POLLUTION IN MANILA BAY AQUACULTURE FARMS

STATUS, IMPACT, AND REMEDIAL OPTIONS

A SPECIAL ISSUE OF THE PHILIPPINE JOURNAL OF FISHERIES

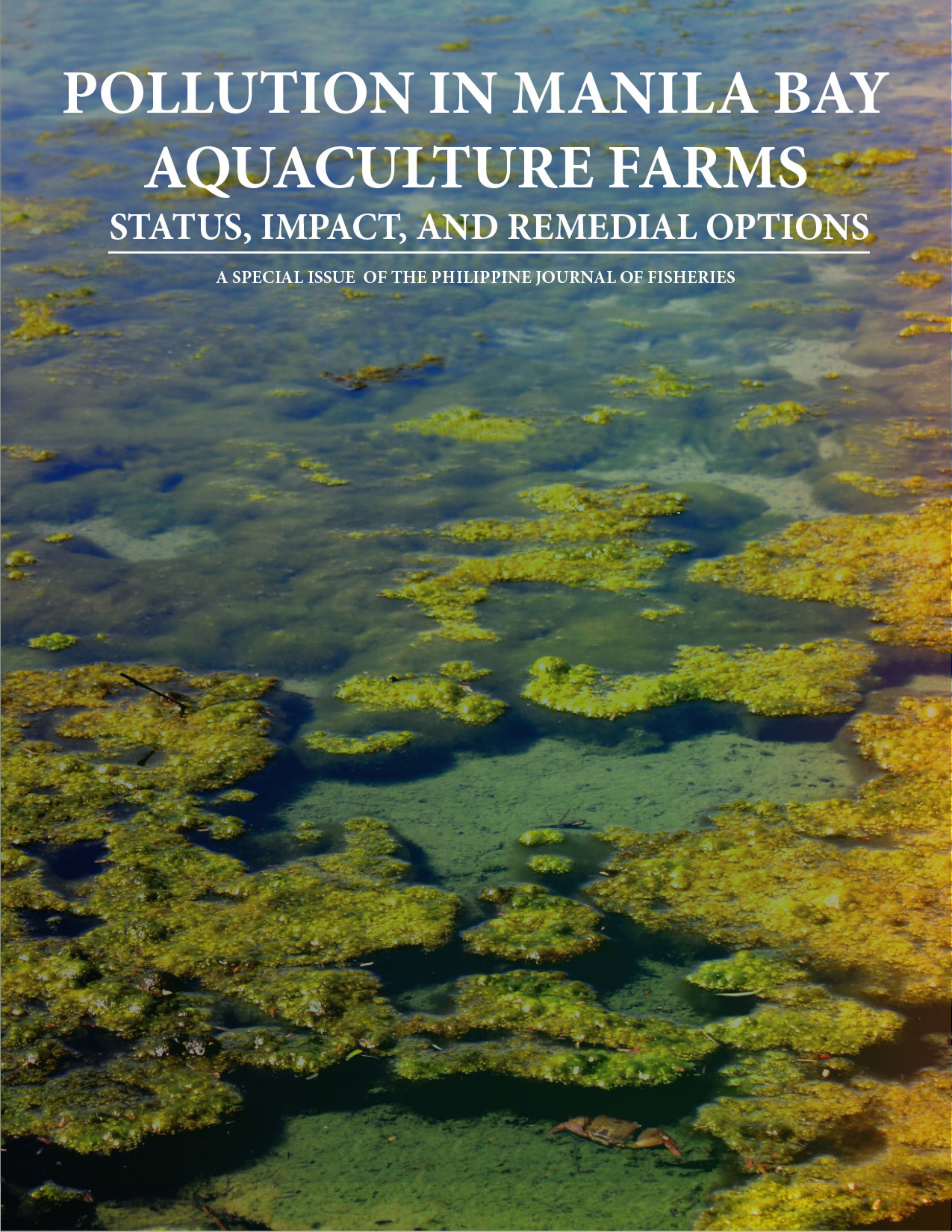

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Photo by: Heherson G. Baun

POLLUTION IN MANILA BAY AQUACULTURE FARMS

STATUS, IMPACT, AND REMEDIAL OPTIONS

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Philippines

Message

Allow me to congratulate our partners and colleagues at the BFAR National Fisheries Research and Development Institute (NFRDI) for the successful publication and launch of the book "Pollution in Manila Bay Aquaculture Farms: Status, Impact and Remedial Options."

With an area of 1,994 km2 (769.9 sq mi) and a coastline of 190 km (118.1 mi) straddling three regions (NCR, Region III, and Region IV-A), Manila Bay provides cheap source of protein and nutrition for the people and contributes more than half of the country's GDP by generating employment for millions of coastline residents, increasing export earnings, and offering both the local and foreign investors the best harbors and the most awesome sunsets and tourist attractions.

Yet, over the past decades. There has been a growing concern over the serious threats to the Bay's precious resources. Population expansion and rapid urbanization, uncontrolled coast and basin development, poorly managed shipping and tourism activities, pollution and destruction of the marine life habitats, the red tide phenomenon and resource mismanagement have all led to the continued degradation and depletion of the Bay's natural riches.

Thus, we in the new DA leadership welcome the efforts of BFAR and all the other Manila Bay stakeholders in and out of government to address these challenges through the "Manila Bay Clean-up and Rehabilitation Program." Pursuant to Sec. 65 of RA 8550, the program seeks to improve and restore marine life in the Manila Bay in order to further develop its fishing industry.

This research project and publication are therefore part of the contribution of the DA and BFAR, through the NFRDI, to the task of judiciously managing Manila Bay's resources by collecting, synthesizing and disseminating scientific and technical papers on: (1) Review of aquaculture practices and anthropogenic activities in Manila Bay aquaculture farms; (2) Spatial and seasonal nutrient trends in Manila bay aquaculture farms; (3) Heavy metal contamination of fish and fishery resources from the Manila Bay; and (4) Contamination of coliform bacteria in water and fishery resources in Manila Bay aquaculture farms.

The DA, together with BFAR, is confident that this book would help Manila Bay's stakeholders come up with strategic approaches to address the impact of the coastal and marine ecosystems heavy metal contamination and pollution. I urge policy makers, the scientific and teaching community, the fisher folk and fishing entrepreneurs, and other stakeholders to seriously study and explore the various management options that this book offers from (1) standardization of good agricultural practices for each culture species, (2) education and capacity building, (3) establishment of standard limits, (4) strict enforcement of compliance, and (5) establishment of baseline information and continuous monitoring.

As I congratulate all the researchers and writers in coming up with this important work, I exhort all the Manila Bay stakeholders and all the books' readers to make full use of the heaps of information found in this new milestone in fishery research.

MABUHAY KAYONG LAHAT!

EMMANUEL F. PIÑOL
Secretary
Department of Agriculture

Message

The Department of Agriculture – Bureau of Fisheries and Aquatic Resources (DA-BFAR) congratulates the Save Manila Bay Project Committee and the National Fisheries Research and Development Institute (NFRDI) for coming up with this book titled Pollution in Manila Bay Aquaculture Farms: Status, Impact, and Remedial Options. We thank NFRDI for continuing its assessment in the state of fisheries resource of Manila Bay particularly the effect of aquaculture farms in the bay area.

For so many years, Manila Bay has been the source of food and livelihood for communities around the area. However, rapid and massive urban development gravely affected the bay's biodiversity. This serious concern in fact, prompted the Supreme Court to issue a Writ of Mandamus compelling concerned government agencies including DA-BFAR to collaborate in restoring the ailing marine life of Manila Bay.

The DA-BFAR's effort to restore Manila Bay is aligned with the Department of Agriculture's main thrust. Under the esteemed leadership of Secretary Emmanuel Piñol, the Department seeks to ensure food sufficiency and improve the livelihood of the fisherfolk through the implementation of various marine conservation programs in the country's major fishing grounds including Manila Bay.

May this book, which presents research results showing the impact of aquaculture in Manila Bay, be an effective information tool that we hope to awaken the consciousness of resource uses and stakeholders to be responsible in doing aquaculture and other similar farming activities. The book also contains technical paper which can serve as ready reference for policy formulation and program development.

Let us continue to rehabilitate the Manila Bay not because there is an existing Mandamus. We must save the Bay because it is our responsibility to leave a legacy of safe and clean marine environment that the future generations deserve.

Maraming salamat po at mabuhay kayo!

COMMODORE EDUARDO B GONGONA PCG (Ret)
Undersecretary for Fisheries and concurrent BFAR National Director

Preface

The Department of Agriculture through the Bureau of Fisheries and Aquatic Resources (DA-BFAR) is one of the mandamus agencies tasked to revitalize the marine life in Manila Bay. Within the scope of the High Court's directive to the DA-BFAR is the scientific assessment of the aquaculture activities in the coastal zones of the bay. The primary purpose is to gauge the impact of fish farming activities to water pollution loading. Information resulting from the study will be used to formulate appropriate management policies that would help address the impact of aquaculture in the waters of Manila Bay.

In fulfillment of this particular task, the DA-BFAR through its research arm, the National Fisheries Research and Development Institute conducted the required scientific study and presents its results in a more popularized form by writing this book. This publication entitled **"Pollution in Manila Bay Aquaculture Farms: Status, Impact, and Remedial Options"** mainly features the first comprehensive report of the status of pollution in the aquaculture farms surrounding Manila Bay. It provides an account of the present situation and highlights information that can be helpful to concerned national government agencies, local government units, Fisheries and Aquatic Resources Management Councils (FARMCs), non-government organizations, and other stakeholders in their collaborative effort to make the marine environment of Manila Bay teeming with life again.

The publication of this book is part of the overall initiatives of the DA-BFAR to accomplish the tasks given by the Supreme Court. We hope that more than aiding the search for baseline information, this book would be a tool to help people become more informed, empowered, and engaged in the government's effort to conserve and protect our marine resources.

Drusila Esther E. Bayate
Flordeliza D. Cambia
Ulysses M. Montojo

Acknowledgment

We are grateful to the MBCO for providing guidance, and to the BFAR management for the funding support. Our heartfelt appreciation is also expessed to the contributors to this volume and all program members, including BFAR - Fisheries Resource Management Division, BFAR Regional Office III, and IV-A, for their ideas, efforts, and time throughout the study.

We would like to express our sincerest gratitude to Prof. Roman C. Sanares for being constantly available for consultation whenever we needed to make statistically valid decisions and to Dr. Maripaz L. Perez, Dr. Macrina T. Zafaralla and Dr. Maria Rowena R. Eguia for taking the time in reviewing the book despite their busy schedules. They had made brilliant comments and suggestions that had enhanced the content of this publication.

The steady support given by the staff of the Provincial Offices of Bataan, Bulacan, Cavite and Pampanga, headed by their governors: Hon. Albert S. Garcia; Hon. Wilhelmino M. Sy-Alvarado; Hon. Juanito Victor C. Remulla; and Hon. Lilia G. Pineda, through their Provincial Agriculture Officer: Imelda D. Inieto; Gloria S.F. Carillo; Lolita C. Pereña; and Edilberto E. Salenga, respectively, are also greatly appreciated.

We are very thankful to the Municipal Mayors and Agriculture Officers of the following municipalities: (1) Limay, Bataan: Hon. Lilivir B. Roque & MAO Felipe Mendoza; (2) Orion, Bataan: Hon. Antonio L. Reymundo, Jr. & MAO Cardina C. Generillo; (3) Abucay, Bataan: Hon. Ana D. Santiago & MAO Caridad Rubiano; (4) Orani Bataan: Hon. Efren E. Pascual, Jr. & MAO Arturo Matias; (5) Samal, Bataan: Hon. Generoso M. Dela Fuente & MAO Nora A. Medina; (6) Hermosa, Bataan: Hon. Danilo C. Malana & MAO Susan Macalanao, (7) Hagonoy, Bulacan: Hon. Raulito T. Manlapaz, Sr. & MAO Dioscoro Francisco, (8) Obando Bulacan: Edwin C. Santos & MAO Freddy Sta. Maria; (9) Bulakan, Bulacan: Hon. Patrick Neil F. Meneses & MAO Elizabeth M. Salvador; (10) Meycauayan, Bulacan: Hon. Joan V. Alarilla & MAO Elvira B. de Asis; (11) Calumpit, Bulacan: Hon. Jessie P. de Jesus & MAO Carina S. Bernardo; (12) Malolos, Bulacan: Hon. Christian D. Natividad & MAO Estelita Mendoza; (13) Paombong, Bulacan: Hon. Isagani G. Castro & MAO Imelda Tolentino; (14) Marilao, Bulacan: Hon. Juanito H. Santiago & MAO Hilario L. Francisco; (15) Ternate, Cavite: Hon. Hermino C. Lindo & MAO Elizabeth Nazareno; (16) Bacoor, Cavite: Hon. Strike B. Revilla & MAO Ella P. Guinto; (17) Naic, CaviteL Hon. Junio C. Dualan & MAO Merlita Tamboong; (18) Cavite City, Cavite: Hon. Bernardo S. Paredes & MAO Solita Campit; (19) Guagua, Pampanga: Hon. Dante D. Torres & MAO Catalina Roman; (20) Sasmuan, Pampanga: Hon. Nardo M. Velasco & Marita Ocampo; (21) Macabebe, Pampanga: Hon. Annette B. Flores-Balgan & MAO Hermes Castro; (22) Lubao, Pampanga: Hon. Mylyn P. Cayabyab & MAO Maria David; (23) Masantol Pampanga: Hon. Danilo S. Guinto & MAO Arlene Bonifacio; and (24) Minalin, Pampanga: Hon. Edgardo G. Flores & MAO Edna Manlapat. Special thanks is also expressed to Ms. Cynthia Saratan of the Binakayan Shellfish Demo Center. They, together with their dedicated and accommodating staff, had assisted our team in locating aquaculture farms and coordinating with farm owners, operators and caretakers during the conduct of the project's sampling area validation, periodic sample collections, and other field activities.

Lastly, this project would not be possible without the continuous cooperation of the owners, operators, and caretakers of the aquaculture farms we have surveyed and sampled. They did not fail to make us feel welcome whenever we conducted our sample collections and site surveys. Their responsiveness is greatly acknowledged.

Photo: Shellfish-growing Area, Bataan
By: Heherson G. Baun

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Photo: Sun drying of Pond, Bataan
By: Heherson G. Baun

List of Acronyms

ATI	-	Agricultural Training Institute
BAI	-	Bureau of Agricultural Industry
BAS	-	Bureau of Agricultural Statistics
BFAR	-	Bureau of Fisheries and Aquatic Resources
BMP	-	Best Management Practices
BOD	-	Biological Oxygen Demand
BPI	-	Bureau of Plant Industry
BSWM	-	Bureau of Soils and Water Management
CNFIDP	-	Comprehensive National Fisheries Industry Development Plan
DA	-	Department of Agriculture
DENR	-	Department of Evironment and Natural Resources
DMR	-	Dry Matter Ratio
DO	-	Dissolved Oxygen
EC	-	European Commisision (European Union)
EMB	-	Environmental Management Bureau
FAO[1]	-	Fisheries Administrative Order
FAO[2]	-	Food and Agriculture Organization
FCR	-	Feed Conversion Ratio
FDA	-	Food and Drug Administration
FPA	-	Fertilizer and Pesticide Authority
GAqP	-	Good Aquaculture Practices
IMO	-	International Maritime Organization
IUCN	-	International Union for Conservation of Nature
LDC	-	Local Development Council
MPCA	-	Minnesota Pollution Control Agency
NFRDI	-	National Fisheries Research and Development Institute
NMIS	-	National Meat Inspection Service

List of Acronyms

NSO	-	National Statistics Office
PFDA	-	Philippine Fisheries Development Authority
PEMSEA	-	Partnerships in Environmental Management for the Seas of East Asia
PHILMINAQ	-	Mitigating Impact from Aquaculture in the Philippines
PhilRice	-	Philippine Rice Research Institute
PNS-BAFS	-	Philippine National Standards - Bureau of Agriculture and Fisheries Standards
PSA	-	Philippine Statistics Authority
RFO	-	Regional Field Office
SEAFDEC	-	Southeast Asian Fisheries Development Center
SRA	-	Sugar Regulatory Administration
UNDP	-	United Nations Development Program
USAID	-	United States Agency for International Development
US-EPA	-	United States - Environmental Protection Agency

Photo: Paddle Wheel Aerator, Bataan
By: Heherson G. Baun

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Photo: Crabs, Bataan
By: Heherson G. Baun

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Executive Summary

Summary of Findings

Aquaculture farms around Manila Bay and the species reared therein are contaminated with nutrients, heavy metals, and coliform bacteria. The levels of ammonia, nitrite, nitrate and phosphorus varied widely in fish ponds and coastal areas including shellfish growing areas and fish pens of the different blocks. Ammonia levels, which ranged from 0.0781 - 3.7455 μg/ml, were higher compared to the other nutrient species. Next is total phosphorus (range of 0.0080 - 2.0116 μg/ml), then nitrate (range of ND - 0.4459 μg/ml), and lastly, nitrite (range of ND - 0.1290 μg/mL). In addition, all of the aquaculture farms monitored failed to meet the standard for ammonia in water by 1.56 to 74.91 times while 11.53% exceeded standard for total phosphorus in water by 1.04 to 4.02 times (DAO 2016-08).

Preliminary determination of nutrient loading showed ammonia and TKN concentrations were significantly higher ($p<0.05$) during the flooding (ranges = 0.81 – 4.63 μg/mL and 1.72 – 6.76 μg/mL, respectively) compared to the draining (range = 0.79 – 2.43 μg/mL and 1.56 – 2.91 μg/mL, respectively) while nitrate levels were significanlty higher ($p<0.01$) during the draining (range = 0.06 – 1.34 μg/mL) compared to the flooding (range = ND – 0.97 μg/mL). On the other hand, nitrite levels during the draining (range = 0.03 – 0.06 μg/mL) and flooding (range = 0.01 – 0.06 μg/mL) were comparable with each other. Ammonia levels in both flooding and draining exceeded the standard by 15.8 to 92.6 times while nitrate levels are within the standard (DAO 2016-08). Phosphorus was not detected in any of the samples collected.

Heavy metals, specifically mercury analysis of pond water samples showed that 3 out of 46 sampling sites, all from Southern Bataan during the wet season and 14 out of 47 sites (three from Eastern Bulacan, one in Western Bulacan, Northern Bataan, and Southern Bataan, and eight in Cavite) during the dry season failed to meet the DENR regulatory limit (DAO No.34, 1990). For lead, 3 out of 46 samples exceeded the regulatory limit, all from Bataan during the wet season. In contrast, all sites were below the regulatory limit for cadmium. On the other hand, 1 out of 12 milkfish and 1 out of 13 oyster samples, collected from Pampanga and Cavite, respectively, were found to exceed the regulatory limit for mercury (EC 1881/2006). Analysis of lead in aquaculture commodities showed that 2 out of 12 milkfish samples, collected from Eastern Bulacan and Pampanga, and 1 out of 9 tilapia samples, from Pampanga, failed to meet the regulatory limit.

Total coliform, fecal coliform, and *Escherichia coli* were also detected in water with levels ranging from <1.8 to >160,000 MPN/mL, <1.8 to 54, 000 MPN/100mL, and <1.8 to 49, 000 MPN/100mL, respectively. Moreover, 6.67% of the milkfish samples, 16% of the tilapia samples, 24.44% of the shrimp samples, 8.89% of the crab samples, 14.67% of the oyster samples, and 25% of the mussel samples exceeded the standards for *E. coli* concentration in seafood (FDA, 2013).

These relatively high levels of contaminants were attributed to the incompliance of most farmers to the proper guidelines on good aquaculture practices as reviewed also by the team. For example, 66% of the aquafarms do not observe proper buffer zoning. Moreover, 72% of the pond owners do not monitor water quality or utilize improper monitoring methods due to lack of proper equipment. Important pond preparation activities, like soil testing, are also bypassed by 89% of the respondents. Use of illegal and noxious chemicals, e.g. cyanide, were also noted in 47% of the sites. Farmers domesticated and/or allowed wading of animals on pond embankments that could cause contamination in the water and culture species. Anthropogenic activities such as sewage and garbage disposal, industry and agriculture could have also contributed to the water quality deterioration. This is further complicated by the fact that available guidelines are nonspecific and vague. In addition, fish farming manuals available in the country contain varying techniques.

River tributaries where farmers source their water may be already contaminated before entering the aquaculture farms. The statistically similar contamination levels in pond and water source in most of the sites imply that equilibrium in both locations is either due to the amount of contaminants coming in from the river during pond water intrusion or going out to the river during water discharge. Moreover, amount of rainfall, temperature, tidal state, water quality parameters, climatic conditions and other external factors may also have an effect on the levels of contaminants.

Management Options

Standardization of GAqP (Good Aquaculture Practices) for each Culture Species

Assessment of aquaculture performance was found difficult because of the diversity of aquaculture activities and potential impacts that varied with area, culture facilities, and species. This is further complicated by the fact that available guidelines are nonspecific and vague. Fish farming manuals available in the country also contains varying techniques. In this regard, it is recommended that standardized GAqP for each culture species be established. Standardized GAqP should identify the allowed and standard amount of fertilizers, pesticides, limes, feeds and other inputs applied to prevent unnecessary application which could lead to contamination.

IEC and Capacity Building

Upon standardizing the aquaculture techniques as discussed above, seminars and trainings on GAqP should be conducted. Impacts of pollution should also be discussed in these seminars. Environmental-friendly techniques on farming may also be introduced to the farmers such as the use of biofilters and biomanipulators, reservoirs and settling ponds, closed recirculation systems and other techniques to lessen the pollution and its impacts.

Production of IEC materials such as brochures, posters, manuals and leaflets containing guidelines on the implementation of good aquaculture practices and environmental-friendly techniques can also be created for distribution to the farmers.

Establishment of Standard Limits

Difficulties in the assessment of the status of pollution indicators and other contaminants were experienced due to the absence of regulatory standard limits for brackish water to compare the results of nutrients such as, ammonia, nitrite, nitrate and phosphorus, and indicator bacteria, fecal coliform, *E. coli* or *Enterococci,* in fishery waters in the country. Without the standard limits which set as reference levels, regulation and control of pollution indicators and other contaminants levels in fishery waters will also be more challenging.

Strict enforcement of compliance on policies, guidelines and ordinances

Policies, guidelines and ordinances on proper zoning and land use, effluent water quality standards and solid waste management should be strictly enforced in these areas to minimize water quality deterioration.

Establishment of Baseline Information and Continuous Monitoring

Guidelines and policies alone will not be adequate to resolve this current environmental crisis and, food and consumer safety issues in Manila Bay aquaculture farms. Monitoring programs on chemical and microbiological contaminants on farmed fishery and water resources should be institutionalized to assess the geographic and periodic variation of contaminants, the pollution indicators in the bay, and to determine the effects of the different interventions to reduce the effects of aquaculture activities on the environment. Establishment of baseline information on more chemical and microbiological contaminants, as well as further studies on nutrient loading, are also necessary for more effective management.


Photo: Fishpond, Bataan
By: Heherson G. Baun

Introduction

CHAPTER 1

Manila Bay, Aquaculture and the Supreme Court Mandamus

Manila Bay

Manila Bay is a semi-enclosed estuary located at the southwest part of Luzon, a major island in the Philippines. The bay is 60 km long with a coastline of approximately 190 km wide and a surface area of about 1800 km^2 (PEMSEA, 2001, 2006; Jacinto *et al*, 2006). It is surrounded by Cavite in the south, Metro Manila in the east, Bulacan and Pampanga in the north, and Bataan in the west and northwest (Jacinto *et al*, 2006; Manila Bay Atlas, 2007). Water from approximately 17, 000 km^2 of watersheds consisting of 26 catchment areas, which include the huge Pampanga river basin, drains into Manila Bay (PEMSEA, 2001). Most of the river systems in the province of Pampanga, Bulacan, and Nueva Ecija drain into the Pampanga River, contributing approximately 49% of the net freshwater influx into the bay (PEMSEA, 2001; Jacinto *et al*, 2006).

Around 30% of the country's population is found in the Manila Bay area, which increasingly contributes to the national gross domestic product from 53% in 2007 to 62.7% in 2013 (PSA, 2007-2013; PEMSEA, 2006). As of 2010, more than 34 million Filipinos reside in the Manila bay area. Around 20 million of this population lives in the coastal areas while the rest comprise the non-coastal population (PSA, 2010). Population in the bay is projected by NSO (2010) to escalate up to 39 million in the year 2020.

Manila bay is highly regarded in the Philippines for its historical, cultural, social, and economic significance. In the study conducted by PEMSEA-UNDP-IMO in 2005, the economic valuation of the uses of Manila Bay as well as the benefits derived from the bay habitats is as high as 8.3 billion pesos per year. Two of the main uses of Manila bay are shipping and navigation and tourism with an economic value of 10% and 23%, respectively. Evidence to this is the presence of the Manila International Container Terminal (MICT), Manila North and South Harbors, and Port of Limay, and of such extensively preserved historical remnants, monuments, centuries-old churches, natural parks, beaches, and national shrines. In fact, Manila Bay regions such as the NCR, Region IVA, and Region 3 ranked 2^{nd}, 3^{rd}, and 4^{th}, respectively, among the top tourism destinations in the country.

Aside from shipping/navigation and tourism, 67% of the Manila Bay value is accounted for by the fisheries industry alone (PEMSEA, 2006). Eight percent of that is credited to offshore fisheries, which includes commercial and municipal fisheries. The bay is known as one of the premier fishing grounds in the country making fishing as the primary source of livelihood around the area (PEMSEA, 2006). Commonly caught fishes in the bay include sardines, mackerel, mullet, threadfin bream, squid, blue crab, round scad, and caesio while commonly fished resources in inland water bodies of the bay area include tilapia, goby, mudfish, carp, prawn, and catfish (BAS, 2012). In spite of a fluctuating trend, Figure 1.1 shows that offshore fisheries production as well as estimated production value of regions around the bay increased from 2005 to 2014 with 359, 220 MT (PhP 18 million) to 386, 360 MT (PhP 20 million), respectively (BFAR, 2005-2014). However, a decreasing catch per unit effort (CPUE) from 1947 (44.0 kg/hr) to 1993 (10.0 kg/hr) was reported by BFAR. This indicates a decline in the fishery resources attributed to overfishing and pollution in the bay (as cited in PEMSEA, 2006).

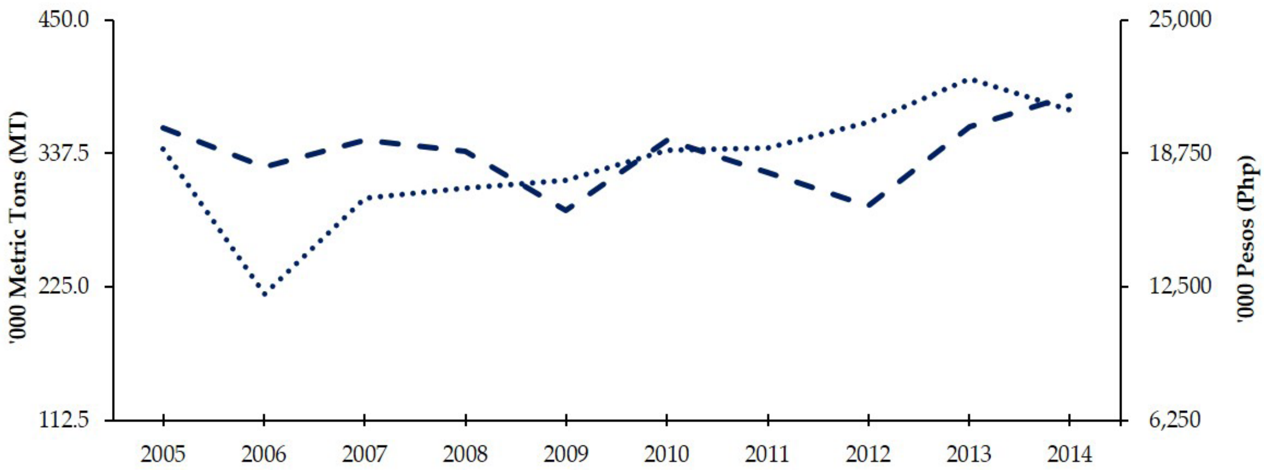

Figure 1.1. Manila Bay offshore fisheries production (— —) and estimated production value (••••••) from 2005-2014 (BFAR, 2005-2014).

Aquaculture in Manila Bay

Besides offshore fisheries, coastal and inland water resources, which account for 59% of the total economic value of Manila Bay, are considered to be among the most productive aquaculture areas in the country. With numerous fishponds, fish pens, and shellfish growing areas in Nueva Ecija, Bulacan, Pampanga, Tarlac, Cavite, Bataan, NCR, and Laguna Lake, these water resources accounts for almost 60,000 hectares of aquaculture area (PEMSEA, 2001; Perez *et al*, 1999). The commonly farmed species in the provinces surrounding the bay are milkfish, tilapia, mud crabs, prawns, oysters, mussels, and seaweeds (BAS, 2012). Overall, there has been an increasing trend in aquaculture production from 302, 287 MT to 410, 908 MT, with an average yearly increase of 3.13% (Figure 1.2). The corresponding production value has likewise increased from approximately 20 million in 2005 to 37 million in 2014 with an average increment of 6.04% per year. From 2005 to 2014, aquaculture sector production and value, which comprise an average of 54.3% and 63.7% of the total fisheries production, respectively, also exceeded that of the offshore fisheries recorded at 45.7% and 36.3%, respectively.

Increased aquaculture production especially coming from pond systems entails intensified intervention/inputs during pond fertilization, liming, stocking, and feeding. These specifically involve the use of feeds and fertilizers as well as other chemicals, which may compromise food safety and pose threats to the environment. Inorganic fertilizers may cause eutrophication and may contribute to an increase in ammonia toxicity in the natural waters (Boyd & Massaut, 1999). Manure, grass, and human wastes, on the other hand, may deplete oxygen in the water and introduce pathogens and heavy metals into the water and to the culture species (Boyd & Massaut, 1999; Golez, 2009; US EPA, 2013).

Although liming materials do not cause environmental and food safety problems, these are caustic and can be hazardous to workers if precautions are not taken (Boyd & Massaut, 1999). Pesticides, aside from being highly toxic, are bioaccumulative and can contaminate the final product thus unnecessarily exposing the consumers to food safety concerns according to the same author above. Wasted feed or unconsumed feed, in addition to pre-

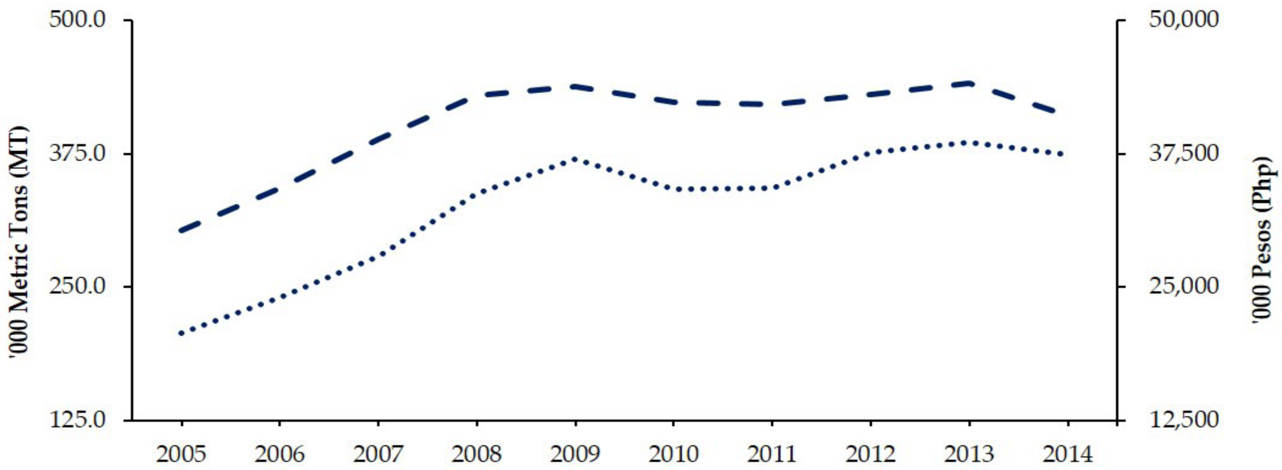

Figure 1.2. Manila Bay aquaculture production (— —) and estimated production value (••••••) from 2005-2014 (BFAR, 2005-2014).

disposing fish to disease-causing pathogens, is also a major contributor to the discharge of nutrients and organic matter from fish farms leading to eutrophication of the environment (PHILMINAQ, 2008; Boyd *et al*, 2007). Another serious threat emanating from the aquaculture is biological pollution or the accidental introduction of non-endemic species into bodies of waters. Escapees from aquaculture facilities may impair wild fish population through competition and interbreeding, or by spreading disease-causing organisms. In addition, aquaculture activities can alter aquatic system landscapes, which results in habitat modification (Erondu & Anyanwu, 2005).

The interactions involving both point and non-point sources of water pollution in pond management are diagrammatically shown by way of two schemes presented (Figures 1.3 and 1.4). The schemes reflect the cumulative impact of all kinds of aquaculture inputs on the Manila Bay. Figure 1.3 is the scheme for a closed system, while Figure 1.4 is for an open system. In both systems, pollutants are shown to enter into the fishponds and exit in the Manila Bay. The inputs from aquaculture practices like those used in disinfection, liming fertilization, and feeding contribute significant amounts of pollutants, if and when proper pond management is not observed. Entry into the Manila Bay takes place during pond water discharge/exchange. To address these possible impacts of aquaculture, one of the strategies of the Comprehensive National Fisheries Industry Development Plan (CNFIDP) Medium-Term 2016-2020 is to institutionalize Good Aquaculture Practices (GAqP) for key commodities and promote sustainable aquaculture (BFAR, 2015).

The Supreme Court Mandamus

In 2008, concerned residents of Manila Bay filed a complaint against the inaction of the government in improving the bay's condition. Several government agencies including the Department of Agriculture (DA) passed a petition against the complainants, which however, were denied. This led to the Supreme Court En Banc (G.R. Nos. 171947-48) for the Manila Bay Clean up, Rehabilitation and Restoration, ordering the DA, as one of

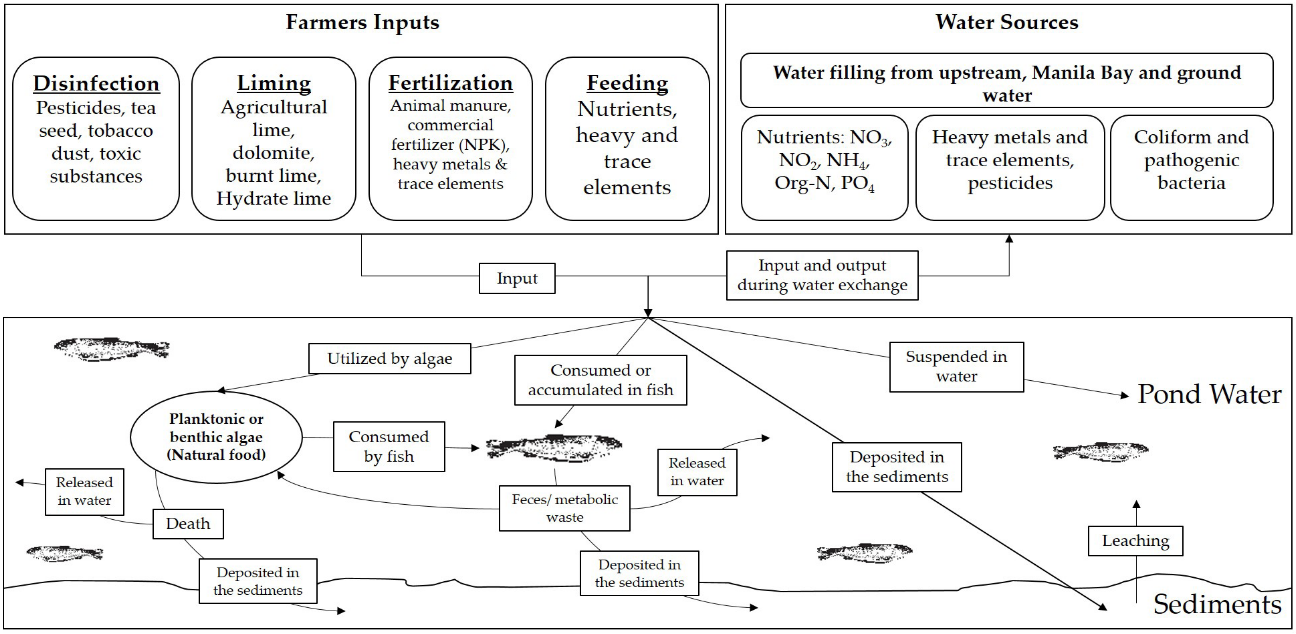

Figure 1.3. Interrelationships in pond management leading to the possible loading of pollutants from fish ponds (closed system) into the Manila Bay system.

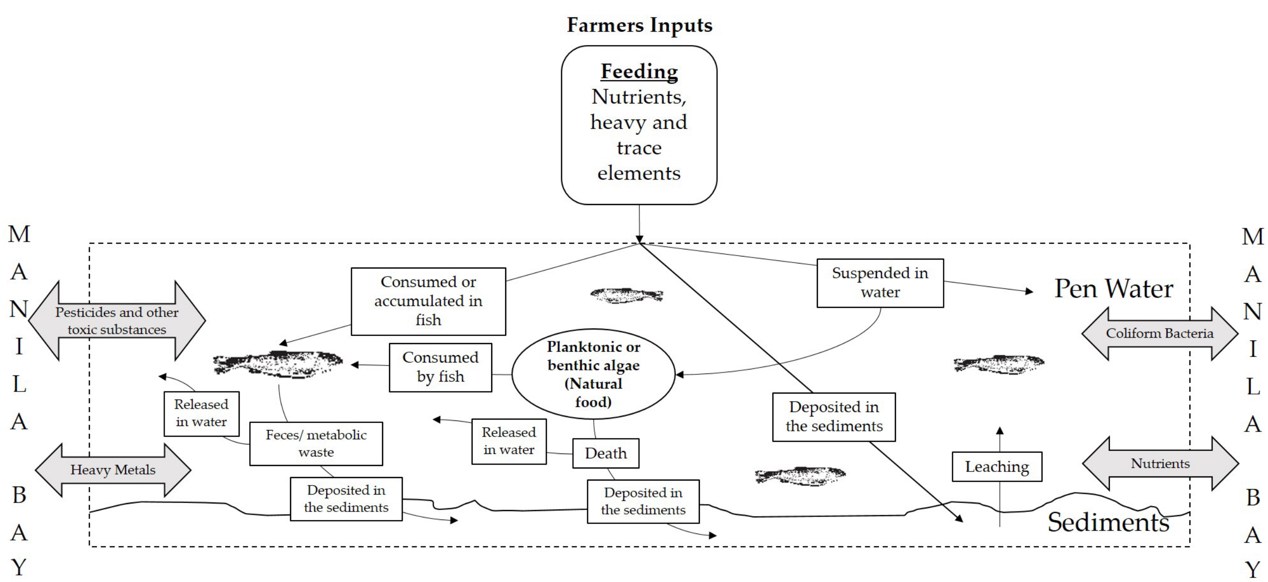

Figure 1.4. Interrelationships of aquaculture practices and pollutant loading of Manila Bay (open system).

the mandamus agencies, to "submit to the Court the baseline data on pollution loading into the Manila Bay system from agricultural and livestock sources.

In response to this, the DA instructed BFAR-NFRDI together with BSWM, BPI, FPA, BAI, PFDA, PhilRice, ATI, LDC, NMIS, SRA, and RFOs III and IVA to implement measures towards improving the overall management of agricultural activities (croplands, livestock, and aquaculture farms) within the Manila Bay region and to conduct IEC activities for the current period.

This compelled the DA-BFAR to conduct a study to assess the possible contribution of aquaculture farms and the fisheries sector to the pollution loading of Manila Bay. Specifically, it aimed to establish a baseline data on the pollution levels in Manila Bay aquaculture farms, and to formulate appropriate interventions/measures.

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Photo: Microbiological Analysis of Water
By: Heherson G. Baun

SUB-STUDIES

CHAPTER 2

Review of Aquaculture Practices and Anthropogenic Activities in Manila Bay Aquaculture Farms

Opinion, April Grace R. and Raña, Joan A.

Abstract

Environmental problems arising from aquaculture activities have been a concern worldwide, especially in Manila Bay. Thus, this study was implemented to identify the aquaculture practices of fish farmers and assess whether these practices are in accordance with the good aquaculture practices (GAqP) guidelines. Information was collected through survey and interview of fishpond operators from provinces around the bay. The study found that most fish farmers around the Bay do not observe the guidelines on GAqP evidenced by their high non-compliance with buffer zone, lack or utilization of improper water quality monitoring methods, non-implementation of important steps in pond preparation (e.g. soil testing), and application of illegal and noxious chemicals (e.g. cyanide). The calculated FCR, DMR and WPR in selected farms greatly varied. Farmers domesticated and/or allowed wading of animals on pond embankments predisposing contamination of both water and culture species. Anthropogenic activities such as sewage and garbage disposal, industry, and agriculture possibly added to deterioration of water quality in the fish ponds. On the other hand, notable practices were also observed in the areas such as the adoption of polyculture and semi-intensive fish farming methods, and the stocking of seeds from hatcheries.

Keywords: aquaculture, GAqP, anthropogenic activities, Manila Bay

Introduction

Aquaculture in the Philippines is believed to have started as early as the 14th century and involved the culture of several finfish and invertebrate species employing different farming practices applied in diverse ecosystems. Since 1976, production from aquaculture has been continuously augmenting compensating the unstable and plateauing fish supply from capture fisheries (BFAR, 2005; 2013). It has started to surpass the production of municipal and commercial fishery sectors since 1996. According to statistical data, production in 2003 corresponding to 1, 454, 503 MT rose to 2, 541, 965 MT in 2012, giving an increase equivalent to roughly 57% in just a decade (Lopez, 2006; BFAR, 2012). In 2013, aquaculture production was approximately 2, 373, 386 MT or 50.46% of the total fisheries production of the country (BFAR, 2013).

The sustained increments in aquaculture production have benefitted the country, especially the low income consumers, who continue to subsist on fish as a major protein source. Aquaculture adequately provides the supply needs of both local and international markets. In fact, approximately 18% of the food fish supply currently comes from aquaculture (PHILMINAQ, 2008). Commercially viable businesses have also been opened in the country ranging from small-scale wet market trading to large scale fish processing plants. With these businesses, employment has been generated especially in rural areas where work opportunities are scarce. According to the 2002 Census of the National Statistics Office (NSO), 226,195 individuals are directly employed in the aquaculture sector (BFAR, 2013). However, the industry estimates employment generation to be higher since the Seaweed Industry Association of the Philippines (SIAP) claims that in 2002, around 1, 017, 925 individuals are engaged in the seaweed industry alone (Monzales, 2003). In addition, the national fish export of the country has increased. BFAR data of 2013 showed that there was a 101% increase in the fishery export volume of the country, as seen in the comparison of volumes produced from 2012 to 2013 (165, 324 vs. 333, 465 MT). The difference is attributed to the increase in seaweeds and shrimp/prawn production, which mainly come from the aquaculture sector. Seaweeds ranked 2nd in terms of export value with a 64% increase from US $185.6 million in 2012 to US $218.7 million in 2013. On the other hand, shrimp/prawn ranked 3rd in 2013 with a total contribution of US $67.5 million to the total export value, or 55% higher than 2012's export earnings of US $37.3 million (BFAR, 2013).

In an effort to optimize benefits from aquaculture, several laws and administrative orders were passed. For example, to further boost aquaculture production, Presidential Decree (P.D.) 704 of 1975 provides for the (a) establishment of fish hatcheries, nurseries, and demonstration fishponds; (b) conduct of experiments and technical demonstrations on the culture of fishery products; and (c) issuance of Fishpond Lease Agreements (FLAs) and permits to operate fish pens and set aside public lands to be subdivided into family-sized fishponds for leasing. The conversion of mangrove areas to fishponds was legalized thru P.D. 705, which stipulates that suitable mangrove areas for fishpond purposes be placed under the administrative jurisdiction of the Bureau of Fisheries and Aquatic Resources (BFAR). Meanwhile, in 1991, the cutting of any mangrove species was prohibited by virtue of Republic Act (R.A.) 7161. Moreover, BFAR Fisheries Administrative Order (FAO) 125 converted fishpond lease agreements from 10 years to 25 years.

Several environmental risks and hazards of aquaculture have been identified as follows: (1) biological pollution or the release of non-endemic species into the water bodies; (2) habitat modification, which happens when aquaculture activities alter landscapes of aquatic systems resulting in habitat destruction and loss of biodiversity; (3) chemical; and (4) organic pollution mainly from the different inputs during the aquaculture activities (Erondu & Anyanwu, 2005). The expansion of the aquaculture sector in the country therefore has not been without problems. Environmental problems arising from aquaculture activities have been a major concern in different areas in the country, including the Manila Bay area.

Manila Bay is an important water resource in the country as it is used for various purposes including aquaculture, which accounts for as much as 59% of the total economic value of the bay (PEAMSEA, 2006). This becomes more and more evident as fishponds, fish pens, and shellfish pens continue to proliferate along provinces surrounding the bay namely Bulacan, Bataan Pampanga, Cavite, and the northern Metro Manila coastlines, which cover an aquaculture area of almost 60,000 hectares (Perez *et al*, 1999).

Several reports on the possible harmful environmental effects of aquaculture in the bay have been released. In July 2013, for instance, the Philippine Daily Inquirer reported on the biological pollution of an exotic species of tilapia, also known as *tilapiang Gloria* or black chin tilapia (*Sarotherodon melanotheron*) in Bataan. It was believed that the species was introduced into the aquaculture areas of Bataan several years ago. In addition, approximately 63.6% of mangrove loss from 1995 to 2006 was primarily attributed to the rampant conversion of mangrove swamps into aquaculture areas (PEMSEA, 2006). This is probably one of the reasons for the decline in the catch of wild shrimps and crabs in the rivers around the area. Chang *et al* (2009), also reported that Manila Bay is highly eutrophic due to organic pollution that yield nutrients like nitrogen. Mendoza (unpublished data, 2010) also observed that heavy metals such as As, Cd, Cu, Mn, Ni, Zn, Pb, Cr, and Hg have been detected in milkfish, tilapia, prawns, green mussel, clam *"paros"*, and oysters collected from fishponds in Bulacan.

Cognizant of the environmental problems emanating from aquaculture activities and in an attempt to address and limit these problems, BFAR issued FAO[1] 214 or the Code of Practice for Aquaculture (BFAR, 2001), in response to Section 47 of R.A. 8550 (as amended by RA 10654). The code outlines the general principles and guidelines for environmentally-sound design and operation for the sustainable development of the aquaculture industry. The Code of Good Aquaculture Practices (GAqp) released by the Philippine National Standard – Bureau of Agriculture and Fisheries Standards (PNS-BAFS, 2014), on the other hand, aims to minimize aquaculture risks.

It is against this backdrop that this study was implemented to generally identify fish farming practices employed by pond owners and assess whether or not these are in accordance with the guidelines on good aquaculture practices. Aquaculture ecological indicators such as feed conversion ratio (FCR), dry matter ratio (DMR), and waste production ratio (WPR), were also estimated. Possible support and interventions to enhance the adoption of good aquaculture practices among pond operators in and around Manila Bay were also recommended.

Methodology

Sampling Scheme

Sampling of the different aquafarms in provinces around Manila Bay followed a blocking strategy since Manila Bay is an estuary with a gradient of anthropogenically modified environments. Block 1 is Eastern Bulacan nearest to the National Capital Region. It is hypothesized to have more contamination. Block 2 is Western Bulacan with Angat River and Pampanga River delta. Block 3 is-Northern Bataan, adjacent to Pampanga River delta. Block 4 is Southern Bataan, towards the mouth of Manila Bay. Block 5 and Block 7 are in Cavite and Pampanga areas, respectively. Figure 2.1 shows the blocking scheme and the number and location of different sampling sites in each block.

The number of sampling sites per municipality was computed using the confidence interval method expressed as a precision for small samples (10% precision) based on the data on location provided by the LGUs. Using this approach, the total number of sites for the whole area was 160. Divided among 6 blocks, this gave 27 sites per block. Due to budgetary considerations, the minimum sampling size was set at 60% of the computed sampling size per block. This is equivalent to 16 minimum sampling sites per block. However, this

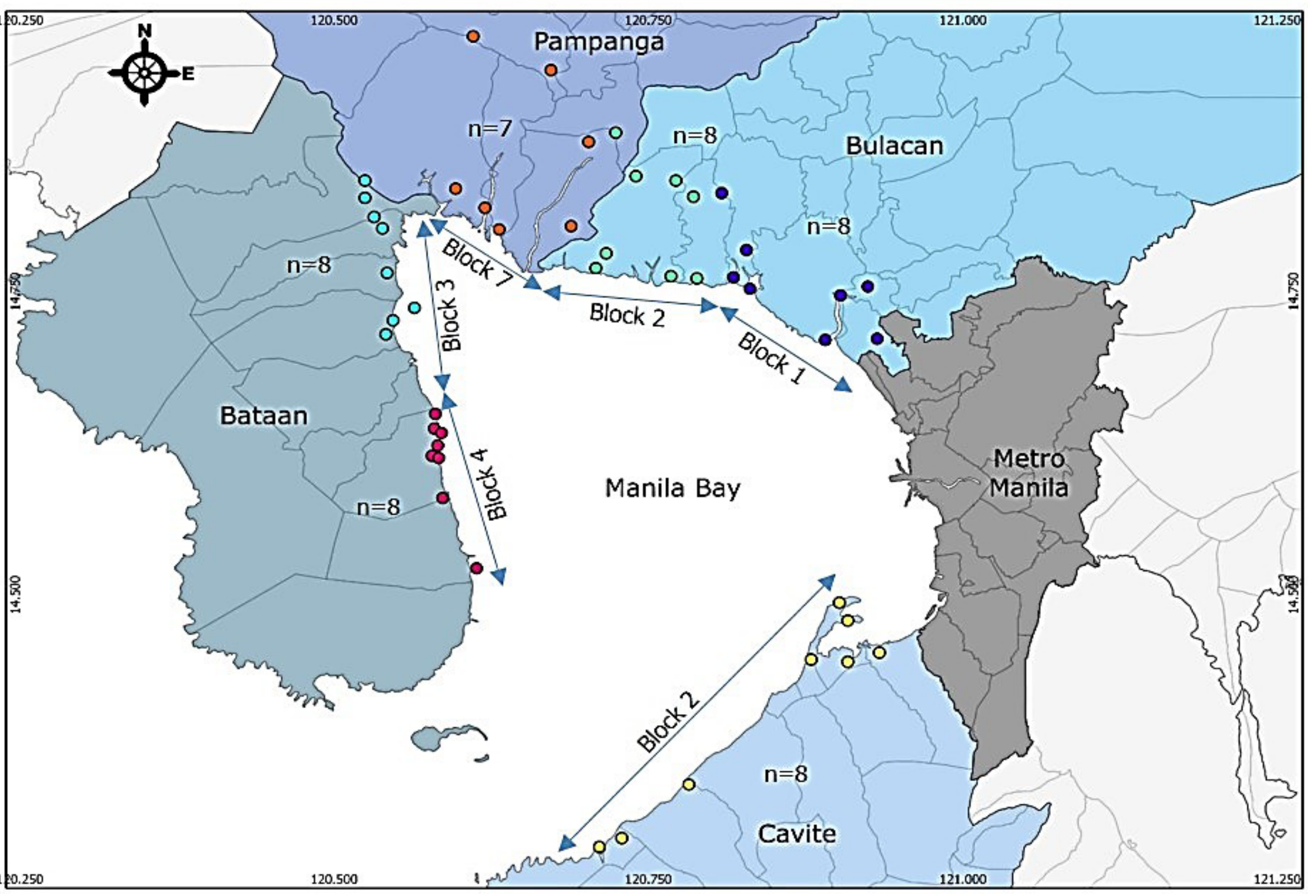

Figure 2.1. Blocking scheme and the number and location of sampling sites in each block.

was decreased or increased depending on the result of the preliminary sampling. The formula shown is used for the said method.

$$T = \frac{\mathrm{X} - \mu}{\sqrt{\mathrm{S}^2/n}} \cong \frac{\rho \mathrm{X}}{\sqrt{\mathrm{S}^2/n}}$$

$$n \cong \frac{\mathrm{T}^2\mathrm{S}^2}{(\rho \mathrm{X})^2}$$

Where:

T = distribution probability (set at n-1 degrees of freedom);
p = precision estimate (set at 10%);
n = sample number; and
S^2 = variance

During the preliminary sampling, the laboratories were not able to process the load of samples from the 16 sampling sites within the holding time – 48 hours. Thus, the number of sampling sites was reduced to 7 – 8 sites per block. Based on statistical analyses, the reduced number of sites is sufficient to determine the differences in water quality parameters between blocks.

Information Collection and Respondents

A total of 47 sites were surveyed. There were eight sites in Blocks 1 to 5 and seven sites in Block 7. Each site considered one respondent. The sampling sites are listed in Table 2.1.

Information on (1) site and farm, (2) water quality management, (3) pond preparation activities, (4) culture species, (5) feeding management, and (6) other anthropological activities were obtained through personal interview of fishpond operators, owners, and/or caretakers. Information obtained during the interview and the site survey were recorded in the Aquafarm Information Sheet (Appendices A and B).

Results and Discussion

The most common approach to minimize the negative environmental impacts of aquaculture is by improving the production practices through a) the use of better management practices (BMPs), b) good aquaculture practices (GAqP), and c) responsible and sustainable aquaculture (IUCN, 2009, Boyd *et al*, 2007, Howerton, 2001). However, the assessment of aquaculture performance against these could be difficult due to the diversity of activities and potential impacts that also vary with area, culture facilities, and species (Boyd *et al*, 2007). Moreover, available guidelines are nonspecific and vague. In the study, there were issues met in securing comprehensive information on aquaculture practices observed by operators. And so to simplify matters, the study attempted to compare the common practices observed in the different aquafarms around Manila Bay with local and international principles and guidelines, as well as results of previous studies on responsible aquaculture.

Basic Farm Information

Three farm types were observed: fish pens, fishponds, and shellfish growing areas. Artificial earthen fishpond (74%) is the most widely operated type of farm in the different provinces around Manila Bay (Figure 2.2). Shellfish growing areas (19%) are also present in the different blocks, except in Pampanga. Fish pens (6%) made of net and wooden materials are present only in Eastern and Western Bulacan. Farms size ranged from 0.3 to 125 hectares.

Table 2.1. List of sampling area around Manila Bay.

Block 1 (Eastern Bulacan)			Block 2 (Western Bulacan)		
Code	**Barangay**	**Municipality**	**Code**	**Barangay**	**Municipality**
B1-01	Binuangan (Coastal)	Obando	B2-01	Masukol (Coastal)	Paombong
B1-02	Paliwas	Obando	B2-02	San Roque	Paombong
B1-03	Ubihan	Meycauayan	B2-03	Sta. Cruz	Paombong
B1-04	Matungao	Bulakan	B2-04	San Isidro II	Paombong
B1-05	Pamarawan (Coastal)	Malolos	B2-05	San Agustin	Hagonoy
B1-06	Pamarawan	Malolos	B2-06	Meyto	Calumpit
B1-07	Calero	Malolos	B2-07	San Roque (Coastal)	Hagonoy
B1-08	Tawiran	Obando	B2-08	San Roque	Hagonoy
Block 3 (Northern Bataan)			**Block 4 (Southern Bataan)**		
B3-01	Almacen	Hermosa	B4-01	Limay (Coastal)	Orion
B3-02	Palihan	Orani	B4-02	Camachile	Orion
B3-03	Kabalutan	Orani	B4-03	Camachile	Orion
B3-04	Samal (Coastal)	Samal	B4-04	Balut	Orion
B3-05	Ibaba	Samal	B4-05	Capunitan	Orion
B3-06	Capitangan	Abucay	B4-06	Camachile	Orion
B3-07	Highway	Orani	B4-07	Capunitan	Orion
B3-08	Wawa	Abucay	B4-08	Sta. Elena	Orion
Block 5 (Cavite)			**Block 7 (Pampanga)**		
B5-01	San Juan I	Ternate	B7-01	Bangkal Sinubli	Lubao
B5-02	Timalang Balsahan	Naic	B7-02	Sapang Kawayan	Masantol
B5-03	San Rafael 4	Noveleta	B7-03	Consuelo II	Macabebe
B5-04	Bacoor Bay (Coastal)	Cavite City	B7-04	Batang 2	Sasmuan
B5-05	Marulas	Kawit	B7-05	Bangkal Pugad	Lubao
B5-06	Bacoor Bay (Coastal)	Bacoor	B7-06	San Antonio	Guagua
B5-07	Mabolo II	Bacoor	B7-07	Mani-ano	Minalin
B5-08	Bucana (Coastal)	Ternate			

Brackish water fish farming is the most common type of farming employed. Salt water and freshwater are derived from the Manila Bay and the different river tributaries, respectively. However, 12.5% of the farms in Northern Bataan obtain freshwater from the ground because of inaccessibility of river tributaries.

Site Selection and Design

Studies have shown that inappropriate and unplanned siting of farms result in production failures as well as environmental degradation (Howerton, 2001). As indicated in Section 3 code of GAqP of the PNS-BAFS, farms should be in an environmentally suitable area where risks to food safety from chemical, biological, and physical hazards from air, soil, and water are minimized. Furthermore, aquafarms should be properly selected to avoid negative impacts on the environment (SEAFDEC, 2009). Proper location can be achieved by conducting an environmental impact assessment (EIA) prior to the construction and development of the farms (PNS-BAFS, 2014; BFAR, 2001). During the survey the farmers were not asked if they have had an EIA conducted prior to locating their farms. The researchers entertained skepticism in being shared this information by the farmers. From the look of things, it was just presumed that since most farms surveyed are not registered, it is likely their owners did not conduct any EIA before the ponds were constructed.

FAO[1] 214 requires the maintenance of buffer zones or the space between the aquacul-

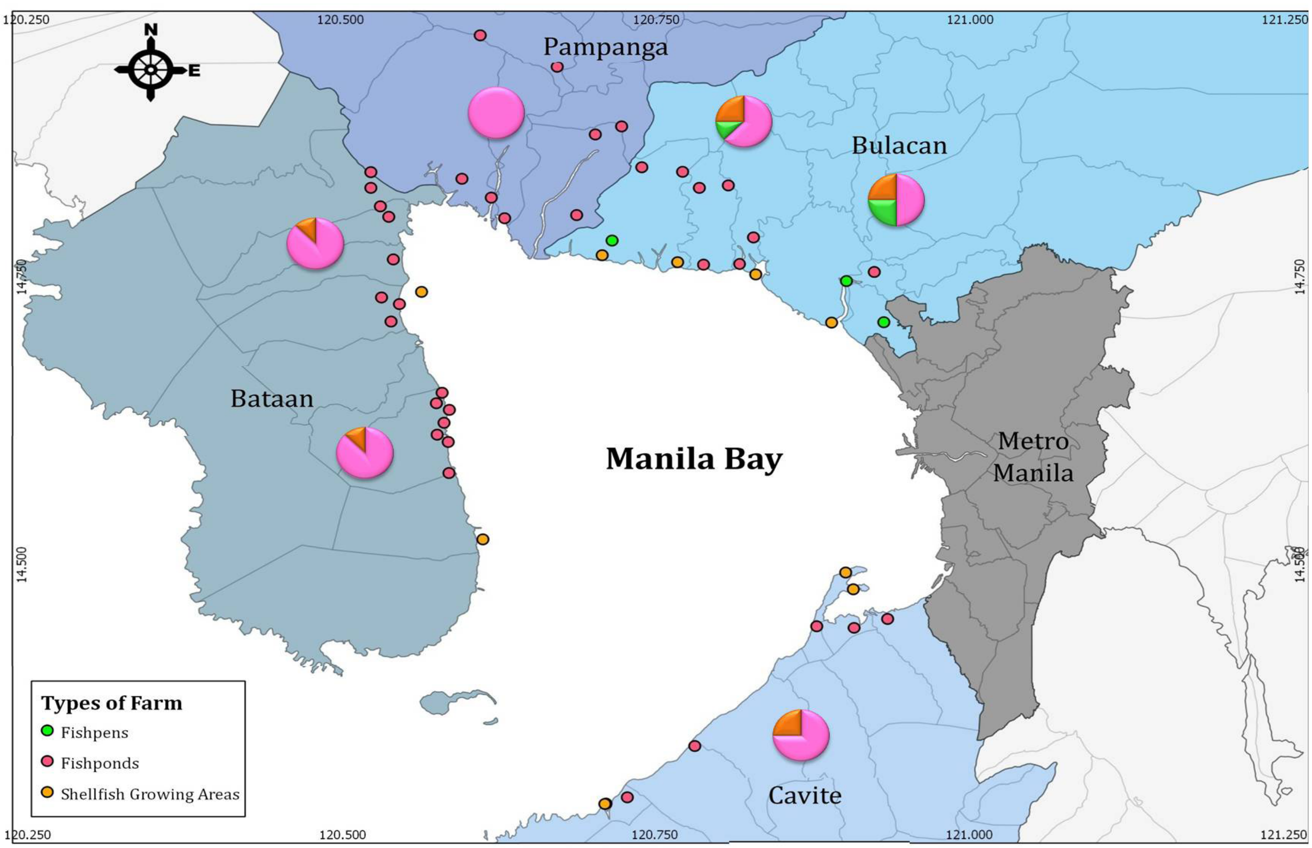

Figure 2.2. Map of the types of farms surveyed in the blocks (pie graph).

ture area and the sensitive ecosystems. These areas serve as environmental buffers to prevent direct settling of waste discharges such as uneaten food, fecal matter, chemical contaminants, and other effluents to the ecosystem (SEAFDEC, 2009; Ahmad *et al*, 2012). However, as seen in Figure 2.3, majority of the farmers do not have this system. Reasons include area constraints and costs associated with dike and canal constructions. Some areas were observed to share embankments with river bodies where water is obtained and discharged.

prevent aquaculture loss.

Section 6.2 of GAqP emphasizes the need for a regular and accurate water quality monitoring program to ensure that water parameters are within advisable limits (PNS-BAFS, 2014). Due to the lack of proper equipment, regular monitoring is not practiced or is incorrectly carried out by 37 - 100% of the pond owners from the different blocks (Figure 2.4). Furthermore, 20% and 100% of the farm ers in Eastern Bulacan and Northern Bataan, respectively, who checks the salinity of the

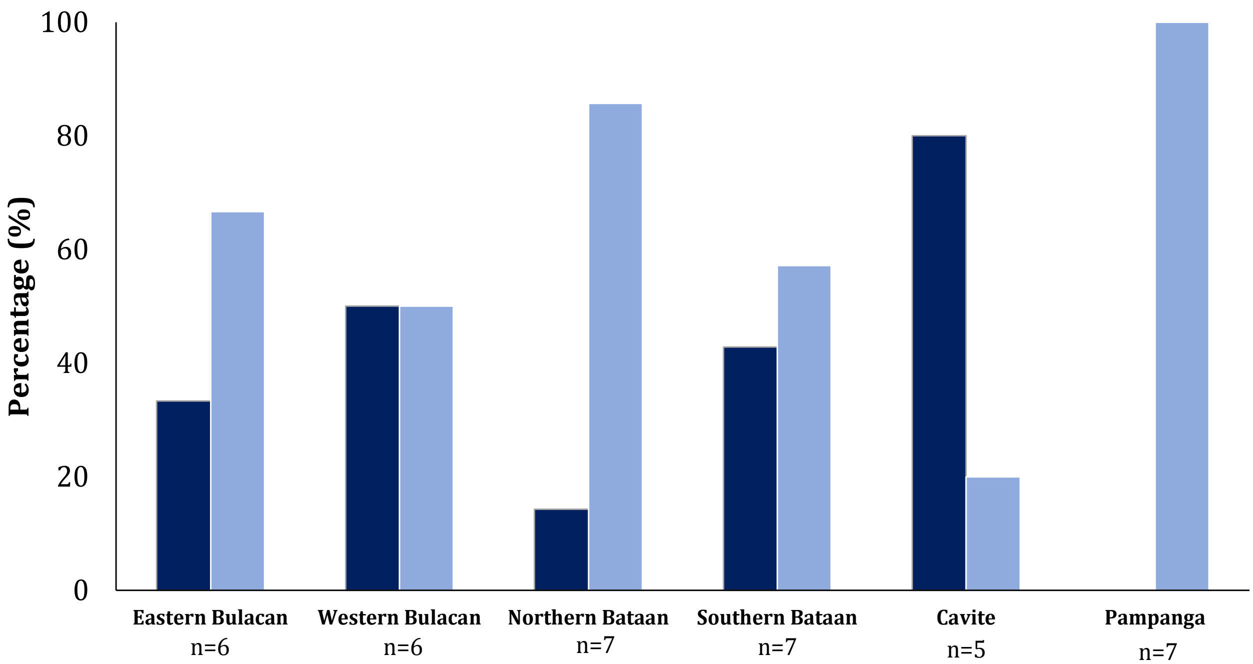

Figure 2.3. Percentage of pond owners that are compliant to proper buffer zone regulations.
Compliant Non-compliant

Water Quality Management

Water in the aquafarm has a profound effect on the health and growth of the culture species (Howerton, 2001). The water quality may deteriorate considerably over the culture period due to several factors including inputs and weather conditions (Boyd & Tucker, 1998); thus, monitoring and control is necessary to

pond use practical but inaccurate techniques such as the “taste method”. Only the intensive shrimp farm in Southern Bataan determines and records water quality parameters daily and tests for ammonia and nitrite concentrations as well as for water hardness twice a week. Twenty five per cent of the farmers in Cavite, on the other hand, depend on water monitoring assistance from the BFAR regional

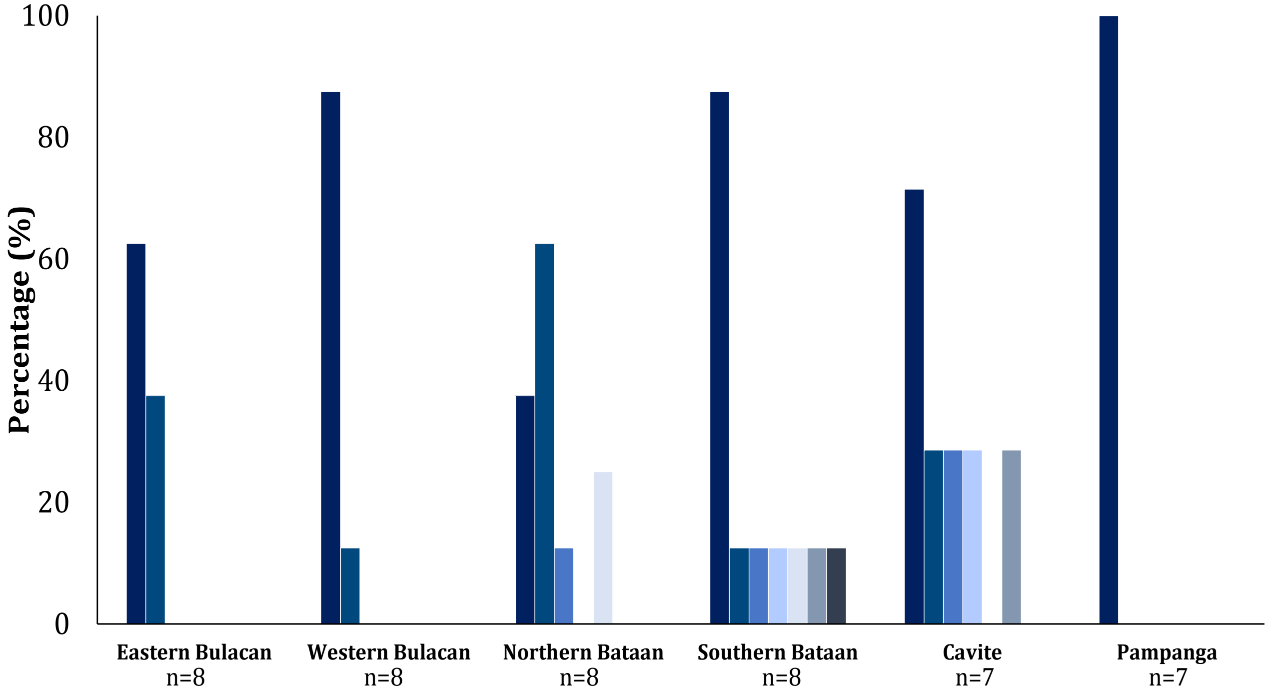

Figure 2.4. Percentage of pond owners that monitors selected water quality parameters: Not Monitoring Salinity pH Temperature Dissolved Oxygen Turbidity Others

office, which are conducted at random.

It was also noted that the primary means of farmers to improve water quality in the pond was through water exchange carried out by flushing nutrients and organic matter from the pond to the river tributaries. However, this practice tends to pollute receiving water bodies since large amounts of nutrients are discharged (Boyd, 2003b). Section 5 of FAO[1] 214 states that discharged water should meet water quality standards, which should be determined qualitatively and quantitatively. But, qualitative standard is relative as it is dependent on the judgment of the farmer and quantitative determination is hindered by the lack of proper equipment of the farmers.

Mechanical aeration can be an alternative option in improving water quality. Actually, it is more effective than water exchange in increasing pond production (Boyd *et al*, 2008). It also lessens the need for large amounts of water. However, mechanical aeration is not practiced by most of the farmers as it entails additional production cost. Only 13% of the farmer respondents in Northern and Southern Bataan use the spray type and paddle wheel aerators, respectively (Figure 2.5).

Another effective method of water quality improvement adopted by 25% of the farmers in Northern and Southern Bataan is the use of milkfish as biofilters or of organisms that ingest impurities from the water resulting in ponds that meet the required optimum physical and microbiological conditions for the cultured species (FAO[1] 214). Milkfish, being filter feeders, feed on the algae induced to grow by the accumulated nutrients coming from uneaten feeds. Presence of milkfish as biofilters somehow prevents algal blooms that

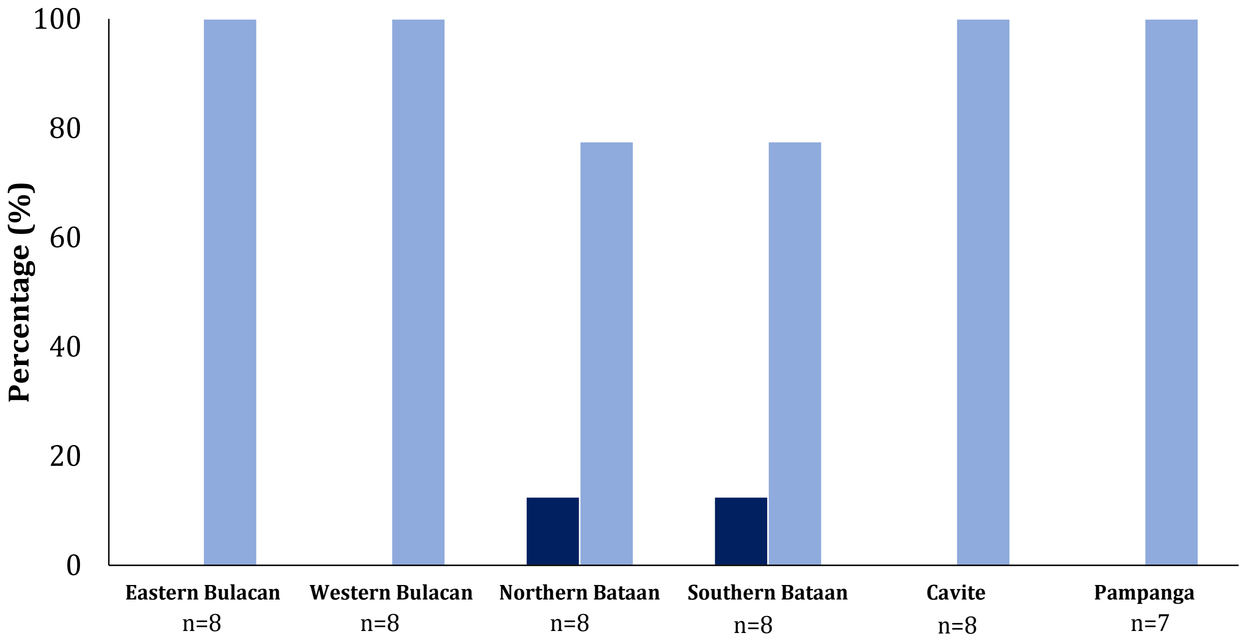

Figure 2.5. Percentage of pond owners that employ aeration system to improve water quality.

Uses Aerator   No Aerator

eventually die-off and deplete the dissolved oxygen resulting in a fish kill. This method of using milkfish as a biofilters ïs considered eco-friendly (Guererro, unpublished), and is more advantageous than using chemicals and substances to treat the water.

Pond Preparation Activities

Figures 2.6a to 2.6h show the sites in which several pond preparation activities were followed consistent with the recommended practices of SEAFDEC (2009). These steps that include soil testing (11%), pond drying (68%), soil scraping (53%), water flushing (38%), pest eradication (70%), liming (34%), and fertilization (53%) are usually performed by fishpond owners after every cropping.

It was noted that fish farmers bypassed several of the important steps during pond preparation because of the extra time, money, and effort involved. For example, soil testing is important in getting a prior knowledge of the fertilizer and lime requirements of the pond (SEAFDEC, 2009). However, it was discovered that 89% of the farmers skipped soil testing and yet performed fertilization and/or liming which leads to most likely erroneous application doses.

Farmers also bypassed pond drying (32%), soil scraping (47%), and water flushing (62%), resulting in the accumulation of nutrients from residual wastes, which in turn causes eutrophic pond water. The resulting accumulated sediments will also consume more oxygen, produce higher levels of ammonia and hydrogen sulfide, trigger propagation of pathogenic bacteria, and eventually cause disease outbreaks in the next production cycles (SEAFDEC, 2009).

Sodium cyanide (NaCN) is used by

46.8% of the farmers to eradicate unwanted species that prey on or compete with the culture species (Figure 2.7). In addition, organic and selective pesticides such as tobacco dust (nicotine) and teaseed (saponin) are used by 10.6% and 25.5% of the farmers, respectively. Inorganic pesticides – wood lice pesticide and ammonia – are used by a farmer in Kawit and a farmer in Bacoor, respectively.

The use of cyanide as a sound pest control measure is still debatable. Section 92 of RA 10654 states that "poisonous or noxious chemicals, including cyanide, which are used in aquaculture in accordance with accepted scientific practices shall not be construed as illegal fishing". The GAqP of PNS-BAFS gives some guidelines on the use of veterinary drugs and chemicals, which includes only the antibiotics, and other disease-controlling drugs and chemicals. FAO[1] 214 is silent on the use of cyanide, but it recommends the use of only biodegradable indigenous materials, like derris roots, teaseed, and tobacco dust which are actually used by some of the farmers interviewed. Recommendations are in place regarding the use of non-biodegradable compounds. Most of the banned and regulated chemicals and substances used in aquaculture in the Philippines are confined to disease control.

Technically, under natural conditions, cyanide does not persist or accumulate in soil and water because it is highly volatile (Canadian Council of Ministers of the Environment, 1999). However, the amount applied and the application procedure could be erroneous; posing threat of contamination of receiving water through water discharges that can cause high mortalities among the wild species. Moreover, cyanide use puts the health of farmers at risk to such health problems as thyroid condition, nerve damage and cancer (US EPA, 2009). Guidelines on the safe use and / or application of cyanide should be established.

Pond fertilization to improve primary productivity was carried out by 55% of the respondents. The commonly used fertilizers ware: chicken manure (13%), urea (46-0-0) (36%), complete fertilizer (14-14-14) (11%), and ammonium phosphate (16-20-0) (11%). Figure 2.8 shows the site-specific percentage of farmers applying these fertilizers. In one case out of 47, the farmer utilized other fertilizers including compost material or plant or animal manure. Reportedly, the fertilizer used for each cropping varied with availability and cost. The amount of fertilizer applied also varied with location.

The use of chicken manure in aquaculture is a way of utilizing the wastes of the poultry industry. However, large amounts of manure are needed to fertilize the pond as it is not concentrated like inorganic fertilizers. Application of large amount of manure tends to deplete the oxygen in the water or cause harmful substances to accumulate during decomposition (SEAFDEC, 2009). Manure provides a favorable environment for pathogens (US EPA, 2013), which can adversely affect the water and the culture species. Nutrient content of manure is also inconsistent and long-time storage is impractical, unlike inorganic fertilizers.

In computing for the actual fertilizer requirement, the amount of nutrient required to grow natural food and the percentage of such nutrient in the fertilizer is needed. Unlike agricultural crops, there are no references on the recommended nutrients for growing natural food like *lablab* and *lumot* in the pond. Considering that nutrient content is proportional to the amount of fertilizer, the chances of under or over fertilization is high. The amount of fertilizer recommended by several manuals may not be sufficient or may be excessive as the nutrient content of the sediments and the water in different sites varies.

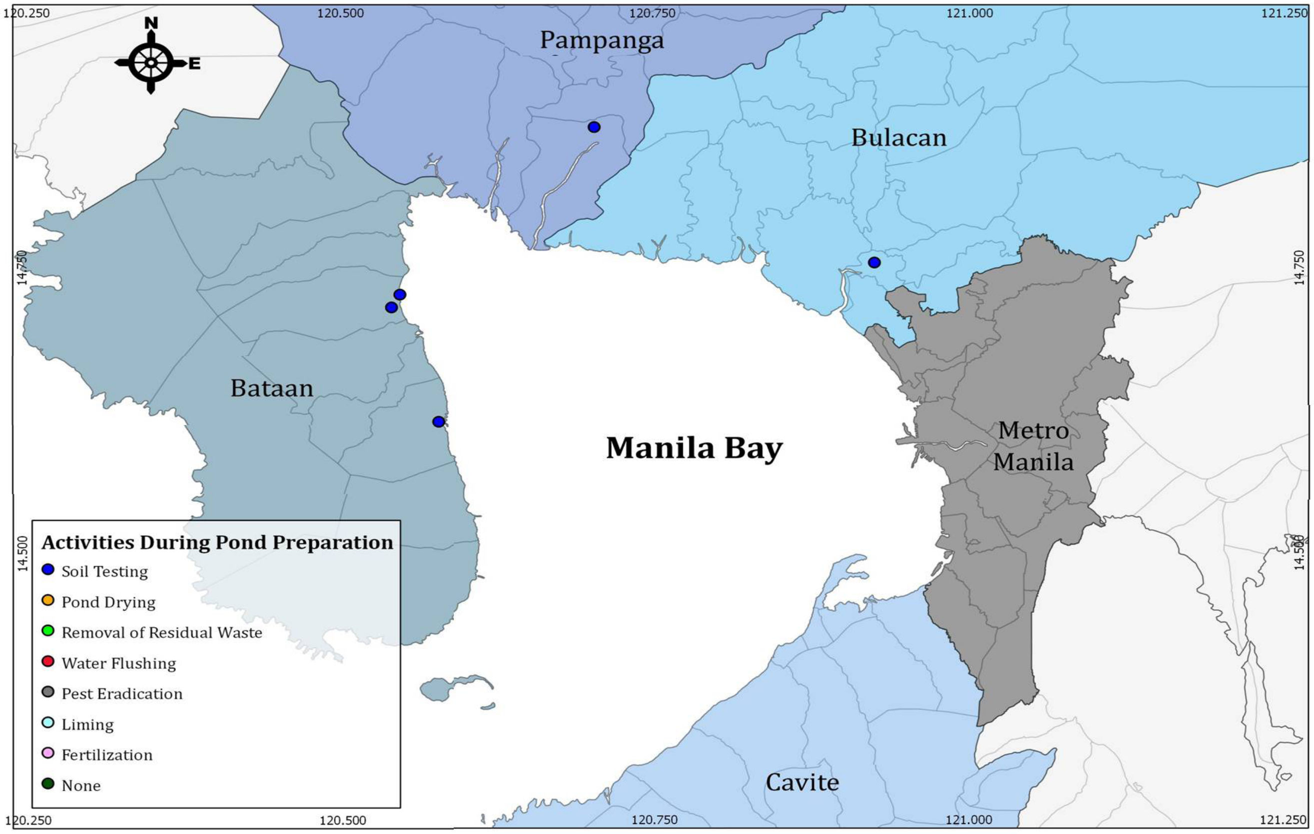

Figure 2.6a. Map highlighting the sites that perform soil testing.

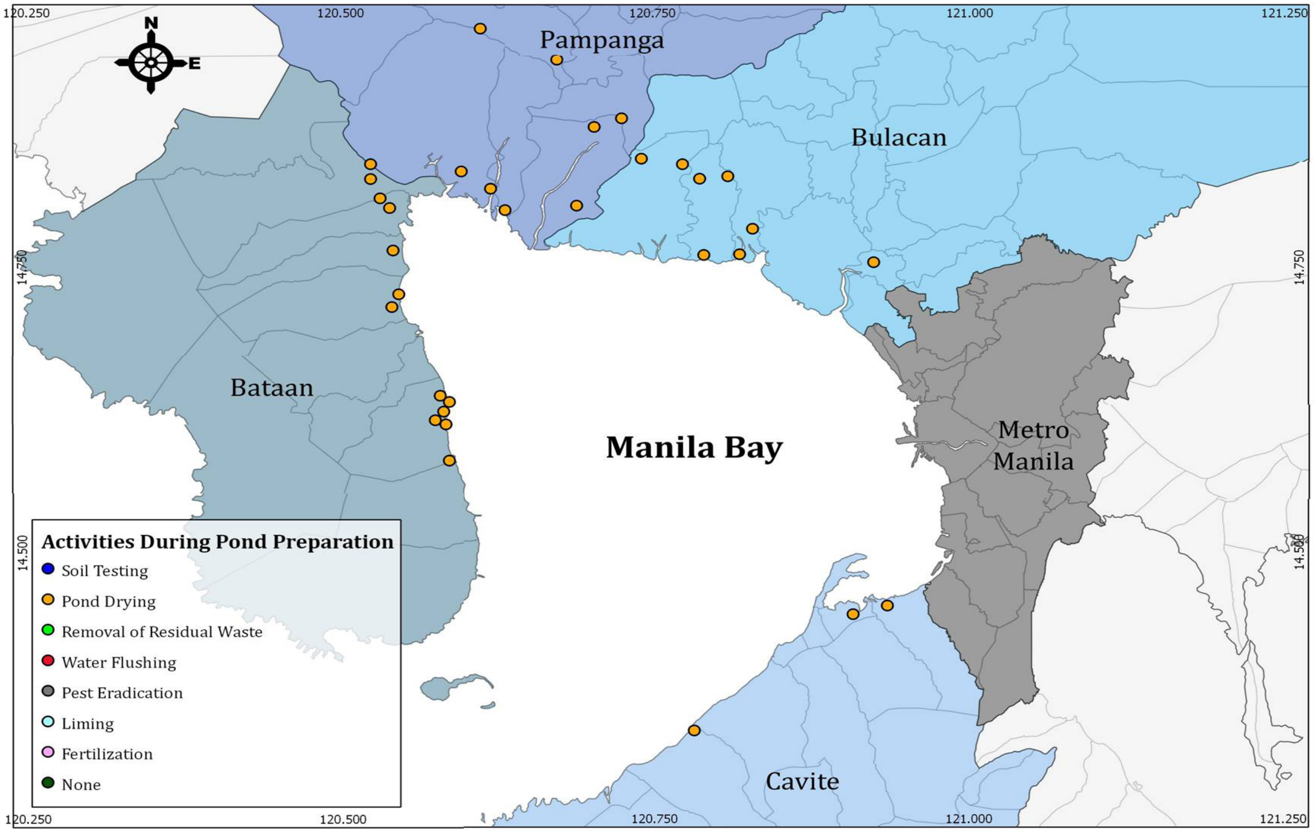

Figure 2.6b. Map highlighting the sites that perform pond drying.

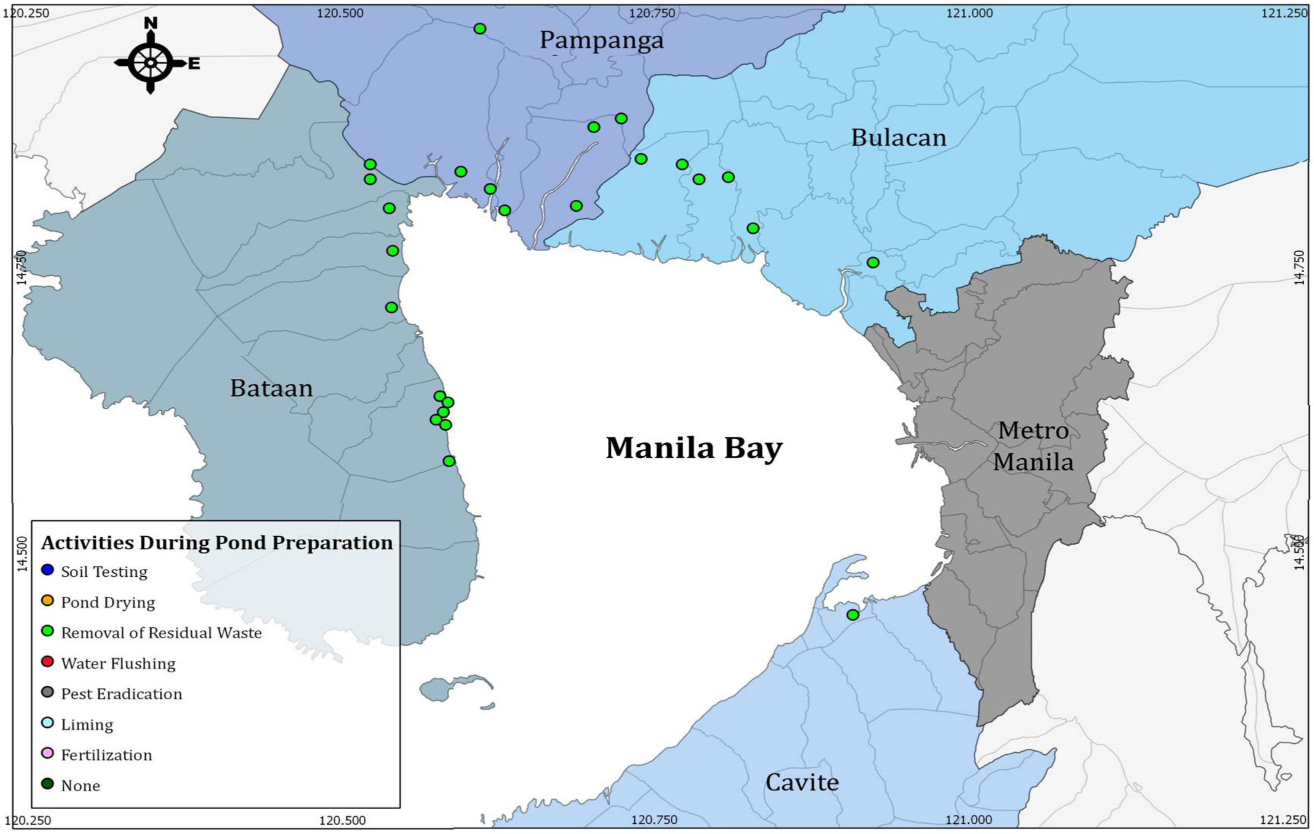

Figure 2.6c. Map highlighting the sites that perform soil scraping.

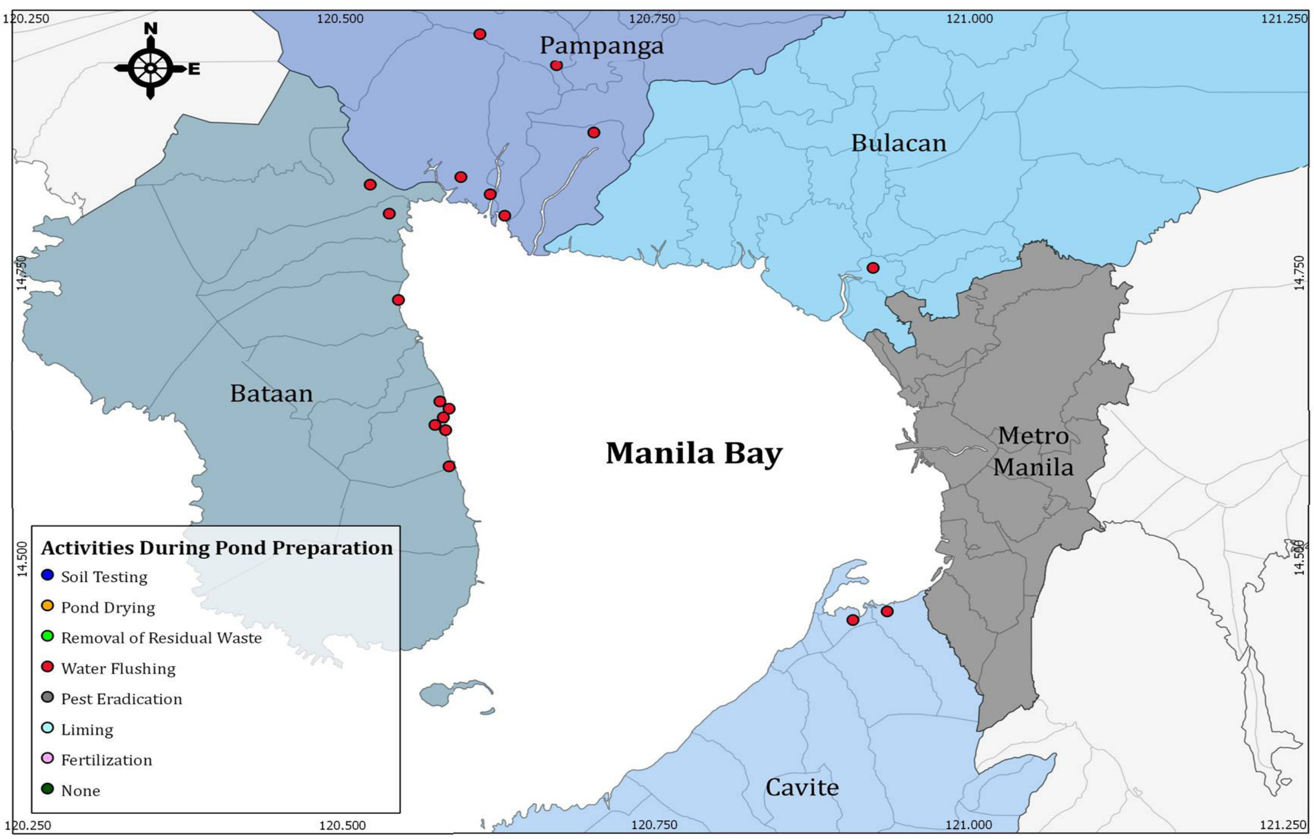

Figure 2.6d. Map highlighting the sites that perform water flushing.

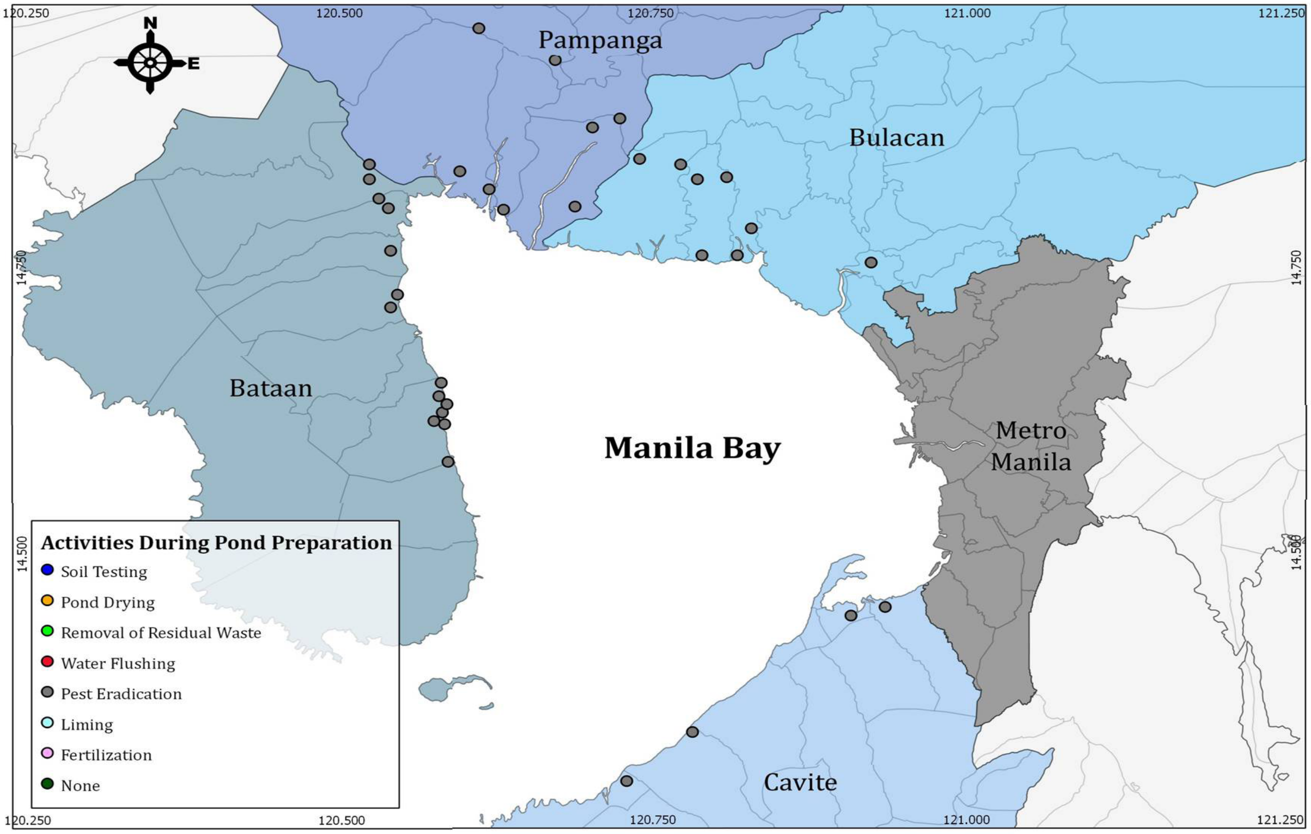

Figure 2.6e. Map highlighting the sites that perform pond disinfection or eradication of pests.

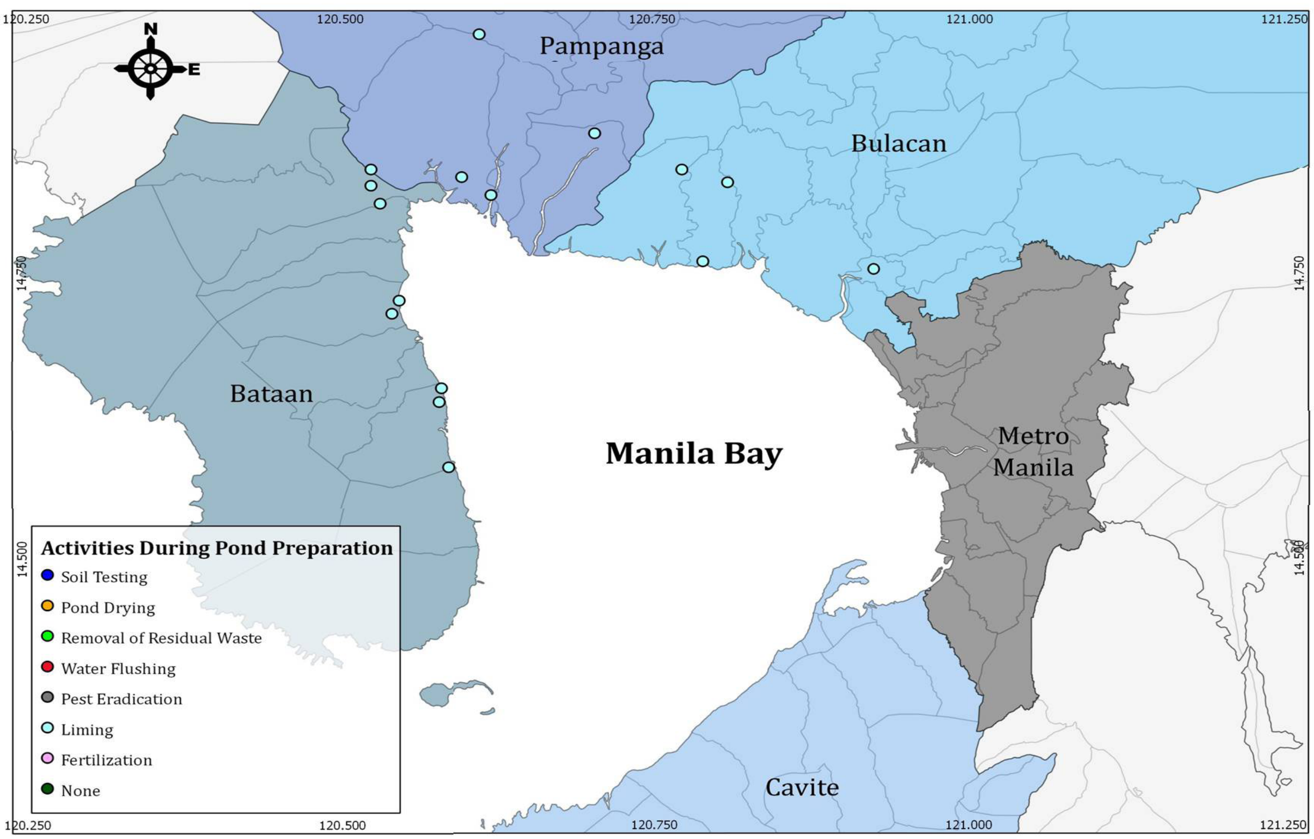

Figure 2.6f. Map highlighting the sites that perform liming.

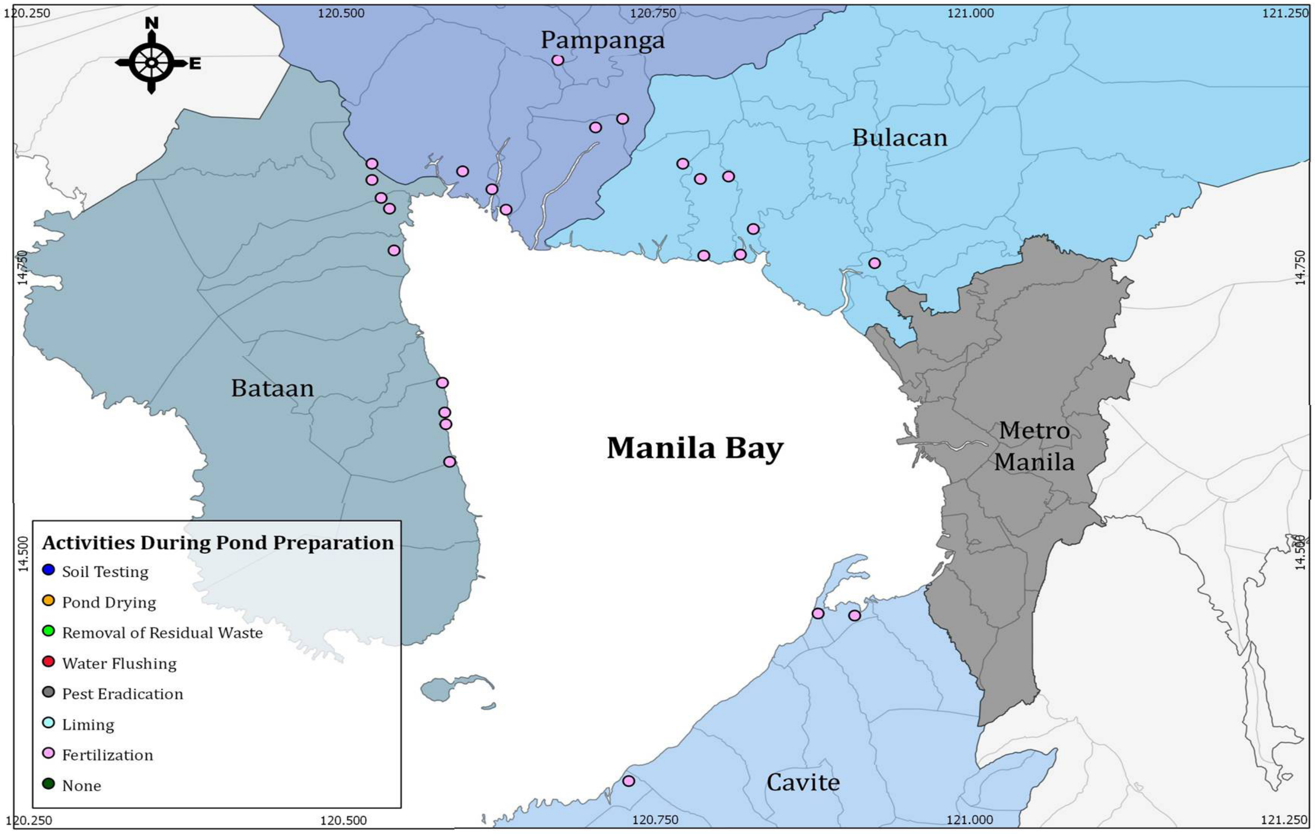

Figure 2.6g. Map highlighting the sites that perform pond fertilization.

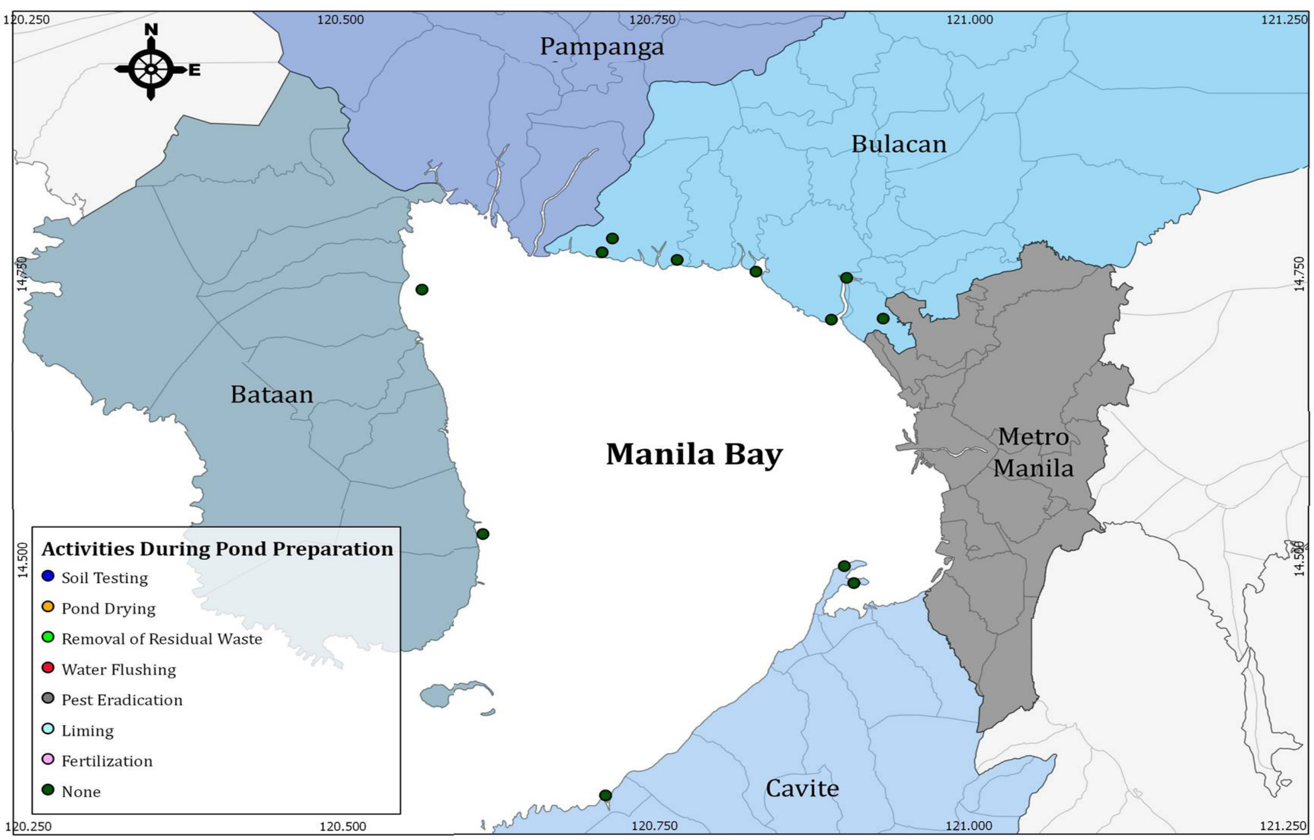

Figure 2.6h. Map highlighting the sites that do not perform any pond preparation activity.

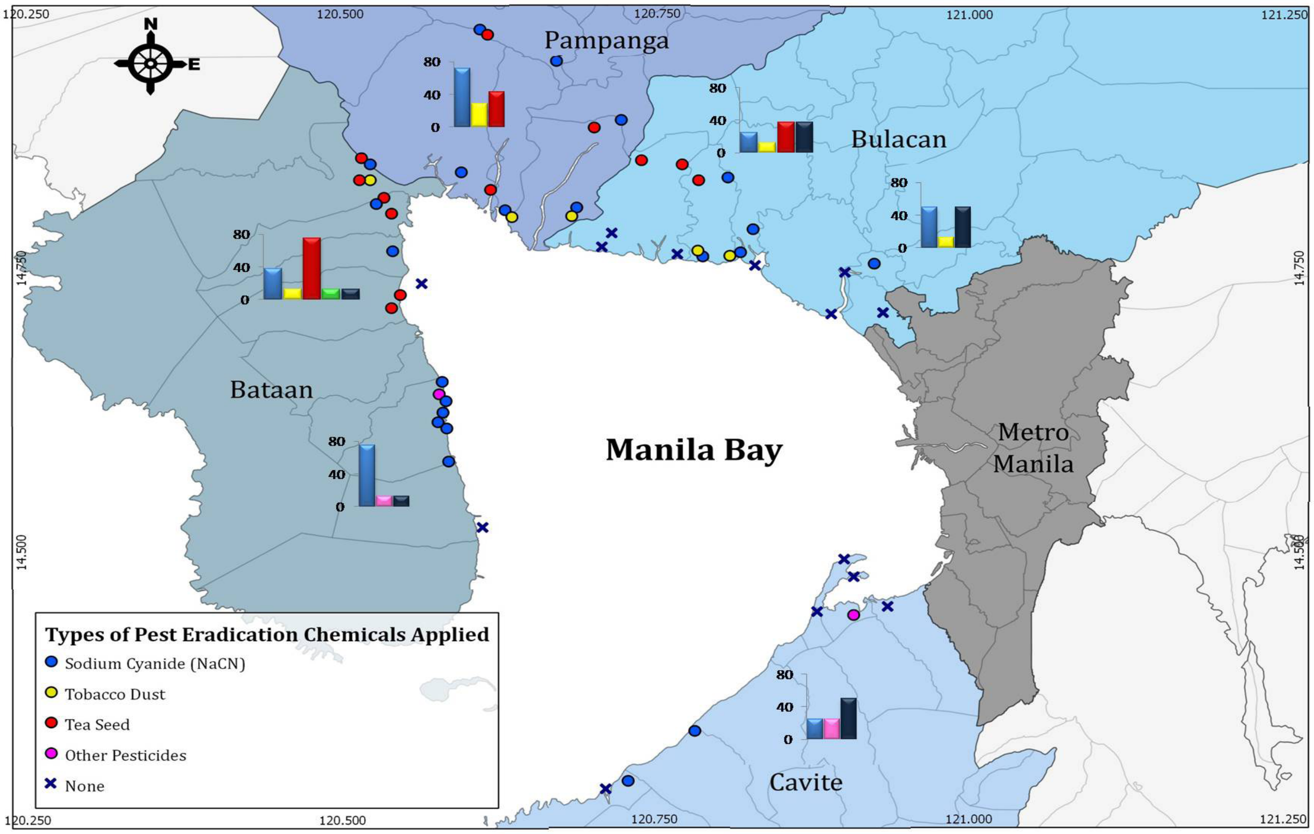

Figure 2.7. Map highlighting the sites (colored circles) and the percentage (bar graph) of farmers in different blocks that apply certain types of pesticides to eradicate unwanted species in the pond.

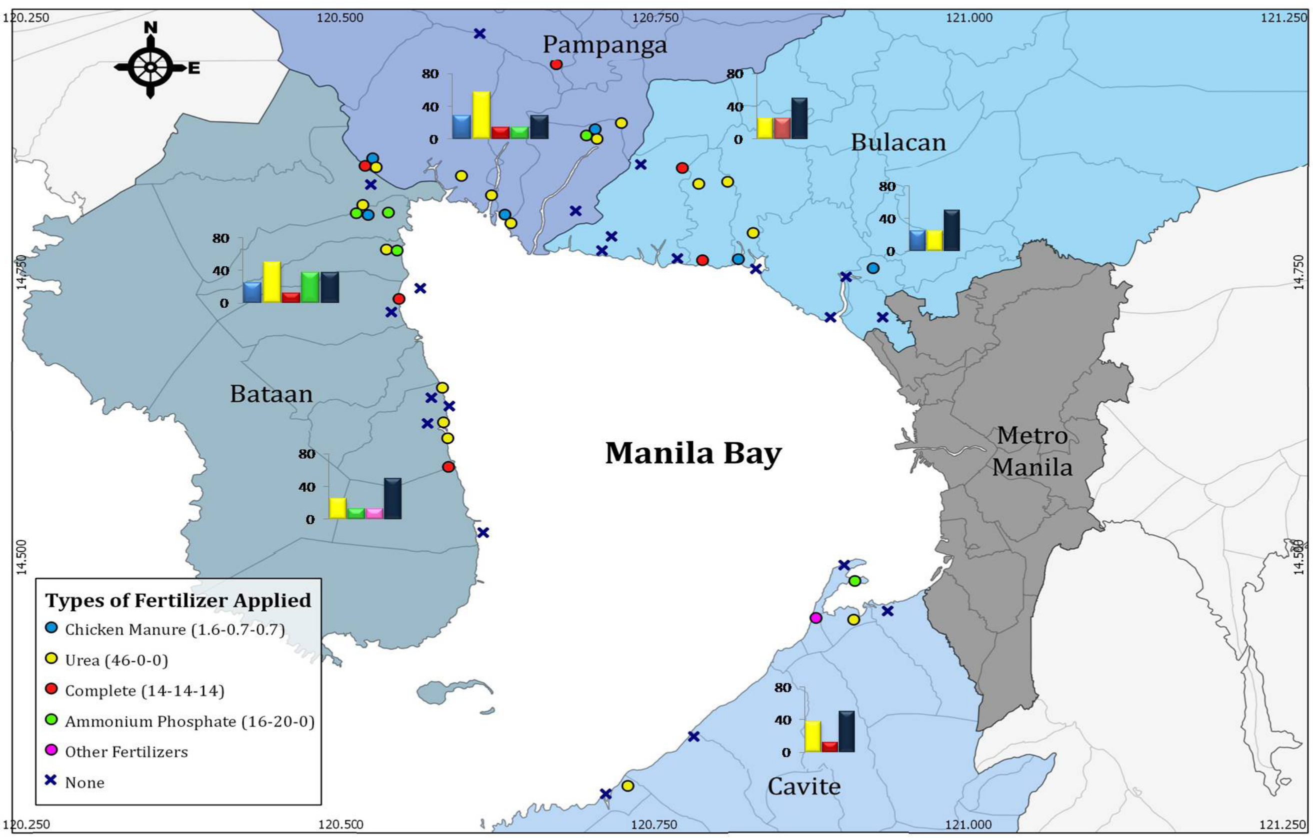

Figure 2.8. Map highlighting the sites (colored circles) and the percentage (bar graph) of farmers in different blocks that apply certain types of fertilizer to enhance primary productivity in the pond.

Culture Species

Figure 2.9 shows that the most common species cultured in the different areas around Manila Bay, namely milkfish (68%), shrimp (53%), crab (29%) and tilapia (25%). Tilapiang arroyo (*Sarotherodon melanotheron*), considered an intruder species from the wild, was present in 58% of the tilapia farms. It poses a major problem, for it is quite invasive and prolific, and a competitor of the primary culture organism. Grouper, trevally or *talakitok* and lady fish or *bidbid* are the high-value species also cultured in the ponds of Northern Bulacan and Cavite. Shellfish like mussel (14.9%) and oysters (12.8%), are cultured by farmers in Cavite and Bulacan and in Bataan.

The stocking of good quality fry is essential to the success of any aquaculture farm. Economic losses incurred due to infectious diseases caused by pathogenic viruses, bacteria, fungi and parasites in cultured fish and shrimp can be prevented through selection of only the clinically healthy stocks as prescribed in Section 9 of GAqP of BAFS (SEAFDEC, 2009; PNS-BAFS, 2014). FAO[1] 214 (BFAR, 2001) and PHILMINAQ (2008) encourage the use of hatchery fry and fingerlings for culture rather than those caught in the wild because there is a lot more chance for the latter to get infected with pathogenic organisms. It was discovered in this study that a high percentage of milkfish, shrimp and crab farmers procured fry from the hatchery, while a still higher percentage of tilapia farmers obtained their stocks from the wild (Figure 2.10).

About 82% of the fishpond and fish pen farmers employed a polyculture farming

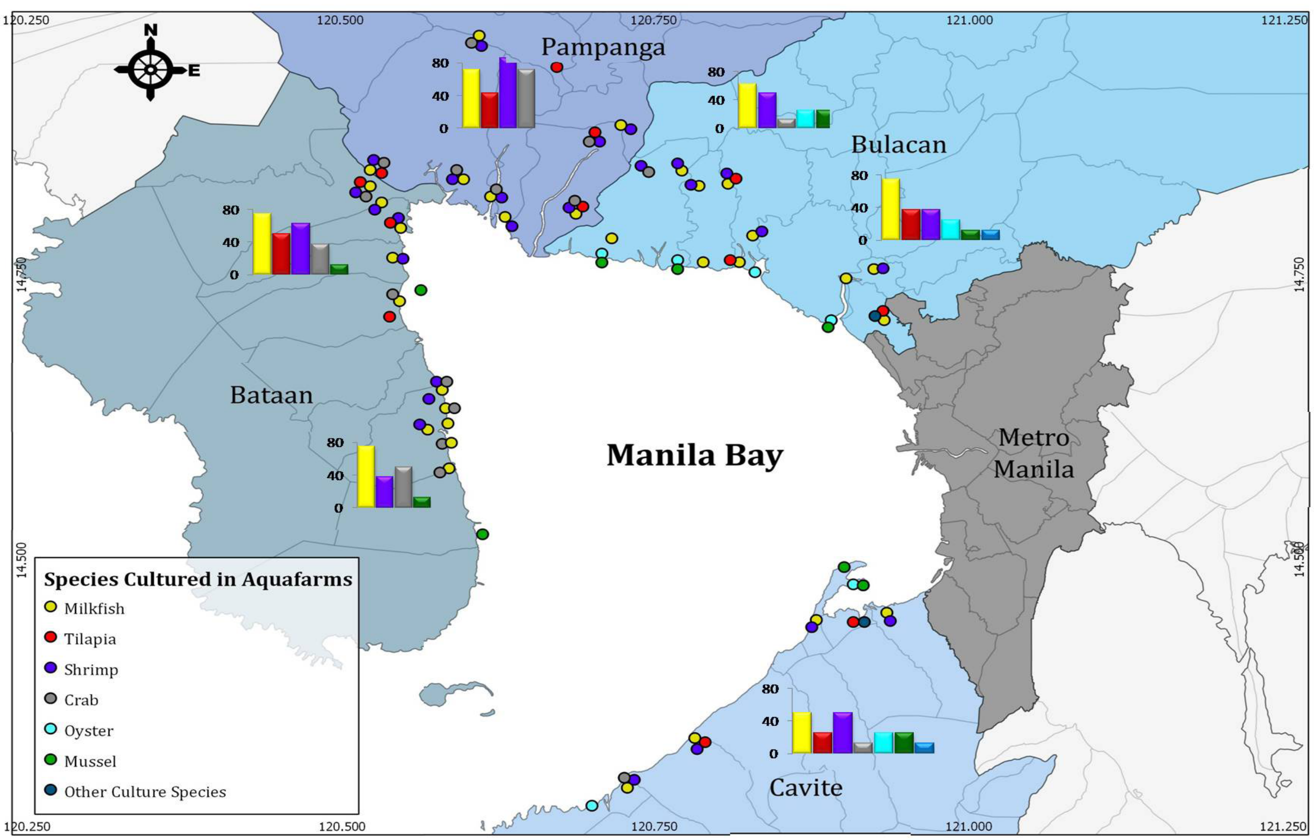

Figure 2.9. Map highlighting the sites (colored circles) and the percentage (bar graph) of farmers who are engaged in the culture of different aquatic species in their aquafarms.

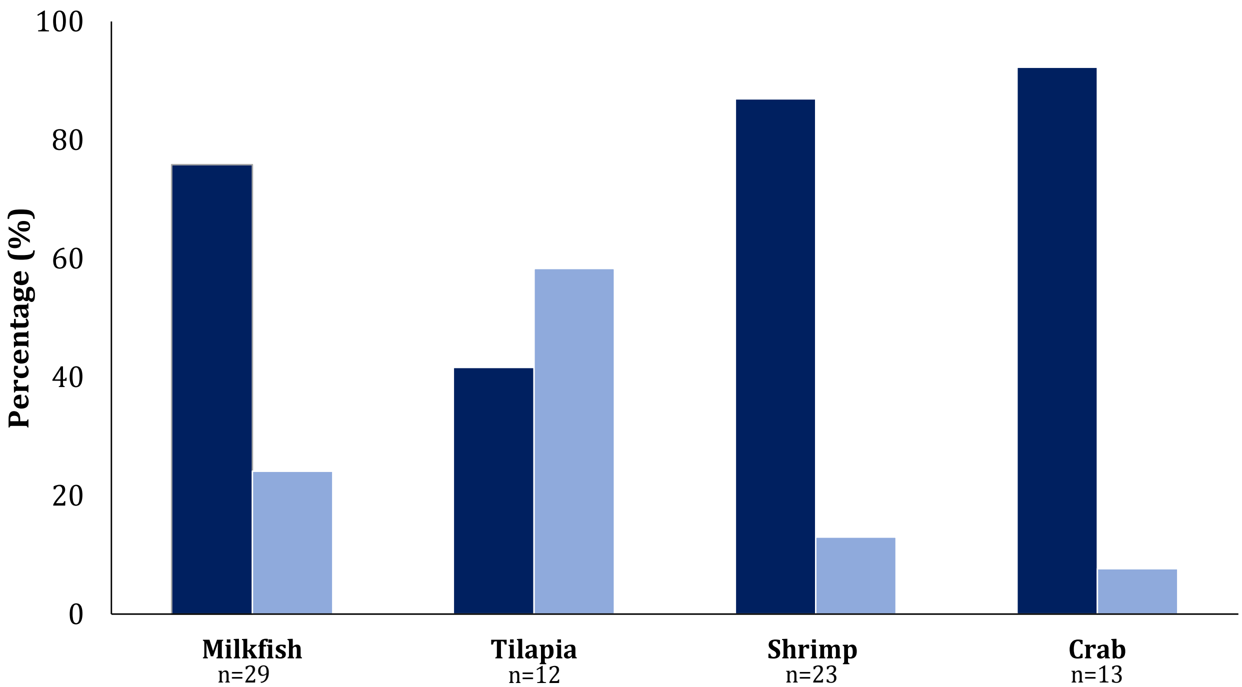

Figure 2.10. Percentage of farmers sourcing stock from hatchery ( ) and wild ( ).

system, in which two or more species are cultured in the same pond. Most of the polyculture farms (77%) had a combination of tilapia and/or milkfish and crustacean species like crab and/or shrimp. A smaller 10% of them employed a finfish-finfish polyculture, while a still smaller 3% utilized a crustacean-crustacean combination. About 10% of the farmers combined at least two species of finfish and crustacean in their ponds.

Polyculture is an effective way to maximize benefits from the available natural food in a pond (SEAFDEC, 2009). It is considered as the most efficient food production system ever devised (Boyd *et al*, 2007). However, pond management in polyculture becomes quite difficult when each stock used has its own requirements for good fertilization and feeding practices (Rahman, Varga, and Chowdhury, 1992). Although manuals on polyculture systems are available, a standard code of practice has not yet been established.

Feeding Management

Aquafeeds usually are the most costly aquacultural input, and every effort should be made to ensure efficient utilization of these (SEAFDEC, 2009). Wasted feed affects water quality and predisposes fish to disease. Wasted feed is a major contributor to discharged nutrients and organic matter from fish farms leading to eutrophication (PHILMINAQ, 2008; Boyd *et al*, 2007). There is much concern expressed over the wasteful use of increasingly scarce resources as one-third of capture fisheries are converted into fish meal for livestock and farmed-fish, rather than for direct human consumption (USAID, 2013). Thus, control and rationalization of feeds and feeding in modern fish farming is of critical importance in maintaining cost-effective and environmentally sound aquaculture operations.

Figure 2.11 shows that the respondents commonly fed natural food, like *lumot* (59%)

and *lablab* (25%) to the culture species. This is advantageous because algae assimilate the nutrients in water at the same time that they increase its oxygenation through photosynthesis. Commercial feeds, specifically extruded or floating feeds, is recommended by FAO[1] 214 and is used by 36% of the respondents. Old bread was used by 15% of the farmers for the purpose of fattening before harvest. On the other hand, shellfish farmers, depend on surface or natural plankton in the area. The other feeding materials used by about 6.4% of the farmers included duckweed, corn, *quiapo* and *darak.* rate and stability. Although low value feed contains high quality protein, the problem with it is that it tends to disintegrate rapidly in water and thus, readily releases its nutrient content leading to water quality degradation in the place where water is discharged (Edwards *et al*, 2004; SEAFDEC 2009). Moreover, issues arise regarding the sustainability of the use of trash fish in aquaculture. For example, the capture of trash fish from the finite stocks of capture fisheries has been observed to produce enormous impacts on local coral reefs through the depletion of fish populations (USAID, 2008). Furthermore, this type of feed can po-

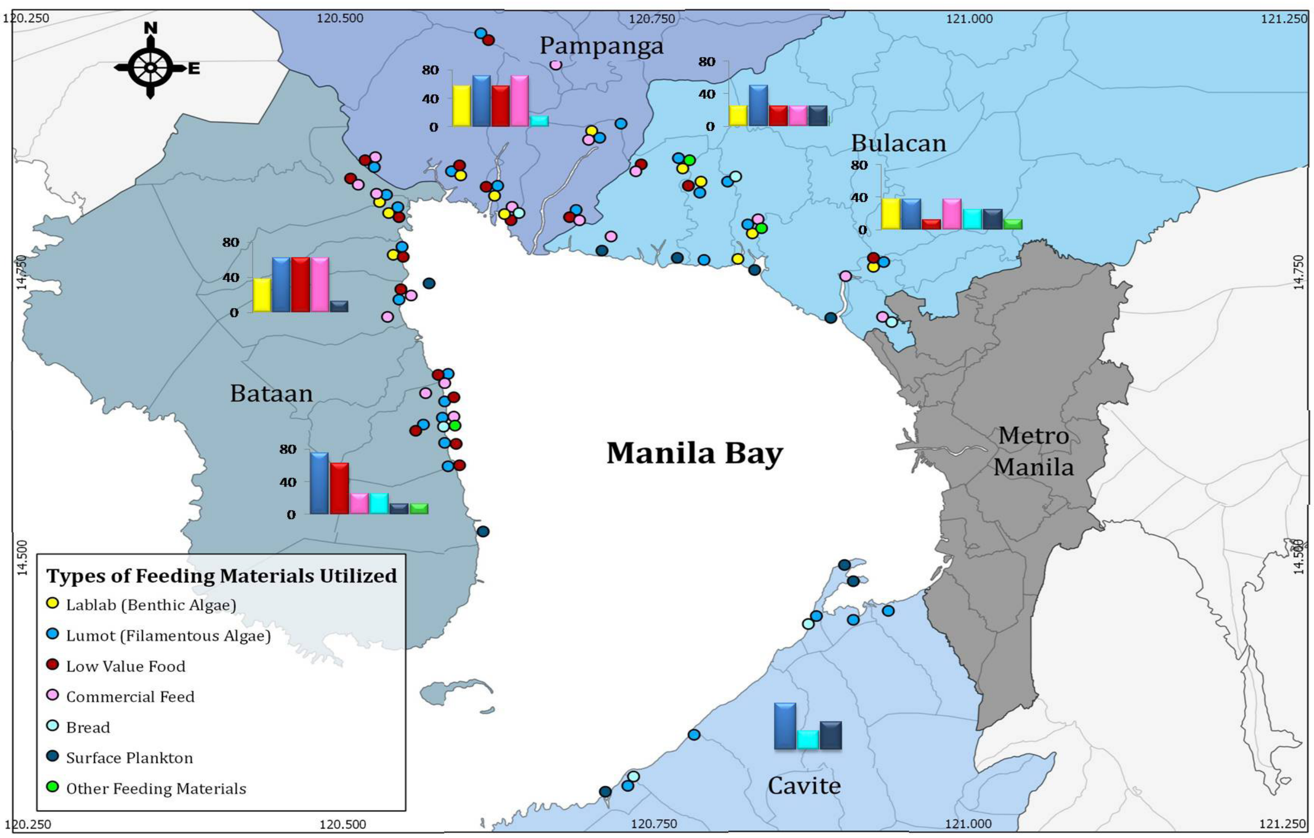

Figure 2.11. Map highlighting the sites (colored circles) and the percentage (bar graph) of farmers in the different blocks that use certain types of feeding materials in their farms.

Low value feed (trash shellfish and fish), an unstable feeding material, is also used by some 36% of the respondents. Its use is inconsistent with the FAO[1] 214 recommendation to use only those feeds with a high utilization tentially introduce pathogenic bacteria, viruses and parasites into the culture area. Section 7 of GAqP of BAFS, however, states that this type of feeding material is acceptable, provided that the use, type and mode of preparation of the feed and its proper storage is observed.

The level or method of fish culture (extensive, semi-intensive or intensive) in the farms was also evaluated based on feeding management. Culture intensity is an important factor for consideration, because it is associated with the input and level of technology required, and with effluent loads (Howerton, 2001). Higher density farms may produce greater environmental impacts, including increased discharge of pollutants, increased tendency to use chemicals, and increased risks to ecosystem health (USAID, 2008; SEAFDEC, 2009). The culture systems used in the different Manila Bay aquafarms include the extensive (21.1%), semi-intensive (57.9), and intensive (21.1%) systems. The most common system employed was the semi-intensive culture which requires only moderate inputs. The effluent loading of the semi-intensive system may be higher than the extensive, but lower than the intensive system (BFAR-PHILIMINAQ, 2007; USAID, 2008). In most cases, according to the USAID (2008), some level of the semi-intensive culture would prove most profitable, for it uses less space and does not require too high a level of technology.

Computation of FCR, DMR and WPR

Feed conversion ratio (FCR) is the most widely used indicator of production and feed use efficiency in aquaculture (Boyd *et al*, 2007; Boyd, 2003a). FCR is the amount of feed used to increase the biomass by one kilogram. The lesser the FCR the better is the feed conversion efficiency of the feed.

Based on the data given by the farmers, the FCR was computed using the formula below.

$$\mathbf{FCR} = \frac{\text{Total Amount of Feed Used (kg)}}{\text{Net Aquacultural Production (kg)}}$$

Table 2.2 shows the computed FCRs of selected farms and compared with the usual or average FCR for a specific culture species. Results showed that in 43% of the selected farms, the FCR exceeded the typical FCR which means some feeds given to the organisms were not absorbed but rather were wasted. Consequently, there is loss of profit and the pond environment is degraded. Improper feeding management, undesirable range of water quality parameters, unhealthy fish condition, and low quality feeds are the possible reasons to a high FCR (Klontz, unpublished).

Fifty seven percent of the computed FCR was below the typical FCR. Interestingly, Table 2.2 shows most of the FCR as below the typical are lesser than 1:1. Theoretically, FCR below 1:1 is not possible because feeding 0.03kg of low value feed to shrimp, as in Ubihan, Meycauyan, would not result in a 1 kg increase in the biomass. It is possible that the presence of natural food and high primary productivity in areas with FCR less than 1:1 enabled fish growth. The data provided by the farmers may not be accurate vis-a-vis what they practice thus, the atypical FCR results.

Boyd (2005), claims that FCR can be a misleading ecological indicator since the attainment of 1.0:1 FCR does not imply that no feed was wasted during the rearing period. Feeds typically contain about 90% dry matter and 10% water, while live fish products usually contain around 25% dry matter and 75% water. Therefore, an FCR = 1.0 indicates that 1 kg of feed produced 1 kg fish or shrimp; however disregarding moisture, 0.9 kg dry matter in feed produced only 0.25 kg dry matter in fish, indicating that production of 1kg live biomass resulted in 0.65kg waste (Boyd, 2005; Boyd *et al*, 2007). With this, Boyd *et al* (2007) came up with dry matter ratio (DMR) or the amount of dry matter needed to produce 1 kg dry matter of fish. The DMR of the fishponds was computed using the formula below.

Table 2.2. Estimated FCR, DMR, and WPR computed based on the information provided by the respondents.

Culture species	Pond Location	Type of Feed	FCR[1]	Typical FCR[2]	DMR	WPR
Milkfish	Ubihan, Meycauayan	Commercial Feed	2.07:1	1.75:1*	7.45	1.61
	Calero, Malolos	Commercial Feed	9.60:1		34.56	8.39
	Tawiran, Obando	Commercial Feed	3.66:1		13.17	3.04
	Matungao, Bulakan	Commercial Feed	4.28:1		15.41	3.60
	San Roque, Hagonoy	Commercial Feed	0.54:1		1.94	0.23
	Sta. Elena, Orion	Low value feed and commercial feed	0.37:1		0.89	---
	SapangKawayan, Masantol	Low Value Feed	1.75:1		2.8	0.45
	Consuelo II, Macabebe	Commercial Feed	2.60:1		9.36	2.09
	Batang II, Sasmuan	Low Value Feed	6.4:1		10.24	2.31
	BangkalPugad, Lubao	Commercial Feed	1.6:1		5.76	1.19
Tilapia	San Antonio, Guagua	Commercial feed	0.05:1	1.8:1**	0.18	---
Shrimp	Ubihan, Meycauayan	Low Value Feed	0.03:1	2.0:1**	0.05	---
	Calero, Malolos	Low Value Feed	83.33:1		133.33	33.08
	San Agustin, Hagonoy	Low value feed and Commercial feed	1.95		4.68	0.92
	Camachile, Orion	Low value feed	0.23:1		0.37	---
	Capunitan, Orion	Low value feed	0.46:1		0.74	---
	BangkalSinubli, Lubao	Commercial feed	0.53:1		1.908	0.28
	SapangKawayan, Masantol	Commercial feed	2.8:1		10.08	2.27
	Consuelo II, Macabebe	Low value feed and Commercial feed	3.54:1		8.50	1.87
	BangkalPugad, Lubao	Commercial feed	6.32:1		22.75	5.44
	Mani-ano, Minalin	Low value feed	23.95:1		38.32	9.33
Crab	Camachile, Orion	Low value feed	0.58:1	5.2:1***	0.93	---
	Camachile, Orion	Low value feed	0.65:1		1.04	0.01
	Sta. Elena, Orion	Low value feed	4.82:1		7.71	1.68
	SapangKawayan, Masantol	Low value feed	1.11:1		1.78	0.19
	Mani-ano, Minalin	Low value feed	1.43:1		2.29	0.32
Catfish	Matungao, Bulakan	Low value feed	3.16:1	2.2:1**	5.06	1.01

[1]*Computed FCR: Red font: exceeded typical FCR; Black font: below typical FCR*
[2]*Typical FCR derived from: *Tucker, 1998; **Boyd & Polioudakis, 2006; ***Allan & Fielder, 2003*

$$\mathbf{DMR} = \mathrm{FCR} \times \frac{\%\ \text{Dry Matter in Feeds}}{\%\ \text{Dry Matter in Fish}}$$

Proximate analysis of the feed and culture species was not done in this research so the dry matter percentages were presumed to be at the usual range indicated in different publication. The dry matter composition of commercial feed (dry feed), low value feed (wet feed), and combination of both feed (semi-moist feed) and fish was assumed to be at 10% (Boyd *et al*, 2007), 40% (Lucas and Southgate, 2012), 60% (New, 2002) and 25% (Boyd *et al*, 2007), respectively. As seen in Table 2.2, the DMR computed ranged from 0.18 to 133.33, implying that 0.18 to 133.33kg of the dry matter of feeds is needed to produce 1kg dry matter of fish.

From the DMR, Boyd *et al* (2007) were able to develop the waste production ratio (WPR), or the amount of waste that would be generated to produce 1kg fish, which can be computed using the formula below.

$$\mathbf{WPR} = (\mathrm{DMR} - 1) \times \%\ \text{Dry Matter in Fish}$$

Results (Table 2.2) showed that the WPR of fishponds ranged from 0.01 to 33.08, meaning that for each kg of live aquaculture product, 0.01 to 33.08kg of waste (dry matter basis) would be produced.

Anthropogenic Factors

Human activities can worsen the effects of inappropriate aquaculture practices. In this connection, different anthropogenic factors around the aquafarm that could have an effect on the water quality of the ponds and water sources were identified. Distance of aquafarm from the nearest residential area was estimated by pace method. As seen in Figure 2.12, 72.3% of the sites are located 0 to 50 meters (sum of 40.4% and 31.9% for <10m range- and 11 to 50m range, respectively) away from the nearest residential area.

Aside from distance, the population of the nearest residential area was also estimated. It was observed that although the distance is not that far, 74.6% of sites surveyed had populations that ranged from 1 to 1,000 individuals considered as relatively sparse compared to other residential areas in the provinces (Figure 2.13). This situation is primarily due to the remoteness of the area from town centers where supplies come.

All farmers domesticated and/or allowed animals to wander in pond embankments except for an intensive pond in Southern Bataan. This practice is runs counter to a guideline in Section 3 of GAqP of BAFS, which says that wild and domestic animals should be excluded from pond and harvesting areas. Farmed, domesticated and feral animals wandering on pond banks can be minimal causes of water degradation (Boyd, 2003b). Moreover, the excreta of livestock and poultry might contain certain contaminants, including pathogenic organisms and antimicrobial-resistant bacteria, which can infect the water, and eventually, the culture species (US EPA, 2013). Figure 2.14 shows that chicken is the most common animal domesticated by 44.7% of the farmers. There were 2-25 chicken per site. Ducks (23.4%), pigs (8.5%), and goats (4.25%) were also domesticated; the number per site ranging from 1 to 14.

As for house pets, 76.6% of the respondents raised dogs in their farm which numbered from 1 to 12 per site (Figure 2.15). Accordingly, dogs are helpful in securing property from poachers and trespassers. Cats, 1 to 10 per site, were also domesticated by 21.3% of the respondents, while wild birds were found in 21.3% of the sites surveyed.

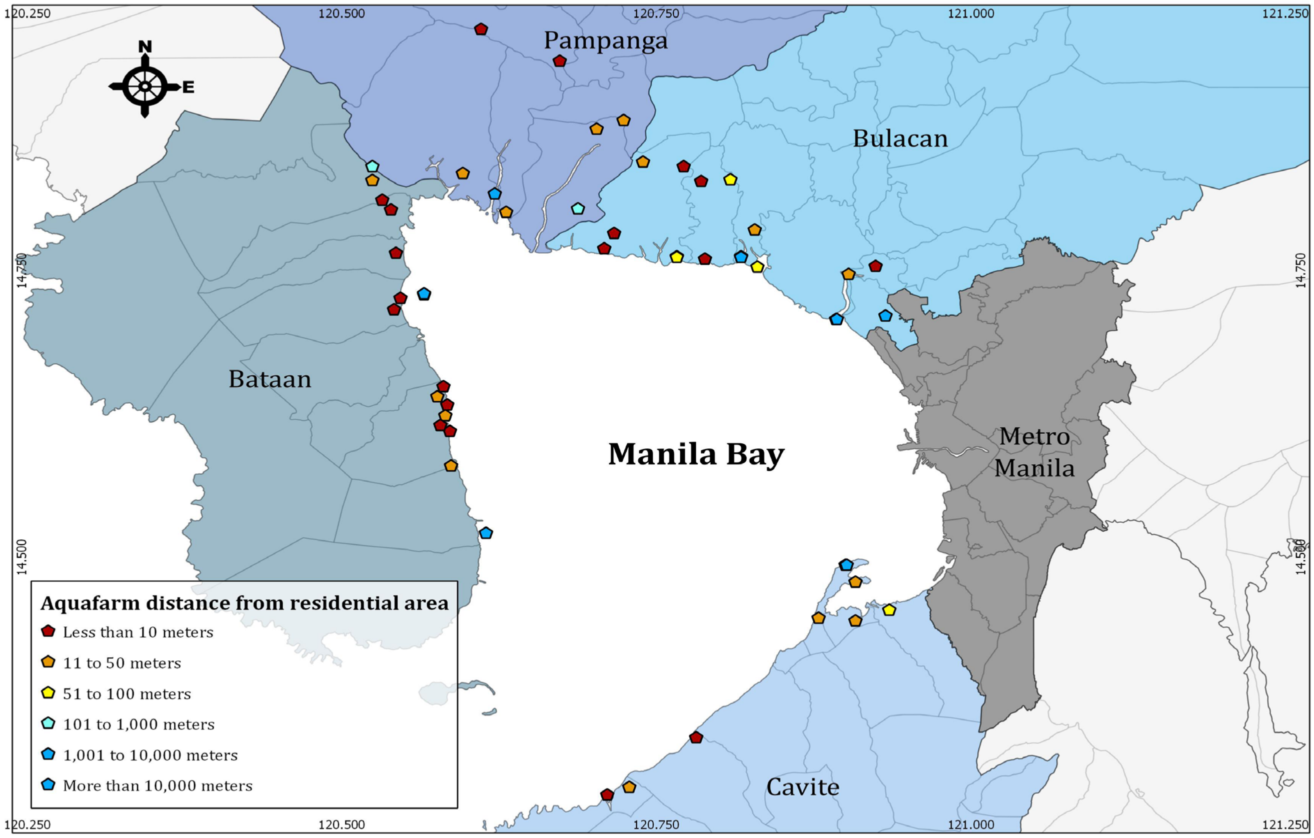

Figure 2.12. Map highlighting estimated distance of aquafarm from the nearest residential area.

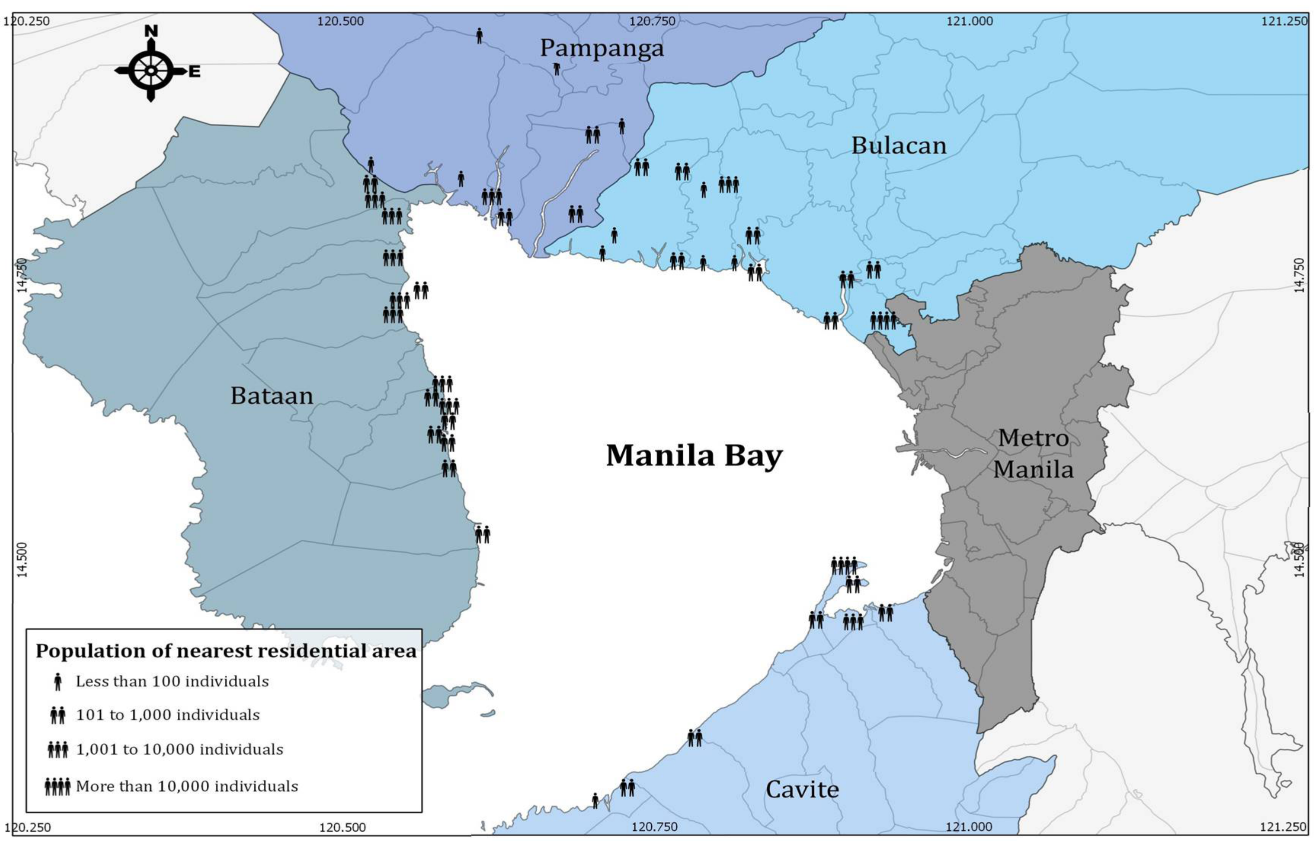

Figure 2.13. Map highlighting estimated population of the nearest residential area to the aquafarm.

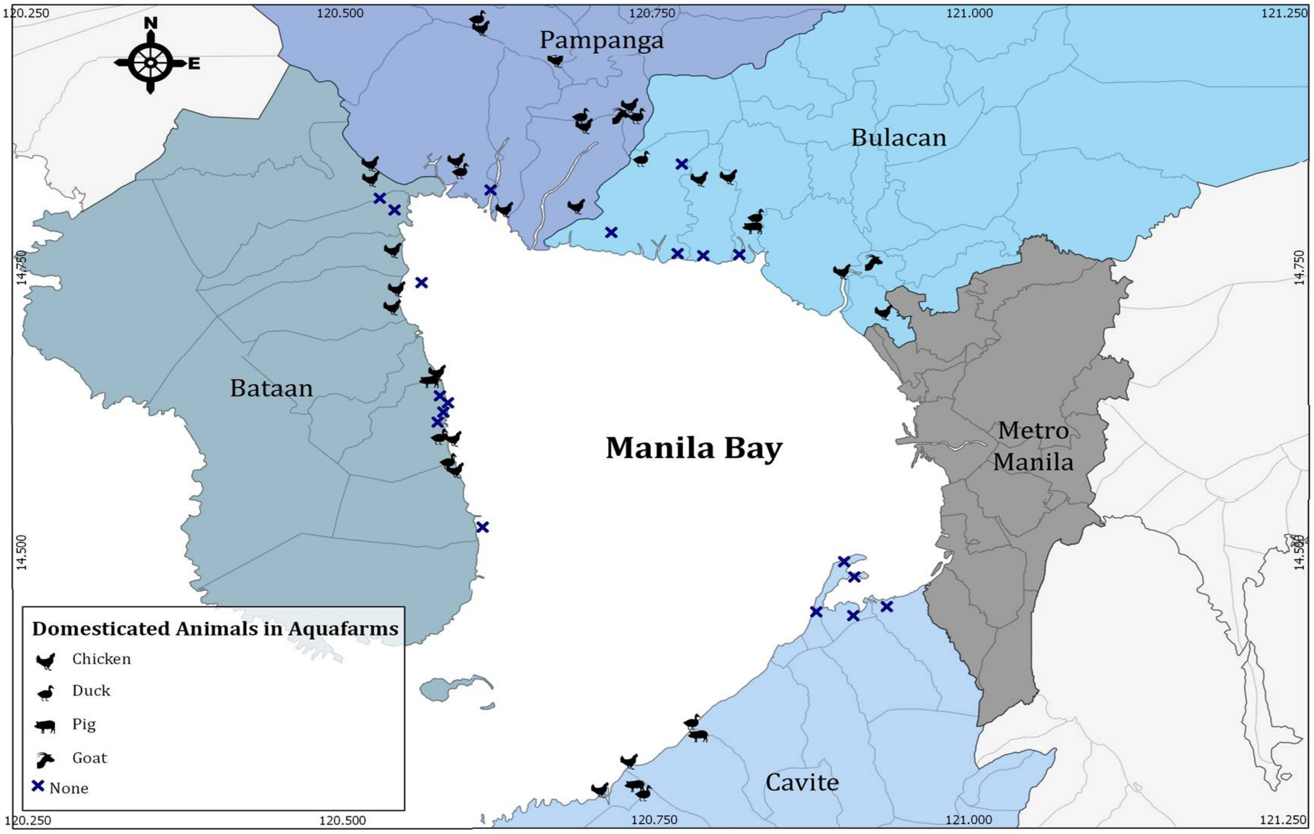

Figure 2.14. Map highlighting sites where farmer-residents domesticate different livestock and poultry animals.

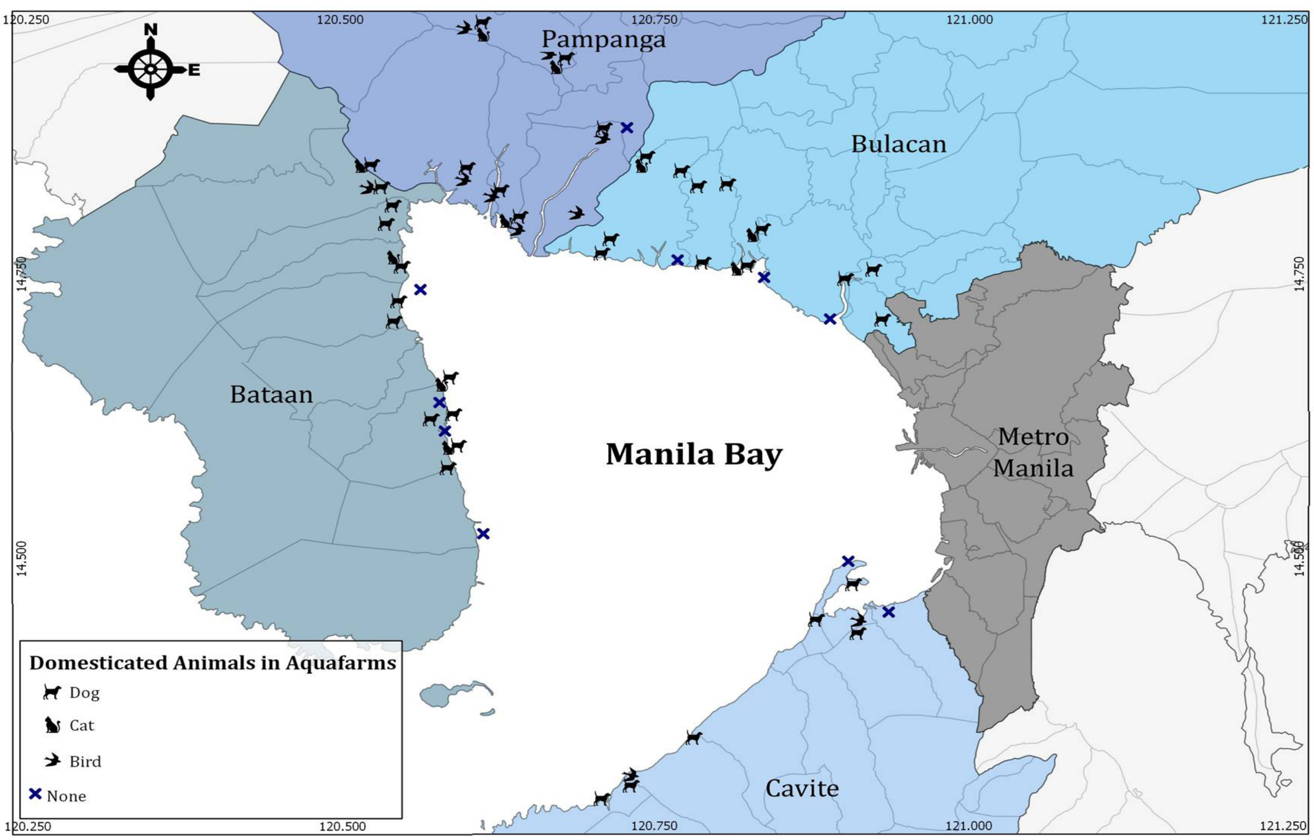

Figure 2.15. Map highlighting sites where farmer-residents domesticate different pets.

Conclusion

Most fish farmers in Manila Bay do not observe the guidelines on good aquaculture practices. Evidences to this contention were uncovered in the study as follows: a) non-compliance with requirement for a buffer zone, b) lack of the necessary equipment and/or use of improper water quality monitoring methods, c) neglect of some important steps in pond preparation (e.g., soil testing); and d) application of noxious and illegal chemicals, like cyanide.

Forty-three percent of the selected farms had FCR values exceeding the typical, meaning some feeds were wasted or not absorbed. The 57% of farms whose FCR fell below 1:1 seems unlikely and might have resulted from the inaccurate data supplied by the farmers. Nonetheless, it is possible that fish growth in ponds with < 1:1 FCR may have resulted from consumption of the natural food supply which came from a high primary production. Proximate analysis of feeds and culture species was not done; dry matter percentages were assumed to be within the range reported by earlier publications. Based on the presumed dry matter composition of commercial feed, low value feed and the combination of both feeds, the DMR computed had a range of 0.18 – 133.33, which is the range of dry matter of feeds needed to produce 1 kg dry matter fish. The computed WPR range, or the amount of waste generated in the production of a kilo live aquaculture product, was 0.01 – 33.08.

Farmers domesticated and/or allowed animals to wander on pond embankments and wade in the water predisposing both water and culture species to contamination. Anthropogenic activities like sewage and garbage disposal by household, liquid effluent from an oil refinery and agriculture possibly compounded the deterioration of water quality in the fish ponds.

On the other hand, the notable practices in the surveyed areas were the following: a) adoption of the sustainable aquaculture practice of polyculture, b) adoption of semi-intensive fish farming, and c) sourcing of seed stocks from primarily from hatcheries.

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CHAPTER 3

Spatial and Seasonal Nutrient Trends in Manila Bay Aquaculture Farms

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Abstract

Sampling was done in duplicates during dry (May 2014 and February 2015) and wet season (September 2014 and November 2014) following the blocking scheme for the nutrient trends. As for the nutrient loading, water samples were collected in three ponds after flooding (water intrusion) and prior to draining (water release). Colorimetric analyses by UV-Vis Spectroscopy following the US EPA standard methods were used to determine the samples' nutrient levels specifically, ammonia, nitrate, nitrite, and phosphorus. Results showed that ammonia had the highest levels followed by phosphorus, nitrate, and nitrite. Geographically, higher concentration of nitrogen and phosphorus were observed in Eastern Bulacan aquaculture farms, which is attributed to the farmers' disregard of the important pond preparation activities. Varying seasonal trends were noted among nutrient species due to the different reactions of each analyte under changing climatic conditions. Nutrient levels in sediments were several folds higher than that in the water column. Results of correlation analyses of nutrients in water and sediments showed: a) a good correlation for phosphorus, b) weak correlation for ammonia, and c) no correlation for nitrites and nitrates, implying that sediments maybe a possible contributor of phosphorus and ammonia in water but not nitrite and nitrate. Ammonia and TKN were significantly higher during the flooding suggesting that water coming in to the pond already contains high levels of said nutrients possibly due to higher organic load. Conversely, nitrite and nitrate levels were significantly higher during the draining suggesting transformation of ammonia into these less toxic substances by nitrifying bacteria.

Keywords: aquaculture, nitrogen, phosphorus, Manila Bay

Introduction

Nutrients, such as nitrogen and phosphorus, are indispensable elements in growing all forms of aquatic life, including algae, fish, crustaceans, mollusks, microbes and other organisms. They are constituents of various coenzymes, nucleic acids, amino acids, lipids, ATP (energy carrier) and some macromolecules that are important in the biological processes of different culture organisms (Hardy & Gatlin, 2002; Olsen *et al*, 2008). Phosphorus is also a structural component of fish bones, teeth, scales and skin (Chow & Schell, 1980; Olsen, 2008). Moreover, these nutrients increase primary productivity of the aquatic culture environment which eventually increases the total productivity as it forms the base of the food chain and improves the water quality by the augmented production of dissolved oxygen in water (Conte, 2000; Golez, 2009; Tucker & Hargreaves, 2012).

Despite their essentiality, nutrients are often present in short supply (Havens & Frazer, 2012). In order to increase aquaculture production to support the food demand of the growing human population, phosphorus and nitrogen must be applied in the culture system through fertilization and feeding. Fertilizers used in aquaculture may either be organic (e.g. animal manure, molasses from sugar cane, composted vegetation and different by-products of other industries) or inorganic fertilizer, which is synthetically produced with concentrated and known amount of nitrogen and phosphorus (Golez, 2009). Feed ingredients from animal or fish by-products also contain relatively high levels of phosphorus and nitrogen coming from bones and protein component of these ingredients (Hardy & Gatlin, 2002). Aside from the inputs, the culture species itself may release such nutrients that are not absorbed by the body through faeces and other wastes (Science for Environment Policy, 2015; Hardy & Gatlin, 2002).

These nutrients occur in several forms in water: nitrogen can be in the form of soluble organic N, ammonium (NH_4-N), ammonia (NH_3-N), nitrate (NO_3-N) and nitrite (NO_2-N), while phosphorus exists as orthophosphate and undifferentiated organic phosphates (Ongley, 1996; Kutty, 1986; Golez, 2009).

However, along with the global increase in fish production from aquaculture, several environmental concerns regarding the nutrients released by fish farms have been raised. In fact, the United States Environmental Protection Agency (US-EPA) identified nutrients as a significant problem contributing to water pollution (MPCA, 2008). Excessive nitrogen and phosphorus in water (from different sources including aquaculture) result in eutrophication (Smith *et al*, 2006), which is the leading cause of water quality impairment around the world (Diaz *et al*, 2012). Eutrophication leads to series of adverse impacts on aquatic ecosystems. Perhaps the most commonly observed is the accumulation of nuisance levels of algal biomass (Smith *et al*, 1999) which eventually reduce light penetration and lead to a loss of submerged aquatic vegetation, including seagrass beds and coral reefs (Carpenter *et al*, 1999; Diaz *et al*, 2012). The imbalance of nutrient ratios may also lead to a shift in phytoplankton species composition, which also alters the aquatic food webs creating conditions favorable to the dominance of toxic algal blooms (Smith *et al*, 1999; Smith *et al*, 2006; Diaz *et al*, 2012). Worse, the decomposition process of these algal blooms require dissolved oxygen which results in oxygen shortages in the water thus causing massive fish kills (Carpenter *et al*, 1998).

Aside from its impact to the environment, an excessive amount of nutrients also adversely affects the culture organisms. A slight increase in ammonia concentration, for example, can already impair fish growth rate and morphological development, while extreme levels may cause severe convulsions, coma and death (Mueller & Helsel, 1996). Excess nitrite, on the other hand, can lead to brown blood disease or a condition wherein the oxygen carrier hemoglobin in the blood is converted to the non-oxygen carrier methemoglobin when combined with nitrite which eventually leads to fish suffocation even at sufficient oxygen (Kroupova, 2005; Durborow *et al*, 1997). Too much nitrate can also cause hypoxia (depletion of dissolved oxygen) and can become toxic to warm-blooded animals under certain conditions (US-EPA, 2012).

Manila bay is a semi-closed bay system populated with approximately 20 million people (PSA, 2010) along its coastal provinces (Metro Manila, Bulacan, Bataan, Pampanga and Cavite). The bay is also used for various purposes including aquaculture which accounts for as much as 59% of its total economic value (PEAMSEA, 2006). According to Perez *et al* (1999), fish, fish pens and shellfish pens proliferate in the provinces surrounding the bay such as Bulacan, Bataan Pampanga, Cavite, and the northern Metro Manila coastlines, covering an aquaculture area of almost 60,000 ha.

The bay has been plagued with several environmental problems including increased organic and nutrient loading coming from several sources like aquaculture, which eventually resulted to episodic hypoxic conditions of its water, intermittent fish kills of cultivated and wild stocks, increased incidence of toxic and nuisance algal blooms and higher suspended material in the water column (Jacinto *et al*, 2008; Reichardt, 2007). Furthermore, Chang *et al* (2009), reported that Manila Bay is highly eutrophicated with organic nutrients such as nitrogen. Phosphate in the water column in all areas of the Bay has also exceeded the recognized marine water quality criterion of a healthy ecosystem of 0.015 mg/L by almost twofold (PEMSEA, 2006).

It has been hypothesized that the existence of different aquaculture activities along the bay is one of the contributors to water quality deterioration in the area. A review of aquaculture practices of farmers revealed that proper guidelines in good aquaculture practices is not followed. Estimation of nutrient levels in aquaculture farms as well as the nutrient loading in Manila Bay are essential in the assessment of the possible pollutants coming from various aquaculture activities. Therefore, the main purpose of this study is to establish baseline data on the spatial and seasonal levels of nutrients in aquaculture ponds, river streams, fish pens, and coastal areas; and to determine whether effluents from the aquaculture farms significantly contribute to nutrient pollution in the river and eventually out into Manila Bay.

Methodology

Sampling Sites and Collection of Samples

NUTRIENT TRENDS

Identification and standardization of sampling sites were described in the methods section of Chapter 2 in this publication.

Forty nine (49) pre-identified aquaculture farms and coastal areas were sampled twice for each season – May 2014 and February 2015 for the dry season and September 2014 and November 2015 for the wet season. As for the sediments, samples were collected only once for each season – May 2014 for the dry season and September 2014 for the wet season. River tributaries/water sources of the aquaculture farms were also sampled for comparison.

Water samples of approximately one (1) liter were collected for each analysis (nitrogen and phosphorus) while one hundred (100) grams of sediments were taken for the study. Manual grab sampling using intermediate container was the method used for collecting water and sediments (DENR-EMB, 2008).

Storage and preservation of the samples were done following the methods described by the US-EPA (Industrial Waste Resource Guidelines, 2009). All samples were analyzed within the specified holding time to avoid microbial buildup and chemical deterioration of a specific analyte.

NUTRIENT LOADING

Three fish ponds in Capitangan, Abucay, Bataan were sampled for the project. The ponds were empty at the beginning of the study to make sure nutrient concentrations in each step of the rearing period can be determined. Figure 3.1 shows the sampling site for the study.

Water samples of approximately one (1) liter were collected subsequent to water intrusion and prior to water discharge. Samples were collected in composite from three different points in the pond – near the water intrusion gate, at the middle, and at the end.

Storage and preservation of the samples were done following the methods described by the US-EPA (Industrial Waste Resource Guidelines, 2009). All samples were analyzed within the specified holding time to avoid microbial buildup and chemical deterioration of a specific analyte.

Water quality parameters such as pH, dissolved oxygen, temperature, and salinity were also measured using HORIBA U-50 multi-parameter water quality meter in each of the sampling points water samples were collected. Interview was also conducted every sample collection to determine the input of the farmers in the pond – feeds, fertilizers, and other chemicals.

Method of Analysis

Total nitrogen and orthophosphate contents were analyzed using the Colorimetric by UV-Vis Spectroscopy following the standard methods of the US Environmental Protection Agency (Method numbers: 350.2, 351.3, 352.1, 354.1, and 365.2) and AOAC Official Methods of Analysis for water and sediments. The method detects total nitrogen and phosphorus with ranges 0.1 to 10 mg N/L and 0.02 to 2 mg P/L, respectively.

Nitrogen and phosphorus concentrations were calculated from the external standard calibration. Total nitrogen was reported as the sum of nitrate (NO_3-N), nitrite (NO_2-N), ammonia (NH_3-N), and organic nitrogen while total orthophosphate as the sum of dissolved and suspended orthophosphates (PO_4-P). Minimum internal quality control schemes such as method/reagent blanks, recovery of fortified samples, and mid standards were applied during the analysis to ensure quality of analytical test results.

Statistical Analysis

NUTRIENT TRENDS

Nutrient levels among blocks all throughout the sampling months were compared using one-way ANOVA while nutrient levels between the ponds and their water sources were compared using t-test. One-way ANOVA was also used to compare the nutrient concentrations among the sampling months.

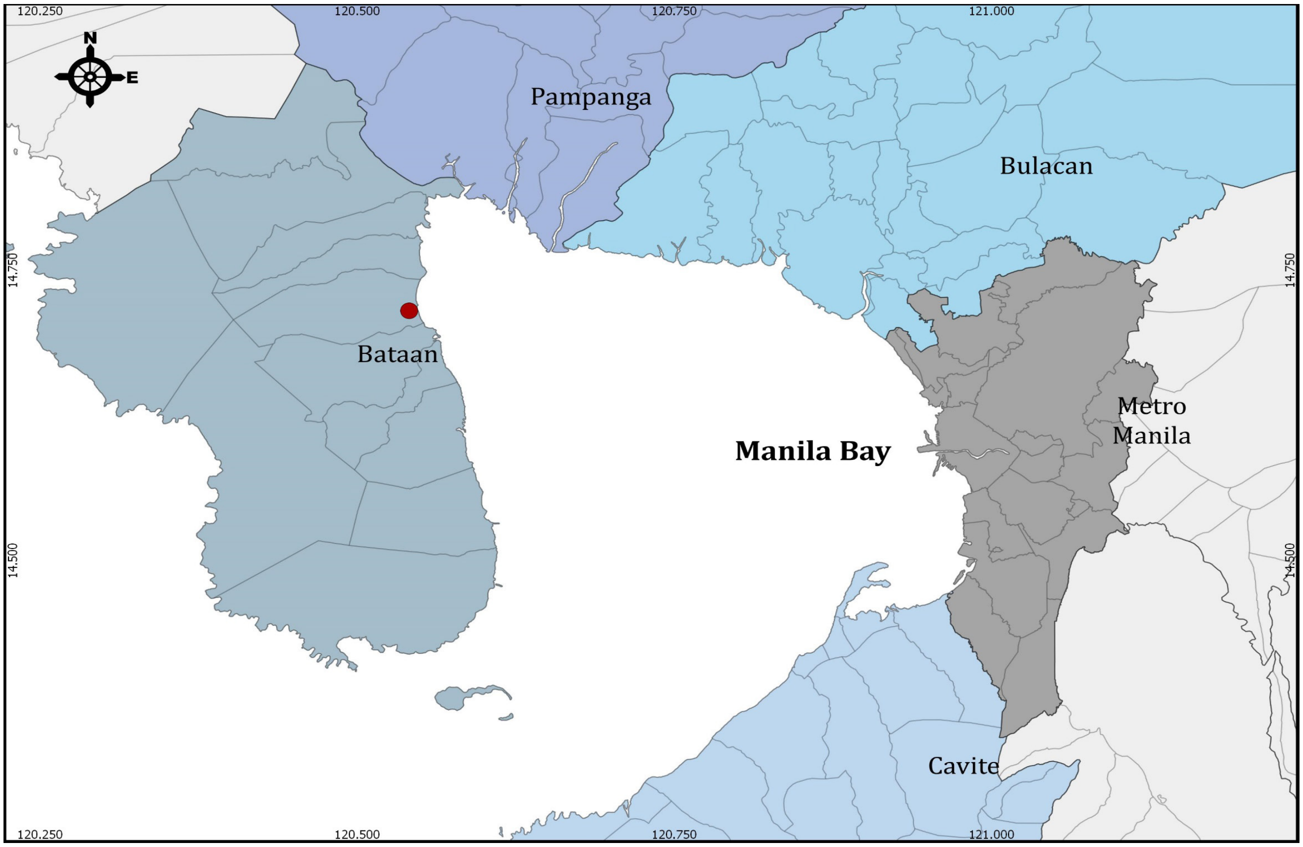


Figure 3.1. Location of sampling sites for nutrient loading.

Due to the absence of replicates, cluster analysis was used to compare the nutrient levels in each site within a block. Cluster analysis determines the group that are homogeneous in terms of several variables that characterize it, in this case, these are the nutrients. Clustering within each block was done for the months of May 2014, September 2014, November 2014, and February 2015 in the ponds and water sources.

NUTRIENT LOADING

Due to the limited number of cooperators, sampling periods will serve as replicates for each pond. A paired t-test comparing nutrient concentrations subsequent to water intrusion and prior to water discharge will be employed to determine whether the pond contributes significantly to the nutrient level in the river.

Results

Nutrient Level in Water Source, Coastal Area/Fish pens, and ponds

Levels of ammonia, nitrite, nitrate and phosphorus varied widely in relation to water source, coastal areas (shellfish growing areas), and fish ponds in the different blocks. Ammonia levels, which ranged from 0.0781 to 23.0909 μg/ml, were found higher compared to the other nutrients. In the case of nitrite, concentrations were relatively lower than other nutrients as it only ranged from 0 to 0.1290 μg/ml. Nitrate, on the other hand, were observed to be relatively higher than nitrite but relatively lower than ammonia, which ranged from 0 to 0.8183μg/ml. As for the total phosphorus, the concentration ranged from 0.0080 to 3.7969μg/ml.

In order to assess the extent of nutrient runoff into the bay, an empirical model that provides an estimate of pollution load will be based on the long term measurements of the concentrations of total nitrogen and phosphate in both pond and water source. When the concentration of nutrient in aquaculture pond is higher than its water source (river), then it is assumed that fish ponds are a source of nutrient overload in the bay. On the other hand, when the concentration of nutrient in water source is higher than the pond water, then the loading comes mainly from the upstream or the source itself.

AMMONIA

Ammonia levels during the dry and wet seasons in the coastal areas, fishponds, and water sources in the different blocks are shown in Figures 3.2a to 3.2d. Concentrations varied widely among the sampling sites in the different blocks. Ammonia levels of aquafarms (fishponds and coastal areas) in Eastern Bulacan, which ranged from 0.3911µg/ml to 3.7455µg/ml, with the extremes in February 2015 and May 2014, respectively, were significantly higher ($p<0.01$) than other blocks all throughout the sampling months. Concentrations of ammonia in Cavite, Southern Bataan and Pampanga were comparable and significantly lower ($p<0.01$) than those in Eastern Bulacan. Ammonia levels in Cavite and Pampanga, on the other hand, ranged from 0.0781µg/ml to 1.7933µg/ml and 0.2040µg/ml to 1.1768µg/ml, respectively, with extremes observed in February 2015 and May 2014. Southern Bataan ammonia ranged from 0.2310µg/ml (September 2014) to 1.3419µg/ml (November 2014). In Northern Bataan and Western Bulacan, significantly lower ($p<0.01$) ammonia levels than those in the blocks mentioned above were found, which ranged from 0.1340µg/ml (February 2015) to 1.4780µg/ml (May 2014) and 0.2990µg/ml (February 2015) to 1.1620µg/ml (September 2014), respectively.

Ammonia concentration in the water sources, which ranged from 0.1493 to 21.4217µg/ml with the highest level obtained in Cavite during the February 2015 sampling, showed no significant difference ($p>0.05$) when statistically compared to the fishponds ammonia levels.

NITRITE

Figures 3.3a to 3.3d show the levels of nitrites in the different blocks during the sampling months. Comparing the nitrite levels of aquafarms in the different blocks, it was noted that Eastern Bulacan and Cavite samples have significantly higher concentration ($p<0.05$) than those collected from the other blocks. The levels ranged from 0 to 0.1203µg/ml and 0.0006 to 0.1290µg/ml, respectively, with maximum values noted in November 2014. Nitrite levels in Western Bulacan followed, which ranged from 0 to 0.0435µg/ml peaking in November 2014. Pampanga, Northern Bataan and Southern Bataan nitrite levels were found comparable and significantly lower ($p<0.05$) than the other blocks. In Northern Bataan, nitrite ranged from 0.0014 to 0.0526µg/ml with peak observed in May 2014. Nitrite levels in Pampanga were almost the same as in Northern Bataan, which is 0.0014 to 0.0525µg/ml, with the highest value observed in February 2015. Southern Bataan, on the other hand, ranged from 0 to 0.0280µg/ml (May 2014).

Similar to ammonia, nitrite levels in water source, which ranged from 0 to 0.1132µg/ml with maximum value noted during September 2014 in Cavite, showed no significant difference ($p>0.05$) when statistically compared to the nitrite concentration of fishponds.

NITRATE

Figures 3.4a to 3.4d show the nitrate levels of aquafarms in different blocks. Ni-

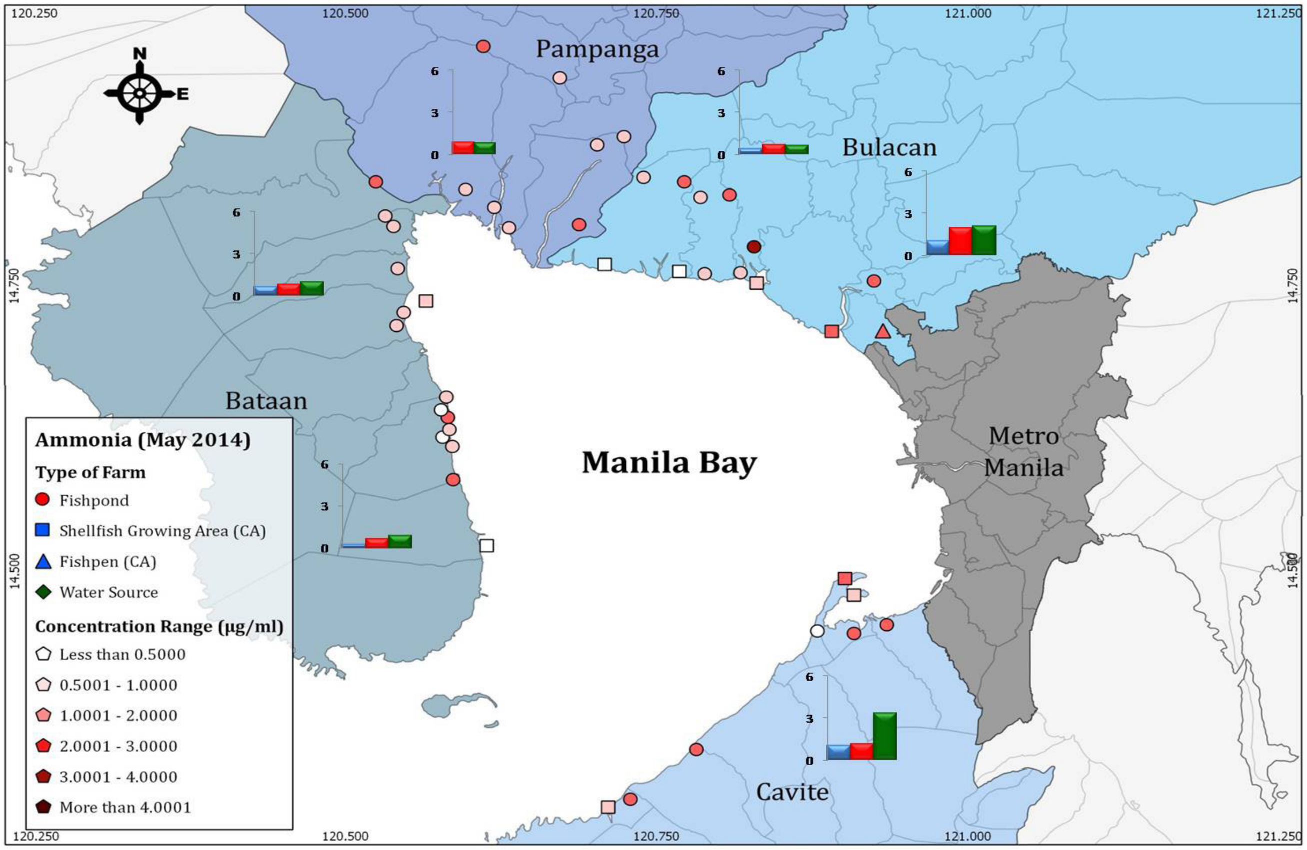


Figure 3.2a. Levels of ammonia in coastal areas/fish pens, ponds, and water sources in May 2014.

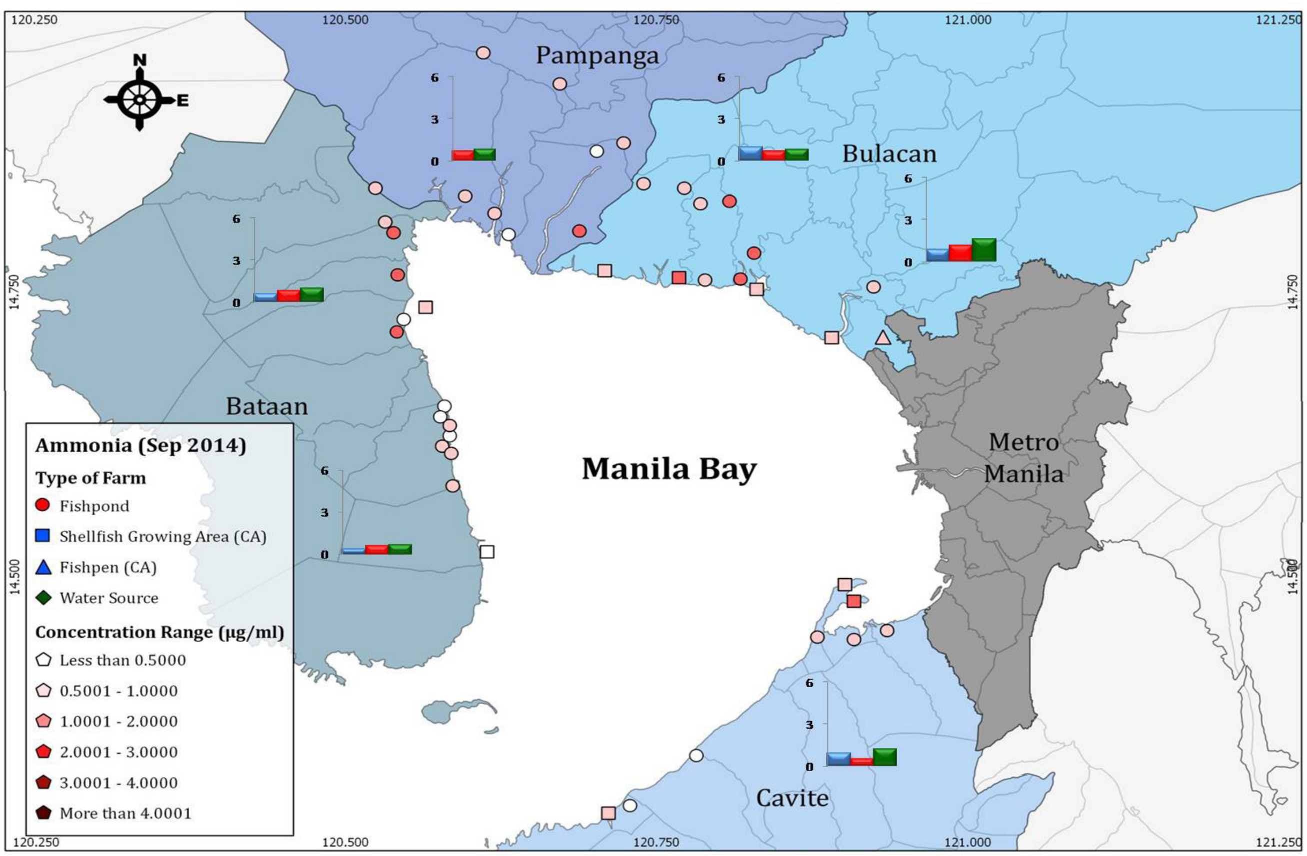


Figure 3.2b. Levels of ammonia in coastal areas/fish pens, ponds, and water sources in September 2014.

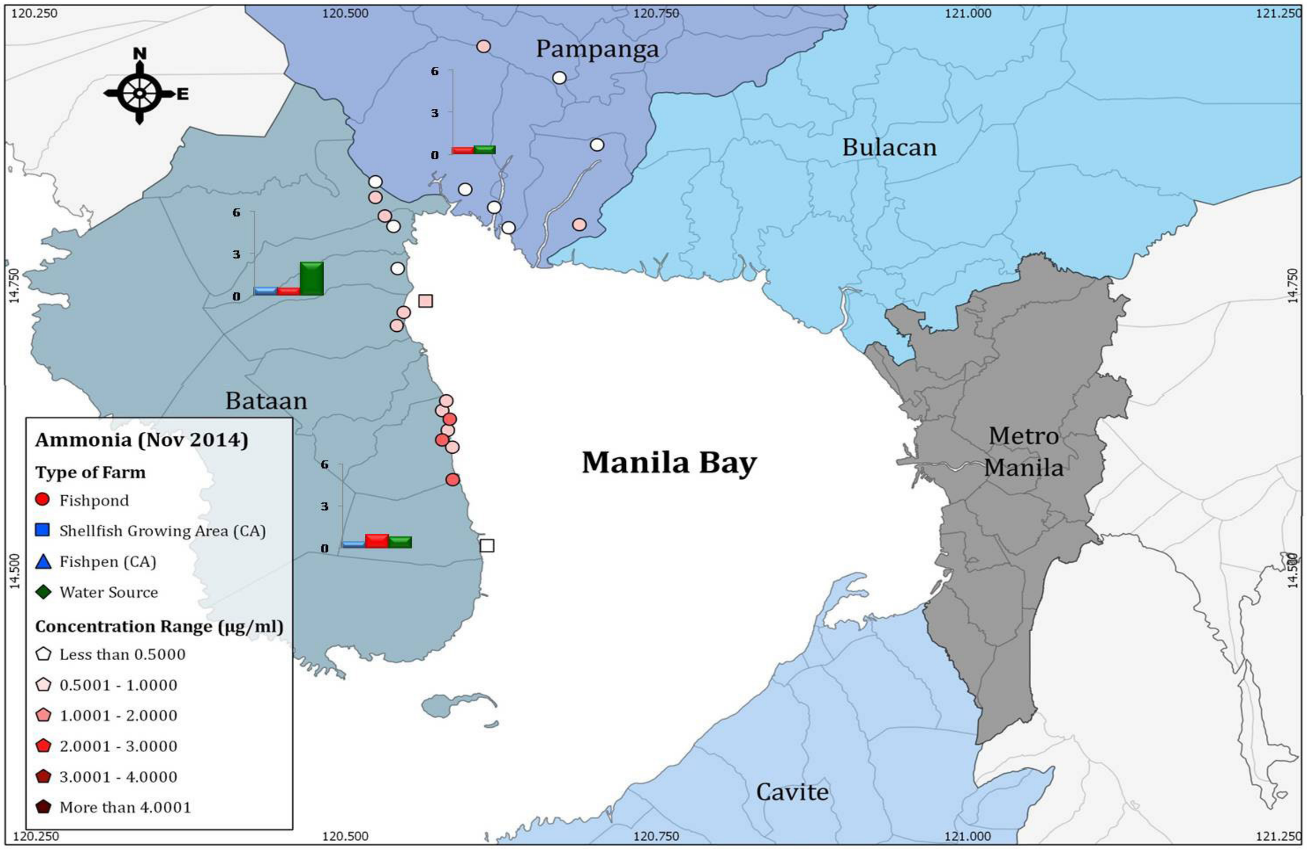


Figure 3.2c. Levels of ammonia in coastal areas/fish pens, ponds, and water sources in November 2014.

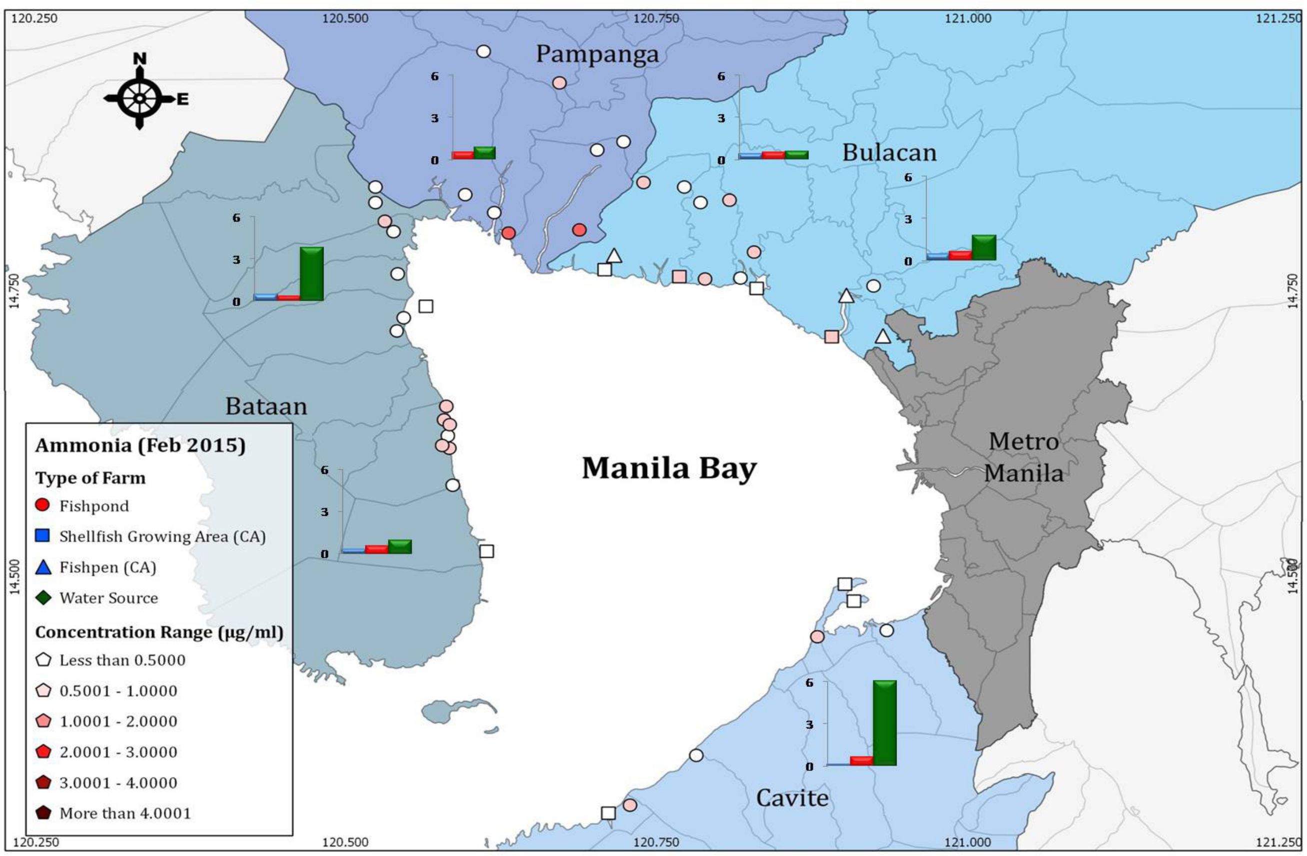


Figure 3.2d. Levels of ammonia in coastal areas/fish pens, ponds, and water sources in February 2015.

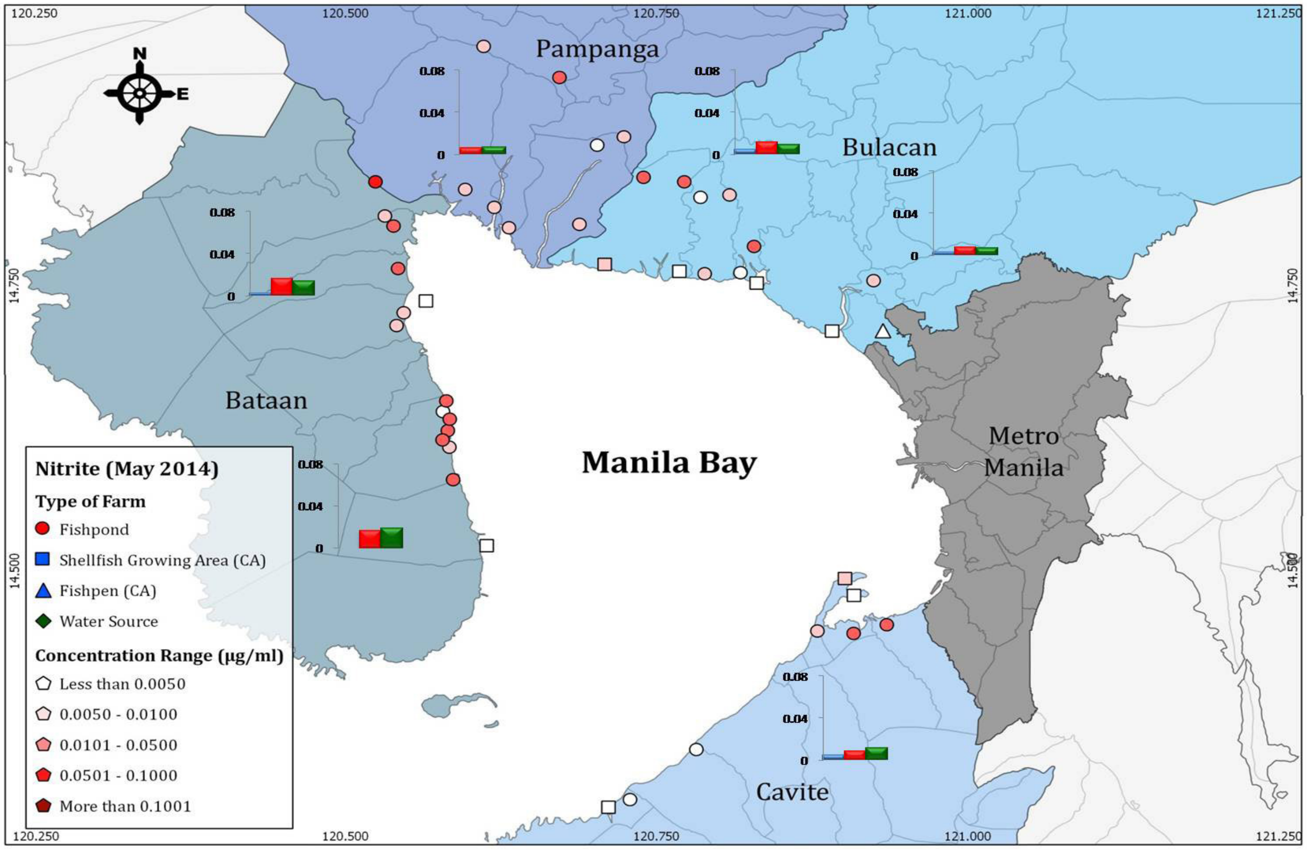


Figure 3.3a. Levels of nitrite in coastal areas/fish pens, ponds, and water sources in May 2014.

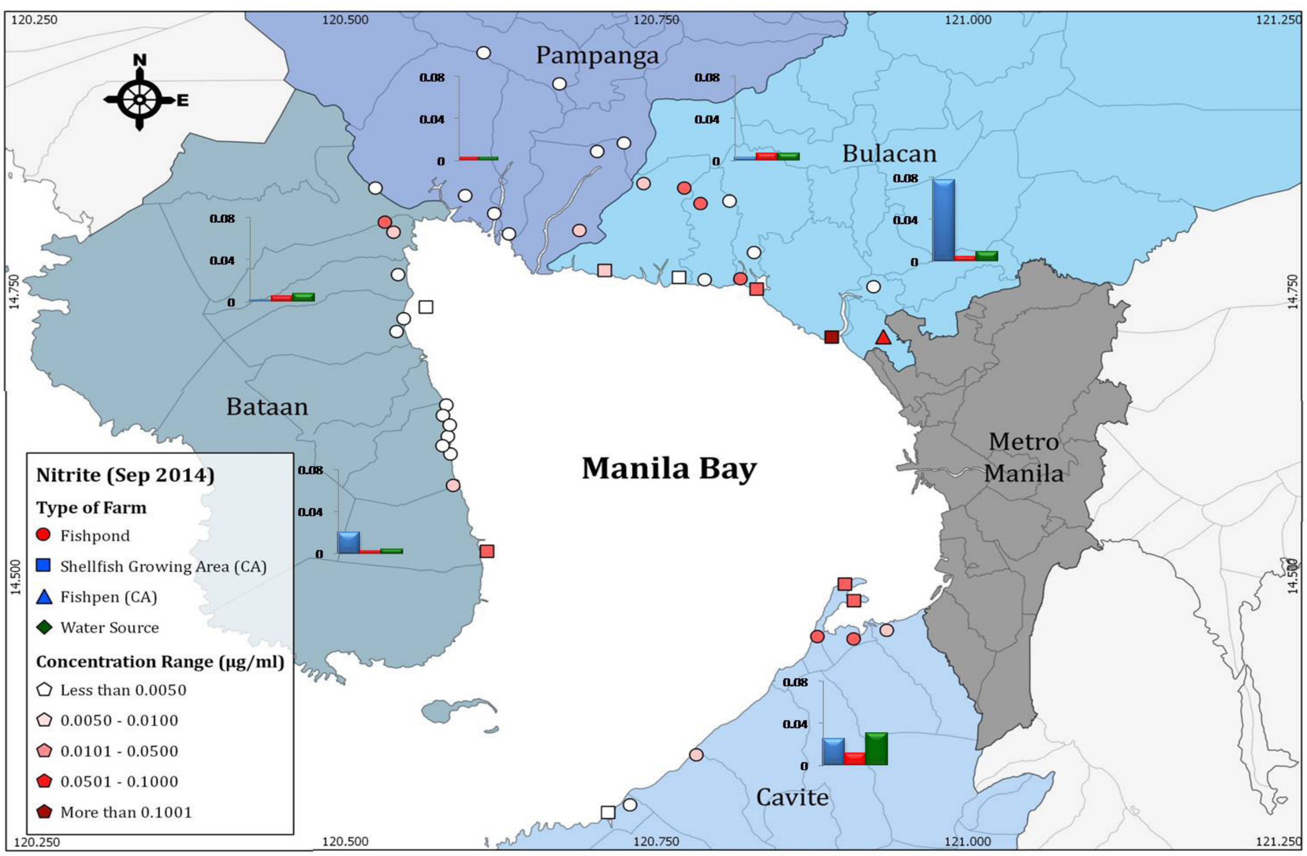


Figure 3.3b. Levels of nitrite in coastal areas/fish pens, ponds, and water sources in September 2014.

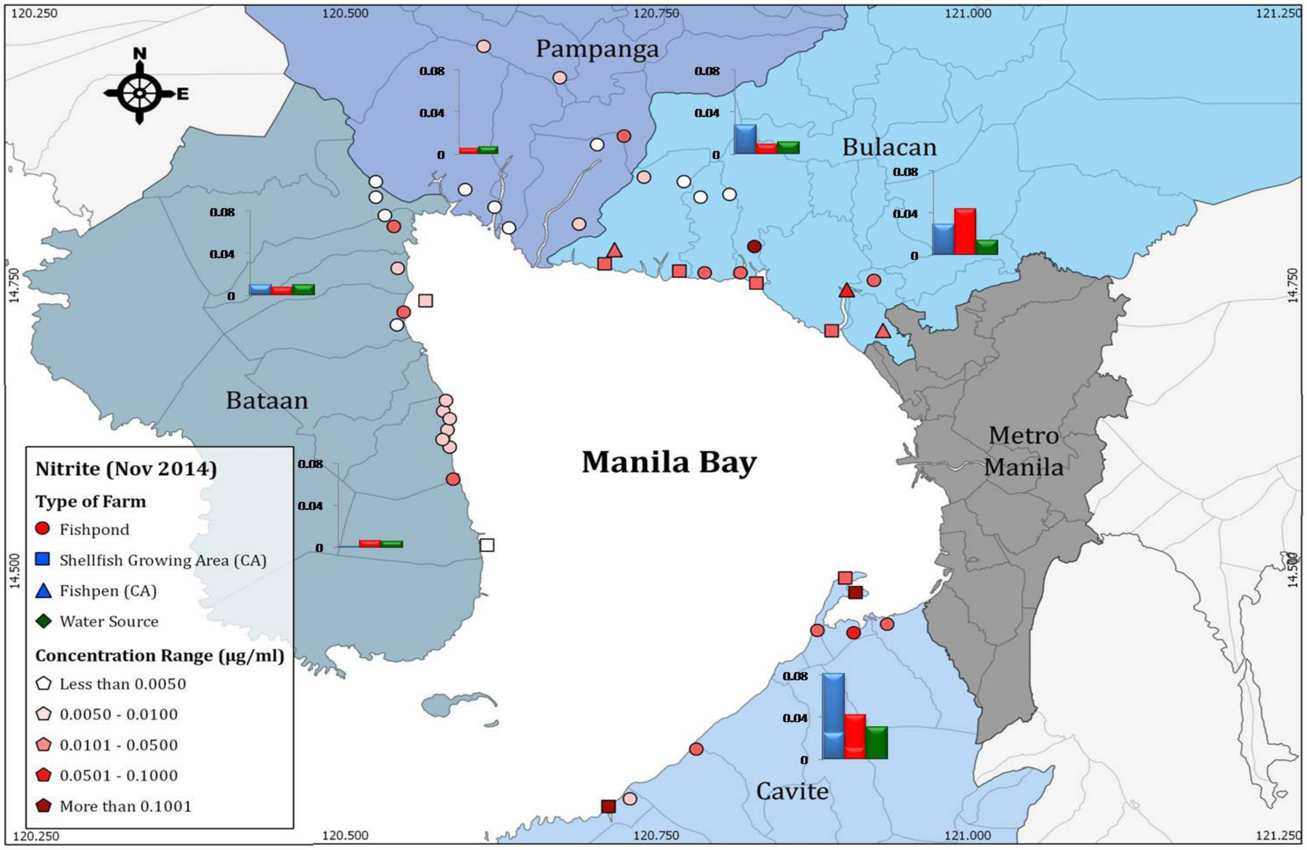


Figure 3.3c. Levels of nitrite in coastal areas/fish pens, ponds, and water sources in November 2014.

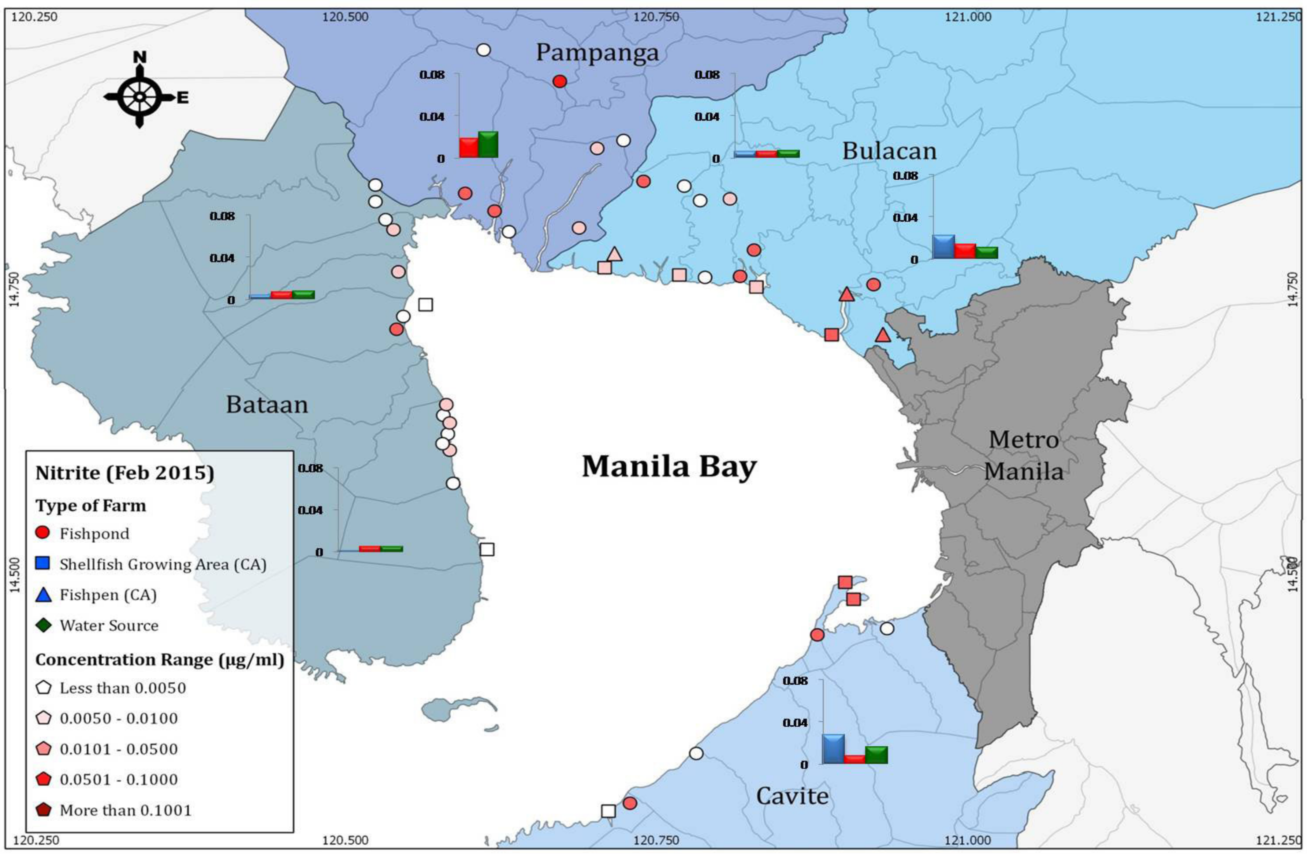


Figure 3.3d. Levels of nitrite in coastal areas/fish pens, ponds, and water sources in February 2015.

trate concentration of aquafarms in Cavite had significantly higher ($p<0.01$) concentrations, which ranged from 0.0203 to 0.4459 µg/ml, with maximum value in the coastal area (November 2014). Nitrate levels in Pampanga, Northern Bataan, Eastern Bulacan, and Western Bulacan were comparable, with the highest recorded value at 0.2943 µg/ml (September 2014), 0.2837 µg/ml (February 2015), 0.2752 µg/ml (September 2014), and 0.2303 µg/ml (September 2014), respectively. Aquafarms in Southern Bataan have relatively lower levels of nitrate, which ranged from 0 to 0.1632 µg/ml, with extremes noted in November 2014.

It was further observed that levels of nitrate in the water source, which ranged from 0 to 0.8183 µg/ml with maximum value in Cavite during September 2014, were relatively higher compared than those noted in fishponds. However, results of statistical analyses showed that the difference is only significant ($p<0.05$) in Southern Bataan in September 2014 and February 2015.

PHOSPHORUS

Seasonal levels of phosphorus in aquafarms are shown in Figures 3.5a to 3.5d. Phosphorus in Eastern Bulacan exhibited significantly higher ($p<0.01$) concentrations compared to the other blocks with a range of 0.0257 to 2.0116 µg/ml with peak in May 2014. Pampanga phosphorus levels, which ranged from 0.1093 to 1.3635 µg/ml and with extremes noted in November 2014 and February 2015, were secondary to that of Eastern Bulacan. Cavite and Northern Bataan had nearly similar levels ranging from 0.0307 to 0.8200 µg/ml and 0.0089 to 0.7605 µg/ml, respectively, their peaks were in November 2014 and February 2015, respectively. Western Bulacan phosphorus levels had a range of 0.0213 (September 2014) to 0.4462 µg/ml (February 2014), while Southern Bataan, from 0.0080 (May 2014) to 0.3758 µg/ml (September 2014).

Phosphorus in the water sources ranged from 0.0222 to 3.7969 µg/ml, peaking at Cavite in February 2015. Statistical analysis results showed that water source were significantly lower ($p<0.05$) than that in the fishponds in Northern Bataan (November 2014) and Southern Bataan (February 2015).

SEASONAL VARIATION

Seasonal variation in the nutrient levels of aquafarms in the different provinces is shown in Figure 3.6. Overall, ammonia concentration decreased from May 2014 (0.1797 to 3.7455 µg/ml) to Feb 2015 (0.0781 to 1.1529 µg/ml). Nitrite levels were highest in November 2014 with a range of 0.0018 to 0.1290 µg/ml, and lowest in February with a range of 0.0011 – 0.0525 µg/ml.

In the case of nitrate, levels were found lowest in May 2014 (range of 0 – 0.1390 µg/ml) and highest in February 2015 (range of 0.0028 – 0.2932 µg/ml). Seasonal change in phosphorus concentration was relatively insignificant. However, comparing the sampling months, phosphorus tended to be highest in May 2014, ranging from 0.0080 – 2.0116 µg/ml, and lowest in November 2014, ranging from 0.0121 to 1.3483 µg/ml.

Nutrients in Sediments

Nutrient levels in sediments in May and September 2014 are shown in Figures 3.7 to 3.10. Ammonia levels in sediments (range of 26.38 – 311.18 µg/g), were found higher compared to the other nutrients. Next to ammonia is nitrate (range of 1.51 – 220.28µg/g). Total phosphorus and nitrite had the least concentrations which ranged from 0.27 to 47.64µg/g

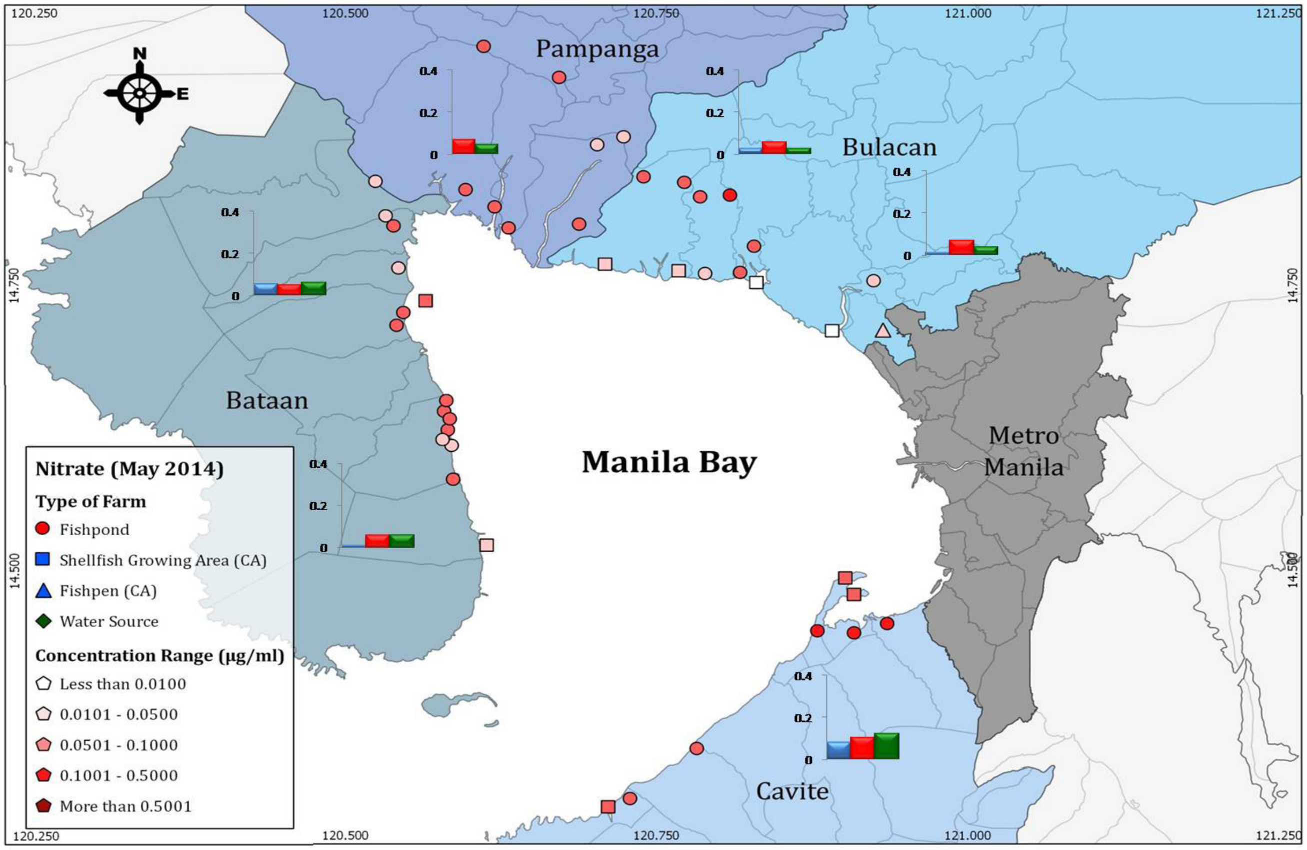


Figure 3.4a. Levels of nitrate in coastal areas/fish pens, ponds, and water sources in May 2014.

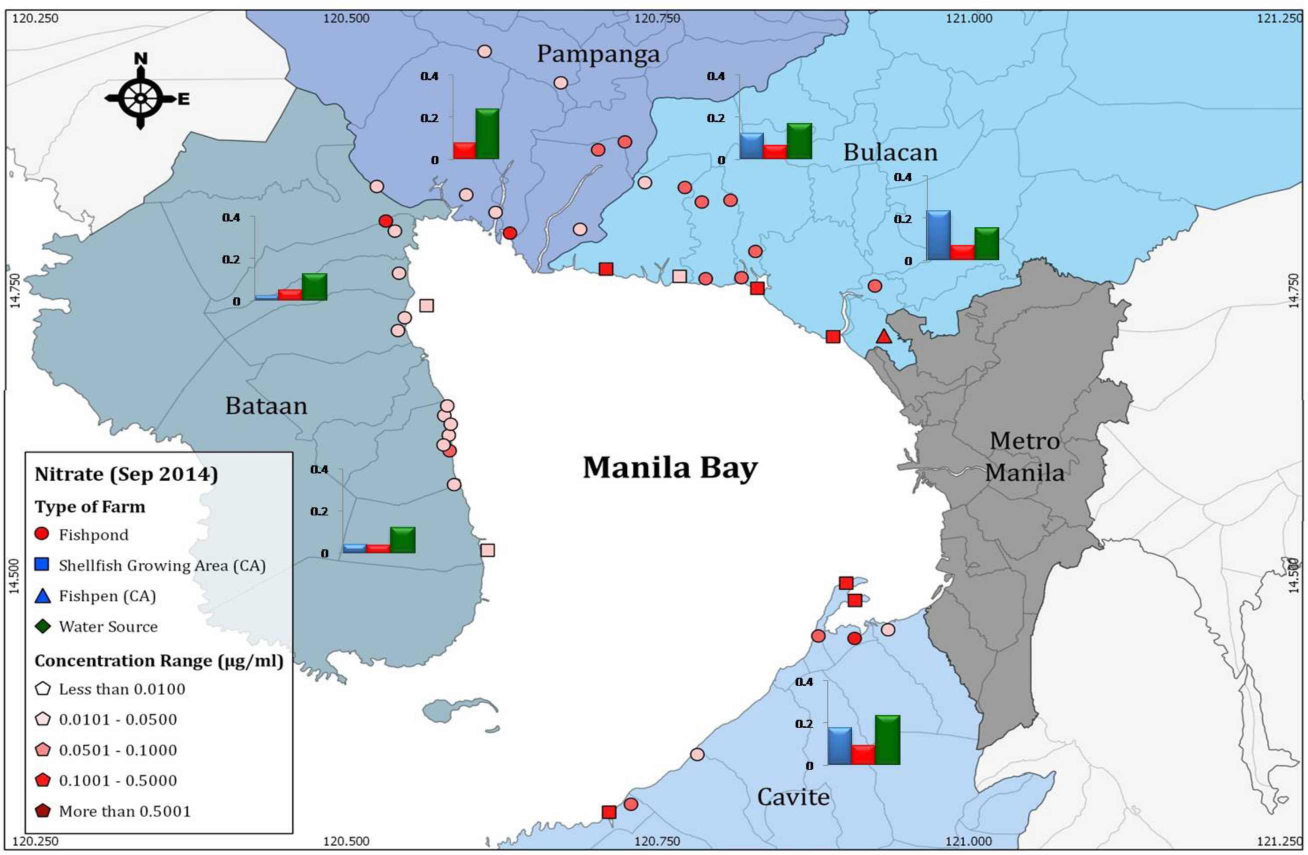


Figure 3.4b. Levels of nitrate in coastal areas/fish pens, ponds, and water sources in September 2014.

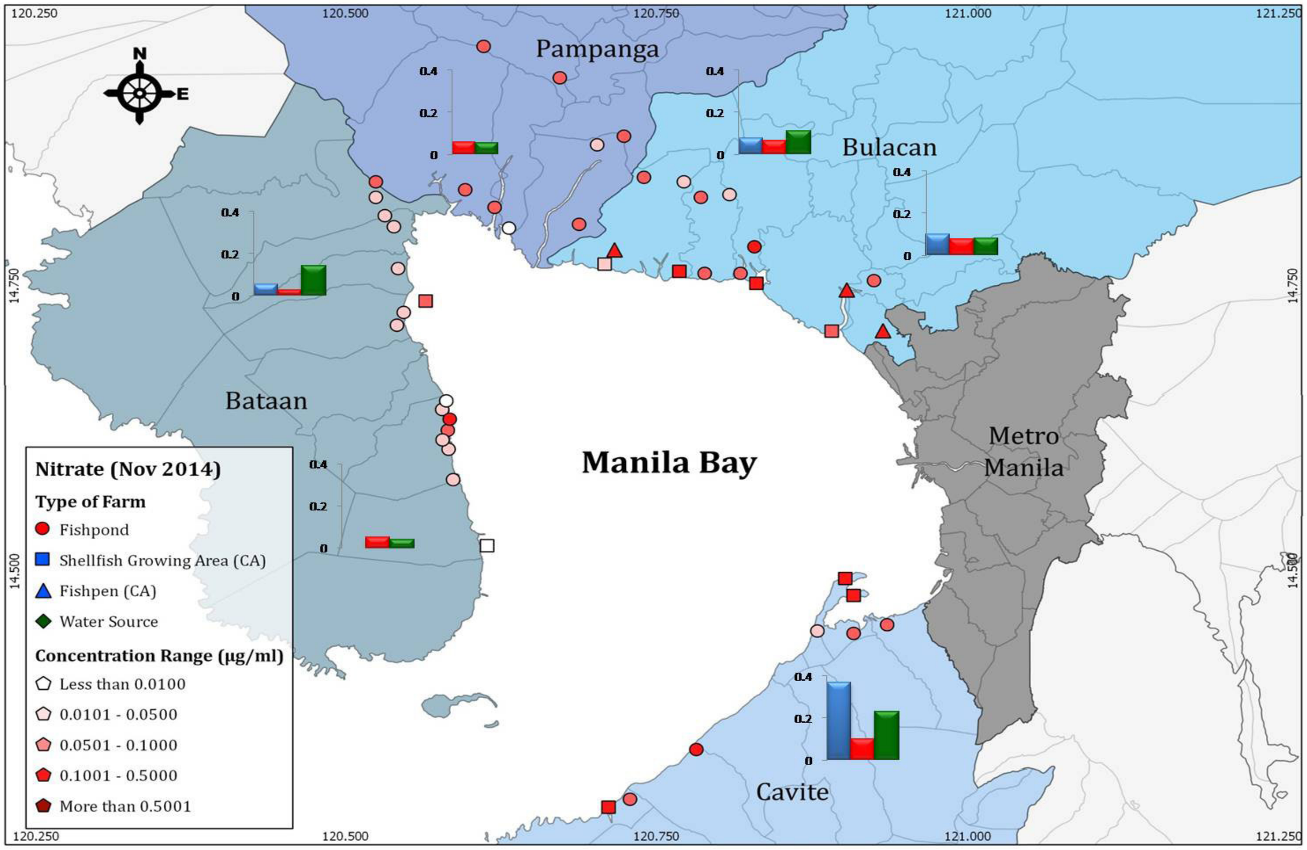


Figure 3.4c. Levels of nitrate in coastal areas/fish pens, ponds, and water sources in November 2014.

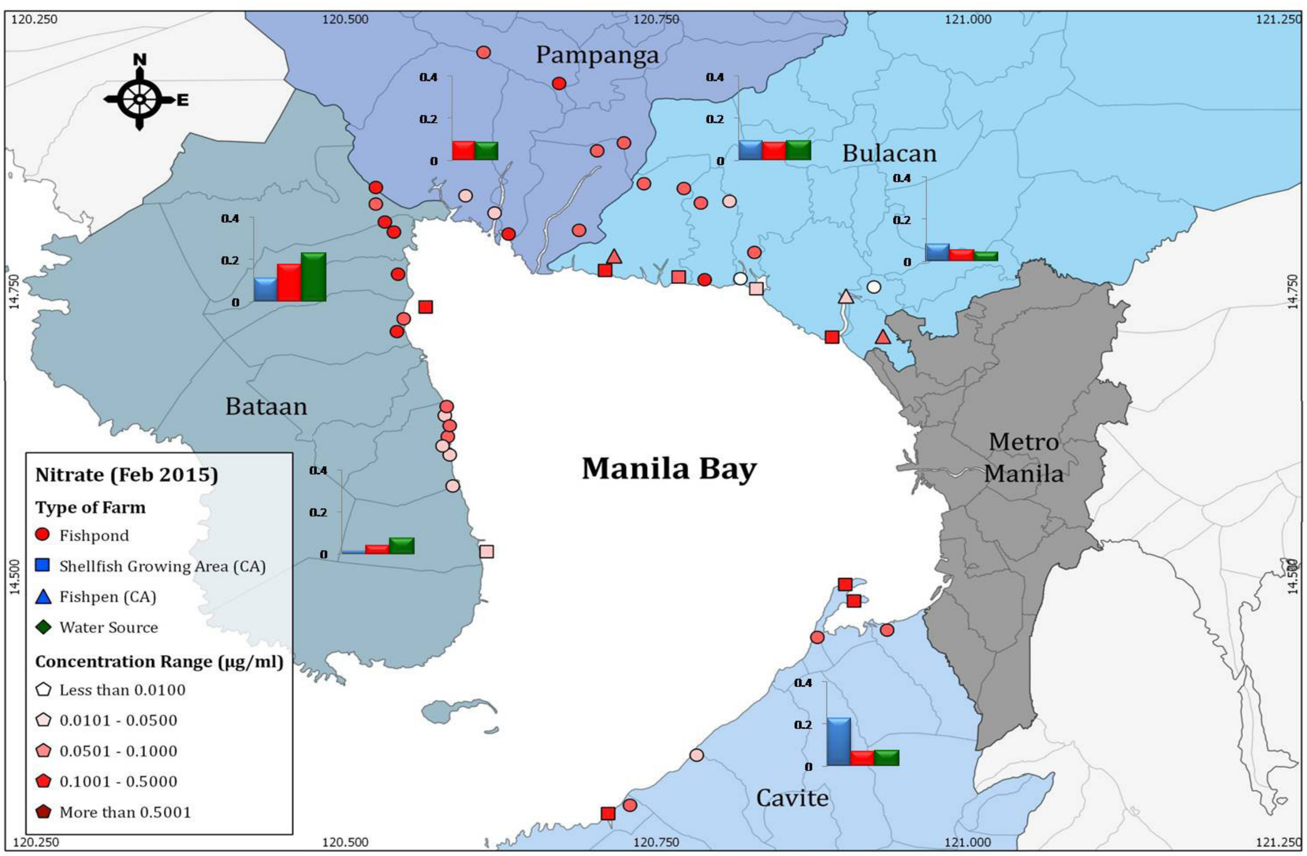


Figure 3.4d. Levels of nitrate in coastal areas/fish pens, ponds, and water sources in February 2015.

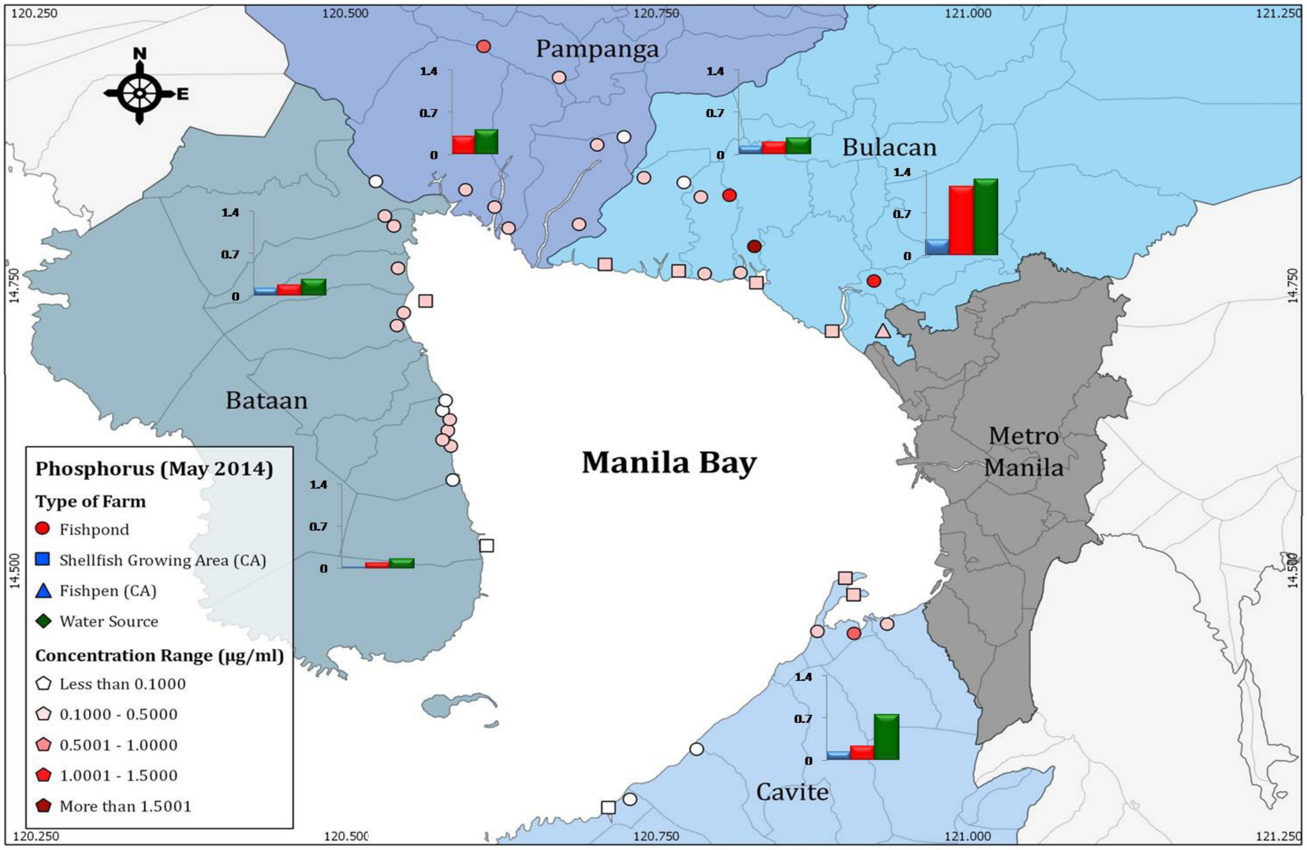


Figure 3.5a. Levels of phosphorus in coastal areas/fish pens, ponds, and water sources in May 2014.

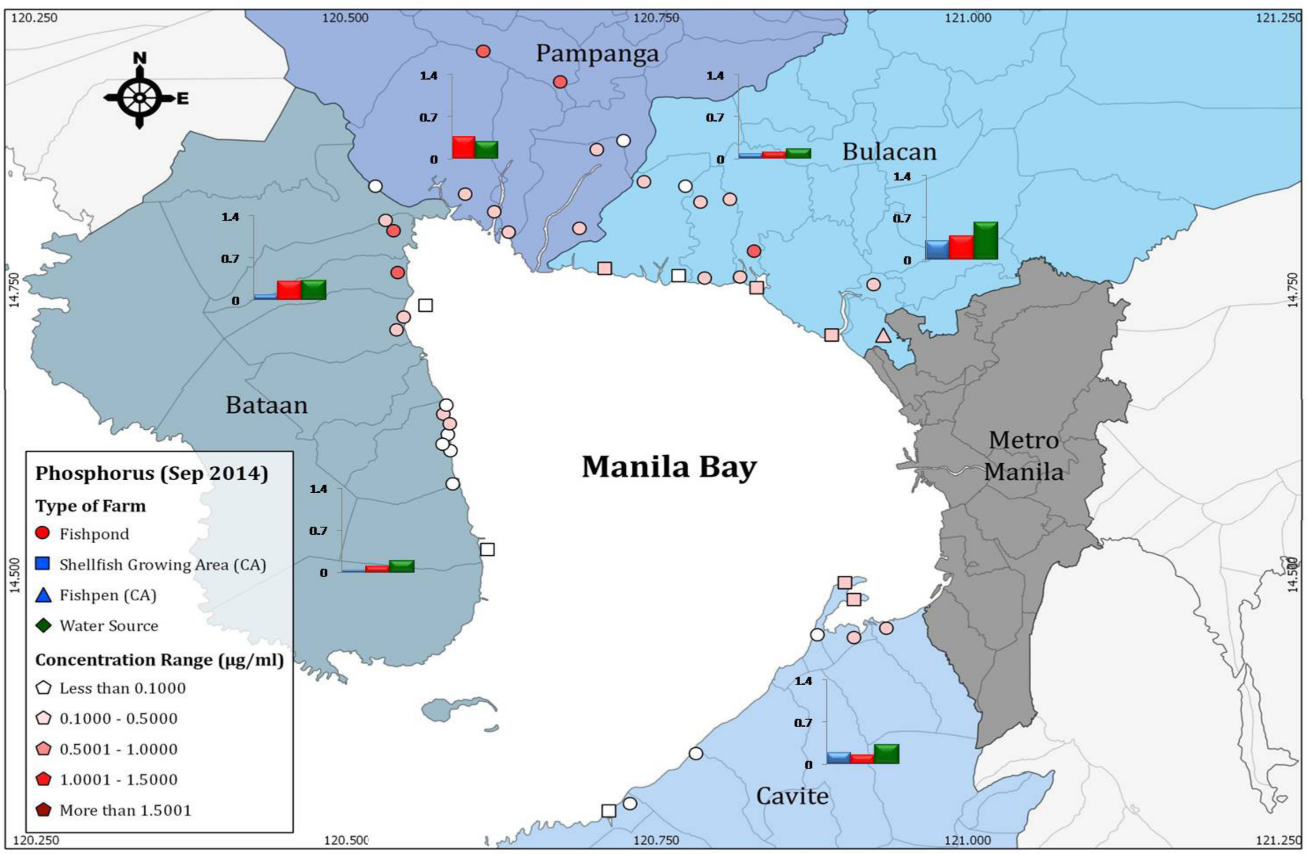


Figure 3.5b. Levels of phosphorus in coastal areas/fish pens, ponds, and water sources in September 2014.

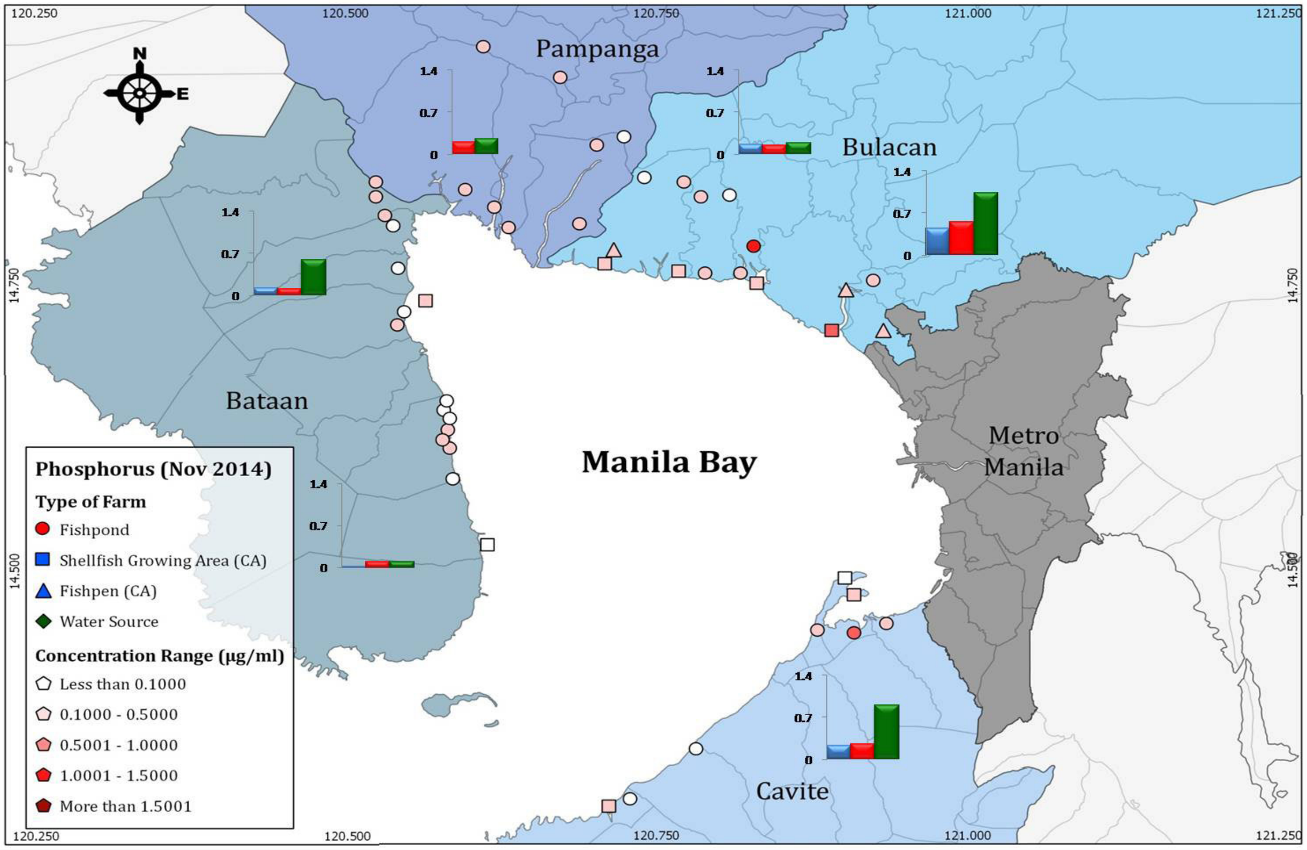


Figure 3.5c. Levels of phosphorus in coastal areas/fish pens, ponds, and water sources in November 2014.

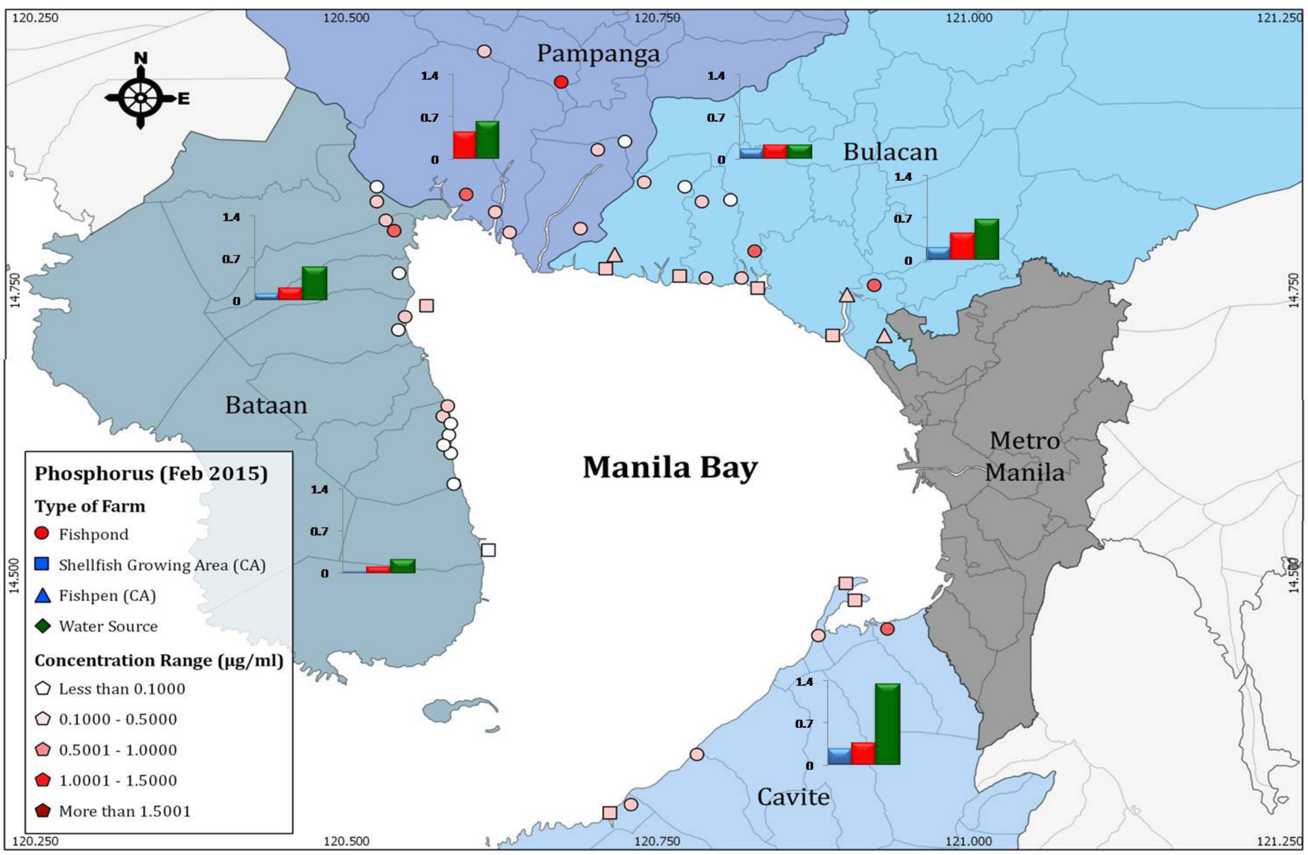


Figure 3.5d. Levels of phosphorus in coastal areas/fish pens, ponds, and water sources in February 2015.

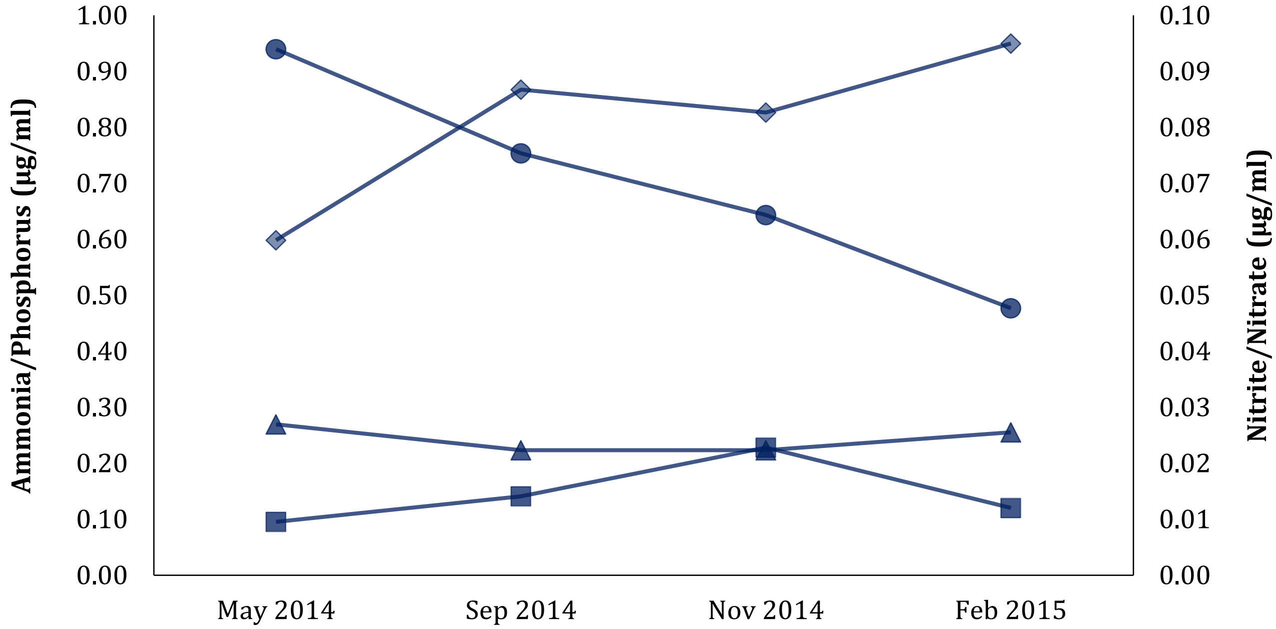


Figure 3.6. Seasonal trend of nutrient levels (mean concentrations in all blocks) in aquafarms.
Ammonia Phosphorus Nitrite Nitrate

and 0 to 6.19µg/g, respectively.

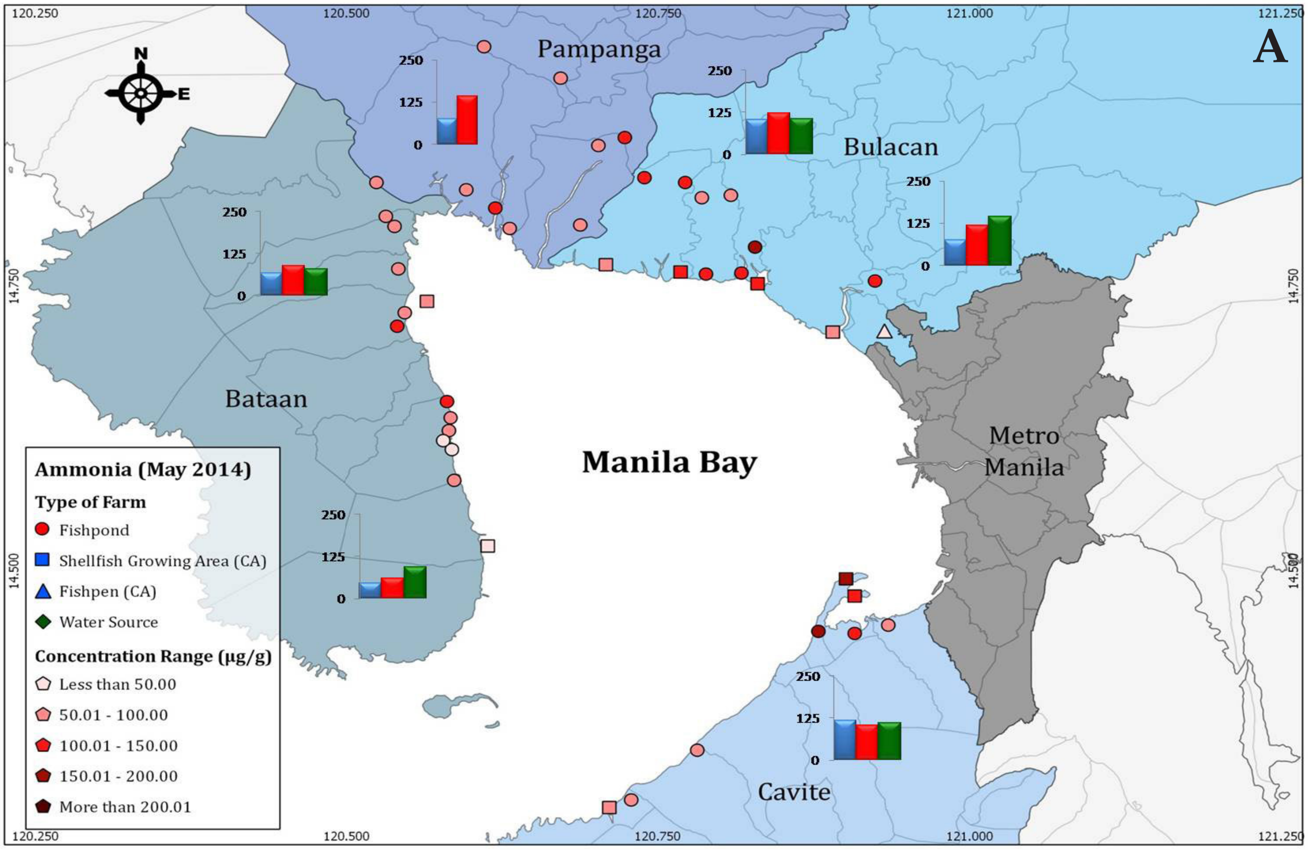
Comparing the sampling months, it can be further observed that the levels of ammonia, nitrate, and total phosphorus were high in September 2014 whereas, nitrite was high in May 2014. Moreover, the concentrations of nitrogen and phosphorus in fish ponds in most blocks were relatively higher compared to fish pen and shellfish growing areas.

Linear Correlation of Nutrient in Sediment with Water

Figures 3.11 and 3.12 show the correlation of nutrients in water and sediments during the May and September sampling. It can be observed that there is a weak correlation of ammonia in sediments and in water. Nitrates and nitrites did not correlate. But, phosphorus gave a good correlation between sediments and water in May.

Pond Clusters

Results of cluster analyses are shown in Figures 3.13a to 3.13d. Each block was analyzed separately, so the characteristics of the cluster numbers of a certain block may differ from one another. For example, sites under cluster 1 of Eastern Bulacan may have different characteristics as that of the same cluster in Western Bulacan. As seen in the figure, the number of clusters for each block throughout the season varied from two to five with the most number of clusters in Southern Bataan during the November 2014 sampling. This indicates a heterogeneous nutrient profile among its sites. It was also observed that, even though the number of clusters in a certain block remained the same for consecutive sampling periods, the sites composed therein are dissimilar. Western Bulacan, for instance, has the three clusters in May 2014 and September 2014, but the sites in the clusters in May 2014 were distinct from those in September 2014.



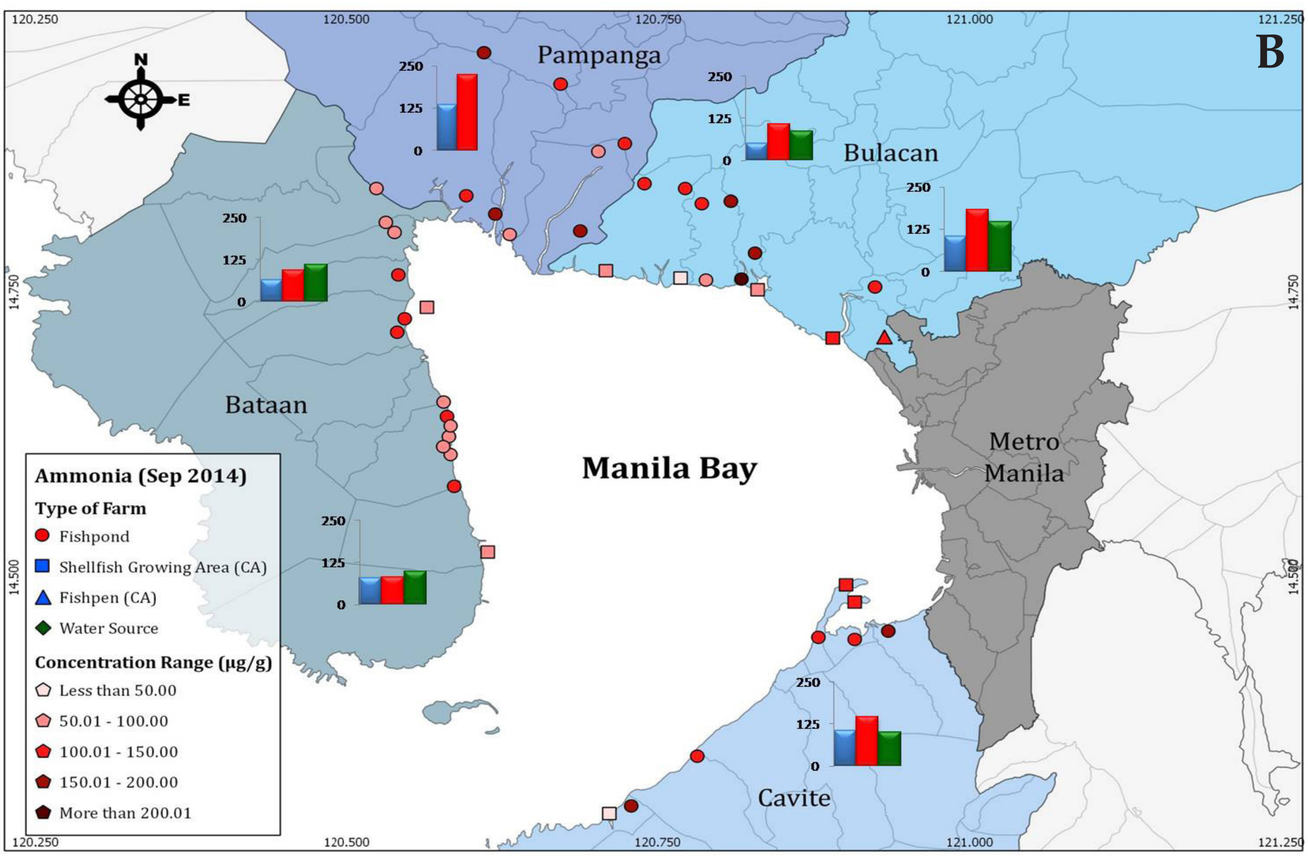


Figure 3.7. Levels of ammonia in sediments in May 2014 (A) and September 2014 (B).

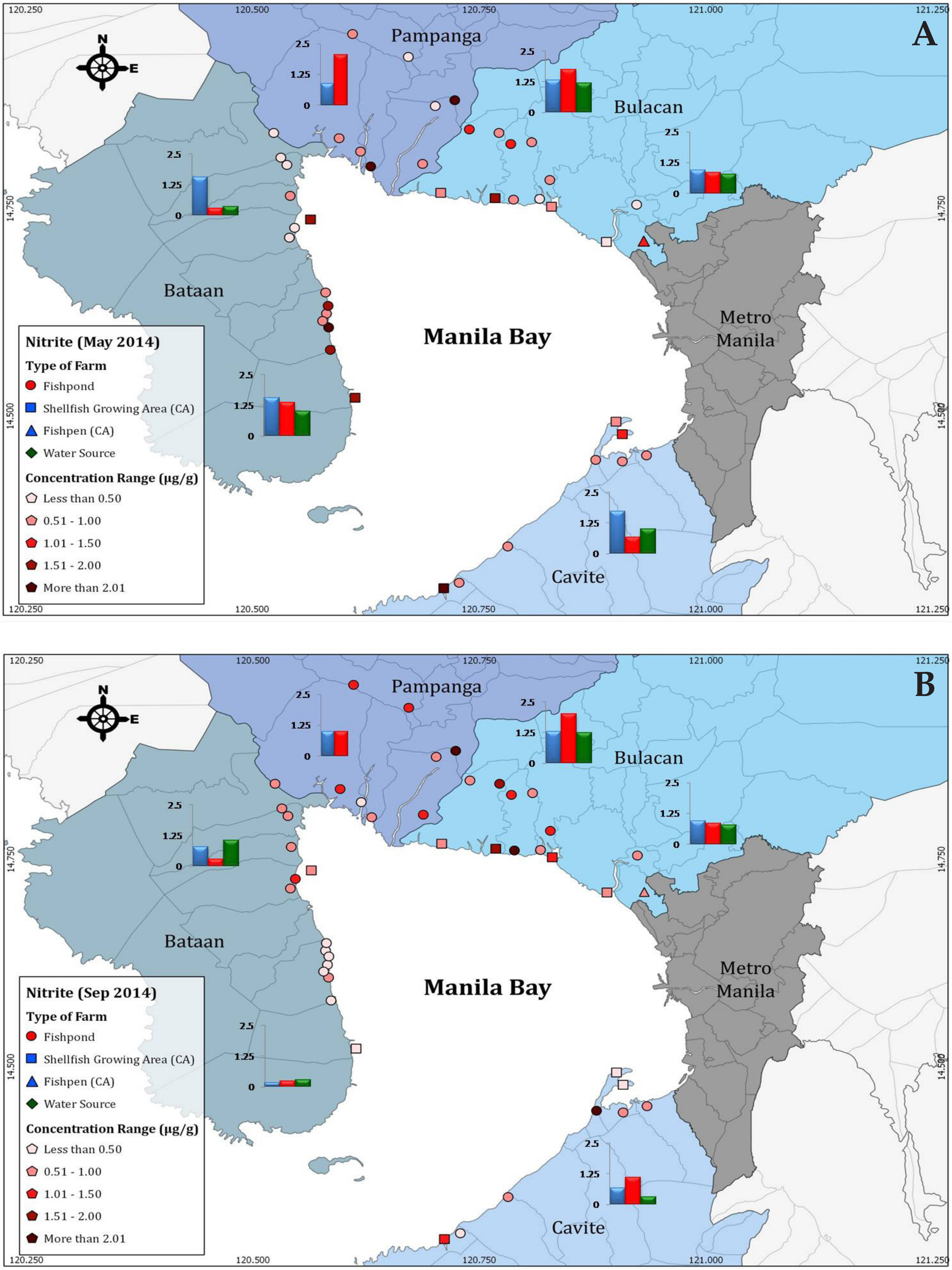
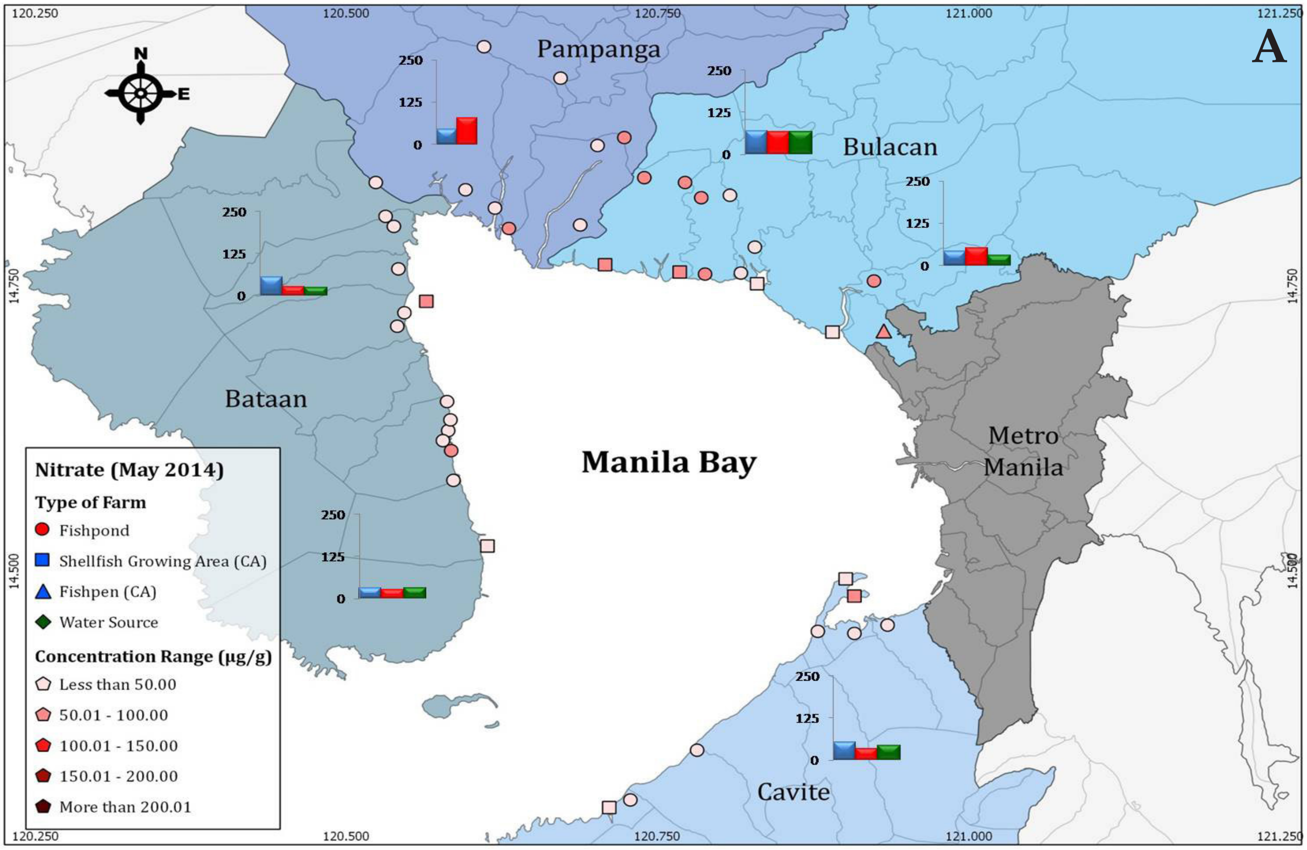


Figure 3.8. Levels of nitrite in sediments in May 2014 (A) and September 2014 (B).



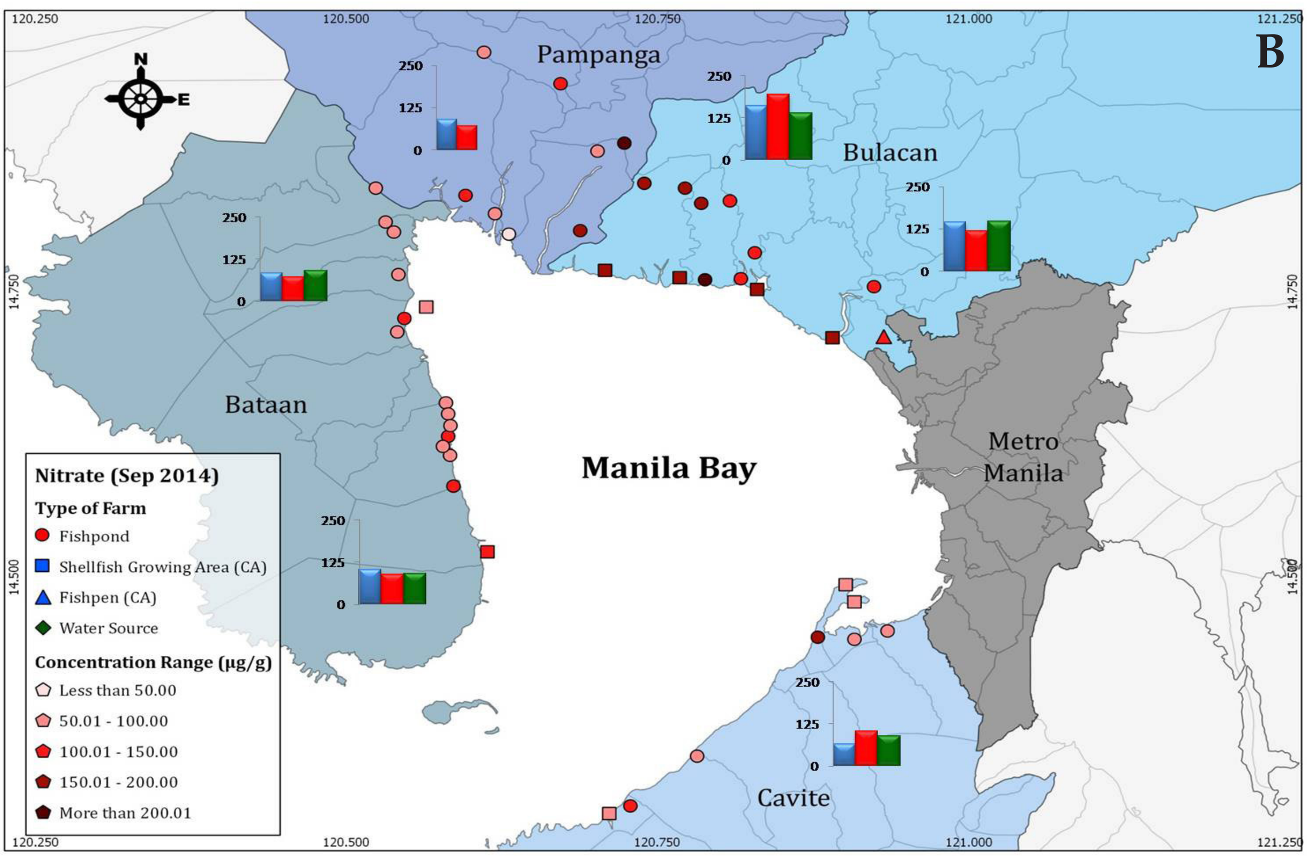


Figure 3.9. Levels of nitrate in sediments in May 2014 (A) and September 2014 (B).

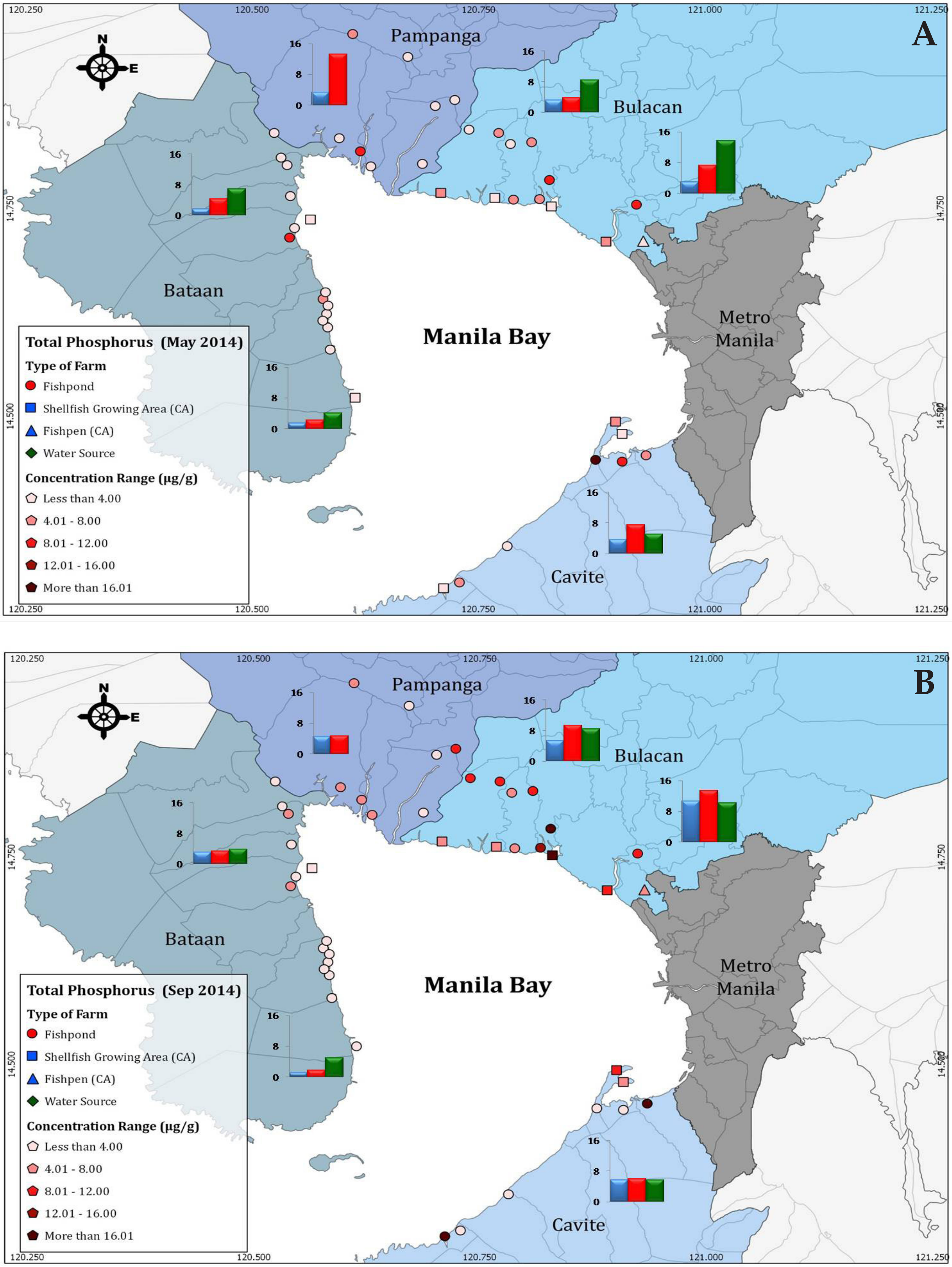


Figure 3.10. Levels of total phosphorus in sediments in May 2014 (A) and September 2014 (B).

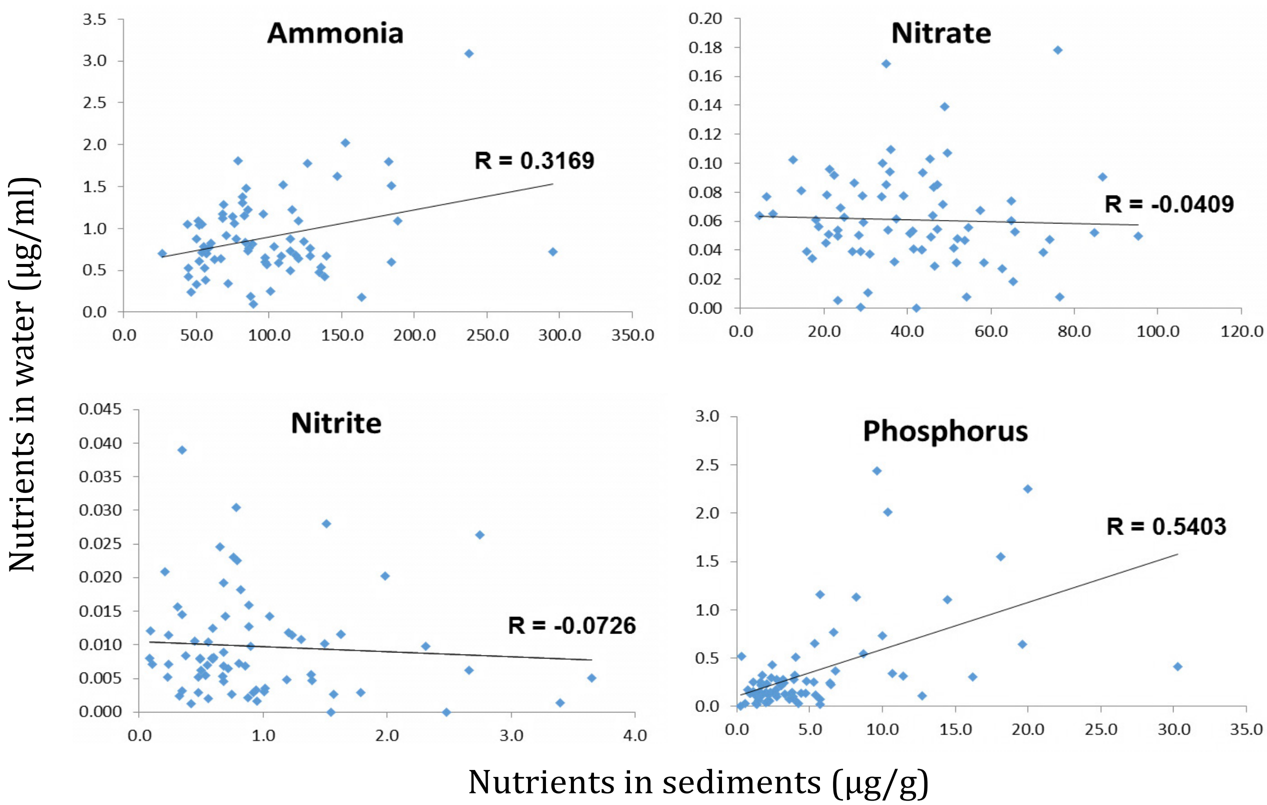


Figure 3.11. Correlation of ammonia, nitrite, nitrate, and phosphorus in sediments and water in each block (May 2014).

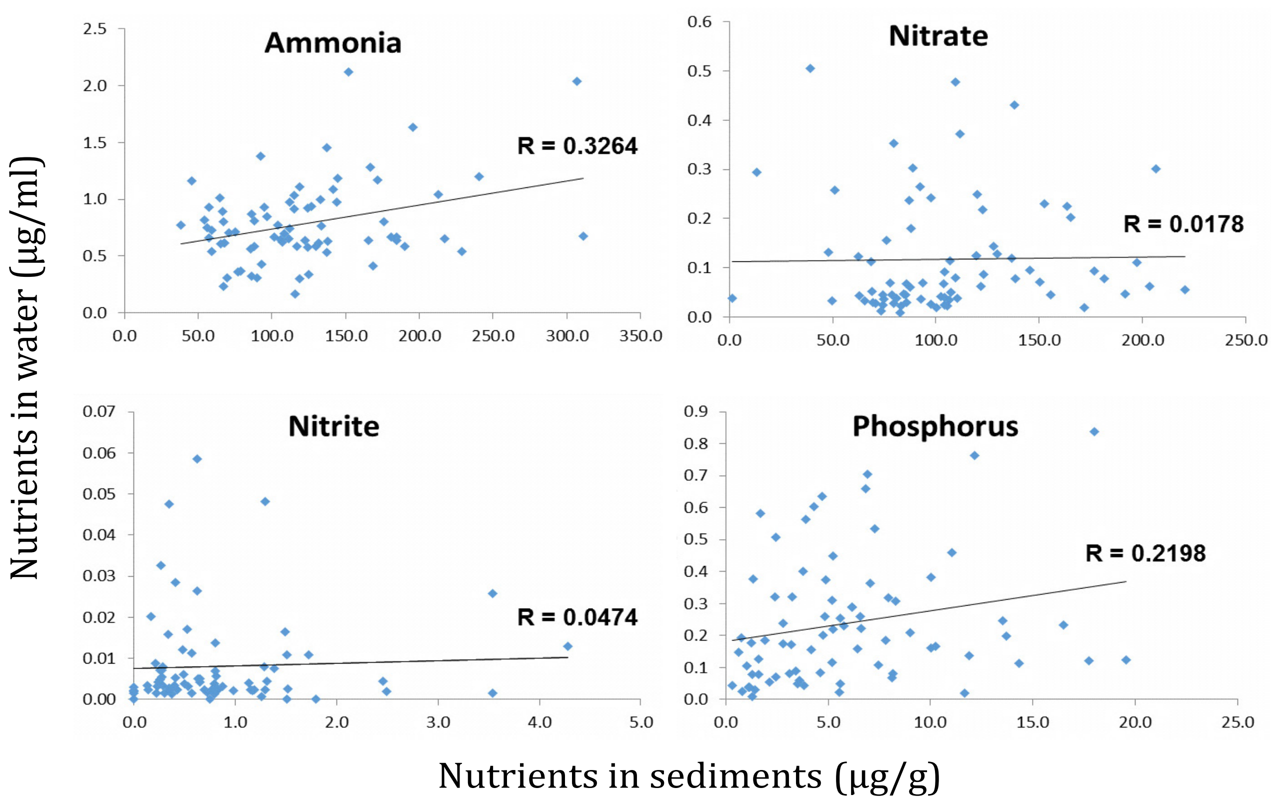


Figure 3.12. Correlation of ammonia, nitrite, nitrate, and phosphorus in sediments and water in each block (September 2014).

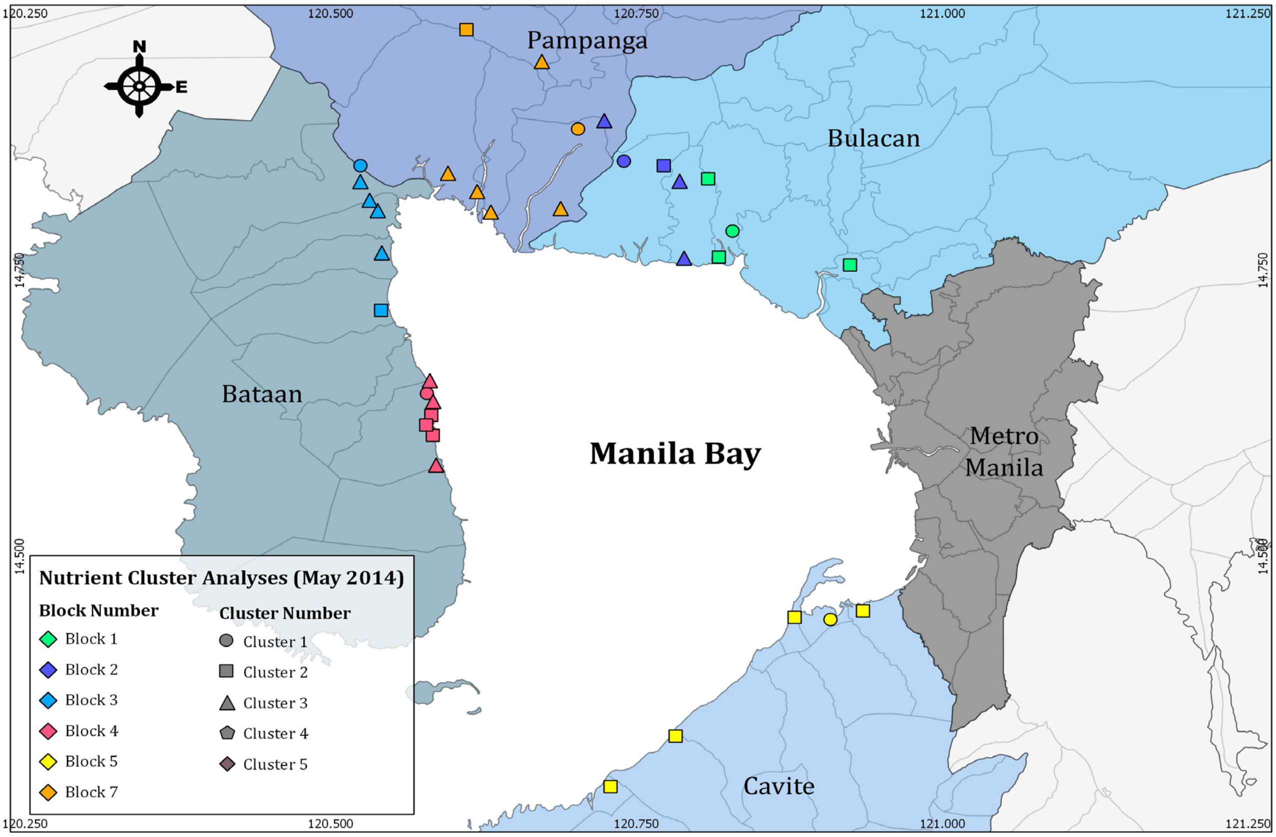


Figure 3.13a. Results of cluster analyses in the different blocks in May 2014.

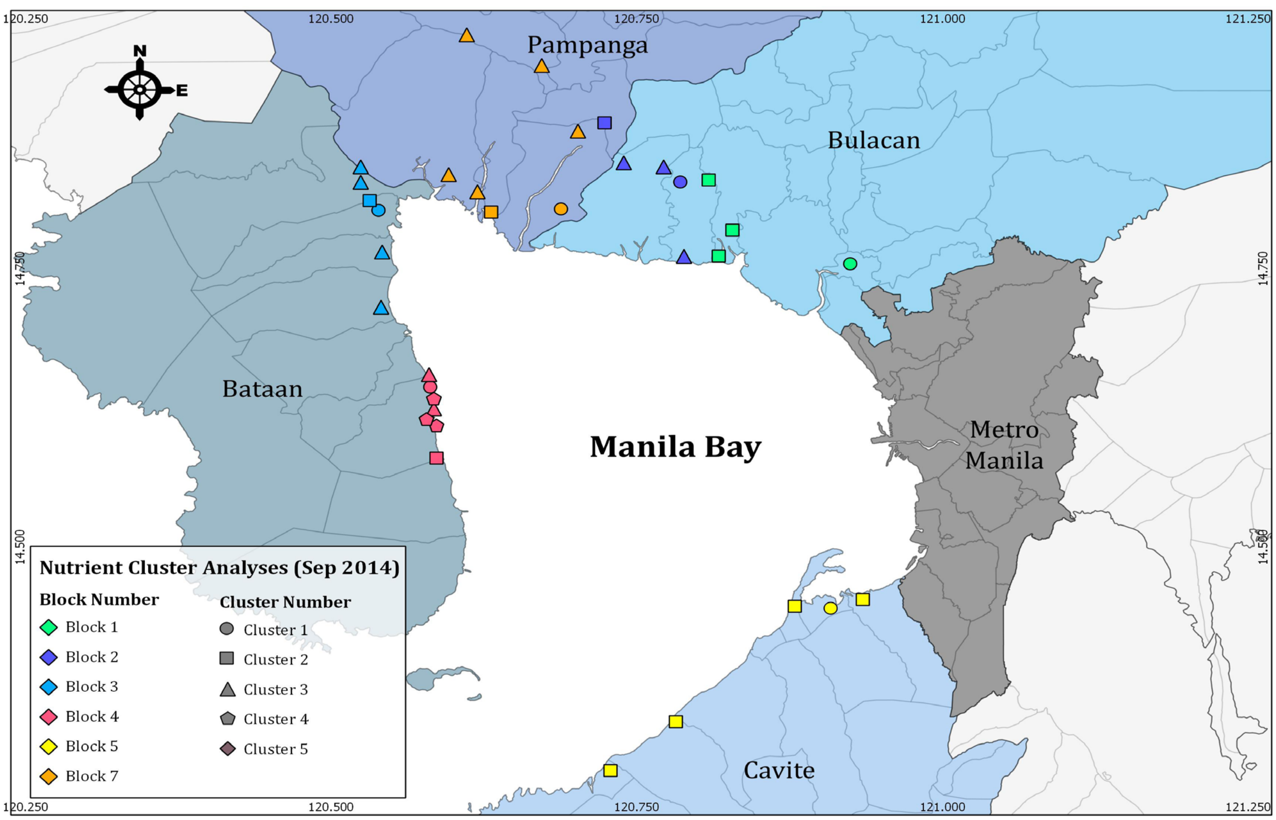


Figure 3.13b. Results of cluster analyses in the different blocks in September 2014.

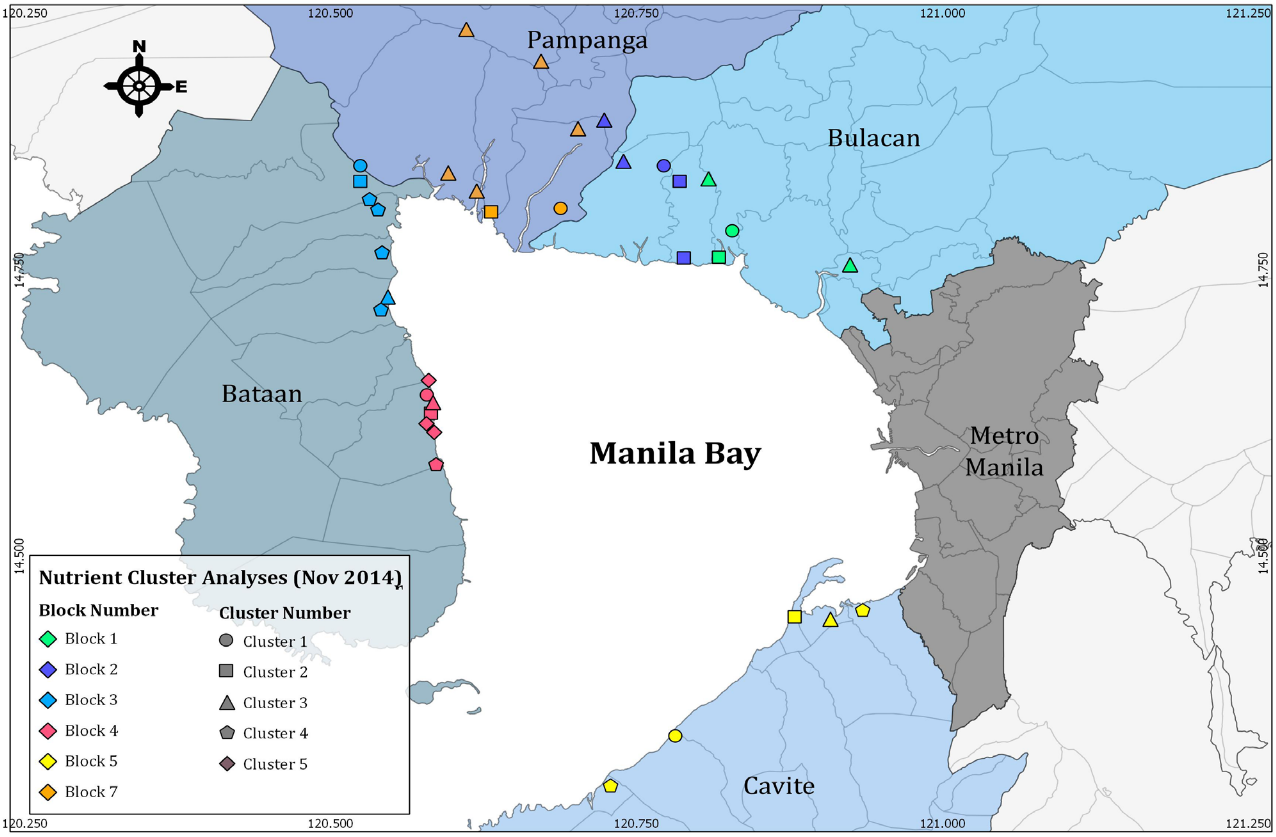


Figure 3.13c. Results of cluster analyses in the different blocks in November 2014.

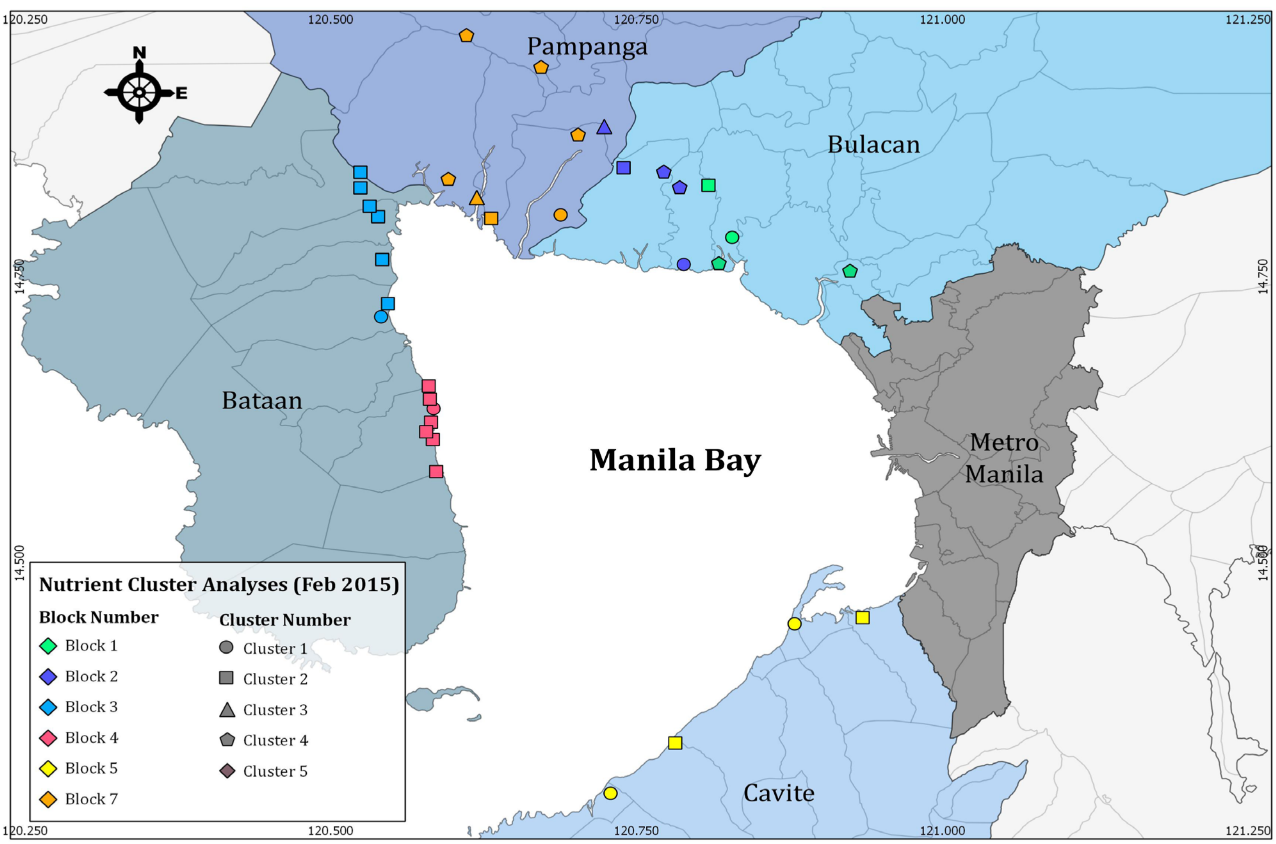


Figure 3.13d. Results of cluster analyses in the different blocks in February 2015.

Nutrient Loading

Ammonia, nitrate, nitrite, and TKN levels in the three ponds during flooding and draining are presented in Figure 3.14. Phosphorus was not detected in any of the samples analyzed. The following are the ranges of nutrients arranged in descending order: TKN (1.56 – 6.76 μg/mL); ammonia (0.79 – 4.63 μg/mL); nitrate (ND – 1.34 μg/mL); and nitrite (0.01 – 0.06 μg/mL). Ammonia levels in both flooding and draining exceeded the regulatory limit of 0.05 μg/mL (DAO 2016-08) by 15.8 to 92.6 times while nitrate levels are within the regulatory limit (10 μg/mL).

Levels of ammonia and TKN during the flooding, which ranged from 0.81 – 4.63 μg/mL and 1.72 – 6.76 μg/mL, respectively, were significantly higher ($p<0.05$) compared to the levels during the draining, which ranged from 0.79 – 2.43 μg/mL and 1.56 – 2.91 μg/mL, respectively. On the other hand, nitrate levels during the draining with levels that ranged from 0.06 – 1.34 μg/mL were significantly higher ($p<0.01$) compared to levels during the flooding that ranged from ND – 0.97 μg/mL. Nitrite levels during the draining (range of 0.03 – 0.06 μg/mL) and flooding (range of 0.01 – 0.06 μg/mL) are comparable with each other. Phosphorus was not detected in any of the samples collected.

As for the water quality parameters, results are reflected in Figure 3.15. Dissolved oxygen ranged from 2.38 to to 6.66 mg/L, highest concentration noted in pond A and lowest in pond C during the third and first water discharge, respectively. Salinity, on the other hand, ranged from 21.10– 47.40 ppt, highest in

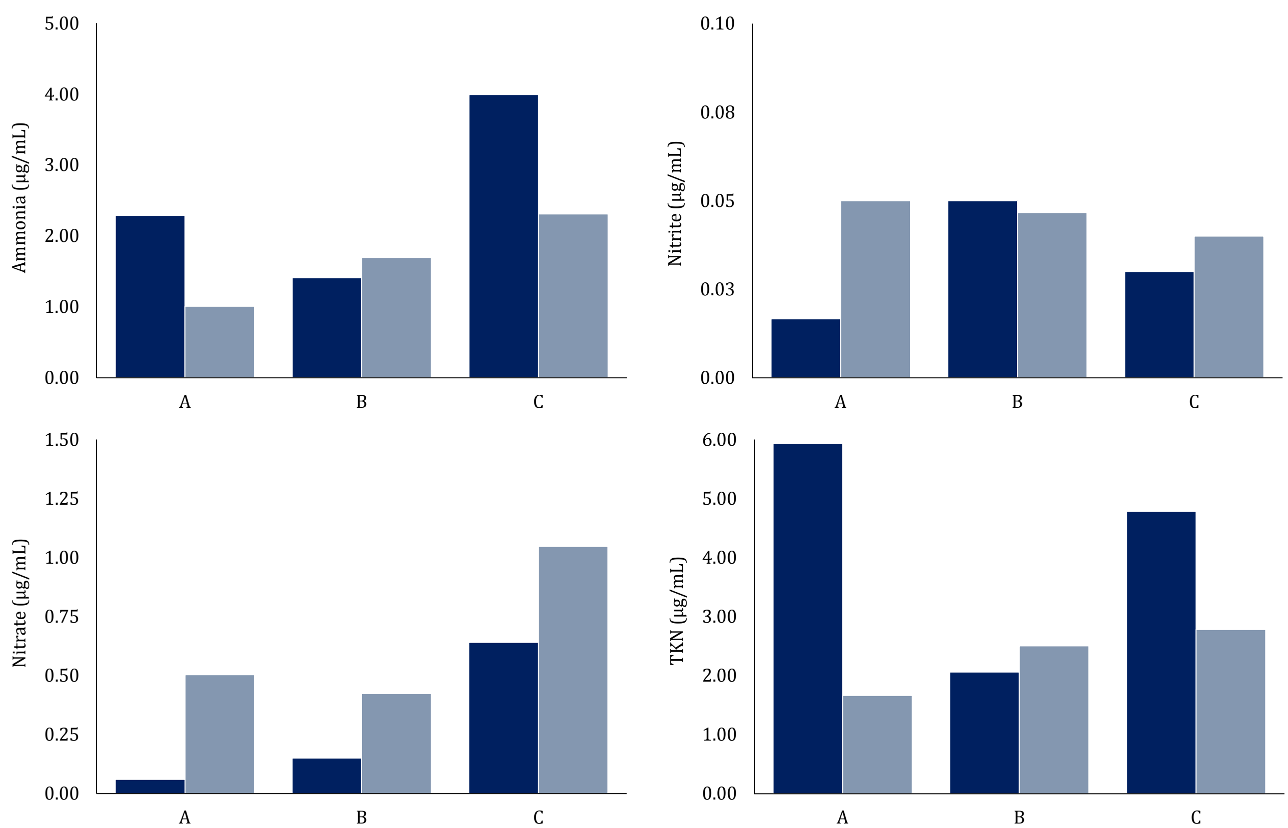


Figure 3.14. Ammonia (A), nitrite (B), nitrate (C), and TKN (D) levels during the flooding ( ) and draining ( ).

pond B and lowest in pond C during the first discharge and flooding, respectively. Temperature (range of 30.90 – 34.68°C) and pH (range of 7.08 – 8.34) were both highest in pond C during the first and third flooding, respectively, and lowest in ponds A (third discharge) and pond B (third flooding), respectively.

T-test results show that dissolved oxygen ($p<0.05$) and temperature (<0.01) are both significantly higher during the flooding compared to the draining. In contrast, pH and salinity during the flooding and draining are comparable.

The correlation between the water quality parameters and the nutrient levels in the ponds was also determined. Results are shown in Figures 3.16 to 3.19. Ammonia had a weak correlation with dissolved oxygen ($R^2 = 0.3336$) and temperature ($R^2 = 0.2243$) but had none with salinity and pH. Nitrate had no correlation with dissolved oxygen, salinity, and pH but had a weak correlation with temperature ($R^2 = 0.2275$). Nitrite and TKN, on the other hand, were both observed to have a good correlation with salinity (R^2 values of 0.5293 and 0.5448, respectively) but none with the other water quality parameters.

It was also noted that other than teaseed, which was used to eradicate pests, the farmers did not apply anything else during the cropping period. Their feeding materials include low value feed, *kabayo/sulib/isda*, *lumot*, and bread, which they only feed one week before the harvest.

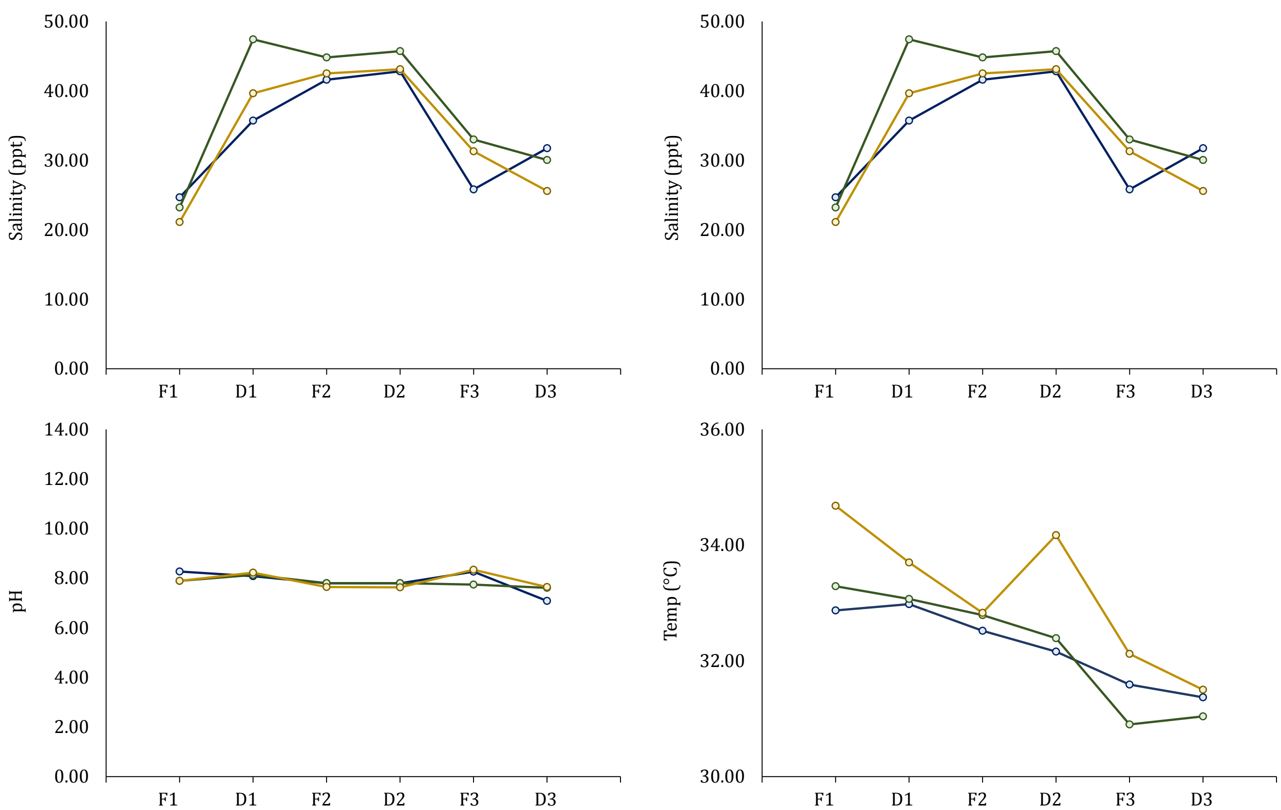


Figure 3.15. Water quality parameters of the fish ponds during the different water exchange activities.

NL310_A NL310_B NL310_C

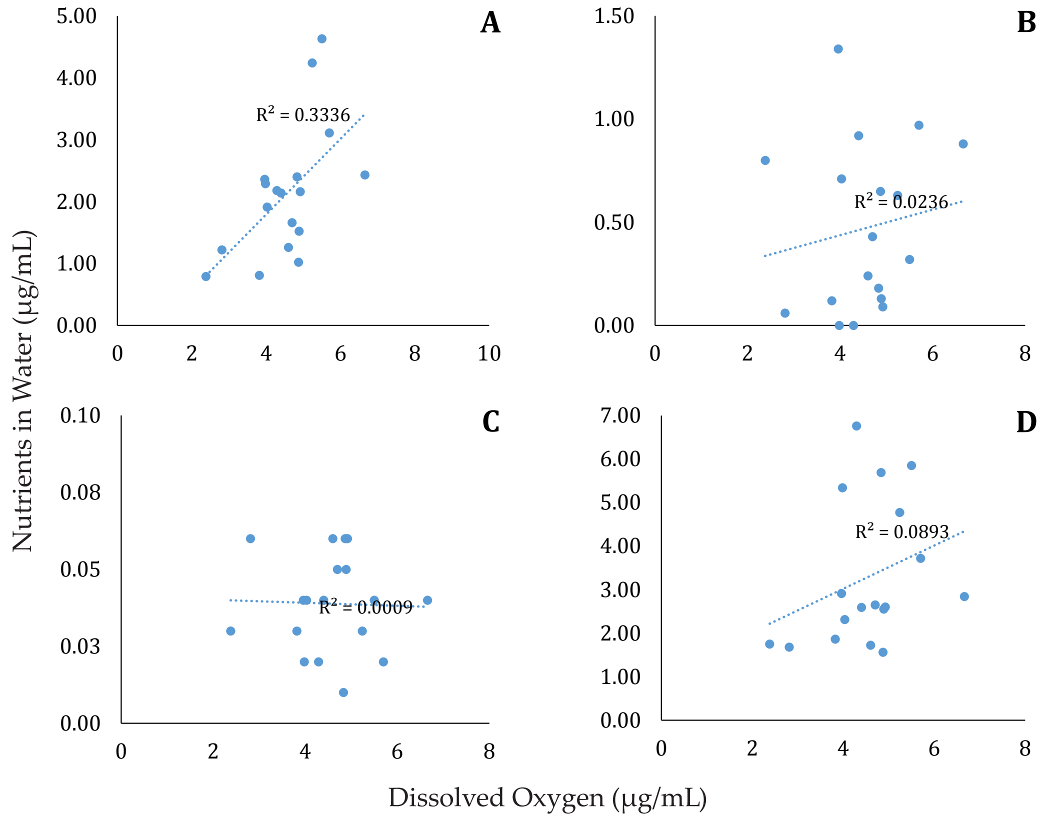


Figure 3.16. Correlation of ammonia (A), nitrate (B), nitrite (C), and TKN (D) levels in water and dissolved oxygen.

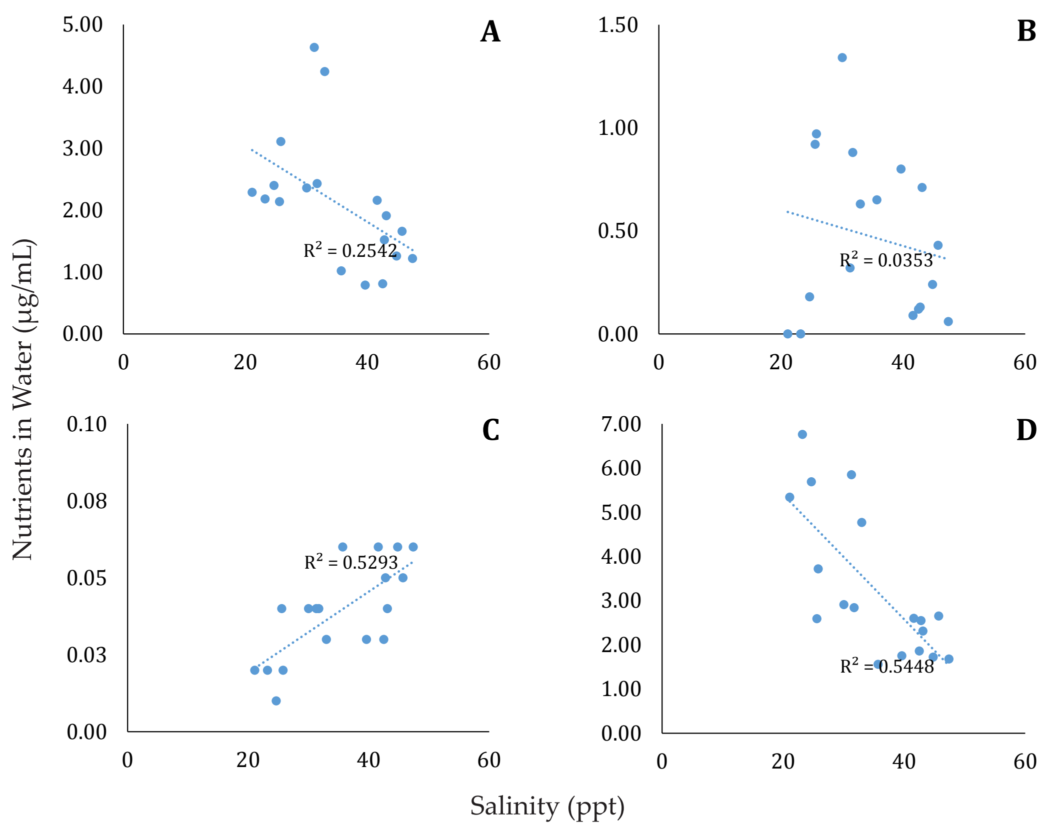


Figure 3.17. Correlation of ammonia (A), nitrate (B), nitrite (C), and TKN (D) levels in water and salinity.

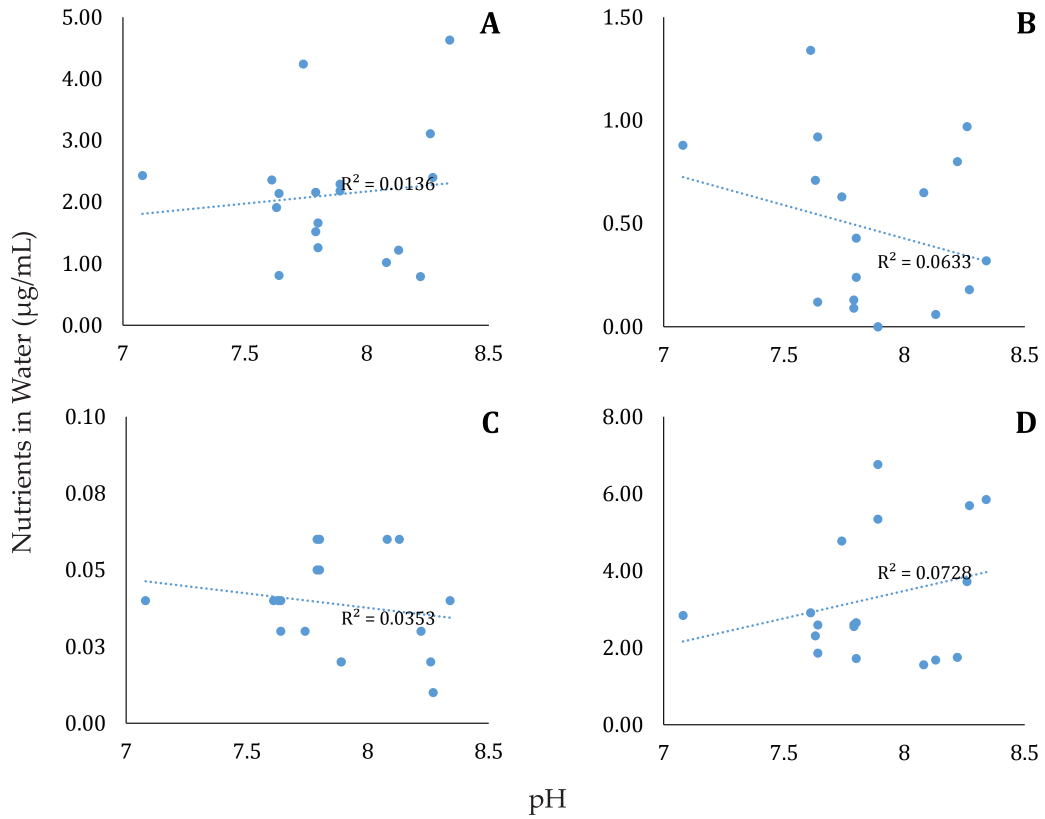


Figure 3.18. Correlation of ammonia (A), nitrate (B), nitrite (C), and TKN (D) levels in water and pH.

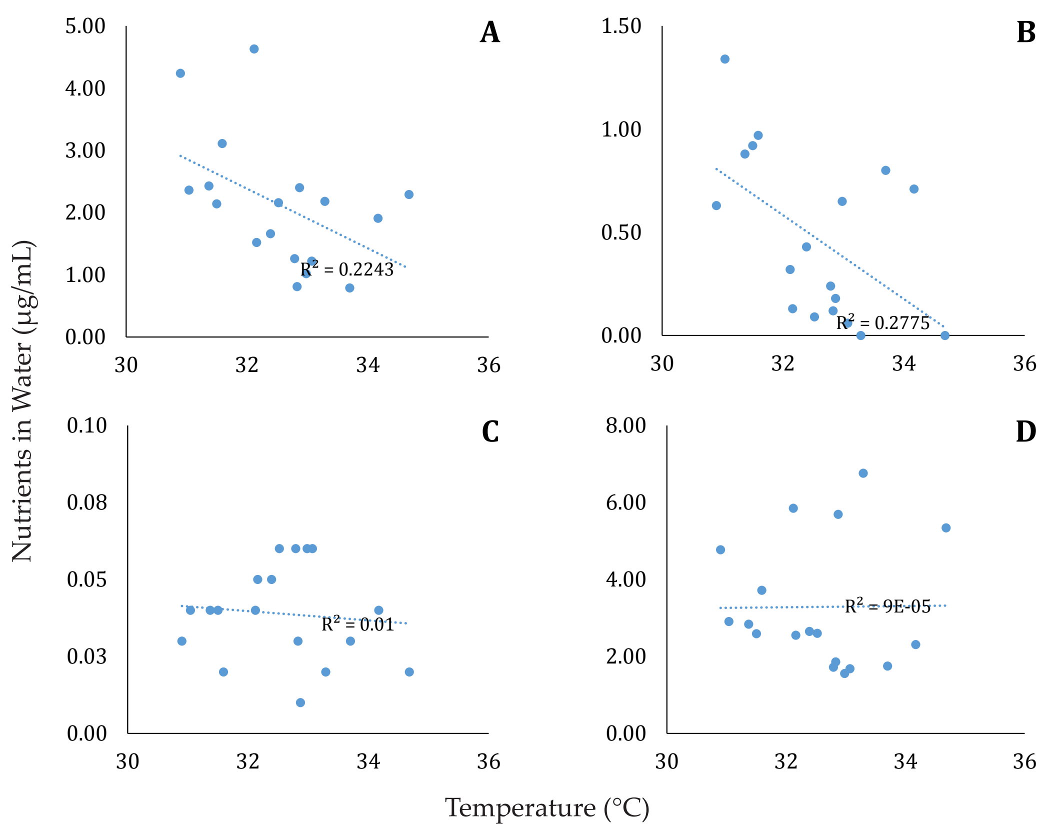


Figure 3.19. Correlation of ammonia (A), nitrate (B), nitrite (C), and TKN (D) levels in water and temperature.

Discussion

Nutrient Levels in Water

Results showed that ammonia levels in water samples were higher compared to other nutrient species. This is apparently because ammonia (compared to other nutrients) accumulates easily in aquatic systems due to deposition, it is the principal metabolic waste product of fish (Floyd *et al*, 2015; Lucas & Southgate, 2012). Decomposition of uneaten feed or dead algae and aquatic plants, which were evident in most of the sites present in the study, further augment ammonia in the pond (Durborow *et al*, 1992; Floyd *et al*, 2015). Nitrite, on the other hand, had the least concentration since this does not accumulate in pond water because of its fast turnover rate, meaning it is easily converted to the least toxic nitrate (Gruber, 2008). Nitrate levels were found intermediate with ammonia and nitrite possibly because nitrate levels are a) dependent on the ammonia levels in the water (nitrification) and b) it has lower turnover rate than nitrite.

SPATIAL NUTRIENT LEVEL

Overall, Eastern Bulacan came out to be highly contaminated since it had the highest levels of nitrogen and phosphorus during dry and wet seasons. Relating the nutrient levels to the aquaculture practices of the farmers, it can be noted that the inputs of the farmers in Eastern Bulacan were not that intense compared to the inputs in the other blocks. Farmers in Eastern Bulacan employed the least variety of pesticides, fertilizers, and feeding materials (see Figures 2.7, 2.8, and 2.11). In fact, a high percentage of farmers did not apply any fertilizer and pesticide at all. Furthermore, the feeding materials used in Eastern Bulacan were mostly natural food, such as *lablab*, *lumot*, and surface plankton, which yield lesser nutrients. However, although their inputs were not intensive, the high percentage of farmers in Eastern Bulacan by-passed important pond preparation activities, such as drying of pond, soil scraping, and water flushing. Consequently, nutrients from residual wastes tended to accumulate in the pond. The location of Eastern Bulacan, being adjacent to Metro Manila, could also be a factor to the increase in nutrient levels. The same may be said of Cavite where nutrient level was second to that of Eastern Bulacan.

High nutrient-producing fertilizer and feeds, such as urea, low value feed and commercial feeds were applied in the aquafarms of Northern Bataan, Southern Bataan, and Pampanga which had lower nutrient levels because the high percentage of the respondents in the said blocks performed important pond preparation activities preventing the accumulation of nutrients.

Furthermore, the difference between the nutrient levels in the water source and fishponds was mostly insignificant. This means that the nutrients in the pond did not significantly contribute or affect the nutrient content of the water source or the river tributaries. The same is true in the case of water source contribution to fishponds.

SEASONAL NUTRIENT LEVEL

Ammonia was highest in May 2014 and lowest in February 2015. These results did not coincide with the normal seasonal variation i.e., it should be lower during summer or dry season (like that of May 2014), possibly because of the assimilation of ammonia by plankton and other aquatic plants (Suter, 2012; Durborow *et al*, 1992). Moreover, higher water temperature would hasten nitrification, or the conversion of ammonia to nitrate, resulting in a lesser available ammonia in the water (Hargreaves & Tucker, 2004). However, these information are from researches performed in temperate countries where differences in temperature are higher than in a tropical country, like the Philippines. The metabolic rate of the culture species in relation to temperature, on the other hand, can be a factor influencing the ammonia levels in water. Increasing temperature during dry season, including the month of May, results in an increase in ammonia production due to increased metabolic rates and a switch to greater protein utilization (Chew *et al*, 2006).

Nitrite seasonal variation, on the other hand, was the similar to the results obtained by Manikannan *et al* (2011) in his study in Indian bodies of water where maximum nitrites were observed during October to December, and minimum during January to March. This can be attributed to the difference in temperature of the sampling months. Temperature range during November was found intermediate as it is a transition period from the wet and dry season of the country. This concurs with the finding of Hargreaves and Tucker (2004) that nitrite levels peak at intermediate temperature when maximum nitrification rates are favored. February, on the other hand, is considered the coldest month in the country which might cause nitrification rates to be lower resulting in lower nitrite production.

Nitrate seasonal variation is contrary to that of ammonia as discussed earlier. Technically, since the formation of nitrate involves a series of reactions, the seasonal variation should be similar to that of ammonia. However, this does not imply a non-conversion of ammonia to nitrate during that time. Possibly, ammonia production due to the increased metabolic rate (as a result of augmented temperature) exceeded the ammonia loss through nitrification and assimilation by algae in May 2014. Nitrate produced during nitrification is also taken up readily by aquatic plants (Chow, 2012) due to the increased temperature and sunlight during May, the warmest month of the year. February, on the other hand, exhibits its high levels of nitrate because it is the coldest month and nitrate assimilation by plants might have been suppressed.

In the case of phosphorus, the seasonal change is relatively insignificant. This might be because phosphorus rapidly disappears from water upon assimilation by phytoplankton, macrophytes and bacteria, as well as adsorption by sediments (Boyd, 1971). Comparing the sampling months, however, phosphorus spiked in May 2014. This is probably because a process of leaching from the sediments to the water column took place (Kutty, 1987) as supported by the high correlation value obtained between sediments and water during that time.

Nutrient Profile in Fishpond Sediments

Results showed that the ammonia, nitrite, nitrate, and phosphorus contents of the sediments were several folds higher than their concentration in water. This is apparently due to nitrogen and phosphorus from fertilizers (i.e., chicken manure and urea) and uneaten feeds deposited in the sediments. Moreover, it was observed that the texture of sediments is more like sludge indicating a high content of organic matter (Avnimelech and Ritvo, 2003). It should be noted that pond owners did not practice scraping of sediments during pond preparation. Apart from this, metabolic wastes from the culture species as well as wastes upstream, entering the pond through runoffs, may have been deposited into the sediments.

Between fishponds and coastal areas, the former had higher concentrations of nitrogen and phosphorus than the fish pens and shellfish growing areas. This result is expected since fishponds, unlike fish pens and shellfish growing areas, are closed or stagnant systems. As such, they tend to accumulate nutrients in the water and sediments. Furthermore, pollution indicators are more concentrated in fish ponds because volumes of water in these are smaller than in fish pen and shellfish growing areas.

Pond Cluster

Most sites under the same cluster were adjacent to each other. As such, they had similarities in environmental, climatic and anthropogenic conditions that could affect the nutrient levels in the ponds. Moreover, these sites also probably obtained their water from the same source or river system.

However, not all sites were clustered based on distance among them. The clustering may also be attributed to the diverse activities performed by the farmers in their respective ponds. The results of cluster analyses were correlated to the aquaculture activities of farmers. Cluster analyses results during February 2015 (Figure 3.13d) were only compared since the survey was conducted during that time. The inputs in May, September, and November 2014 were most probably different from that of February 2015.

No trend was apparent from the comparison of the cluster analyses results regarding the fertilizer and pesticide/disinfectant inputs of the farmers. This is most probably because sampling was done during the rearing period, and so the nutrients from the fertilizers and pesticides/disinfectants had already been used up or washed away. However, feeding inputs were somehow correlated with the cluster results of some sites as these were administered into the pond when the sampling was conducted. In Cavite, for example, all sites under cluster 1 used bread and *lumot* as feeding material, while all sites under cluster 2 uses *lumot* only. In Northern Bataan, on the other hand, all sites in cluster 2 used both low value feed and commercial feed combined with natural food (*lumot* or *lablab*), whereas a site in cluster 1 used commercial feed only. Four out of six sites under cluster 2 of Southern Bataan used the same feeding materials, namely *lumot* and low value feed. In Western Bulacan, all sites under cluster 4 used three types of feeding input which included both *lablab* and *lumot*, and differed only in the third feeding material.

The nutrient profile of other sites, where the same feeding material was used but belonged to different clusters, may have been affected by a combination of factors such as climatic, anthropogenic and other related aspect that might influence nutrient content. For instance, two sites in Pampanga used similar feeds, namely *lablab*, *lumot*, and low value feed. These sites, however, were clustered differently, most probably because the other site had a denser population than the other. Consequently, there was more waste, hence higher nutrient levels. The same could be true for sites belonging to the same cluster but given different aquaculture practice.

Clustering identifies sites that are similar in terms of nutrient levels. It is useful in reducing the number of sampling sites in each block for monitoring.

Nutrient Loading

Significantly higher levels of ammonia and TKN observed during the flooding suggest that the water entering the pond contain higher levels of both nutrients than the water initially present in the pond. Since ammonia is known to be the by-product of bacterial decomposition of organic matter such as feces and dead planktons (Auburn University, 2008), higher levels of this nutrient implies higher organic load, which in this case denotes that the water source has higher organic load. The accumulation of ammonia in the pond is toxic to fish, causing gill damage, lethargy, and eventually death (Hargreaves & Tucker, 2004). Fortunately, phytoplanktons use it as a direct nutrient source and/or is broken down by nitrifying bacteria into nitrite and then into nitrate, which is less toxic. This means that the amount of ammonia in the pond positively affects the concentration of nitrite/nitrate in the medium. As can be observed from the results, nitrate and nitrite levels are significantly higher during the draining compared to the flooding. This indicates that ammonia initially present in the pond and from the water source have been transformed into nitrite/nitrate prior to draining.

Significantly higher levels of dissolved oxygen were recorded during the flooding implying that water coming in to the pond is more aerated than water going out of it. This is because water sources are continuously flowing, which means there is continuous mixing of the water column while water during draining has been stocked in the pond for a while, oxygen used up by the reared aquaculture commodity as well as by the algae, phytoplankton, and bacteria during metabolic processes.

The amount of nitrite was found positively correlated with salinity ($R^2 = 0.5293$). This may be attributed to the inhibitory property of salt to nitrite oxidizers, blocking the conversion of nitrite species into nitrate. It is known that the oxidation of ammonia into nitrate cannot be carried out completely by a single species of bacteria, rather, it is a sequential activity accomplished by two groups of bacteria – ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (Madigan *et al*, 2012). The population of nitrifying bacteria and the nitrification process are affected by environmental factors such as temperature, dissolved oxygen concentration, pH, available substrate, product inhibition, and inhibitory compounds (Hellinga *et al*, 1998; Moussa *et al*, 2006). In a study by Dincer and Kargi (1999) and Vredenbregt *et al* (1997), it was concluded that the accumulation of nitrite at higher salt concentrations imply that nitrite oxidizers are more affected than ammonia oxidizers at increased salinity. As for the pH, Hellinga *et al* (1998) reported that at a low pH, nitrite oxidizers are predicted to grow faster and in turn increasing the amount of nitrate in the medium. However, results of the study conducted showed no correlation between pH and the amount of nutrients in the ponds collected. This may be attributed to the fluctuating pH in the pond due to the continuous water exchange activities. Temperature, on the other hand, although weak, was negatively correlated ($R^2 = 0.2775$) with nitrate. In the same study conducted by Hellinga *et al* (1998), it was found out that nitrite oxidizers grow faster at normal temperatures (5-20°C) resulting in complete oxidation of ammonium into nitrate. However, at elevated temperatures, ammonia oxidizers grow faster than nitrite oxidizers implying that nitrite, instead of nitrate, is produced more. Lastly, dissolved oxygen increases the nitrification process, which suggests that as the concentration of dissolved oxygen increases, nitrate should also increase (Tan *et al*, 2013). However, results of the study show no correlation between nitrate and/or nitrite to dissolved oxygen but was positively correlated ($R^2 = 0.3336$) with the level of ammonia. This may be due to the already high amounts of ammonia present in the water entering the pond. And as was discussed earlier, water coming in to the pond has higher dissolved oxygen due to the consistent mixing of the water column.

Conclusions

Overall, the levels of nutrients varied widely among the water sources, coastal areas, and fishponds around Manila Bay. Ammonia levels were highest followed by phosphorus, nitrate, and nitrite. Comparing the different blocks, aquafarms in Eastern Bulacan were more contaminated evidenced by higher levels of nitrogen and phosphorus which may have resulted from farmers' bypassing important pond preparation activities. Nutrients had varying seasonal trends possibly due to the variable reactions of the nutrient species under changing climatic conditions. Furthermore, levels of ammonia, nitrite, nitrate, and phosphorus in the sediments were found several folds higher than that in the water column. Correlation analyses results of nutrients in water and sediments exhibited that only phosphorus has a good correlation. Ammonia, on the other hand, has a weak correlation, while, nitrites and nitrates have no correlation at all. These results suggest that sediments maybe a contributor of phosphorus and ammonia in water but not of nitrite and nitrate. Ammonia and TKN were significantly higher during the flooding, which suggests that water coming in to the pond already contain high levels of such nutrients. In contrast, significantly higher levels of nitrite and ntirate were observed during the draining suggesting that ammonia in the medium has been transformed into less toxic forms - nitrite and nitrate - by nitrifying bacteria.

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CHAPTER 4

Heavy Metal Contamination in Water and Fishery Resources in Manila Bay Aquaculture Farms

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Abstract

Heavy metals (HM) are high-atomic weight elements hazardous at very low concentrations. Despite the health risk HM contamination brings, studies conducted were only confined to the offshore and marine portions of the bay. Hence, this study was conducted to establish baseline information and compare the spatial and seasonal distribution of heavy metal contamination in water and fishery resources in aquaculture farms and coastal areas in Manila Bay. Water and major aquaculture commodities were collected in November 2014, February 2015 and April 2015 and were analyzed for lead (Pb), cadmium (Cd), and mercury (Hg) using the following methods: (1) Graphite Furnace Atomizer-Atomic Absorption Spectrophotometry (GFA-AAS) for Pb and Cd and (2) Mercury Vaporizing Unit-AAS (MVU-AAS) for Hg. Cd (Bataan, Cavite, Bulacan, and Pampanga) and Hg (Cavite and Pampanga) in water were found significantly higher during the dry season ($p<0.05$). In contrast, Pb in water was relatively higher during the wet season but levels were not significantly different with those in the dry. Several sites in Bataan, Bulacan, and Cavite, exceeded DENR regulatory limits for Pb and Hg in water by 1.35 to 1.8%. As for the fishery commodities, 2/12 milkfish samples and 1/9 exceeded regulatory limit for Pb in finfish (0.3 mg/kg) by 1.03 to 3.57% while 1/12 milkfish samples and 1/13 oyster samples exceeded the limit for Hg in bivalves (0.5 mg/kg) by 0.45 to 0.75%.

Keywords: heavy metals, aquaculture, Manila Bay

Introduction

Heavy metals (HM) are high-atomic weight elements hazardous at very low concentrations. These primarily include cadmium (Cd), lead (Pb), and mercury (Hg) (Tiimub & DzifaAfua, 2013). Heavy metals can enter the aquatic ecosystem from different natural and anthropogenic sources such as domestic wastewater, pesticides and inorganic fertilizers, geologic weathering as well as shipping and harbor activities (Krishna *et al*, 2014). Heavy metal pollution creates an immense threat owing to their persistence in the aquatic environment and accumulation in the organisms. Their entering the food chain increases public health risks (Su *et al*, 2009).

Fish absorbs metals through ingestion of contaminated water, sediments, suspended solids, and prey organisms. Heavy metals have also been observed to undergo bioaccumulation and bio-magnification in the tissue of aquatic organisms (Ebrahimi and Taherianfard, 2011). The rate of bioaccumulation of HM in aquatic organisms depends on several factors like fish species, ability to digest the metals, feeding habits, age of fish, lipid content in the tissue, and the concentration of such metal in the area (Eneji, 2011; Khayatzadeh and Abbasi, 2010; Su *et al*, 2009). Heavy metals accumulate mainly in kidneys, adrenal glands, liver, lungs or gills in fish, hair and skin (Martin & Griswold, 2009).

Heavy metals, in trace amounts, have different roles for aquatic organisms' proper physiological functions (Khayatzadeh and Abbasi, 2010). However, water bodies with a high amount of these negatively affect the aquatic organisms. Heavy metal pollution primarily inhibits the growth of fish and other aquatic organisms. It also reduces the survival of fish larvae, which may considerably reduce the fish population or result in their extinction.

Furthermore, contaminated sediments may cause death in benthic organisms which implies reduced food availability for larger animals such as fish. Behavioural anomalies may also develop such as impaired locomotors performance resulting in increased susceptibility to predators or structural damages, mainly vertebral deformities. In humans, exposure to copper, for example, inhibit skeletal ossification, while lead cause scoliosis (Khayatzadeh and Abbasi, 2010; Sharma *et al*, 2014).

Human consumption of contaminated fish and other aquatic organisms may result in transfer and accumulation of metals in the human body posing a threat on consumer health and safety. Accumulation of high amounts of heavy metals may cause high blood pressure, cancerous changes, and damage to kidneys, liver, and brain. In some cases they may also lead to mental disorders and loss of brain function (Martin & Griswold, 2009).

Accumulation of cadmium, for example, affects the liver, kidney, lung, bones, placenta, brain, and the central nervous system. Other damages such as reproductive and development toxicity, hepatic, haematological, and immunological effects in character may also result (Morais *et al*, 2012). Moreover, chronic lead intoxication in adults can cause anaemia, cancer, reproductive harm in males, and hormonal imbalance of vitamin D metabolism (WHO, 1995). Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and even the developing fetus (Morais *et al*, 2012).

Manila Bay is known as one of the premier fishing grounds in the country making fishing as the primary source of livelihood around the bay. Surrounding coastal communities are benefited by the bountiful aquatic resources. Moreover, the once varied ecological habitats like sea grasses, corals, and mangroves in the bay area serve as the breeding grounds of fish life and also serve indirect ecological functions (e.g. shoreline stabilization, storm barriers, and carbon storage) (PEMSEA, 2006).

Aquaculture in the provinces around Manila Bay was first documented in 1932 with the introduction of oyster farming in Binakayan, Cavite (Yap, 1999), one of the most productive aquaculture areas in the country. Manila Bay accounts for an average of almost 50% of the national production of tiger prawn and oyster (PEMSEA, 2006). As reported by BAS (2012), the volume of aquaculture production in Manila bay provinces (Bataan, Pampanga, Bulacan, Cavite, and NCR) in 2010 has reached 335,985.80 MT, 3% higher than 2009 production of 314,110.74 MT. However, all these resources, which are quite beneficial to all Filipinos especially those living near the bay, are constantly under threat. Population expansion, rapid urbanization, uncontrolled coast, and basin development and mismanagement of resources are apparent in many areas around the bay. Products of modernization are considered major threats to the bay's sustainability and productivity because these products result in water pollution, including HM pollution.

Several studies have been conducted to assess the contamination of HM in Manila bay. According to Su *et al* (2009), HM particularly total chromium, total lead and total cadmium were evident in the bay waters, fish and macroinvertebrates. The study of Velazquez *et al* (2010) showed that the total dissolved copper and cadmium were labile while total dissolved zinc was organically bound. Elevated levels of these metals near point sources suggest anthropogenic inputs in the bay. Results of the risk assessment conducted by PEMSEA and MBEMP TWG-RRA (2004) showed that the concentrations of HM in water around river mouths were higher than in the water inside the bay, suggesting that the contribution of land-based human activities is a major source of pollution. Similar results were observed in the case of HM in the sediments. In the case of HM concentration in the organisms' tissues, scad (0.067 mg/kg), sardines species (1.39 mg/kg), and crevalle (0.296 mg/kg) are among the pelagic fish that appear to have a high bioaccumulation of metals. HM in shellfish (mussels and oysters) from Manila Bay, specifically from the mouth of the Pasig River, may pose a relatively significant risk to human health since concentrations showed high RQ values of 3.8 to 7 for lead (Pb).

These studies were confined to the offshore and marine portions of the bay only. It was only lately that Dr. Marlo Mendoza (unpublished) obtained data concerning HM concentrations in freshwater and brackishwater aquaculture species in areas surrounding Manila Bay. Results of monitoring conducted from January to December 2008 showed that heavy metals, such as As, Cd, Cu, Mn, Ni, Zn, Pb, Cr, and Hg were present in milkfish, tilapia, prawns, green mussel, clam "*paros*", and oysters collected from the area. The levels ranged from non-detectable (ND) to several folds higher than the standard limits set by BFAR (Fisheries Administrative Order 210 Series of 2001) and US Environmental Protection Agency, EPA (2000). Based on standards set by Codex Alimentarius Commission (CAC 206), European Union EC1881-206 and US FDA Centre for Food Safety and Applied Nutrition (CFSAN), however, these are acceptable. These results prompted BFAR to conduct a verification study, which also aims to generate baseline information on the possible contamination of heavy metals in fish and fishery products from Manila bay and adjacent aquaculture areas.

This study aims to establish baseline information and compare the spatial and seasonal distribution of heavy metal contamination in water and fishery resources in aquaculture farms and coastal areas in Manila Bay.

Methodology

Sampling Sites and Collection of Samples

Blocking strategy and sampling sites used in Chapter 2 was employed in this study. Forty-seven pre-identified aquaculture farms were sampled each season –November 2014 for the wet season and February and April 2015 for the dry season. River tributaries of the aquaculture farms were also sampled for comparison.

WATER SAMPLES

Water samples about one liter (1L) were collected in composite for heavy metal analyses and were placed in appropriate container, preserved with acid, labeled and transported to the laboratory in a temperature-controlled cooler. Storage and preservation of collected water samples were done according to US-Environmental Protection Agency (EPA), Sampling and Analysis of Waters, wastewaters soils and Wastes (2009).

Eighty one (81) water samples (35 pond, 35 water source, 2 pen, and 9 coastal area) were collected during the wet season (November 2014), while 82 water samples (35 pond, 35 water source, 3 pen, and 9 coastal area) were collected during the dry season (February and April 2015).

FISH SAMPLES

Six kinds of farmed fishery resources were collected from the aquaculture farms for the study – tilapia, milkfish, shrimp, crab, mussel, and oyster. However, sample species and number of samples collected per site were variable, as sample collection is solely dependent on the availability of samples.

Fifty six (56) samples of aquaculture commodities (12 milkfish, 9 tilapia, 7 crab, 5 shrimp, 13 oyster, and 10 mussel) were collected throughout the sampling period. Freshly harvested fish and shellfish of at least one kilogram (1kg) per species were put separately in an appropriately-labeled re-sealable polyethylene bags and placed in a temperature-controlled cooler kept at 0 to 4°C for transport at laboratory for analyses.

Fish samples were cleaned and dissected, composited and homogenized to make up at least 200 grams. Homogenized samples were stored in re-sealable polyethylene bags and placed in the ultralow freezer (-80°C) prior to analysis.

Method of Heavy Metal Analyses

Water samples were analyzed for total mercury (Hg), lead (Pb), cadmium (Cd) and hexavalent chromium (Cr^{+6}). As for the fish and fishery resources, all the above-mentioned HM analyses were conducted except for the hexavalent chromium. The method used for both water and fish was the closed vessel-microwave-assisted-acid digestion adopting the method of the manufacturer, followed by analytical determinations of metals using the following instruments: (1) Graphite Furnace Atomizer-Atomic Absorption Spectrophotometer (GFA-AAS) for Pb and Cd and (2) Mercury Vaporizing Unit AAS (MVU-AAS) for Hg. The reference methods used for the analysis of heavy metals in water were US-EPA 3015a, 2007(Cd, Hg, and Pb) and US-EPA 7196a 1992, (hexavalent chromium) and for fish samples, modified AOAC 2013.06 was used.

To ensure the accuracy and reliability of analytical results, the laboratory performed quality assurance and quality control schemes (e.g. mid-standards, recovery of fortified samples, and method/reagent blanks) and other performance characteristics for method validation (e.g. repeatability, reproducibility and accuracy using Certified Reference Materials).

Statistical Analyses

The levels of HM in the aquaculture farms and water sources during the wet and dry seasons were compared using t-test, as well as its seasonal variations. On the other hand, HM in the fishery commodities collected during the wet and dry seasons were compared using Analysis of Variance (ANOVA). All statistical analyses were carried out using SPSS version 20.

Results

Levels of Heavy Metals in Water

Levels of heavy metals in the aquaculture farms varied among the different blocks. The following are the ranges of the levels listed from the most abundant to the least: lead (ND to 0.0759 mg/L); cadmium (ND - 0.0028 mg/L); mercury (ND to 0.0065 mg/L); and hexavalent chromium (ND).

LEAD

Table 4.1 summarizes the values of lead concentration obtained for the both sampling periods, while its spatial distribution in aquaculture farms are presented in Figure 4.1.

Lead levels in the aquaculture farms for the both sampling season were found to be variable, with values ranging from ND to 0.0759 mg/L. The highest concentration was observed in a pond water sample from Southern Bataan (November 2014).

During wet season, levels of lead ranged from ND to 0.0759 mg/L, with three sampling sites (one in Northern Bataan and two in Southern Bataan) exceeding DENR regulatory limit of 0.05 mg/L, and were found relatively higher compared to the dry season, with levels of Lead ranging from ND to 0.0251 mg/L and all sites conforming with the limit. However, there was no significant difference in the levels of lead in the different blocks in both sampling periods ($p > 0.05$, refer to Table 4.2). In addition, statistical analysis showed that lead concentration in aquaculture farm (pond, pen and coastal waters) and their respective water sources had no significant difference ($p>0.05$), as shown

Table 4.1. Lead concentrations in aquaculture farms for wet (November 2014) and dry season (February and April 2015.

BLOCK	Mean Lead Concentration (ppm, mg/L)	Lead Concentration Range (ppm, mg/L)
Block 1 (Eastern Bulacan)	0.0143	0.0022 – 0.0251
Block 2 (Western Bulacan)	0.0085	0.0014 – 0.0185
Block 3 (Northern Bataan)	0.0129	ND – 0.0502
Block 4 (Southern Bataan)	0.0232	0.0035 – 0.0759
Block 5 (Cavite)	0.0089	ND – 0.0178
Block 7 (Pampanga)	0.0078	ND – 0.0308

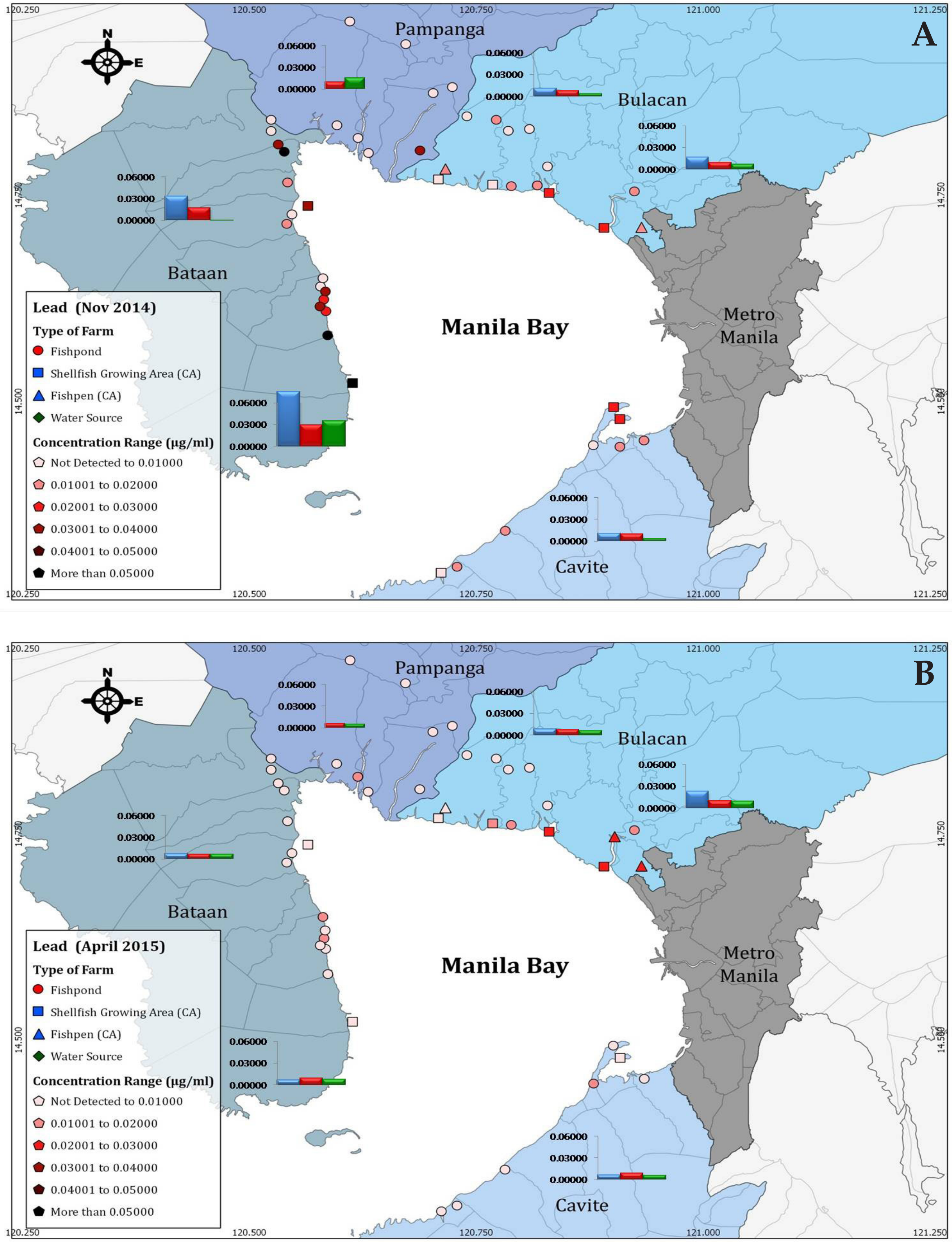


Figure 4.1. Levels of lead in coastal areas/fish pens, ponds, and water sourcesin November 2014 (A) and April 2015 (B).

Table 4.2. Seasonal comparison of heavy metal concentration in aquaculture farms.

Sampling Sites	Cd, mg/L		Hg, mg/L		Pb, mg/L	
	Wet	Dry	Wet	Dry	Wet	Dry
B1	0.00016	0.00020	0.00010	0.00100	0.0092	0.00844
	P = 0.681		P = 0.298		P = 0.863	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B2	0.00016	0.00076	0.0007	0.0007	0.0075	0.0077
	P = 0.098		P = 0.172		P = 0.927	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B3	0.00007	0.00027	0.0004	0.0006	0.01746	0.0061
	P = 0.004		P = 0.529		P = 0.249	
Significance, $\alpha_{0.05}$	Significant		Not Significant		Not Significant	
B4	0.00042	0.00088	0.0012	0.0005	0.02953	0.0097
	P = 0.009		P = 0.342		P = 0.069	
Significance, $\alpha_{0.05}$	Significant		Not Significant		Not Significant	
B5	0.0002	0.0012	0.00004	0.00374	0.0098	0.0088
	P = 0.012		P = 0.000		P = 0.773	
Significance, $\alpha_{0.05}$	Significant		Significant		Not Significant	
B7	0.0003	1.4934	0.0001	0.0012	0.0096	0.006
	P = 0.016		P = 0.000		P = 0.394	
Significance, $\alpha_{0.05}$	Significant		Significant		Not Significant	

in Table 4.3. This indicates that neither the aquaculture farm nor the water source affected the level of lead in the other.

CADMIUM

Reflected in Figure 4.2 are the levels of cadmium in the different sampling sites during the wet and the dry seasons. On the other hand, Table 4.4 shows the cadmium concentrations obtained throughout the sampling period.

Cadmium levels during the dry season (range of ND - 0.0028 mg/L) were significantly higher ($p<0.05$) compared to the wet season were comparable with all the blocks. All sam-(range of ND - 0.0023 mg/L). Cadmium concentrations in Cavite and Pampanga were significantly higher ($p<0.01$) than in Western Bulacan, Eastern Bulacan, and Northern Bataan. Those in Southern Bataan, on the other hand, pling sites passed the DENR regulatory limit of 0.01 mg/L for cadmium in water.

T-test results showed that cadmium levels in the aquaculture farms in Northern Bataan with an average cadmium concentration of 0.00017 mg/L, were significantly higher ($p<0.05$) than the water source (mean = 0.00015 μg/ml)during wet season, as indicated in Table 4.3.

MERCURY

Levels of mercury in the aquaculture farms during the wet and dry seasons are presented in Figure 4.3. On the other hand, Table 4.5 shows the mercury concentrations obtained throughout the sampling period.

Table 4.3. Comparison of concentrations of heavy metal in pond collected from different sites of Manila Bay during wet and dry seasons.

WET

Sampling Sites	Cd, mg/L		Hg, mg/L		Pb, mg/L	
	Pond	River	Pond	River	Pond	River
B1	0.0002	0.0002	0.0012	0.0011	0.0101	0.0096
	P = 0.802		P = 0.215		P = 0.652	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B2	0.0008	0.0011	N.D.	N.D.	0.0077	0.0033
	P = 0.0574		No variance		P = 0.151	
Significance, $\alpha_{0.05}$	Not Significant				Not Significant	
B3	0.0001	0.00001	0.0003	0.0002	0.0175	0.0004
	P = 0.018		P = 0.449		P = 0.044	
Significance, $\alpha_{0.05}$	Significant		Not Significant		Significant	
B4	0.0004	0.0003	0.0012	0.0002	0.0295	0.0352
	P = 0.578		P = 0.142		P = 0.739	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B5	0.0002	0.0002	0.00004	0.00012	0.0098	0.0036
	P = 0.423		P = 0.545		P = 0.125	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B7	0.0003	0.0009	0.0001	0.00006	0.0096	0.00155
	P = 0.105		P = 0.337		P = 0.224	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	

DRY

Sampling Sites	Cd, mg/L		Hg, mg/L		Pb, mg/L	
	Pond	River	Pond	River	Pond	River
B1	0.0002	0.0002	0.0012	0.0011	0.0101	0.0096
	P = 0.539		P = 0.619		P = 0.915	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B2	0.0002	0.00005	0.0007	0.0002	0.0075	0.0064
	P = 0.487		P = 0.667		P = 0.579	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B3	0.0003	0.0003	0.0006	0.0004	0.0064	0.0064
	P = 0.562		P = 0.200		P = 0.835	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B4	0.0009	0.0009	0.0005	0.00003	0.0098	0.0079
	P = 0.906		P = 0.378		P = 0.388	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B5	0.0012	0.0011	0.0037	0.0031	0.0088	0.0046
	P = 0.857		P = 0.164		P = 0.341	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B7	0.0015	0.0012	0.0012	0.00043	0.006	0.0051
	P = 0.643		P = 0.006		P = 0.565	
Significance, $\alpha_{0.05}$	Not Significant		Significant		Not Significant	

Table 4.4. Cadmium concentrations in aquaculture farms for wet (November 2014) and dry season (February and April 2015.

BLOCK	Mean Cadmium Concentration (ppm, mg/L)	Cadmium Concentration Range (ppm, mg/L)
Block 1 (Eastern Bulacan)	0.0003	0.00004 – 0.00050
Block 2 (Western Bulacan)	0.0007	ND – 0.0027
Block 3 (Northern Bataan)	0.0002	ND – 0.0004
Block 4 (Southern Bataan)	0.0006	0.0002 – 0.0011
Block 5 (Cavite)	0.0008	0.0001 – 0.0019
Block 7 (Pampanga)	0.0009	ND – 0.0028

Mercury levels during the dry season (range of ND - 0.0065 mg/L) were higher than in the wet season (range of ND - 0.0037 mg/L). Significantly higher mercury levels during the dry season were observed in Cavite and Pampanga ($p<0.05$).

Three out of 46 sampling sites, all noted in Southern Bataan during the wet season exceeded DENR regulatory limit of 0.002 mg/L for mercury in water. For the dry season, 14 out of 47 sites – three from Eastern Bulacan, one in Western Bulacan, Northern Bataan, and Southern Bataan, and eight in Cavite – failed to meet the DENR regulatory limit.

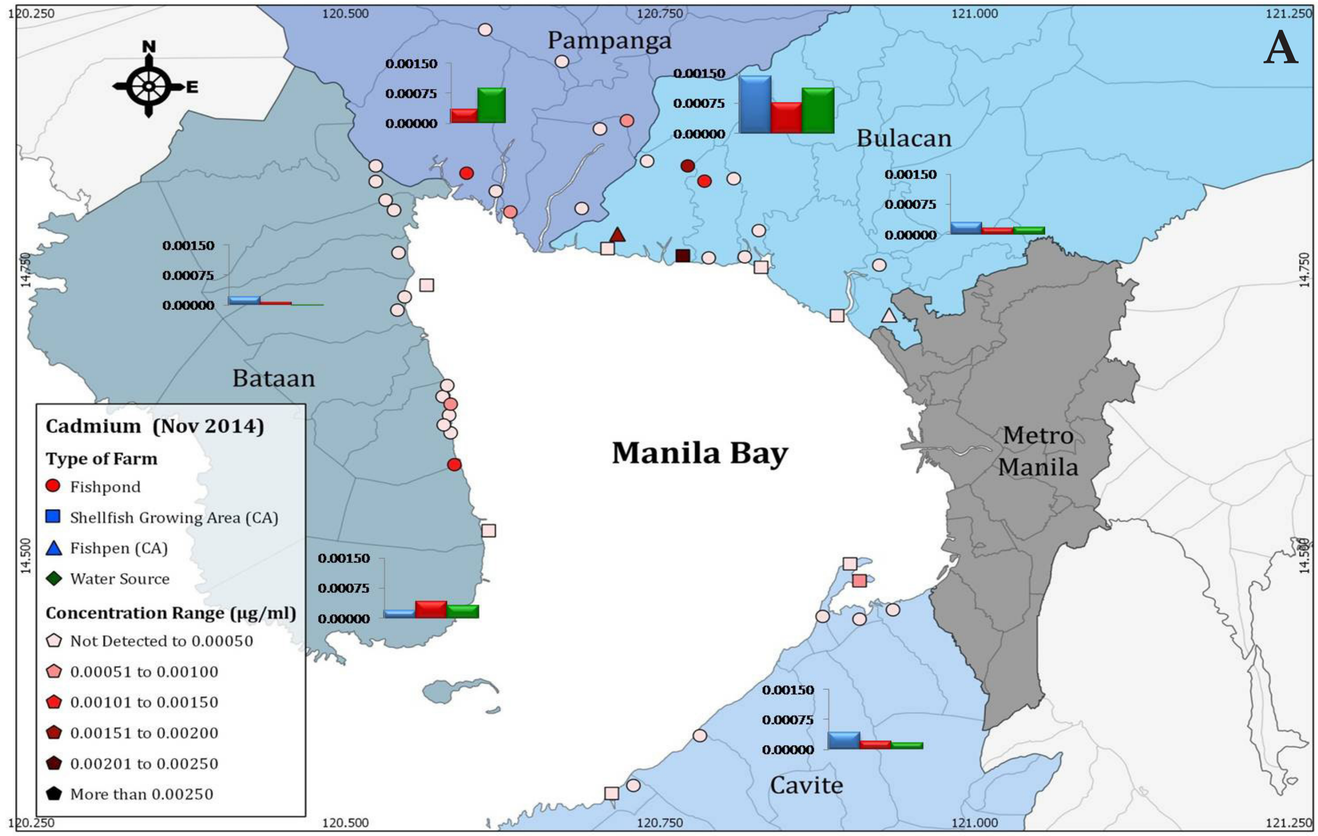
T-test results showed that only Pampanga had significantly higher ($p<0.05$) mercury levels in the aquafarm than its water source, as indicated in Table 4.3.

Levels of Heavy Metals in Fish and Fishery Resources

Heavy metals in fish and fishery resources had no significant difference ($p>0.05$) during the wet (November) and dry (February and April) seasons except for lead in crab (refer to Table 4.6). The following are the ranges and mean values of heavy metals in aquaculture commodities collected arranged in descending order: lead (ND - 1.0723 mg/kg; 0.1463 mg/kg); cadmium (ND - 0.5526 mg/kg; 0.0606 mg/kg); and mercury (ND - 1.1063 mg/kg; 0.0567 mg/kg).

Table 4.5. Mercury concentrations in aquaculture farms for wet (November 2014) and dry season (February and April 2015.

BLOCK	Mean Mercury Concentration (ppm, mg/L)	Mercury Concentration Range (ppm, mg/L)
Block 1 (Eastern Bulacan)	0.0008	ND – 0.0034
Block 2 (Western Bulacan)	0.0004	ND – 0.0022
Block 3 (Northern Bataan)	0.0005	ND – 0.0024
Block 4 (Southern Bataan)	0.0010	ND – 0.0037
Block 5 (Cavite)	0.0022	ND – 0.0065
Block 7 (Pampanga)	0.0006	ND – 0.0014



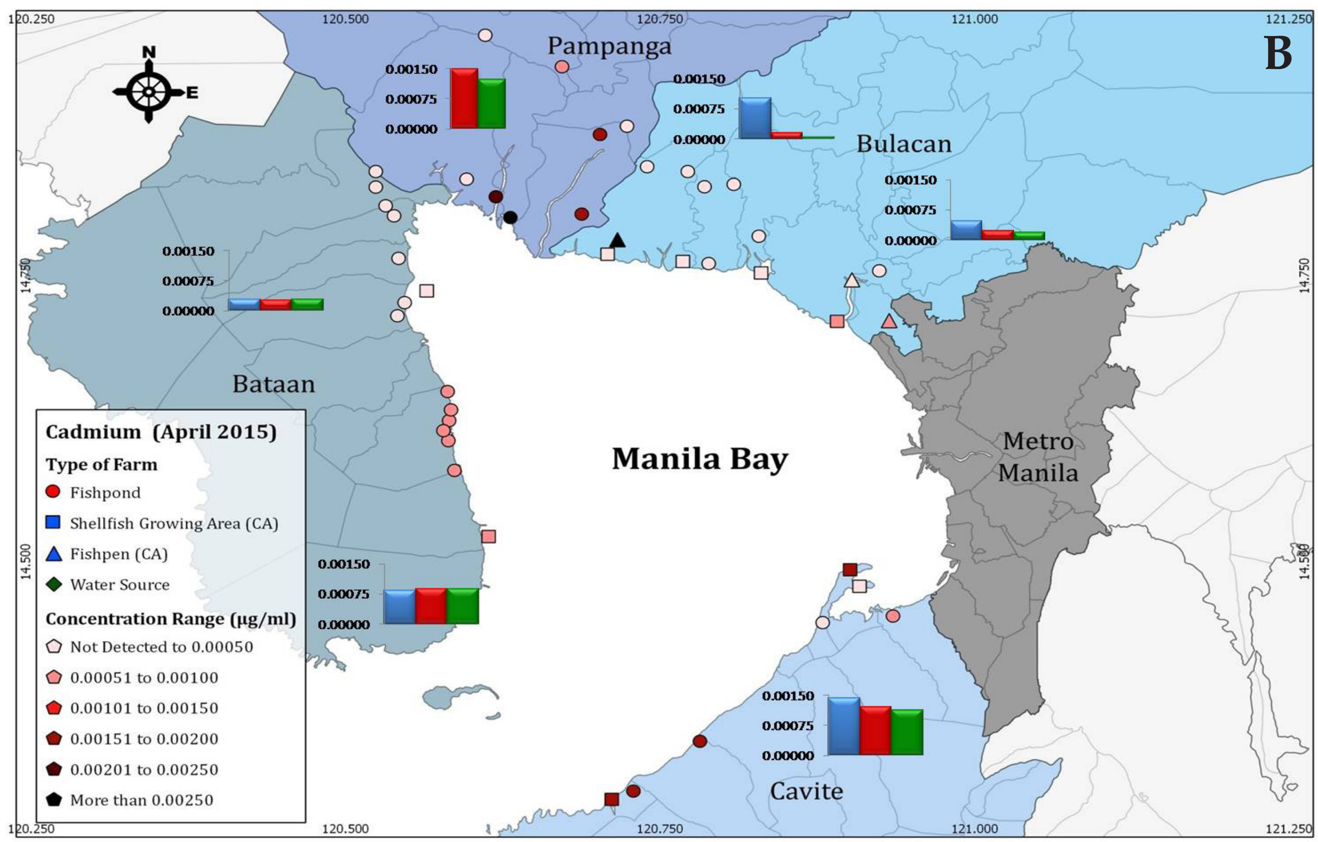


Figure 4.2. Levels of cadmium in coastal areas/fish pens, ponds, and water sourcesin November 2014 (A) and April 2015 (B).

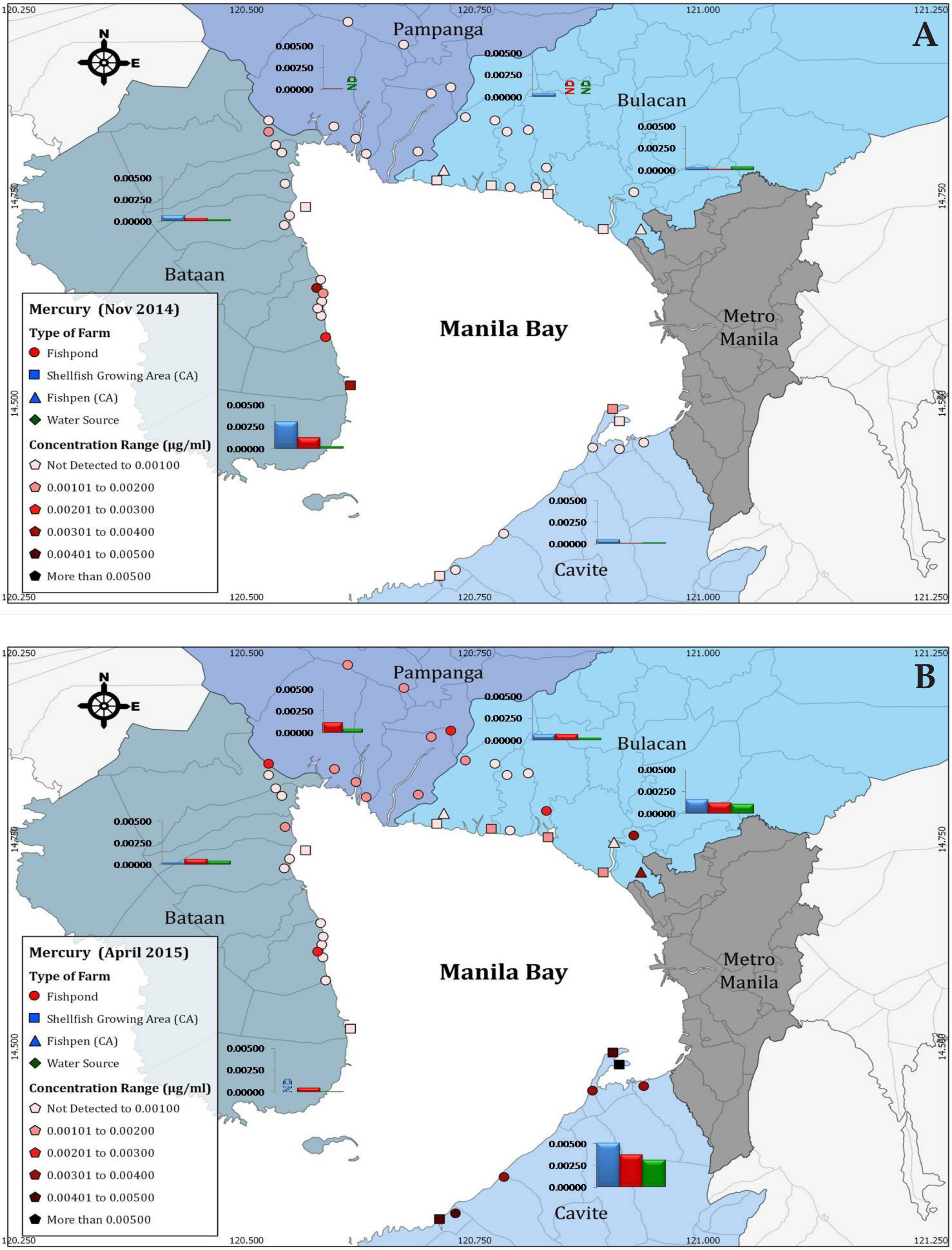


Figure 4.3. Levels of mercury in coastal areas/fish pens, ponds, and water sourcesin November 2014 (A) and April 2015 (B).

Table 4.6. Seasonal comparison of heavy metal concentrations in crab.

SEASON	**HEAVY METAL CONCENTRATIONS (ppm, mg/kg)**		
	Cd	Hg	Pb
Wet	0.0174	0.0122	0.0609
Dry	0.0191	0.0581	0.2640
	P = 0.997	P = 0.570	P = 0.030
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Significant

Table 4.7. Seasonal comparison of heavy metal concentrations in milkfish.

SEASON	**HEAVY METAL CONCENTRATIONS (ppm, mg/kg)**		
	Cd	Hg	Pb
Wet	0.0159	0.1568	0.1986
Dry	0.0105	0.0269	0.1384
	P = 0.068	P = 0.699	P = 0.788
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.8. Seasonal comparison of heavy metal concentrations in mussel.

SEASON	**HEAVY METAL CONCENTRATIONS (ppm, mg/kg)**		
	Cd	Hg	Pb
Wet	0.0269	0.0179	0.1781
Dry	0.0384	0.0844	0.2079
	P = 0.546	P = 0.534	P = 0.761
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.9. Seasonal comparison of heavy metal concentrations in oyster.

SEASON	**HEAVY METAL CONCENTRATIONS (ppm, mg/kg)**		
	Cd	Hg	Pb
Wet	0.0804	0.5590	0.1203
Dry	0.2044	0.2249	0.1515
	P = 0.153	P = 0.449	P = 0.663
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.10. Seasonal comparison of heavy metal concentrations in shrimp.

SEASON	**HEAVY METAL CONCENTRATIONS (ppm, mg/kg)**		
	Cd	Hg	Pb
Wet	0.0158	0.0076	0.0914
Dry	0.0091	0.0091	0.1652
	P = 0.327	P = 0.810	P = 0.654
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.11. Seasonal comparison of heavy metal concentrations in tilapia.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0122	0.1021	0.1242
Dry	0.0121	0.0637	0.2223
	P = 0.978	P = 0.776	P = 0.205
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

LEAD

Results for lead concentration in aquaculture commodities collected are reflected in Figure 4.4. Lead concentrations in samples collected in the wet season ranged from ND to 1.0723 mg/kg, with the highest noted in a milkfish sample from Eastern Bulacan, while dry season lead concentrations ranged from ND to 0.3953 mg/kg, the highest noted in a mussel sample from Cavite.

Lead concentrations in milkfish samples ranged from ND to 1.0723 mg/kg, highest in a sample collected in Eastern Bulacan, while lead levels in tilapia samples ranged from ND to 0.3087 mg/kg, highest in sample from Pampanga. Two out of twelve milkfish samples, collected from Eastern Bulacan (wet season) and Pampanga (dry season), and one out of nine tilapia samples, collected from Pampanga (dry), failed to meet the regulatory limit of 0.3 mg/kg (EC 1881/2006).

Crab samples had lead concentrations ranging from ND to 0.2688 mg/kg, highest in sample collected from Cavite while shrimp samples had lead levels that ranged from ND to 0.3065 mg/kg, highest in sample collected from Pampanga. Lead concentrations in all the crustacean samples were within the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Lead in oyster and mussel samples ranged from ND to 0.3147 mg/kg and 0.0726 to 0.3953 mg/kg, respectively, both maximum values noted in Cavite. All oyster and mussel samples collected had lead concentrations within the regulatory limit of 1.5 mg/kg (EC 1881/2006).

CADMIUM

Cadmium levels in aquaculture commodities collected during the wet and the dry seasons are presented in Figure 4.5. Cadmium concentrations in the dry season (range of 0.0034 - 0.5526 mg/kg) were relatively higher compared to cadmium concentrations in wet season (range of ND - 0.1283 mg/kg). Highest concentrations for both seasons were noted in oyster samples from Western Bulacan.

Cadmium in milkfish samples ranged from ND to 0.0178 mg/kg, the highest concentration noted in a sample collected from Pampanga, while the levels in tilapia ranged from ND to 0.0191 mg/kg, the highest observed in a sample from Northern Bataan. All finfish samples had levels within the regulatory limit of 0.05 mg/kg (EC 1881/2006).

Crab and shrimp samples had cadmium concentrations ranging from 0.0047 to 0.0392 mg/kg and 0.0034 to 0.0158 mg/kg, respectively. The highest levels in both species were noted in samples collected from Pampanga. Cadmium concentrations in crustacean samples were within the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Cadmium levels in oyster samples,

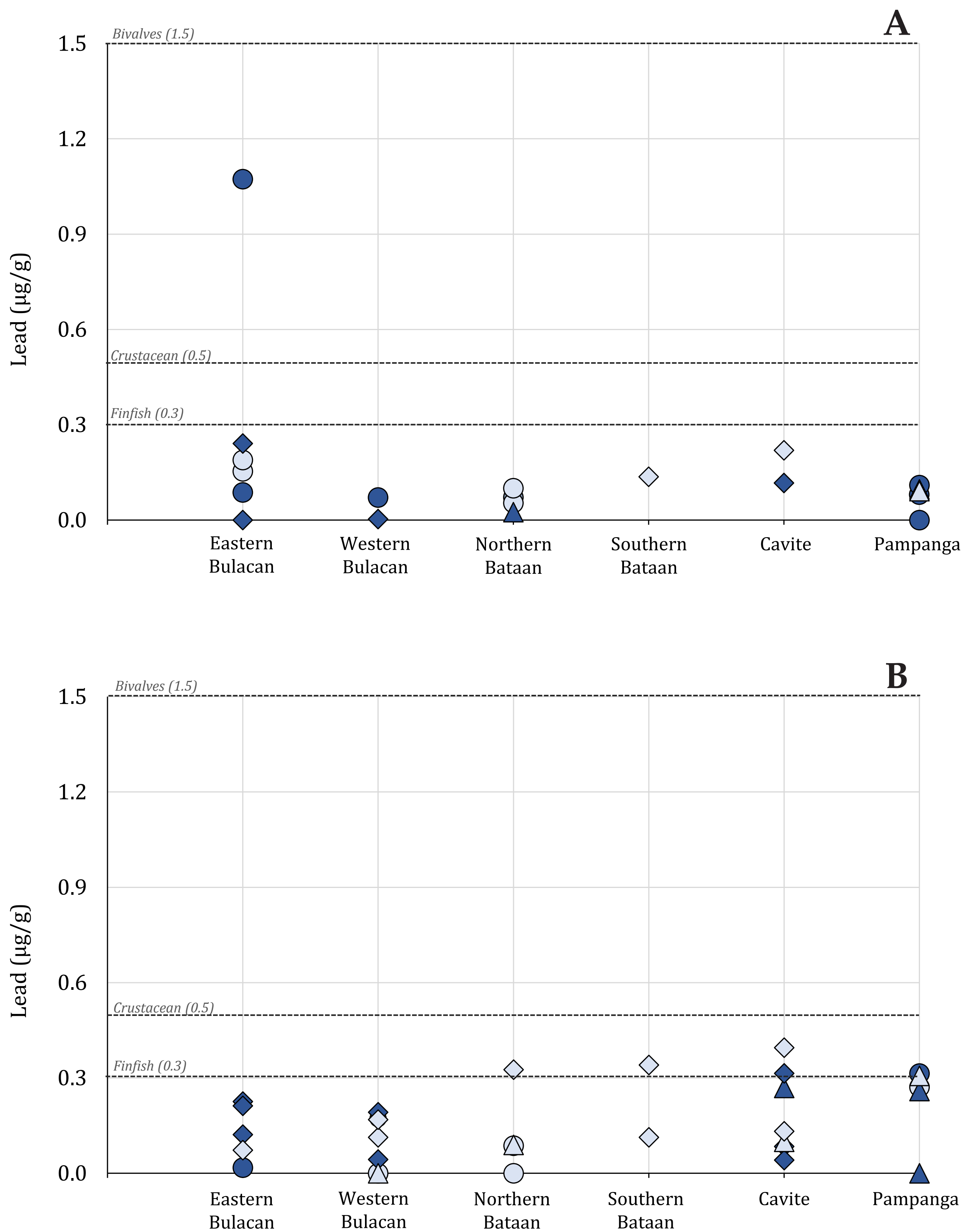


Figure 4.4. Lead levels in aquaculture commodities collected in wet season (A) and in the dry season (B).
Milkfish (MF) Tilapia (TI) Crab (CR) Shrimp (SH) Oyster (OY) Mussel (MU)

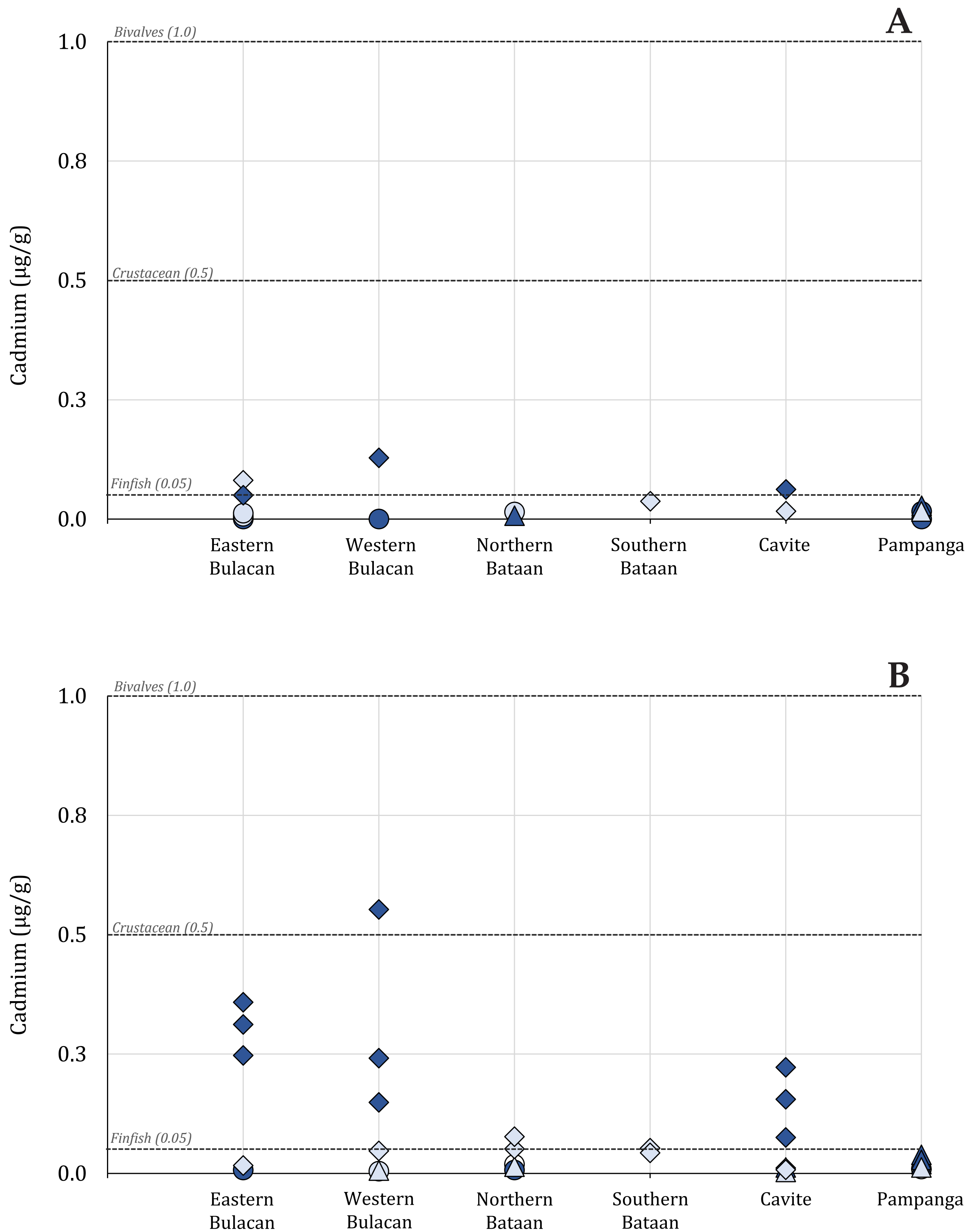


Figure 4.5. Cadmium levels in aquaculture commodities collected in the wet season (A) and in the dry season (B).
Milkfish (MF) Tilapia (TI) Crab (CR) Shrimp (SH) Oyster (OY) Mussel (MU)

ranging from 0.0500 to 0.5526 mg/kg, were highest in a sample collected in Western Bulacan. Oyster had higher levels than mussels whose range was from 0.0077 to 0.0769 mg/kg, and highest in a sample from Northern Bataan. All bivalve samples had cadmium concentrations within the regulatory limit of 1.0 mg/kg (EC 1881/2006).

MERCURY

Mercury in aquaculture commodities collected in the wet and the dry seasons are presented in Figure 4.6. As shown, the wet season samples had mercury levels ranging from ND to 1.1063 mg/kg, while dry season samples had levels ranging from ND to 0.1271 mg/kg. Maximum mercury concentrations were noted in oyster (wet) and mussel (dry) samples both collected from Eastern Bulacan.

Milkfish samples had mercury levels ranging from ND to 0.6662 mg/kg, highest in a sample collected from Pampanga, while tilapia samples had mercury concentrations ranging from ND to 0.2930 mg/kg, highest in sample collected from Eastern Bulacan. One out of twelve milkfish samples (collected from Pampanga, wet season) failed to meet the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Mercury in crab samples ranged from ND to 0.1257 mg/kg, highest in a sample collected from Western Bulacan, while those in shrimp ranged from ND to 0.0846 mg/kg, highest in a sample collected from Northern Bataan. All crustacean samples had mercury levels within the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Oyster and mussel samples had mercury concentrations ranging from ND to 1.1063 mg/kg and ND to 0.1271 mg/kg, respectively. Maximum values in both were noted in Eastern Bulacan. One out of thirteen oyster samples (collected from Eastern Bulacan, wet season) failed to meet the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Method Validation

The methods for the determination of total cadmium (Cd), lead (Pb), and mercury (Hg) were validated by using spiked concentrations of 1 μg/L Cd, 5 μg/L Pb, and 0.1 μg Hg in different fishery resources such as finfish (n=9), crustaceans (n=7), and mollusk (n=9). Validation were also done in certified reference material (CRM) sample, DORM-3 (NRC-CNRC Fish Protein Certified Reference Material for Trace Metals) with certified values of 0.290±.020 mg/kg for cadmium, 0.395±0.050 mg/kg for lead, and 0.382±0.060 mg/kg for mercury. The calculations for the method detection limit (MDL) and limit of quantification (LOQ) were based on signal-to-noise ratio since the analytical procedure exhibited a baseline noise. The determination of method MDL and LOQ was done by comparing the measured signal of the spiked blank sample with those of the signal of the blank sample alone. Results of the method validation are summarized in Table 4.12.

Discussion

Spatial and Seasonal Distribution of Heavy Metals in Water

Heavy metals are known to exist in trace amounts in the environment. These are naturally harmless (Shremati & Varma, 2010). However, continuous urbanization and industrialization along with the increase in population and different anthropogenic activities result in the increment of these (Oluyemi et al, 2008) to alarming levels necessitating that regulatory standards are to be set.

Rainfall causes urban runoff, a non-

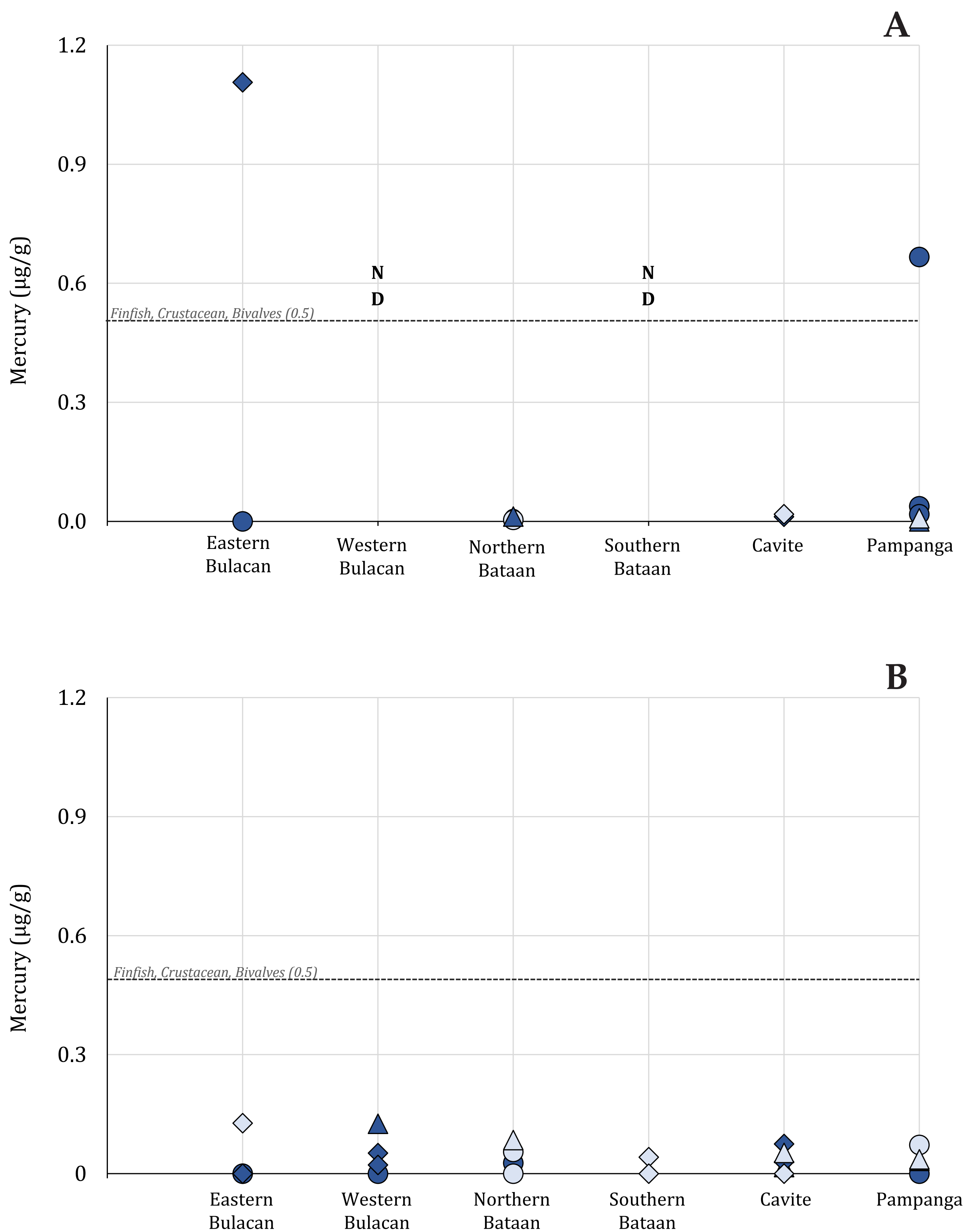


Figure 4.6. Mercury levels in aquaculture commodities collected in the wet season (A) and in the dry season (B).
Milkfish (MF) Tilapia (TI) Crab (CR) Shrimp (SH) Oyster (OY) Mussel (MU)

Table 4.12. Summary of the method validation results for total cadmium, lead, and mercury.

Performance Characteristics and its Accepted Criteria	**CADMIUM:** Microwave digestion followed by Graphite Furnace	**LEAD:** Microwave digestion followed by Graphite Furnace	**MERCURY:** Microwave digestion followed by Mercury Vapor Unit
MDL *	0.0011 (Fish) 0.0033 (Mollusk) 0.0034 (Crustaceans)	0.0334 (Fish) 0.0480 (Mollusk) 0.0205 (Crustaceans)	0.0353(Fish) 0.0291 (Bivalves)
LOQ ¥	0.0036 (Fish) 0.0109 (Mollusk) 0.0113 (Crustaceans)	0.1114 (Fish) 0.1598 (Mollusk) 0.0683 (Crustaceans)	0.1176 (Fish) 0.971 (Bivalves/ Mollusk)
Mean Concentration $^{\delta}$	0.0001±0.00002	0.0005±0.00007	
Mean % RSD $^{\alpha}$	1.68%	12.98%	
Accuracy: Spike % Recovery (40-120% for Pb and Cd; 80-110 for Hg) $^{\beta}$	87.49% 78.46-101.77%	77.06% 72.06-81.17%	76.7-113.4%
Accuracy: CRM % Recovery (80 -110%) $^{\beta}$	100.10% 92.81-106.65%	98.47% 88.39-110.73%	80.47-116.62% (Day 1) 88.38-125.84% (Day2)

All concentrations are expressed as μg/g total cadmium, lead and mercury

* *MDL is calculated as 3 X standard deviation*

¥ *LOQ is calculated as 3.33 X LOD*

δ *Mean concentration is the average concentration of the number of samples analyzed*

α *% Relative standard deviation, calculated as: (standard deviation divided by mean concentration) X 100*

β *% Recovery is calculated as: (difference of concentration of the spiked and unspiked samples divided by the theoretical concentration) X 100; reported % recovery range corresponds to number of samples analyzed.*

point source of pollution, that carries several contaminants, including heavy metals (US EPA, 2016), draining into river systems and enters aquaculture farms through water exchange activities (Srivanasa *et al*, 2007). It is known that water exchange activity in ponds is more frequent during the wet season since water levels in river tributaries or water sources increases with rainfall, thus more water is available for the farmers (Yoo& Boyd, 1994). This only means that rainfall results in an increase in heavy metal concentration in pond water due to the continuous influx of water. This is evident in the high concentrations of lead during the wet season where rainfall is greater.

Contrary to the case of lead, cadmium and mercury concentrations were found higher during the dry season. This can be attributed to slow water exchange owing to the limited supply from water sources, and/or due to an increase in temperature, which increases the evaporation rate. In a study by Nartey *et al* (2011), mercury concentrations in the river increased with increased evaporation rate of surface water. Cadmium, on the other hand, is a very soluble heavy metal easily leached from the sediments and dissolved in water (Rajan *et al*, 2013).

Aside from the observed seasonal distribution of heavy metal contamination in aquaculture farms, spatial distribution was also observed. Cadmium was found highest in Pampanga, where farming, fishing, manufacturing, handicrafts, poultry and swine, food processing industries, as well as ceramics and metalworking are the main sources of livelihood (pampanga.gov.ph). Wetlands, swamplands, agricultural lands, clay, gravel, sand, and copper are the natural resources there (alviera.ph). Pampanga is adjacent to Zambales, which houses Mount Pinatubo, an active volcano. Volcanic action is known as one of the natural sources of cadmium not only in the atmosphere but also in the soil, which in turn gets washed off into river systems (Buat-Ménard *et al*, 1987; Hutton *et al*, 1987). Quarrying and coal combustion, two of the anthropogenic activities that emit cadmium, are practiced in the province following the 1991 eruption of Mount Pinatubo as reported by the Greenpeace Southeast Asia and Orejas in 2014. Some aquaculture farmers do not observe proper buffer zone nor monitor water quality; they had the highest population of chicken and wild birds among the provinces, and used chicken manure and urea as fertilizers, as reported in the previous study conducted by the group, *"Review of Aquaculture Practice and Anthropogenic Activities in Manila Bay Aquaculture Farms."* Inorganic fertilizers such as urea as well as chicken manure may contain trace metals such as cadmium, which are deposited in pond sediment (Benson *et al*, 2014; Boyd & Masaut, 1999). Buffer zones filter out wastes, fertilizers, pesticide runoffs, and other contaminants (chemical and microbiological) from entering the ponds (DeFries, Karanth, & Pareeth, 2010). The lack of these may result in higher contamination of the pond.

Mercury was found highest in Cavite where the population is highest among the provinces sampled (NSO, 2010). Mercury is primarily used in dental amalgams, fluorescent lights, thermometers, electric switches, batteries, insecticide, disinfectant, rat poisons, and even in skin ointments (The Columbia Electronic Encyclopedia, 2012). Since the province has the highest population, it is safe to assume that production as well as usage of above-mentioned products is highest in the province and so is mercury emission. Aside from anthropogenic activities already discussed, mining and quarrying, huge contributors to mercury contamination, are major components of production land-use in the province (cavite.gov.ph). Mercury is discharged into the air (gets deposited into the ground through precipitation) or water through point sources (Lindberg *et al*, 1987). This in turn gets into aquaculture farms by way of river systems. In addition, aquaculture farmers in Cavite bypass residual waste removal and water flushing – two activities that reduce contamination in the pond – during pond preparation as reported in the previous study conducted by the group entitled, *"Review of Aquaculture Practice and Anthropogenic Activities in Manila Bay Aquaculture Farms."*

Lead, which is known to come from gasoline, batteries, ammunition, paint, ceramics, and even in cosmetics (US EPA, 2015) was found highest in Bataan, where oil and gasoline companies, refineries, and feed mills are the major industry sectors. The province, which will soon be considered a special defense economic zone, has also been manufacturing ammunition for nearly 60 years (Rivera, 2015). Aquaculture farmers in the province use urea, an inorganic fertilizer that may contribute to the concentration of lead in the water. In a study by Benson *et al* in 2013, lead concentration was found highest among the other trace elements in the urea fertilizer.

Heavy Metals Concentration in Fish and Fishery Resources

Aquaculture commodities such as milkfish, tilapia, shrimp, crab, mussel, and oyster differ in several ways – size, production cycle, feeding habit, etc. It is only logical to assume that the uptake of heavy metals in the above-mentioned commodities differ from species to species. In fact, findings in a study by Su *et al* (2009) suggest that accumulation of heavy metals in aquatic organisms is species-dependent. In the present study, heavy metal accumulation in aquaculture commodities is as follows: oyster > mussel >tilapia > shrimp > crab > milkfish.

Heavy metal contamination in bivalves, which was observed to be higher compared to the other aquaculture commodities analyzed, may be attributed to their feeding habit of filter feeding (Garrido-Handog, 1990; Aypa, 1990). Filter feeders siphon the water in which they are suspended through filters that retain suspended matter, like phytoplanktons (Barker Jørgensen, 1990). This siphoning and filtration processes cause heavy metals in the water column to pass through the organism, ultimately to get deposited in different cellular compartments (Rodríguez de la Rúa *et al*, 2005). Oysters and mussels were collected from coastal areas. It is known that all effluents, treated and untreated, flow to the bay as the bay is the catch basin for all water systems in Manila Bay. As shown in the results on the heavy metals in water, although inconsistent, concentration of heavy metals came out to be high in coastal areas. This implies that higher heavy metal concentration in the water column causes a higher contamination in aquaculture commodities.

Heavy metals in aquaculture commodities were found higher during the dry season than the wet season. Milkfish, tilapia, shrimp, and crab feeding habits depend on temperature; as temperature increases, feeding frequency also increases (FAO[2], 2011; Garcia, 1990; Greenfield *et al*, 2005; Benitez, 1984) and so does their heavy metal uptake.

Conclusion

Lead was found higher during the wet season while cadmium and mercury, during the dry season. The following are the sites that failed to meet DENR regulatory limits for heavy metals in water in wet season: Northern Bataan (1) and Southern Bataan (2) for lead (0.05 μg/mL); Northern Bataan (3) for mercury (0.002 μg/mL); while the following are the sites that failed DENR regulatory limits in the dry season: Eastern Bulacan (3), Western

Bulacan (1), Northern Bataan (1), Southern Bataan (1), and Cavite (8) for mercury (0.002 μg/mL). Cadmium was found highest in Pampanga, while mercury and lead in Cavite and Bataan, respectively. Two out twelve milkfish samples and one out of nine tilapia samples failed to meet the regulatory limit for lead in finfish (0.3μg/g) while one out of twelve milkfish samples and one out of thirteen oyster samples exceeded the limit for mercury in finfish (0.5 μg/g) and bivalves (0.5 μg/g), respectively.

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CHAPTER 5

Contamination of Coliform Bacteria in Water and Fishery Resources in Manila Bay Aquaculture Farms

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Abstract

The coliform group of bacteria is widely used as an indicator of pollution related to the presence of pathogenic bacteria linked to fecal contamination, which poses great health risk. This study aimed to establish baseline information on the coliform contamination in water and fishery resources in Manila Bay aquaculture farms. Water samples and major aquaculture commodities were collected twice per season from representative aquafarms in the coastal provinces of the bay and were analyzed for total coliform (TC), fecal coliform (FC), and E. coli *(EC) using the Multiple Tube Fermentation method of the Bacteriological Analytical Manual. TC, FC, and EC in water were found higher during the wet season, their average concentrations being 8,747, 2,808, and 1, 216 MPN/100mL, respectively; while those in the dry being 6,255, 1,223, and 286 MPN/100mL, respectively. More samples exceeded the DENR Standard Limit for TC (5,000 MPN/100mL) in the wet season than in the dry season (roughly 25% vs 10%). Farmed fishery resources, on the other hand, had higher EC concentrations during the dry season. The following are the percentages of samples that exceeded DENR Standards: 25% of mussels, 24.44% of shrimps, 16% of tilapia, 14.67% of oysters, 8.89% of crabs, and 6.67% of milkfish.*

Keywords: Manila Bay, farmed fishery resources, coliforms, aquaculture farms

Introduction

Coliforms are facultative anaerobic, gram-negative, lactose-fermenting, non-spore-forming rods present in the environment and in the intestinal tract of humans and other animals in large numbers (Madigan *et al*, 2012). Widely used as an indicator of microbial contamination, the coliform group of bacteria includes both the pathogenic and non-pathogenic forms (Forsythe, 2010).

Total coliforms, which encompass bacteria common in soils, plants, and animals, including both fecal coliforms and *Escherichia coli*, react to the natural environment and treatment processes similar to pathogens; the reason they are the primarily used indicators of contamination simple enough to identify (Treyens, 2009). Taking a closer look at the coliform bacteria gives an estimate of the number/concentration of pathogenic bacteria in the sample (Henze *et al*, 2008).

The US Environmental Protection Agency in 2012 had recommended the use of thermo-tolerant group of coliforms linked to the presence of fecal matter commonly known as fecal coliforms to monitor water quality standards. While fecal coliforms do not necessarily cause illness, its presence in high numbers suggests higher risk of contracting disease-causing bacteria and/or viruses including pathogenic *Escherichia coli* (Oram, 2014). The US Environmental Protection Agency recommends the determination of *Escherichia coli* or *Enterococci* to further identify health risk.

Escherichia coli, commonly known as *E. coli*, is a typically harmless bacterium naturally found in the intestines of warm-blooded animals including humans, and plays a vital role in the digestion, absorption of essential nutrients, and production of Vitamin K and B (Hayhurst, 2004). Characterized by its ability to utilize sugars as a source of energy, *E. coli* can live with or without oxygen (Snyder, 2008).

Although mutualistic with its host, certain *E. coli* strains are pathogenic and can cause illnesses including urinary tract infections, sepsis/meningitis, and enteric/diarrheal diseases in immunosuppressed hosts (Nataro and Kaper, 1998). With a minimum infectious dose of 104 cells and four enterovirulent classes, namely Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC), Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), and Enterohemorrhagic *E. coli* (EHEC), it can be a cause of diarrhea. Enteropathogenic *E. coli* (EPEC) causes severe diarrhea in infants 17-72 hours upon ingestion, which can last for over two weeks, resulting in death, if dehydration is severe. Enteroinvasive *E. coli* (EIEC), with an infectious dose of 104 to 105 cells has symptoms similar to shigellosis – chills, fever, headache, muscle pain, abdominal cramps, and profuse diarrhea – evident 8-24 hours upon infection. Enterotoxigenic *E. coli* (ETEC), commonly known as traveler's diarrhea has an infectious dose of 108 to 1010 cells within 8-44 hours, causing severe diarrhea without fever leading to dehydration. Enterohemmorraghic *E. coli* (EHEC), causes bloody diarrhea, central nervous system involvement in which patients develop blood clots in the brain and frequently results in death, and uremic syndrome in children, the leading cause of kidney failure in children, and may ultimately lead to death (Centers for Diseases Control and Prevention, 2015).

Manila Bay is a semi-enclosed estuary highly regarded for its usefulness to different industries including aquaculture. Fish, fish pens, and shellfish-growing areas are widespread in provinces along the bay covering an aquaculture area of almost 60,000 ha (Perez *et al*, 1999). According to the valuation study of PEMSEA (2006), 59% of Manila Bay's current economic value of 8.3 billion pesos is accounted for by aquaculture alone. However, the bay is currently beset with environmental problems due to pollution. Runoffs from agricultural, domestic, and industrial wastes are concentrated in the bay area most likely indicating presence of pathogenic microorganisms in the water as well as in the fishery resources therein.

Reports on bacterial contamination in several parts the bay had been recorded in the past years. PEMSEA (2006) reported that fecal coliform levels at the eastern part of Manila Bay has exceeded the criteria set by the Department of Environment and Natural Resources (DENR DAO 34) for non-contact recreation of 5,000 MPN/100mL by 150 times and an alarming 900 times for swimming (1,000 MPN/100mL). The western side of the bay where values were much lower than those at the eastern side, also failed the criteria occasionally. In addition, shellfish collected from Bulacan, Bacoor, Kawit, Naic, and Parañaque exceeded the European Union Guideline for fecal coliform of 300 MPN/100g by 1.3 to 2, 667 times, or 52 times on average. In 2014, Parco reported that fecal coliform levels of more than 100,000 MPN/100mL, have greatly exceeded the DENR Standard of 200 MPN/100mL for Class B Sea Water, in Manila Bay.

The presence of pathogenic bacteria in the bay can affect post-harvest quality, not just of wild fishery resources, but also of aquaculture commodities, as the water for fish farming is also sourced from the bay and its river tributaries. Health of the consumers, therefore, is greatly at risk especially since people tend to consume more fishery resources because of its healthier reputation. In a report by the BFAR (2011), fish and fishery products consumption amounts to 38 kilograms per year per capita, even higher than the consumption of meat products, which only amounts to 22 kilograms per year per capita. However, few studies have been done to assess the microbial levels of aquaculture commodities as well as the water where it is farmed. In fact, gaps on the water quality standards of the Philippines, given by the lack of standard limits for *E. coli* and/or *Enterococci* in spite of its risks, have been noticed.

This study aims to detect and quantify coliform, fecal coliform, and *Escherichia coli* levels in water and fishery resources in aquaculture farms around Manila Bay. Results gathered will be compared with existing standards and will serve as a significant baseline data for the establishment of limits for fecal coliform and *E. coli* in fishery water. The study also intends to determine and compare the spatial and seasonal (wet and dry) microbial distribution in water and fishery resources in aquaculture farms around Manila Bay.

Methodology

The blocking strategy and sampling sites used in Chapter 2 were employed in this study. Forty-seven pre-identified aquaculture farms and coastal areas were sampled twice for each season – September and November 2014 for the wet season, and February and April 2015 for the dry. River tributaries of the aquaculture farms were also sampled for comparison.

Water samples of about 250 mL were collected in composite from the deepest sections of the area using an improvised water collector and transferred in appropriately-labeled sterile 250 mL borosilicate bottles containing 0.25 mL 3% sodium thiosulfate. Aseptic technique was observed throughout the collection. Samples were placed in a temperature-controlled cooler kept at 0 to 4°C for transport. Samples were analyzed in the laboratory within 6 hours from the time of collection (APHA, AWWA, and WEF, 1999).

Freshly harvested fish and shellfish samples of at least 500 grams per species were put separately into appropriately-labeled sterile re-sealable polyethylene bags and placed in a temperature-controlled cooler kept at 0°C to 4°C for transport. Aseptic technique was observed throughout the collection. Samples were analyzed in the laboratory within 24 hours from the time of collection.

Six kinds of farmed fishery resources were collected from the aquaculture farms for the study, namely tilapia, milkfish, shrimp, crab, mussel, and oyster. However, sample species and number of samples collected per site were variable as sample collection was solely dependent on availability. Table 5.1 shows the summary of fishery commodities collected.

Multiple tube fermentation method of analysis for total coliform, fecal coliform, and *Escherichia coli* was employed, as is recommended in DAO 34 (APHA, AWWA, & WEF, 1999; BAM, 2002). Results were interpreted using the Most Probable Number (MPN) table and expressed as MPN/g for the fish and fishery resources, and MPN/100mL for the water samples.

T-test was used to compare results for the microbiological analyses - total coliform, fecal coliform, and *Escherichia coli* – in pond and river for each block in each season. The same test was employed in determining significant differences in bacterial level between the wet and the dry season in each of the blocks. Analysis of Variance (ANOVA) was utilized for the comparison of results of each fishery commodity in different sources or sites during a particular season, given that fishery commodity was taken from more than two sites. Otherwise, the t-test was used. All values were transformed to log10 for analysis. Raw data were used for the summary of the means.

Table 5.1. Summary of the fishery commodities collected for the study.

TYPE	COMMODITY	NUMBER OF SAMPLES	
		WET	DRY
Finfish	Milkfish	70	20
	Tilapia	40	35
Crustacean	Shrimp	20	25
	Crab	20	25
Bivalves	Oyster	35	40
	Mussel	20	40

Results

Seasonal and spatial distribution of coliform bacteria in pond water and water sources

TOTAL COLIFORM

Figures 5.1a to 5.1d show total coliform concentrations in Manila Bay aquaculture farms and coastal areas in September 2014, November 2014, February 2015, and April 2015, respectively.

Total coliform levels recorded in September 2014 ranged from 49 MPN/100mL to 54, 000 MPN/100mL, highest in Calero and Tawiran, Eastern Bulacan and Sapang Kawayan, Pampanga; and lowest in Camachile, Southern Bataan. Thirty four percent of the samples collected in this month failed to meet DENR Standard Limit for Total Coliform Concentration for Class SC Water of 5, 000 MPN/100mL (DAO 34) broken down as follows: 4.55% in Eastern Bulacan, Western Bulacan, and Northern Bataan; 13.64% in Cavite; and 6.82% in Pampanga.

November 2014 total coliform levels ranged from 6.8 MPN/100mL to 160, 000 MPN/100mL, the highest noted in San Antonio, Pampanga and the lowest in Sta. Elena, Southern Bataan. Sixteen percent of the samples collected in this month exceeded the Standard Limit for Total Coliform: 2.27% in Western Bulacan and Southern Bataan, 6.82% in Cavite, and 4.55% in Pampanga. Total coliform levels in this month were relatively lower with 22.73% of the aquaculture farms within the <1.8 – 100 MPN/100 mL range as compared to 9.09% of the aquaculture farms within the same range for September 2014. A majority of the results, 22.73%, were within 235 – 800 MPN/100mL.

February 2015 total coliform levels with only 8.51% of the results exceeded the DENR Standards for total coliform with a range of <1.8 MPN/100mL to >160, 000 MPN/100mL. The highest was observed in Batang 2, Pampanga and the lowest in Samal (Coastal Area), Northern Bataan. Samples that exceeded DENR standards were observed in Northern Bataan (2.12%), Cavite (2.12%), and Pampanga (4.26%). Total coliform levels during this month were relatively lower with 34% of the aquaculture farms within the range of <1.8 – 100 MPN/100mL as against only 29.55% of the aquaculture farms within the same interval on April 2015.

Total coliform levels in April 2015 ranged from 7.8 MPN/100mL to >160, 000 MPN/100mL, highest noted in Capitangan, Northern Bataan and lowest in San Roque (Coastal Area), Western Bulacan. Eleven percent of the collected samples failed to meet the DENR Standard Limit for Total Coliform: 2.22% in Northern Bataan and 4.44% in both Southern Bataan and Cavite.

To determine if total coliform concentrations in the ponds are significantly different from that of their water sources, simultaneous collection of samples from the water sources of the ponds was performed. The difference in the total coliform concentration in the pond and its river tributary or water source is indicative of the flow of bacterial contamination. A higher total coliform concentration in the pond suggests that the pond may contaminate its river tributary, which in turn flows into Manila Bay, while a higher total coliform concentration in the river tributary or water source suggests otherwise. Figures 5.2 and 5.3 show the total coliform concentrations of the ponds, coastal areas, and their river tributaries or water sources of all the blocks during the first and second replicates of the dry season (February and April 2015), respectively. As can be observed, total coliform concentrations were consistently lower in the pond than in their river tributaries in all the blocks in both

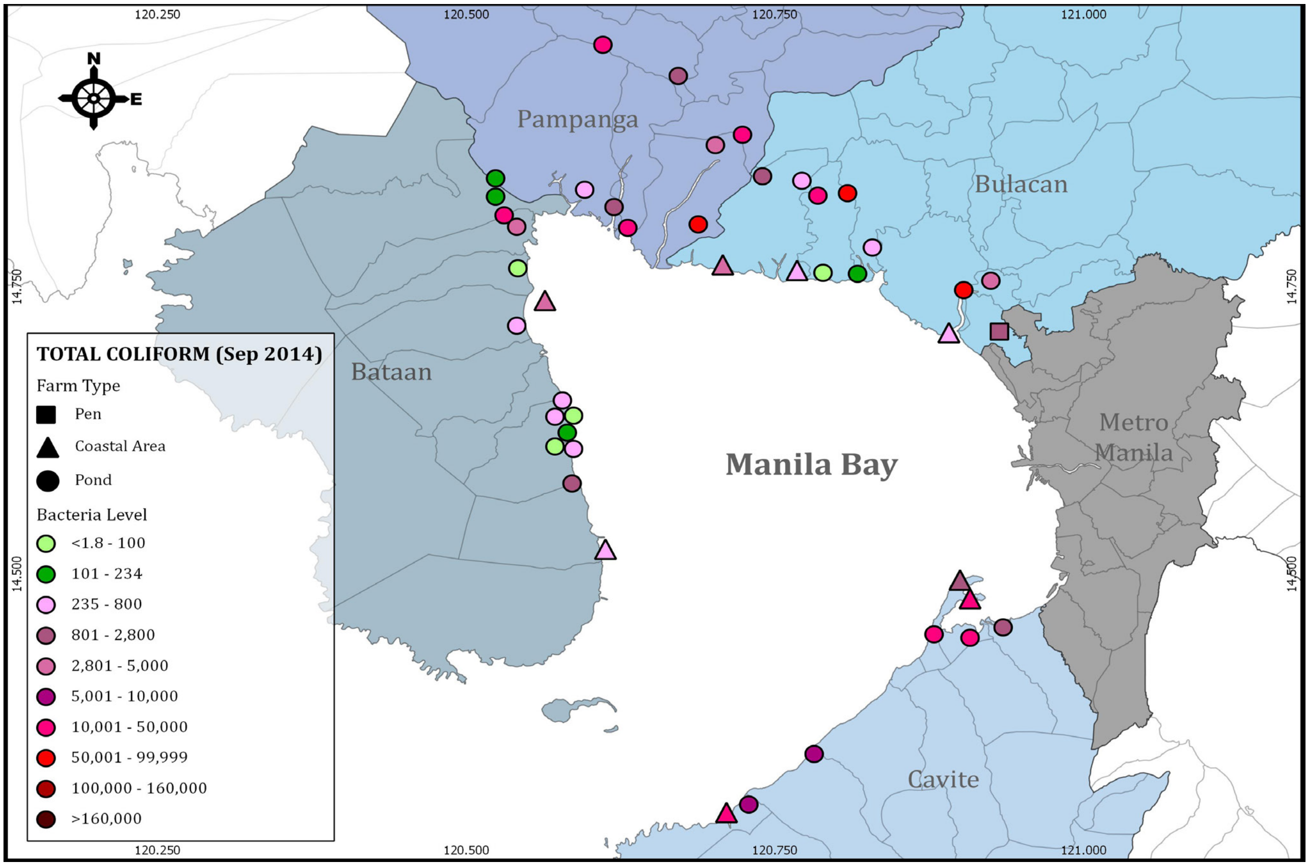

Figure 5.1a. Total coliform levels in Manila Bay aquaculture farms in September 2014.

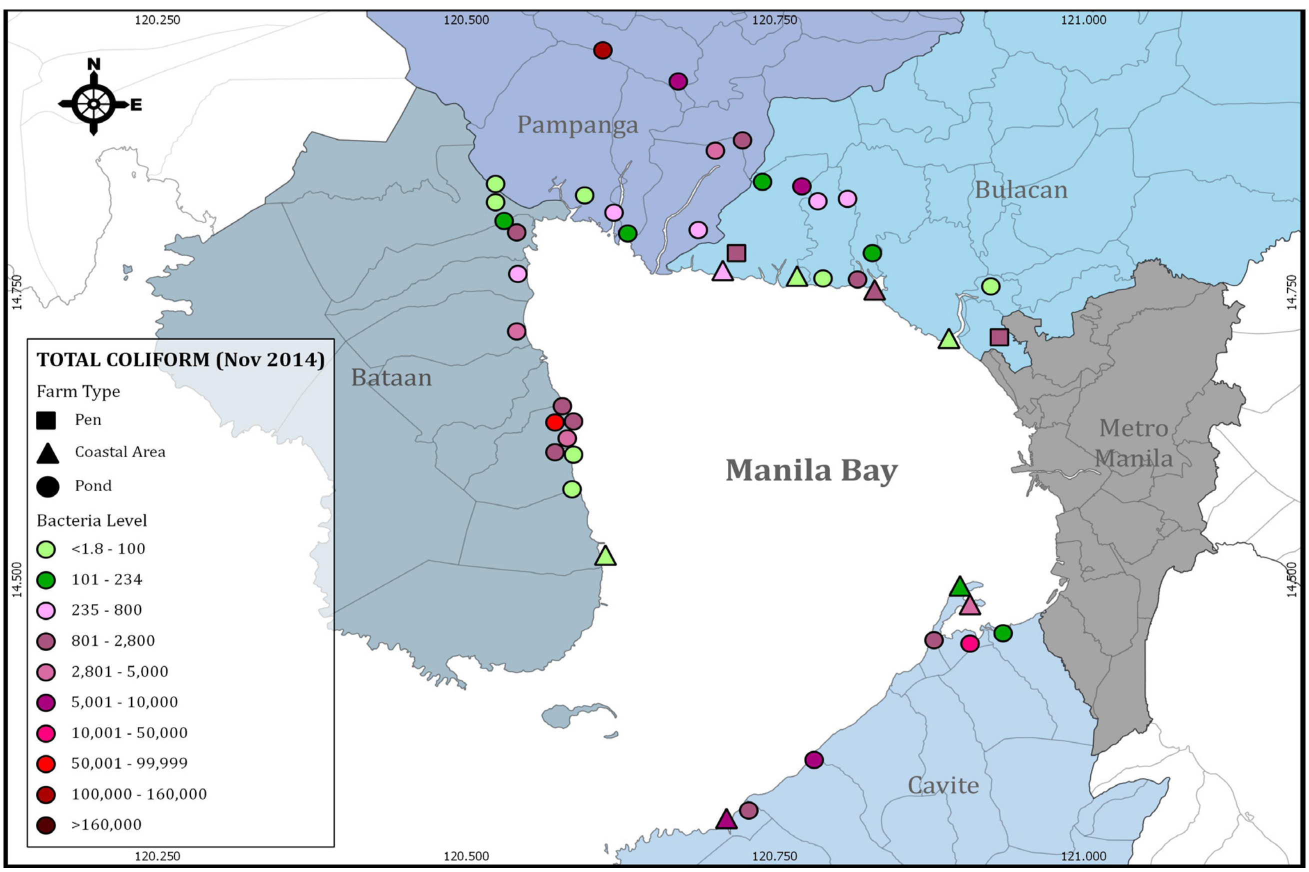

Figure 5.1b. Total coliform levels in Manila Bay aquaculture farms in November 2014.

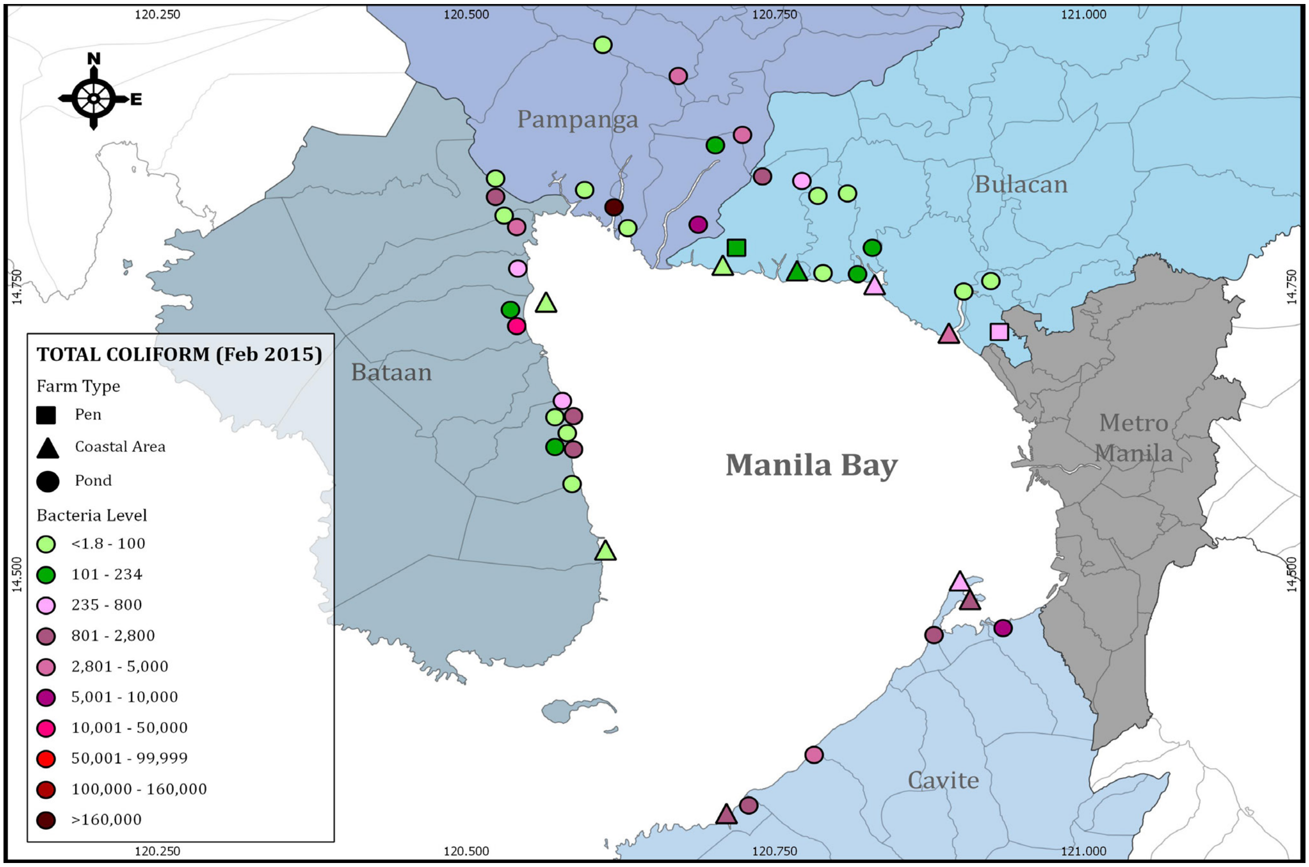

Figure 5.1c. Total coliform levels in Manila Bay aquaculture farms in February 2015.

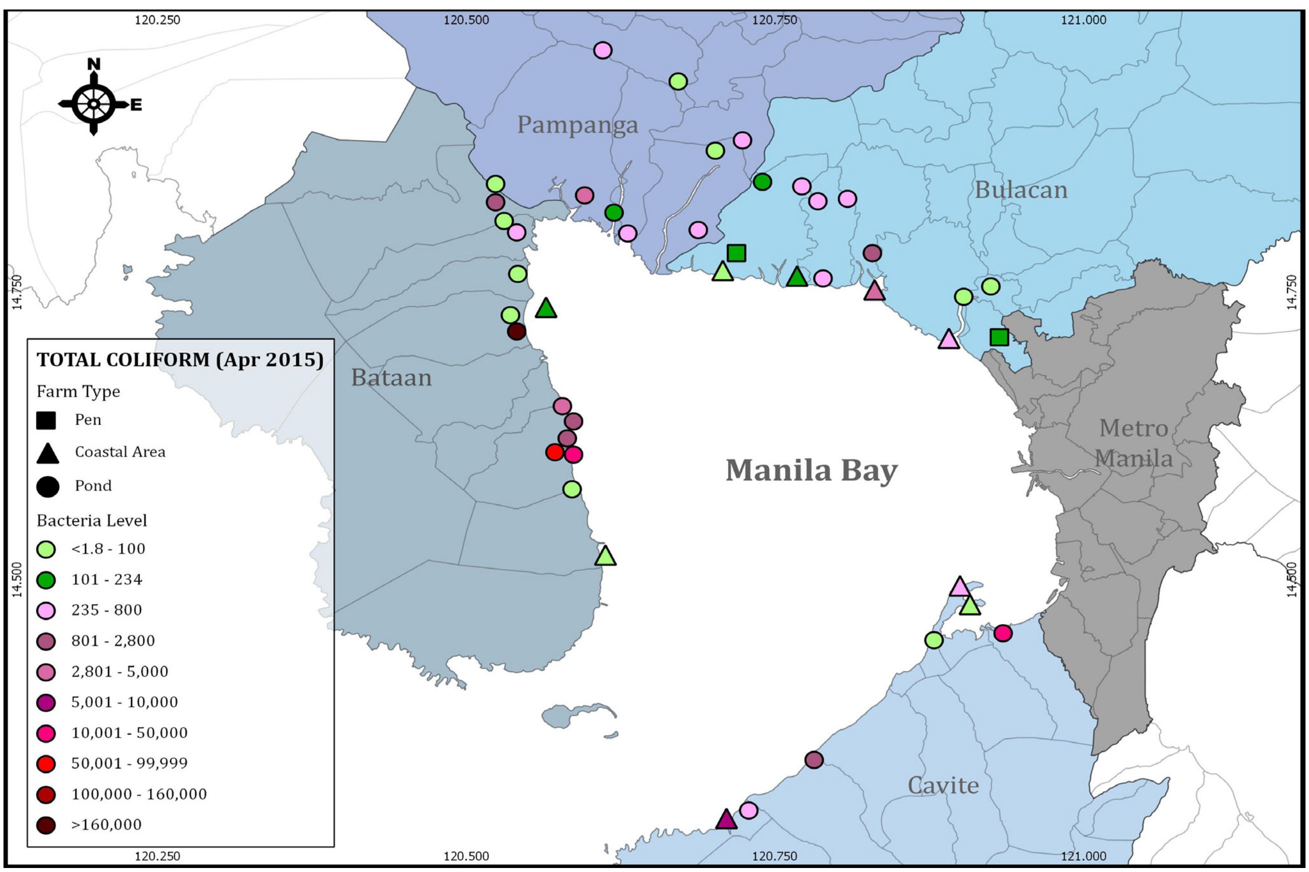

Figure 5.1d. Total coliform levels in Manila Bay aquaculture farms in April 2015.

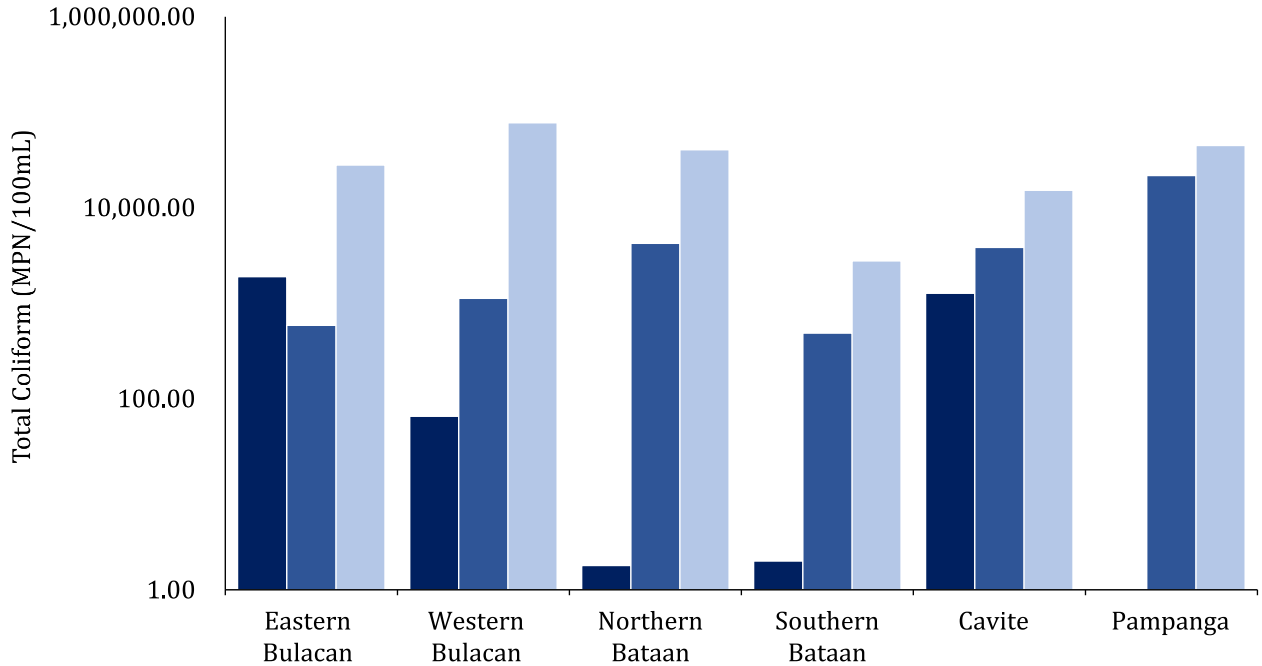

Figure 5.2. Total coliform levels in ponds, coastal areas, and river tributaries or water sources in February 2015. ■ Coastal Area ■ Pond ■ Water Source

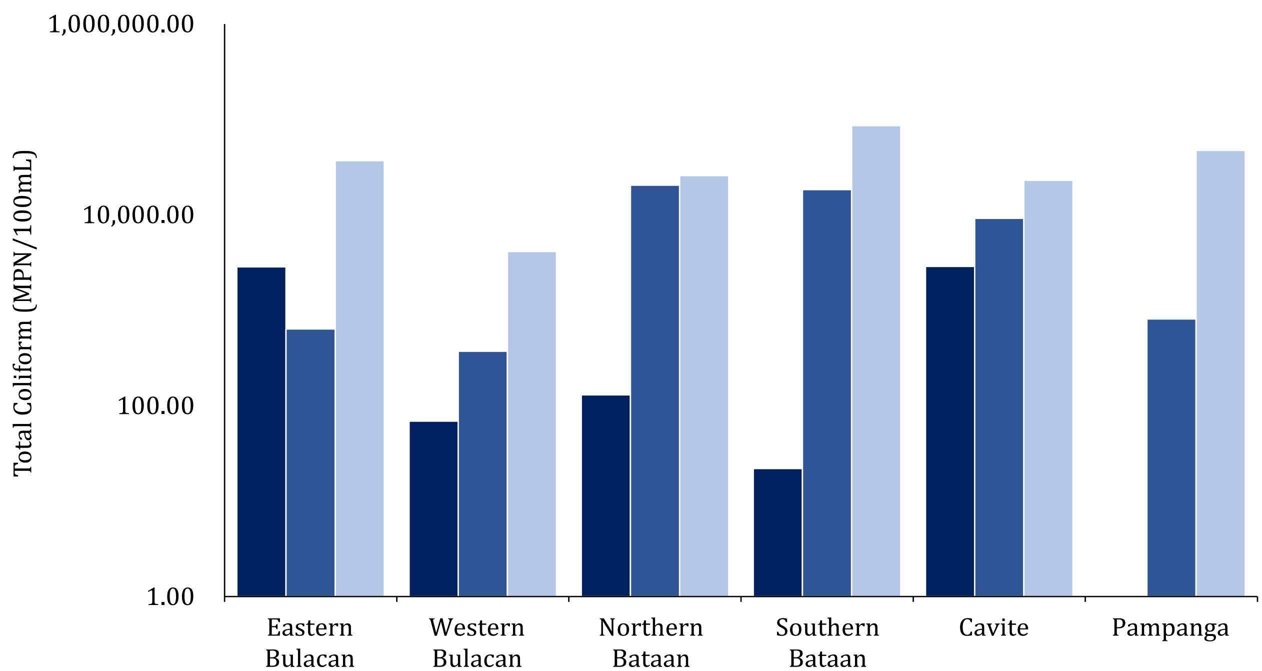

Figure 5.3. Total coliform levels in ponds, coastal areas, and river tributaries or water sources in April 2015. ■ Coastal Area ■ Pond ■ Water Source

replicates of the dry season. Comparison of the total coliform levels between the water sources and the ponds of the following areas showed that the former are significantly higher than the latter: Eastern Bulacan ($p<0.01$) and Northern Bataan ($p<0.05$) in February and April 2015; Southern Bataan ($p<0.05$) in February 2015; and Western Bulacan, Cavite, and Pampanga ($p<0.05$) in April 2015.

FECAL COLIFORM

Fecal coliform levels in Manila Bay aquaculture farms and coastal areas for September 2014, November 2015, February 2015, and April 2015 are shown in Figures 5.4a to 5.4d, respectively.

September 2014 fecal coliform levels ranged from 11 to 49, 000 MPN/100mL, the highest noted in Consuelo II, Pampanga and lowest in Wawa, Northern Bataan and Camachile, Southern Bataan. Southern Bataan had the lowest fecal coliform concentrations among the blocks with a mean concentration of 153 MPN/100mL, while Pampanga with an average concentration of 14, 537 MPN/100mL had the highest.

The November 2014 fecal coliform levels, which ranged from <1.8 MPN/100mL to 7, 900 MPN/100mL, highest were found in San Isidro II, Western Bulacan and Marulas, Cavite, and lowest at Wawa, Northern Bataan and Sta. Elena, Southern Bataan. Highest concentrations were in Cavite (average, 1, 936 MPN/100mL), while the lowest were noted in Southern Bataan (average, 248 MPN/100mL).

February 2015 fecal coliform in Manila Bay aquaculture farms and coastal areas were from <1.8 to 7, 900 MPN/100mL, highest in Sapang Kawayan, Pampanga and lowest in Samal (Coastal Area), Northern Bataan, Camachile, Southern Bataan, and Consuelo II and San Antonio, Pampanga. The levels in this month were the lowest observed among all the sampling months. Lowest concentrations were observed in Northern Bataan (average, 202 MPN/100mL). Highest levels were noted in Pampanga (average, 1, 664 MPN/100mL).

The levels in April 2015 ranged from <1.8 to 54, 000 MPN/100mL, highest in Capitangan, Northern Bataan and lowest in Bangkal Pugad, Pampanga. Those observed in Western Bulacan (average, 67 MPN/100mL) were the lowest, while the concentrations in Southern Bataan (average, of 3, 377 MPN/100mL) were the highest.

Fecal coliform concentrations of the ponds, coastal areas, and the river tributaries or water sources of all the blocks in February and April 2015 are shown in Figures 5.5 and 5.6, respectively. It should be noted that fecal coliform concentrations in water sources were consistently higher than those of ponds in all the blocks in both replicates of the dry season. A comparison of the fecal coliform levels between the water sources and the ponds of the following areas showed that the former were significantly higher than the latter in February 2015: Eastern Bulacan ($p<0.01$), Western Bulacan ($p<0.05$), Northern Bataan ($p<0.01$), Southern Bataan ($p<0.05$), Cavite ($p<0.05$), and Pampanga ($p<0.05$). On the other hand, only Eastern Bulacan, Northern Bataan, and Pampanga had significantly higher fecal coliform concentrations in the water sources than in the ponds in April 2015 ($p<0.05$).

Escherichia coli

Escherichia coli concentrations in Manila Bay Aquaculture farms and coastal areas in September 2014, November 2014, February 2015, and April 2015 are shown in Figures 5.7a to 5.7d, respectively.

E. coli concentrations in September 2014 (range, <1.8 to 49, 000 MPN/100mL) were

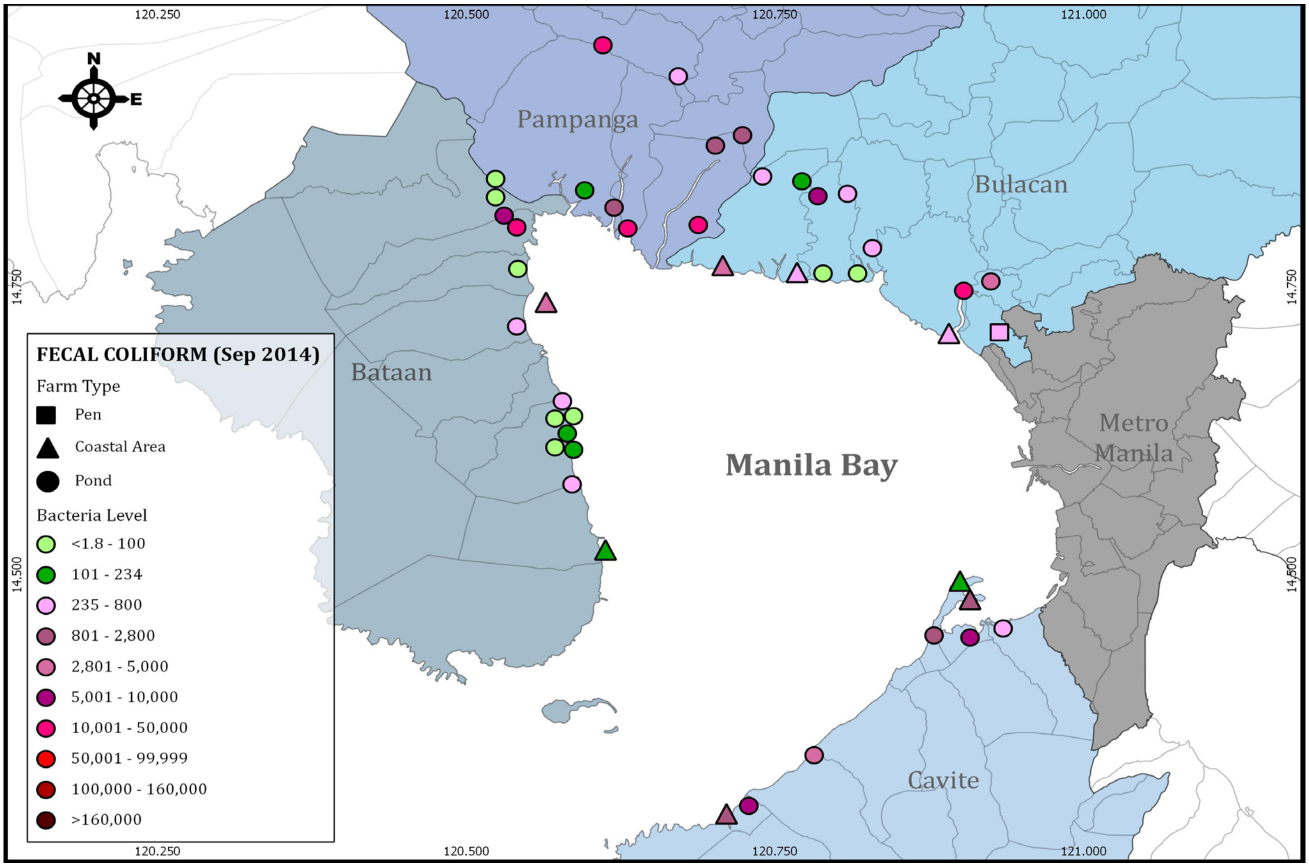

Figure 5.4a. Fecal coliform levels in Manila Bay aquaculture farms in September 2014.

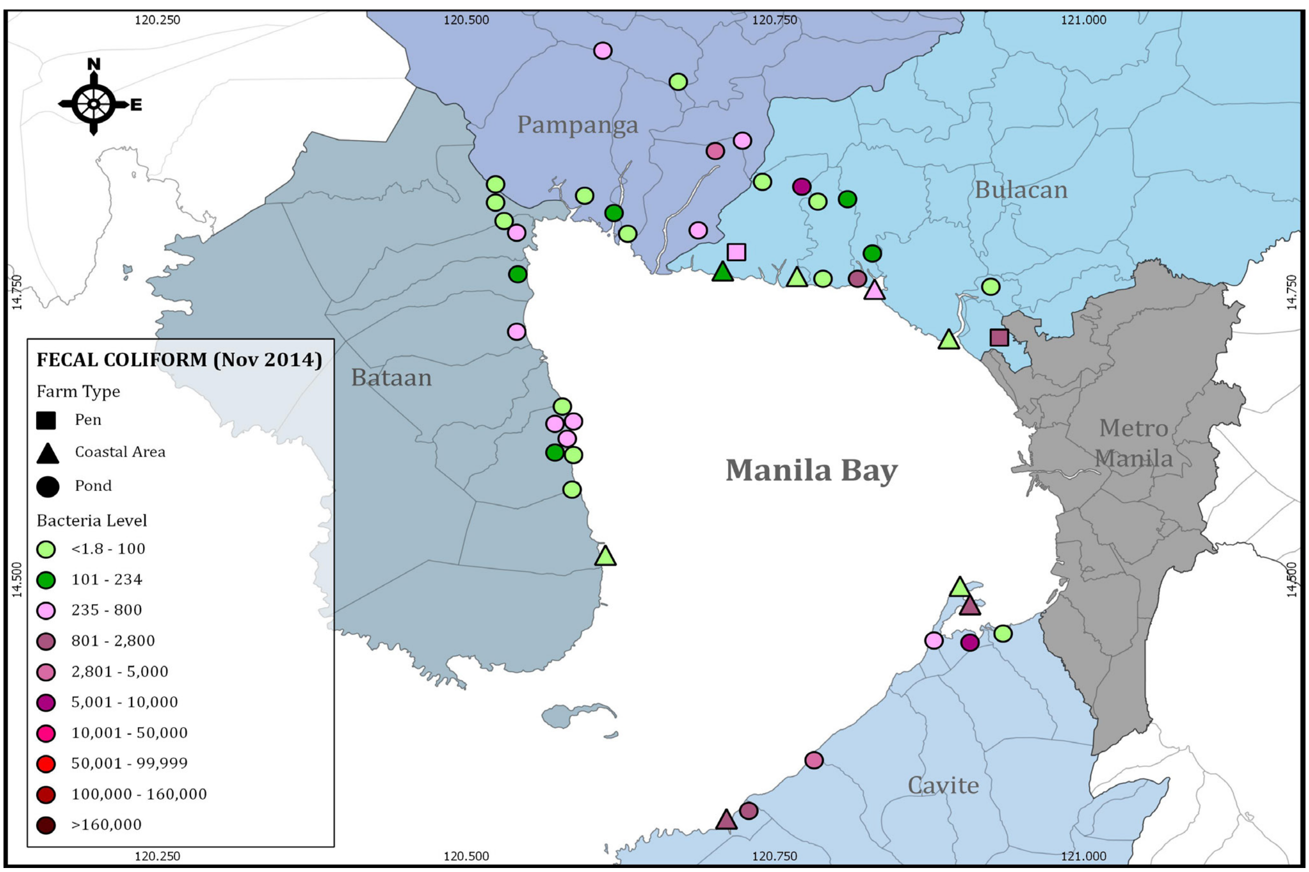

Figure 54b. Fecal coliform levels in Manila Bay aquaculture farms in November 2014.

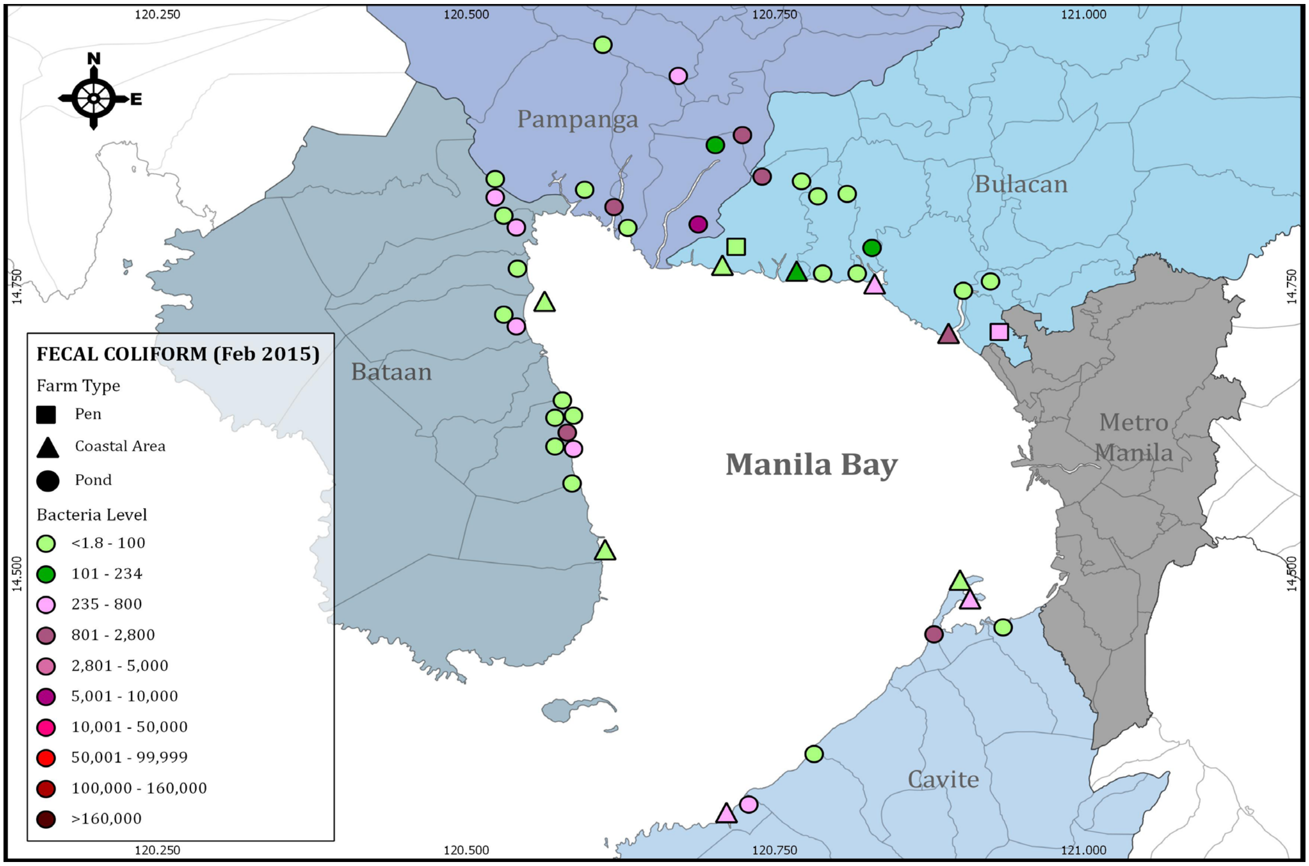

Figure 5.4c. Fecal coliform levels in Manila Bay aquaculture farms in February 2015.

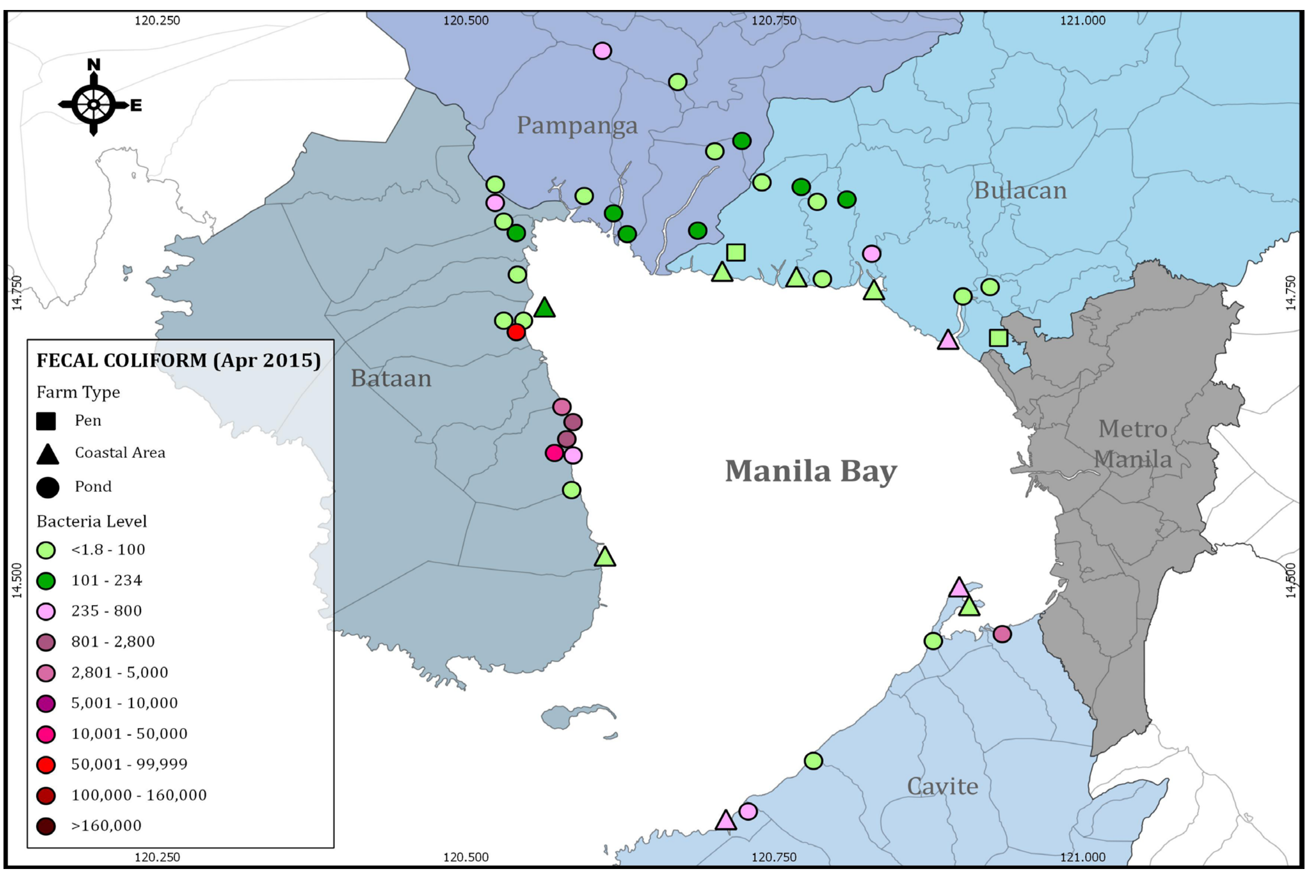

Figure 5.4d. Fecal coliform levels in Manila Bay aquaculture farms in April 2015.

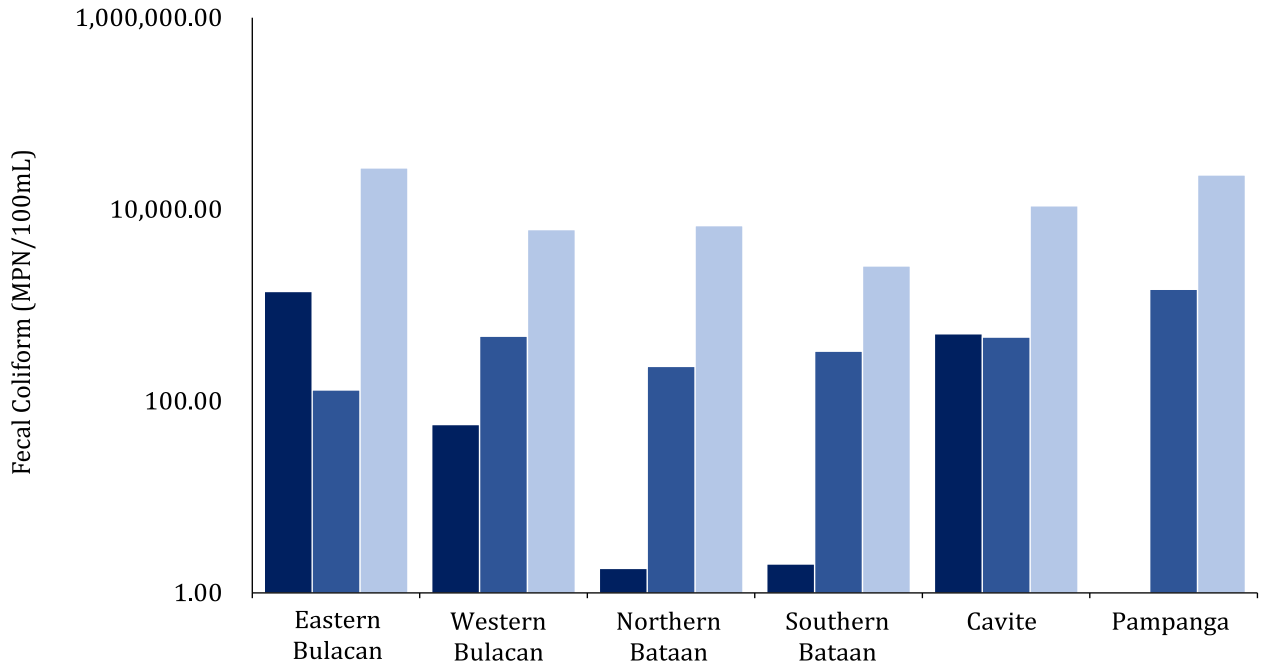

Figure 5.5. Fecal coliform levels in ponds, coastal areas, and river tributaries or water sources in February 2015. Coastal Area Pond Water Source

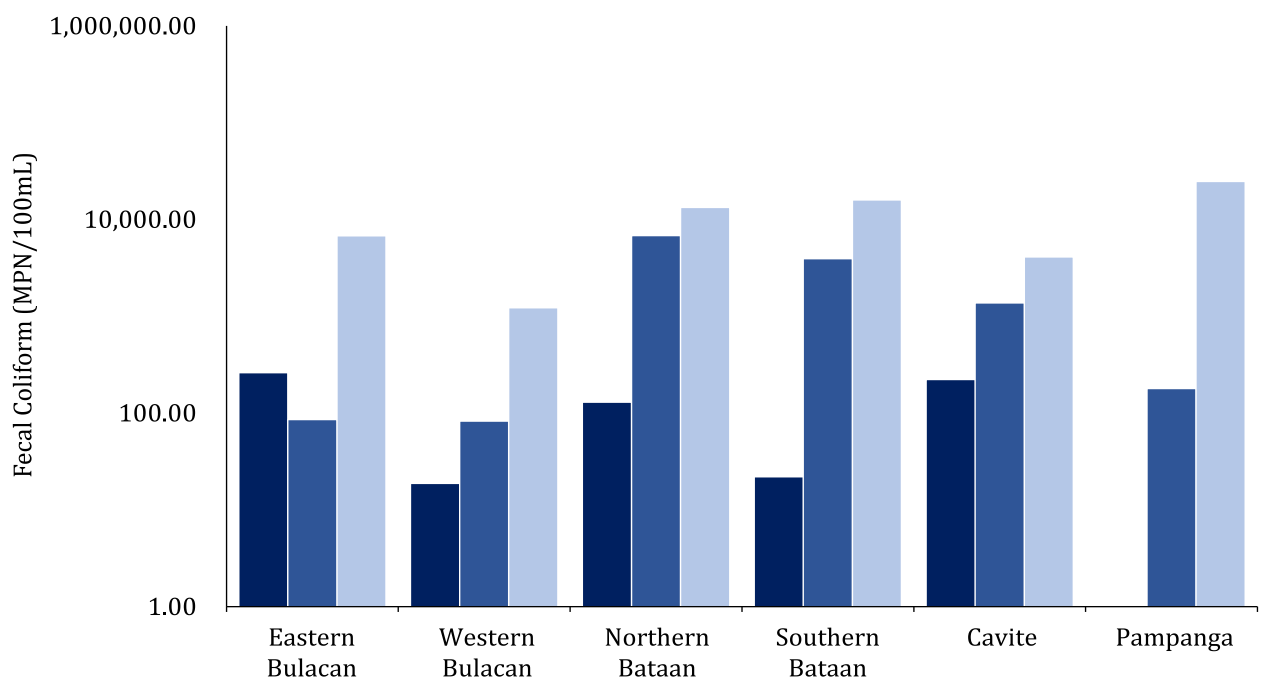

Figure 5.6. Fecal coliform levels in ponds, coastal areas, and river tributaries or water sources in April 2015. Coastal Area Pond Water Source

highest in Consuelo II, Pampanga and lowest in San Agustin, Western Bulacan. Those of this month were the highest *E. coli* levels among the sampling months. Of the blocks sampled, Southern Bataan had the lowest levels (average, 18 MPN/100mL) while Cavite had the highest (average, 8, 341 MPN/100mL).

The November 2014 *E. coli* concentrations ranged from <1.8 to 4, 900 MPN/100mL, the highest recorded in Bangkal Sinubli, Pampanga and the lowest in Sta. Elena, Southern Bataan. Southern Bataan had the lowest (average, 58 MPN/100mL), while Pampanga had the highest (average, 772 MPN/100mL).

In February 2015, the range was <1.8 to 7, 900 MPN/100mL, highest in Sapang Kawayan, Pampanga and lowest in Tawiran, Eastern Bulacan, Samal (Coastal Area) and Ibaba, Northern Bataan, Camachile, Southern Bataan, and Consuelo II and San Antonio, Pampanga. *E. coli* levels during this month were the lowest among all the sampling periods. Western Bulacan (average, 12 MPN/100mL) had the lowest *E. coli* concentrations among the blocks, while Pampanga had the highest levels (average, 1, 489 MPN/100mL).

April 2015 *E. coli* levels ranged from <1.8 to 4, 700 MPN/100mL, lowest in Tawiran, Eastern Bulacan and Bangkal Pugad, Pampanga and highest in Capitangan, Northern Bataan. Lowest levels were observed in Western Bulacan (average, 40 MPN/100 mL), while highest were noted in Southern Bataan (average, 487 MPN/100mL).

Figures 5.8 and 5.9 show the *E. coli* concentrations of the ponds, coastal areas, and their river tributaries or water sources of all the blocks during the first and second replicates of the dry season (February and April 2015), respectively. It can be seen in the figures that *E. coli* concentrations in the water sources were consistently higher than those in ponds in all the blocks in February and April 2015. Comparison of the *E. coli* levels between the water sources and the ponds of the following areas showed that the former were significantly higher than the latter in February 2015: Eastern Bulacan, Western Bulacan, Northern Bataan, and Pampanga ($p<0.01$), and Southern Bataan ($p<0.05$). In contrast, only Pampanga was found to have significantly higher *E. coli* level in the water source than the pond in April 2015 ($p<0.05$).

Seasonal distribution of coliform bacteria in farmed fishery resources

E. coli IN FINFISH

Results for the *E. coli* levels in the finfish samples collected are reflected in Figures 5.10, 5.11, 5.12, and 5.13.

E. coli concentrations in September and November 2015 ranged from 3 MPN/g to 75 MPN/g with the highest recorded in milkfish sample collected from Batang 2, Pampanga (Fig. 5.10 & 5.12). Twelve out of 110 finfish samples or 10.91% exceeded the FDA Standard Limit for Fishes of 11 MPN/g. Of the 70 milkfish samples collected, 8.57% (all samples collected from Pampanga) failed to meet FDA Standard Limit, while 15% (6/40) of the tilapia samples (both 2.5% from Western Bulacan and Cavite, and 10% from Pampanga) had *E. coli* levels greater than 11 MPN/g.

On contrary, all milkfish samples collected in February and April 2015 conformed with the FDA Standard Limit for *E. coli* concentration in finfish of 11 MPN/g, while 17.14% of the tilapia samples failed to meet FDA Standard Limit (Fig. 5.11 & 5.13). *E. coli* levels during this sampling period ranged from <3 to 23 MPN/g, with the maximum value observed in tilapia samples collected from Batang 2 and Mani-ano, Pampanga.

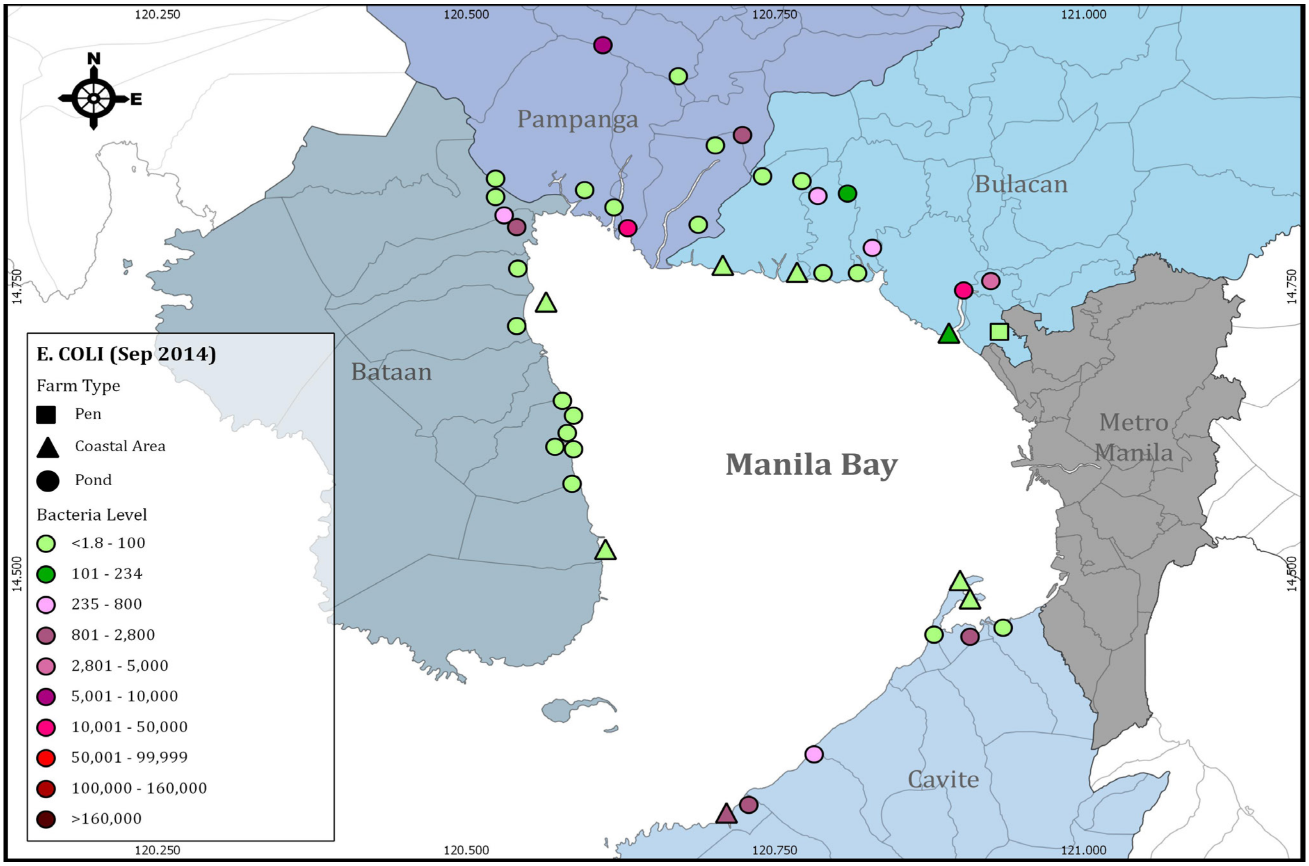

Figure 5.7a. *E. coli* levels in Manila Bay aquaculture farms in September 2014.

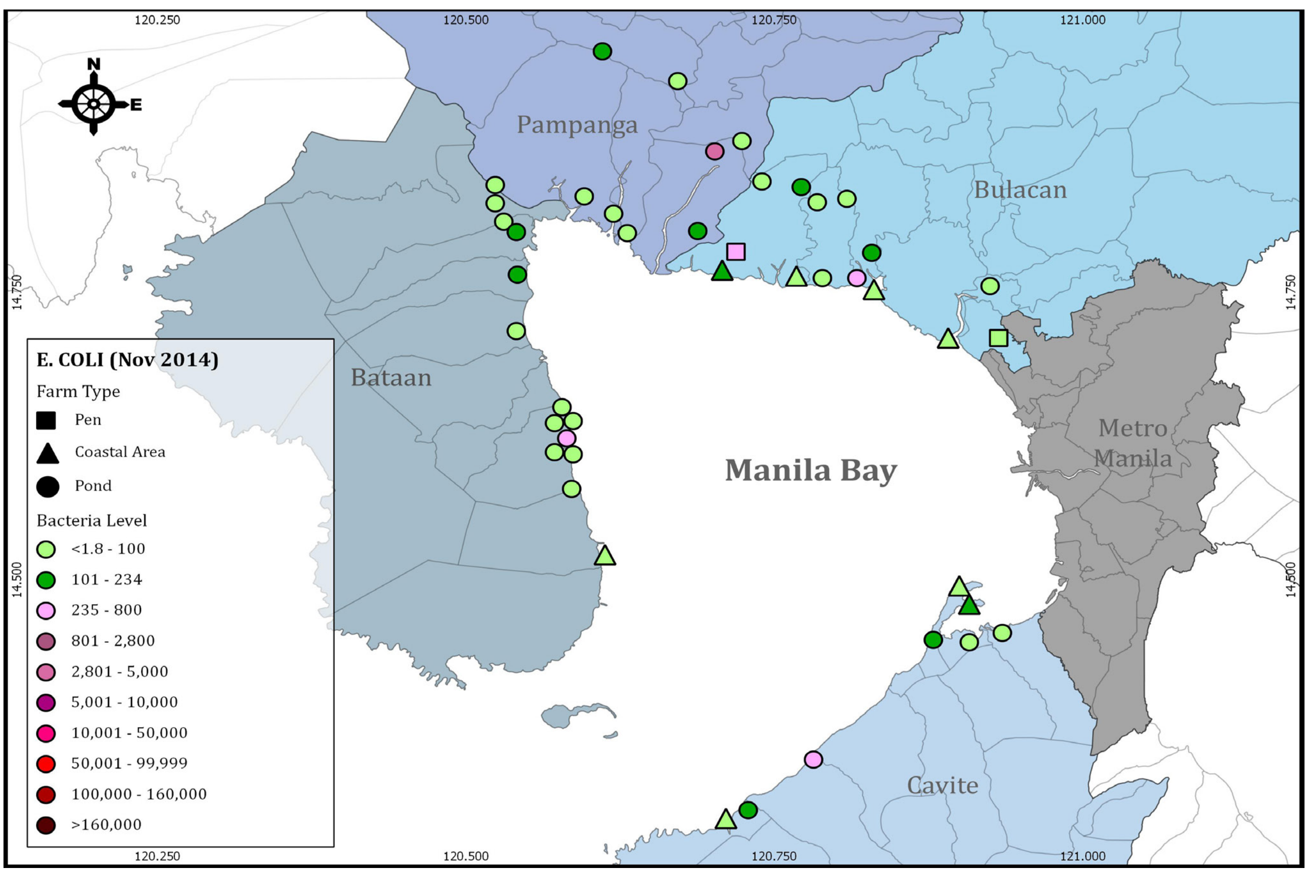

Figure 5.7b. *E. coli* levels in Manila Bay aquaculture farms in November 2014.

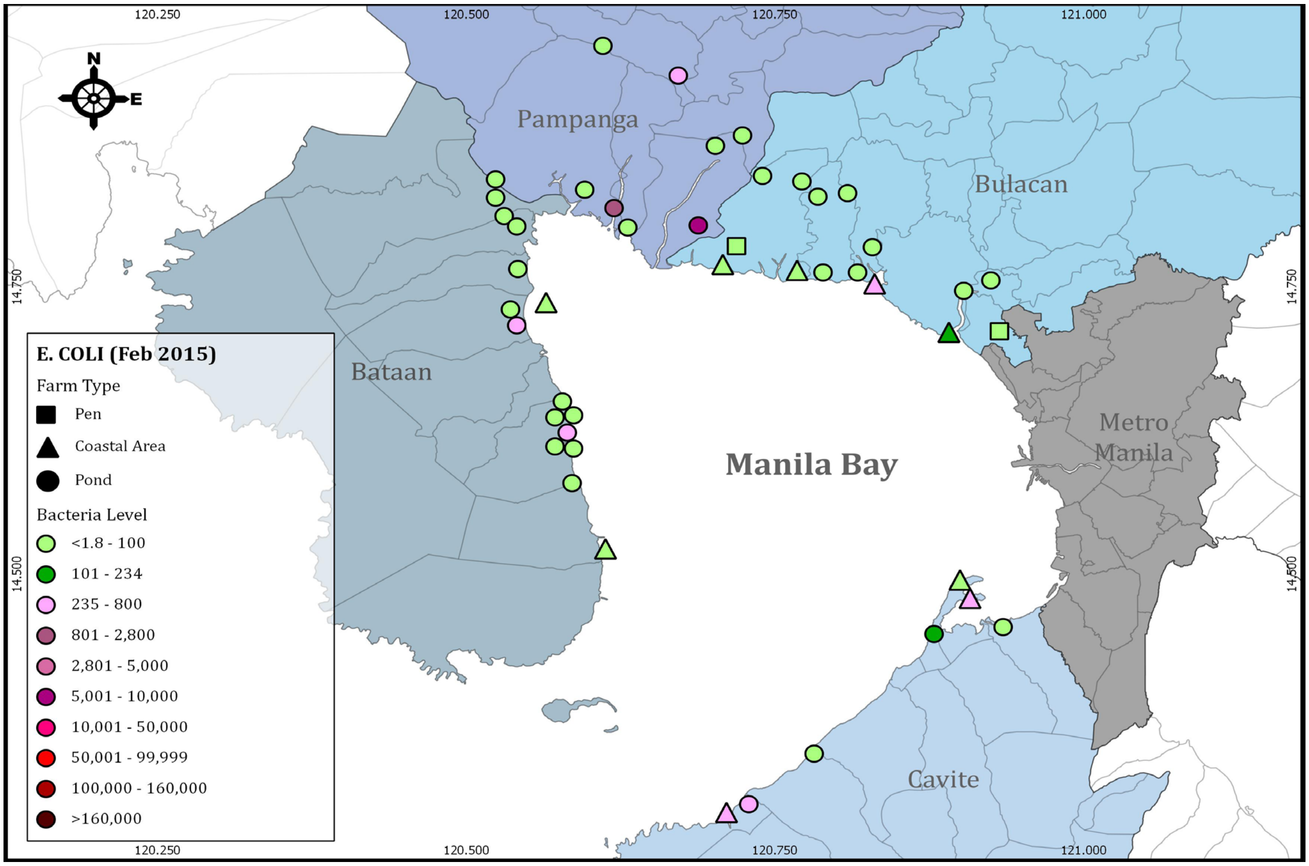

Figure 5.7c. *E. coli* levels in Manila Bay aquaculture farms in February 2015.

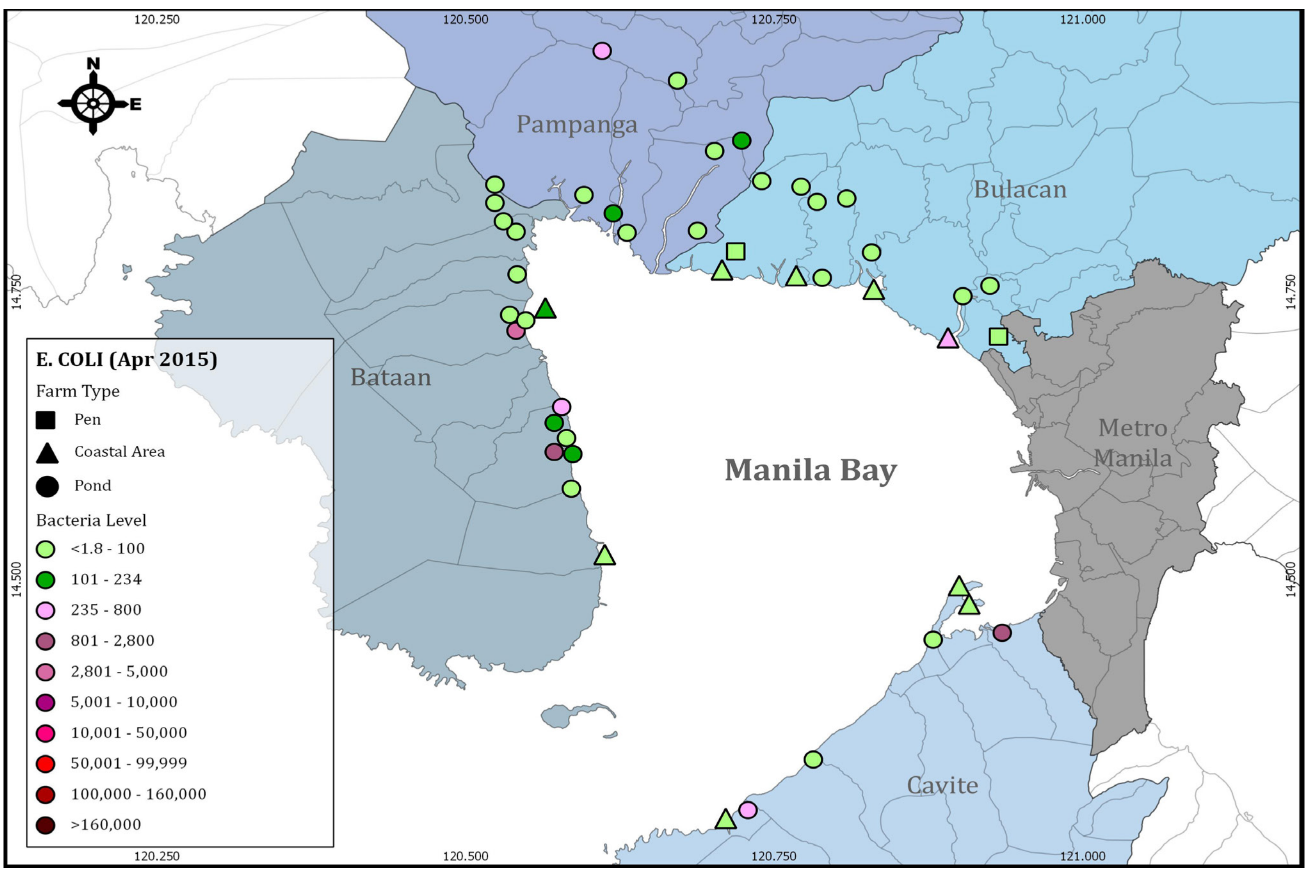

Figure 5.7d. *E. coli* levels in Manila Bay aquaculture farms in April 2015.

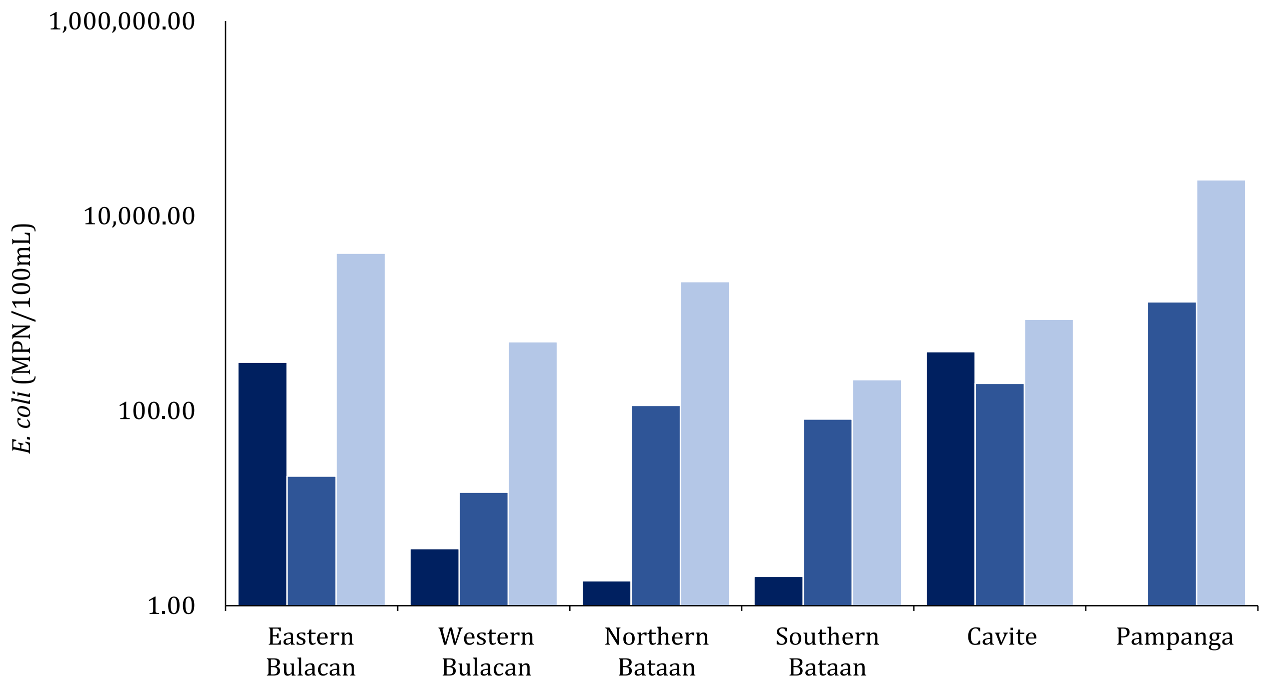

Figure 5.8. *E. coli* levels in ponds, coastal areas, and river tributaries or water sources in February 2015.

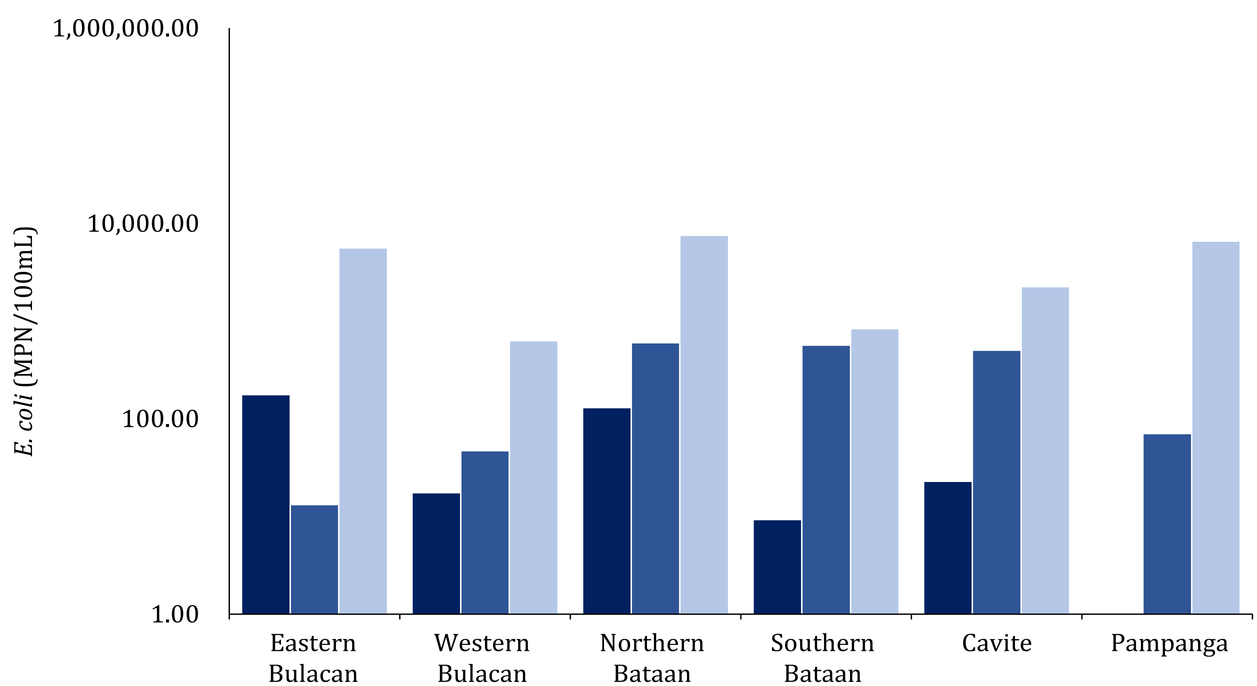

Figure 5.9. *E. coli* levels in ponds, coastal areas, and river tributaries or water sources in April 2015.

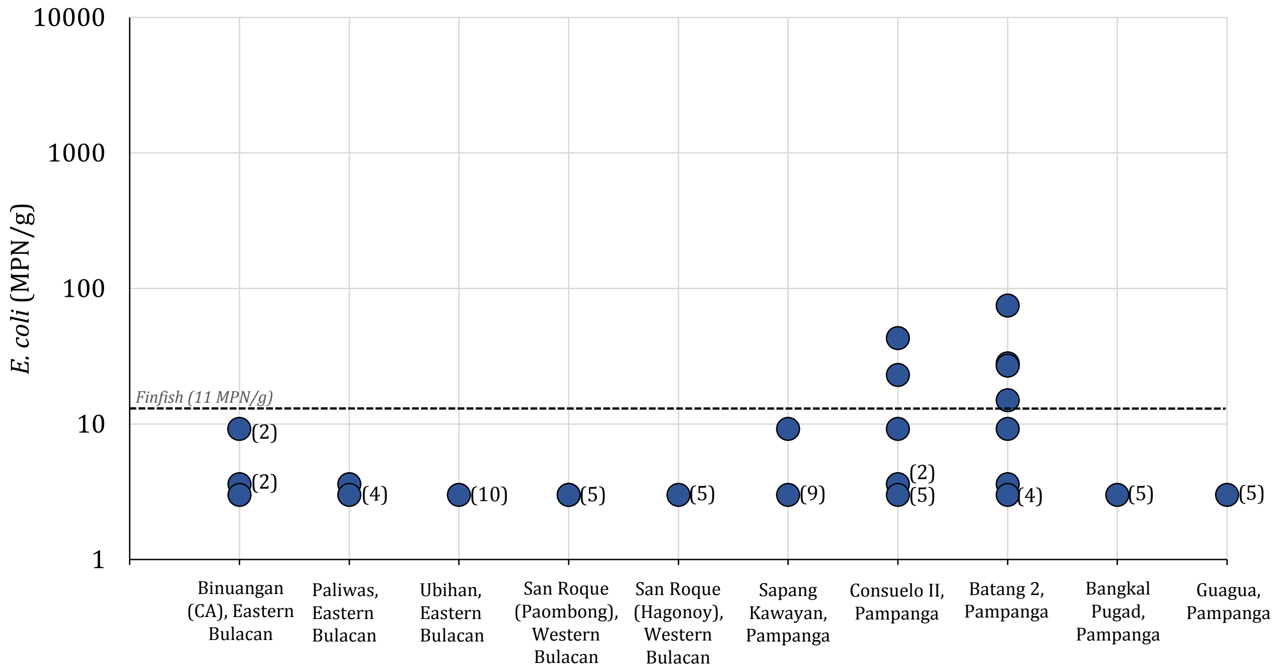

Figure 5.10. *E. coli* levels in milkfish samples collected from aquaculture farms along Manila Bay in September and November 2014.

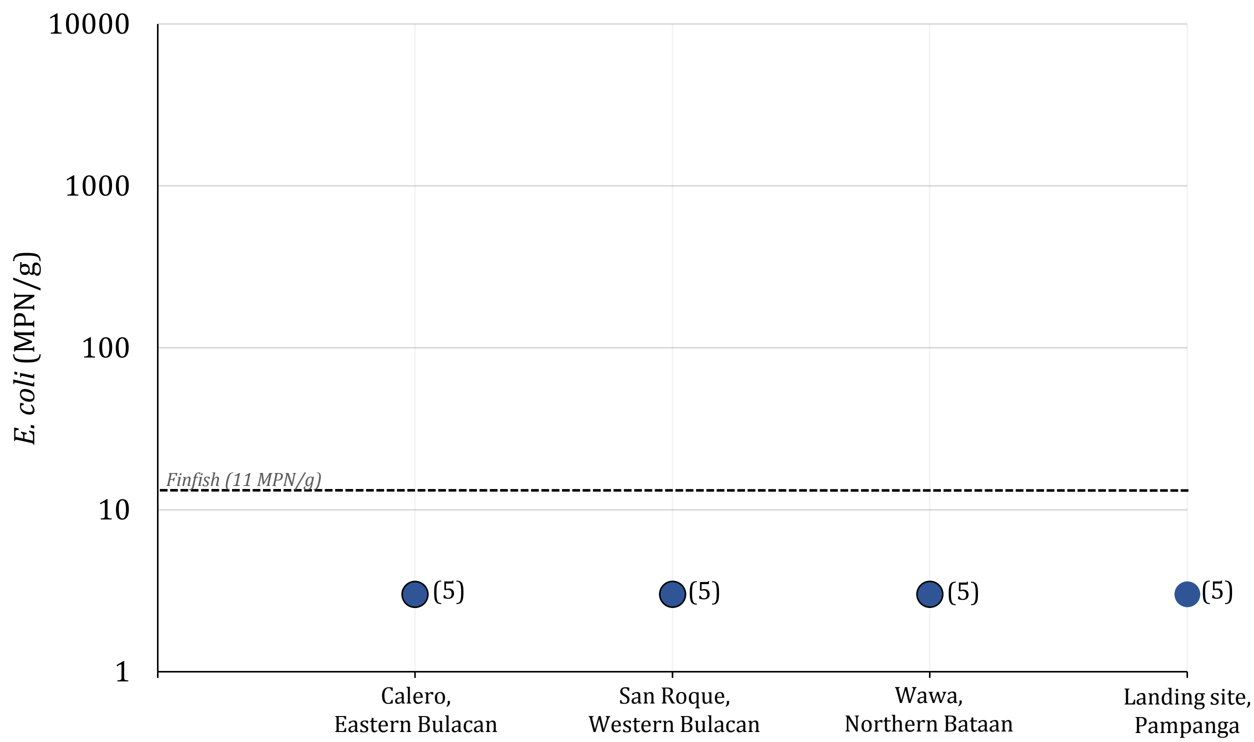

Figure 5.11. *E. coli* levels in milkfish samples collected from aquaculture farms along Manila Bay in February and April 2015.

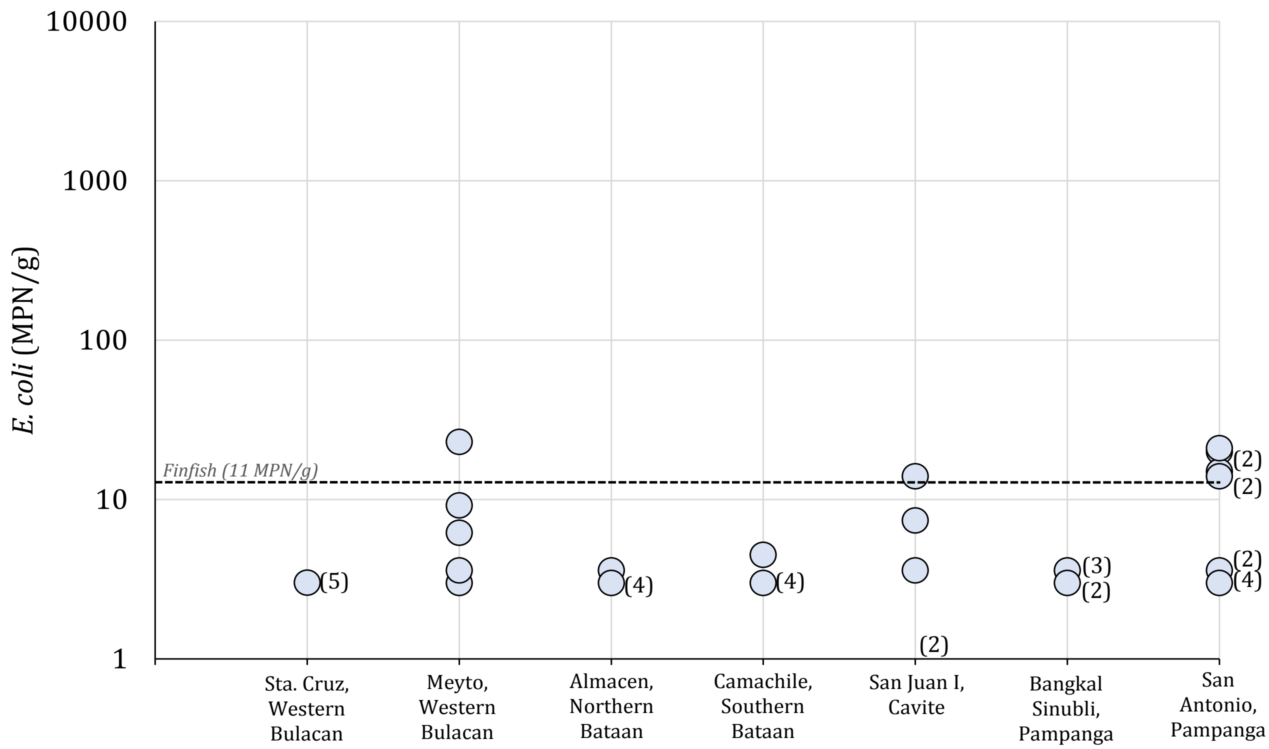

Figure 5.12. *E. coli* levels in tilapia samples collected from aquaculture farms along Manila Bay in September and November 2014.

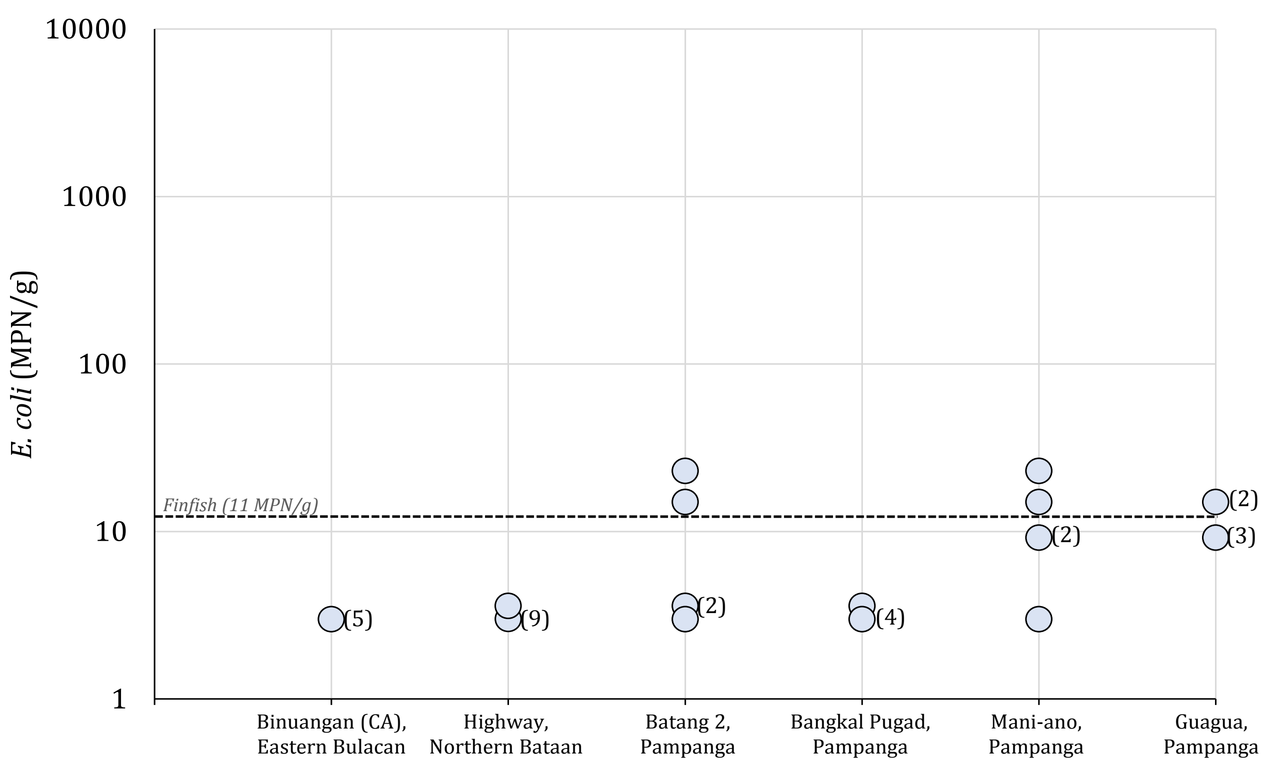

Figure 5.13. *E. coli* levels in tilapia samples collected from aquaculture farms along Manila Bay in February and April 2014.

E. coli IN CRUSTACEAN

Results for the *E. coli* levels in the crustacean samples collected are reflected in Figures 5.14, 5.15, 5.16 and 5.17.

E. coli levels in September and November 2014 ranged from <3 MPN/g to 120 MPN/g; the highest value noted in shrimp sample collected from Tawiran, Eastern Bulacan, where three out of five samples collected in the site failed to meet the FDA Standard Limit for *E. coli* concentration in crustaceans of 11 MPN/g (Fig. 5.14 & 5.16). Out of the 40 samples collected, a total of 7 crustacean samples (17.5%) – 4 shrimp and 3 crab samples – exceeded the FDA Standard Limit. Four out of 20 (20%) shrimp samples – 15% from Tawiran, Eastern Bulacan and 5% from Consuelo II, Pampanga – failed to meet this limit, while only 15% of the crab samples (10% from San Agustin and 5% from Sta. Cruz, both from Western Bulacan) had *E. coli* levels greater than 11 MPN/g.

On the other hand, 28% of the shrimp samples in February and April 2015 failed to meet the FDA Standard Limit (8% from Binuangan, Eastern Bulacan (CA) and 20% or 5/5 from Bangkal Sinubli, Pampanga); while 4% of the crab samples (collected from Bangkal Sinubli, Pampanga) exceeded the standard (Fig. 5.15 & 5.17). *E. coli* levels in February and April 2015 ranged from <3 MPN/g to 240 MPN/g, with maximum value recorded in shrimp sample from Bangkal Sinubli, Pampanga.

E. coli IN BIVALVES

Results for the *E. coli* levels in the bivalve samples are reflected in Figures 5.18, 5.19, 5.20, and 5.21.

Of the 35 oyster samples collected in September and November 2014, four (11.43%) exceeded the FDA Standard Limit for *E. coli* concentration in bivalves of 16 MPN/g (5.71% from Pamarawan, Eastern Bulacan, 2.86% in both Bacoor Bay and Bucana, Cavite) (Fig. 5.18). On the other hand, 15% of the mussel samples collected (all samples from Bacoor, Cavite) failed to meet the FDA Standard Limit for bivalves (Fig. 5.20). *E. coli* levels in the samples collected in September and November 2014 ranged from <3 MPN/g to 1, 100 MPN/g with the highest value observed in oyster samples collected from Pamarawan, Eastern Bulacan.

On the contrary, 17.5% of the oyster samples collected in February and April 2015 failed to meet FDA Standard Limit for bivalves of 16 MPN/g (12.5% from Pamarawan, Eastern Bulacan and 5% from Bucana, Cavite), while 30% of the mussel samples collected had *E. coli* levels greater than 16 MPN/g (5% from Binuangan, Eastern Bulacan, 12.5% from San Roque, Western Bulacan, 10% from Samal, Northern Bataan, and 2.5% from Bacoor Bay, Cavite (Fig. 5.19 & 5.21). *E. coli* levels in the samples collected in February and April 2015 ranged from <3 MPN/g to 1,100 MPN/g with the maximum value noted in oyster samples from Pamarawan, Eastern Bulacan where five out of five samples exceeded FDA Standard Limit for bivalves.

Discussion

Seasonal and spatial distribution of coliform bacteria in pond water and water sources

Several factors such as rainfall, population, livestock, pets, presence of waterfowls, and other aquaculture practices affect the concentration of these microorganisms (Oram, 2014).

Lower levels of total coliform, fecal coliform, and *E. coli* observed during the months of February and April 2015 (dry season) compared to September and November 2014

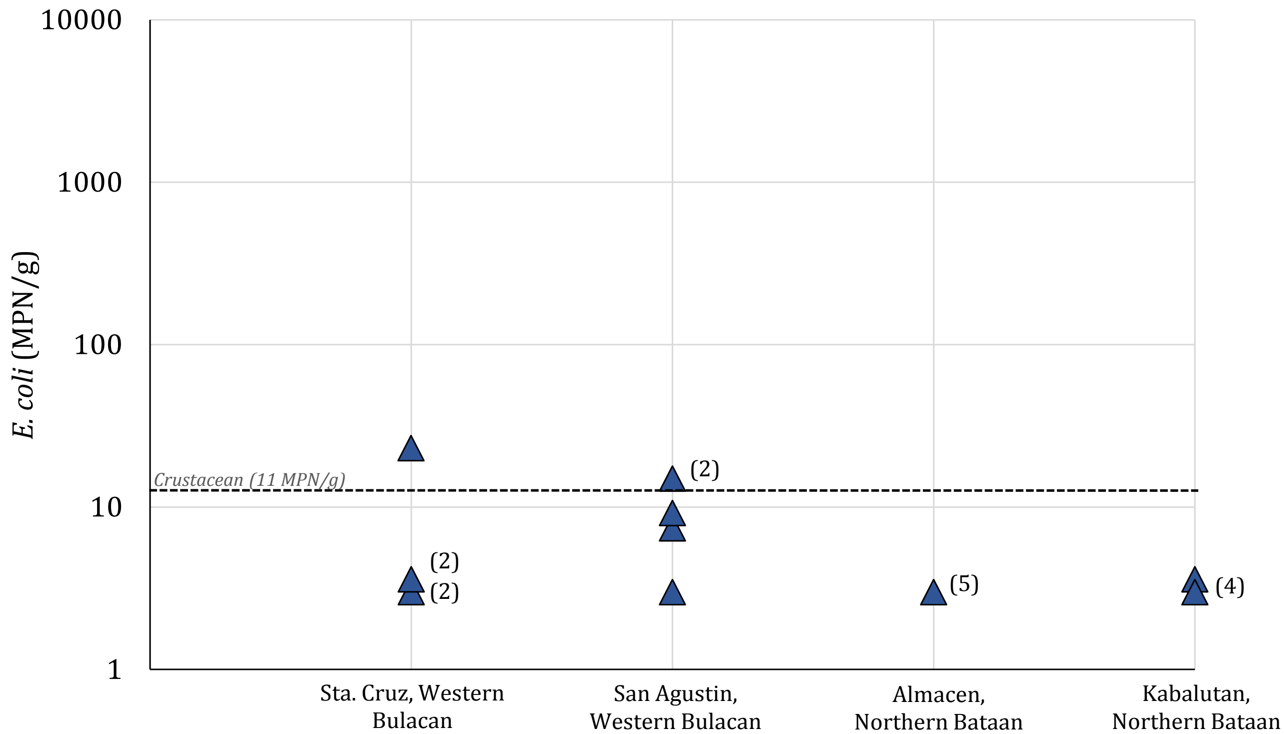

Figure 5.14. *E. coli* levels in crab samples collected from aquaculture farms along Manila Bay in September and November 2014.

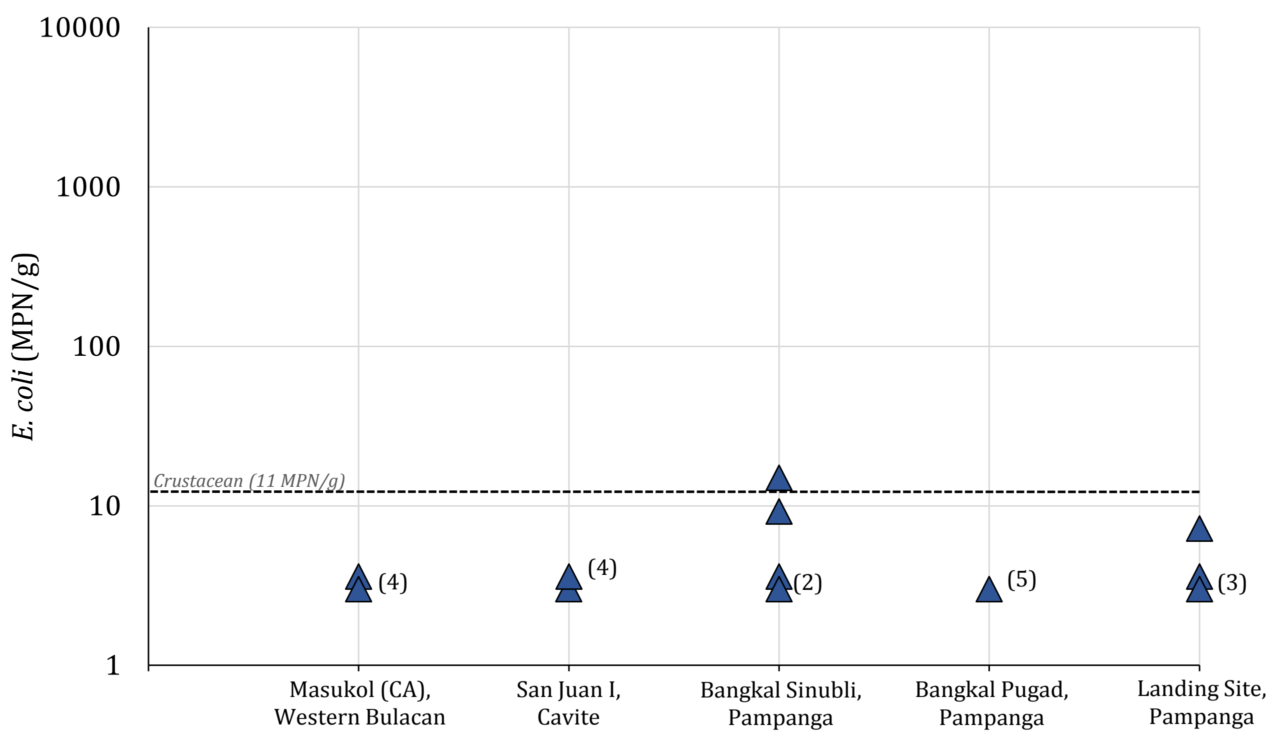

Figure 5.15. *E. coli* levels in crab samples collected from aquaculture farms along Manila Bay in February and April 2014.

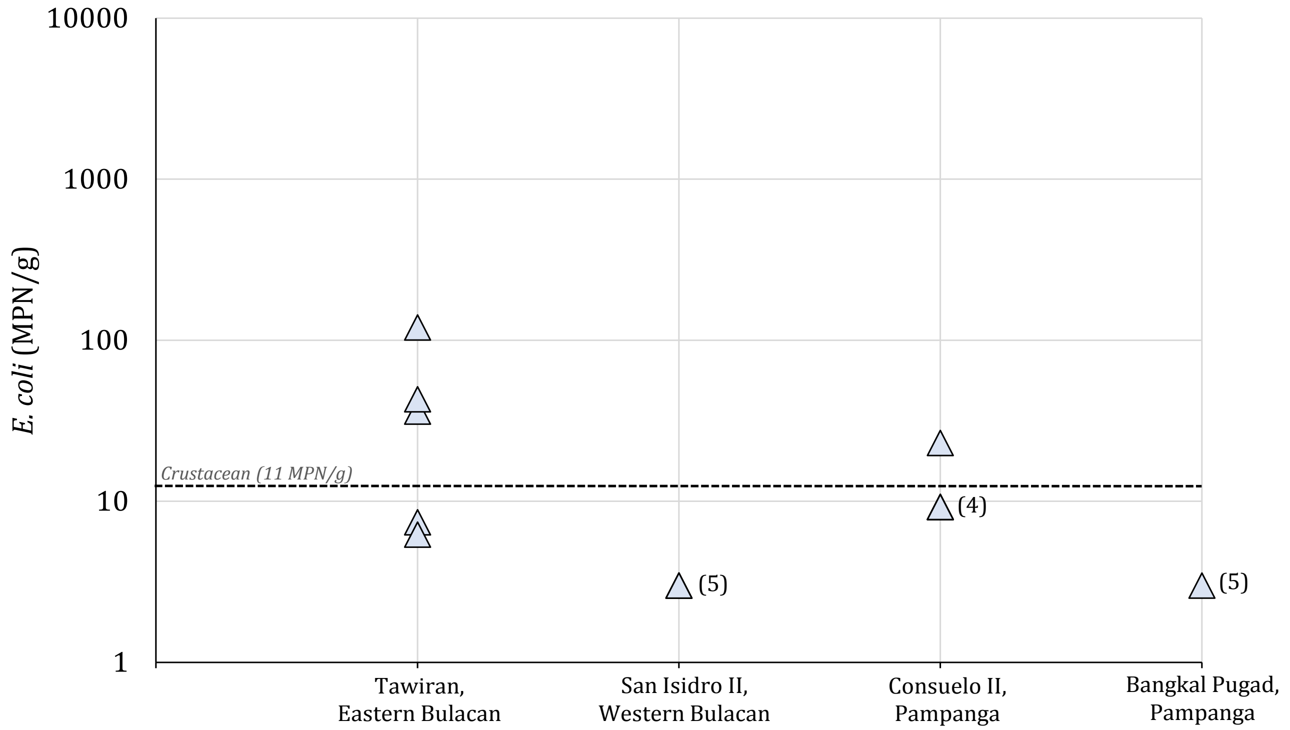

Figure 5.16. *E. coli* levels in shrimp samples collected from aquaculture farms along Manila Bay in September and November 2014.

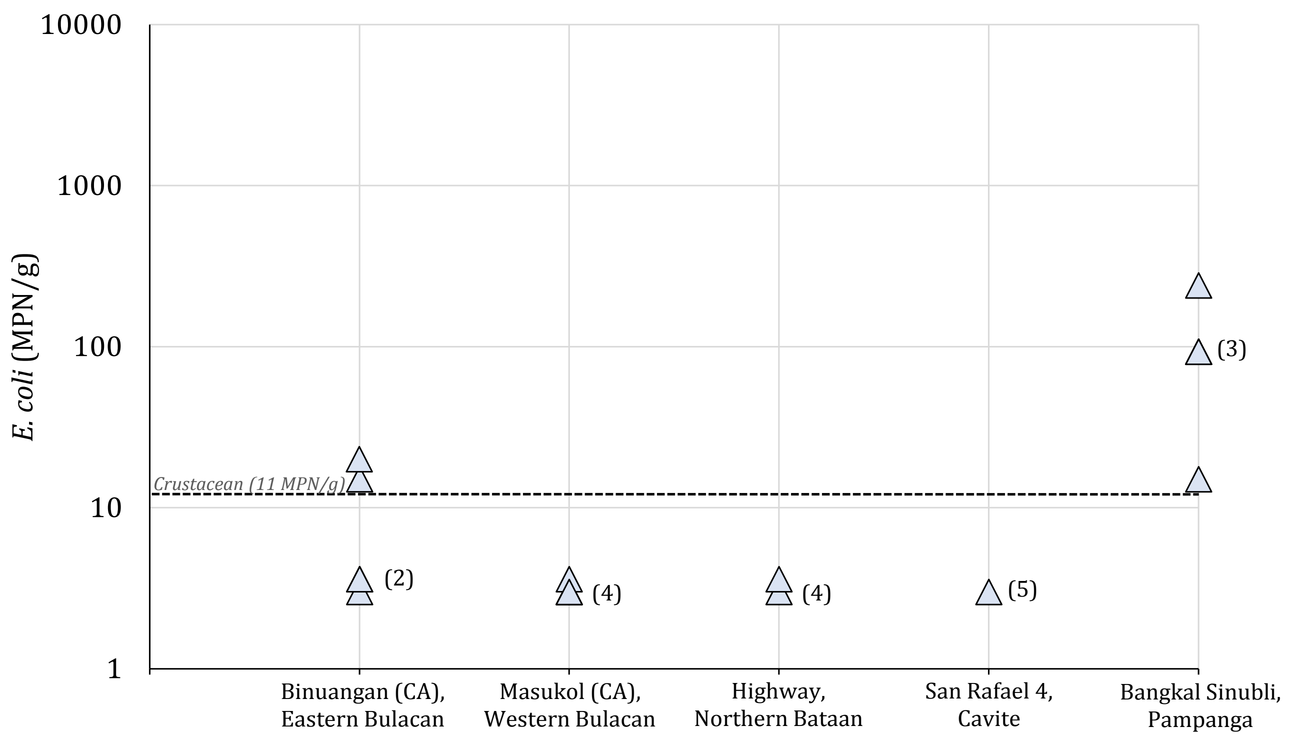

Figure 5.17. *E. coli* levels in shrimp samples collected from aquaculture farms along Manila Bay in February and April 2014.

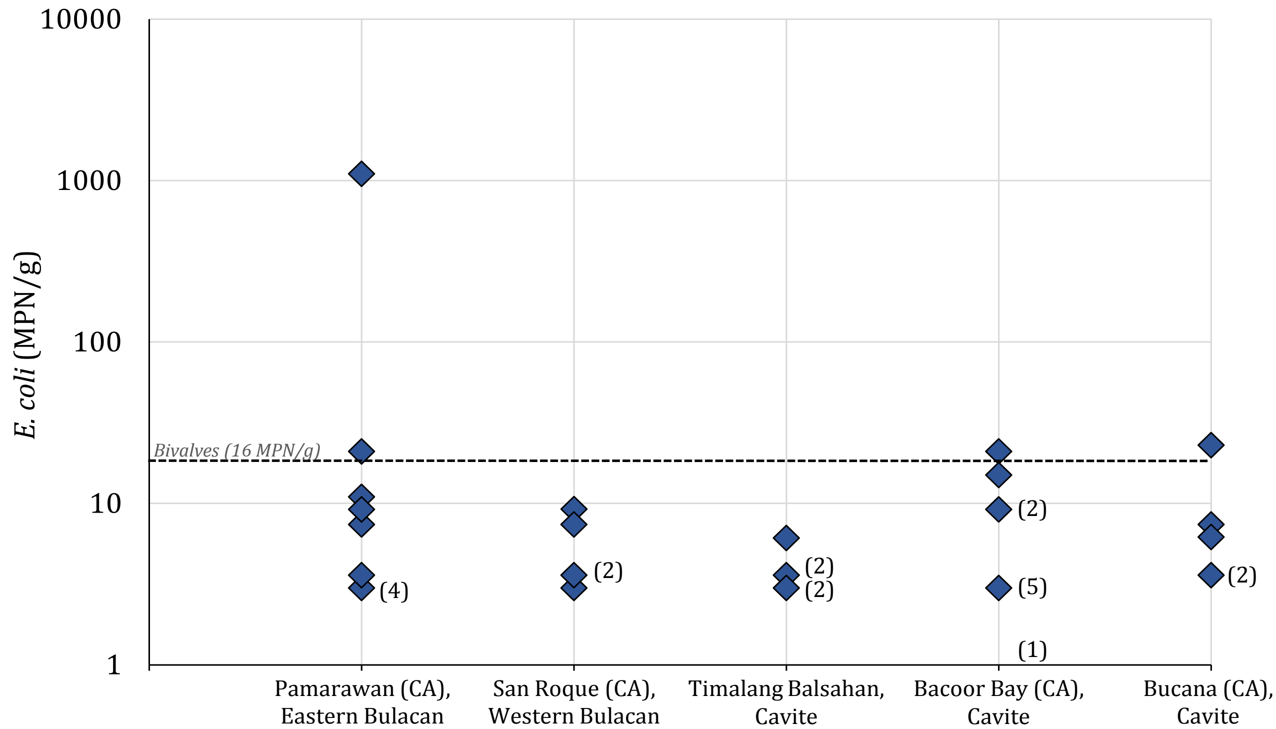

Figure 5.18. *E. coli* levels in oyster samples collected from aquaculture farms along Manila Bay in September and November 2014.

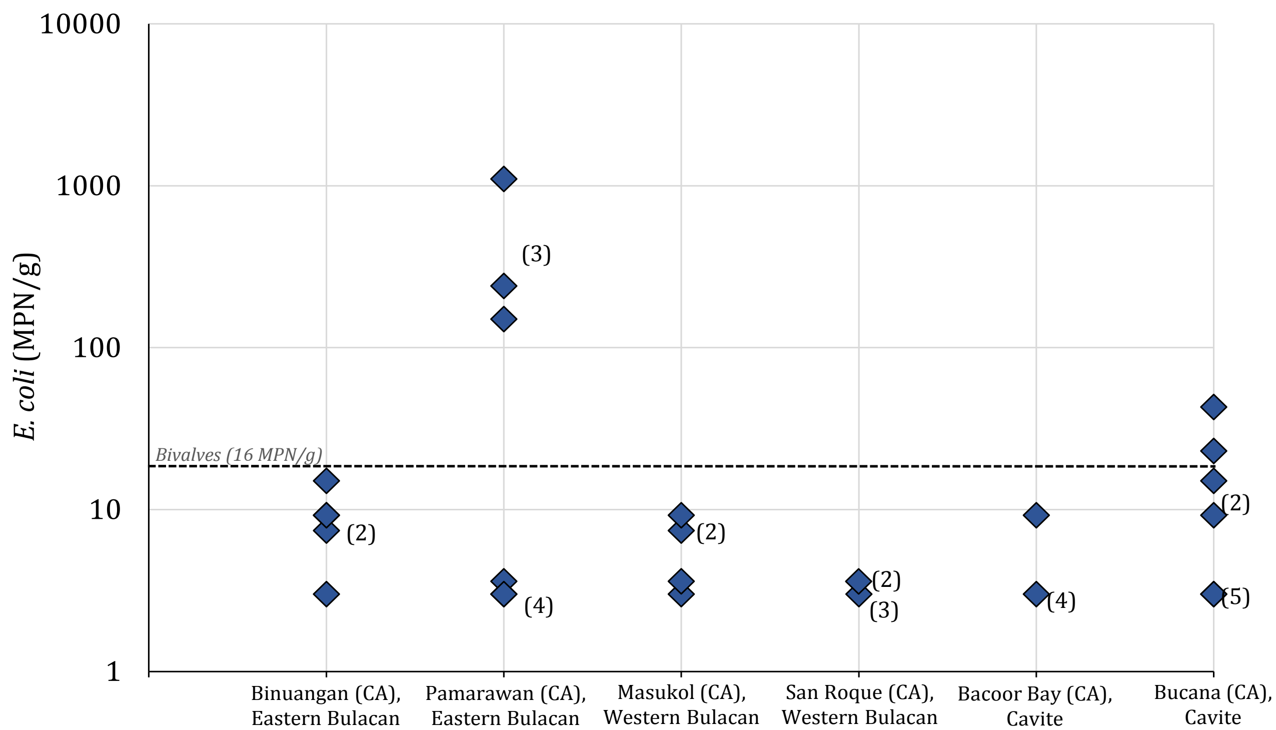

Figure 5.19. *E. coli* levels in oyster samples collected from aquaculture farms along Manila Bay in February and April 2014.

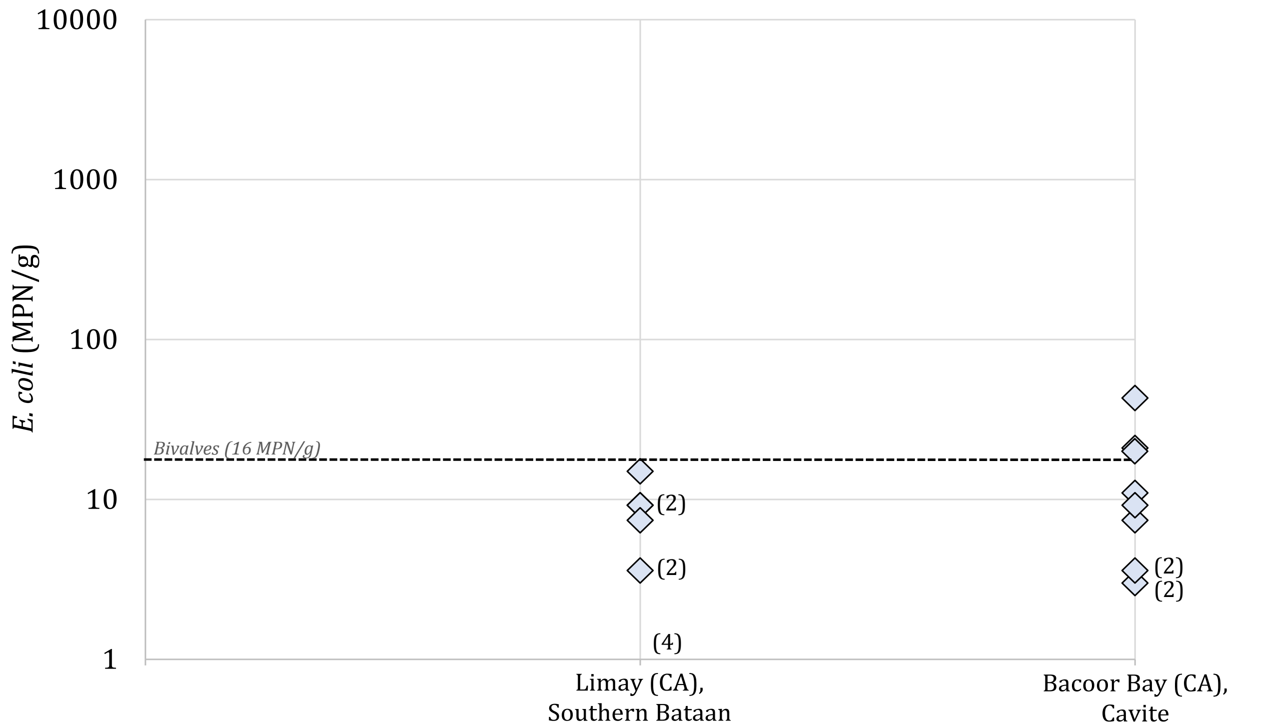

Figure 5.20. *E. coli* levels in mussel samples collected from aquaculture farms along Manila Bay in September and November 2014.

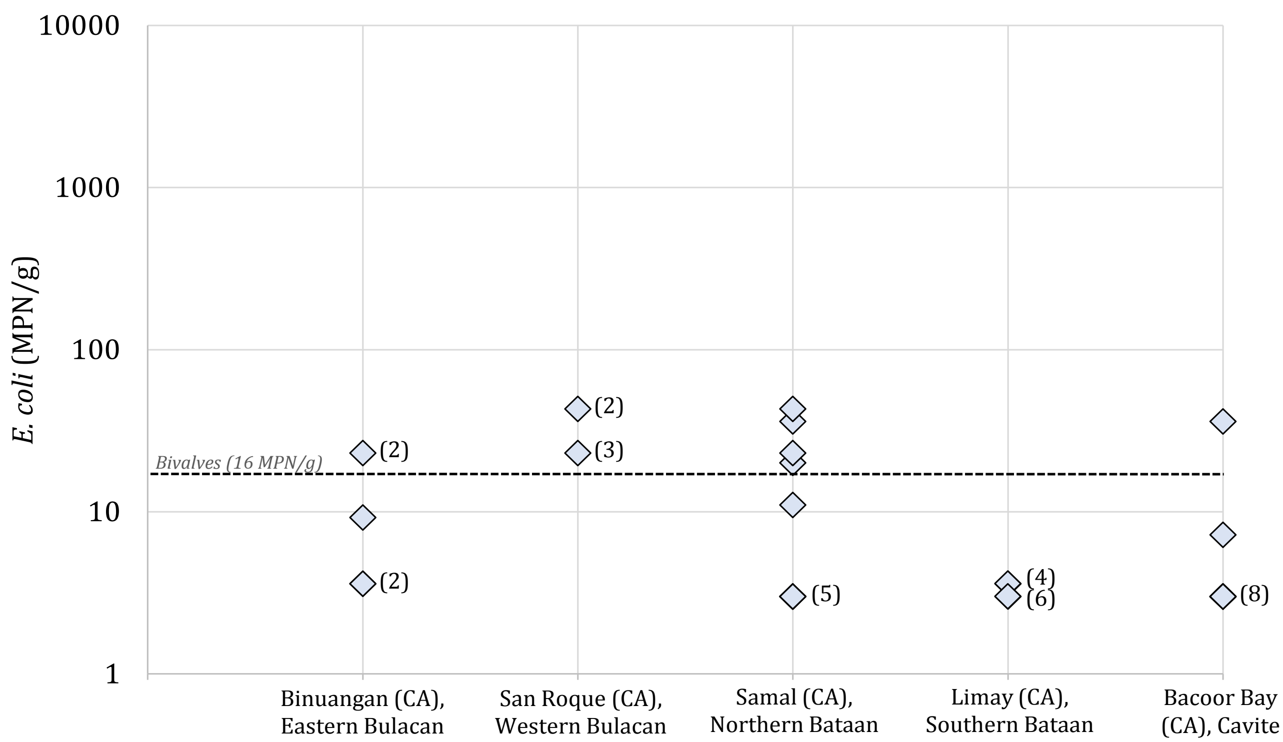

Figure 5.21. *E. coli* levels in mussel samples collected from aquaculture farms along Manila Bay in February and April 2014.

(wet season) may be attributed to the differences in rainfall. Average rainfall in the sampled provinces during the wet season (233.5375) is 16.45 times greater than that of the dry season (13.5875). Rainfall causes non-point sources of pollution, like surface runoffs that carry contaminants, which include sediments, nutrients, bacteria from animal and human wastes, pesticides, metals, and petroleum by-products from the land (USGS, 2015; US EPA, 2016).

Although with some inconsistencies, data on livestock and poultry, pets, and waterfowls – presence and number – from a separate study on aquaculture activities and anthropogenic activities in the aquaculture farms simultaneously conducted by the team (Chapter 2)– associate with the data on the total coliform, fecal coliform, and *E. coli* levels in the aquaculture farms; sites with a relatively higher concentration of these microorganisms also had a relatively higher number of livestock and poultry, pets, and waterfowls. This may be due to the fact that total coliform, fecal coliform, and *E. coli* are found in the intestines and feces of warm-blooded animals, such as pets, livestock, poultry, wild animals, and humans (Washington State Department of Health, 2011; Meals *et al*, 2013).

It was also observed that aquaculture farms less than 10 meters from the residential area had relatively high coliform concentrations. The distance of the ponds to the residential areas might be a factor for the levels of microorganisms in the ponds since surface runoffs may collect contaminants from residential areas through faulty or not properly maintained septic tanks, pet droppings, and other wastes (Harvey, 2016).

Some of the aquaculture farms that performed liming, pond drying, removal of waste, and disinfection had relatively lower coliform concentrations compared to those that did not employ these activities. Aquaculture farms that used urea (46-0-0) and complete (14-14-14) fertilizers had relatively higher coliform levels compared to those that utilize chicken manure, which unexpectedly had relatively lower coliform concentrations. The increase in the available nutrients in the aquaculture farms owing to the application of fertilizers, may have favored the growth of bacteria. This is supported by the studies of Baluyut (1989) and Stander (2012) where they had mentioned that liming, pond drying, removal of waste, and disinfection eradicated unwanted or wild species along with undesirable microorganisms, while fertilization stimulated and maintained growth of natural food.

Among the aquaculture farms that administered the following feeding materials: *lablab*, *lumot*, low value feed, commercial feed, bread, and surface plankton; those that utilize natural food – *lablab* and *lumot* – had a lower coliform bacterial content as compared to those that utilized bread. This maybe because the broadcasting of bread in the pond attracts wild bird species and water fowls; and where waterfowls and birds flock, fecal coliform counts can surge. Data on the use of low value feed, commercial feed, and surface plankton did not correlate with the data on the coliform levels in the ponds.

As can be observed in the maps, highest total coliform, fecal coliform, and *E. coli* levels in the aquaculture farms and coastal areas were noted mostly in Pampanga and Cavite. This may be caused by the differences in the spatial segregation of the blocks. Pampanga River, the major river system of the Pampanga province, had failed the standards for DO and BOD of 5mg/L and 10mg/L, respectively, in all sampling stations as reported in the Manila Bay Atlas in 2007. Dissolved oxygen (DO) is oxygen dissolved in water. It is necessary for the sustenance of fish life and other aquatic organisms. Low DO suggests incapacity to harbor life (Lee, 2005). On the other hand, BOD or biological oxygen demand represents the amount of oxygen (mg/L) needed by bacteria and other microorganisms to oxidize organic matter in an area; higher BOD means lower available oxygen for aquatic organisms (Palanna, 2009). The Angat River, which passes through NCR merges with the Pampanga River before discharging into the bay. This means that possible pollutants picked up by the Angat River will add to what is already present in the Pampanga River. Furthermore, upland Pampanga has the highest agricultural and fishpond areas. Agriculture runoff is considered a non-point source of pollution and a major contributor of contamination (US EPA, 2012).

It is interesting that Cavite, which is located south of Manila Bay where the bay connects to the sea and contamination is supposedly minimal, was observed to have the highest bacterial levels. This may be attributed to the fact that Cavite has the highest population and most built-up areas among the coastal provinces of the bay (PSA, 2010; Manila Bay Atlas, 2007). The increasing population and urbanization in the province increases the nonpoint sources of pollution such as urban runoffs, which are usually contaminated with organic matter and bacteria, among other pollutants. In addition, urbanization also increases combined sewer flow, which may also cause contamination (US EPA, 1980).

Seasonal distribution of coliform bacteria in farmed fishery resources

E. coli levels in farmed fishery resources were higher during the dry season, with the exception of crab and milkfish. *Escherichia coli* is a typical mesophile, which grows optimally at 39°C with a minimum of 8°C and a maximum of 40° (Madigan *et al*, 2012). This suggests that as the temperature increases, growth of *E. coli* is favored.

The decrease in the *E. coli* levels in crab and milkfish samples during the dry season may be attributed to the *E. coli* levels in the water column. Fish, being cold-blooded animals, cannot be a natural host to *E. coli*, as mentioned in the study of Apun et.al. (as cited in Barbosa *et al*, 2104). It only acquires the microorganisms when ingesting food contaminated with fecal matter and/or presence of *E. coli* in the water, which may enter the fish through ingestion and penetration through lacerations (Zhuikov, 2008; Missouri Department of Natural Resources, 2014). This means that *Escherichia coli* level in fishery commodities is associated to the *E. coli* level in the water medium; higher *E. coli* concentration in the water may result in higher *E. coli* concentrations in the fishery commodities and vice versa.

However, some aquaculture species do not have the ability to regulate their body temperature and are easily influenced by the temperature around them (Fishresearch.org, 2009). This means that as the temperature of the medium increases, the temperature of their body also increases, which in turn causes an increase in their *E. coli* levels during warmer temperatures (dry season) as was demonstrated by the increase in the number of samples that failed to meet the standard limits during the dry season.

Conclusion

Total coliform, fecal coliform, and *E. coli* in water tended to increase in the wet season, their approximate average concentrations during the study being 8,747 MPN/100mL, 2,808 MPN/100mL, and 1,216 MPN/100mL, respectively. Those in the dry were lower at around 6,255 MPN/100mL, 1,223 MPN/100mL, and 286 MPN/100mL, respectively. More water samples tended to exceed the DENR Standard Limit for Total Coliform of 5,000 MPN/100mL in the wet season than in the dry season (roughly 25 vs. 10%). Farmed fishery resources, on the other hand, were more contaminated in the dry season favored by the warmer temperature. The following are the rough percentages of exceedance of the FDA standard limits in decreasing order: 25% of the mussel samples, 24% of shrimp, 16% of tilapia, 14.67% of oyster, 8.89% of crab, and 6.6% of the milkfish.

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GLOSSARY

Aeration – is the mixing of air and water by wind action, or by air forced through water, generally refers to a process by which oxygen is added to water.

Algae – is a diverse group of aquatic organisms that can range from the microscopic (microalgae) to large seaweeds (macroalgae) that have the ability to conduct photosynthesis.

Algal bloom – is a high density or rapid increase in abundance of algae.

Amino acid – is a class of chemical of chemicals containing both an amino and an acidic group in the molecule. They are present in the uncombined form in tissues, and when combined with each other constitute the building blocks of proteins and peptides.

Ammonia – is the gas NH_3 with a characteristic pungent, irritating, odour.

Ammonium – is the ionized form of ammonia, NH_4^+.

Ammonium phosphate – is a type of inorganic fertilizer commonly used in aquaculture with N-P-K concentration of 16-20-0 (16%N-20%P-0%K).

Anthropogenic – is used to describe changes in nature made by people or human activities.

Antibiotics – are type of antimicrobial drug, also called antibacterials, used in the treatment and prevention of bacterial infection, by either killing or inhibiting the growth of such bacteria.

Aseptic technique – is a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by microorganisms.

Aquaculture – is fishery operations involving all forms of propagating, raising, and breeding fish and other fisheries species in fresh, brackish, and marine water impoundments.

Aquafarm/Aquaculture farm – includes facilities such as fishponds, shellfish growing areas, and fish pens, installed in either coastal or inland areas, used for controlled rearing and growing of aquatic organisms.

Benthic organism – is an organism also known as benthos that lives in and on the bottom sediment of the aquatic environment.
Bioaccumulation- refers to the gradual build-up of a chemical from the environment in a living organism.

Bio-filter – is a living organism used capture and biologically degrades pollutants thereby aiding in controlling pollution.

Biological Oxygen Demand (BOD) – is the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in a given water sample at a certain temperature over a specific time period.

Biological pollution – is the movement of organisms, either accidentally or deliberately, from the places where they evolved to new environments where a lack of natural competition permits explosion of their population.

Bio-magnification – refers to the increase in concentration of a substance in a food chain, or the tendency of a chemical to concentrate as they move from one trophic level to the next.

Brackishwater – is a mixture of fresh and seawater with salinity values ranging from 0.5ppt to 32 ppt.

via canoes, rafts or motorized outboard boats.

Buffer zone – is a space between the aquaculture area and the sensitive ecosystem.

Capture Fisheries – involves fishing or catching of aquatic resources in fresh and marine waters using various instruments or devices and accessories

Carcinogenic – is used to describe any substance or chemical which has the potential to cause cancer.

Certified reference material – is a standard or control to check the quality or metrological traceability of products, to validate analytical measurements methods, or for the calibration of instruments.

Chronic – refers to a condition that is persisting for a long time or constantly recurring.

Coenzyme – is a substance that usually contains a vitamin or mineral that works with an enzyme to initiate or aid the function of that enzyme.

Commercial Fisheries – involves fishing operations outside the municipal waters, which is beyond 15 kilometers from the coastline, using vessels 3 gross tones or larger.

Complete fertilizer – is a type of inorganic fertilizer commonly used in aquaculture containing all N-P-K nutrients at a concentration of either 14-14-14 (14%N-14%P-14%K) or 15-15-15 (15%N-15%P-15%K).

Cropping – refers to the cycle of activities related to the growth and harvest of aquatic culture species. These activities include pond preparation, fertilization, liming, stocking, feeding, and harvesting, among others.

Derris root – contains selective poison called rotenone readily affecting the finfishes but not the shrimps at certain concentrations.

Dissolved oxygen (DO) – is the amount of elemental oxygen, O_2, in solution under existing atmospheric pressure and temperature. It comes from the mixing of oxygen from the atmosphere with the water through wave actions and through the photosynthesis of plants in the water.

Dry matter ratio – is the ratio of the amount of dry matter of feeds needed to produce one kilogram dry matter of fish.

Dry season – is a period of low rainfall which starts from November to May.

Effluent – is the liquid discharge from a rearing facility, treatment plant, or industry
Enteric Organisms- are organisms normally or pathogenically occurring in the intestines of human and other animals

Environmental Impact Assessment (EIA) – is a multidisciplinary approach to determine the positive or negative impact a project might have on its location's surrounding environment.

Eutrophication – is the enrichment of an ecosystem with chemical nutrients, typically compounds containing nitrogen and phosphorus, causing a dense growth of algae which eventually dies. Decomposition of dead algae results to oxygen depletion which leads to massive death of fish and other fishery resources.

Extensive Culture – is a culture system with low densities of fish and which depends on food items present in the culture system.

Facultative anaerobic organism – is an organism that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent.

Feed Conversion Ratio (FCR) – is the ratio of the amount of feed used to increase the biomass by one kilogram.

Fortified sample – is an aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory.

Good aquaculture practices – are series of considerations, procedures, and protocols designed to foster efficient and responsible aquaculture production and expansion and help ensure final product quality, safety, and environmental sustainability.

Haematological/haematology – is a term relating to blood or blood producing organs. It is a branch of medicine concerned with the study diagnosis, treatment, and prevention of diseases related to blood.

Half-life – is the time required for a entity to reduce to half its initial value/quantity.

Hardness – is the ability of water to neutralize soap, due to the presence of calcium and magnesium, usually expressed as parts per million equivalents of calcium carbonate. It refers to the calcium and magnesium ion concentration in water on a scale of very soft (0-20ppm as $CaCO_3$), soft (20-50ppm), hard (50-500ppm) and very hard (55+ ppm).

Hatchery – is a facility where the hatching of fish or other fishery resources is artificially controlled for commercial purposes.

Hazardous – is a term to describe substance, materials, and activities that involves risk or danger.

Heavy metals – are metals with relatively high densities, atomic weights, or atomic numbers. Some heavy metals are either essential nutrients (such as iron, cobalt, and zinc), or relatively harmless (typically ruthenium, silver, and indium) but can be toxic in large amounts (including cadmium, mercury, lead, and arsenic).

Hemoglobin – is a red protein responsible for transporting oxygen in the blood of vertebrates.

Hepatic – is a term of or relating to the liver.

Hydrogen sulfide – is an odorous, soluble gas, H_2S, resulting from anaerobic decomposition of sulfur-containing compounds, especially proteins.

Hyperkeratosis – is the thickening of the outermost layer of the epidermis (stratum corneum), often associated with the presence of an abnormal quantity of keratin, and also usually accompanied by an increase in the granular layer.

Hyperpigmentation – is a condition in which patches of skin become darker in color than the normal surrounding skin due to the excess of melanin.

Hypopigmentation – is a condition in which skin color is loss due to the absence of normal amounts of melanin caused by disease, injury, burns or other trauma to the skin.

Hypoxia – is an environmental phenomenon where the concentration of dissolved oxygen in the water column decreases to a level than can no longer support living aquatic organisms.

Immunological/immunology – is a branch of biomedical science that covers the study of immune systems in all organisms.

Immunosuppressed/immunosuppression – is a reduction of the activation or efficacy of the immune system.

Infectious dose – is the amount of pathogen required to cause an infection in the host.

Inorganic – is a term used to describe a material or compound which does not have the structure or characteristics of living organisms or products without a carbon basis.

Intensive Culture – rearing of fish at densities greater than can be supported in the natural environment; utilizes high water flow or exchange rates, aeration, and requires the feeding of formulated feeds

Intoxication – is an abnormal state that is essentially a poisoning.

Lablab – is an algal mat complex with some species of zooplankton grown on the pond bottom to serve as food for the culture species. The mat includes species of *Oscillatoria, Lyngbia, Spirulina*, *Anabaena*, *Diatoms*, protozoans, copepods, polychaete worms, and mollusk larvae.

Liming – is the application of calcium and magnesium compounds to the soil for the purpose of reducing soil acidity.

Lipid – is a group of organic compounds that do not interact appreciably with water, including fats, oils, hormones, and certain components of membranes.

Lumot – are filamentous algae belonging to the species *Chaetomorpha linum, Enteromorpha intestinalis, Cladophora, Lyngbia*, and *Spirogyra sp.* grown in the pond to serve as natural food for the culture species.

Macromolecules – are very large molecule commonly created by polymerization of smaller subunits (monomers), including carbohydrates, proteins, lipids, and nucleic acids.

Macrophytes – is an aquatic plant visible to the naked eye that grows in or near water and is either emergent, submergent, or floating.

Mesophile – is an organism (mainly microorganisms) that grows best in moderate temperature, typically between 20 and 45 °C.

Metabolic rate – is the rate of at which an organisms' body burns calories. It is the amount of energy used by an organism to sustain itself and perform its various activities.

Methemoglobin – is a form of heamoglobin that is incapable of carrying oxygen, sometimes found in the blood after certain poisonings, such as nitrite.

Method validation – is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability, and consistency of analytical results.

Municipal Fisheries – involves fishing operations in coastal waters, within 15 kilometers from the coastline, with or without the use of vessels less than 3 gross tons.

Mutualistic – is a symbiotic interaction between different species in which each individual benefits from the activity of the other.

Natural food – is a type of food found naturally in the pond which can be enhanced by liming and fertilization. It may in clude detritus, bacteria, plankton, worms, insects, snails, aquatic plants and fish.

Nitrate – is the NO_3^- ion.

Nitrification – is a process (normally biological) through which ammonia is biologically oxidized to nitrite and then nitrate.

Nitrite – is the NO_2^- ion.

Nitrogen – is an odorless, gaseous element that makes up 78% of the Earth's atmosphere, and is a constituent of all living tissue.

Nucleic acids – are complex organic molecules consisting of many nucleotides linked in a long chain, essential for all forms of life. There are two types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

Nutrient – is a substance that provides nourishment essential for growth and the maintenance of life.

Organic – is term used to describe materials or compounds which have the characteristic of living organisms or products having a carbon basis.

Orthophosphate – is a compound containing the trivalent group PO_4^-. It is the simplest in a series of phosphate, and usually just called phosphate.

Pathogenic/pathogen – is an organism that is capable of causing diseases, directly or by excreting a toxin. Examples are viruses, bacteria, parasites, and moulds.

Periorbital swelling – is the appearance of swelling in the tissues around the eyes, called orbits.

Pesticide – is a substance used for destroying organisms harmful to cultivated plants or animals.

pH – is an expression of the acid-base relationship designated as the negative of the molar concentration of hydrogen ion in solutions; the value of 7.0 expresses neutral solutions; values below and above 7.0 represent increasingly acidic and basic solutions, respectively.

Phosphate – is the PO_4^- ion.

Phosphorus – is a chemical element with symbol P and atomic number 15.

Phytoplankton – is a microscopic plant suspended in water with little or no capability for controlling its position in the water mass; frequently referred to as algae.

Plankton – is mostly microscopic, aquatic plant and animal with little or no capability for controlling its position and serve as food for larger aquatic animals.

Point source – is a single identifiable source of air, water, thermal, noise or light pollution. It has a negligible extent, distinguishing it from other pollution source geometries.

Pollution – is the introduction of contaminants into the natural environment causing adverse change.

Polyculture – is the simultaneous cultivation of two or more non-competitive fish and/or other fishery resources in the same pond.

Productivity – is an average measure of the efficiency of production which can be expressed as the ratio of output to inputs in the production process.

Protein – is a large biomolecule consisting of one or more long chains of amino acid residues. It is essential in all living organisms as it performs a vast array of functions, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another.

R^2 value – is also known as the coefficient of determination. It is a statistical measure of how close the data are to the fitted regression line. 0% indicates that the model explains none of the variability of the response data around its mean.

Residual wastes – refers to waste which is not fit for prevention, re-use or recycling, and needs to be sent for disposal.

RQ value (risk quotient) – is calculated from an estimated exposure, divided by an estimated effect. This ratio is a simple, screening-level estimated that identifies high- or low-risk situations. If a value less than 1, then there is an acceptable risk. However, if the RQ greater than 1, there is an unacceptable level of risk and measures to reduce exposure should be taken.

Runoff – is the movement of landwater to the oceans, chiefly in the form of rivers, lakes, and streams.

Salinity – is the concentration of salt including sodium, potassium, magnesium, calcium, bicarbonate, carbonate, sulfate, and halides (chloride, fluoride, bromide) in water, usually expressed in parts per thousand (ppt).

Seasonal distribution – refers to variations in the occurrence of a certain phenomenon, substance, compound, and contaminant within a time series.

Semi-intensive Culture – depends largely on natural food which is increased over baseline levels by fertilization and/or use of sup-

Siphon – is a tubelike part especially of a mollusk (as a clam) usually used to draw in or squirt out water.

Sodium cyanide – is a highly toxic inorganic compound with the formula NaCN. It is used as an aid in capturing fish and eradicating unwanted species in fishponds.

Soluble – is used to describe a property of a solid, liquid, or gaseous chemical substance to dissolve in a solid, liquid or gaseous solvent.

Spatial distribution – refers to how a resources, activities, human demographics, and contaminants are arranged across a certain geographical surface. It is the physical location of salient features of a place.

Spiked concentration – is a known concentration of standard analyte added into a sample and run in the instrument. The resulting concentration, or "recovery" of the spiked material, demonstrates If the expected value can be measured accurately.

Sustainability – is the property of biological systems to remain diverse and productive indefinitely.

Teaseed – is a residue from oil processing of *Camellia sp*. seed containing 10-15% saponin, a chemical widely used to eradicate finfishes without toxic effect on crustaceans especially shrimps.

Temperature – is the measure of the hotness or coldness.

Thermo-tolerant – is used to describe organisms that are able to tolerate, but not thriving in, high temperatures.

Tobacco dust – contains nicotine which is nonselective type of poison used to eradicate unwanted species in the pond.

Trace amount – refers to extremely low or small concentration of a chemical component.

Trash fish – refers to captured fish having little or no market value as human food but used in the production of fish meal

Turbidity – is the measure of the presence of suspended or colloidal matter or planktonic organisms that reduces light penetration of water.

Urea – is a type of inorganic fertilizer commonly used in aquaculture with N-P-K concentration of 45-0-0 (45%N-0%P-0%K).

Uremic syndrome – is a serious complication of chronic kidney disease and acute kidney injury, which occurs when urea and other waste products build up in the body because the kidneys are unable to eliminate them.

Waste production ratio – refers to the ratio of the amount of waste that would be generated for every kilogram of fish produced.

Water flushing – is an essential activity during pond preparation which is done by allowing water into the pond and draining completely after holding for a minimum of 24 hours. Wastes are flushed out of the pond during draining.

Water Source – are water bodies, such as rivers, streams, seas, bays, and ground waters, where aquaculture farms obtain their water for culturing aquatic organisms.

Wet season – also known as rainy season, is the time of the year (starts at June and lasts till October) when most rainfall occurs.

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Aquafarms Info Sheet

NFRDI
5th/6th Flr., Corporate 101 Bldg., Mother Ignacia Ave., Quezon City

NATIONAL FISHERIES RESEARCH AND DEVELOPMENT INSTITUTE

Document Code :
Effectivity Date:
Revision No.: **0**

Block No.:	**Date:**	**Time:**	**Interviewer:**
Barangay/Municipality:			
Coordinates:			

CONTACT PERSONS

Name of Owner:	**Contact Number:**
Name of Caretaker:	**Contact Number:**
LGU/BFAR Representative:	**Contact Number:**
MFARMC Representative:	**Contact Number:**
Boat Operator:	**Contact Number:**

SITE AND FARM INFORMATION

Year when farm was established:	**Size of Pond:**

Type of Farm	**Source of Water/River Tributaries and Description**
☐ Pond Type	☐ Manila Bay: ____________
☐ Pen Type	☐ Saltwater other than Manila Bay: ____________
☐ Cage Type	☐ River: ____________
☐ Shellfish Growing Area	☐ Freshwater: ____________
	☐ Groundwater: ____________

Observe proper buffer zone from the sea or the river tributaries?	☐ Yes	☐ No
Use proper slopes and compaction to minimize erosion potential on embankments?	☐ Yes	☐ No
Provide grass cover on watersheds and gravel on farm roads and tops of embankments?	☐ Yes	☐ No
Presence of settling ponds (☐) or reservoir ponds (☐) or use wastewater treatment (☐) prior to water discharge?	☐ Yes	☐ No
Area prone to floods, storms, earthquakes and other natural calamity?	☐ Yes	☐ No
Allow livestock/poultry/wild/birdsanimals to walk on embankments or to wade in pond?	☐ Yes	☐ No

PRODUCTION METHODOLOGY

Water Quality Management

Water Discharge		**Water Intrusion**	
Date of Last Water Discharge:		**Date of Last Water Intrusion:**	
Frequency of Water Discharge	**Quantity/Amount of water:**	**Frequency of Water Intrusion**	**Quantity/Amount of water:**
☐ Daily ☐ Weekly		☐ Daily ☐ Weekly	
☐ Twice per Month	**Quality/Condition of water:**	☐ Twice per Month	**Quality/Condition of water:**
☐ Once per Month ☐ Others ____________		☐ Once per Month ☐ Others ____________	

Water Quality Monitoring (WQM)			**Pond Aeration**
Parameters Checked	**Equipment/Method Used**	**Frequency of WQM**	☐ Yes ☐ No
☐ Temperature ☐ pH ☐ Salinity ☐ Dissolved Oxygen ☐ Turbidity ☐ Others: ____________	____________ ____________ ____________ ____________ ____________ ____________ ____________	☐ Daily ☐ Weekly ☐ Twice per Month ☐ Once per Month ☐ Yearly ☐ Others ____________	**Type of Aerators *(if yes)*:** **Amount of Aerator per pond *(if yes)*:** **Operating Schedule *(if yes)*:**

POND PREPARATION

Activities during Pond Preparation	**Pond Preparation Frequency**	**Liming**
☐ Soil Testing ☐ Drying of Pond ☐ Removal of Residual Waste/Scraping ☐ Water Flushing/Jet Flushing ☐ Eradication of Unwanted Species	☐ Monthly ☐ After every cropping ☐ Once a Year ☐ Never ☐ Others: ____________	**Type of Lime Used:** **Amount of Lime Applied:**
☐ Liming ☐ Fertilization ☐ Others: ____________	**Date of Last Pond Preparation:**	**Liming Frequency:**

Fertilization			**Disinfection**		
Fertilizer	**Amount/ha**	**Frequency**	**Disinfectant**	**Amount/ha**	**Frequency**
☐Chicken Manure	________	☐ Monthly	☐Sodium Cyanide	________	☐ Monthly
☐Urea (46-0-0)	________	☐ After every cropping	☐Tobacco Dust:	________	☐ After every cropping
☐Complete (14-14-14)	________	☐ Once a Year	☐Teaseed	________	☐ Once a Year
☐$(NH_4)_3PO_4$	________	☐ Never	☐Antibiotic/Probiotic	________	☐ Never
☐Others: ________	________	☐ Others: ______	☐Others: ________	________	☐ Others: ______
☐None	________	____________	☐None	________	____________

APPENDIX B

NFRDI 5th/6th Flr., Corporate 101 Bldg., Mother Ignacia Ave., Quezon City	**Aquafarms Info Sheet** NATIONAL FISHERIES RESEARCH AND DEVELOPMENT INSTITUTE	Document Code : Effectivity Date: Revision No.:**0**

CULTURE SPECIES

Culture Species	Stocking Density (ind/ha)	Size at Stocking (g)	Source of Stock	Date of Stocking	Current size of stock
☐ Milkfish	______	______	______	______	______
☐ Tilapia	______	______	______	______	______
☐ Shrimp/Prawn	______	______	______	______	______
☐ Crab	______	______	______	______	______
☐ Seabass	______	______	______	______	______
☐ Oyster/Mussel	______	______	______	______	______
☐ Others: ______	______	______	______	______	______
☐ Others: ______	______	______	______	______	______

Culture Species	Cropping per Year	Date of Harvest	Harvest Method	Individual Size at Harvest (kg)	Total Production per cropping (kg)
☐ Milkfish	______	______	______	______	______
☐ Tilapia	______	______	______	______	______
☐ Shrimp/Prawn	______	______	______	______	______
☐ Crab	______	______	______	______	______
☐ Seabass	______	______	______	______	______
☐ Oyster/Mussel	______	______	______	______	______
☐ Others: ______	______	______	______	______	______
☐ Others: ______	______	______	______	______	______

FEEDING MANAGEMENT

Feeds	Feeding Species	Frequency	Amount per day	Amount per cropping	Feeding Method
☐ Lablab	______	______	______	______	______
☐ Lumot	______	______	______	______	______
☐ Low Value Fish	______	______	______	______	______
☐ Commercial Feed/Brand	______	______	______	______	______
☐ Bread	______	______	______	______	______
☐ Others: ______	______	______	______	______	______
☐ Others: ______	______	______	______	______	______
☐ None	______	______	______	______	______

ANTHROPOLOGICAL ASPECT

Pond from Residential Area	Estimated Population	Livestock/Poultry: Number	Pet: Number
☐ <10 meters	☐ < 100 individuals	☐ Pig: ______	☐ Dog: ______
☐ 10-50 meters	☐ 100-1,000 individuals	☐ Cow: ______	☐ Cat: ______
☐ 51-100 meters	☐ 1,000- 10, 000 ind.	☐ Chicken: ______	☐ Bird: ______
☐ 101-1,000 meters	☐ >10,000 individuals	☐ Duck: ______	☐ Others: ______
☐ 1,001- 10, 000 meters		☐ Others: ______	
☐ >10,001 meters			

Comfort Room	Other possible sources of contaminants observed in the area:
☐ Direct ☐ Pit (Indirect) ☐ Concrete Septic Tanks (Indirect)	

Employment Status

Number of Workers Employed:	**Origin of Workers:**
Pay Scale/Incentives for Workers:	**Worker Training:**

Community Relations

Assistance provided by local government:

Procedures used to communicate with local government:

Local fisherfolk/fish farmer organization in the area:

On-going conflicts:

Other fishery related issues and concern:

POLLUTION IN MANILA BAY AQUACULTURE FARMS
STATUS, IMPACT, AND REMEDIAL OPTIONS

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