ORIGINAL ARTICLE

Identification of aquatic consumer trophic pathways in four volcanic tropical lakes using fatty acid biomarkers

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ABSTRACT

Food web studies rely heavily on the use of traditional gut content analysis or the fairly popular stable isotope analysis rather than fatty acid analysis (FAA) despite its straightforward process, and ability to identify and characterize more diverse trophic pathways. In this study, we employed fatty acid (FA) biomarkers as a preliminary attempt to trace and characterize trophic pathways in food webs of four tropical lakes of volcanic origin in Luzon Island, Philippines - three clustered maar lakes (Yambo, Pandin and Calibato) and a large caldera lake (Taal). These lakes have a long history of human disturbance but limited ecological data. Knowledge of basal food sources and existing trophic dynamics of organisms are generally non-existent. Particulate organic matter (POM), zooplankton, and fish species were collected in August 2019 from the study lakes as representative of three trophic guilds. Non-metric multidimensional scaling (NMDS) and principal component analysis (PCA) were conducted to analyse FA profiles and characterize trophic relationships between representative organisms. For the POM, within lakes comparison of taxon-specific FA profiles showed a significant difference between the surface and near bottom depths, with the former dominated by photoautorophs and the latter by chemotrophs, suggesting the ability of FAA to effectively delineate between micro-organisms. Between lakes comparison also showed significant difference between the caldera and maar lakes, with the latter containing higher composition of bacterial FA, reminiscent of the considerably smaller lakes' response to the impact of unmitigated organic loadings from anthropogenic activities. Taken together with the primary consumers' FA profiles, analysis confirmed the ability of FAA to discriminate between FA profile sources. PCA explained >70% of the variance in the FA compositions for three trophic guilds in the two deepest lakes, which delineated both zooplankton and fish species food selectivity in each lake, alluding to FAA's capacity to characterize dietary reliance of various species in an environment with numerous food sources. Although certain limitations were encountered, such as the specificity of the sampling depths for POM, and the small sample size of the representative species of the third trophic level, this study demonstrated the effectiveness of FAA as a powerful ecological tool for disentangling intricate lake food webs comprising various food sources. Overall, this study provided baseline information on basal food sources and trophic pathways of representative organisms from four tropical lakes. Taken together, FAA studies have wide application in understanding food webs, including anthropogenically-threatened lake ecosystems.

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Key words: FAA; food web; NMDS; trophic dynamics; tropical lakes.

Contributions: All the authors made a substantive intellectual contribution, read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: The authors declare that they have no competing interests, and all authors confirm accuracy.

Citation: de Leon JR, Fujibayashi M, Petilla FM, *et al.* Identification of aquatic consumer trophic pathways in four volcanic tropical lakes using fatty acid biomarkers. *J Limnol 2022;81:2070.*

Edited by: Diego Copetti, *CNR-IRSA Brugherio, Italy.*

Received: 2 February 2022.
Accepted: 4 July 2022.

Licensee PAGEPress, Italy
J. Limnol., 2022; 81:2070
DOI: 10.4081/jlimnol.2021.2070

INTRODUCTION

Food web ecology, which focuses on the transfer of energy from primary producers (*e.g.*, phytoplankton, periphyton, and macrophyte) to higher trophic levels (*e.g.*, zooplankton, zoobenthos, and fish), is fundamental for the understanding of the trophic dynamics of aquatic ecosystems (Burns *et al.*, 2011). Trophic relationships among these primary producers have been traditionally investigated by inspecting gut contents. However, this approach is limited in delineating the complete picture of aquatic food webs. This dietary analysis is less applicable to micro-organisms, even for some macro-organisms, and only takes a snapshot of their diets, making the assessment mainly qualitative (Traugott *et al*., 2013). To overcome this weakness, biochemical, immunological, and stable isotope techniques have been developed and applied to food web studies (Napolitano, 1999). Among these analytical methods, the use of certain lipid species, specifically fatty acids (FAs), has attracted attention because its principle is simply underlain by standard metabolic processes (Napolitano, 1999). The utility of FAs as trophic biomarkers has been recognized and applied to studies that look at the distinct FA profiles of consumers, which result from their assimilated diets for a given time (Napolitano, 1999; Alfaro *et al*., 2006; Torres-Ruiz *et al*., 2007; Fujibayashi *et al*., 2019).

FA profiles vary among species and taxonomic groups, making FAs effective biomarkers as they often produce taxon-specific FAs. These specific FA profiles, often incorporated unmodified into organisms through their diet, serve as sources of energy (Dalsgaard *et al*., 2003) or are retained as structural components of cell membranes and other organelles (Burns *et al*., 2011). These essential fatty acids (EFAs) are known to significantly affect the nutrition, growth, reproduction, immune response, and adaptation of primary and secondary consumers (Burns *et al*., 2011; Lau *et al*., 2012).

EFAs include polyunsaturated fatty acids (PUFAs) and highly unsaturated FAs, which are vital biomolecules that function as fundamental materials for the structural components of cells, tissues, and organs and synthesis of other biologically active substances (Ander *et al*., 2003). Certain PUFAs cannot be synthesized by the consumer, so instead, they are assimilated from the consumption of organisms from lower trophic levels (Lau *et al*., 2012; Fujibayashi *et al*., 2018). For example, the PUFAs linoleic acid (LA; 18:2ω6) and α-linolenic acid (α-LIN: 18:3ω3) are produced in great amounts by green algae and cyanobacteria, and arachidonic acid (ARA; 20:4ω6) is produced by higher plant species and green algae. Eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) are prominent PUFAs produced by diatoms and dinoflagellates (Napolitano, 1999; Burns *et al*., 2011).

Furthermore, for aquatic ecosystems, FA profiles have been utilized in identifying microbes specific to a certain water column depth (Kenyon, 1972; Mancuso *et al*., 1990). Epilimnetic particulate organic matter (POM) is composed of diverse obligately photoautotrophic algae and heterotrophic bacteria (Raven and Maberly, 2009), while hypolimnetic POM is mainly composed of heterotrophic and chemotrophic bacteria (Newton *et al*., 2011; Kurt, 2019). Based on POM FA depth profiles, Mancuso *et al*. (1990) observed a vertical shift in the dominant taxa of microbial communities from PUFA-producing microeukaryotes in shallow layers to sulfur-reducing bacteria (*i.e*., 10Me16:0) in deeper layers.

Among the chemotrophs, methane oxidizing bacteria (MOB) play an important role in lake food webs as an alternative carbon source for consumers through the biofiltration of dissolved methane generated from lake bottoms (Deines and Fink, 2011; Jones and Grey, 2011; Sanseverino *et al*., 2012). MOB subsidize zooplankton production in oligotrophic to mesotrophic lakes (Bastviken *et al*., 2004; Kankaala *et al*., 2010; Ho *et al*., 2016) and sometimes provide the dominant carbon pathway for zooplankton during unproductive seasons (Taipale *et al*., 2007). The MOB are divided into three major taxa, Type I, Type II, and NC10, which are differently distributed both vertically and geographically (Kobayashi *et al*., 2016; Ghashghavi *et al*., 2019). Since they produce taxon-specific FAs based on different metabolic pathways for the assimilation of formaldehyde (Hanson and Hanson, 1996), these specific FAs can be used as tracers for aquatic consumers' methane-derived trophic pathways (Sanseverino *et al*., 2012).

Taken together, taxon-specific FA profiles can be used as biomarkers of basal food sources for aquatic consumers (Tab. 1). LA and α-LIN serve as biomarkers for algae and cyanobacteria together with ARA, while EPA and DHA serve as biomarkers for diatoms and dinoflagellates, re-

Tab. 1. Fatty acid biomarkers for basal taxa.

Taxa	Fatty acid biomarker
Green algae and cyanobacteria	18:3ω3 (α-LIN), 18:3ω6 (GLA), 18:2ω6c (LA)
Diatoms	20:5ω3 (EPA)
Dinoflagellates	22:6ω3 (DHA)
Terrestrial plants	SAFAs 24:0–31:0
MOB Type I	16:1ω5t, 16:1ω7t, 16:1ω8, 16:1ω5c
MOB Type II	18:1ω8
Bacteria (all other)	i-15:0, a-15:0, i-16:0, i17, a-17:0,18:1ω7

α-LIN, α-linolenic acid; GLA, gamma-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SAFAs, saturated fatty acids; MOB, methane oxidizing bacteria. Source: Burns et al. (2011), Fujibayashi et al. (2019), Itoh et al. (2017), Napolitano (1999).

spectively. Most very-long chain saturated fatty acids (SAFAs; $\geq$25 carbon atoms) are terrestrial plants biomarkers, while short-chain SAFAs and mono-unsaturated FAs with $\leq$19 carbons (*i.e.*, 16:1n7t and 18:1ω7) are bacterial biomarkers (Napolitano, 1999). Due to the inability of aquatic consumers to synthesize EFAs, FA profiles serve as natural tracers that can be used to understand trophic ecology and the physiology of consumer species (Lau *et al*., 2012). In aquatic food webs, zooplankton have long been regarded as a pivotal ecological link between primary producers and predators, and studies have shown that zooplankton naturally acquire FAs through diet (Burns *et al*., 2011). Generally, herbivorous zooplankton contain more saturated FAs than PUFAs and assimilate ω3-FA derived from phytoplankton more efficiently than carnivorous zooplankton (Brett *et al*., 2009; Ravet *et al*., 2010). Persson and Vrede (2006) indicated that copepods, in particular, tend to accumulate EPA and DHA. EPA, along with ARA, serve as precursors for prostaglandins, which in turn regulate molting and reproduction and maintain cell membrane functions, while DHA facilitates neural tissue development (Simopoulos, 2002). Among copepod species, calanoids have a moderate preference for DHA, while cladocerans prefer to assimilate EPA. The assimilation of PUFAs by zooplankton may temporally vary due to seasonal successions of phytoplankton communities (Hartwich *et al*., 2013) and abiotic effects on seston PUFA concentrations (Thompson *et al*., 1990).

For lake fish, some FAs, such as DHA, EPA, and ARA, can only be assimilated from microbes and zooplankton (Lau *et al*., 2012). DHA and EPA can also be obtained by converting α-LIN from seston; however, this pathway may be inefficient and energy costly compared to direct consumption via food sources (Lau *et al*., 2012). Considering consumer reliance on basal taxa-specific FAs, fatty acid analysis (FAA) is a more powerful tool for identifying diverse consumer trophic pathways in intricate lake food webs than stable isotope analysis, which can only identify two major trophic pathways derived from planktonic and benthic algae (France, 1995). Taking advantage of FAA, we attempted to characterize trophic pathways in tropical lake food webs in the Philippines where ecological data are very limited. Four tropical lakes of volcanic origin on Luzon Island were selected: three of the Seven Maar Lakes (SMLs); Lakes Yambo, Pandin, and Calibato; and a large caldera lake, Lake Taal. These lakes are of great importance to communities around the lakes' perimeter as they are utilized for various economically important activities (Tab. 2) (Mendoza *et al*., 2019a, 2019b). First, we made a within- and between lake comparison of basal food sources based on the FA profiles of surface and near bottom POM using non-metric multidimensional scaling (NMDS). We then performed principal component analysis (PCA) for the FA profiles of the three trophic guilds, POM, zooplankton, and fish, to examine zooplankton food selectivity and characterize trophic pathways for fish species in the deepest two of the four lakes. Finally, we discuss the characteristics and uniqueness of tropical lake food webs in developing countries under anthropogenic disturbance.

METHODS

Study lakes

Samples were collected from four tropical southern Luzon Island lakes during August 2019: three of the seven maar lakes (SMLs) in Laguna province, namely Lakes Yambo, Pandin, and Calibato, and a large caldera lake, Lake Taal in Batangas province (Fig. 1). Lake Taal was formed by the explosion of Taal Volcano (Papa and Mamaril Sr., 2011), and the SMLs were formed by the explosion of Mount Cristobal (Brillo, 2016a). Two of the SMLs, Lakes Yambo and Pandin, are referred to as twin lakes due to their proximity to each other, and unlike Lakes Calibato and Taal, these twin lakes are not heavily used for aquaculture but are popular tourist destinations (Brillo, 2016b). Lake Calibato, the deepest of the SMLs (Tab. 2), is used for fisheries and aquaculture, the main source of the local community's livelihood (Brillo, 2016a), while Lake Taal, the third largest lake in the Philippines, has multiple resource uses, including tourism, recreation, and aquaculture and fisheries essential to the local community. Due to their economic importance, these

Tab. 2. Physical characteristics of the four study lakes. Based on De Leon *et al.* (2022) and Mendoza *et al.* (2019a).

Lake	Yambo	Pandin	Calibato	Taal
Maximum depth (m)	27.60	61.75	156.00	198.00
Surface area (km^2)	0.31	0.24	0.43	268.00
Elevation (m asl)	160	160	1706	2.5
Trophic condition	E	E	E	M-E
Settlement population	2,000	8,500	8,500	286,358
Usage	T	T	F, T	F, T, N

E, eutrophic; M, mesotrophic; T, tourism; F, fishery; N, navigation.

lakes have been at the center of lake ecosystem management programs to mitigate human disturbances (Mendoza *et al*., 2019a, 2019b). According to the Philippine Fisheries Code (Republic Act 8550), only 10% of the total lake surface area (SA) can be occupied by fish cages. The twin lakes have the lowest fish cage to SA ratio among the SMLs with Lake Yambo having 2.07% of its total SA occupied by fish cages, while Lake Pandin only has 1.80% (Mendoza *et al*., 2019a). Unfortunately, this was greatly exceeded by Lake Calibato, with 22.36% of its SA occupied by fish cages (Mendoza *et al*., 2019a). On the other hand, an ordinance of the Philippines' Department of Agriculture stipulated that Lake Taal is only allowed to have 6,000 units of fish cages. This standard was eventually met by 2011 after conducting a significant 38.09% reduction of fish cages (Mendoza *et al*., 2019b).

Sample collection and processing

Three types of samples were collected to represent the three trophic levels to be analyzed, namely: water samples for POM, zooplankton, and fish samples. POM and Zooplankton samples were collected at established sampling points through assessment of total lake area and maximum depth (Tab. 2). One sampling point was established for each of the SMLs (Yambo 14.118685 ºN, 121.367941 ºE; Pandin 14.11490 ºN, 121.36864 ºE; Calibato 14.10357 ºN, 121.37732 ºE), while two points were selected for Lake Taal to represent its north (14.06586 ºN, 121.01919 ºE) and south (13.96746 ºN, 121.00947 ºE) basins (Fig. 1). Water samples (5 L) were collected from 5 m below the surface and 5 m above the lake bottom using a Niskin water sampler (General Oceanics). The water samples were filtered through a 5 µm mesh plankton net, then through a 0.7 µm GF/F filter (Whatman) precombusted at 500°C in the laboratory with a filtration volume of 1 L/filter. A total of 5 filter papers per depth were then stored in corresponding vials and refrigerated.

Collection of zooplankton samples was designed to target calanoid copepods. Coincidentally, the neotropical *Arctodipatomus dorsalis* (Marsh, 1907) is the only calanoid species in the SMLs and it is the dominating calanoid in Lake Taal (Papa *et al*., 2012; De Leon *et al*.,

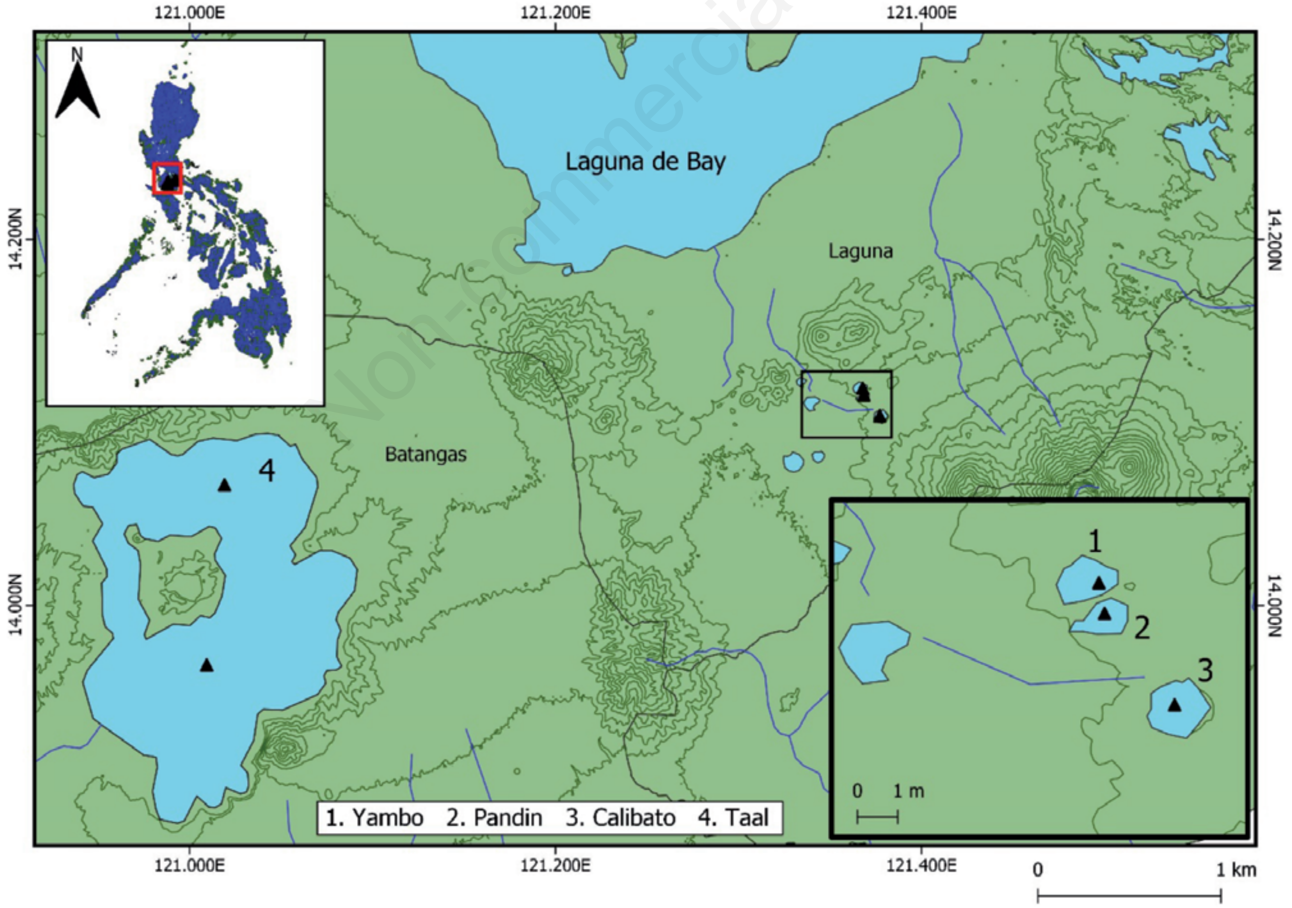

Fig. 1. Map of the four southern Luzon Island, Philippines, study lakes showing the provinces they are located in. Lakes are numbered (1 = Yambo, 2 = Pandin, 3 = Calibato, 4 = Taal) based on maximum depth. Triangle markers indicate sampling points for each lake.

2020). Samples were collected using conical 100 µm mesh plankton net towed vertically from near bottom to the surface at a constant speed. To ensure target species dominated sample aggregate, these were cleaned of debris and unwanted planktonic species by sieving through 94 µm mesh aided by tweezers and a wash bottle filled with lake water. Resulting aggregates were stored in labelled falcon tubes.

Wild-caught fish species were collected through opportunistic sampling by using gill nets set-up by fishermen. Samples were only caught in Lake Calibato and from the south basin of Lake Taal. Fish species collected from Lake Taal included *Ambassis* sp. (n=1), *Sardinella tawilis* (Herre, 1927) (n=4), and *Oreochromis niloticus* (Linn.) (n=1), while *Leiopotherapon plumbeus* (Kner, 1864) (n=1) and *O. niloticus* (n=1) were collected from Lake Calibato. Fish muscle tissue (~1-2 mg dry weight) was extracted by careful dissection of each specimen in fresh condition and then stored in vials. All three types of samples were quickly frozen at −20°C in a laboratory prior to FAA.

Fatty acid analysis

All frozen samples were subjected to lyophilization (Operon FDB-5502; Gyeonggi-do, Korea) for 24 hours at the University of Santo Tomas, Analytical Services Laboratory. The freeze-dried samples were then transported frozen to Kyushu University, Fukuoka, Japan, for FAA following the slightly modified one-step method of Abdulkadir and Tsuchiya (2008).

An internal standard was prepared by dissolving 10 mg of tricosanoic acid (23:0) to 100 mL of hexane. Zooplankton and fish tissue samples were then finely ground and the surface of the GF/F samples that retained POM were peeled and used for further analysis. These samples were prepared for esterification; the samples were combined with 2 mL of hexane and 1 mL of internal standard solution in a 10 mL centrifuge tube. Then, 0.8 mL of 14% BF_3 in methanol was added and the tube was flushed with nitrogen gas and then tightly closed with a Teflon-lined screw-cap. The resulting solution was heated on a hot plate at 100°C for 120 min. Then, 0.5 mL of hexane was added followed by 1 mL of distilled water after cooling to room temperature. The solution was shaken vigorously for 1 min and centrifuged for 3 min at 2500 rpm. The upper phase of the resulting mixture, the hexane layer containing fatty acid methyl esters (FAMEs), was transferred into a clean sample vial for FAME analysis by gas chromatography-mass spectrometry (5977; Agilent). A 100 × 0.25 mm i.d. capillary column (Supelco SP2560; Sigma-Aldrich) was used for the separation, with helium as the carrier gas. The initial column temperature of 70°C was elevated to 110°C at a rate of 10°C min^{-1}. The temperature was increased to 161°C at 1°C min^{-1}, then further increased to 240°C at 5 °C min^{-1} and held for another 20 min. The peaks of the FAMEs were identified by comparison with the retention time of the standard and by the mass spectrum.

Data analysis

Data for the compositions of selected FA biomarkers (Tab. 1) in the POM collected from each lake (or each lake basin) at two depths were incorporated into non-metric multidimensional scaling (NMDS) to visualize between- and within-variations in the microbial community compositions based on ordination plots with the following factors: sampling site (lake), collection depth (surface *vs* near bottom), and lake type (caldera *vs* maar). Stress values were used in the assessment of the produced ordination plot, with stress value =/< 0.05 considered to be a good fit. Permutational multivariate analysis of variance (PERMANOVA) was performed to test for significant differences among the lakes and between the sampling depths based on goodness of fit. Data for the zooplankton sample FA biomarkers were then added to the ordination plot in the NMDS to visualize food selectivity from available food sources and between- and within-variations in the FA profiles. Significant differences were tested using PERMANOVA.

In the two deepest lakes, Lakes Taal and Calibato, in which POM, zooplankton, and fish species were collected as representative of three trophic guilds, principal component analysis (PCA) was performed to visualize the trophic pathways of each consumer in the lake food webs, with FA biomarker composition data as vectors. All the data analyses were carried out using R (R Core Team, 2013), the vegan (Oksanen *et al*., 2020) and ggplot2 (Wickham, 2016) packages.

RESULTS

Fatty acid profiles

All the FAs detected in the samples from the representative trophic guilds are shown in Tab. S1. Taxon-specific FA compositions are summarized in Tab. 3, based on identification shown in Tab. 1. For POM as a basal food source, bacteria-specific FA was the most abundant in all the lakes followed by green algae/cyanobacteria-specific FA. Dinoflagellate-specific FA was not detected in either the surface or near bottom samples from all the lakes. For the three SMLs, Type I MOB was common in both the surface and near bottom samples, whereas Type II-specific FA was only detected in near bottom samples. In contrast, no MOB was detected in either the surface or near bottom samples from Lake Taal.

Using NMDS analysis, the POM in the surface and near bottom samples from the four lakes was plotted based on

Tab. 3. Compositions of taxon-specific fatty acid (FA) biomarkers based on Tab. 1 for surface and near bottom particulate organic matter (POM), zooplankton, and fish samples from Lakes Yambo (Y), Pandin (P), Calibato (C), and Taal (T).

Taxa producing specific FAs	Surface POM				Near bottom POM				Zooplankton				*Oreochromis niloticus*		*Sardinella tawilis*	*Ambassis* sp.	*Leiopotherapon plumbeus*
	Y	P	C	T (n=2)	Y	P	C	T (n=2)	Y	P	C*	T (n=2)	C	T	T (n=4)	T	C
Green algae and cyanobacteria	4.68	6.66	8.55	6.41 (0.35)	2.09	1.10	3.36	3.34 (0.46)	9.01	3.39	8.16	5.67 (1.61)	2.22	3.38	1.66 (0.52)	4.95	2.80
Diatom	0.77	–	1.20	1.23 (0.11)	-	-	-	–	13.05	2.63	0.96	7.65 (3.53)	3.08	0.58	0.61 (0.14)	6.21	0.08
Dinoflagellate	–	–	–	–	–	–	–	–	9.22	8.57	3.03	1.97 (0.94)	15.29	3.51	1.28 (0.33)	7.68	1.92
Terrestrial plants	2.38	1.75	3.26	1.36 (0.33)	1.41	–	2.03	1.65 (0.06)	0.42	–	–	0.62 (0.15)	0.45	–	0.70 (0.08)	0.48	0.07
MOB Type I	0.64	0.49	0.69	-	0.36	0.16	0.40	–	0.41	0.40	-	0.32 (0.14)	0.42	0.09	0.27 (0.02)	0.26	0.37
MOB Type II	–	–	–	–	0.20	0.08	0.10	–	0.14	0.03	–	–	–	–	–	–	–
Bacteria (all other)	8.06	1.75	14.04	4.49 (0.62)	9.98	26.88	14.57	4.78 (0.45)	26.56	16.91	10.27	17.44 (4.50)	5.43	3.03	7.87 (0.44)	4.24	7.89

**Small sample size, mean % and standard deviation in parentheses, dashes (–) represent below detection limit.*

the similarity of the FA biomarker compositions (Fig. 2). There was a significant difference in the FA biomarker compositions between lake types (PERMANOVA caldera vs. maar lakes, p=0.022) and sampling depths (surface vs. near bottom, p=0.034). Vectors with significant contribution to the ordination plot were cyanobacteria ($r^2 = 0.83$, p=0.002), diatom ($r^2 = 0.83$, p=0.002), bacteria ($r^2 = 0.96$, p=0.001), and MOB Type II ($r^2 = 0.57$, p=0.049). The cyanobacteria and diatoms, which are autotrophs, were observed to be more related to the surface euphotic habitat, while the bacteria, which function as both chemotrophs and heterotrophs, were more related to the near bottom aphotic habitat (Fig. 2). The bacteria vector alone appeared to generate the dissimilarity of the POM FA biomarker compositions between the caldera and maar lakes (Fig. 2).

The zooplankton FA biomarkers from the four lakes showed that bacteria-specific FAs accounted on average for 17.80% of the total FA content. This was followed by green algae/cyanobacteria (6.60%) and diatom-specific FA (6.07%) (Tab. 3). Terrestrial plants and MOB-specific FAs constituted only a small part of the zooplankton FA content (<0.50%). Taken together with the POM as the available food sources, an ordination plot of the zooplankton showed a distinct discrimination between trophic guilds (PERMANOVA zooplankton *vs* POM, p=0.001; Fig. 3) but no significant variation among lakes (p=0.392), suggesting the conservatism of zooplankton FA compositions compared to the wider variation in the POM FA compositions. The ordination plot indicated that zooplankton had an increased proportion of cyanobacteria- ($r^2 = 0.78$, p=0.001), dinoflagellate- ($r^2 = 0.58$, p=0.006), and bacteria-specific FAs ($r^2 = 0.65$, p=0.005) compared to the POM FAs (Fig. 3).

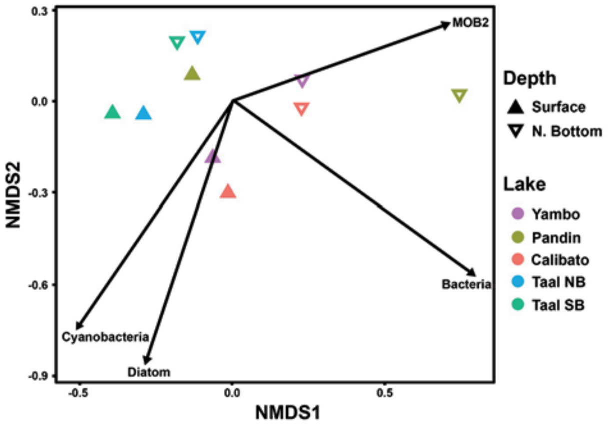

Fig. 2. Ordination plot (stress value = 0.013) with significant vectors to account for variations in the fatty acid biomarker compositions of particulate organic matter (POM) samples collected from Lakes Yambo, Pandin, Calibato, and Taal north (NB) and south (SB) basins.

The FA biomarker compositions of the fish sampled from Lakes Taal and Calibato are summarized in Tab. 3. In general, fish samples had a higher proportion of dinoflagellate- and bacteria-specific FAs and a minimal proportion of MOB-specific FAs. However, the compositions of the FA biomarkers varied greatly among the fish species in each lake. In Lake Taal, *Ambassis* sp. had a high proportion of dinoflagellate-specific FA (7.68%) and diatom-specific FA (6.21%). Within the same lake, coexisting *S. tawilis* relied heavily on bacteria (7.87%) and *O. niloticus* on dinoflagellates (3.51%) and green algae/cyanobacteria (3.38%). In Lake Calibato, *L. plumbeus* had the highest proportion of bacteria-specific FA (7.89%), similar to *S. tawilis* in Lake Taal. In contrast, coexisting *O. niloticus* had the highest proportion of dinoflagellate-specific FA (15.29%).

Trophic pathways

For Lakes Taal (south basin) and Calibato, the three trophic guild FA biomarker compositions were compared: POM, zooplankton, and fish. For the Lake Taal food web, the PCA of the FA profiles explained 71.67% of the total variance, with eigenvalues of PC1 = 2.84 and PC2 = 1.45. A biplot of the PCA showed that both surface and near bottom POM FA biomarkers were more associated with terrestrial plants, while zooplankton relied on diatoms and bacteria (Fig. 4a). Among the fish species, the FA profile of *Ambassis* sp. was characterized by dinoflagellate- and Type I MOB-specific FAs. In contrast, the FA profiles of *O. niloticus* and *S. tawilis* were not characterized by specific FA biomarkers but were observed to be negatively related to cyanobacteria-specific FA.

For the Lake Calibato food web (Fig. 4b), the PCA of the FA profiles explained 72.85% of the total variance, with eigenvalues of PC1 = 3.47 and PC2 = 1.63. Surface POM FA biomarkers were more associated with terrestrial plants, while those of near bottom POM were more associated with Type II MOB. Zooplankton FA profile was shown to be negatively associated with Type I MOB. Among the fish species, *L. plumbeus* had a FA profile similar to that of the zooplankton. In contrast, the FA profile of *O. niloticus* had a positive association with diatom- and dinoflagellate-specific FAs. When comparing *O. niloticus* between these two lakes, the FA profiles were characterized by different basal food sources (Fig. 4 a,b).

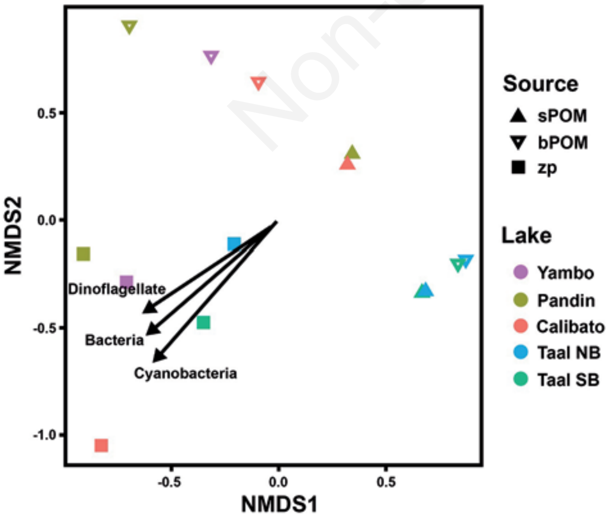

Fig. 3. Ordination plot (stress value = 0.040) with species vectors for surface particulate organic matter (sPOM), near bottom particulate organic matter (bPOM), and zooplankton (zp) from Lakes Yambo, Pandin, Calibato, and Taal north (NB) and south (SB) basins.

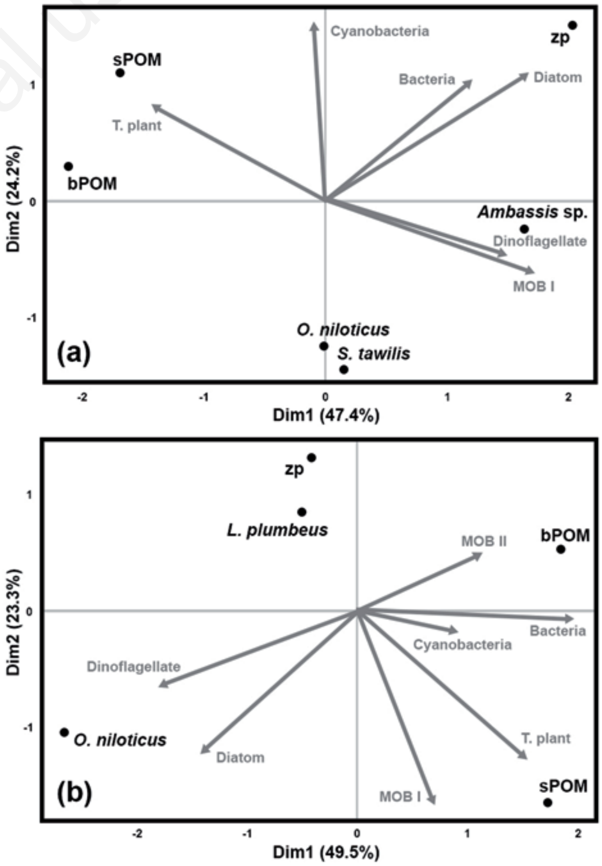

Fig. 4. Biplot of particulate organic matter (POM), zooplankton, and fish collected from Lakes (a) Taal and (b) Calibato. *Oreochromis niloticus*, *Sardinella tawilis*, *Ambassis* sp, and *Leiopotherapon plumbeus*. sPOM, surface particulate organic matter; bPOM, near bottom particulate organic matter; zp, zooplankton.

DISCUSSION

Fatty acid analysis showed that the POM from the study's tropical volcanic lakes was mainly composed of bacteria-specific FAs. Although bacterial production is expected to be low in the tropics due to constant high temperatures, the dominating presence of bacteria in the study's lakes may have been due to increased nutrient inputs, both anthropogenic or from terrestrial runoff. This has been shown to be the primary guiding factor for bacterial growth in similar environments (Scofield *et al.*, 2015; Freitas *et al.*, 2017), and many studies have acknowledged the critical role of bacterial communities in freshwater food webs (Newton *et al.*, 2011). Based on our results, near bottom POM was characterized more by bacteria- and Type II MOB-specific FAs, while surface POM was associated with cyanobacteria and diatoms (Fig. 2). Such a vertical pattern can be simply determined by the light environment: cyanobacteria and diatoms are common autotrophs in the euphotic layer (Falkowski, 1994), while bacteria are regarded as heterotrophs, active in both aphotic and euphotic layers (Newton *et al.*, 2011).

In freshwater food webs, MOB serve not only as heterotrophs but also as chemotrophs and provide aquatic consumers with methane-derived carbon as an alternative food source (Kankaala *et al.*, 2010; Sanseverino *et al.*, 2012). In temperate and boreal lakes, Type I MOB often dominate in the suboxic boundary layers of the hypolimnion due to their oxygen and anaerobically produced methane requirements (Sundh *et al.*, 2005; Rissanen *et al.*, 2018). In contrast, the dominance of Type II MOB has been reported in many tropical and sub-tropical lakes (Dumestre *et al.*, 2001). In the study's tropical lakes however, Type I showed greater abundance in the surface than in the near bottom waters and was more abundant than the Type II, with vertical distribution limited to the near bottom (Tab. 3). This was also in contrast to tropical and subtropical lakes, in which Type II is widely distributed throughout the oxic layer (Zigah *et al.*, 2015; Kobayashi *et al.*, 2016). Although MOBs are commonly present through the whole water column (Lew and Glinska-Lewczuk, 2018), they have low methane oxidation activity in surface waters due to the inhibitory effect of strong light (Dumestre *et al.*, 1999; Murase *et al.*, 2005). In deeper small maar lakes (i.e., the SMLs), since the near bottom layer is euxinic (Mendoza *et al.*, 2021), it can retard methane oxidation by aerobic MOB, such as Type I and II (Rahalkar *et al.*, 2009; Schubert *et al.*, 2010), suggesting that their optimal growth condition may exist in the mid-depth suboxic layer, from which we did not collect any POM samples. A reason why the dominant group and vertical pattern of the MOB in our study were inconsistent with those of other lakes.

A significant factor that generated the discrimination of the POM FA compositions between the caldera (Lake Taal) and maar lakes (Lakes Yambo, Pandin, and Calibato) was the relative abundance of bacteria, with the maar lakes having higher concentrations of bacterial FA (Tab. 3 and Fig. 2). Bacterial dominance in microbial communities can be determined by the availability of organic nutrients relative to inorganic nutrients (Jansson *et al.*, 2006) and increased phosphorus concentration (Coveney and Wetzel, 1992; Farjalla *et al.*, 2009). Since the 1970s, the study's lakes have been heavily impacted by unregulated fish farming, with increased organic loads driven by fish pellet input (Papa and Mamaril Sr., 2011; Brillo, 2016a). In terms of the fish cage area relative to the lake surface area, the organic load was presumed to be heavier in the SMLs than the larger Lake Taal, resulting in more frequent fish kills due to anoxia in the former (Papa *et al.*, 2011; Brillo, 2016a; Papa and Briones, 2017; Mendoza *et al.*, 2019b). Although the LLDA, a governmental agency for the environmental protection of Laguna Lake and its watershed, recently implemented an ordinance to reduce the total area of fish cages in the SMLs, some of the lakes are still regarded as eutrophic (Mendoza *et al.*, 2019a). In addition, Mendoza-Pascual *et al.* (2021) recently updated the trophic status of three of the study lakes based on total phosphorus concentrations and classified them as eutrophic to hypereutrophic lakes. In contrast, in the Lake Taal food web, the POM FA compositions were characterized by terrestrial plants (Fig. 4). Since this lake has a considerably large catchment area (682.8 km^2) and the land cover is dominated by forests and croplands, the lake basin can receive more allochthonous inputs from its watershed through river inflows and terrestrial runoffs, as is often the case in lakes with less impacted catchments (Carpenter *et al.*, 2005; Scofield *et al.*, 2015).

In all the study's lakes, zooplankton had the highest proportion of bacterial FAs (Tab. 3). The PCA revealed that the zooplankton preferentially assimilated bacteria-, cyanobacteria-, and dinoflagellate-specific FAs among the available POM food sources (Fig. 3). However, dinoflagellate-specific FA was not found in the POM from any of the lakes; nevertheless, this may have been an artefact of the sampling method. In this study, the POM was prefiltered through a 5 µm mesh plankton net to remove small zooplankton, such as nanoflagellates and ciliates. However, such pre-filtration also screens out large-cell phytoplankton. In Lake Taal, for example, the dinoflagellate *Ceratium furcoides*, which was observed to dominate during the southwest monsoon, have a cell size ≥40 µm (Rott *et al.*, 2008). The pre-filtration might have then excluded this otherwise dominant phytoplankton from the POM sample. Furthermore, in both the SMLs and Lake Taal, phytoplankton assemblages are often dominated by a colony-forming cyanobacteria *Microcystis* sp. (LLDA, 2008; Mercurio *et al.*, 2016), which is a unicellular organism that forms large-size colonies, ranging from 300 to

1000 µm in diameter (Xiao et al. 2018). The proportions of FAs specific to large-size phytoplankton in the POM may have been underestimated, resulting in the positive food selectivity of the zooplankton for these taxa.

In our study lakes, it has been reported that zooplankton communities are dominated by a calanoid copepod, *A. dorsalis*, which has become a common species in Philippine inland waters (Papa *et al*., 2012; De Leon *et al*., 2020). *A. dorsalis* is known as a selective phytoplankton grazer that prefers diatoms over cyanobacteria and green algae among phytoplankton assemblages (Cisneros *et al*., 1991). Its food preference accounts for the FA composition of the zooplankton collected from the less impacted Lake Taal (Fig. 4a) but not the more impacted and eutrophic Lake Calibato (Fig. 4b). Although the zooplankton in Lake Taal also strongly relies on bacterial FA, *A. dorsalis*, which is a raptorial feeder, has difficulty directly grazing on small bacteria. One potential trophic pathway to Lake Taal's *A. dorsalis* population is the microbial loop, which occurs when bacteria and meso-zooplankton are trophically linked through bacterivorous micro-zooplankton, such as heterotrophic nanoflagellates and ciliates (Nakano *et al*., 1998, 2001). However, this more detailed trophic pathway could have been traced should we conduct a FAA for size-fractionated 20-100 µm POM samples, which covers the size range of these suspected micro-zooplankton.

In contrast, in Lake Calibato, the zooplankton showed no positive food selectivity to any basal food sources, but was observed to show a negative selectivity against Type I MOB, and terrestrial plants in the surface POM (Fig. 4b). Although terrestrial-derived POM is regarded as difficult for aquatic consumers to assimilate, it can sometimes subsidize zooplankton in lake ecosystems (Pace *et al*., 2004). In the more impacted Lake Calibato, however, autotrophic and heterotrophic organic products boosted by anthropogenic nutrient loadings may be much more easily available to zooplankton. The availability of MOB may also be low because of their small cell size (<0.5-3.0 µm; Bowman *et al*., 1994) without the intermediation of micro-zooplankton.

The FA biomarker compositions of the wild-caught fish as predators showed great within- and between lake variations. Among the fish species in Lake Taal, *Ambassis* sp. selectively assimilated dinoflagellates and Type I MOB (Fig. 4a). This fish is widely distributed in shallow coastal waters in tropical and subtropical regions (Martin and Blabber, 1984), and is regarded as a predominant carnivore that feeds on planktonic crustaceans in coastal food webs (Martin and Blaber, 1983). It has also been reported that in Lake Taal, this species preys not only on copepods and cladocerans but also on chironomid larvae (Ariyaratne *et al*., 2008; Mendoza *et al*., 2015). Dinoflagellates are the main prey item for most copepod and cladoceran species (Persson and Vrede, 2006), while the chironomids rely heavily on MOB (Eller *et al*., 2005; Jones *et al*., 2008). Considering that these microbes are too small to be directly grazed by *Ambassis* sp., its selective assimilation of their FAs can be explained by primary consumer-mediated trophic interactions rather than direct trophic interactions.

The two other species from Lake Taal, *S. tawilis* and *O. niloticus*, did not show any positive selectivity for any FA biomarkers (Fig. 4a). However, *S. tawilis*, which in Lake Taal is an endemic freshwater sardine, has been reported to be predominantly zooplanktivorous (Papa *et al*., 2008), but our PCA did not support the assumption that the fish would rely on its main prey item, zooplankton (Fig. 4a). In the current study, the zooplankton samples were collected from the whole water column. Considering that *S. tawilis* had the highest proportion of bacteria-specific FAs, it might feed on zooplankton specific to the aphotic habitat where bacterial production is dominant. In contrast, *O. niloticus* is well known to have a wider range of food items, such as phytoplankton, zooplankton, insects, and fish larva. It has also been reported to undergo temporal shifts in food habits in accordance to its ontogeny and food availability (Zenebe *et al*., 1998; Nijiru *et al*., 2004; Rumisha and Nehemia, 2013; Mendoza *et al*., 2015). Such omnivory and food habit flexibility may be a cause of the dissociation with specific basal food sources. Rather, it should be noted that these fish showed negative selectivity against cyanobacteria. This pattern may be due to prey avoidance, as reported in a previous study in which *O. niloticus* was observed to detect and avoid toxic strains of *Microcystis* sp., the dominant phytoplankton species of Lake Taal (Mercurio *et al*., 2016), in an environment where other non-toxic food items were available (Beveridge *et al*., 1993).

Unlike Lake Taal, *O. niloticus* from Lake Calibato showed a positive selectivity for diatoms and dinoflagellates (Fig. 4b). *O. niloticus* is generally regarded as an omnivore, but it also behaves as an algal feeder (Getachew and Fernando, 1989; Dempster *et al*., 1993; Nijiru *et al*., 2004). Using stable isotope analysis, Briones *et al*. (2016) showed that *O. niloticus* from Lake Sampaloc (one of the SMLs) relies strongly on periphyton compared to other fish species. It has also been reported that diatoms and dinoflagellates are the main components of diet for *O. niloticus* (Rumisha and Nehemia, 2013), further supporting our analytical result.

In contrast, *L. plumbeus*, which is endemic to Laguna Lake, had no selectivity to any basal food sources considered in the study, whereas it had the same vector as that of the zooplankton (Fig. 4b). This species is associated with rocky, vegetated lacustrine waters; the majority of its diet is composed of microcrustaceans, diatoms, and algae; and it is regarded as an omnivore (Delmendo, 1968; Quilang *et al*., 2007; Corpuz *et al*., 2016; De Leon *et al*., 2017). Kock *et al*. (2000), in contrast, reported that *L. plumbeus* relies heavily on zooplankton, such as cladocerans and various species of copepods, making up to 50%–70% of its diet. The strong association with the zooplankton FA compositions suggested that *L. plumbeus* may act as zooplanktivores in the food web of Lake Calibato.

CONCLUSIONS

Taking advantage of FA biomarkers, we demonstrated that trophic pathways from basal to predatory species spatially vary in tropical lakes. In particular, FAA coupled with NMDS or PCA was able to visualize how the compositions of basal species vary within and between lakes. Such spatial variations were, in turn, trophically translated to consumers, such as zooplankton and fish. Further, the FAA was also useful for detecting consumer food selectivity, especially in cases where general patterns are not readily observed. The establishment of taxon-specific FA markers for other basal taxa whose metabolic pathways are unknown can increase the resolution of food web analysis. The capability of FAA to detect structural changes in food webs and identify their driving forces makes it a powerful tool for lake ecosystem management, particularly for highly-impacted systems.

ACKNOWLEDGEMENTS

This work was supported by The Philippine Department of Science and Technology—Accelerated Science and Technology Human Resource Development Program; The PADI Foundation; the Japan Society for the Promotion of Science under Grants [16H05774]; The RIHN Project under Grant [D06-14200119] and Bilateral Joint Research Projects under Grant [JPJSBP120208602].

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