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COMPARISON OF THE FUNGAL PROFILE OF AQUACULTURE AND NON-AQUACULTURE WATER IN TAAL LAKE, BATANGAS, PHILIPPINES THROUGH PCR-DGGE OF THE 18S rRNA GENE

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ABSTRACT – Profiling of fungal communities is now an emerging tool for assessing the effects of stress in aquatic ecosystems. In the present study, we determined if there is a difference in fungal community structure in the Taal Lake ecosystem as a result of intensive aquaculture activities. Fungal profiles of aquaculture (Bañaga area) versus non-aquaculture waters (Gonzales area) along Taal Lake were obtained through PCR-DGGE of the 18S rRNA gene. Three indices were determined by DGGE band analysis using Bio-Rad QuantityOne™ software. Dice's Similarity Coefficient was used to determine similarity. Both the Shannon and Simpson indices were generated to compare the diversity of the two sites. The Dice's Similarity Coefficients between the two sites were low, with a mean value of 39.58. Mean values among aquaculture samples (36.74) and non-aquaculture samples (41.83) were also low, suggesting difference in species composition were present in sample replicates. The values of Shannon Diversity (2.73 vs 2.67) and Shannon Evenness (0.87 vs 0.85) indices suggest a more diverse non-aquaculture fungal community compared to the aquaculture community. Cultural and morphological characterization revealed culturable fungal species, such as *Aspergillus niger*, *A. terreus* and *Penicillium* sp. Sequencing of distinct DGGE bands also revealed the presence of several unknown and uncultured fungal species.

Keywords: fungi, aquaculture, PCR-DGGE, Taal Lake. Dice similarity index, diversity indices

INTRODUCTION

Fisheries and aquaculture play a major role in the livelihoods of millions of people around the world, especially in developing countries where the bulk of production comes from. Freshwater fishery is a prime source of food for inland communities in the Philippines and its neighboring countries (Fisheries and Aquaculture 2010). Aquaculture is the fastest growing animal-based food-producing sector. It provides nearly one third of the total fish products since its production is derived from a variety of aquatic animals and plants such as fish, crustaceans, mollusks, and seaweeds. China and other Asian

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countries provide the largest production (Duffy 2010).

The rapid growth of the aquaculture industry is often cited as one of the causes of environmental damage in some areas. Aquaculture activities were found to have negative effects on levels of biodiversity in water ecosystems (Beardmore *et al.* 1997). Escape from aquaculture pens has a high probability of leading to invasiveness especially in areas where the cultured species are non-native (Diana 2009). Also, both the introduction of feeds and the high amount of organic matter accumulated due to intensive fish production have negative effects on the local biodiversity of aquaculture communities (Havens 1994; Jiang and Paul 1994; Laws and Redalje 1982). The various fungal species residing in the aquatic environments are the least studied among the greatly affected communities.

There is an urgent need for better documentation of the numerous undescribed fungal species, especially in aquatic habitats (Shearer *et al*, 2007). Profiling fungi present in aquaculture and non-aquaculture communities will provide information on the fungal species present, their functions, and their contributions to ecological stability that might pose risks on fishes and other aquatic organisms in the community, consequently affecting fish production. Establishing the fungal structure of the water in Taal Lake will be useful in improving aquaculture techniques. It will also aid in rapid decision-making with regard to adequate treatment and other major interventions aimed at preventing the harmful effects these fungal species might pose.

This study aimed to compare the fungal profile between aquaculture and non-aquaculture communities and relate the differences to the physicochemical properties of the water in the two communities.

METHODOLOGY

Water Sample Collection

Sample collection was done in Barangay Gonzales, Tanauan City for the non-aquaculture community (NA) and Barangay Bañaga, Agoncillo for the aquaculture community (AQ) (Figure 1). In each of the chosen sampling areas, three 30-meter transect lines were set up perpendicular to the shore. Physicochemical characteristics of water measured *in situ* comprise pH, temperature, conductivity, salinity, dissolved oxygen, total dissolved solids (TDS), phosphorus, and nitrogen content. Climatic conditions were also noted.

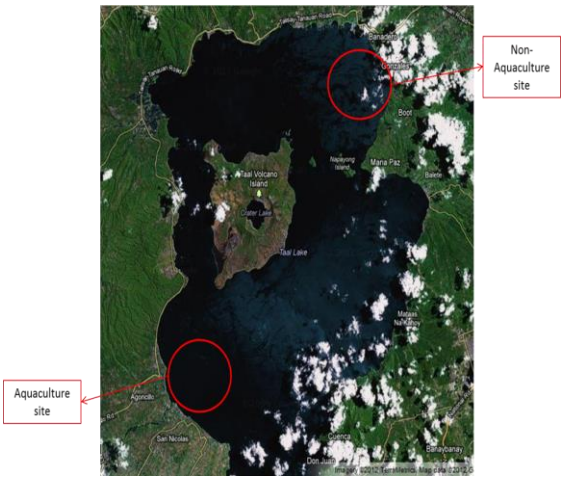


Figure 1. Sampling areas within Taal Lake, Batangas, Philippines.

Five-liter plastic bottles were rinsed three times prior to collection of water samples. The bottles, with the cap removed, were immersed below the water's surface and filled until half full. The caps were then replaced, the bottles were removed from the water, shaken and the water was poured out.

Water was collected by submerging the rinsed plastic bottles underwater, opening them underwater until they were completely filled, and then closing them while still underwater. Five liters of water were collected from five randomly designated points in the sampling site of one community. The total collected water (25 L) was mixed in a big plastic container from which five liters of composite water were taken as sample from that community. The samples were then placed in a cooler and transported to the

laboratory where they were stored in a freezer until ready for flocculation.

Identification of Culturable Fungal Species

Water samples were plated to determine the culturable fungal species present and to provide supporting data as to the possible species present for both AQ and NA sites. One milliliter from each of the samples was mixed with nine milliliter nutrient broth, and incubated at 37°C overnight. For spread plating, 0.01 mL of incubated sample was inoculated onto acidified Potato Dextrose Agar plates. The plates were then incubated for 2 - 3 days at 37°C. After the incubation period, isolated colonies were observed under the microscope. A small portion of each of the isolates was placed on a glass slide. A few drops of lactophenol were added as mounting medium. Culturable fungal species were identified based on morphology observed under a light microscope.

Concentration of Fungal Spores using Skimmed Milk (SM) Flocculation Procedure

Fungal spores from the freshwater sample were concentrated using the Skimmed Milk Flocculation procedure with slight modifications (Calgua *et al*. 2008).

Pre-flocculated skimmed milk solution (1 % w/v) was prepared by dissolving 10 g skimmed milk powder (Difco) in 1 L distilled water and adjusting the pH to 3.5 with 1 N HCl. One hundred mL of this solution was added to each of the previously acidified (pH 3.5) 5-L water samples to a final concentration of skimmed milk of 0.01 % w/v. Samples were stirred using a mechanical mixer for eight hours at room temperature and flocs were allowed to settle by gravity for another eight hours. Supernatants were removed using a vacuum pump without disturbing the sediment. The final volume of approximately 500 mL of the sediment was centrifuged at 7,000 × g for 30 min at 12°C. The supernatant was decanted and the pellet was re-suspended in 8 mL of 0.2 M phosphate buffer at pH 7.5. Once the pellet was completely dissolved, phosphate buffer was added to get a final volume of 10 mL. This concentrate was stored at –20°C.

Fungal DNA Extraction using CTAB Method

Genomic DNA was isolated from the concentrated samples (10 for each site) using modified cetyltrimethylammonium bromide (CTAB) method.

The fungal concentrate was re-suspended in 200 µL Tris-EDTA (TE) buffer. The mixture was vortexed for 20 seconds. Twenty-five µL of 10% SDS and 5 µL 20 mg mL^{-1} protease K were added to break the cells. The tubes were incubated with gentle rocking (30 rpm) at 37ºC for one hour until a clear and viscous solution was achieved. Forty-five µL of 5 M NaCl was added and mixed thoroughly using a vortex mixer for 10 seconds. Forty µL of CTAB solution (10% CTAB in 0.7 M NaCl) was added and mixed by vortexing for 20 seconds. The tubes were incubated at 65ºC for 20 minutes in a heating block. After incubation, 315 µL of chloroform:isoamyl alcohol mixture (24:1) was added and mixed thoroughly. The mixture was left to stand for 30-45 minutes at room temperature. It was then centrifuged at 15,000 rpm for 10 minutes at room temperature. The aqueous phase was transferred to a clean microcentrifuge tube. One µL RNAse K (10µg $µL^{-1}$) was added and incubated for 30 minutes at 37ºC in a heating block. An equal volume of cold isopropanol was added and the tube was gently inverted up and down until a stringy white DNA precipitated out of solution and condensed into a tight mass. The isopropanol was removed and 1 mL of cold 70% ethanol was added to the tube. The suspension was again subjected to centrifugation for 5 minutes at 14,000 rpm. Ethanol was decanted and the isolated DNA was air-dried for 1 to 1 ½ hours in laminar flowhood. One hundred µL DNA grade water was added and the remaining DNA was dissolved by gently flicking the tube. After dissolution, the samples were stored at -20ºC.

Amplification of 530bp 18S rRNA Gene

A 530-bp region of the 18S rRNA gene was amplified by polymerase chain reaction (PCR) from the extracted genomic DNA. A pair of fungus 18S rDNA-specific primers, namely – EF4f (5'- GGA AGG GRT GTA TTT ATT AG-3') and Fung5r (5'-GTA AAA GTC CTG GTT CCC-3'), were used for the amplification.

For the first round PCR condition, the PCR mixture contained 7.5 µL of 2× Taq Master

Mix, 1 µL of 5 µM EF4f primer, 1 µL of 5 µM Fung5r primer, 2 µL of PCR H_2O, and 50 ng DNA template.

The samples were amplified in a MJ Research™ PTC 200 thermal cycler under the following conditions: initial denaturation at 94°C for 3 minutes, followed by 40 cycles with a denaturation step at 94°C for 30 seconds, an annealing step at 53°C for 1 minute, and an extension step at 72°C for 1 minute, followed by 1 cycle at 72°C for 5 minutes for final chain elongation.

Amplification of 230bp 18S rRNA Gene

A second PCR was done to amplify a 230-bp nested region of the 18SrRNA gene amplified from the first PCR. The primers used were NS2f (5'-GGC TGC TGG CAC CAG ACT TGC-3') and Fung5r-GC (GC clamp + Fung5r). The second round PCR mixtures contained 1 µL of first-round PCR product with 20 µL of 2× Taq Master Mix, 1.5 µL of 5 µM NS2f primer, 1.5 µL of 5 µM Fung5r-GC primer, and 16 µL of PCR H_2O. The conditions involved a touch-down program which decreased annealing temperature by 2°C every two rounds and then followed by 25 cycles at 50°C. Reactions involved 1 cycle at 94°C for 3 minutes, followed by 30 cycles with a denaturation step at 94°C for 30 seconds, an annealing step at 60-50°C for 1 minute, and an extension step at 72°C for 1 minute, followed by 1 cycle at 72°C for 5 minutes.

Agarose Gel Electrophoresis

Electrophoresis was done to determine presence of amplicons. Seven µL of the PCR product was mixed with 2 µL of 6× gel loading dye before loading into each well of 1% agarose gel. 1kb Vivantis™ DNA ladder was used as molecular weight standard. Electrophoresis was performed at 100V for 30 to 45 minutes using 0.5× TAE buffer. The gel was stained with ethidium bromide for 1 minute and then de-stained with sterile distilled water for 15 minutes. The bands were viewed using the BioRad™ Gel Documentation System under a short wavelength UV light of 302 nm. DNA concentrations were determined spectrophotometrically using a NanoDrop™ instrument.

Denaturing Gradient Gel Electrophoresis

For DGGE, a 30-60% and 35-65% linear denaturing gradients were prepared in 8% (w/v) polyacrylamide gels. The 100% denaturing solution was prepared by mixing 7 M urea, deionized formamide, 40% Acrylamide/Bis, 50× Tris-Acetate EDTA (TAE) buffer, and distilled water. The 0% denaturing solution was made of the same components as the 100% solution with the exception of formamide and urea. The High (60 and 65%) and Low (30 and 35%) solutions were prepared by mixing amounts of the 100% and 0% solution relative to their concentrations. The DGGE gel was prepared by first placing the 100% denaturing solution, followed by the simultaneous addition of the High and Low concentration gels, and then followed by the 0% denaturing solution. The amplicons of the second PCR were first subjected to DGGE in 1× TAE buffer (40 mM Tris, 20 mM acetic acid and 1mM EDTA) at pH 8, ran at 100 V for 10 minutes, then at 60V and at 60°C for 15 hours in a BioRad™ D Code DGGE apparatus. After the 15-hour run, the gels were stained for 1 minute with 5 µg mL^{-1} ethidium bromide and destained for 15 minutes using sterile distilled water. The gels were visualized through ultraviolet transillumination. Digital images of the DGGE gel profiles were taken and recorded.

Generation of Dice's Similarity Coefficient of the Two Communities

The resulting denaturing gradient gel images were scanned and analyzed using Quantity One Software package (BioRad™). The software was used in detecting and matching bands on assigned gel lanes. The Dice Similarity Coefficients were determined by matching the bands in different lanes with the reference lane. This allowed analysis of the similarity of the generated banding patterns representing the fungal profiles. The profile was used to determine the core fungal population in lake water and to compare the unique fungal species present in each community.

Computation of Diversity Indices

Shannon index of general diversity (H') was calculated using the equation: $H' = -\sum Pi \cdot \ln Pi$. The diversity index was calculated on the basis of the number and the relative intensities of bands in the gel tracks. Using the same data, evenness was

calculated by the following function: EH = H'/ lnS, where S is the number of bands in gel tracks. The Simpson Index of Diversity was computed as SID = 1-$\sum Pi^2$ (Xiaoxu and Fuqiang,2011).

Sequencing of Selected DGGE Bands

Distinct bands from the DGGE gels were excised, solubilized in HPLC-grade water, and re-amplified using NS2f and Fung5r primer pair. The PCR products were sent to Macrogen sequencing facility in Korea for 18S rDNA sequencing.

RESULTS AND DISCUSSION

Physicochemical Parameters of Sampling Sites

Based on the data for the physicochemical parameters (Table 1), pH values for the non-aquaculture and aquaculture sites belonged to the alkaline range (Gonzales (NA) 8.08; and Bañaga (AQ) 8.97, with the AQ site having a pH above the standard. Total nitrogen (N) was far above the minimum quality standard for freshwater environments. Gonzales had a total N of 40.87 units while Bañaga had 36.68. Total P values in both sites were above the maximum standard value (0.173 units in Gonzales and 0.713 units in Bañaga), and are 7× more in AQ than in the NA site. Moreover, the TDS value was higher for the AQ site than the NA site (Bañaga 1,508.22 ppm; Gonzales 1,361.67 ppm). Although most parameters measured were higher in the AQ site than in the NA site, the values obtained indicate that the sampled waters from both areas are nutrient-rich, implying a highly eutrophic state. This condition may lead to shifts and decreases in local biodiversity, confer increased competitive advantage of invasive species and increased incidence of algal blooms (US EPA 2008).

Table 1. Physicochemical properties of water in the non-aquaculture site (Table adapted from Garcia and Salonga, 2012).

Parameter	Non-aquaculture	Aquaculture	Standard
DO (ppm)	7.41	13.96	Min of 5
Conductivity (mS/cm)	1377.45	1510.22	1500-5000
Salinity (ppm)	694.34	744.06	Min of 350
pH	8.08	8.97	6.65-8.5
Temperature (°C)	27.68	30.56	26-28
TDS (ppm)	1361.67	1508.22	--
Total N	40.87	36.68	Min of 10
Total P	0.173	0.713	Max of 0.05

PDA Culture

Five culturable fungal species were identified from morphological characteristics observed both macroscopically and microscopically. These species are *Aspergillus niger, Aspergillus terreus, Penicillium* sp., *Syncephalastrum racemosum* and *Trichoderma* sp.

DNA Extraction

Isolated fungal genomic DNA from the 20 samples (10 from each site) showed light smearing in the agarose gel. NanoDrop™ readings of isolated DNA from AQ and NA samples showed concentrations of 126.4 ng μL^{-1} and 26.2 ng μL^{-1}, respectively, suggesting greater abundance of fungi in AQ than in NA samples.

Amplification of the 18S rRNA gene

The two rounds of PCR for amplification of the fungal 18S rRNA gene from all 20 samples were successfully carried out, giving an average yield of 50 ng μL^{-1} of DNA amplicon based on NanoDrop™ readings.

Comparison of Fungal Diversity between Aquaculture and Non-Aquaculture Communities through Denaturing Gradient Gel Electrophoresis

DGGE analysis of the amplified 230 bp portion of the 18S rRNA gene from the 20 samples manifested characteristic fingerprints of the fungal communities based on their migration behavior on the polyacrylamide gel (Figures 2 and

3). In the DGGE electrophoresis profile, the green bands represent the reference lane. The red bands from the other lanes indicate matched bands relative to the reference lane. All yellow bands are unmatched when compared to the reference lane, and they represent unique bands.

The DGGE profile obtained using 35-65% gradient gel (Figure 2) shows a diverse fungal community for both AQ and NA water as shown by the numerous bands, both common and unique for each community. The highest number of bands can be seen in Lane 3 or NA4 having 32 bands, followed by Lane 4 or NA6 having 31 bands.

Thirteen bands, comprising approximately 41% of matched fungal identities and 15% of all resolved bands, are found to be common for both AQ and NA communities. These bands (on lanes 7, 10, 11, 12, 13, 17, 18, 20, 21, 25, 27, 28, 29) may represent fungal identities that comprise the core lake water fungal communities. The percentage of the putative core fungal species in the AQ group is approximately 21% (13 out of 61 bands) and approximately 23% (13 out of 56 bands) in the NA group.

The AQ group showed 30 yellow bands while the NA, 24 yellow bands. These represent unique fungal identities in each community.

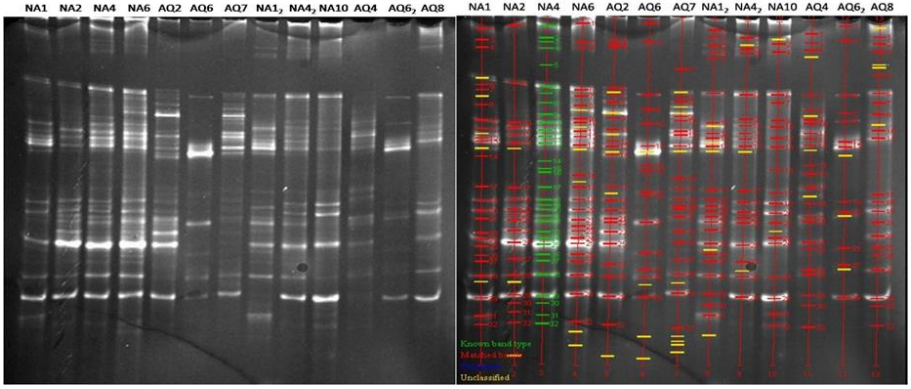


Figure 2. DGGE Profile of Amplified 18S rRNA Gene from Aquaculture and Non-Aquaculture Sites using 35-65% Gradient Gel. (Left) Unprocessed image and (Right) Image after processing using Bio-Rad™ Quantity One Software.

A significant number of matched bands among the AQ and NA samples suggest that there are fungal species present common to both communities.

The DGGE profile obtained using 30-60% gradient gel (Figure 3) has only seven lanes, with three lanes for NA samples and four lanes for AQ samples. The profile shows a relatively lower average number of bands compared to the 35-65% gradient gel. It shows a diverse fungal community of both AQ and NA as shown by the numerous bands, both common and unique for each community. Lane 6 or AQ4 has the highest number of bands (30 bands) followed by Lane 7 or AQ2 with 27 bands. A significant number of matched bands can also be seen among the aquaculture and non-aquaculture samples suggesting that there are fungal species present and are common to both communities.

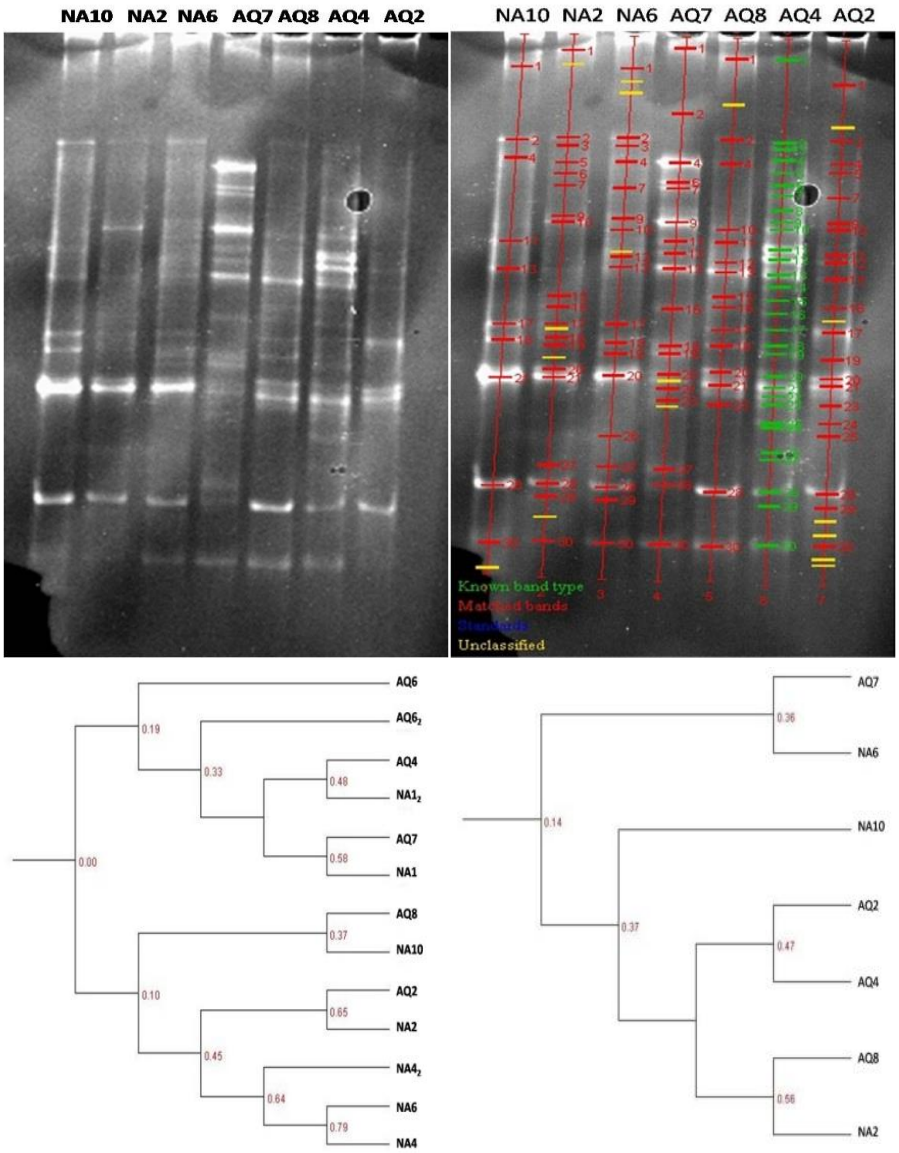


Figure 3. DGGE Profile of Amplified 18S rRNA Gene from Aquaculture and Non-Aquaculture Sites using 30-60% Gradient Gel. (Left) Unprocessed Image and (Center) Image after processing using Bio-Rad™ Quantity One Software.

(**Legend**: **green bands** – reference bands; **red bands** – bands matched to reference bands; **yellow bands** – unclassified bands.) Dendrogram of Dice's similarity coefficient comparing Aquaculture and Non-Aquaculture communities as shown in the DGGE profile of (upper right) 35-65% Gradient Gel and (lower right) 30-60% Gradient Gel

Eighteen bands, comprising approximately 53% of matched fungal identities and 38% of all resolved bands were found to be common for both AQ and NA communities. These bands (1, 2, 4, 7, 9, 10, 11, 12, 13, 16, 17, 18, 19, 20, 21, 24, 28, 30) may represent fungal identities that comprise the core lake water fungal communities. The percentage of the putative core fungal species in the aquaculture group is approximately 46% (18 out of 39 bands) and approximately 56% (18 out of 32 bands) in the non-aquaculture group.

The number of bands present in the DGGE profiles of AQ and NA samples suggests that diverse fungal communities are present in Taal Lake. There are more unique bands than common bands to both sites.

Similarity Coefficient among and between Aquaculture and Non-Aquaculture Sites

The Similarity Coefficients computed from the two gradient gels are summarized (Table 2) and graphically represented by the dendrogram generated from Ward's algorithm (Figure 3: upper right and lower right). The similarity coefficients were generated based on the banding patterns on the DGGE profiles of each run. Each lane was scored based on the presence or absence of each band on the DGGE profile when compared to other banding patterns of other samples. The Dice similarity index was used to provide a pairwise computation of shared bands between samples. Values of Dice coefficient ranged from 0-1, with 1.0 indicating that all bands (100%) are common between samples and 0.0 indicating that no bands (0%) are shared (Rabie 2010). A similarity coefficient value of less than 50% indicates that the two communities being compared are more different than similar. A similarity coefficient value of 50% or more indicates similarity between the samples. The mean similarity coefficient was computed from the generated similarity coefficients of individual samples to reduce bias in presenting the results.

Table 2. Mean Dice's Similarity Coefficient among and between Aquaculture and Non-aquaculture Sites

Groups Compared	Mean Similarity Coefficient (35-65% Gradient)	Mean Similarity Coefficient (30-60% Gradient)	Mean Similarity Coefficient of the two gels
NA vs. NA	51.46	32.2	41.83
AQ vs AQ	31.53	41.95	36.74
NA vs AQ	39.81	39.35	39.58

Based on upper right side of Figure 3, most AQ and NA samples are grouped together and have high similarity coefficients between them. This indicates that these samples from the two communities have a high number of similar fungal identities. Only NA6 and NA4, and the replicate $NA4_2$ grouped with them have a high similarity coefficient (64-79%) that belongs to the same community.

In the lower right portion of Figure 3, are the AQ samples that grouped with other NA samples. They have a low (36 - 56%) similarity coefficient between them. This indicates that these samples have different fungal profiles compared with the NA samples. Interestingly, there is also a low (47%) similarity coefficient between AQ samples (AQ2 and AQ4). This could indicate high variability among samples, possibly pointing to a highly heterogeneous environment.

The generated mean similarity coefficients of the 35-65% gradient gel show a low (31.53%) similarity coefficient among AQ samples. In NA, a higher (51.46%) similarity coefficient was found among the samples pointing to a more highly heterogeneous environment in AQ than in NA. This result is consistent with a previous study showing that more nutrient-rich aquatic environments exhibit higher heterogeneity than oligotrophic systems (Monchy *et al*. 2011).

There is also a low (39.81%) mean similarity coefficient between the AQ and NA communities which indicates that the fungal communities in these two sites are more different than similar.

In the 30-60% gradient gel, a low mean similarity coefficient was found among AQ samples (41.95%) and among NA samples (32.2%). A low (39.35%) mean similarity coefficient is also seen between the NA and AQ communities.

The similarity coefficients between the AQ and NA fungal communities produced from the two different gradient gels were 39.81% and 39.35%, respectively. These values indicate a low percentage of similarity between the two communities. The Dice similarity index compares two profiles based on the number of shared bands; the higher the number of similar bands present in both communities, the higher the similarity coefficient. With a low mean similarity coefficient of 39.58%, it shows that aquaculture and non-aquaculture communities are more dissimilar than similar. The shared or common fungal inhabitants between them represent the putative core fungal population in the Taal Lake water.

Diversity Indices of Fungal Communities present in Aquaculture and Non-aquaculture Water

Table 3 shows that there are small differences in the computed indices between the two communities. It manifests that the NA community has higher diversity and evenness/equitability values than the AQ community.

Both communities have a fairly high species richness indicating a high level of ecosystem stability (Biodiversity 2012). The NA community, however, exhibited relatively higher species richness, with a mean of 24 species, compared to the AQ community. This signifies a healthier and more stable fungal community in the non-aquaculture water.

Table 3. Mean Diversity Indices of Aquaculture and Non-aquaculture fungal communities based on Denaturing Gradient Gel Electrophoresis (DGGE) banding patterns.

	Mean Indices from the banding patterns in the two DGGE gels	
Index	Non-aquaculture	Aquaculture
Species Richness	24	23.6
Shannon Diversity	2.73	2.67
Shannon Evenness	0.87	0.85
Simpson Dominance	0.10	0.10
Simpson Diversity	0.90	0.90
Simpson Equitability	0.49	0.47

In terms of the fungal diversity, both communities showed moderate diversity. However, samples from the NA community exhibited a slightly higher diversity. NA community has higher Shannon (2.73) diversity index than the 2.67 index of the aquaculture community. Previous studies show that higher biological diversity is observed in less nutrient-rich aquatic communities (Monchy *et al*. 2011), and in less polluted sites (Au et al. 1992; Tan and Lim 1984). Both communities showed high evenness, which means that the species present have similar abundance and there is no dominating species in the two communities. It was also supported by the low dominance index values of 0.0985 in non-aquaculture and 0.1009 in aquaculture community. Furthermore, it has been previously shown that the Evenness Index is able to discriminate between different levels of eutrophication in aquatic communities (Karydis 2009). The slightly higher Evenness observed in NA community (0.87) indicates a lower eutrophic state than the AQ community (0.85). This is consistent with the results of the physicochemical parameter measurements suggesting the higher eutrophic state of the AQ site.

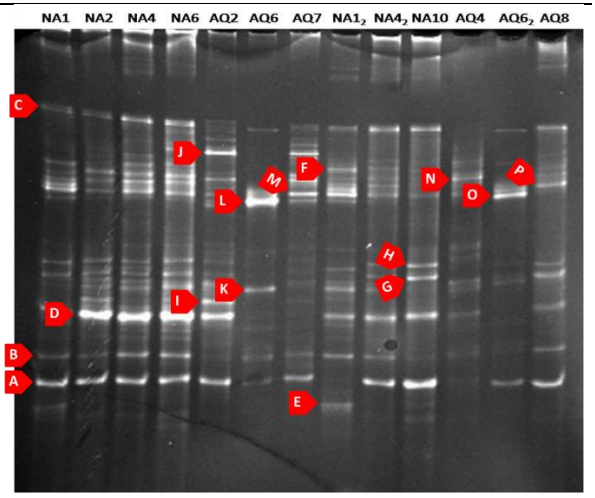
Fungal Identities based on 18S rRNA Gene Sequencing

Table 4 shows the summary of the fungal identities of sequenced DGGE bands from NA and AQ water samples. Specific bands were obtained from both samples based on their distinct appearance on the gels. It was observed that the two samples shared common bands except for E, F and J. Bands E and F seem to be unique to non-aquaculture samples, while band J seems to be unique to the aquaculture samples. Almost half of the sequenced bands (44%) shown in Table 4 are those of uncultured fungi. One band from the NA community gave no significant similarity indicating that it is possibly a previously undocumented fungal species. These suggest that many new species are yet to be reported from Philippine freshwater ecosystems, most likely because few studies address this area of research.

Apparently, the species isolated from PDA plates (*Aspergillus, Penicillium, Syncephalastrum,* and *Trichoderma* species) are not the dominant microflora. None of the thick and distinct DGGE bands that were sequenced belong to these genera, illustrating the bias with relying solely on culture-dependent techniques. On the other hand, the presence of these species would not have been documented by using DGGE only, since faint or thin bands that are difficult to manually excise from the gels could represent them. Therefore, culture-based techniques can complement molecular techniques to obtain more information on microbial diversity.

Table 4. Sequenced fungal identities of selected DGGE gel bands from lanes of Non-aquaculture and Aquaculture water samples.

Non-aquaculture Lane	Aquaculture Lane
A. Ascomycete	I. Uncultured Ascomycota
A. Fusarium oxysporum	J. Uncultured Basidiomycota
A. Fungal sp.	K. *Aspergillus ustus*
A. Uncultured Basidiomycota	L. *Thamnostylum repens*
A. No significant similarity	M. *Cladosporium langeronii*
A. Uncultured fungus 1	N. *Mucoromycotina*
A. Uncultured fungus 2	O. *Colletotrichum*
A. Uncultured fungus 3	P. Uncultured Pleosporaceae



Hydrodynamics of Taal Lake

The small difference in the diversity indices of the aquaculture and non-aquaculture water does not necessarily mean that the two communities are the same. It does not reflect the low similarity of the two sites as seen in the Dice coefficient. The differences may be attributed to the fact that they are actually several kilometers apart, even if the two communities are situated in the same lake and share the same body of water. Although mixing of the water's components from the two sites or between them and other aquaculture sites is highly possible due to water currents, microbial community assemblages can vary greatly due to heterogeneity of microenvironments that have a direct impact on the fungal communities (Subrekropp 1995; Grattan and Suberkropp 2001).

Fungal abundance and activity are affected by several factors. The water quality affects the composition, biomass and activity of fungal communities (Suberkropp 1998). Moreover, fungal activity is usually greater with higher concentrations of dissolved nitrogen and phosphorus (Subrekropp 1995; Grattan and Suberkropp 2001). This is supported by the physicochemical parameters from the two communities. Both revealed a eutrophic environment as evidenced by the high values of nitrogen and phosphorus content. This supports the claim that there has been horizontal mixing of water from the aquaculture and non-aquaculture sites. Horizontal motions or water currents are driven by wind and density differences in horizontal directions (McGinnis and Wuest, 2012). These movements create conditions favorable for fungal proliferation.

Aside from the horizontal mixing of water, mixing processes in the lake may also happen through vertical exchange (Cowan et al 1996). Vertical exchange happens when sediments are re-suspended releasing nutrients into the water column. This can provide up to 94% of the N and 83% of the P required by phytoplankton. After a heavy rainfall, dynamic mixing of the whole water column caused by the introduction of a large volume of rainwater runoff lead to increased particulate matter (mainly carbonates) in the water body (Zaw and Chiswell 1998). This whole mixing process is evident in the measurements of physicochemical parameters. Although the NA site is several kilometers away, the values obtained were not very different from the AQ site.

The temporal dynamics in the lake water mixture also occur and these are mainly due to heavy rainfall and runoff, river flooding, exchange with an adjacent lake, groundwater exchange, and evaporation (Lesack and Melack 1995). Periodic storms, floods and tidal actions disturb the sediments underwater, mixing the components with the water (Hallare *et al* 2009). The prevailing winds and thermal stratification of the lake's deep water bring loads of organic sediment to the surface. The large organic load in the aquaculture site could be due to excess feed and fish wastes from tilapia fish cages that have proliferated in the lakes of Agoncillo, Laurel and Leviste in Batangas City. The unconsumed food settles at the bottom of the lake but gets mixed with the water (Hallare *et al* 2009) especially during seasonal changes. A better picture of the mixing of nutrients in Taal Lake can be obtained if measurements at different depths and at different times of the year are made, and relating these to the changes in microbial assemblages.

Aside from aquaculture, run-offs from terrestrial ecosystems such as residential, agricultural, and industrial areas may also contribute to the eutrophic state of the lake as well as to the aquatic microbial diversity. In this case, measurements from different inlets may be done to compare it with the values obtained from the lake water. This will help pinpoint the source of excess nutrients and help identify critical control points.

CONCLUSION

The DGGE profiles of aquaculture and non-aquaculture water samples reveal a diverse fungal population for both communities. The Dice similarity coefficient implies that the two communities exhibit low similarity between them, meaning that the fungal species present between the two communities are more different than similar. Relatively higher values of both Shannon Diversity and Evenness indices in the non-aquaculture community further signify a higher fungal diversity compared to the aquaculture community. The difference in the fungal profile could be attributed to the difference in the

physicochemical properties of the water from the two communities. Despite the low Dice's coefficient, it must be noted that there are similar fungal species present in both communities. This was shown in the number of matched bands in the denaturing gradient gels, and by the fungal species identified through culture plating, microscopy, and sequencing of selected bands.

STATEMENT OF AUTHORSHIP

Ms. Charize Mae U. Galan and Mr. Lance Dominic D. Gamiao did the sampling, sample processing, data analysis, preparation of tables and figures, and writing of the paper. Dr. Charina Gracia B. Banaay did sample processing, data analysis, preparation of tables and figures, writing, and editing of the final version of the paper. Prof. Marilen P. Balolong, Dr. Leslie Michelle M. Dalmacio, and Prof. Arnold V. Hallare conceptualized the study, and contributed to sample processing, data analysis, writing, and editing of the paper.

REFERENCES

Abel, T. R. and Barlocher, F. 1984. Effects of cadmium on aquatic hyphomycetes. *Applied and Environmental Microbiology* 48: 245-251.

Beardmore, J.A., Mair, G.C., and Lewis, R.I. 1997. Biodiversity in aquatic systems in relation to aquaculture. *Aquaculture Research* 28: 829-839.

Chamier, A. C. and Tipping, E. 1997. Effects of aluminum in acid streams on growth and sporulation of aquatic hyphomycetes. *Environmental Pollution* 96: 289-298.

Cowan, J. L. W., Pennock, J. R., and Boynton, W. R. 1996. Seasonal and interannual patterns of sediment-water nutrient and oxygen fluxes in Mobile Bay, Alabama (USA): Regulating factors and ecological significance. *Marine Ecology Progress Series* 141:229–245.

Diana, J.S. 2009. Aquaculture production and biodiversity conservation. *BioScience* 59(1): 27-38.

Duffy, E. 2010. "Fisheries and aquaculture". In: Encyclopedia of Earth. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment). [First published in the Encyclopedia of Earth February 21, 2010; Last revised Date February 21, 2010; Retrieved 23 May 2011 at http://www.eoearth.org/article/Fisheries_and_aquaculture

Garcia, R. and Salonga, A . 2012. Macroalgae and aquatic plant biodiversity and productivity between aquaculture and non-aquaculture sites within Taal Lake, Philippines. Unpublished B.S. Thesis. University of the Philippines, Manila.

Grattan, R.M., and Suberkropp, K. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *J N Am Benthol Soc* **20**:33-43.

Hallare, A., Factor, P., Santos, E. and Hollert, H. 2009. Assessing the Impact of Fish Cage Culture on Taal Lake (Philippines) Water and Sediment Quality Using the Zebrafish Embryo Assay. *Philippine Journal of Science* 138(1):91-104.

Havens. 1994. Experimental perturbation of a freshwater plankton community - a test of hypotheses regarding the effects of stress. *Oikos* 69:147-153.

Jiang, S. C. and Paul, J. H. 1994. Seasonal and diet abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. *Marine Ecology Program Series* 104:163-172.

Karydis, M. 2009. Eutrophication assessment of coastal waters based on indicators: A literature review. *Global NEST Journal* 11(4): 373-390.

Laws, E. A., and Redalje, D. G. 1982. Sewage diversion effects on the water column on a subtropical estuary. *Marine Environment Res* 6:265-79.

Lesack, L. and Melack, J. 1995. Flooding Hydrology and Mixture Dynamics of Lake Water Derived from Multiple Sources in an Amazon Floodplain Lake. *Water Resources Research* 31(2):329-345.

Madsen, J., Chambers, P., James, W., Koch, E., and Westlake, D. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia* 444:71-84

Maltby, L. and Booth, R. 1991. The effect of coal mine efiluent on fungal assemblages and leaf breakdown. *Water Research* 25: 247-250.

Monchy S, Sanciu G, Jobard M, Rasconi S, Gerphagnon M, Chabe M, Cian A, Meloni D, Niquil N, Christaki U, Viscogliosi E, and Sime-Ngando T. 2011. Exploring and quantifying fungal diversity in freshwater lake ecosystems using rDNA cloning/sequencing and SSU tag pyrosequencing. *Environmental Microbiology* Doi:10.1111/j.1462-2920.2011.02444.x.

Rabie, T. 2010. Implementation of some similarity coefficients in conjunction with multiple UPGMA and neighbor-joining algorithms for enhancing phylogenetic trees. *Egypt Poult Sci* 30:607-21.

Sagara, N. 1975. Ammonia fungi – a chemoecological grouping of terrestrial fungi. *Contributions of the Biology Lab of Kyoto* 24:205-76.

Shearer, C., Descals, E., Kohlmeyer, B., Kohlmeyer, J. 2007. Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation* 16:49–67.

Suberkropp, K. 1998. Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. *Mycol. Res* 102: 998–1002

Tan, T. K. and Lim, G. 1984. A comparison of Fungi from polluted water. *Environmental pollution*. Series A 35: 57-65.

Tsui, K. M., Fryar, S. C., Hodgkiss, U., Hyde K. D., Poonyth, A. D. and Taylor, J. E. 1998. The effect of human disturbance on fungal diversity in the tropics. *Fungal Diversity* 1: 19-26.

US EPA United States Environmental Protection Agency. 2008. Nutrient Criteria Technical Guidance Manual. Retrieved from http://www2.epa.gov/sites/production/files/documents/wetlands_full.pdf

Xiaoxu, F. and Fuqiang, S. 2011. Dynamics of fungal diversity in different phases of *Pinus* litter degradation revealed through denaturing gradient gel electrophoresis (DGGE) coupled with morphological examination. *African Journal of Microbiology Research* 5(31):5674-5681.

Yamanaka, T. 2003. The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia in vitro. *Mycologia* 95(4):584-9

Zaw, M. and Chiswell, B. 1998. Iron and manganese dynamics in lake water. *Water Research* 33(8):1900-1910.

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