

Influence of Biofloc Technology on Water Quality Parameters in Aquaculture: A Meta-analysis

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Session: 2021-2022



Fisheries and Marine Resource Technology Discipline

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Khulna-9208, Bangladesh

November 2022

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A Thesis

By

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Session: 2021-2022

The thesis submitted to the Fisheries and Marine Resource Technology Discipline in partial
fulfillments of the requirements for the degree of

Master of Science (MS)

in

Aquaculture

Fisheries and Marine Resource Technology Discipline

Khulna University

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November 2022

DECLARATION

Influence of Biofloc Technology on Water Quality Parameters in Aquaculture: A Meta-analysis

The results presented in this thesis with the above-mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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DEDICATED To

My BELOVED PARENTS



Acknowledgement

At first, all my admiration goes to supreme creator and ruler Almighty **ALLAH**, whose boundless blessings enabled me to successfully complete the thesis work for the degree of Master of Science in Fisheries and Marine Resource Technology from Khulna University under the Fisheries and Marine Resource Technology Discipline.

I wish to express my profound appreciation and heartfelt gratitude to my honorable supervisor, **Dr. Muhammad Abdur Rouf**, Professor, Fisheries and Marine Resource Technology Discipline, Khulna University, for his patient guidance, intense supervision, worthy suggestions, constructive criticism and inestimable help during my thesis write up.

I like to express my profound gratitude to my respected teacher **Dr. Md. Moshir Rahman**, Professor, Fisheries and Marine Resource Technology Discipline, Khulna University, for his cordial support, hearty inspiration, constructive criticism during the research period and manuscript preparation of the research work. I am also grateful and thanks to my teacher **Shaikh Tareq Arafat**, Associate Professor, Fisheries and Marine Resource Technology Discipline, Khulna University, for his scholastic guidance and sustained support to execute this thesis.

My gratitude also goes to **Dr. Md. Golam Sarower**, Professor and Discipline Head, Fisheries and Marine Resource Technology Discipline, Khulna University, for allowing me to use departmental facilities throughout my research and for his sympathy and every possible favorable attitude for providing necessary facilities to complete this research work as Head of the Department.

I am especially thankful to my classmate Afrin Sultana and Jannatul Ferdose for their effective help and cordial co-operation and suggestion throughout my research work.

Finally, I express my deepest sense of gratitude to my parents for their immeasurable blessing, continuous inspiration and financial support throughout the study.

Airin Sultana
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Abstract

Biofloc technology (BFT) is an environmentally friendly aquaculture technique in which water quality parameters maintenance and monitoring are the essential practices aiming at the success of the growing cycles. Studies revealed that improving water quality parameters in BFT and maintaining the safety range of the parameters could help to increase the growth performance of cultured species. Following a systematic review of the literatures, a meta-analysis was performed to explore how some important water parameters such as pH, dissolved oxygen (DO), nitrite ($\text{NO}_2\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), ammonia ($\text{NH}_3\text{-N}$), total ammonia nitrogen (TAN), total suspended solids (TSS), and alkalinity were influenced by different BFT systems. The PRISMA screening process was followed and 33 studies were finally eligible for the meta-analysis. The meta-analyses showed that $\text{NO}_2\text{-N}$ and TSS were significantly affected by BFT, while pH, DO, $\text{NO}_3\text{-N}$, $\text{NH}_3\text{-N}$, TAN, and alkalinity were not significantly influenced by this system. The analyses revealed that $\text{NO}_2\text{-N}$ had a significant negative effect because of BFT, whereas TSS showed a significant positive effect. The study also revealed some publication bias in which few experiments of some studies showed extremely positive and negative effect sizes because of BFT application in the system. Overall, the findings suggest a clear evidence of profound influence of BFT on the water quality parameters in different aquaculture systems, suggesting for the future development of BFT for sustainable and environmentally friendly aquaculture production.



1. Introduction

Biofloc technology (BFT) has been driven towards increased fish production with sustainability in the last decade (Abakari *et al.*, 2021). BFT is a technique for improving water quality by incorporating additional external carbon sources in conjunction with high levels of aeration in order to produce high quantities of microbial bacterial floc in aquaculture systems (Crab *et al.* 2012; Ahmad *et al.* 2017). Recently, it has received a lot of attention as a method that is affordable, sustainable, and environmentally benign (Khanjani & Sharifinia, 2020). An important benefit of this system is that there is no water exchange, which significantly decreases water use and prevents environmental harm from effluent releases (de Souza *et al.*, 2014). Biosecurity seems to be a prime concern nowadays to prevent vertical infections given the widespread distribution of viruses worldwide. Enhancing biosecurity can be accomplished using the BFT limited water exchange culture approach (Taw 2010), besides minimize the effluent discharge, and protecting the surrounding areas. The use of the same water for multiple production cycles with no adverse environmental effects is made possible by the heterotrophic bacteria in BFT that assimilate nitrogen molecules (Krummenauer, 2014). In such a system, a symbiotic community of microbes, algae, and protozoa, together with detritus and dead organic particles, forms in the water column, improving water quality and generating microbial protein for aquatic organisms. The resulting highly concentrated microbial population can act as a system for improving the quality of system water, and the produced microbial protein can be fed to animals as food (Cardona *et al.*, 2016).

The world human population is increasing exponentially, which has a negative impact on the environment and the fish stocks in lakes, rivers, and oceans. In order to encourage sustainable farming, aquaculture production must be increased without significantly consuming the necessary natural resources (Avnimelech, 2009). However, the development of aquaculture industry has been restricted due to the environmental contamination caused by the discharge of waste products containing a lot of organic nitrogenous component (Bakar *et al.*, 2015). The formation of toxic inorganic nitrogen compounds such as NH_4^+ and NO_2^- in the water results in an undesirable water quality, which is one of the major problems confronting the intensive aquaculture sector. The excretion of concentrated ammonia by cultured aquatic organisms with high commercial value such fish and shrimp has reportedly led to an accumulation in the rearing

system or pond (Avnimelech, 1999). There are several solutions for water quality improvement such as traditional method (Jackson *et al.*, 2003) with increasing water exchange, recirculating aquaculture system (RAS) (Kolarevic *et al.*, 2012) with waste treatment infrastructures, and BFT with zero-water exchange (Avnimelech, 2006). RAS provide fish farmers with a number of significant benefits over open pond culture. These include a technique to increase production on a finite amount of land and water, and practically total environmental control to increase fish development (Helfrich & Libey, 1991). But increasing water exchange increases operating expenses because it uses more energy and water, and reduces the duration that nutrients are retained in the culture system (Cang *et al.*, 2019). Aquaculture practice with the addition of BFT could be a solution rather than other systems to this problem by allowing effective removal of nutrients while simultaneously producing beneficial microbial biomass of high protein content (Cang *et al.*, 2019). By recycling feed waste and effluents, increasing food production, minimizing pathogen transmission, enhancing immunity, and preventing disease outbreaks, BFT decreases or eliminates the need for the frequent replacement of the water in the enclosures (Otoshi *et al.*, 2008; Crab *et al.*, 2010). Microbial flocs are created when these bacteria join forces with organic residue particles and other microbes. Microalgae and bacteria recycle excess nutrients in the water such as phosphorus and nitrogen, and also take up these substances, which help to keep harmful substances like ammonia under control (Brito *et al.*, 2016). Temperature, dissolved oxygen (DO), pH, salinity, solids [total suspended solids (TSS) and settling solids], total ammonia nitrogen (TAN), alkalinity, and orthophosphate are some examples of parameters that should be continuously monitored in BFT (Emerenciano *et al.* 2017). The comprehension and understanding of water quality parameters and its interactions in BFT are crucial to the correct development and maintenance of the production cycle. For example, the recommended ranges of pH, DO, TAN, TSS, and alkalinity lead a healthy growth and avoid mortalities of cultured species. (Emerenciano *et al.* 2017).

A lot of studies are available about the influence of biofloc technology on water quality parameters in aquaculture. When compared to the control system, BFT effectively removed inorganic nitrogen from culture water and significantly increased the growth parameters of rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (*Cirrihinus mrigala*) in polyculture mode (Deb *et al.*, 2020). Due to the high stocking density and high amounts of suspended particles, BFT did not increase growth performance of *Farfantepenaeus duorarum* when compared to a traditional

clear-water water exchange system (Emerenciano et al., 2013). Maintaining water quality and possibly utilizing the microbial population of the system as a food source will enable appropriate hepatopancreas development and better nutritional conditions, leading to healthier post-larvae shrimp (*Litopenaeus vannamei*) (Suita et al., 2015a). Toxic inorganic nitrogen is consumed by the microbial biomass growing in the biofloc, which then transforms it into helpful protein that is then made available to the cultivated shrimp (*Penaeus vannamei*) (Kumar et al., 2018). The benefits of BFT technology are supported by the noticeably lower TAN and NO₂-N concentrations as well as the improved growth, biomass output, and food and protein conversion efficiency of shrimp (Luis-Villaseñor et al., 2015). After maintaining water quality and the planktonic profile, the BFT system did not improve the growth performance of piracanjuba (*Brycon orbignyanus*) in comparison to RAS clear water (Sgnaulin et al., 2018).

Although some studies are available but no studies have been conducted yet to compile the findings of improved water parameters because of BFT. The outcomes of these studies can be compiled through review, systematic review, synthetic analysis and meta-analysis. Therefore, the present study has been designed to compile the findings of previous studies systematically using the meta-analysis and to analyze how the water quality parameters influence the biofloc technology in aquaculture.

Meta-analysis is a statistical procedure that combines the findings of numerous unrelated research that are considered to be "combinable." It is best to think of meta-analysis as an empirical observational research. In comparison to traditional narrative reviews, well-conducted meta-analyses allow for a more objective assessment of the data, provide a more accurate estimate of a treatment effect, and may help to explain the differences in results between different research (Egger *et al.*, 1997). Complete coverage of all pertinent studies is the aim of a good meta-analysis. They also seek for heterogeneity and use sensitivity analysis to investigate the robustness of the key findings (Crombie & Davies. 2009).

1.1. Objectives

- To systematically review different studies of water quality parameters those have been done on the development and application of BFT.
- To compile and analyze the findings using the meta-analysis of different studies conducted for water quality improvement through BFT.
- To make this information available for researchers and give an overview of the current status for management of different water quality parameters using BFT.

2. Methodology

2.1. Systematic literature search

A systematic literature search was done to obtain relevant studies testing for the influence of biofloc technology on water quality parameters in aquaculture according to PRISMA guidelines (Page et al., 2021). Search results for each stage of the PRISMA workflow are provided in Figure 1; further study-by-study details are provided in Table 1.

An initial database search was conducted in Web of Science using 7 different keyword strings (Figure 1). Search results for each keyword string were exported as CSV files, formatted for consistency (i.e., with respect to columns and relevant information), and merged into a single Microsoft Excel file after which duplicates were removed.

Each article was then screened for relevance by reading the titles and abstracts and selecting only those studies that experimentally tested the influence of biofloc technology on eight water quality parameters in aquaculture such as alkalinity, dissolve oxygen (DO), ammonium ($\text{NH}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), pH, total ammonia nitrogen (TAN) and total suspended solid (TSS). Biofloc related endpoints were then assessed for eligibility, including only those studies (or experiments within studies) that directly measured only water quality parameters which are directly related to the effects of biofloc technology on aquaculture water parameters. We further excluded studies, or experiments within studies, if

the experimental water quality parameters were not relevant to biofloc technology, no standard deviation or standard error was mentioned. This selection process finally resulted in 33 studies eligible for the meta-analysis (Figure 1).

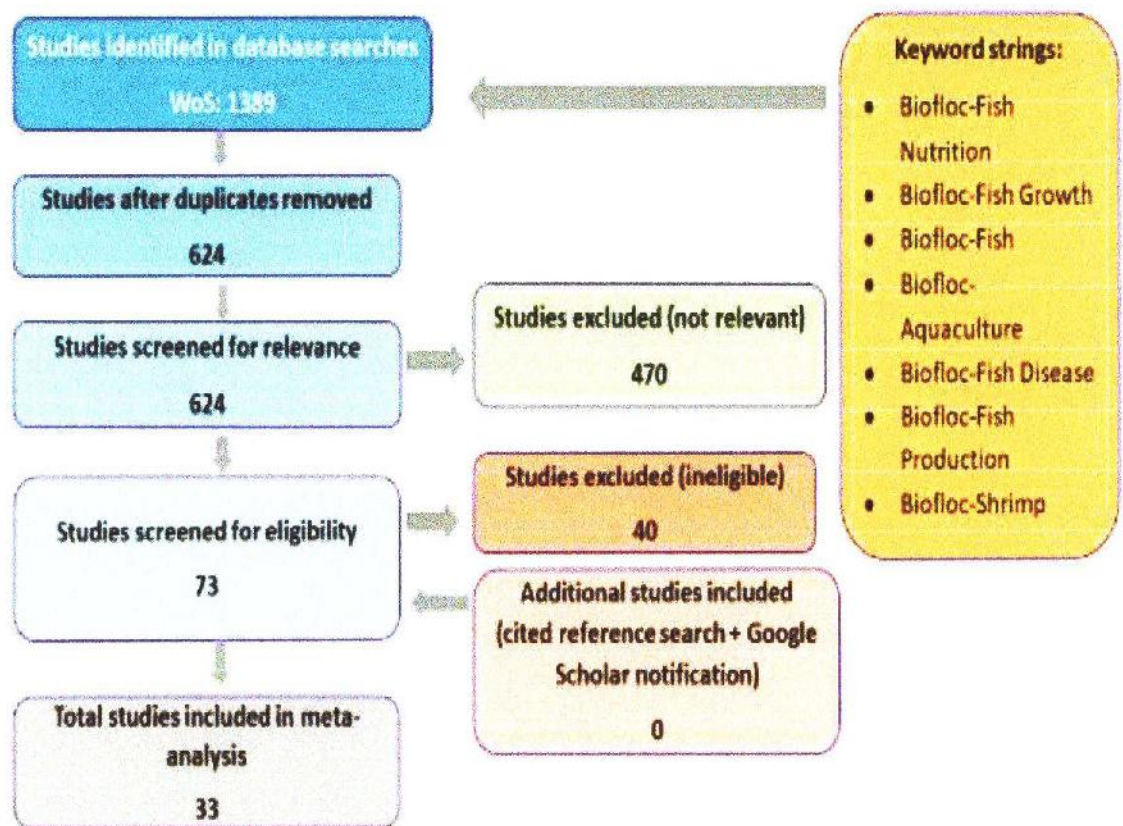


Figure 1. PRISMA flow diagram depicting the results of each step in the systematic literature search. Detailed information for each unique paper found in the literature search, including reasons for exclusion, are presented in Table 1.

Table 1. Studies that assessed different water quality parameters in various aquaculture systems

Water quality	Multilevel effect size	Pooled effect size	Study species	Life stage of study species	Aquaculture system	BF compounds	Control compounds	References
Alkalinity (mg/L)	-6.01	-6.01	<i>Litopenaeus vannamei</i>	Juvenile	Traditional aquaculture	Molasses	Clear water	de Souza et al., 2019
	1.06	1.06	<i>Oreochromis niloticus</i>	Fingerling	BFT	Sugar and powdered diet	RAS	Jatobá et al., 2019
	-1.35-0.95	-1.13	<i>L. vannamei</i>	Post-larvae	BFT	Dextrose	Traditional nursery with water renewal	Suita et al., 2015
	2.79-12.16	7.47	<i>Penaeus semisulcatus</i>	Juvenile	Feeding trial	Molasses	Clear water	Kaya et al., 2020
	-0.49	-0.49	<i>Penaeus vannamei</i>	Post larvae	BFT	Molasses, sugar & wheat	Feed based control pond	Kumar et al., 2018

	1.05	1.05	<i>Brycon orbignyanus</i>	Juvenile	BFT	Crustacean, wheat meal & molasses	Clear water	Sgnaulin et al., 2018
	-0.34	-0.34	<i>L. Vannamei</i>	Post-larvae	BFT	Molasses	Control system	Tepaamorndech et al., 2020
	-0.76--0.72	-0.74	<i>Tinca tinca</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
	-0.76--0.72	-0.74	<i>Mugil cephalus</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
Dissolved oxygen (mg/L)	-0.46	-0.46	<i>L. vannamei</i>	Post larvae	BFT	Molasses	Conventional pond system	Brito et al., 2016
	-0.33	-0.33	<i>L. stylirostris</i>	Post larvae	BFT	Molasses	Clear water	Cardona et al., 2016
	-1.54	-1.54	<i>L. vannamei</i>	Juvenile	BFT	Molasses	Clear water	Chan-Vivas et al., 2019
	-3.96	-3.96	<i>L. vannamei</i>	Juvenile	Traditional	Molasses	Clear	de Souza et al.,

						aquaculture	ess	water	2019
0	0				<i>Cyprinus carpio specularis</i>	Juvenile	Feeding trial	No carbon sources and microorganisms	Du et al., 2018
0	0				<i>Hypophthalmichthys molitrix</i>	Juvenile	Feeding trial	No carbon sources and microorganisms	Du et al., 2018
0	0				<i>Aristichthys nobilis</i>	Juvenile	Feeding trial	No carbon sources and microorganisms	Du et al., 2018
0.27	0.27				<i>Farfantepenaeus duorarum</i>	Juvenile	BFT	Clear-water (CW)	Emerenciano et al., 2013
0.13-0.6	0.36				<i>O. niloticus</i>	Fingerling	BFT	RAS	Jatobá et al., 2019

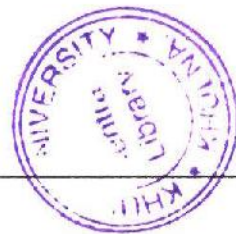
D.

-1.34	-1.34	<i>Labeo rohita</i>	Fingerling	BFT	Molasses	Without Molasses	Kamiliya et al., 2017
-26.39	-26.39	<i>Metapenaeus monoceros</i>	Juvenile	BFT	Feeding trial	BFT with 50% daily water exchange	Kaya et al., 2019
-0.23-0.46	0.11	<i>P. semisulcatus</i>	Juvenile	Feeding trial	Molasses	Clear water	Kaya et al., 2020
-1.74	-1.74	<i>L. vannamei</i>	Post-larvae	BFT	Renewed seawater at 50%	Clear seawater	Kim et al., 2014
-1.01-0.47	0.36	<i>L. vannamei</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-1.01-0.47	0.36	<i>Fenneropenaeus chinensis</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-1.01-0.47	0.36	<i>Marsupenaeus japonicus</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015

3.58	3.58	<i>P. vannamei</i>	Post larvae	BFT	Molasses, sugar & wheat	Feed based Control pond	Kumar et al., 2018
-6.74	-6.74	<i>L. vannamei</i>	Post larvae	BFT	Glucose	Seawater	Kumar et al., 2020
0.41	0.41	<i>Procambarus clarkii</i>	Juvenile	BFT	Seaweed (<i>Vallisneria spiralis</i>)	Commercial diet	Li et al., 2019
-7.74	-7.74	<i>O. niloticus</i>	Juvenile	BFT	Glucose	Not receive glucose	Long et al., 2015
-76.35	-76.35	<i>L. vannamei</i>	Juvenile	BFT	Molasses	Seawater	Martins et al., 2015
0.34	0.34	<i>Macrobrachium rosenbergii</i>	Juvenile	Feeding trial	<i>Bacillus subtilis</i> , molasses & blood meal	Clear water	Nguyen et al., 2019
0.30	0.30	<i>O. niloticus</i>	Juvenile	Feeding trial	<i>Bacillus subtilis</i>	Clear water	Nhi et al., 2018

NH ₃ -N (mg/L)	-0.32	-0.32	<i>L. vannamei</i>	Juvenile	Feeding trial	Soybean meal & wheat flour	CW-35%	Xu et al., 2012a
	-0.24	-0.24	<i>L. vannamei</i>	Juvenile	Feeding trial	Feeding trial	CW-40%	Yun et al., 2016
	-0.82-1.39	0.28	<i>P. semisulcatus</i>	Juvenile	Feeding trial	Molasses	Clear water	Kaya et al., 2020
	-32.87	-32.87	<i>P. vannamei</i>	Post larvae	BFT	Molasses, Sugar & wheat	Feed based Control pond	Kumar et al., 2018
	0.12	0.12	<i>P. clarkii</i>	Juvenile	BFT	Seaweed (<i>Vallisneria spiralis</i>)	Commercial diet	Li et al., 2019
	-1.03	-1.03	<i>L. vannamei</i>	Juvenile	BFT	Commercial	Shrimp feed	Luis-Villaseñor et al., 2015
	-0.42	-0.42	<i>L. vannamei</i>	Juvenile	BFT	Molasses	Seawater	Martins et al.,

NO ₂ -N (mg/L)	0.23	0.23	<i>L. vannamei</i>	Post-larvae	BFT	Molasses	Control system	2015
	2.63-2.65	2.64	<i>T. tinca</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
	2.63-2.65	2.64	<i>M. cephalus</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
	-3.86	-3.86	<i>Carassius auratus</i> gibelio × <i>C. carpio</i>	Fingerling	BFT	Molasses	Aerated freshwater	Cang et al., 2019
	-3.77	-3.77	<i>L. vannamei</i>	Juvenile	Traditional aquaculture	Molasses	Clear water	de Souza et al., 2019
	0.78-5.07	2.61	<i>L. rohita</i>	Fingerling	BFT	Tapioca flour	Without biofloc	Debet et al., 2020
	0.78-5.07	2.61	<i>Catla catla</i>	Fingerling	BFT	Tapioca flour	Without biofloc	Debet et al., 2020
	0.78-5.07	2.61	<i>Cirrhinus mrigala</i>	Fingerling	BFT	Tapioca flour	Without biofloc	Debet et al., 2020
	-12.19	-12.19	<i>C. carpio specularis</i>	Juvenile	Feeding trial	Glucose, starch with	No carbon sources	Du et al., 2018



-0.13	-0.13	<i>P. clarkii</i>	Juvenile	BFT	Seaweed (<i>V. natans</i>)	Commercial diet	Li et al., 2019
-5.96	-5.96	<i>O. niloticus</i>	Juvenile	BFT	Glucose	Not receive glucose	Long et al., 2015
-8.41	-8.41	<i>L. vannamei</i>	Juvenile	BFT	Commercial	Shrimp feed	Luis-Villaseño et al., 2015
-1.82	-1.82	<i>L. vannamei</i>	Juvenile	BFT	Molasses	Seawater	Martins et al., 2015
0.19	0.19	<i>O. niloticus</i>	Juvenile	Feeding trial	<i>B. subtilis</i> , molasses, blood meal	Clear water	Nhi et al., 2018
6.00	6.00	<i>L. vannamei</i>	Post larvae	BFT	Foam fructonator	RAS	Ray et al., 2017
0.39	0.39	<i>B. orbignyanus</i>	Juvenile	BFT	Commercial, wheat meal, molasses	Clear water	Sgnaulin et al., 2018

-6.72--3.96	-5.55	<i>L. vannamei</i>	Juvenile	Feeding trial	Sucrose, cassava dregs, cassava with enzyme	Without carbon source	Shang et al., 2018
-117.06	-117.06	<i>L. vannamei</i>	Post-larvae	BFT	Molasses	No-Biofloc	Soto-Alcalá et al., 2020
-0.43-0.23	-0.06	<i>L. vannamei</i>	Post-larvae	BFT	Dextrose	Traditional nursery with water renewal	Suita et al., 2015
-0.18	-0.18	<i>L. vannamei</i>	Post-larvae	BFT	Molasses	Control system	Tepaamorndech et al., 2020
-0.16-0.42	0.13	<i>T. tinca</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
-0.16-0.42	0.13	<i>M. cephalus</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
0.98	0.98	<i>L. vannamei</i>	Juvenile	Feeding	Soybean	CW-	Xu et al.,

NO ₃ -N (mg/L)							trial	n meal and wheat flour	35%	2012a
	-0.62-0.49	-0.06	<i>L. vannamei</i>	Juvenile	BFT	Brown sugar	Xu et al., 2013			
	0.18	0.18	<i>L. vannamei</i>	Juvenile	Feeding trial	Feedin g trial	Yun et al., 2016			
	-1.53	-1.53	<i>M. japonicus</i>	Postlarvae	BFT	Sucros e	Zhao et al., 2012			
	4.38	4.38	<i>L. vannamei</i>	Juvenile	Tradition al aquacultu re	Molass es	de Souza et al., 2019			
	-11.12-13.92	0.93	<i>L. rohita</i>	Fingerling	BFT	Tapioc a flour	Deb et al., 2020			
	-11.12-13.92	0.93	<i>C. catla</i>	Fingerling	BFT	Tapioc a flour	Deb et al., 2020			
	-11.12-13.92	0.93	<i>C. mrigala</i>	Fingerling	BFT	Tapioc a flour	Deb et al., 2020			
	-0.63	-0.63	<i>C. carpio specularis</i>	Juvenile	Feeding trial	Glucos e, starch with <i>Bacillu s</i> sp.	Du et al., 2018	No carbon sources and microor ganisms		

-0.63	-0.63	<i>H. molitrix</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
-0.63	-0.63	<i>A. nobilis</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
1.18	1.18	<i>Farfantepenaeus duorarum</i>	Juvenile	BFT	Molasses and wheat bran	Clear water (CW)	Emerenciano et al., 2013
-1.20	-1.20	<i>L. rohita</i>	Fingerling	BFT	Molasses	Without Molasses	Kamiliya et al., 2017
9.38	9.38	<i>M. monoceros</i>	Juvenile	BFT	Feeding trial	BFT with 50% daily water exchange	Kaya et al., 2019
-3.96--3.91	-3.94	<i>P. semisulcatus</i>	Juvenile	Feeding	Molasses	Clear	Kaya et

					trial	s	water	al., 2020
6.36	6.36		<i>L. vannamei</i>	Post-larvae	BFT	Renewed seawater at 50%	Clear seawater	Kim et al., 2014
4.81-12.20	7.59		<i>L. vannamei</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
4.81-12.20	7.59		<i>F. chinensis</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
4.81-12.20	7.59		<i>M. japonicus</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-4.28	-4.28		<i>P. vannamei</i>	Post larvae	BFT	Molasses, sugar & wheat	Feed based Control pond	Kumar et al., 2018
19.83	19.83		<i>L. vannamei</i>	Post larvae	BFT	Glucose	Seawater	Kumar et al., 2020
1.76	1.76		<i>P. clarkii</i>	Juvenile	BFT	Seaweed (<i>V. natans</i>)	Commercial diet	Li et al., 2019
-56.33	-56.33		<i>O. niloticus</i>	Juvenile	BFT	Glucose	Not receive glucose	Long et al., 2015

-7.42	-7.42	<i>L. vannamei</i>	Juvenile	BFT	Commercial	Shrimp feed	Luis-Villaseño et al., 2015
4.65	4.65	<i>L. vannamei</i>	Juvenile	BFT	Molasses	Seawater	Martins et al., 2015
2.97	2.97	<i>L. vannamei</i>	Post larvae	BFT	Foam fructio nator	Clear-water (CW) RAS	Ray et al., 2017
0.32	0.32	<i>B. orbignyana</i>	Juvenile	BFT	Commercial, wheat meal, molasses	Clear water	Sgnaulin et al., 2018
-12.52--3.72	-8.70	<i>L. vannamei</i>	Juvenile	Feeding trial	Sucrose, cassava dregs, cassava with enzyme	Without carbon source	Shang et al., 2018
-8.57	-8.57	<i>L. vannamei</i>	Post-larvae	BFT	Molasses	No-Biofloc	Soto-Alcalá et

-0.14	-0.14	<i>C. carpio specularis</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
-0.14	-0.14	<i>H. molitrix</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
-0.14	-0.14	<i>A. nobilis</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
-0.31	-0.31	<i>F. duorarum</i>	Juvenile	BFT	Molasses and wheat bran	Clear-water (CW)	Emerenciano et al., 2013
-0.36	-0.36	<i>O. niloticus</i>	Fingerling	BFT	Sugar and powdered diet	RAS	Jatobá et al., 2019

0.05	0.05	<i>L. rohita</i>	Fingerling	BFT	Molasses	Without Molasses	Kamiliya et al., 2017
1.93	1.93	<i>M. monoceros</i>	Juvenile	BFT	Feeding trial	BFT with 50% daily water exchange	Kaya et al., 2019
0-0.19	0.10	<i>P. semisulcatus</i>	Juvenile	Feeding trial	Molasses	Clear water	Kaya et al., 2020
-4.54	-4.54	<i>L. vannamei</i>	Post-larvae	BFT	Renewed seawater at 50%	Clear seawater	Kim et al., 2014
-7.18	-7.18	<i>L. vannamei</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-7.18	-7.18	<i>F. chinensis</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-7.18	-7.18	<i>M. japonicus</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-13.96	-13.96	<i>P. vannamei</i>	Post larvae	BFT	Molasses	Feed based	Kumar et al., 2018

	-6.36--6.32	-6.34	<i>L. vannamei</i>	Juvenile	BFT	flour	Brown sugar	Clear water	Xu et al., 2013
	1.60	1.60	<i>L. vannamei</i>	Juvenile	Feeding trial	Feeding trial	Feeding trial	CW-40%	Yun et al., 2016
TAN (mg/L)	-3.71	-3.71	<i>L. vannamei</i>	Juvenile	Traditional aquaculture	Molasses	Molasses	Clear water	de Souza et al., 2019
	-6.33--0.81	-3.43	<i>L. rohita</i>	Fingerling	BFT	Tapioca flour	Tapioca flour	Without biofloc	Deb et al., 2020
	-6.33--0.81	-3.43	<i>C. catla</i>	Fingerling	BFT	Tapioca flour	Tapioca flour	Without biofloc	Deb et al., 2020
	-6.33--0.81	-3.43	<i>C. mrigala</i>	Fingerling	BFT	Tapioca flour	Tapioca flour	Without biofloc	Deb et al., 2020
	0.04	0.04	<i>C. carpio specularis</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
	0.04	0.04	<i>H. molitrix</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i>	Glucose, starch with <i>Bacillus</i>	No carbon sources and microorganisms	Du et al., 2018

0.04	0.04	<i>A. nobilis</i>	Juvenile	Feeding trial	s sp.	ganisms	Du et al., 2018
0.8	0.8	<i>F. duorarum</i>	Juvenile	BFT	Molasses and wheat bran	Clear-water (CW)	Emerenciano et al., 2013
0.8	0.8	<i>L. rohita</i>	Fingerling	BFT	Molasses	Without Molasses	Kamiliya et al., 2017
38.62	38.62	<i>M. monoceros</i>	Juvenile	BFT	Feeding trial	BFT with 50% daily water exchange	Kaya et al., 2019
-2.01	-2.01	<i>M. rosenbergii</i>	Juvenile	Feeding trial	<i>B. subtilis</i> , molasses & blood	Clear water	Nguyen et al., 2019

1.34	1.34	<i>O. niloticus</i>	Juvenile	Feeding trial	B. subtilis, molasses & blood meal	Clear water	Nhi et al., 2018
8.76	8.76	<i>L. vannamei</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
8.76	8.76	<i>F. chinensis</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
8.76	8.76	<i>M. japonicus</i>	Post-larvae	BFT	Feeding trial	Seawater	Kim et al., 2015
-19.87	-19.87	<i>P. vannamei</i>	Post larvae	BFT	Molasses, sugar & wheat	Feed based Control pond	Kumar et al., 2018
-0.81	-0.81	<i>L. vannamei</i>	Post larvae	BFT	Glucose	Seawater	Kumar et al., 2020
1.80	1.80	<i>P. clarkii</i>	Juvenile	BFT	Seaweed (<i>V. natans</i>)	Commercial diet	Li et al., 2019

1.79	1.79	<i>O. niloticus</i>	Juvenile	BFT	Glucose	Not receive glucose	Long et al., 2015
-7.36	-7.36	<i>L. vannamei</i>	Juvenile	BFT	Commercial	Shrimp feed	Luis-Villaseño et al., 2015
-0.08-0.20	0.04	<i>L. vannamei</i>	Post-larvae	BFT	Dextrose	Traditional nursery with water renewal	Suita et al., 2015
2.19-2.67	2.43	<i>T. tinca</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
2.19-2.67	2.43	<i>M. cephalus</i>	Juvenile	BFT	Glucose	RAS	Vinatea et al., 2018
0.50	0.50	<i>B. orbignyana</i>	Juvenile	BFT	Commercial, wheat meal, molasses	Clear water	Sgnaulin et al., 2018
-7.57--1.38	-5.09	<i>L. vannamei</i>	Juvenile	Feeding trial	Sucrose, cassava	Without carbon	Shang et al., 2018

									va dregs,c assava with enzym e	source	
-3.07	-3.07								Molasses	No-Biofloc	Soto-Alcalá et al., 2020
-0.10	-0.10								Soybean meal & wheat flour	CW-35%	Xu et al., 2012a
-4.32--2.38	-3.35								Brown sugar	Clear water	Xu et al., 2013
-1.45	-1.45								Feeding trial	CW-40%	Yun et al., 2016
-3.80	-3.80								Sucrose	Without sucrose	Zhao et al., 2012
0.25	0.25								Sugar and powdered diet	RAS	Jatobá et al., 2019
7.43	7.43								Molasses	Aerated freshwater	Cang et al., 2019
TSS (mg/L)											

-6.32	-6.32	carpio	Juvenile	Traditional aquaculture	Molasses	Clear water	de Souza et al., 2019
-2.23-13.17	4.60	<i>L. rohita</i>	Fingerling	BFT	Tapioca flour	Without biofloc	Deb et al., 2020
-2.23-13.17	4.60	<i>C. catla</i>	Fingerling	BFT	Tapioca flour	Without biofloc	Deb et al., 2020
-2.23-13.17	4.60	<i>C. mrigala</i>	Fingerling	BFT	Tapioca flour	Without biofloc	Deb et al., 2020
2.32	2.32	<i>C. carpio specularis</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
2.32	2.32	<i>H. molitrix</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i> sp.	No carbon sources and microorganisms	Du et al., 2018
2.32	2.32	<i>A. nobilis</i>	Juvenile	Feeding trial	Glucose, starch with <i>Bacillus</i>	No carbon sources and	Du et al., 2018

10.90	10.90	<i>O. niloticus</i>	Juvenile	BFT	Glucose	Not receive glucose	Long et al., 2015
9.04	9.04	<i>L. Vannamei</i>	Juvenile	BFT	Commercial	Shrimp feed	Luis-Villaseño et al., 2015
4.56-9.08	6.09	<i>L. vannamei</i>	Juvenile	Feeding trial	Sucrose, cassava dregs, cassava with enzyme	Without carbon source	Shang et al., 2018
6.83	6.83	<i>L. vannamei</i>	Post-larvae	BFT	Molasses	No-Biofloc	Soto-Alcalá et al., 2020
0.56-1.41	0.94	<i>L. vannamei</i>	Post-larvae	BFT	Dextrose	Traditional nursery with water renewal	Suita et al., 2015
6.19-7.94	7.07	<i>L. vannamei</i>	Juvenile	BFT	Brown sugar	Clear water	Xu et al., 2013



2.2. Data collection

For each of the 33 studies, we collected various qualitative and quantitative attributes. Specifically, we recorded general bibliographic data (authors, year published), name of the species tested, fish species belong to which group, species life stage, treatment name, treatment level, control variable, water quality parameter response, and response unit. For each eligible experiment, we also obtained the treatment mean & standard deviation/standard error value, control mean & standard deviation/standard error value, sample size (n). These values were obtained directly from the text or tables within the article, from graphs within the article by using ImageJ software. We also added data source (exactly from where data were collected) and if there were any lack of information those were added in the comment section. Then all standard error (SE) were converted to standard deviation (SD) prior to analysis.

2.3. Meta-analysis

The analysis was carried out using the standardized mean difference (SMD) as the outcome measure. The following equations were used to calculate the effect size and variances (Noble et al., 2022):

$$SMD^a = \frac{(M_2 - M_1)}{SD_p} J$$

$$\text{Where, sampling variance} = \frac{N_1 + N_2}{N_1 N_2} + \frac{SMD^2}{2(N_1 + N_2)}$$

Here, M_1 : mean of control group; M_2 : mean of treatment group; SD_p : pooled standard deviation; J : a small sample correction; N_1 : sample size of control group, and N_2 : sample size of treatment group.

$$^a J = 1 - \frac{3}{4(N_1 + N_2 - 2)}; SD_p = \sqrt{\frac{(N_1 - 1)SD_1^2 + (N_2 - 1)SD_2^2}{N_1 + N_2 - 2}}$$

Here, SD1: sample standard deviation of control group; SD2: sample standard deviation of treatment group.

A random-effects model was fitted to the data. The amount of heterogeneity (i.e., τ^2), was estimated using the restricted maximum-likelihood estimator (Viechtbauer, 2005). In addition to the estimate of τ^2 , the Q-test for heterogeneity (Cochran, 1954) and the I^2 statistic (Higgins & Thompson, 2002) are reported. In case any amount of heterogeneity is detected (i.e., $\tau^2 > 0$, regardless of the results of the Q-test), a prediction interval for the true outcomes is also provided (Riley et al., 2011).

The analysis was carried out using R (version 4.0.5) (R Core Team, 2021) and the metafor package (version 3.4.0) (Viechtbauer, 2010). A subset of the qualitative and quantitative factors collected from each experiment were explored as potential explainers of effect size variation such as study, year of publication, study species, life stage of study species, aquaculture system, experimental biofloc compounds and control compounds, etc. Studentized residuals and Cook's distances were used to examine whether studies might have outliers and/or influential in the context of the model (Viechtbauer & Cheung, 2010). Studies with a studentized residual larger than the $100 \times (1 - 0.05 / (2 \times k))^{th}$ percentile of a standard normal distribution were considered potential outliers (i.e., using a Bonferroni correction with two-sided $\alpha = 0.05$ for k studies included in the meta-analysis). Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances were considered to be influential. The rank correlation test (Begg & Mazumdar, 1994) and the regression test (Sterne & Egger, 2005), using the standard error of the observed outcomes as predictor, were used to check for funnel plot asymmetry.

3. Results

3.1. Overview of included studies

A total of 33 studies published between 2012 and 2020 that examined the effects of BFT on different water quality parameters in aquaculture systems were eligible for the meta-analysis database (Fig. 2). The studies were started searching on 8/11/2020 and finished on 10/12/2020. The review found the highest number of eligible studies in 2019 (7 studies) followed by 2018 (6 studies), while the lowest number of eligible publication (1 study) was found in 2014 (Fig. 2).

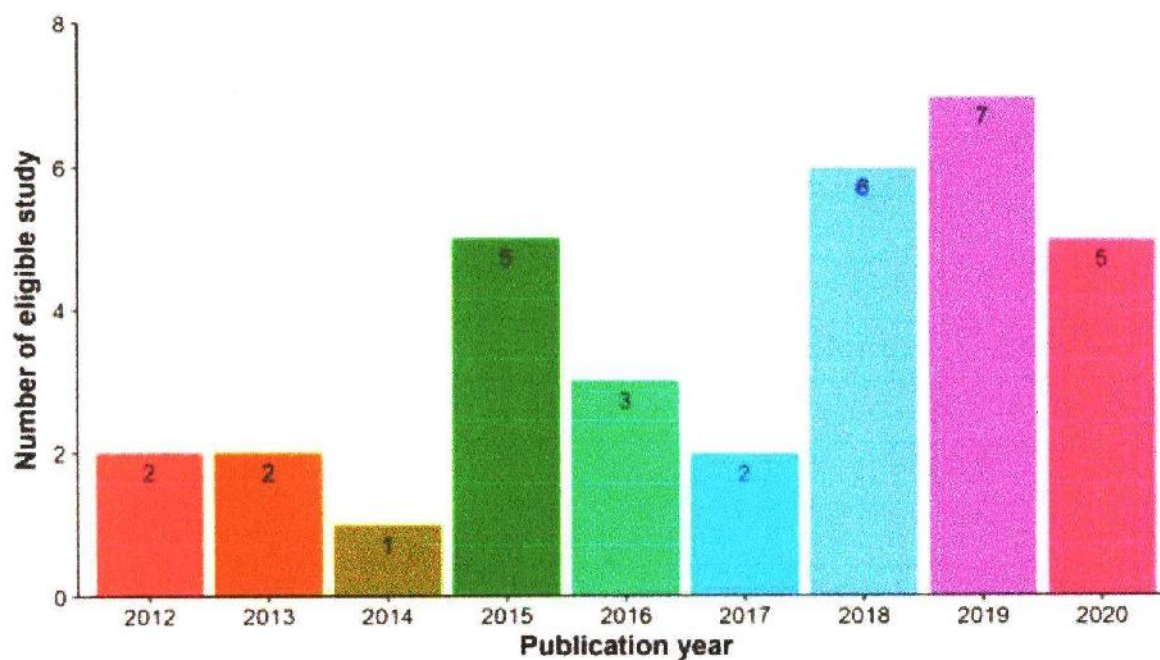


Figure 2. Publication years of the eligible studies for the meta-analysis database.

The number of studies was greatest in Asia (17 studies), followed by Europe (6 studies), South America and North America (5 studies in each continent) (Fig. 3a). The highest number of studies were conducted in China (8 publications), followed by Brazil (5 publications), and other countries (Fig. 3b).

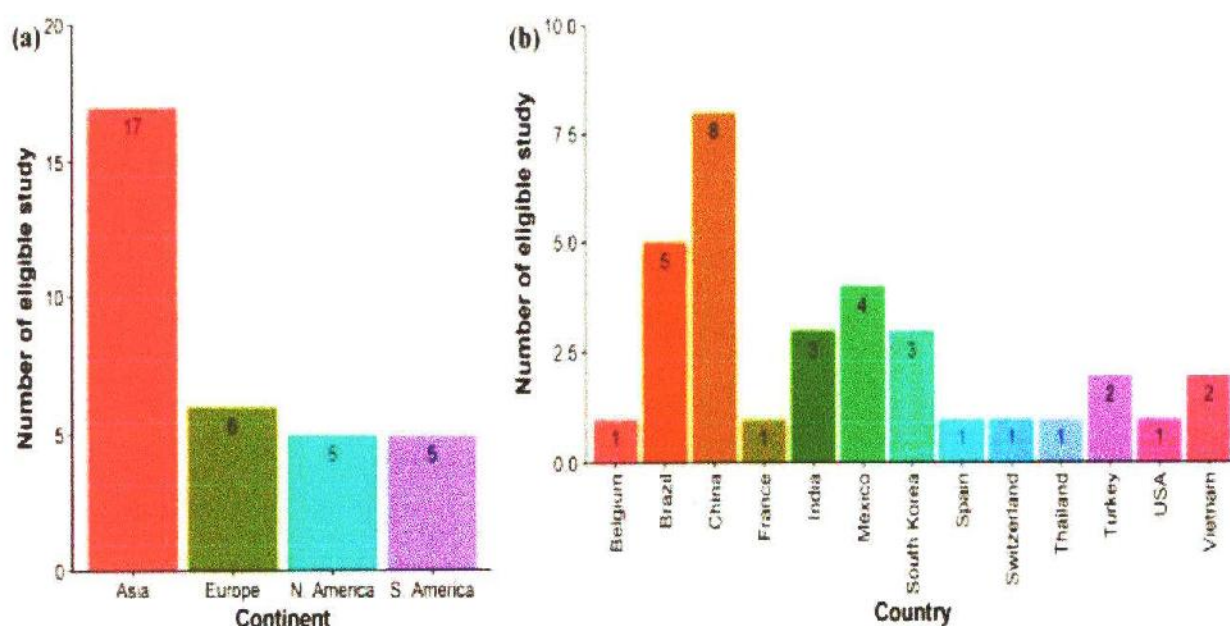


Figure 3. Indication of (a) the continent and (b) country where BFT on water quality experiments were conducted.

The studies included 16 different fish culture systems (monoculture and mixed culture of various species) in which monoculture of *Litopenaeus vannamei* was received the most interest (in 15 studies), followed by the monoculture of *Oreochromis niloticus* (3 studies per species), and carps mixed culture (2 studies) (Fig. 4).

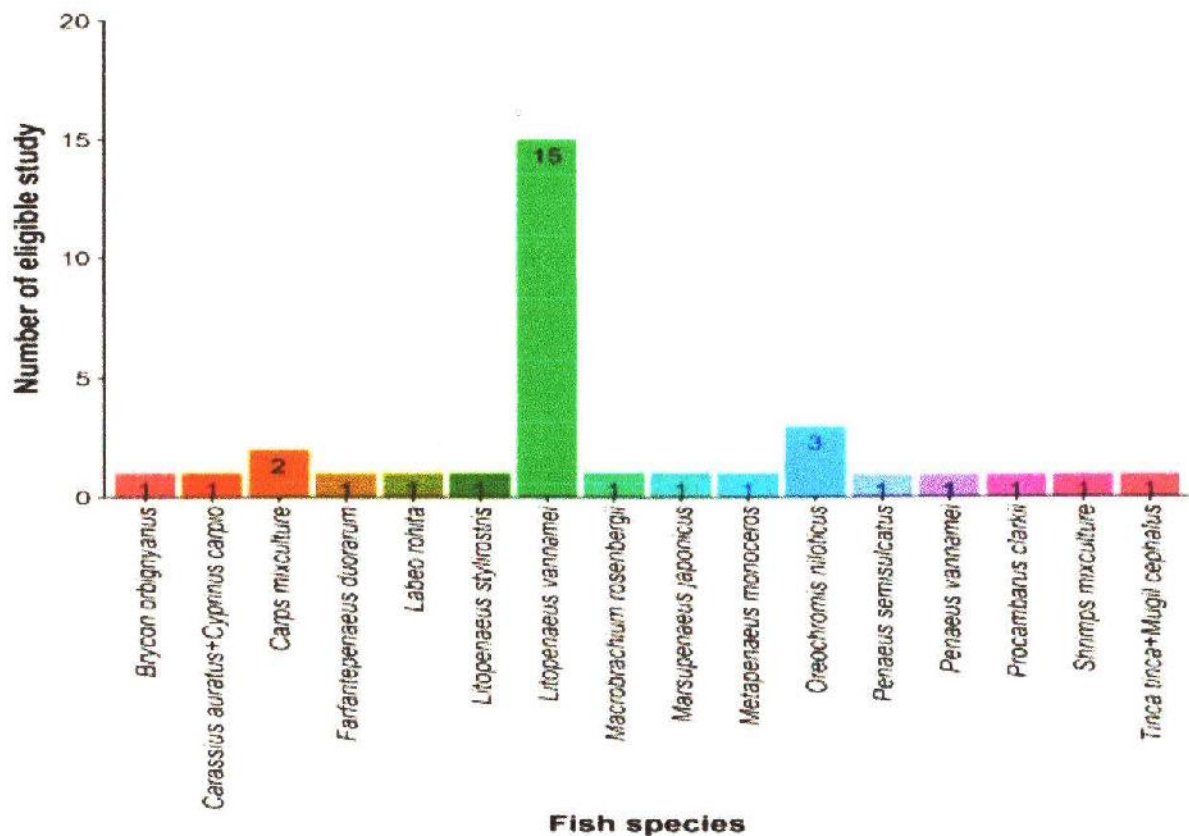


Figure 4. List of different fish species that were used in the eligible studies for meta-analysis.

The review revealed that the highest number of studies (n=8) used only molasses as biofloc compound (n=10) (Fig. 5).

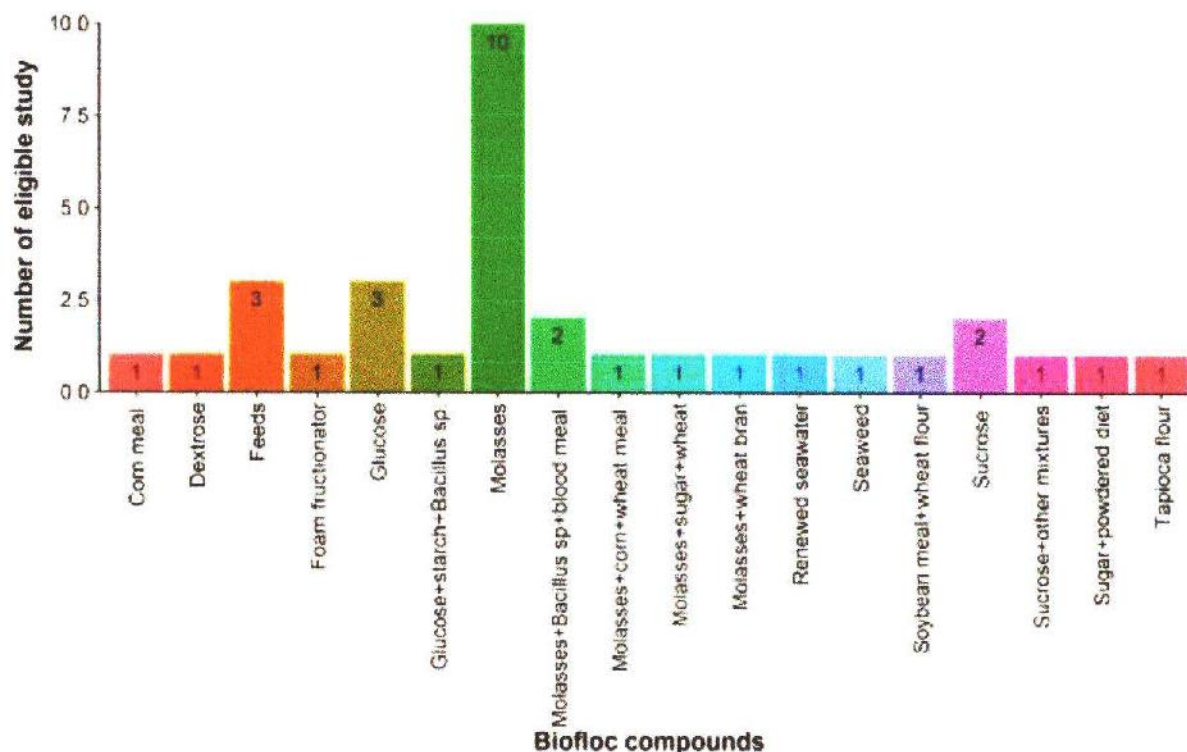


Figure 5. List of biofloc compounds that were used in the biofloc systems for the eligible studies for meta-analysis.

3.2. Effects on water alkalinity

The data from 11 multilevel experiments of 8 studies were included in the analysis. The observed standardized mean differences ranged from -6.01 to 12.16, with the majority of estimates being negative (64%). The estimated average standardized mean difference based on the random-effects model was $\mu=0.53$ (95% CI: -1.78 to 2.84). Therefore, the average outcome did not differ significantly from zero ($z=0.45$, $p=0.65$).

The aggregated analysis of these multiple experiments showed a total of 8 studies were included in the analysis. The observed aggregated outcomes ranged from -6.01 to 7.47, with the majority of estimates being negative (62%). The estimated average outcome based on the random-effects model was $\mu=0.19$ (95% CI: -2.14 to 2.51). Therefore, the average outcome did not differ significantly from zero ($z=0.16$, $p=0.87$).

3.3. Effects on DO

The data from 33 multilevel experiments of 27 studies were included in the analysis. The observed standardized mean differences ranged from -76.35 to 3.57, with the majority of estimates being negative (61%). The estimated average standardized mean difference based on the random-effects model was $\mu=-0.32$ (95% CI: -0.72 to 0.07). Therefore, the average outcome did not differ significantly from zero ($z=-1.59$, $p=0.11$).

The aggregated analysis of these multiple experiments showed a total of 27 studies were included in the analysis. The observed aggregated outcomes ranged from -76.35 to 3.57, with the majority of estimates being negative (59 %). The estimated average outcome based on the random-effects model was $\mu=-0.51$ (95% CI: -1.09 to 0.07). Therefore, the average outcome did not differ significantly from zero ($z=-1.71$, $p=0.09$).

3.4. Effects on NH₃-N

The data from 9 multilevel experiments of 7 studies were included in the analysis. The observed standardized mean differences ranged from -32.87 to 2.65, with the majority of estimates being negative (56%). The estimated average standardized mean difference based on the random-effects model was $\mu=0.46$ (95% CI: -0.49 to 1.42). Therefore, the average outcome did not differ significantly from zero ($z=0.95$, $p=0.34$).

The aggregated analysis of these multiple experiments showed a total of 7 studies were included in the analysis. The observed aggregated outcomes ranged from -32.87 to 2.64, with the majority of estimates being negative (57 %). The estimated average outcome based on the random-effects model was $\mu=0.21$ (95% CI: -0.48 to 0.91). Therefore, the average outcome did not differ significantly from zero ($z=0.61$, $p=0.54$).

3.5. Effects on $\text{NO}_2\text{-N}$

The data from 33 multilevel experiments of 24 studies were included in the analysis. The observed standardized mean differences ranged from -117.06 to 5.99, with the majority of estimates being negative (64%). The estimated average standardized mean difference based on the random-effects model was $\mu=-1.12$ (95% CI: -2.06 to -0.19). Therefore, the average outcome differed significantly from zero ($z=-2.35$, $p=0.02$). A forest plot showing the observed outcomes and the estimate based on the random-effects model is shown in Figure 6.

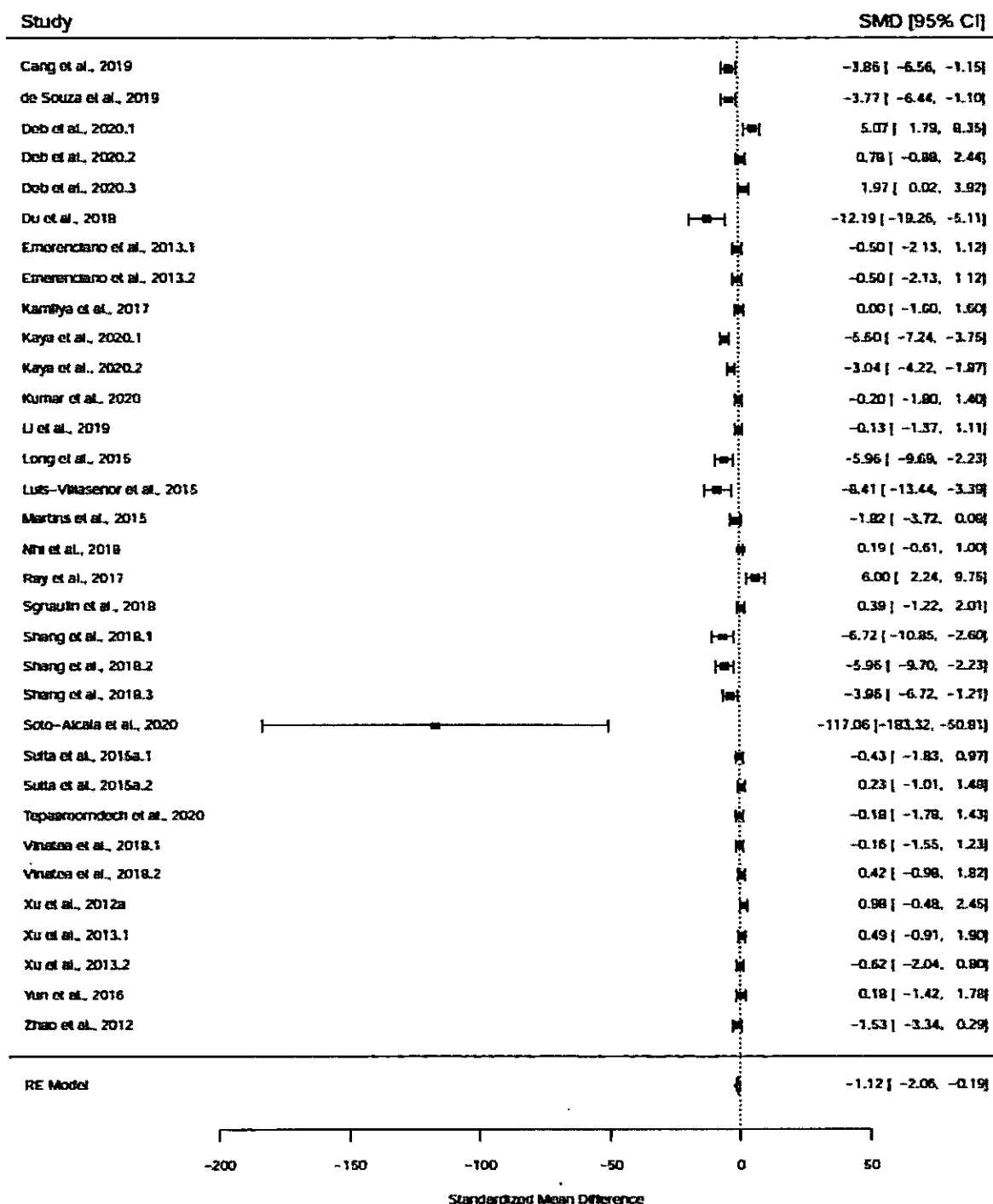


Figure 6. Forest plot reporting the effect size (standardized mean differences) for NO₂-N in different studies of BFT having multiple experimental levels. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (cyan diamond shape). When a study includes multiple treatment levels, it is presented with a 'numeric number' after the publication year.

According to the Q-test, the true outcomes appear to be heterogeneous ($Q(32) = 172.99, p < 0.001, \tau^2 = 5.99, I^2 = 88.83\%$). A 95% prediction interval for the true outcomes is given by -6.01 to 3.76 . Hence, although the average outcome is estimated to be negative, in some studies the true outcome may in fact be positive.

An examination of the studentized residuals revealed that one study (Soto-Alcala et al., 2020) had a value larger than ± 3.00 and may be a potential outlier in the context of this model. According to the Cook's distances, one study (Soto-Alcala et al. 2020) could be considered to be overly influential. A funnel plot of the estimates is shown in Figure 7. Both the rank correlation and the regression test indicated the funnel plot asymmetry ($p < 0.001$ and $p < 0.01$, respectively).

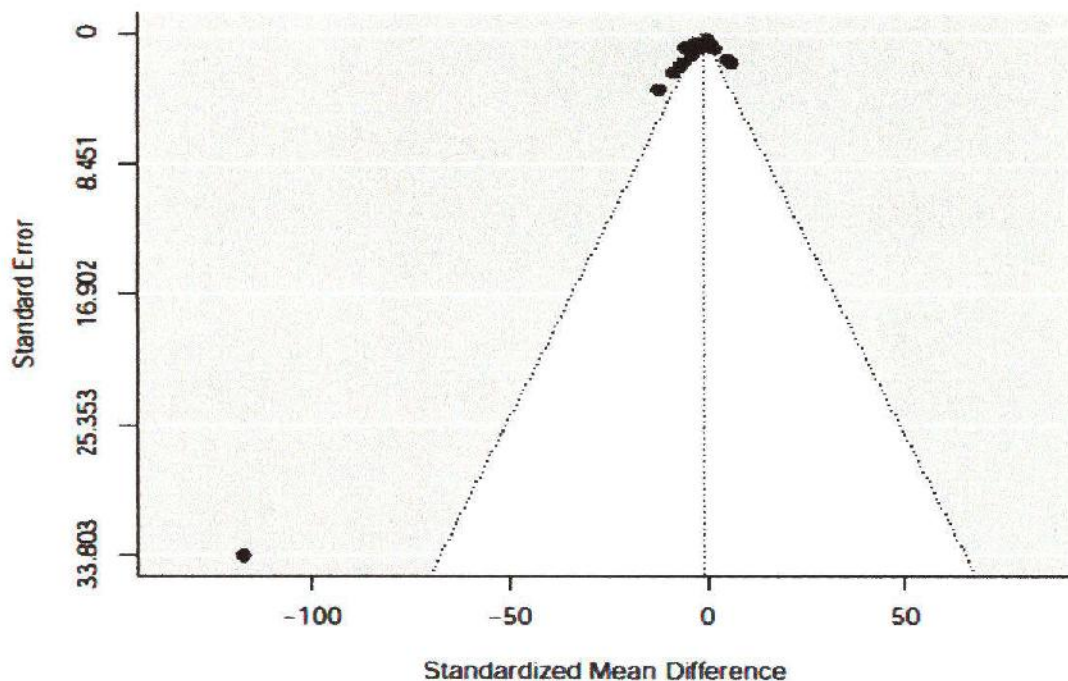


Figure 7. Funnel plot depicting the effect size of each study (x-axis) for NO₂-N in relation to the precision of that study (SE; y-axis). Here points are not scattered symmetrically within the funnel suggesting a publication bias.

The baujat plot also showed the study of Soto-Alcala et al., 2020 has the higher values on x-axis that may be considered to be an overly influential case on overall results (Figure 8). The studies of Kaya et al., 2020.1, Luis-Villaseñor et al., 2015, Du et al., 2018, etc. have the higher values on y-axis that may also be considered to be an overly influential case on overall results (Figure 8).



Figure 8. Baujat plot showing the heterogeneity in NO₂-N meta-analytic data. The plot shows the overall heterogeneity contribution of each effect size in x-axis, while the y-axis shows the influence of each effect size on the pooled result.

The sensitivity analysis was done by removing the outliers which showed almost the same significant negative overall effect size $\mu = -1.10$ (95% CI: -2.03 to -0.16), $z = -2.30$, $p = 0.02$).

The aggregated analysis of these multiple experiments showed a total of 24 studies were included in the analysis. The observed aggregated outcomes ranged from -117.06 to 5.99 , with the majority of estimates being negative (67%). The estimated average outcome based on the random-effects model was $\mu = -1.15$ (95% CI: -2.23 to -0.07). Therefore, the average outcome differed significantly from zero ($z = -2.09$, $p = 0.04$). A forest plot showing the observed outcomes and the estimate based on the random-effects model is shown in Figure 9.

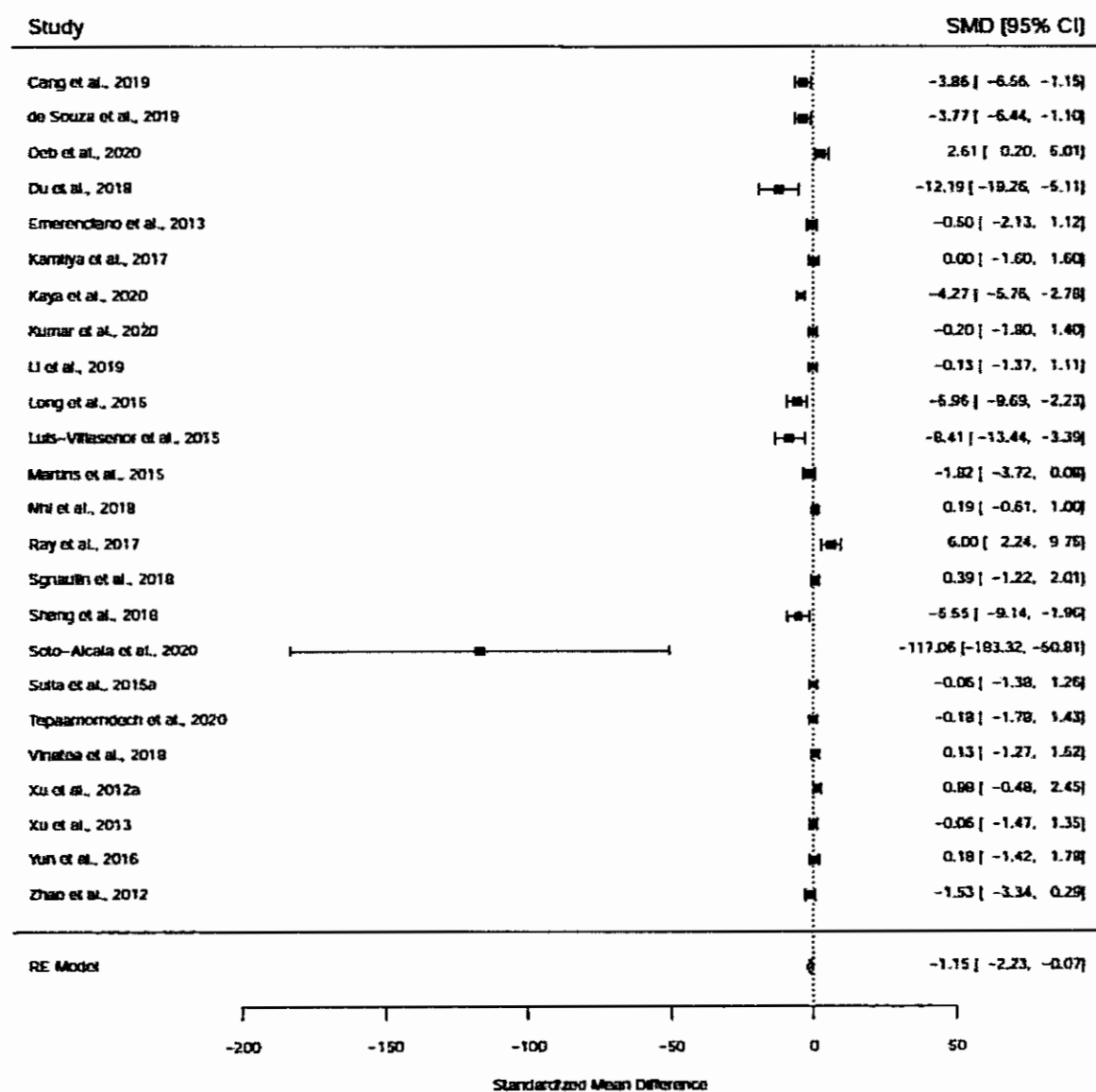


Figure 9. Forest plot reporting the aggregated effect size (standardized mean differences) for NO₂-N in different studies of BFT. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (blue diamond shape).

The sensitivity analysis was done for this aggregated effect size model by removing the outliers which showed almost the same significant negative overall pooled effect size $\mu=-1.12$ (95% CI: -2.19 to -0.05), $z=-2.04$, $p=0.04$).

3.6. Effects on NO_3-N

The data from 32 multilevel experiments of 23 studies were included in the analysis. The observed standardized mean differences ranged from -56.33 to 19.83, with the majority of estimates being negative (56%). The estimated average standardized mean difference based on the random-effects model was $\mu=0.69$ (95% CI: -1.51 to 2.90). Therefore, the average outcome did not differ significantly from zero ($z=0.62$, $p=0.54$).

The aggregated analysis of these multiple experiments showed a total of 23 studies were included in the analysis. The observed aggregated outcomes ranged from -56.33 to 19.83, with the majority of estimates being negative (61 %). The estimated average outcome based on the random-effects model was $\mu=0.88$ (95% CI: -1.35 to 3.12). Therefore, the average outcome did not differ significantly from zero ($z=0.77$, $p=0.44$).

3.7. Effects on pH

The data from 33 multilevel experiments of 26 studies were included in the analysis. The observed standardized mean differences ranged from -15.95 to 31.92, with the majority of estimates being negative (64%). The estimated average standardized mean difference based on the random-effects model was $\mu=-0.75$ (95% CI: -1.82 to 0.33). Therefore, the average outcome did not differ significantly from zero ($z=-1.36$, $p=0.17$).

The aggregated analysis of these multiple experiments showed a total of 26 studies were included in the analysis. The observed aggregated outcomes ranged from -15.95 to 31.92, with the majority of estimates being negative (65 %). The estimated average outcome based on the random-effects model was $\mu=-0.97$ (95% CI: -2.15 to 0.21). Therefore, the average outcome did not differ significantly from zero ($z=-1.61$, $p=0.11$).

3.8. Effects on TAN

The data from 32 multilevel experiments of 24 studies were included in the analysis. The observed standardized mean differences ranged from -19.86 to 38.62, with the majority of estimates being negative (56%). The estimated average standardized mean difference based on the random-effects model was $\mu = -0.70$ (95% CI: -2.08 to 0.67). Therefore, the average outcome did not differ significantly from zero ($z = -0.99$, $p = 0.32$).

The aggregated analysis of these multiple experiments showed a total of 24 studies were included in the analysis. The observed aggregated outcomes ranged from -19.86 to 38.61, with the majority of estimates being negative (50 %). The estimated average outcome based on the random-effects model was $\mu = -0.19$ (95% CI: -2.28 to 1.89). Therefore, the average outcome did not differ significantly from zero ($z = -0.19$, $p = 0.85$).

3.9. Effects on TSS

The data from 24 multilevel experiments of 16 studies were included in the analysis. The observed standardized mean differences ranged from -6.32 to 16.66, with the majority of estimates being positive (92%). The estimated average standardized mean difference based on the random-effects model was $\mu = 4.81$ (95% CI: 3.05 to 6.56). Therefore, the average outcome differed significantly from zero ($z = 5.37$, $p < 0.0001$). A forest plot showing the observed outcomes and the estimate based on the random-effects model is shown in Figure 10.

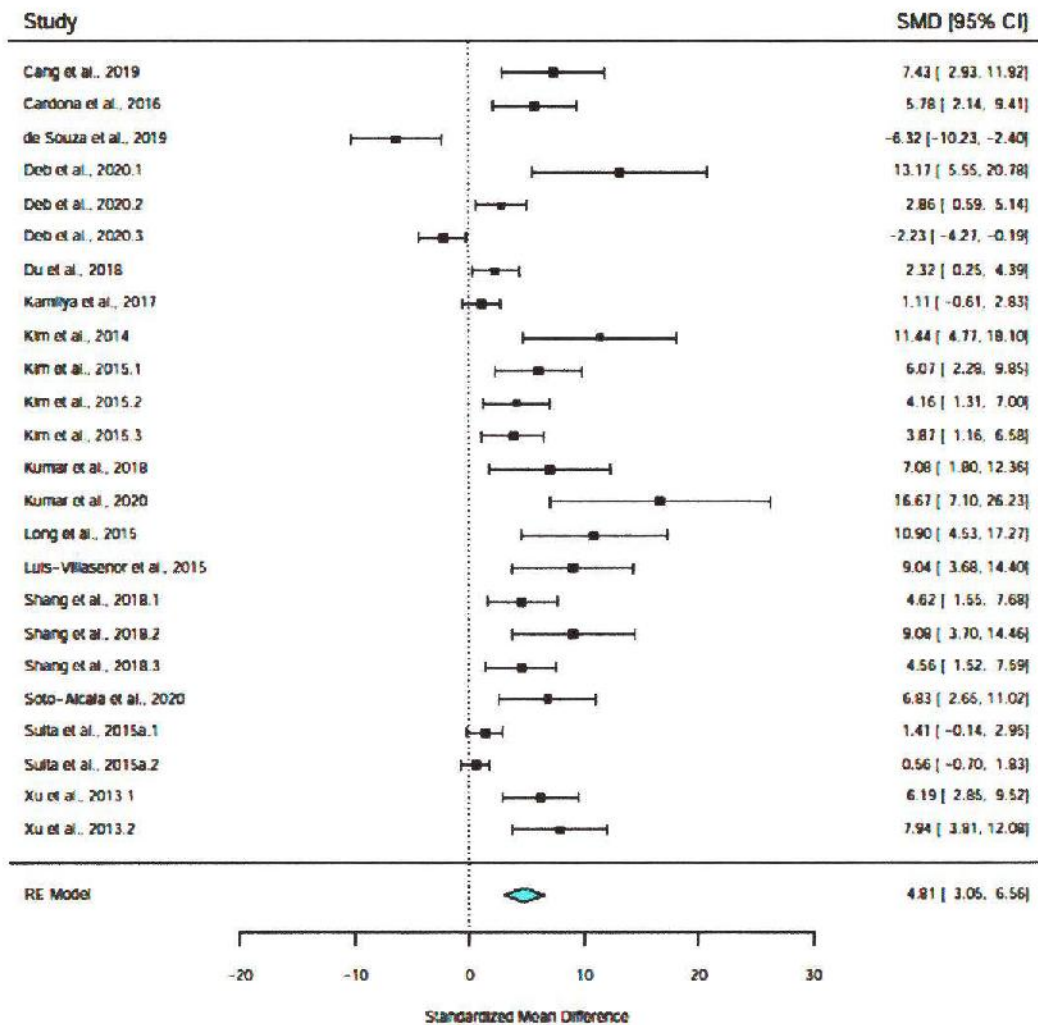


Figure 10. Forest plot reporting the effect size (standardized mean differences) for TSS in different studies of BFT having multiple experimental levels. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (cyan diamond shape). When a study includes multiple treatment levels, it is presented with a ‘numeric number’ after the publication year.

According to the Q-test, the true outcomes appear to be heterogeneous ($Q(23) = 130.13$, $p < 0.0001$, $\tau^2 = 14.99$, $I^2 = 87.82\%$). A 95% prediction interval for the true outcomes is given by -2.98 to 12.59 . Hence, although the average outcome is estimated to be positive, in some studies the true outcome may in fact be negative.

An examination of the studentized residuals revealed that none of the studies had a value larger than ± 3.08 and hence there was no indication of outliers in the context of this model. According to the Cook's distances, none of the studies could be considered to be overly influential. A funnel plot of the estimates is shown in Figure 11. Both the rank correlation and the regression test indicated potential funnel plot asymmetry ($p < 0.0001$ and $p < 0.0001$, respectively).

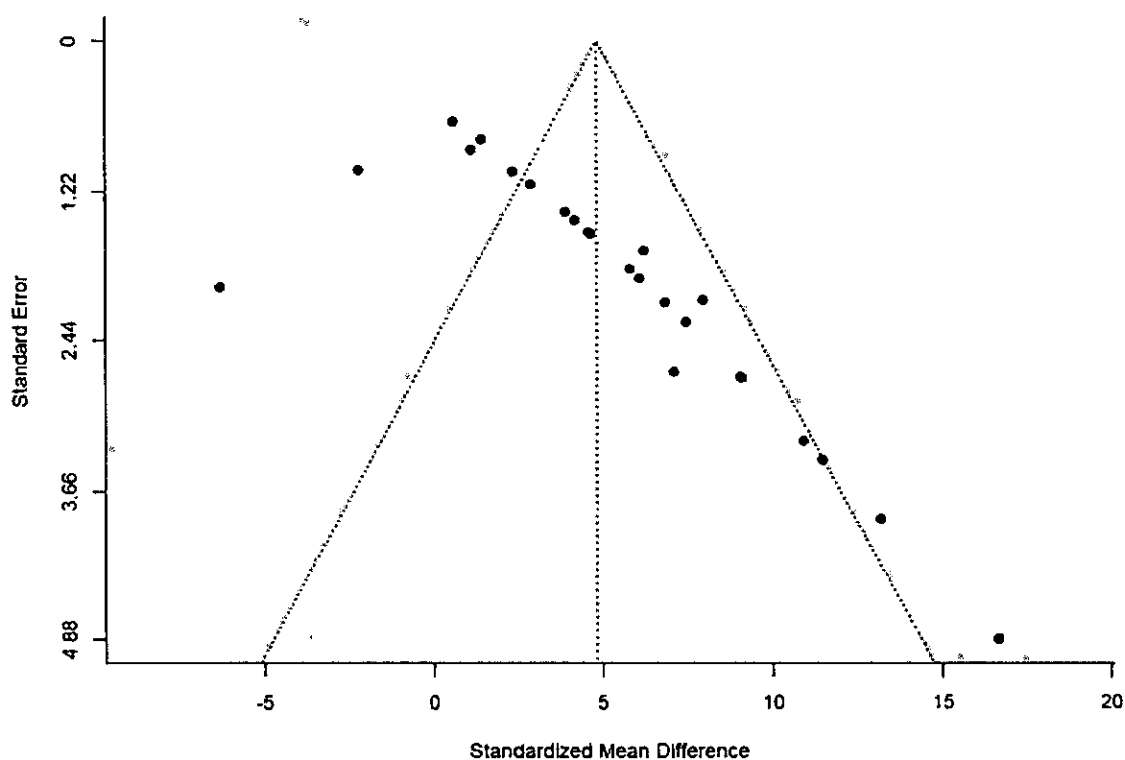


Figure 11. Funnel plot depicting the effect size of each study (x-axis) for TSS in relation to the precision of that study (SE; y-axis). Here points are not scattered symmetrically within the funnel suggesting a publication bias.

The baujat plot also showed the study of de Souza et al., 2019 has the higher values on x-axis that may be considered to be an overly influential case on overall results (Figure12). The studies of Deb et al., 2020.3, Kumar et al., 2020, Deb et al., 2020.1, etc. have the higher values on y-axis that may also be considered to be an overly influential case on overall results (Figure12).

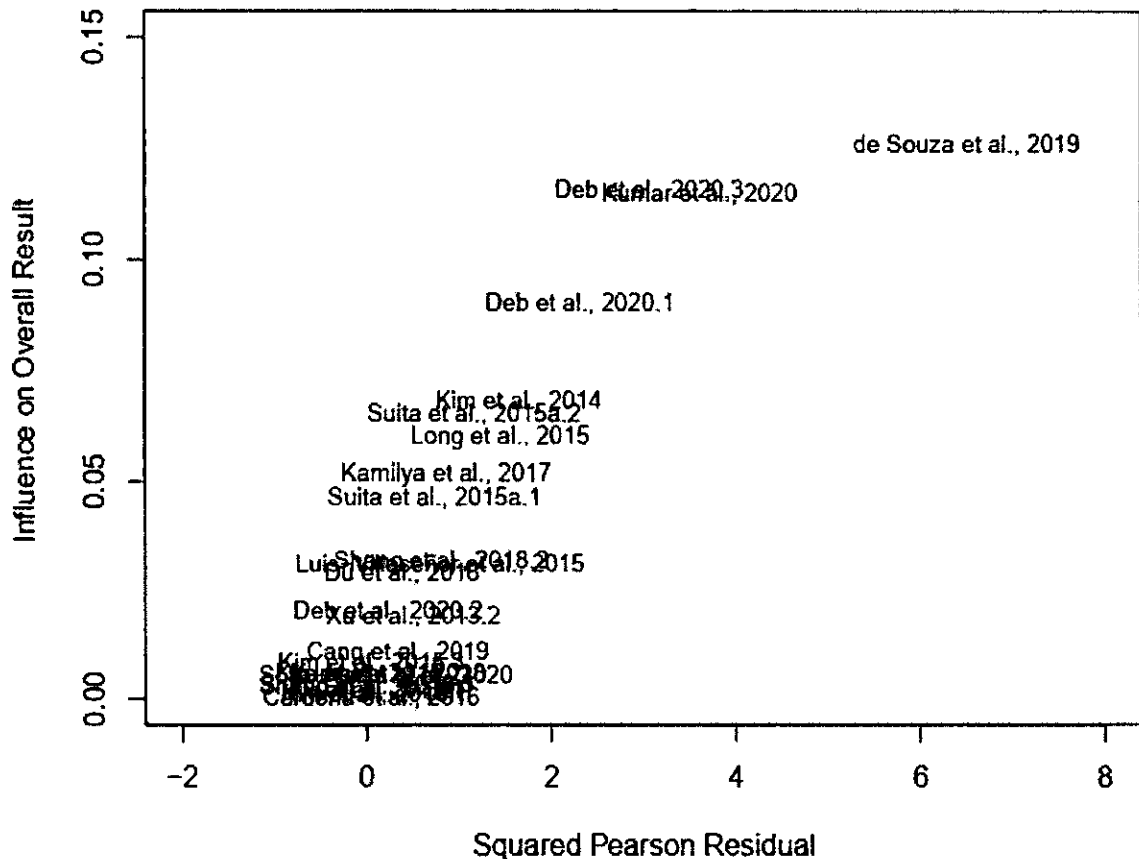


Figure 12. Baujat plot showing the heterogeneity in TSS meta-analytic data. The plot shows the overall heterogeneity contribution of each effect size in x-axis, while the y-axis shows the influence of each effect size on the pooled result.

The sensitivity analysis was done by removing the outliers which showed almost the same significant positive overall effect size $\mu=5.08$ (95% CI: 3.54 to 6.63), $z=6.46$, $p<0.0001$).

The aggregated analysis of these multiple treatments showed a total of 16 studies were included in the analysis. The observed aggregated outcomes ranged from -6.32 to 16.66, with

the majority of estimates being positive (94%). The estimated average outcome based on the random-effects model was $\mu=5.25$ (95% CI: 2.97 to 7.53). Therefore, the average outcome differed significantly from zero ($z=4.51$, $p<0.0001$). A forest plot showing the observed outcomes and the estimate based on the random-effects model is shown in Figure 13.

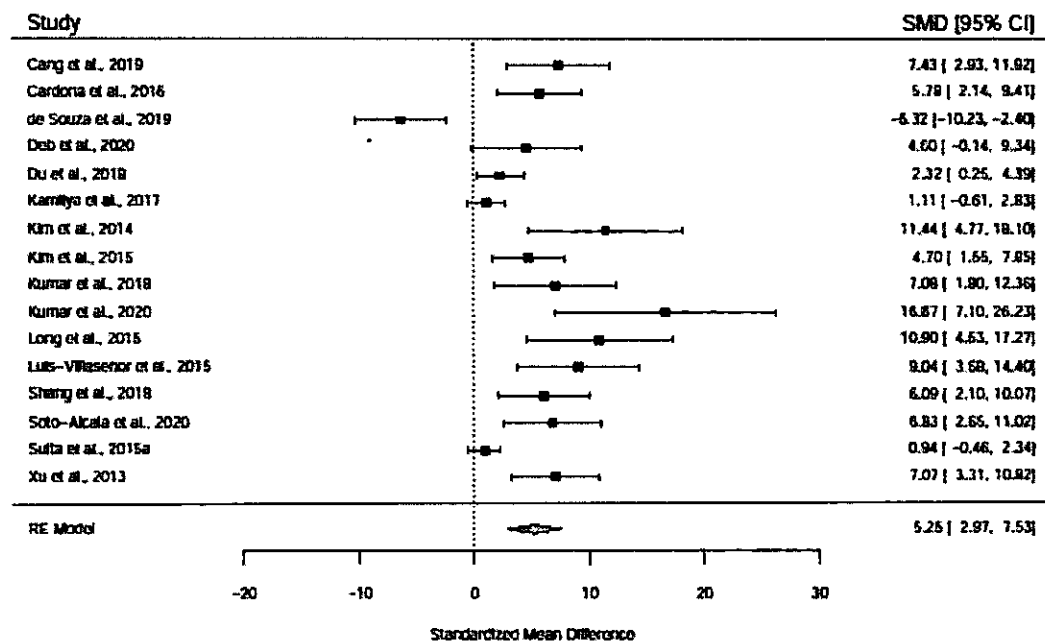


Figure 13. Forest plot reporting the aggregated effect size (standardized mean differences) for TSS in different studies of BFT. Mean and 95% confidence interval (CI) effect sizes are reported. The estimated summary effect size is indicated at the bottom of the figure (blue diamond shape).

The sensitivity analysis was done for this aggregated effect size model by removing the outliers which showed almost the same significant positive overall pooled effect size $\mu=5.68$ (95% CI: 3.87 to 7.49), $z=6.15$, $p<0.0001$).

4. Discussion

Maintaining and monitoring water quality in aquaculture is crucial for the performance of the production cycles in BFT. In aquaculture, organic matter and nitrogen wastes are a major issue for a good and sustainable production (Emerenciano et al., 2017). High carbon-to-nitrogen (C:N) ratios are used to maintain water quality parameters because heterotrophic bacteria can readily absorb nitrogenous byproducts for both maintenance (respiration, feeding, motility, digestion, etc.) as well as for growth and to form new bacterial cells (Emerenciano et al., 2017). The stability of zero or minimal water exchange in BFT depends on the dynamic interaction among communities of bacteria, microalgae, fungi, protozoans, nematode, rotifer, etc. Such consortia of microorganisms usually help the water quality for maintenance and recycling wastes to produce a high-value fish-food (Emerenciano et al., 2017).

The rise in the number of publications in recent years (Fig. 2) revealed the attention of researchers about the influence of biofloc technology on water quality parameters in aquaculture. Based on the analysis, Asian countries take the lead in research on this particular topic (Fig. 3a) which is not surprising because these Asian countries have suitable opportunities and modern research facilities as well as they invest a lot of money for this kind of research. Among various countries, China ranked the highest in publication (8 studies) on this topic followed by Brazil (5 studies), Mexico (4 studies), and other countries (Fig. 3b). Unfortunately, no African country was found to conduct any research on this topic that has been eligible for this meta-analysis. Therefore, poor countries (especially in African region) having valuable fisheries resources should collaborate with the interested rich countries to explore the influence of biofloc technology on water quality parameters of different fish species culture. Although 16 species were investigated during these studies (Fig. 4), more species should be included in the future research particularly many commercially important species are required to know how BFT can influence the water quality parameters for the sustainable production of these species. The review showed the use of a variety of biofloc compounds in the eligible studies including molasses, glucose, sucrose, tapioca flour, cornmeal, seaweed, etc. The analysis revealed that the majority of these studies were utilized molasses as biofloc compound (Fig. 5), however, further research should be conducted by using the locally available, cheap and environment friendly BFT compounds for sustainable aquaculture.

In the present study, a significant negative effect of $\text{NO}_2\text{-N}$ on BFT has been revealed. Most of the reviewed studies showed that $\text{NO}_2\text{-N}$ was significantly lower in BFT than the control group. For examples, Du et al. (2018) used glucose and starch along with heterotrophic bacteria (*Bacillus* sp.) as the organic carbon sources in BFT treatment and found that $\text{NO}_2\text{-N}$ was significantly lower in BFT than that of control group. It might be the cause of the fact that in the BFT system, heterotrophic bacteria can convert ammonia into microbial biomass, which could remove more dissolved nitrogen. Heterotrophic bacteria will increase their need for nitrogen as a result of increased organic carbon intake, which encourages competition for nitrogen between heterotrophic bacteria and phytoplankton (Martínez-Córdova et al., 2014). Luis-Villaseñor et al. (2015) used cornmeal as carbon source and found $\text{NO}_2\text{-N}$ concentration because the addition of cornmeal to the carbon cycle preserves the proper C:N ratio required by bacteria to convert these toxic nitrogen molecules into single cell proteins. For biofloc formation, sugar beet molasses (*Beta vulgaris*) was used to stimulate the growth of heterotrophic bacteria by Kaya et al. (2020) and found lower $\text{NO}_2\text{-N}$ concentration in BFT system than that of control system. Cang et al. (2019) used molasses and found $\text{NO}_2\text{-N}$ was increased gradually and reached the highest value (0.15 mg L^{-1}) at day 12, and then decreased to undetectable level with the development of bioflocs in BFT group. By including a carbon source in the BFT, heterotrophic bacteria are encouraged to flourish, which aids in the conversion of these harmful N-compounds into single-cell proteins (Shang et al., 2018). In the present study, the study of Soto-Alcala et al. (2020) was found to be overly influential. They used molasses as a carbon source for biofloc formation and found lower $\text{NO}_2\text{-N}$ concentration in BFT than control group. $\text{NO}_2\text{-N}$ is a crucial element in determining the success of aquaculture. Fish are severely harmed by $\text{NO}_2\text{-N}$, even at low concentrations (Montealegre et al., 2002). Boyd and Pillai (1985) reported that $\text{NO}_2\text{-N}$ levels above 1.0 mgL^{-1} in pond water might cause death to the cultured fish. They found overall negative effects of $\text{NO}_2\text{-N}$ in BFT, however, some reviewed studies showed positive effects. For example, Ray et al. (2017) used foam fractionator to manage solids concentration and an external bio-filter for nitrogen control and found that $\text{NO}_2\text{-N}$ contributed a significantly higher concentration in the biofloc treatment. In systems with a functioning nitrifying bacterial population, nitrogen concentration did not appear to accumulate as would be predicted; this could mean that the floc community was not fully developed. This is not clear why such continuous accumulation did not take place (Ray et al., 2017). Sgnaulin et al. (2018) used corn

meal, wheat meal and molasses as carbon source and found that $\text{NO}_2\text{-N}$ concentration was higher than control group. Most serious effects in fish exposed to nitrite are increased plasma methemoglobin and nitrite levels, resulting in hypoxia, stress, reduced feed intake and reduced growth (Arillo et al., 1984; Hilmy et al., 1987; Woo and Chiu, 1997).

The meta-analysis revealed a significant positive effect of TSS in BFT. An overall positive effect was shown by different studies. For example, Kumar et al. (2020) used glucose in BFT and found that TSS recorded significantly higher in biofloc group as compared to clear water group, and Luis-Villaseñor et al. (2015) also used glucose and found TSS significantly higher in biofloc group than control group. Long et al. (2015) used glucose as carbon source and found that TSS level in the BFT group increased gradually throughout the experimental period. The average concentration was 24.61 mgL^{-1} in the control group, while 484.48 mgL^{-1} in the BFT treatment (Long et al., 2015). Ray et al. (2010) demonstrated that the TSS concentration in the BFT tanks should be well controlled because the anti-clogging of fish gills and water quality are intimately connected. Kim et al. (2015) stated that TSS was significantly higher in all biofloc treatments compared to seawater because an intensive shrimp production system with zero exchange provided the water source for biofloc treatment every day throughout this trial. Cang et al. (2019) used molasses as carbon source and found TSS increased with the development of bioflocs in BFT system and was much higher than that in control group due to water exchange in control. TSS is heavily reliant on pond conditions and management techniques including feed, water quality parameters, stocking density, and culture period (Deb et al., 2020). TSS is related with bioflocs formation in BFT group. TSS concentration of $400\text{-}600 \text{ mgL}^{-1}$ was considered suitable for super-intensive culture of *L. vannamei* (Gaona et al., 2017). When TSS was lower than 100 mgL^{-1} , water quality was difficult to maintain well (Schveitzer et al., 2013). However, high TSS ($\geq 800 \text{ mgL}^{-1}$) might become a stressor to respiration of the shrimp since gills were clogged with suspended solids (Schveitzer et al., 2013). But de Souza et al. (2019) showed negative result where TSS was recorded significantly lower in biofloc group as compared to clear water group after using molasses as carbon source in BFT. It is unclear why such biofloc production did not occur in the BFT system.

Although no significant effect of alkalinity on BFT has been revealed in this study, an overall negative effect was shown by different studies. For example, de Souza et al. (2019) and Tapaamorndech et al. (2020) used molasses, Kumar et al. (2018) used molasses, sugar and

wheat, Suita et al. (2015) used glucose, Vinatea et al. (2018) used glucose as a carbon source in BFT, and found the values obtained for alkalinity were lower in the BFT system than the control. This was most likely caused by nitrification, which increased the amount of carbon dioxide in the water used in the biofloc treatment, and by the respiration of heterotrophic organisms (Wasiolesky Jr. et al., 2006; Furtado et al., 2011). On the other hand, there are some studies which showed positive effect sizes. Kaya et al. (2020) used molasses and showed alkalinity parameters of both trials were higher in BFT tanks compared to control tanks. Jatoba et al. (2019) used sugar and powdered diet, while Sgnaulin et al. (2018) used cornmeal, wheatmeal and molasses as carbon source who also showed higher alkalinity in BFT tanks compared to control tanks.

No significant effect of DO on BFT has been revealed in this study, an overall negative effect of DO on BFT has been revealed in this meta-analysis. For example, Kaya et al. (2019) showed that DO values were lower in the biofloc groups compared with the control groups. This was most likely caused by the higher oxygen requirements of heterotrophic bacteria in biofloc tanks (Emerenciano et al., 2012, Xu and Pan., 2013). Long et al. (2015) used glucose as carbon source and found that DO was maintained at a level greater than 6 mgL⁻¹ and differed significantly between BFT and control group. This likely resulted from the higher respiration rates caused by the bacteria and other microorganisms in the BFT group (Emerenciano et al., 2012, Kim et al., 2014). However, the DO level in the BFT treatment was well within an acceptable range for the survival and growth of fish. Similar results were recorded by Martins et al. (2015), Kumar et al. (2020), de souza et al. (2019), and Chan-Vivas et al. (2019). But there are some studies which showed positive results. For example, Vinatea et al. (2018) showed that the levels of DO in the BFT tanks of *M. cephalus* were significantly higher than those registered in the RAS due to the incorporation of pure oxygen to cover the high oxygen demand caused by the bacterial respiration of the bioflocs, especially during the application of glucose (Avnimelech, 1999). However, only in the mullet BFT tanks did pure oxygen addition become necessary. This is likely because the mullet's swimming activity was significantly higher than that of the tench, and the mullet approached the surface to receive food with great excitement while the tench remained listless at the tank bottom (Vinatea et al., 2018). Kumar et al. (2018) showed significantly higher DO in the biofloc pond, but within acceptable range for culture of *P. vannamei*, indicating the impact of aeration.

No significant effect of $\text{NH}_3\text{-N}$ on BFT has been revealed in this study. An overall negative effect of DO on BFT has been revealed in this meta-analysis. For example, Kumar et al. (2018) used molasses, sugar and wheat as carbon source and showed that $\text{NH}_3\text{-N}$ concentration decreased significantly in the biofloc pond when compared with the control. It was apparent that the addition of carbon increased total heterotrophic bacterial population in the biofloc pond having the ability to consume $\text{NH}_3\text{-N}$, and $\text{NO}_3\text{-N}$ for their growth and multiplication (Avnimelech, 1999). Ammonia cannot be completely eliminated from the system because of the increased unaccounted nitrogen loss (denitrification and ammonia volatilization) (De Schyver and Verstraete, 2009). Similar results are found by Martins et al. (2015). Some studies showed positive effect of $\text{NH}_3\text{-N}$ on BFT. For example, Vinatea et al. (2018) used glucose as carbon source and found that $\text{NH}_3\text{-N}$ concentration was higher in BFT group than control group. In biofloc-based cultures, ammonia can be controlled by the application of carbohydrates (Avnimelech, 1999). However, because of the limited amount of water used and the high protein content of the feeds, the oxygen concentration was impaired due to the high glucose requirements. This remained true even after spreading the addition of glucose out over the course of the day (two in the morning, at 08 and 12 h, and two in the afternoon, at 15 and 18 h) (Vinatea et al., 2018). Kaya et al. (2020) experimented two trials with different stocking densities (trial I: 20 shrimp/0.24m², trial II: 10,20,30,40 shrimp/m²). In trial I, $\text{NH}_3\text{-N}$ concentration was lower in BFT than control group, and in trial II, $\text{NH}_3\text{-N}$ concentration was higher in BFT than control group. Because tanks were being stocked at higher densities, the higher ammonia level in Trial II might be related to an increase in organic waste.

Although no significant effect of $\text{NO}_3\text{-N}$ on BFT has been revealed in this study, an overall negative effect of $\text{NO}_3\text{-N}$ on BFT has been revealed in this meta-analysis. For example, Kumar et al. (2018) showed that $\text{NO}_3\text{-N}$ concentration was lower in BFT than control group. It was apparent that the addition of carbon increased total heterotrophic bacterial population in the biofloc pond having the ability to consume $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ for growth and multiplication. Similar results were found by Shang et al. (2018), and Soto-Alcalá et al. (2020). Long et al. (2015) showed that the $\text{NO}_3\text{-N}$ concentration in the control group fluctuated throughout the culture period and a similar trend was observed in the BFT treatment. The highest $\text{NO}_3\text{-N}$ level in the BFT was 5.45 mg/L, observed in week 3, which then gradually decreased over time. The $\text{NO}_3\text{-N}$ concentration was significantly higher in the control and showed a tendency to

accumulate in the first 5 weeks of the culture period to levels of 11.64 mg/L. In week 8, the level first dropped and then increased once again. The immobilization by heterotrophic bacteria, which impeded the nitrification process, is most likely what caused the decrease in NO₃-N from the third week until the end of the experiment. In addition, denitrification may have occurred during the experiment. Some studies showed positive result. For example, Martins et al. (2015) used molasses and found higher nitrate concentration in BFT than control group. Lower nitrate levels were observed in control where 80–90% of water was renewed every 48 h and in BFT, where no water renewal was performed. Kim et al. (2014) showed that NO₃-N concentration was higher in BFT than control due to the extensive nitrification. Xu et al. (2013) showed that the accumulation of NO₃-N concentration occurred in the two bioflocs treatments. This demonstrated the presence of nitrifying bacteria in the bioflocs and suggested that both heterotrophic and autotrophic TAN removal mechanisms might have taken place in the system. Similar results were found by Kim et al. (2015), de souza et al. (2019), Ray et al. (2017), and Yun et al. (2016).

No significant effect of TAN on BFT has been revealed in this study, an overall negative effect of TAN on BFT has been revealed in this meta-analysis. For example, Kumar et al. (2018) used molasses, sugar and wheat as a carbon source and showed that the TAN concentration in the biofloc system gets effectively reduced after the formation of biofloc by both heterotrophic assimilation and autotrophic nitrification when carbon and nitrogen were well balanced. de Souza et al., (2019) also showed that TAN was also lower in BFT tanks due to the nitrification process performed by the microorganisms present in this system. Luis-Villaseñor et al. (2015) showed low TAN concentration because the additional carbon source cornmeal maintains an appropriate C:N ratio for bacterial transformation of these toxic N compounds into single cell protein. Xu et al. (2013) used brown sugar and showed the same result, indicating that nitrifying bacteria were present in the bioflocs and both heterotrophic and autotrophic TAN removal processes might have occurred in the system. Similar results were found by Zhao et al. (2012), Nguyen et al. (2019), and Deb et al. (2020). However, some studies showed positive results. The study of Kaya et al. (2019) could be considered to be overly influential. In this study, reduced feeding rates and effects of the fasted were evaluated comparatively in BFT and control groups. The levels of TAN values were higher in the biofloc groups. The aquatic species being raised, and the sources of the diet's basic materials were hypothesized to affect the rates of the nutrient components of the biofloc product. Vinatea et al. (2018) showed that TAN concentration

was always higher in BFT rearing tanks in both grey mullet and tench than in RAS cultures. In Long et al. (2015) the difference of TAN concentrations was fairly consistent over time between the two groups. The TAN concentration in the biofloc group remained stable from week 1 to the end of the experiment. During the 8-week experimental period, no water was discharged from the BFT treatment. By adding a carbon source (Glucose) to stimulate the growth of heterotrophic bacteria, TAN can be used and stored by the formation of biofloc microbes (Asaduzzaman et al., 2008, Avnimelech, 1999, Ebeling et al., 2006, Emerenciano et al., 2012), which may explain why the TAN concentration did not exhibit significant difference between the clear water control group and zero water exchange BFT treatment.

Although no significant effect of pH on BFT has been revealed in this study, an overall negative effect of pH on BFT has been revealed in this meta-analysis. For example, de Souza et al. (2019) showed that the value obtained for pH was lower in the BFT system than in control. This was most likely caused by the respiration of heterotrophic organisms, which raised the carbon dioxide concentration in the biofloc treatment water (Wasielesky Jr. et al., 2006; Furtado et al., 2011). Kumar et al. (2018) also showed that pH was lower in the BFT because of dominance of heterotrophic over autotrophic bacteria in the system and the impact of carbon dioxide excretion by microbes during respiration. In Kim et al. (2014), the pH in the biofloc group was lower than that of the control, most likely as a result of substantial nitrification and the respiration of heterotrophic organisms, which increased the water's carbon dioxide concentration during the biofloc treatment (Tacon et al., 2002; Wasielesky et al., 2006). Similar results were found by Kim et al. (2015), Kumar et al. (2020), and Xu et al., (2013). However, some studies showed positive results. For example, Martins et al., (2015) showed that BFT system possessed significantly higher values for pH compared with control but were within acceptable limits for the survival and growth of *L. vannamei*. Long et al. (2015) showed higher value of pH than control but there was no significant difference between the systems. Kaya et al. (2019) also showed higher value of pH than control but there was no significant difference between the systems.

5. Conclusion

The current literature review and meta-analysis is about the influence of biofloc technology on water quality parameters in aquaculture. In general, the findings of meta-analysis ensure that BFT improves the water quality of different aquaculture systems significantly by maintaining some physico-chemical parameters such as $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_3\text{-N}$, TAN, TSS, pH, DO, and alkalinity etc. Microbial community is able to rapidly utilize dissolved nitrogen leached from cultured shrimp or fish feces and uneaten food and convert it into microbial protein, and thus, maintaining the water quality. The physical, chemical, and biological interactions that occur into the BFT are complex. The findings of this study could provide information available for researchers and interested stakeholders, and give an overview of the current status and make recommendations for proper maintenance and management of different water quality parameters in different aquaculture systems using BFT.

6. References

- Abakari, G., Luo, G., Kombat, E. O., Alhassan, E. H., 2021. Supplemental carbon sources applied in biofloc technology aquaculture systems: types, effects and future research. *Aquaculture*. 13(3), 1193-1222.
- Ahmad, I., Babitha Rani, A. M., Verma, A. K., Maqsood, M., 2017. Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture*. 25, 1215–1226.
- Arillo, A., Gaino, E., Margiocco, C., Mensi, P., Schenone, G., 1984. Biochemical and ultrastructural effects of nitrite in rainbow trout: liver hypoxia as the root of the acute toxicity mechanism. *Environ*. 34(1), 135-154.
- Asaduzzaman, M., Wahab, M. A., Verdegem, M. C. J., Huque, S., Salam, M. A., Azim, M. E., 2008. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*. 280, 117-123.
- Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*. 176 (3–4), 227–235.
- Avnimelech, Y., 2006. Bio-filters: The need for a new comprehensive approach. *Aquaculture*. 34, 172-178.
- Avnimelech, Y., 2009. Biofloc technology. A practical guide book. The World Aquaculture Society. Baton Rouge. 182.
- Azim, M.E., Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*. 83, 29-35.
- Bakar, N. S. A., Nasir, N. M., Lananan, F., Hamid, S. H. A., Lam, S. S., Jusoh, A., 2015. Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish (*Clarias gariepinus*) utilizing Bioflocs Technology. *Int. Biodeterior. Biodegradation*. 102, 100-106.

- Begg, C. B., Mazumdar, M., 1994. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. 50(4), 1088–1101.
- Bossier, P., Ekasari, J., 2017. Biofloc technology application in aquaculture to support sustainable development goals. *Microb. Biotechnol.* 10, 1012–1016.
- Boyd, C.E., Pillai, V., 1985. Water Quality management in aquaculture. *CMFRI Special*. 22, 1-44.
- Brito, C., do Valle, B., Interaminense, J., Peixoto, S., Lima-Filho, J. V., Soares, R., 2016. Microbiological quality of *Litopenaeus vannamei* culture using conventional and biofloc systems. *Aquaculture*. 47(10), 3098-3108.
- Brito, L. O., Chagas, A.M., da Silva, E.P., Soares, R.B., Severi, W., Galvez, A.O., 2016. Water quality, *Vibrio* density and growth of Pacific white shrimp *Litopenaeus vannamei* (Boone) in an integrated biofloc system with red seaweed *Gracilaria birdiae* (Greville). *Aquaculture*. 47, 940–950.
- Cang, P., Zhang, M., Qiao, G., Sun, Q., Xu, D., Li, Q., Liu, W., 2019. Analysis of Growth, Nutrition and Economic Profitability of Gibel Carp (*Carassius auratus gibelio*♀× *C. carpio*♂) Cultured in Zero-water Exchange System. *Pak. J. Zool.* 51(2), 619.
- Cardona, E., Gueguen, Y., Magré, K., Lorgeoux, B., Piquemal, D., Pierrat, F., Saulnier, D., 2016. Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system. *BMC Microbiol.* 16(1), 1-9.
- Chan-Vivas, E., Edén, M. G., Maldonado, C., Escalante, K., Gaxiola, G., Cuzon, G., 2019. Does biofloc improve the energy distribution and final muscle quality of shrimp, *Litopenaeus vannamei* (Boone, 1883). *Aquaculture*. 50(2), 460-468.
- Clements, J., George, M., 2021. Ocean acidification and bivalve byssus: explaining variable responses using meta-analysis.
- Cochran, W. G., 1954. The combination of estimates from different experiments. *Biometrics*. 10(1), 101–129.

- Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W., 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture*. 41, 559–567.
- Crab, R., Defoirdt, T., Bossier, P., Verstraete, W., 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*. 356, 351–356.
- Crombie, I. K., Davies, H. T., 2009. What is meta-analysis. *What is*, 1(8).
- da Silva Martins, Á. C., Artigas Flores, J., Porto, C., Wasielesky Junior, W., Monserrat, J. M., 2015. Antioxidant and oxidative damage responses in different organs of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) reared in a biofloc technology system. *Marine and Freshwater Behaviour and Physiology*. 48(4), 279–288.
- De Schryver, P., Verstraete, W., 2009. Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. *Bioresour. Technol.* 100(3), 1162–1167.
- de Souza, D. M., Suita, S. M., Romano, L. A., Wasielesky Jr, W., Ballester, E. L. C., 2014. Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system. *Aquaculture*. 45(2), 270–277.
- Deb, S., Noori, M. T., Rao, P. S., 2020. Application of biofloc technology for Indian major carp culture (polyculture) along with water quality management. *Aquaculture*. 91, 102–106.
- Du, X., Almeida, D., Song, D., Zhao, Z., Luo, L., Li, J., Xu, Q., 2018. Effects of organic carbon addition on water quality and phytoplankton assemblages in biofloc technology ponds. *Aquaculture*. 497, 155–163.
- Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*. 257 (1–4), 346–358.
- Egger, M., Smith, G. D., Phillips, A. N., 1997. Meta-analysis: principles and procedures. *Bmj*. 315(7121), 1533–1537.

- Ekasari, J., Suprayudi M.A., Wiyoto, W., Hazanah, R.F., Lenggara, G.S., Sulistiani, R., Alkahfia, M., ZairinJra, M., 2016. Biofloc technology application in African catfish fingerling production: the effects on the reproductive performance of broodstock and the quality of eggs and larvae. *Aquaculture*. 464, 349-356.
- Emerenciano, M. G. C., Martinez-Cordova, L.R., Martinez-Porchas, M., Miranda-Baeza, A., 2017. Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture. *Water Quality*. 5, 92-109.
- Emerenciano, M., Ballester, E. L., Cavalli, R. O., Wasielesky, W., 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture*. 43(3), 447-457.
- Emerenciano, M., Cuzon, G., Paredes, A., Gaxiola, G., 2013. Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc. *Aquaculture*. 21(6), 1381-1394.
- Espírito Santo, C.M., Pinheiro, I.C., Jesus, G.F.A., Mouriffo, J.L.P., Vieira, F.D.N., Seiffert, W.Q., 2017. Soybean molasses as an organic carbon source in the farming of *Litopenaeus vannamei* (Boone, 1931) in a biofloc system. *Aquaculture*. 48 (4), 1827-1835.
- Furtado, P. S., Poersch, L. H., Wasielesky Jr, W., 2011. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. *Aquaculture*. 321(1-2), 130-135.
- Gaona, C.A.P., Almeida, M.S.D., Viau, V., Poersch, L.H. Wasielesky, Jr. W., 2017. Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. *Aquaculture*. 48, 1070-1079.
- Helfrich, L. A., Libey, G. S., 1991. Fish farming in recirculating aquaculture systems (RAS). Virginia Cooperative Extension.
- Higgins, J. P. T., Thompson, S. G., 2002. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21(11), 1539-1558.

- Hilmy, A. M., El-Domiaty, N. A., Wershana, K., 1987. Acute and chronic toxicity of nitrite to *Clarias lazera*. Comparative Biochemistry and physiology. C, Comparative Pharmacology and Toxicology. 86(2), 247-253.
- Jackson, C.N., Preston, P.J. Thompson, M., 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture. 218, 397-411.
- Jatobá, A., Borges, Y. V., Silva, F. A., 2019. BIOFLOC: sustainable alternative for water use in fish culture. Arq Bras Med Vet Zootec. 71, 1076-1080.
- Kamilya, D., Debbarma, M., Pal. P., Kheti. B., Sarkar, S., Singh, S.T., 2017. Biofloc technology application in indoor culture of *Labeo rohita* (Hamilton, 1822) fingerlings: the effects on inorganic nitrogen control, growth and immunity. Chemosphere. 182, 8-14.
- Kaya, D., Genc, E., Genc, M. A., Aktas, M., Eroldogan, O. T., Guroy, D., 2020. Biofloc technology in recirculating aquaculture system as a culture model for green tiger shrimp, *Penaeus semisulcatus*: Effects of different feeding rates and stocking densities. Aquaculture. 528, 735526.
- Kaya, D., Genc, M. A., Aktas, M., Yavuzcan, H., Ozmen, O., Genc, E., 2019. Effect of biofloc technology on growth of speckled shrimp, *Metapenaeus monoceros* (Fabricus) in different feeding regimes. Aquaculture. 50(10), 2760-2768.
- Khanjani, M. H., Sharifinia, M., 2020. Biofloc technology as a promising tool to improve aquaculture production. Aquaculture. 12(3), 1836-1850.
- Khatoon, H., Banerjee, S., Guan Yuan, G. T., Haris, N., Ikhwanuddin, M., Ambak, M. A., 2016. Biofloc as a potential natural feed for shrimp postlarvae. Int. Biodeterior. Biodegradation. 113, 304-309.
- Kim, S. K., Guo, Q., Jang, I. K., 2015. Effect of biofloc on the survival and growth of the postlarvae of three penaeids (*Litopenaeus vannamei*, *Fenneropenaeus chinensis*, and *Marsupenaeus japonicus*) and their biofloc feeding efficiencies, as related to the morphological structure of the third maxilliped. J. Crust. Biol. 35(1), 41-50.

- Kim, S. K., Pang, Z., Seo, H. C., Cho, Y. R., Samocha, T., Jang, I. K., 2014. Effect of bioflocs on growth and immune activity of Pacific white shrimp, *Litopenaeus vannamei* postlarvae. *Aquaculture*. 45(2), 362-371.
- Kim, S., Pang, Z., Seo, H., Cho, Y., Samocha, T., Jang, I., 2014. Effect of bioflocs on growth and immune activity of pacific white shrimp. *Aquaculture*. 45, 362-371.
- Kolarevic, J., Selset, R., Felip, O., Good, C., Šnekvik, K., Takle, H., Ytteborg, E., Bæverfjord, G., Åsgård, T., Terjesen, B.F., 2012. Influence of long term ammonia exposure on Atlantic salmon (*Salmo salar* L.) parr growth and welfare. *Aquaculture*. 44(11), 1649-1664.
- Krummenauer, D., Samocha, T., Poersch, L., Lara, G., and Wasielesky Jr, W., 2014. The reuse of water on the culture of Pacific white shrimp, *Litopenaeus vannamei*, in BFT system. *Aquaculture*. 45(1), 3-14.
- Kumar, V. S., Pandey, P. K., Anand, T., Bhuvaneswari, G. R., Dhinakaran, A., Kumar, S., 2018. Biofloc improves water, effluent quality and growth parameters of *Penaeus vannamei* in an intensive culture system. *J. Environ. Manage.* 215, 206-215.
- Kumar, V., Wille, M., Lourenço, T. M., Bossier, P., 2020. Biofloc-based enhanced survival of *Litopenaeus vannamei* upon AHPND-causing *Vibrio parahaemolyticus* challenge is partially mediated by reduced expression of its virulence genes. *Front. Microbiol.* 11, 1270.
- Li, J., Li, J., Li, W., Sun, Y., Liu, X., Liu, M., Cheng, Y., 2019. Juvenile *Procambarus clarkii* farmed using biofloc technology or commercial feed in zero-water exchange indoor tanks: A comparison of growth performance, enzyme activity and proximate composition. *Aquaculture*. 50(7), 1834-1843.
- Long, L., Yang, J., Li, Y., Guan, C., Wu, F., 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*. 448, 135-141.
- Luis-Villaseñor, I. E., Voltolina, D., Audelo-Naranjo, J. M., Pacheco-Marges, M. R., Herrera-Espéricueta, V. E., Romero-Beltrán, E., 2015. Effects of biofloc promotion on water

- quality, growth, biomass yield and heterotrophic community in *Litopenaeus vannamei* (Boone, 1931) experimental intensive culture. Ital. J. Anim. Sci. 14(3), 3726.
- Martínez-Córdova, L.R., Emerenciano, M., Miranda-Baeza, A., Martínez-Porchas, M., 2014. Microbial-based systems for aquaculture of fish and shrimp: an updated review. Rev. Aquaculture. 6, 1–18.
- Montealegre, R.J., Marc, V., Jorge, E.Z., Johan, V., 2002. Organic matter sedimentation and resuspension in tilapia (*Oreochromis niloticus*) ponds during a production cycle. Aquaculture. 26 (1), 1–12.
- Nguyen, N. H., Trinh, L. T., Chau, D. T., Baruah, K., Lundh, T., Kiessling, A., 2019. Spent brewer's yeast as a replacement for fishmeal in diets for giant freshwater prawn (*Macrobrachium rosenbergii*), reared in either clear water or a biofloc environment. Aquac. Nutr. 25(4), 970-979.
- Nhi, N. H. Y., Da, C. T., Lundh, T., Lan, T. T., Kiessling, A., 2018. Comparative evaluation of Brewer's yeast as a replacement for fishmeal in diets for tilapia (*Oreochromis niloticus*), reared in clear water or biofloc environments. Aquaculture. 495, 654-660.
- Noble, D. W., Pottier, P., Lagisz, M., Burke, S., Drobniak, S. M., O'Dea, R. E., & Nakagawa, S., 2022. Meta-analytic approaches and effect sizes to account for 'nuisance heterogeneity' in comparative physiology. Exp. Biol. 225(Suppl_1), jeb243225.
- Otoshi, C. A., Naguwa, S. S. Falesch, F. C., 2008. Commercial-scale Production of Pacific White Shrimp *Litopenaeus vannamei* in a Biosecure, Super-intensive, Recirculating Aquaculture System. Lake Buena Vista. 71–84.
- Page, M., McKenzie, J., Bossuyt, P., Boutron, I., Hoffmann, T., Mulrow, C., Shamseer, L., Tetzlaff, J., Akl, E., Brennan, S., Chou, R., Glanville, J., Grimshaw, J., Hróbjartsson, A., Lalu, M., Li, T., Loder, E., Mayo-Wilson, E., McDonald, S., Moher, D., 2021. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Systematic reviews. 10(1), 1-11.
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

- R Development Core Team, 2021. R: A language and environment for statistical computing, version 4.0.5. R Foundation for Statistical Computing, Vienna, Austria.
- Ray, A. J., Drury, T. H., Cecil, A., 2017. Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. *Aquacultural Engineering*, 77, 9-14.
- Riley, R. D., Higgins, J. P. T., Deeks, J. J., 2011. Interpretation of random effects meta-analyses. *BMJ*. 342, d549.
- Schveitzer, R., Arantes, R., Costódio, P.F.S., do Espírito Santo, C.M., Arana, L.V., Seiffert, W.Q. Andreatta, E.R., 2013. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. *Aquaculture*. 56, 59-70.
- Sgnaulin, T., de Mello, G. L., Thomas, M. C., Garcia, J. R. E., de Oca, G. A. R. M., Emerenciano, M. G. C., 2018. Biofloc technology (BFT): An alternative aquaculture system for piracanjuba *Brycon orbignyanus*. *Aquaculture*. 485, 119-123.
- Shang, Q., Tang, H., Wang, Y., Yu, K., Wang, L., Zhang, R., Wei, C., 2018. Application of enzyme-hydrolyzed cassava dregs as a carbon source in aquaculture. *Sci. Total Environ*. 615, 681-690.
- Soto-Alcalá, J., Álvarez-Ruiz, P., Audelo-Naranjo, J. M., Esparza-Leal, H. M., Luis-Villaseñor, I. E., Estrada-Godínez, J. A., Diarte-Plata, G., 2019. Transcriptional response of immune-related genes in *Litopenaeus vannamei* post-larvae cultured in recirculating aquaculture systems with and without biofloc. *Aquaculture*. 27(1), 209-225.
- Souza, D. M. D., Kutter, M. T., Furtado, P. S., Romano, L. A., Wasielesky, W., Monserrat, J. M., Garcia, L., 2019. Growth, antioxidant system, and immunological status of shrimp in bioflocs and clear water culture systems. *Pesquisa Agropecuária Brasileira*, 54.
- Sterne, J. A., Egger, M., (2005). Regression methods to detect publication and other bias in meta-analysis. *Publication bias in meta-analysis: Prevention, assessment and adjustments*. 99-110.

- Suita, S. M., Cardozo, A. P., Romano, L. A., Abreu, P. C., Wasielesky Jr, W., 2015. Development of the hepatopancreas and quality analysis of post-larvae Pacific white shrimp *Litopenaeus vannamei* produced in a BFT system. *Aquaculture*. 23(2), 449-463.
- Tacon A.G.J., Cody J.J., Conquest L.D., Divakaran S., Forster I.P. Decamp O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* fed different diets. *Aquaculture*. 8, 121-137.
- Taw, N., 2010. Biofloc technology expanding at white shrimp farms. *Global aquaculture advocate*. 24-26.
- Tepaamorndech, S., Nookaew, I., Higdon, S. M., Santiyanont, P., Phromson, M., Chantarasakha, K., Visessanguan, W., 2020. Metagenomics in bioflocs and their effects on gut microbiome and immune responses in Pacific white shrimp. *Fish & Shellfish Immunol*. 106, 733-741.
- Viechtbauer, W., 2005. Bias and efficiency of meta-analytic variance estimators in the random-effects model. *J Educ Behav Stat*. 30(3), 261-293.
- Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. *Statistical Software*. 36(3), 1-48.
- Viechtbauer, W., Cheung, M. W.-L., 2010. Outlier and influence diagnostics for meta-analysis. *Research Synthesis Methods*. 1(2), 112-125.
- Vinatea, L., Malpartida, J., Carbó, R., Andree, K. B., Gisbert, E., Estévez, A., 2018. A comparison of recirculation aquaculture systems versus biofloc technology culture system for on-growing of fry of *Tinca tinca* (Cyprinidae) and fry of grey *Mugil cephalus* (Mugilidae). *Aquaculture*. 482, 155-161.
- Wang, Z.S., Zhao, W.H., Lv, F., Zhang, H. Huang, J.T., 2012. Study on annual nitrogen and phosphorus nutrients changes in gibel carp (*Carassius auratus gibelio*) culture. *Jiangsu Agric. Sci*. 40, 252-254.
- Wasielesky Jr, W., Atwood, H., Stokes, A., Browdy, C. L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*. 258(1-4), 396-403.

- Woo, N. Y. S., Chiu, S. F., 1997. Metabolic and osmoregulatory responses of the sea bass *Lates calcarifer* to nitrite exposure. *Environmental Toxicology and Water Quality*. 12(3), 257-264.
- Xu, W. J., Pan, L. Q., 2013. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in bio-floc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture*. 412, 117-124.
- Xu, W. J., Pan, L. Q., Sun, X. H., Huang, J., 2013. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. *Aquaculture*. 44(7), 1093-1102.
- Xu, W. J., Pan, L. Q., Zhao, D. H., Huang, J., 2012. Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture*. 350, 147-153.
- Yun, H., Shahkar, E., Katya, K., Jang, I. K., Kim, S. K., Bai, S. C., 2016. Effects of bioflocs on dietary protein requirement in juvenile whiteleg shrimp, *Litopenaeus vannamei*. *Aquaculture*. 47(10), 3203-3214.
- Zhao, P., Huang, J., Wang, X. H., Song, X. L., Yang, C. H., Zhang, X. G., Wang, G. C., 2012. The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*. *Aquaculture*. 354, 97-106.