark with untreated water sample and placed into the left opening of the Colour Comparator. The Comparator was held up to a light source while the colour disc was rotated until the colours in the left and right windows match. The nitrite–nitrogen concentration (mg/L) was read through the scale window and Nitrite (NO_2^-) concentration was calculated as:

Nitrite-nitrogen (mg/L) x 3.3 = mg/L nitrite (No_2^-).

Mortality rate of fries

The mortality rate of the experimental fry was calculated thus:

$$\text{Mortality rate} = \frac{F1-F2}{F1} \times 100$$

Where: F_1 = Initial number of fry and F_2 = Final number of fry.

Statistical analysis

The results were reported as mean ± standard deviations of triplicate experiments. Data were subjected to One-Way Analysis of Variance (ANOVA) and considered significant at $P < 0.05$.

Results

Stabilization of pond pH

There was only slight increase in the pH of the ponds inoculated with microalgae during the 10 days (240 h) of experiment. However, in the control ponds (without microalgae) there was significant increase in the pH. The pH of the experimental ponds with *C. lewinii, S. dimorphus* and that with the co-culture of *C. lewinii* and *S. dimorphus* increased slightly from 6.3±0.03 to 6.5±0.2, 6.7±0.6 and 6.4±0.1 respectively during the 240 h of the experiment (Figure 1). The control ponds without microalgae experienced a drastic increase in the pH from 6.3±0.03 (at zero hour) to 9.0±0.06 (at 240 h) as shown in Figure 1.

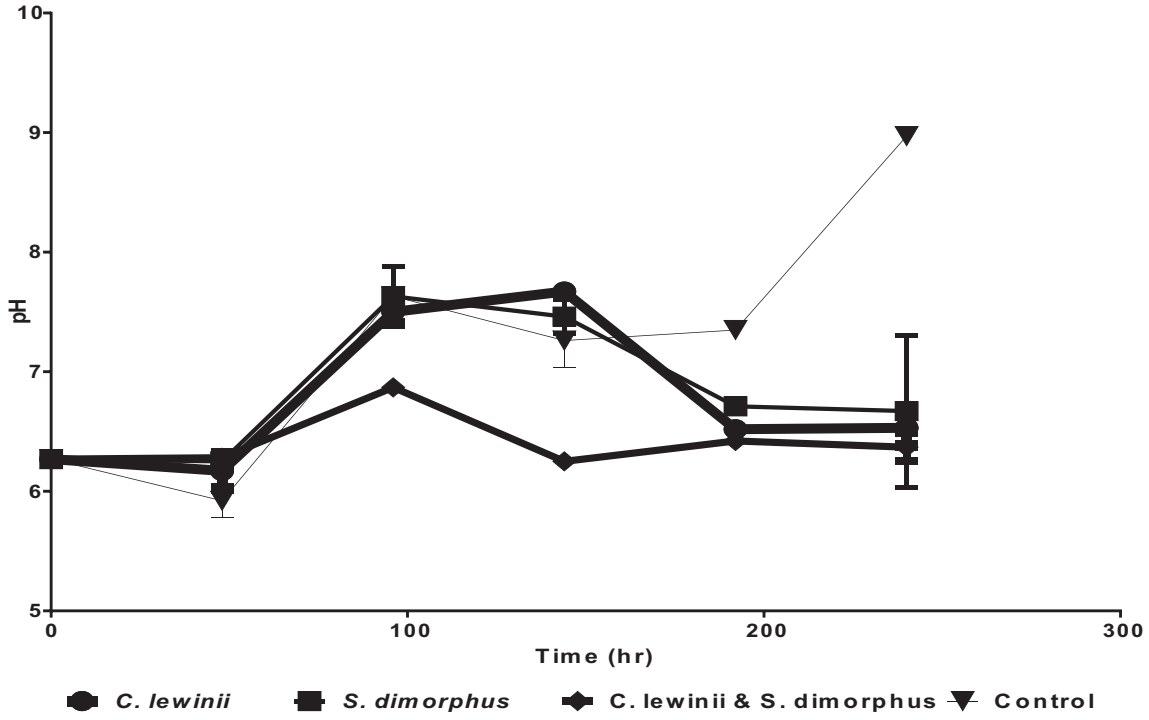

Figure 1: Changes in the pH of aquaculture ponds during the co-culture of fry and microalgae.

Changes in the nitrite concentrations during the culture

There was significant nitrite reduction in the ponds with microalgae compared to the control ponds without microalgae (Table 1). The nitrite concentration in the ponds with *S. dimorphus* decreased to zero mg/L within 144 hours and remained zero until the end of the experiment (240 h). This compared to 0.2310±0.1143 mg/L and 0.0770±0.0381mg/L at 144 h and 240 h respectively in the control ponds. Also, the ponds inoculated with both *C. lewinii* and *S. dimorphus* experienced a gradual drop in the nitrite concentration until it was completely exhausted at the 240th hour of the experiment. In the case of the ponds inoculated with *C. lewinii*, the nitrite concentration decreased to 0.0440±0.0762 mg/L at the 240th hour of the experiment (Table 1).

Table 1: Changes in toxic nitrite concentration (mg/L) in the aquaculture ponds

Time (h)	C. lewinii	S. dimorphus	C. lewinii & S. dimorphus	Control
0	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
48	0.0869±0.0182	0.1639±0.0296	0.1320±0.0330	0.1100±0.0191
96	0.2695±0.0095	0.3190±0.0831	0.3795±0.1004	0.0935±0.0095
144	0.4092±0.0866	0.0000±0.0000	0.4180±0.0762	0.2310±0.1143
192	0.2365±0.0252	0.0000±0.0000	0.1100±0.0831	0.1210±0.0950
240	0.0440±0.0762	0.0000±0.0000	0.0000±0.0000	0.0770±0.0381

Changes in ammonia concentration during the culture

There was oscillation in the concentrations of the total ammonia-nitrogen in the ponds inoculated with microalgae but the oscillation was not apparent in the control ponds. (Table 2). On the whole, the concentrations of toxic ammonia were significantly lower in the ponds containing the microalgae than those in the control ponds. The differences became very pronounced from the 196th hours of the experiment. There were drastic reductions in the concentrations of toxic unionized ammonia (NH_3) in the experimental ponds inoculated with microalgae. In the experimental ponds inoculated with only *C. lewinii* or *S. dimorphus*, the concentrations of the toxic ammonia were successfully reduced to zero mg/L at 192nd h of the experiment and the concentration remained zero all through the 240 h of the experiment (Table 3). In the experimental ponds inoculated with the co-culture of *C. lewinii* and *S. dimorphus*, the concentration of the toxic ammonia was reduced to zero mg/L within 144 h of the experiment (48 h earlier than in ponds separately inoculated with *C. lewinii* or *S. dimorphus*) and remained zero all through the 240th hour of the experiment. On the other hand, the control ponds witnessed rise in the concentration of the toxic ammonia, rising to 0.4832±0.0711 mg/L on the 240th hour of the experiment (Table 3).

Table 2: Changes in total ammonia-nitrogen concentrations (mg/L) in the aquaculture ponds

Time (h)	C. lewinii	S. dimorphus	C. lewinii & S. dimorphus	Control
0	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
48	1.0000±0.2000	0.0967±0.0058	0.5167±0.0289	0.1967±0.0058
96	2.2833±0.1041	1.4333± 0.0289	1.8500±0.1732	1.7000±0.1323
144	0.4333±0.3055	0.0583±0.0144	0.0750±0.0250	1.0667±0.3055
192	1.9000±0.1323	2.7833±0.0764	2.0500±0.1323	2.0333±0.1258
240	0.8000±0.1000	0.1667±0.05774	0.0000±0.0000	1.1667±0.1528

Table 3: Changes in the toxic ammonia concentration in the aquaculture ponds

Time (h)	C. lewinii	S. dimorphus	C. lewinii & S. dimorphus	Control
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
48	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
96	0.0644±0.0183	0.0411± 0.0105	0.0154±0.0014	0.0637±0.0152
144	0.0161±0.0113	0.0016±0.00	0.0±0.0	0.0167±0.0085
192	0.0±0.0	0.0±0.0	0.0±0.0	0.0384±0.0103
240	0.0±0.0	0.0006±0.0010	0.0±0.0	0.4832±0.0711

Mortality rate of fry

The average mortality of the fry was 40% in the ponds inoculated with *C. lewinii*, 37% in the ponds inoculated with co-culture of *C. lewinii* and *S. dimorphus*, 35% in the ponds inoculated with *S. dimorphus* but 80% in the control ponds (without microalgae) .

Relationship between pH and toxic ammonia in the aquaculture ponds

There was a strong correlation between the pH and toxic ammonia concentration in the control aquaculture ponds. The pH of the aquaculture pond increased as the concentration of the toxic ammonia increased (Figure 2). The pH reached a peak of 9.0±0.06 as the toxic ammonia rose to a peak of 0.4832±0.0711 mg/L as shown in Figure 2. This direct proportional relationship was not very apparent in the experimental ponds inoculated with microalgae (Figures 3, 4 and 5).

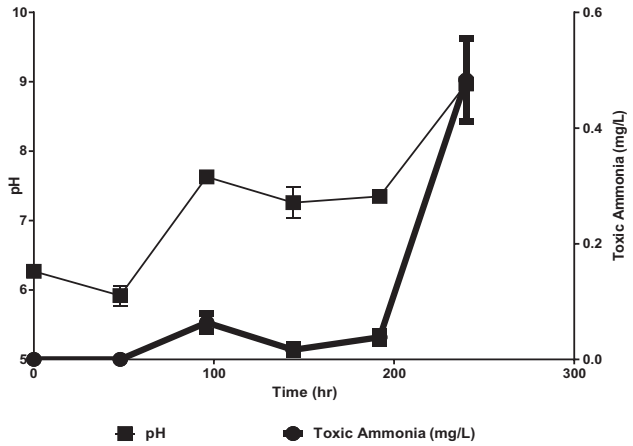

Figure 2: Relationship between pH and toxic ammonia concentration in control ponds

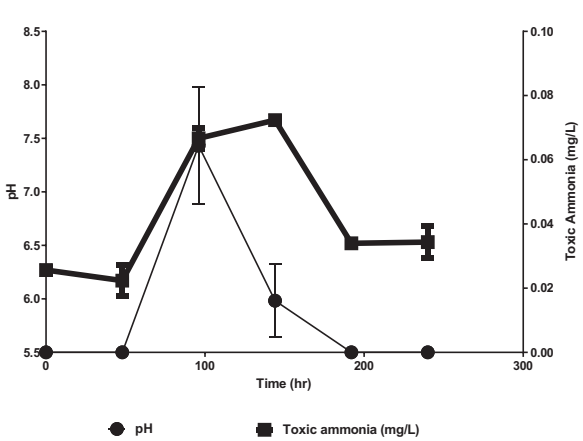

Figure 3: Relationship between pH and toxic ammonia concentration in the experimental ponds with *C. lewinii*

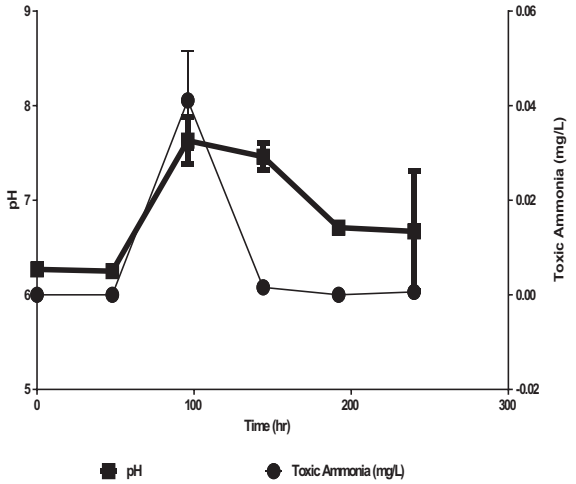

Figure 4: Relationship between pH and toxic ammonia concentration in the experimental ponds with *S. dimorphus*

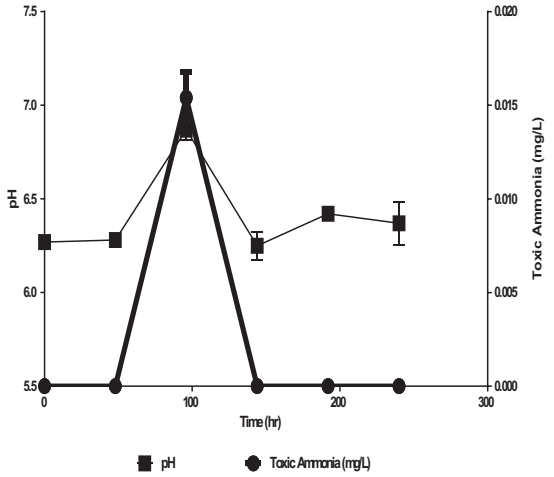

Figure 5: Relationship between pH and toxic ammonia concentration in the experimental ponds with combined cultures of *C. lewinii* and *S. dimorphus*

Discussion

Photosynthetic microalgae proved to be efficient bio-stabilizer of pH in aquaculture ponds. This is mainly attributed to the fact that microalgae use ammonia as their primary source of nitrogen (Artorn et al., 2013; Liu et al., 2017). Therefore, ammonia (NH_3) which is produced from fish excretal and excess uneaten fish feed (Hii et al., 2011; Chen et al., 2012; Somerville et al., 2014; Summerfelt et al., 2016) that ought to dissolve and dissociate in aquaculture water to produce hydroxyl ion (OH^-) (as represented in the equations i & ii below) which consequently increases water alkalinity and pH (Ababio, 2011; Gendel and Lahav, 2013) was assimilated by these microalgae thereby stabilizing the ponds' pH (Hii et al., 2011; Artorn et al., 2013). The pH of the experimental ponds with *C. lewinii, S. dimorphus* and that with the combination of *C. lewinii* and *S. dimorphus* increased slightly from 6.3±0.03 to 6.5±0.2, 6.7±0.6 and 6.4±0.1 respectively during the 240 h of the experiment (Figure 1). These concentrations are all within the normal pH ranges (i.e. 6 - 8 with optimum performance at 7) for fish fry cultivation (Uzoka et al., 2015). The control ponds without microalgae experienced a drastic pH increase from 6.3±0.03 (at 0 h) to 9.0±0.06 (at the 240th hour). This is deleterious to fry growth and survival. This drastic pH increase is as a result of accumulation of toxic unionized ammonia (NH_3) in the control ponds (without microalgae) (Table 3) while the experimental ponds which witnessed relatively little pH change or fluctuations demonstrated the efficacy of microalgae in assimilating and utilizing toxic ammonia (Heidari et al., 2011; Hii et al., 2012), thereby controlling aquaculture pH biologically. The combined co-culture of *C. lewinii* and *S. dimorphus* gave the least overall fluctuation in pH and therefore regarded as the best pH bio-stabilizer, followed by *C. lewinii* and then *S. dimorphus*. The use of microalgae as a pH bio-stabilizer in aquaculture in this research work is quite novel as there is currently no published work on it.

$$NH_3\,(aq) + H_2O\,(l) \upsilon NH_3.H_2O\,(aq) \ldots\ldots (i)$$

$$NH_3.H_2O\,(aq) \upsilon NH_4^+\,(aq) + OH\upsilon\,(aq) \ldots . (ii)$$

Some microalgae are efficient bio-purifiers or nutrient-removers as they can drastically reduce and/or eliminated nitrite and ammonia. This is why microalgae are used in sewage treatment scheme (Muylaert et al., 2015; Taziki et al., 2015; Ge et al., 2016; Kaparapu and Geddada, 2016). In this experiment, nitrite, a transient or ephemeral and very toxic nitrogenous waste (Bhatnagar and Devi, 2013; Taziki et al., 2015), was efficiently and effectively removed by microalgae. There was significant nitrite-nitrogen reduction in the ponds with microalgae compared to the control ponds without microalgae as represented in Table 1. This reduction may be attributed to the production of the enzyme ferredoxin-nitrite reductase (by the experimental microalgae) which converts nitrite directly to ammonia which are then assimilated by microalgae since their direct source of nitrogen is ammonia (Hii et al., 2011; Liu et al., 2017). *S. dimorphus* proved to be the best nitrite-remover as it zeroed toxic nitrite in the aquaculture ponds from the 144th hour of the experiment followed by the combined culture of *C. lewinii* and *S. dimorphus* (in 240th hour) and *C. lewinii*, which was the least nitrite removal (Table 1). There are several works on the use of microalgae in waste water treatment (Ogbonna et al., 2000; Yusoff et al., 2011; Abdel-Raouf et al., 2012; Sriram and Seenivasan, 2012; Martins et al., 2013; Wahid et al., 2013; Banerjee et al., 2015; Muylaert et al., 2015; Nasir et al., 2015; Taziki et al., 2015; Kaparapu and Geddada, 2016; Ramsundar et al., 2017) but there is currently none on the use of photosynthetic microalgae in the removal of nitrite from fish seedlings' aquaculture ponds. However, similar pattern of nitrite assimilation was obtained in shrimp's aquaculture by Ge et al., (2016) when they co-cultured *Platymonas helgolandica*, *Chlorella vulgaris* and *Chaetoceros mulleri* separately with shrimps for 84 days. Nitrite was kept within the recommended levels of = 0.55 mg/L as against the control which peaked at 3.5 mg/L on the 84th day of experiment.

Toxic ammonia was also effectively reduced in the ponds with photosynthetic microalgae compared to the control ponds. This may be attributed to several reasons. Firstly, the primary source of microalgal nitrogen is ammonia (Hii et al., 2011; Artorn et al., 2013; Liu et al., 2017). Secondly, eukaryotic microalgae use nitrogen in the form of non-toxic ionized ammonia (i.e. NH_4^+) (Artorn et al., 2013; Liu et al., 2017) probably because less energy is required for its uptake and assimilation (Chen et al., 2012). It therefore implies that toxic unionized ammonia (NH_3) is converted to NH_4^+ for it to be assimilated by these microalgae (*C. lewinii* and *S. dimorphus*, which are all eukaryotic cells) (Hii et al., 2011). The aquaculture ponds with combined cultures of *C. lewinii* and *S. dimorphus* achieved the best toxic ammonia bio-purification as it reduced ammonia to zero level within 144 hours of the experiment. This was followed by *C. lewinii* (in 192nd hour) and then *S. dimorphus* (Table 3) as against the control ponds where the ammonia concentration increased to a peak of 0.4832±0.0711 mg/L in the 240th hour of the experiment. These results are consistent with those of Kim et al. (2010) in which *Chlorella vulgaris* was used to efficiently remove ammonia from wastewater effluent. Also, Al-Balushi et al. (2012) demonstrated the effectiveness of *Trentepohlia aurea* microalgae to remove nitrite from wastewater. However, there seems to be no reports on the use of photosynthetic microalgae to remove toxic ammonia from fish seedlings' aquaculture ponds. The oscillatory or trajectory patterns of total ammonia nitrogen (TAN) observed in the aquaculture ponds with microalgae (Table 2) may be attributed to the conversion of other forms of nitrogen (especially nitrite and nitrate) to ammonia, which is the primary source of nitrogen to microalgae (Hii et al. 2011; Artorn et al., 2013). Microalgae release ferredoxin-nitrite reductase which converts nitrite to ammonia, and then $NADH_2$-nitrate reductase which converts nitrate to nitrite and then to ammonia with the help of ferredoxin-nitrite reductase (Hii et al., 2011; Liu et al.,

generally frugal in their metabolism, they therefore do not produce enzymes when the enzymes are not needed except in basal or house-keeping level (Willey et al., 2009). Thus, as TAN drops, these enzymes are secreted to produce more ammonia from either nitrite and/or nitrate, and then as ammonia increases, the enzymes production is inhibited or drops drastically until TAN concentration drops again similar to the illustrations by Artorn et al. (2013). Hii et al. (2011) stated that this temporal inhibition of these enzymes in microalgae may either be due to the inactivation of the enzyme system by ammonia or by the by-product of ammonia assimilation. These therefore resulted to the oscillation in the concentration of TAN in the experimental ponds with microalgae as against the control ponds without microalgae.

There was also a direct proportional relationship between toxic ammonia and the pH of the aquaculture ponds (mostly pronounced in the control ponds without microalgae). This is consistent with the report by Hurtado and Cancino-Madariaga (2014); Li and Boyd (2016). This relation is due to the fact that toxic unionized ammonia (NH_3) which is very soluble in water gives hydroxyl ion when it dissolves (as indicated in equations i and ii above) that consequently results in a corresponding increase in the pH of the pond (Ababio, 2011; Hii et al., 2011; Artorn et al., 2013). In the control ponds, a mini and major peaks of 7.6±0.06 and 9.0±0.06 values in pH were obtained respectively as a result of the corresponding mini and major peaks in toxic ammonia of 0.0637±0.0152 mg/L and 0.4832±0.0711 mg/L respectively (Figure 2). Ponds with microalgae only witnessed this direct proportional relationship within the first 96 h of the experiment after which the relationship existed loosely (Figures 3, 4 and 5). This may be because when the concentration of the microalgae increased to a significant level, they assimilated ammonia as soon as it is formed and thus prevented increase in the pH of the ponds.

The reduction in fry's mortality rate in the experimental ponds with microalgae may be attributed to a multiple of factors. Primarily, it may be due to the removal of, and/or reduction in the poisonous nitrogenous waste (i.e. ammonia and nitrite) (Tables 1 and 3) from the ponds and the bio-stabilization of the ponds' pH (Figure 1) by the photosynthetic microalgae; as well as the ample supply of dissolved oxygen into the aquaculture ponds by the photosynthesizing microalgae (data not included). Secondarily, the physical presence of microalgae in ponds also protects fish from direct solar radiations (Hii et al., 2011); and the good nutritive value (such as protein, vitamins and polyunsaturated fatty acids (PUFA)) (Tibbetts et al., 2014; Ge et al., 2016) that the fry may have accumulated from the consumption of microalgae may have added to their health status and the consequent reduction in the mortality of the fry in the ponds co-cultured with microalgae (40%, 37% and 35% for *C. lewinii, C. lewinii* and *S. dimorphus*, and *S. dimorphus* co-cultured ponds) as against the control ponds that doubled to 80%.

C. lewinii and *S. dimorphus* have proven to be effective and efficient pH bio-stabilizers and bio-purifiers in fish fry's aquaculture. The combined culture of *C. lewinii* and *S. dimorphus* were the best pH bio-stabilizer and toxic ammonia bio-purifier followed by *S. dimorphus. S. dimorphus* is the best nitrite bio-purifier closely followed by the combined culture of *C. lewinii* and *S. dimorphus*. Therefore, more research is needed to determine the best proportions of *C. lewinii* and *S. dimorphus* that may be used as efficient bio-stabilizer and bio-purifier. These photosynthetic microalgae can therefore be used in fish seedlings' sustainable aquaculture as replacement to constant water changing or the water re-circulatory system currently in practice, which are very expensive and add to the cost of fish seedlings' production.

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