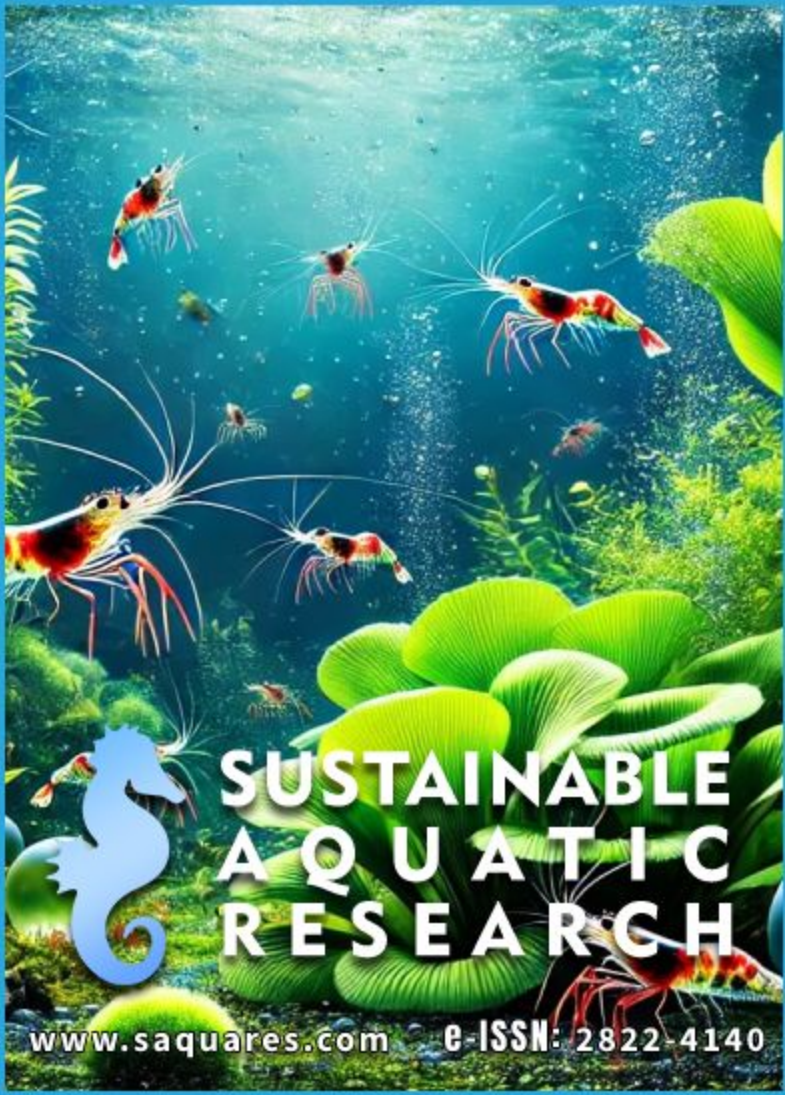
Year: 2024, Volume: 3, Issue: 3



SUSTAINABLE AQUATIC RESEARCH

www.saquares.com e-ISSN: 2822-4140

SUSTAINABLE AQUATIC RESEARCH

2024 ●Volume 3 ● Issue 3
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How sustainable is sustainable living without sustainable aquatic research?

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Aims & Scope

Aims

SUSTAINABLE AQUATIC RESEARCH (SAquaRes) aims to play an important role in advancing and understanding of aquatic sustainability. The most important aim of SAquares is "to put the research on aquatic sustainability at the focus of science. Sustainable life in the world will be realized with a sustainable aquatic ecosystem."

Scope

The scope of SAquaRes includes papers from non-traditional scientific areas such as sustainability science, social-ecological systems, ornamental, conservation, and restoration, and also the traditional priorities of its sections related to aquatic environments *(the list below is given in alphabetical order)*:

- Alternate Aquatic Energy Technologies
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- Seafood Quality and Safety
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- Sustainable Aquatic Ecosystem
- Sustainability assessment and design of aquacultural systems and decision support tools
- Water Quality and Pollution
- Wastewater Treatment
- And more research focused on sustainability

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DOI: 10.5281/zenodo.14555064
e-ISSN: 2822-4140

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RESEARCH PAPER

Fishmeal partial replacement using duckweed (*Lemna minor*) enhances growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) L.

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Citation

Opiyo, M.A., Mbogo, k., Abwao, J., Kyule, D., Amahwa C., Nyonje B.M., and Munguti, J. (2024). Fishmeal partial replacement using duckweed (*Lemna minor*) enhances growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) L. *Sustainable Aquatic Research*, *3*(3), 127-136.

Article History

Received: 29 August 2024
Received in revised form:24 December2024
Accepted: 25 December 2024
Available online: 30 December 2024

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Keywords

Duckweed
Fish feed
Growth
Lemna minor
O. niloticus

Abstract

Nile tilapia (*Oreochromis niloticus*) juveniles were fed experimental diets with duckweed (*Lemna minor*) supplementing fish meal at 0% (LM0), 5% (LM5), and 15% (LM15) and compared to a commercial diet as a positive control (COMM). Growth performance, feed utilization, and body composition were evaluated and compared with the control diet. The final weight and specific growth rate were significantly higher in the fish fed the commercial diet and in LM15 when compared to LM0 ($P < 0.05$). The fish could utilize the *L. minor*-based feed although the feed conversion ratio was significantly lower in fish fed the LM15 and COMM diets than the other diets ($P < 0.05$). Fish body composition was significantly affected by *L. minor*-based diets. Protein content was significantly higher in fish fed on control diet and diet LM10 ($P < 0.05$) compared with other diets. In contrast, lipid content was significantly higher in fish fed *L. minor* -based diets than in the control, with LM15 having the highest levels ($P < 0.05$). Partial replacement of fish meal with *L. minor* at 15% in the diet of *O. niloticus* is therefore recommended because it enhances growth performance, improves feed utilization, and increases the lipid content in *O. niloticus.*

Introduction

Fish feed is the most expensive input in fish farming, constituting more than 60% of the total production cost in an aquaculture enterprise (El-Sayed, 2008; Charo-Karisa et al., 2013). Feed ingredients, especially fish meal, have continuously experienced fluctuating prices and competition from other animal feed industries, thus affecting aquaculture feed production and, consequently, fish production (Shati et al., 2022). Plant-based protein sources from agricultural produce and by-products have been used as

alternatives to animal proteins (Abowei & Ekubo, 2011; Munguti et al., 2012; Montoya-Camacho et al., 2019). However, the sustainability of these plant protein sources is often threatened by unpredictable weather conditions due to climate change (Shati et al., 2022). Aquatic macrophytes are a sustainable source of protein for fish feed production because they can be grown in large quantities in nutrient-rich water lagoons that are not being used by communities and ponds in tropical and subtropical countries (Hassan & Edwards, 1992; Hasan & Chakrabarti, 2009; Chakrabarti *et al.*, 2018; Naseem et al., 2021).

Duckweed (*Lemna minor)*is considered a novel feed ingredient for the replacement of fish meal for omnivorous/herbivorous fish such as Nile tilapia (*Oreochromis niloticus*) (Hasan & Chakrabarti, 2009; Chakrabarti, 2018) and are considered beneficial for increasing the sustainability of small-scale aquaculture (Slembrouck et al., 2018). *Lemna minor* is a free-floating freshwater macrophyte belonging to the family Lemnacea and is found in freshwater ponds, lagoons, ditches, and streams in both tropical and subtropical climates (Culley et al., 1981; Hassan & Edwards, 1992; Young et al., 2006). They have multiple uses, including wastewater treatment, as food for humans, and as feed ingredients for fish and terrestrial animals (Culley et al., 1981; Chakrabarti et al., 2018; Nesan et al., 2020; Sosa et al., 2024). In aquaculture, *L. minor* is readily consumed as a raw macrophyte by *O. niloticus*, the Common carp (*Cyprinus carpio)* and other omnivorous fish (Hassan & Edwards, 1992; Yılmaz et al., 2004). It is reported to contain 35–45% CP, essential amino acid and mineral profiles of the plant dry weight (El-Sayed, 1999). It is also characterized by the availability of essential amino acids, vitamins A, B, and E, and carotenoids, which are required by the fish (El-Sayed, 1999; Cruz et al., 2011; Naseem et al., 2021).

Previous studies have documented the use of *L. minor* as a protein source for the larval stages of various fish species, *O. niloticus* (El-Shafai et al., 2004; Solomon & Okomoda, 2012; Uddin et al., 2014; Cipriani et al., 2021; Achoki et al., 2024), *C. carpio* (Yılmaz et al., 2004), Silver barbs (*Barbonymus gonionotus*) (Noor et al., 2000) and other omnivorous fish. However, the use of *L. minor* to replace fish meal in the diets of grow-out *O. niloticus* cultured in ponds has not been documented. This study aimed to determine the effects of *L. minor* as a replacement for fish meal on *O. niloticus* grow-out in ponds, focusing on growth performance and body composition.

Materials and methods

Experimental design

The study was conducted in cages installed in an 800 m^2 with a depth of 1.5 m pond at the Kenya Marine and Fisheries Research Institute (KMFRI), National Aquaculture Research Development & Training Center (NARDTC), Sagana. Twelve cages whose length, width and height/depth were 2 m × 2 m × 1.2 m respectively were installed in earthen ponds that were previously limed and treated with agricultural lime at 100 g m^{-2}. The cages were stocked with *O. niloticus* juveniles of an average weight of 30.5 g. *L. minor* previously harvested from ponds at KMFRI Sagana was processed by drying under a shed for feed formulation. The other feed ingredients (Table 1) were purchased from local agrovet shops and ground separately into finer particles using a hammer mill (Thomas-Wiley intermediate mill, 3348-L10 series, USA). Three isonitrogenous (approximately 30% crude protein) experimental diets were prepared by replacing levels of fishmeal with dry *L. minor* meal 0% (LM0) (control), 10% (LM10), and 15% (LM15), following *L. minor* inclusion levels by (Opiyo et al., 2022). A commercial diet (COMM) sourced from a local feed manufacturer was used as a positive control. During the production of diets (LM0, LM10, and LM15), the ingredients were mixed thoroughly with water to make a homogenous dough and pelletized using a 2-3 mm commercial pelletizing machine into floating pellets. The pellets were dried, packed, and stored in a clean, dry, and cool environment. The experimental fish were hand-fed twice a day (1000 and 1600 h) to apparent satiation for 84 days.

Water quality monitoring

Water quality parameters were measured weekly using a multiparameter water quality meter model H19828 (Hanna Instruments Ltd., Chicago, USA). Nutrients were analyzed weekly using standard methods (Boyd and Tucker, 1998).

Table 1. Ingredients, formulations, and proximate compositions of the experimental diets

	Diet			
Ingredients (%)	**LM0**	**LM10**	**LM15**	**COMM**
Wheat bran	27	23	20	-
Soybean meal	23	22	18	-
Maize bran	25	24	25	-
Fish meal	20	16	17	-
Lemna minor	0	10	15	-
Soybean oil	1	1	1	-
Monocalcium Phosphate (MCP)	3	3	3	-
Vitamin premix	1	1	1	-
Proximate composition (% of dry weight)				
Dry matter	93.8	94.2	94.1	94.1
Crude protein	30.2	30.1	30.3	30.2
Crude lipids	5.7	4.5	5.5	8.5
Ash	12	14.2	13.3	9
Carbohydrates	52.1	51.2	50.9	52.3

***** LM0 (0 % *L. minor*); LM10 (10% *L. minor*); LM15 (15% *L. minor*); COMM (commercial diet).

Fish sampling for growth parameters and feed utilization

Fish were monitored for growth and mortality were recorded daily. Fish sampling was performed every 21 days. Fish were fasted for 24 h before sampling to allow gut emptying. All the fish in the cage were sedated with clove oil at 20 g L^{-1} and individually weighed with a digital balance (model EHB-3000, China) to the nearest 0.01 g and total length with a measuring board to the nearest 0.1 cm according to Caspers, (1969). At the end of the experiment, the dead fish were subtracted from the number stocked, and the percent survival was calculated. Growth performance and feed utilization were assessed in terms of weight gain, average daily growth, specific growth rates (SGRs) and condition factors as follows:

$$\text{SGR (\%)} = \frac{100\,(\ln(W\text{t}) - \ln(W0)}{t} \quad (1)$$

Where;

$W0$ is the natural logarithm of initial weight (g), Wt is the natural logarithm of final weight (g), and t is the period in days.

$$\text{Daily Weight gain (WG)} = \text{final weight (g)} - \text{initial weight (g)} \quad (2)$$

$$\text{Weight gain (WG\%)} = [(\text{final weight (g)} - \text{initial weight (g)}] \times 100 \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \text{Average feed given (g)} / \text{weight gain (g)} \quad (4)$$

$$\text{Survival (\%)} = \frac{\text{number of fish at end of experiment}}{\text{number of fish stocked}} \quad (5)$$

At the end of the experimental period, a random sample of three fish was collected from each replicate cage (n=9) and euthanized by placing fish in a container with ice water before culling (Lambooij et al., 2008). The fish samples were pooled and homogenized to form one sample per cage (n=3 per diet treatment) for body composition analysis using standard methods.

Proximate composition analysis of feeds and fish

The proximate composition of the experimental diets and fish carcasses was analysed using standard methods by the Association of Official

Analytical Chemists (AOAC, 2023). Dry matter content was measured by gravimetry, and moisture content was determined by oven drying for 12 h at 105 °C to a constant weight. Protein content (N × 6.25) was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas City, USA), lipid content was measured using a Soxhlet extractor (VELP Scientifica, Milano, Italy), and ash content was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, IO, USA) at 550 °C for 12 h. Carbohydrates were determined by subtracting of crude protein, crude lipids, and ash from 100.

Data analysis

The data were cleaned, and normality was determined using the Shapiro-Wilk test. Mean comparisons were performed using one-way analysis of variance (one-way ANOVA) followed by Tukey's HSD post-hoc test to determine the pairwise differences among the diets. Differences were considered statistically significant at $P < 0.05$. Percentage data were arcsine-transformed to normalize the data before analysis. All statistical analyses were performed using Statistical Package and Service Solutions (SPSS version 23).

Results

Water quality

The mean values of the pond water quality parameters were stable with minimal variations during the experimental period. The mean values were as follows: Temperature (26.41 ± 0.23 °C), dissolved oxygen (DO) (5.58 ± 0.08 mg L^{-1}), conductivity (102.46 ± 1.68 µS cm^{-1}), total dissolved solids (64.06 ± 1.09 mg L^{-1}), and pH (8.13 ± 0.02). Low nutrient values were recorded for phosphate (0.3 ± 0.01 µg L^{-1}), nitrite (0.1 ± 0.01 µg L^{-1}), nitrates (0.4 ± 0.03 µg L^{-1}), and ammonium (2.0 ± 0.1 µg L^{-1}). All parameters were within the recommended levels for *O. niloticus* growth and survival when cultured in ponds.

Growth performance and feed utilization

The growth performance parameters are listed in Table 2. Among the fish fed with *L. minor*-based diets, those fed with LM15 containing 15% *L. minor* had significantly higher final weight and specific growth rate (SGR) ($P < 0.05$) than the other diets. Fish fed a commercial diet presented the highest final weight, SGR, and the best FCR among all diets ($P < 0.05$). The final weight of the fish fed LM10 containing 10% *L. minor* was not significantly different from that of fish fed the LM0. The feed conversion ratio (FCR) was low in fish fed commercial feed and was not significantly different from fish fed diet LM15. Fish survival was the highest in fish fed the commercial diet, but no significant differences ($P = 0.543$) were observed among all the diets.

Table 2. Growth parameters of *O. niloticus* fed on *L. minor* diets for 84 days

Parameter	Diet					
	LM0	**LM10**	**LM15**	**COMM**	**P-value**	**F-value**
IL (cm)	11.96±0.07^{a}	11.86±0.08^{a}	11.84±0.07^{a}	11.74±0.07^{a}	$P < 0.005$	6.378
IW (g)	30.46±0.31^{a}	30.50±0.38^{a}	30.45±0.37^{a}	30.48±0.26^{a}	$P > 0.005$	5.230
FL (cm)	16.87±0.19^{a}	16.20±0.17^{a}	18.89±0.17^{b}	19.16 ±0.22^{b}	$P < 0.005$	50.545
FW (g)	100.51±1.99^{a}	94.07±0.02^{a}	121.05±3.10^{b}	148.64±4.50^{c}	$P < 0.005$	59.096
SGR (%)	1.10±0.02^{a}	1.03±0.02^{a}	1.32±0.03^{b}	1.51±0.04^{c}	$P < 0.005$	56.075
DWG	0.66±0.02^{a}	0.59±0.01^{a}	0.89±0.03^{b}	1.13±0.05^{c}	$P < 0.005$	61.635
WG (%)	225.35±8.18^{a}	202.45±6.53^{a}	281.02±11.77^{b}	412.81± 20.13^{c}	$P < 0.005$	54.92
FCR	1.72±0.05^{b}	1.94±0.05^{c}	1.30±0.05^{a}	1.28 ±0.05^{a}	P <0 .005	55.82
Survival (%)	97.33±3.53^{a}	98.67±1.33^{a}	97.83±4.81^{a}	98.67±1.34^{a}	$P = 0.543$	0.77

**Means within the same row with different superscript letters are significantly different at $P < 0.05$. LM0 (0% *L. minor*); LM10 (10% *L. minor*); LM15 (15% *L. minor*); COMM (commercial diet). IL-initial length, IW- Initial weight, FL-Final length, FW-Final weight, SGR-Specific growth rate, DWG-Daily weight gain, WG-Weight gain, FCR- Food conversion ratio.

There was a non-linear relationship between the replacement level of fish meal with *L. minor* and the growth performance of *O. niloticus*. In general, fish fed *L. minor* had a lower SGR and final weight than fish fed a commercial diet. Likewise, there was a higher FCR in fish fed the *L. minor* diet than in fish fed the commercial diet. The FCR in fish fed 15% *L. minor* (LM15) was

statistically the same to fish fed on the commercial diet ($P > 0.05$). LM15 represents the suitable level for the replacement of fishmeal with *L. minor* in *O. niloticus* diets for better feed efficiency and growth performance.

Fish body composition

The proximate composition of *O. niloticus* fed *L. minor* diets is presented in Table 3. The experimental fish had moisture content ranging between 40.23 to 41.47% and was not significantly different ($P > 0.05$). Total lipid content was significantly higher in fish fed LM15 and COMM ($P < 0.05$) compared to LM0 and LM10. The total protein content was significantly higher in fish fed the LM0 and LM10 diets ($P < 0.05$) than the fish fed LM15 and COMM. The ash content did not differ significantly among the treatments ($P > 0.05$). Carbohydrates were also not significantly different ($P > 0.05$) among the four treatments.

Table 3. Whole body composition of *O. niloticus* fed on *L. minor* diets for 84 days

Parameter (% wet weight)	Diet			
	LM0	**LM10**	**LM15**	**COMM**
Moisture	41.46±0.08[a]	41.47±0.06[a]	40.3±0.08[a]	40.23±0.08[a]
Protein	34.01±0.08[a]	33.22±0.04[a]	30.24±0.07[b]	30.46 ± 0.03[b]
Lipid	19.34±0.90[a]	20.32±0.98[a]	24.44±0.20[b]	24.2 ± 0.21[b]
Ash	4.04±0.09[a]	4.03±0.10[a]	4.02±0.13[a]	4.03 ± 0.09[a]
Carbohydrate	0.35±0.07[a]	0.35±0.02[a]	0.35±0.03[a]	0.35±0.04[a]

**Means within the same row with different superscript letters are significantly different at $P < 0.05$. LM0 (0% *L. minor*); LM10 (10% *L. minor*); LM15 (15% *L. minor*); COMM (commercial diet).

Discussion

This study has established that *O. niloticus* fed on *L. minor* at 15% (LM15) had a better growth performance than LM0 and LM10. This indicates that the inclusion of duckweed did not compromise the overall growth potential of the fish, as demonstrated by final body weights and growth rates that were higher than LM0, indicating that *L. minor* can replace 15% of fish meal in *O. niloticus* diets. These results align with previous research demonstrating that *O. niloticus* exhibits promising growth performance when fed diets containing 15% *L. minor* (Yen et al. 2015; Opiyo et al. 2022). This suggests that duckweed can effectively serve as a viable alternative protein source in tilapia diets, without negative effects on growth performance. A study by Uddin et al. (2014) reported significantly high SGR and final weight in Nile tilapia fed *L. minor* as supplementary feed. However, other studies reported higher levels of up to 20% of *L. minor* to replace fish meal for Indian major carp (*Gibelion catla*) (Shafi et al., 2024) and *C. carpio* (Yılmaz et al., 2004) to grow to juvenile size. Contrary to our study, better growth and feed utilization were reported in tilapia (*Sarotherodon galilaeus*) fed on a 33% CP diet containing duckweed as a partial replacement for fish meal (Mbagwu et al., 1990). Low feed utilization have been reported in *O. niloticus* fed high levels of dry or fresh *L. minor* with a 20% replacement level being utilized better than a 40% replacement level with fish meal (El-Shafai et al., 2004). The SGR and FCR ranged between 1.1 - 1.5% and 1.28 - 1.94% respectively. The SGR are within the range reported by (El-Shafai et al., 2004) in Nile tilapia while the FCR in the present study were higher compared to 0.9-1.1% in a related study by El-Shafai et al. (2004). The combination of both fishmeal and *L. minor* has been reported to lead to better FCR when compared to other plant sources (El-Shafai et al., 2004). The FCR in the LM15 were comparable to COMM, indicating that the fish had the same utilization of the feed as the commercial feed. A similar scenario was reported in Nile tilapia fingerlings, where the 15% *L. minor* inclusion had the same FCR to the control diet formulated to mirror the commercial feed (Opiyo et al., 2022). Goswami et al. (2020) reported improved SGR and FCR when fishmeal was partially replaced with duckweed in the diets of *Labeo rohita* fingerlings. Improved final weight, SGR, and FCR were reported in grow-out rainbow trout fed on *Spirodela polyrrhiza* at 12% (Stadtlander et al., 2023).

The survival of the experimental fish was not affected by the replacement of fishmeal in any

diet. This indicated that the nutrients in the feeds supported fish well-being equally, as the survival of the fish was more than 90% in all the treatments. This could also be attributed to the overall experimental management and good health of the fish. The nutritional composition of cultured *L. minor* is sufficient to meet the nutritional requirements of cultured Nile tilapia because it contains essential fatty acids, especially polyunsaturated fatty acids (PUFAs), which are important for fish well-being and performance (Mukherjee et al., 2010; Kumar et al., 2022; Opiyo et al., 2023). The high survival rate and production of *O. nioloticus* fed *L. minor* as a supplementary feed in fertilized ponds was documented by Uddin et al. (2014). Similarly, a study replacing fish meal with fermented *L. minor* at 0, 2.5, 5, and 7.5% reported high survival with no significant differences among the treatments (Herawati et al., 2020). High percent survival has been recorded in Nile tilapia fed diets replacing fish meal with duckweed *Spirodela polyrrhiza* at 5% and 10%, and *C. carpio* fed on *L. minor* at 5% and 10% (Fasakin et al., 1999; Yılmaz et al., 2004) which is contrary to the present study, which had high survival at all the treatments.

The moisture content of the fish was not significantly different. The protein content in the fish body decreased with an increase in *L. minor* in the diets with the fish fed diet LM15, which had the lowest protein content. This is in agreement with studies of Hassan & Edwards (1992), who recorded low protein levels in Nile tilapia fed with whole *Lemna perpusilla* and a study by Opiyo et al. (2022), where *L. minor* inclusion levels ranged from 5-20% in plant based feeds. Lipid levels increased with increasing levels of *L. minor* in the fish diet. This could be a result of the size of fish, which increased with increasing levels of *L. minor* with diet LM15 having significantly bigger fish but were not significantly different from the fish fed on the commercial diet. The high lipid content in diets LM15 and COMM could also indicate that the diets were more energy dense, leading to lipid deposition (Hassan & Edwards, 1992). The ash content was not significantly affected by the diet. A similar trend was reported in silver barbs (*Barbonymus gonionotus*) fed on diets with *L. minor* partially replacing fishmeal at 10, 20, 30 and 35% (Noor et al., 2000). In contrast, El-Shafai et al. (2004) reported a high ash content in tilapia fed on duckweed diets. A reduction in ash content was reported when *O. niloticus* were fed on *S. polyrhiza* (Fasakin et al., 1999) and *L. minor* from 5 to 20% (Solomon & Okomoda, 2012). This study indicates that there were no significant differences in the body composition of the fish fed on *L. minor* at 15% and the commercial diet.

Conclusion

Replacement of fish meal with duckweed (*Lemna minor*) at 15% gave the best growth performance and could be used to replace fish meal in Nile tilapia grow-out diets to obtain similar outcomes to those of commercial feed for growth performance, feed utilization, and fish body composition. The fact that there was no significant effect on the survival of the fish indicated that the fish were in good condition. The use of higher levels of *L. minor* than those in this study requires further processing to improve digestibility due to the high fiber content and antinutritional factors that could be present in the macrophytes. The use of duckweed as a protein source in tilapia diets has important implications for sustainable aquaculture. Duckweed is a fast-growing aquatic plant that requires minimal input and can be cultivated using nutrient-rich water from fish culture systems, making it an environmentally friendly, climate-smart, and sustainable substitute for fishmeal. The collection of duckweed from unknown sources is not recommended because of possible contamination which may be present in the water. Only duckweed cultured with known manure and nutrient-rich water from an aquaculture facility is recommended for use in fish feed. More studies are recommended for high levels of *L. minor* to replace fish meal in tilapia growth after analysis of antinutritional factors which may pose challenges to utilization of *L. minor* by *O. niloticus*.

Acknowledgement

Special thanks go to the technologists at KMFRI Sagana, headed by Mr. Elijah Gichana, for assistance in the experiment.

Credit author statement

Mary A. Opiyo and Kevin Mbogo: funding acquisition, conceptualization, methodology, data collection, data analysis, writing of the original

draft, and revision of the manuscript. Jacob Abwao, Domitila Kyule, Charles Amahwa, Betty M. Nyonje, and Jonathan Munguti: conceptualization, writing of the original draft, and revision of the manuscript.

Ethical statement

The experiment was conducted following the standard operating procedures (SOPs) of the Kenya Marine and Fisheries Research Institute (KMFRI) guidelines for handling animals after ethical review and approval by KMFRI Ethical Review Committee registered with the National Commission for Science, Technology and Innovation (NACOSTI) registration number NACOSTI/2016/05/001. The SOPs comply with the Prevention of Cruelty to Animals Act 1962, CAP 360 (Revised 2012) of the laws of Kenya, and EU regulation (EC Directive 86/609/EEC).

Competing interests

The authors state that there is no conflict of interest to declare.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Funding Information

The study was funded by the Kenya Climate Smart Agriculture Project (KCSAP) through the Kenya Agriculture and Livestock Research Organization (KALRO) under the Applied Research titled "Use of Locally Available Aquatic Macrophytes as Sustainable Ingredients for Fish Feeds for Rural Aquaculture" Grant No. KCSAP/AR02-4/2.

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Sustainable Aquatic Research (2024) 3(3):137-150
DOI: 10.5281/zenodo.14558081
e-ISSN: 2822-4140

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RESEARCH PAPER

Evaluating Water Quality in Northern and Eastern Coastal Zones of Sri Lanka: A Baseline Study for Environmental Monitoring and Conservation

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Citation

Jayawardena, N.K.R.N., Thirukeswaran, S., Weerasekara, K.A.W.S. (2024). Evaluating Water Quality in Northern and Eastern Coastal Zones of Sri Lanka: A Baseline Study for Environmental Monitoring and Conservation. *Sustainable Aquatic Research, 3*(3), 137-150.

Article History

Received: 24 October 2024
Received in revised form:24 December2024
Accepted: 24 December 2024
Available online: 30 December 2024

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Keywords

Water Quality Index
Coastal
Sri Lanka
Northern
Eastern

Abstract

The coastal area of Sri Lanka, spanning approximately 1,650 km, serves as a major source of livelihood, habitat, tourism, aquaculture, and trade. Although the coastal region provides numerous benefits to nearly 55% of Sri Lanka's population, it faces severe threats from factors such as aquatic pollution, coastal erosion, ecosystem degradation, urbanization, and sand mining. To ensure ecosystem stability and conserve the coastal environment, it is crucial to conduct environmental monitoring of coastal waters. However, a significant number of water quality assessments conducted so far have primarily targeted the Western and Southern coastal zones of Sri Lanka. This study, therefore, aims to assess the marine water quality in selected locations (26 locations in total) within the Northern (Mannar, Pooneryn, Kilinochchi, Jaffna, and Mullaithivu) and Eastern (Trincomalee) coastal zones. The results showed statistically significant differences in TDS ($P = 0.004$), COD ($P = 0.036$), and nitrite levels ($P = 0.009$) between all the locations. However, no significant variation in COD was observed in the HSD test. Heat map analysis of the water quality index model indicated that some locations in Mannar, Pooneryn, and Jaffna had very poor water quality, while Trincomalee and Mullaithivu exhibited moderate to good water quality in selected locations. Overall, these findings provide a clear understanding of the current water quality status in each of the selected locations. Therefore, it can be concluded that regular water quality monitoring and the application of the Water Quality Index approach should be conducted in each coastal district. This will help to develop a robust database that can serve as baseline information for coastal ecosystem management, conservation efforts, and emergency mitigation measures, such as oil spills or ship fire incidents.

Introduction

Sri Lanka, an island nation in the Indian Ocean, boasts an extensive coastline that plays a pivotal role in the country's economic well-being, particularly through fisheries, tourism, aquaculture, and maritime trade (Manage et al., 2022). According to the Census of Sri Lanka, around 57% of the total population resides in the coastal zones (Weerasekara et al., 2015). The coastal area also serves as a natural buffer against storm surges and coastal erosion, protecting inland regions and communities (Dong et al., 2024). Especially, the Northern and Eastern coastal regions host a large number of coastal communities relying on coastal resources for their livelihood. Since the ocean is considered as an open-access resource, unrestricted access to coastal resources and ecosystem services has led to significant impacts on marine ecosystems, primarily due to coastal aquatic pollution (Li et al., 2017; Manage et al., 2022). These coastal regions hold substantial ecological and economic importance due to their unique marine ecosystems and strategic locations. They support diverse aquatic life and provide essential resources for local communities.

Despite their importance, these coastal zones are increasingly threatened by various anthropogenic activities, including overfishing, coastal development, urbanization, and pollution from agricultural and industrial sources (Myers et al., 2019; Suresh, 2024). For instance, over 60% of Sri Lanka's small, medium, and large-scale enterprises operate along the coastal areas, often discharging industrial effluents directly into the area with minimal or no treatment (Weerasekara et al., 2015). Additionally, accidental oil spills, waste disposal from ships, mining activities and industrial operations are major contributors to marine pollution in Sri Lanka (Bandara, 2003). These activities raised significant concerns regarding water quality degradation, which poses serious threts to marine biodiversity, aquatic animal health, human health, and the sustainability of local livelihoods (Pires de Souza Araujo et al., 2021). As a result of coastal pollution, Sri Lanka reanked 213 out of 220 coastal countries and territories to Ocean Health Index Scores. This indicates that, goals including clean waters, coastal protection, livelihood and economies, biodiversity, tourism and creation are not being sustainably managed in the country (Ocean Health Index, 2023). Moreover, efforts to combat water contamination have depicted relatively slow in Sri Lanka, despite the laws and government regulations (Bandara, 2003). Therefore, the preservating and sustainably managing these coastal ecosystems is vital for mitigating the impact of climate change and ensuring long-term ecological and economical stability in Sri Lanka.

In order to preserve and manage the coastal environment, it requires a clear understanding of current status of the marine environment, as well as the identifing and acknowledging the potential threats. Continuous water quality monitoring in these regions is particularly necessary to assess the current status, detect pollution, and implement effective measures to mitigate its impacts. However, comprehensive studies on the water quality across these coastal regions in Sri Lanka are limited, particularly in the context of recent environmental changes and development pressures in the Northern and Eastern provinces.

Hence, this study aims to assess the water quality of six key coastal locations within the Northern and Eastern Provinces of Sri Lanka: Mannar, Pooneryn, Jaffna, Kilinochchi, Mullaithivu, and Trincomalee. The water quality parameters were analyzed using a newly developed water quality index model, with data collected from each location providing a snapshot of the current state of the marine environment in these areas. The findings of this study are expected to contribute to the broader understanding of coastal water quality in Sri Lanka and to inform future conservation and management efforts.

Materials and methods

Study Area and Sample Collection

Seawater samples were collected from the off-shore to open sea at Kalpitiya (1 location), Mannar (4 locations), Pooneryn (6 locations), Jaffna (7 locations), Kilinochchi (1 location), Mullaitivu (4 locations), and Trincomalee (3 locations) as shown in Figure 1, in 2023.

Samples were collected according to the National Field Manual for the collection of water quality data (National Field Manual for the Collection of

Water-Quality Data. U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, 2015). The polypropylene vessels were soaked in the 1:4 nitric acid for a few days and then washed using distilled water. The sampling vessels were sealed after collecting the samples. A total of 52 samples were analyzed for a variety of parameters, including physico-chemical and biological variables. The sampling locations were selected representing the environmental conditions present in this study area.

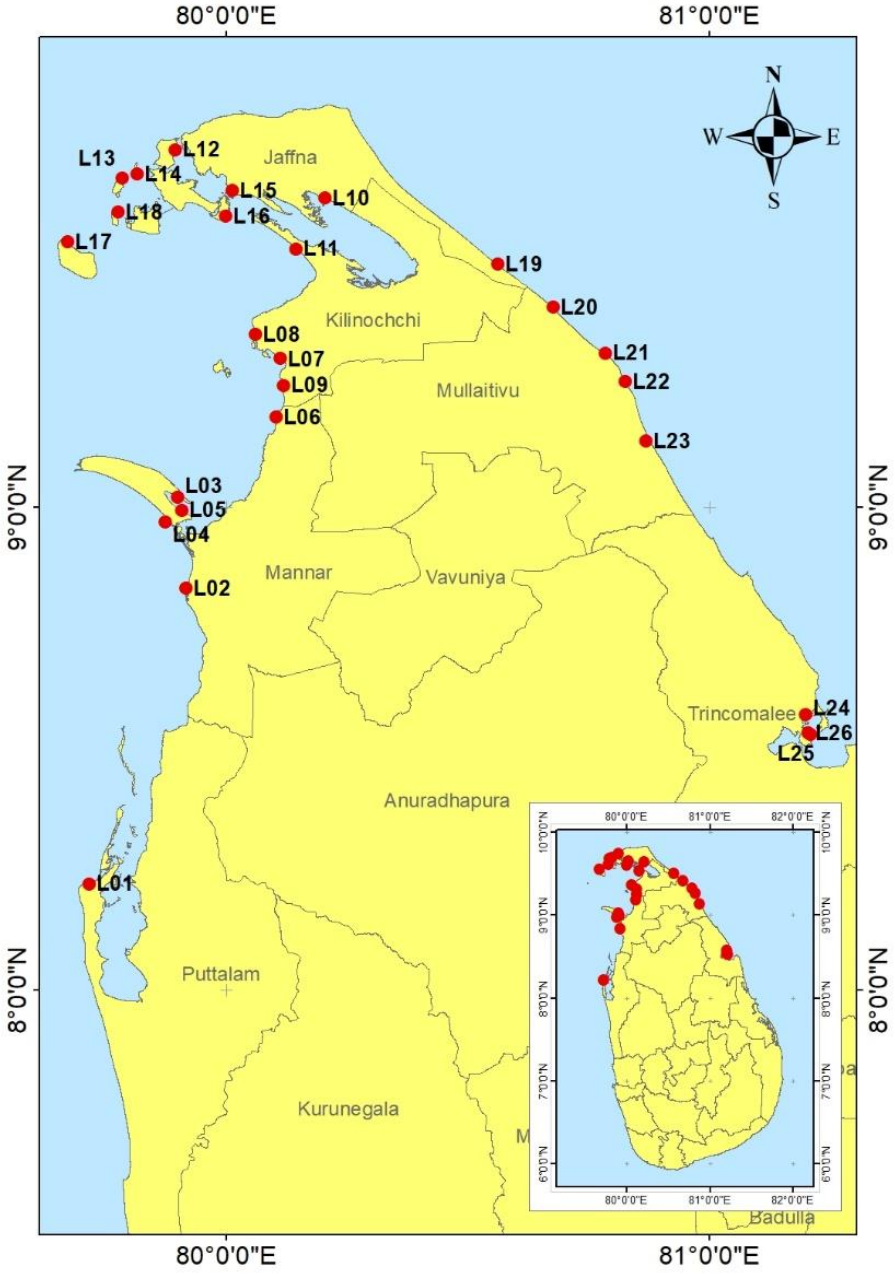

Figure 1. The Study Area

Table 1. Samples collected locations under each study area

No.	Study Area	Locations	No.	Study Area	Locations
1	Kalpitiya 1	Kandakuliya	**15**	Jaffna 3	Eluvathivu
2	Mannar 1	Achchan Kulam	**16**	Jaffna 4	Guru Nagar
3	Mannar 2	Pallimunei	**17**	Jaffna 5	Mandathivu
4	Mannar 3	South Bar	**18**	Jaffna 6	Delft
5	Mannar 4	North Bar	**19**	Jaffna 7	Nainathivu
6	Mannar 5	Iranathivu	**20**	Kilinochchi 1	Chundikkulam
7	Pooneryn 1	Thewampitei	**21**	Mulativu 1	Chalei
8	Pooneryn 2	Kiranchi	**22**	Mulativu 2	Mulliwaikkal
9	Pooneryn 3	Waleypadu	**23**	Mulativu 3	Kalaipadu
10	Pooneryn 4	Nachchikuda	**24**	Mulativu 4	Nayaru
11	Pooneryn 5	Palavi	**25**	Trincomalee 1	Cod-bay
12	Pooneryn 6	Kavuthurumunei	**26**	Trincomalee 2	Marble beach
13	Jaffna 1	Karainagar	**27**	Trincomalee 3	Clappernburge beach
14	Jaffna 2	Analathivu			

Analytical method of sample analysis

The pH, salinity, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO) and turbidity were measured at the site using a DO meter (HANNA, Romania), a Multimeter (HANNA, Romania) and a turbidity meter (LOVIBOND, Germany). The biochemical oxygen demand (BOD) was measured after a 5-day incubation at 20°C in a BOD incubator using Winkler's titration method (APHA, 2019). Total suspended solids (TSS) were determined by filtering 1 L of seawater through pre-dried and pre-weighed filter papers (Millipore GF/C) and washing them with Milli-Q water to remove salt content (APHA, 2019). Phosphate, nitrate, nitrite and chemical oxygen demand (COD) were analyzed using standard methods from APHA 2019.

Statistical Analysis

ANOVA (Analysis of Variance)

ANOVA was employed to determine whether there were statistically significant differences in water quality parameters between different locations (Montgomery, 2013). A significant ANOVA result indicates that at least one location's water quality differs from the others.

Tukey's Honest Significant Difference (HSD) Test

For the significant ANOVA results, the Tukey's HSD test was performed to identify which specific locations had significantly different water quality (Tukey, 1949).

Correlation Analysis

Correlation analysis was conducted to examine the relationships between the selected water quality parameters. The Pearson's correlation coefficient was used to quantify the strength and direction of the relationships between pairs of variables (Mukaka, 2012)

Moran's I Test for Spatial Correlation

The global spatial autocorrelation technique was employed to assess the correlation between adjacent observations, identifying patterns and the extent of spatial clustering across neighboring locations. Moran's I, a statistic analogous to the Pearson correlation coefficient (Tsai et al., 2010) is computed by Eq. 1.

$$I = \frac{n}{W} * \frac{\sum_i \sum_j w_{ij}(x_i - \bar{x})(x_j - \bar{x})}{\sum_i (x_i - \bar{x})^2} \quad (1)$$

Where n represents the number of observations, W denotes the sum of the weights, w_{ij} represents the weight between locations i and j, x_i and x_j are

values at locations i and j and $\bar{x}$ denotes the mean of the values.

Water Quality Index (WQI)

$$WQI = \sum_{i=1}^{n} W_i S_i \quad (2)$$

Where n represents the number of water quality parameters, W_i denotes the weight assigned to the i^{th} parameter, and S_i is the score of the i^{th} parameter. The weight W_i was derived based on the outcomes of the Principal Component Analysis (PCA) and Factor Analysis (FA). The score S_i representing the standardized value of each of the 10 water quality parameters, was determined using Equations 3 and 4. These 10 parameters were categorized into three groups: "the more the better," "the less the better," and "neutral." The "more the better" group included only the Dissolved Oxygen (DO) parameter, the "neutral" group included pH, and the "less the better" group included the remaining 8 parameters. For the "more the better" and "neutral" parameters, the score S_i was calculated following Eq. 3 (Le et al., 2023).

$$S_i = \frac{X_i - X_{min}}{X_{max} - X_{min}} \quad (3)$$

For the "the less the better" parameters, S_i was determined following Eq. 4.

$$S_i = \frac{X_{max} - X_i}{X_{max} - X_{min}} \quad (4)$$

Where X_i, X_{min}, and X_{max} were the analyzed, minimum, and maximum values of the parameter i, respectively.

Assigning accurate weights to each parameter in the WQI is essential, as it signifies the relative significance of each parameter in assessing overall water quality. The raw data were first subjected to Z-score normalization to standardize the parameters, ensuring they are comparable across different measurement scales. Following this, PCA was applied to the normalized dataset to identify the principal components that account for the majority of the variance within the data. The weights for each water quality parameter were derived from the loadings of the principal components that explained the highest proportion of variance. These weights were then utilized to calculate the WQI by aggregating the weighted scores of each parameter for each location and beach. The resulting WQI values, which represent the overall water quality, were expressed as a percentage, enabling comparative analysis across the different beaches.

Results and Discussion

The water quality data across the different study areas (Kalpitiya, Mannar, Pooneryn, Jaffna, Kilinochchi, Mulativu, and Trincomalee) highlight both consistencies and significant deviations in key environmental parameters, offering insights into the varying conditions of these coastal regions. pH levels consistently range between 7.9 and 8.3 across all locations, indicating slightly alkaline waters typical of coastal environments, which is generally favorable for aquatic life (Jiang et al., 2019). However, even minor fluctuations in pH can influence the solubility and toxicity of chemical compounds, potentially impacting ecosystem health. TDS are remarkably stable across all sites, hovering around 28 mg/L, reflecting the salinity of the water in these coastal regions. This consistency suggests limited freshwater intrusion or significant saline contamination, maintaining the typical saline nature of these coastal waters. In contrast, turbidity shows notable variability, particularly in Mannar and Mulativu, with Mannar's Location 2 and Mullaitivu's Location 4 exhibiting elevated turbidity levels of 70.4 NTU and 26.9 NTU, respectively. High turbidity can decrease light penetration, adversely affecting aquatic plants and indicating possible sediment or organic matter presence, which could stem from runoff or local disturbances (Hinga, 2002). This can, in turn, harm aquatic life and increase the risk of fish mortality (Rahmania et al., 2024). DO levels also vary, with most locations maintaining adequate levels for aquatic life, except for some sites in Pooneryn and Jaffna, where lower DO levels compared to other locations (as low as 6.6 mg/L) might indicate localized organic pollution or stagnant water conditions. Conversely, higher DO levels in Mulativu and Trincomalee suggest well-aerated waters, potentially due to increased water movement or photosynthetic activity. Dissolved oxygen levels are typically higher at the water's surface due to the diffusion of oxygen from the air and the process of photosynthesis. As depth increases, the concentration of dissolved oxygen declines due to the reduced occurrence of photosynthetic activity (Rahmania et al., 2024).

The current study provides a complete investigation of water quality along Sri Lanka's Northern and Eastern beaches. Table 2 compares the findings of this study with earlier studies done at Pasikuda (Sivakumar, 2019) and Arugam Bay (Sivakumar, 2016). The WT (29.8±0.5 °C) and pH (8.0±0.1) observed in this study are consistent with previous findings, but higher values were recorded for EC (57.9±0.2 mS/cm), TDS (28.6±0.1 mg/L), and salinity (38.6±0.2 ppt). This could be most likely reflecting spatial or temporal variations in environmental conditions. DO levels had exceeded 4 mg/L in all studies indicating improved oxygenation. Nutrient concentrations, such as nitrates (0.019±0.010 mg/L) and phosphates (0.142±0.127 mg/L), were much lower than previously investigated locations. Nevertheless, it cannot be concluded that water quality has been improved and further monitoring is recommended.

Table 2. The comparison of water quality parameters with previous studies done in Eastern coastal areas

Parameter	**Unit**	**Eastern Coasts (This Study)**	**Eastern Coastal Areas**	
			Pasikuda (Sivakumar, 2019)	Arugam Bay (Sivakumar, 2016)
WT	ºC	29.8±0.5	30.2	30.9±1.9
pH		8.0±0.1	8.0	8.2±0.1
EC	mS/cm	57.9±0.2	53.8	55.3±3.6
TDS	mg/L	28.6±0.1	26.4	27.1±1.7
Salinity	ppt	38.6±0.2	31.6	32.2±0.6
Turbidity	NTU	8.5±6.0	9.9	9.5±0.6
DO	mg/L	8.6±0.8	7.2	7.5±0.3
COD	mg/L	43.3±10.6	-	-
BOD	mg/L	7.5±2.1	-	-
Nitrates	mg/L	0.019±0.010	0.63	0.068±0.075
Nitrites	mg/L	0.004±0.002	-	-
Phosphates	mg/L	0.142±0.127	1.36	2.970±4.350
Ammonia	mg/L	0.406±0.091	-	-
TSS	mg/L	33.7±12.5	-	-

Table 3. The comparison of water quality parameters with previous studies done in Northern coastal areas

Parameter	**Unit**	**Northen Coasts (This Study)**	**Northern Coastal Areas**					
			Mathgal (Gobiraj et al., 2022)	Point Pedro (Gobiraj et al., 2022)	Charty Beach (Gobiraj et al., 2022)	Gurunagar Beach (Anandakrishnan Sivanandan et al., 2023)	Charty Beach (Moksha S. Usgoda et al., 2024)	Nayaru (Jayawardena et al., 2023)
WT	ºC	30.8±1.4	29.3±1.9	29.1±1.6	29.4±1.4	30.3±1.4	29.2±1.4	28.7±0.2
pH		8.0±0.1	8.2±0.2	8.1±0.2	8.1±0.3	-	8.1±0.2	7.2±0.1
EC	mS/cm	57.3±3.9	52.9±6.1	52.7±5.1	53.5±5.1	-	49.4±2.6	47.6±0.7
TDS	mg/L	28.3±2.0	31.8±2.8	31.8±2.4	32.1±2.8	-	30.2±1.5	29.3±0.5
Salinity	ppt	38.0±3.3	32.5±3.3	32.5±2.9	32.9±3.2	36.5±13.5	29.8±1.4	31.3±0.4
Turbidity	NTU	12.1±14.4	-	-	-	27.0±29.1	8.0±0.1	5.8±1.3
DO	mg/L	7.8±1.1	6.8±0.9	9.6±1.3	6.4±1.4	-	6.7±1.8	7.3±0.1
COD	mg/L	35.0±15.9	-	-	-	135.7±154.6	-	-
BOD	mg/L	7.5±1.9	-	-	-	62.3±69.7	1.2±0.6	-
Nitrates	mg/L	0.013±0.008	-	-	-	24.270±31.480	2.660±2.080	0.005±0.001
Nitrites	mg/L	0.013±0.022	-	-	-	-	-	-
Phosphates	mg/L	0.216±0.547	-	-	-	0.853±0.553	0.150±0.060	0.005±0.001
Ammonia	mg/L	0.415±0.275	-	-	-	-	-	-
TSS	mg/L	45.7±20.8	-	-	-	21.3±12.9	-	32.8 ± 22.2

The water quality paramters analysed for Northern region in this study revealed siginificant variations across different coastal regions in the Northern province, which may be attributed to the differences in geographical, and environmental factors (Table 3). The average water temperature (30.8±1.4 ºC) observed in the Northern coastal areas alignsed closely with other studies previously carried out in Charty beach, Gurunagar, Point Pedro and Nayaru (Sivanandan et al., 2023; Gobiraj et al., 2022; Jayawardena et al., 2023; Usgoda et al., 2024). Slight variation in water temperature could have occured due to seasonal differences or localized infleunces. The pH levels of coastal water samples (8.0±0.1) recorded within the slightly alkaline range which are typical for marine environments (Jiang et al., 2019). This value is comparable to the values shown in previous studies (Sivanandan et al., 2023; Gobiraj et al., 2022; Usgoda et al., 2024) except for Nayaru (7.2±0.1) (Jayawardena et al., 2023). In this study, EC (57.3±3.9 mS/cm) values were found relatievly higher compared to other coastal areas. Neverthless (Usgoda et al., 2024) obtained a low EC value in 2024 compared to (Gobiraj et al., 2022) 2022 for the same location. The TDS values are within the similar range with previous studies. The DO had greater values than 4 mg/L in this study as well as the previous studies indicating proper mixing of water. It was observed that BOD and COD were higher in (Sivanandan et al., 2023) compared to this study. (Sivanandan et al., 2023) carried out the sampling at the fishery harbour, Gurunagar and the discards of fish waste could be the reason for having higher BOD and COD values (Weerasekara et al., 2015). The overall findings incidate that Northern coastal stretch exhibit relatively stable water quality with paramteres generally within the acceptable ranges for marine ecosystems as well as similar to the previous studies. However, variation across regions highlight the importance of localized and area based management strategies to mitigate pollution impacts and ensure the sustainability of coastal resources.

ANOVA Test

One way ANOVA test was conducted with the location as the independent variable and the water quality parameters as the dependent variables. This compared the means of a single dependent variable across multiple independent groups or categories to examine whether there are any significant differences in the mean of the water quality parameter between the different locations. The obtained Pr (>F) values for each parameter is listed in Table 4.

Table 4. ANOVA test results

Parameter	DF	Sum Sq.	Mean Sq.	F value	Pr (>F)
pH	6	0.0184200	0.00306900	0.251	0.95300
TDS	6	0.9288000	0.15480000	4.612	0.00474 **
Turbidity	6	733	122.100000	0.617	0.71500
DO	6	5.9610000	0.99340000	0.900	0.51500
COD	6	1121	186.900000	2.871	0.03650 *
BOD	6	2.6300000	0.43800000	0.101	0.99500
Nitrates	6	0.0004255	0.00007092	2.014	0.11400
Nitrites	6	0.0035180	0.00058640	3.948	0.00989 **
Ammonia	6	0.0005590	0.00009312	0.537	0.77400
TSS	6	985	164.200000	0.547	0.76600

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

If the p-value associated with the F-statistic is less than the significance level (0.05), it can be concluded that there is a statistically significant difference between the locations. It can be seen that all the parameters do not have any significant difference between locations except for TDS, COD and Nitrites. Therefore, Tukey's Honest Significant Difference (HSD) test was conducted for the TDS, COD and Nitrites (Table 5).

Tukey's Honest Significant Difference (HSD) test

For TDS, Mannar-Jaffna (p adj = 0.0010434) and Mullaitivu-Mannar (p adj = 0.0228076) had p-values less than 0.05, indicating that the TDS

levels in these pairs of locations are significantly different from each other. But other pair of locations had p-values greater than 0.05, indicating that there is no statistically significant difference in TDS levels. For Nitrites, Mannar-Jaffna (p adj = 0.0061325), Mullaitivu-Mannar (p adj = 0.0291910), Pooneryn-Mannar (p adj = 0.0292759) and Trincomalee-Mannar (p adj =0.0198157) had p-values less than 0.05 indicating a significant difference in mean Nitrites levels between stated locations. The ANOVA test yielded a p-value of 0.0365 for COD, indicating a statistically significant difference in COD levels across the locations overall. However, the subsequent Tukey's HSD test did not reveal any statistically significant differences between specific pairs of locations. This means that although the ANOVA suggests an overall variation in COD levels among the locations, the differences between individual pairs of locations are not substantial enough to reach statistical significance after accounting for multiple comparisons. This outcome implies that the overall difference detected by ANOVA does not translate into significant pairwise differences when using the more conservative Tukey's HSD test.

Table 5. Tukey's Honest Significant Difference (HSD) test results

	Location	Difference*	lower**	upper***	p adj****
TDS	Mannar-Jaffna	-0.58571429	-0.96288311	-0.20854546	0.0010434
	Mullaitivu-Mannar	0.475000000	0.04949618	0.90050382	0.0228076
Nitrites	Mannar-Jaffna	0.032707143	0.007619137	0.057795149	0.0061325
	Mullaitivu-Mannar	-0.030575000	-0.058878088	-0.002271912	0.0291910
	Pooneryn-Mannar	-0.027900000	-0.053737066	-0.002062934	0.0292759
	Trincomalee-Mannar	-0.034750000	-0.065320829	-0.004179171	0.0198157

*The difference in mean TDS levels between the two locations

**lower: The lower bound of the confidence interval for the difference

***upper: The upper bound of the confidence interval for the difference

****p adj: The adjusted p-value for the comparison, indicating whether the difference between the means is statistically significant

Correlation Analysis

Correlation analysis was done to investigate the correlations between different water quality parameters. Several important relationships were identified in correlation analysis. Notably, nitrites and nitrates displayed a moderate positive correlation (r = 0.47), indicating that higher nitrite levels tend to coincide with increased nitrate levels, possibly due to shared sources or nitrogen cycling processes. Similarly, TSS showed a positive correlation with ammonia (r = 0.35), suggesting that particulate matter may be associated with higher ammonia concentrations, potentially from organic materials in the water.

Moran's I Test for Spatial Correlation

The spatial distribution of water quality parameters was assessed using Moran's I statistic to identify any significant spatial autocorrelation. For all the parameters, the Moran's I values were close to zero, with p-values greater than 0.05, indicating no significant spatial clustering or dispersion of water quality measurements across the study area. Specifically, the Moran's I for all the parameters was calculated as -0.04, with an expectation of -0.04 and a p-value of 0.5, confirming the absence of significant spatial autocorrelation. The results suggest that the water quality variations in Northern and Eastern coasts are largely spatially random. These results imply that water quality across different sampling locations does not follow a distinct spatial pattern and may be influenced by local factors rather than regional spatial trends.

Table 6. Moran's I statistic standard deviate and variance for each parameter

Parameter	Moran I statistic standard deviate	Variance
pH	0	4.748806e-17
TDS	-2.0372e-09	4.640385e-17
Turbidity	0	1.864828e-17
DO	1.0782e-09	4.141652e-17
COD	1.0046e-09	4.77049e-17
BOD	0	4.18502e-17
Nitrate	0	4.87891e-17
Nitrite	2.4253e-09	3.274291e-17
Ammonia	0	4.748806e-17
TSS	0	4.943962e-17

Water Quality Index

When calculating the WQI, the threshold values for each parameter were taken from the ASEAN marine water quality guidelines and the wastewater discharge limits defined by the Central Environmental Authority of Sri Lanka. The weights were calculated comprehensively to all the locations using the PCA (Figure 2) and shown in the Table 7.

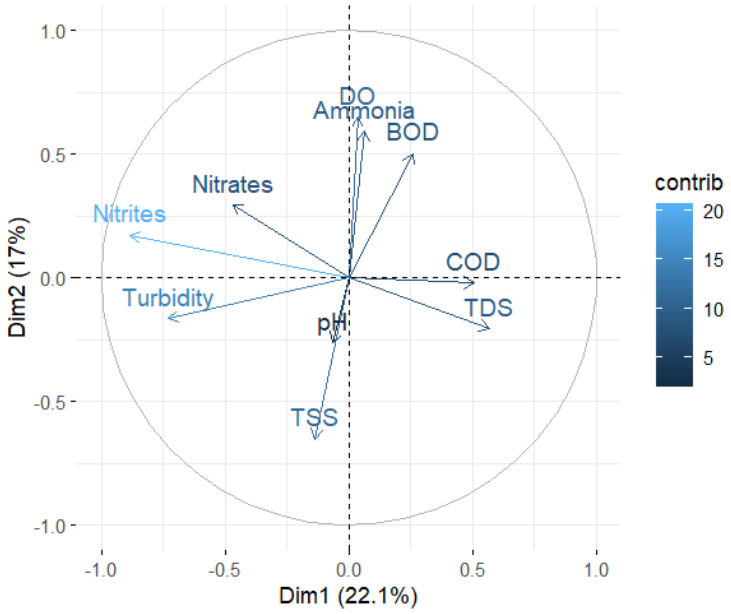

Figure 2. The contribution by parameters for weightage

Table 7. Assigned weightages for each parameter

Parameter	Assigned weightage by PCA
pH	0.0173699
TDS	0.1528968
Turbidity	0.1966145
DO	0.0095867
COD	0.1359924
BOD	0.0698287
Nitrate	0.1263624
Nitrite	0.2375299
Ammonia	0.0165927
TSS	0.0372260
	1.0000000

In order to develop the WQI classification, the distribution of the obtained WQI values for each location were analyzed and the natural breaks were determined. Then the quintiles were used to define the thresholds (Table 8). The minimum quintile was assinged for the lowest obtained WQI value and the maximum quintile was assigned for the highest WQI value. After identifying the WQI per each quantile, the WQI classification was developed (Table 9).

Table 8. Quintiles, WQI and interpretation

Quintiles		WQI	Interpretation
0%	Minimum	-70.65	Same as the minimum value.
20%	1st Quintile	44.45	The WQI value below which 20% of the data fall.
40%	2nd Quintile	60.95	The WQI value below which 40% of the data fall.
60%	3rd Quintile	67.27	The WQI value below which 60% of the data fall.
80%	4th Quintile	77.14	The WQI value below which 80% of the data fall.
100%	Maximum	80.29	Same as the maximum value.

Table 9. WQI Classification

WQI Classification	WQI value
Very Poor	WQI < 44.45
Poor	44.45 ≤ WQI < 60.95
Moderate	60.95 ≤ WQI < 67.27
Good	67.27 ≤ WQI < 77.14
Excellent	WQI ≥ 77.14

The minimum WQI was received for the Location 1 of Mannar (Achchankulam) and the maximum was received for the Location 1 of Kilinochchi (Chundikulam). The heat map shows the WQI for all the locations selected for the study (Figure 3).

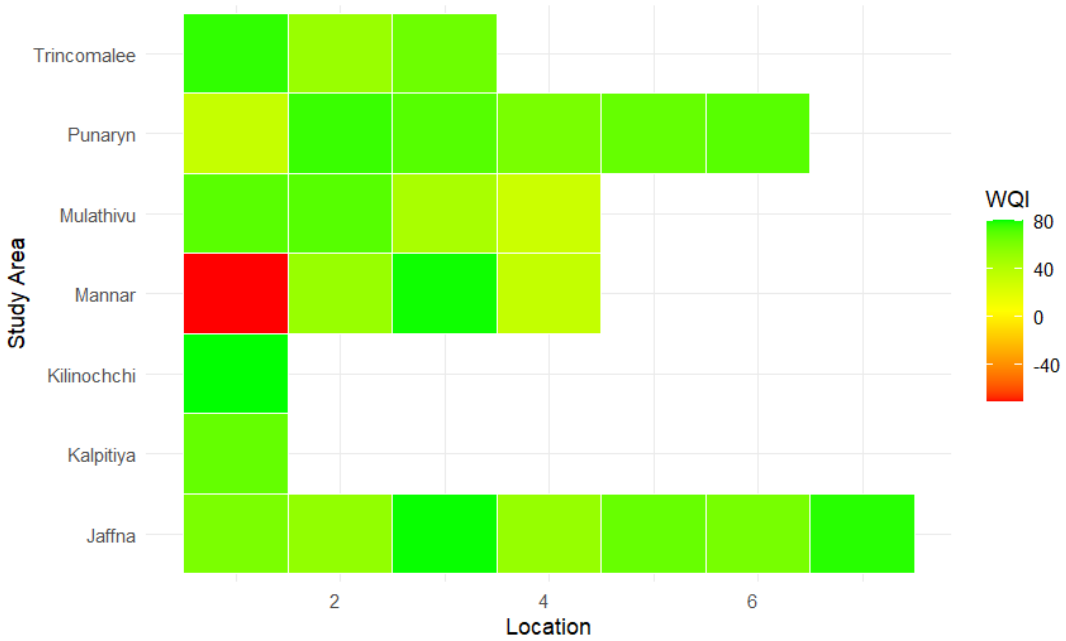

Figure 3. Graphically representation of WQI

It can be observed that the water quality at most of the chosen locations is classified as good or excellent based on the WQI, with the exception of Location 1 in Mannar. The proximity of Achchankulam to the river mouth, which carries a high nutrient load from terrestrial sources to the coastal waters, could explain the poor water quality observed there in terms of WQI.

Conclusions

This baseline study with special reference to the coastal water quality of Northern and Eastern coastal zones provides essential insights into the present status of these vital coastal ecosystems of Sri Lanka. The results reveal that the water quality parameters including DO, Nitrate, Nitrite, and Ammonia assessed from all the locations have not exceeded the standards according to the ASEAN guidelines for coastal waters. However, our study findings highlight significant variations in water quality across these locations for a few parameters including TDS, COD, and Nitrates, mainly influenced by anthropogenic factors such as industrial and fisheries activities. Further, according to the WQI applied in this study, certain locations in Mannar, Jaffna, and Pooneryn were identified with very poor water quality as the study locations are notably associated with fisheries landing sites. The collected data serves as a critical reference point for future conservation efforts and environmental monitoring activities, and this will inevitably enable policymakers and conservationists to prioritize conservation efforts accordingly, the observed trends and variations found in locations emphasize the importance of integrating site-specific pollution control management efforts to safeguard the coastal and marine environment of these selected locations. Moreover, this study underscores the need for more continued and enhanced monitoring activities where the coastal zones are experiencing severe impacts due to anthropogenic activities. To ensure the long-term sustainability of these coastal zones to reap the economic, social, and environmental benefits for the island, this study recommends increasing collaborative efforts both from government, nongovernment, and local coastal communities.

Acknowledgments

We gratefully acknowledge the important contributions of the laboratory staff at the Environmental Studies Division of NARA and Imani Herath of Ocean University, Sri Lanka.

Ethical approval

The author declares that this study complies with research and publication ethics.

Informed consent

Not available

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

Data availability statement

The authors declare that data are available from authors upon reasonable request. In case of unavailable data due to conditionals of funding organizations, etc., a clear explanation should be given.

Funding organizations

This research was funded by the Ministry of Fisheries of Sri Lanka and the National Aquatic Resources Research and Development Agency, Sri Lanka, to the first author is gratefully acknowledged.

Author contribution

Jayawardena, N.K.R.N: Writing original draft, Conceptualization, Investigation, Data curation, Software, Formal analysis, Thirukeswaran, S.: Methodology, Funding acquisition, Writing original draft, Weerasekara, K.A.W.S: Supervision, Validation, Visualization, Project administration, Resources, Review, Editing

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Sustainable Aquatic Research (2024) 3(3):151-160
DOI: 10.5281/zenodo.14559437
e-ISSN: 2822-4140

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RESEARCH PAPER

Gut analysis of the freshwater shrimp *Caridina nilotica* ("Ochong'a") for its conservation in the face of its extensive utilization in aquaculture and climate change in Lake Victoria, Kenya

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Citation

Otachi, E., Osano, A., Ogendo, J. (2024). Gut analysis of the freshwater shrimp *Caridina nilotica* ("Ochong'a") for its conservation in the face of its extensive utilization in aquaculture and climate change in Lake Victoria, Kenya. *Sustainable Aquatic Research, 3*(3), 151-160.

Article History

Received: 11 August 2024
Received in revised form:22 December2024
Accepted: 25 December 2024
Available online: 30 December 2024

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Keywords

Freshwater shrimp
Lake Victoria
Conservation
Aquaculture
Climate change
Kenya.

Abstract

The aquaculture industry is expected to double by the year 2050 but has not yet reached its full potential in East Africa. Tilapia makes up 75% of the freshwater fish production in Kenya, but the high cost of commercial fish feed is limiting the expansion of the industry. The freshwater shrimp *Caridina nilotica* is an established alternative to fishmeal in the production of the commercial feed, but its accessibility is limited to the natural stocks in Lake Victoria. Towards this end, this study investigated the gut contents of *Caridina nilotica*. One hundred specimen of *C. nilotica* were caught from Usoma Beach, in Winam Gulf of Lake Victoria. They were dissected and their gut samples were examined under a microscope. Every observed gut content were photographed and their frequency of occurrence recorded. Our findings showed that the main food for the *C. nilotica* was algae. The algal species were identified based on their morphology, color, and overall shape. The mean total body length of the examined *C. nilotica* was 2.12 ±0.29cm (±SD). Thirteen genera were identified from 6 divisions. The identified genus included; *Microcystis* sp., *Surirella* sp., *Staurostrium* sp., *Synechococcus* sp., *Pediastrium* sp., *Synedra* sp., *Oocystis* sp., *Hantzschia* sp., *Oscillatoria* sp., *Fragilaria* sp., and *Glaucocystis* sp. *Merismopedia* sp. (Cyanobacteria) and *Botryococcus* sp. (Chlorophyta) were abundant. *C. nilotica* mainly feed on algae from the division Chlorophyta, with diatoms and Cyanobacteria also being common. The information obtained in this study can be used to develop protocols for mass *C. nilotica* cultivation.

Introduction

On a global scale, there is an urgent need to address the current challenges of climate change and world population growth. With an estimated 9.7 billion people to feed by the year 2050, agricultural production must increase by 70% to meet this demand (UNDESA, 2022; FAO, 2009a). Currently, aquaculture is the second fastest-growing food sector after biotechnology. The demand for aquaculture products is estimated to

nearly double during this period (Naylor et al., 2021). As it stands now, the fishery industry is not operating in a way that is sustainable or able to be expanded upon virtually. Overfishing, unsustainable practices, pollution, habitat destruction, disease, and the spread of invasive species are significantly reducing the productivity of the fisheries industry and causing substantial harm to surrounding ecosystems. These factors not only deplete fish stocks but also disrupt the balance of aquatic environments, threatening biodiversity and long-term sustainability. Specifically in the tropics, the fisheries industry is vulnerable to the direct and indirect effects of climate change (Pickering et al., 2011).

In Kenya, commercial cultivation of freshwater fish was established in the 1920's and was commonplace by the 1960s (Opiyo et al., 2018). In the 1960s, the "Eat More Fish Campaigns" led to the growth of rural fish farming production, until the 2000's when further investment in the sector caused production in Kenya to quadruple (1000 MT to 4000 MT) (FAO, 2016). Production stayed steady until 2010 when the government introduced the Economic Stimulus Project – Fish Farming Enterprise Productivity Program. Through this program, farmers received subsidies for pond construction, fingerlings, and feed. As a result of the program, production was projected at 24,096 MT in 2014. Unfortunately, the system was not sustainable, and production later decreased to 18,542MT in 2019 (Obwanga et al., 2017; Munguti et al 2021).

Aquaculture in Kenya includes many varieties of fish and utilize different cultivation methods. Freshwater fish production in Kenya is limited to four main species: Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), and the Rainbow trout (*Oncorhyncus mykiss*). Nile tilapia makes up 75% of the fish that is produced through freshwater aquaculture in Kenya (Farm Africa, 2016). Nile tilapia can be farmed in ponds or in cages in Lake Victoria (Opiyo et al., 2018). Outdoor ponds are a common method of cultivation, as they have a wide range of applications and can be modified to different environments by adding Ultraviolet-treated liners (Munguti et al., 2014). Cage farming on the Kenyan side of Lake Victoria is relatively new, taking off in 2013 (Njiru et al., 2019). The only fish currently being cultivated in cages in Lake Victoria is Nile tilapia. As of 2017 there were approximately 3400 cages and was expected to increase (Njiru et al., 2019).

Fish farmers, face barriers to producing a quality product while making a reasonable profit. Quality seed stock is inaccessible and expensive, and the cost of production is high due to the cost of fish feed. As of 2023, the average cost of fish feed is 147ksh/kg. In addition to high cost, the feeds available have decreased in quality due to the high demand (Munguti et al., 2014). As a result, many small-scale farmers formulate their own feed using the available resources or feed their fish with either poultry or livestock feed. These alternative practices do not meet the nutrient requirements for Nile tilapia and can introduce unnecessary antibiotics or hormones to the fish (National Research Council, 1993; Opiyo et al., 2018).

A common source of protein in farmer-formulated fish feed is the freshwater shrimp *Caridina nilotica* found in Lake Victoria. *Caridina nilotica* is a freshwater shrimp species of the family Atyidae. It can be found in Lake Victoria and is known locally as 'Ochonga'. The biomass of *C. nilotica* in Lake Victoria has been estimated at 22,694 metric tonnes (Getabu et al., 2003). Within Lake Victoria, *C. nilotica* is abundant in the littoral region, but can also be found in offshore waters (Fryer, 1960; Lehman et al., 1996). Nearshore populations are benthic, while offshore populations are mainly (86%) planktonic (Lehman et al., 1996). *Caridina nilotica* can reach a maximum length of 2.5 cm and have a slim body form with attenuated appendages (Fryer, 1960). While small individuals are transparent in appearance, the larger individuals tend to be darkly pigmented. Dark blue, black, and an emerald-green individual have been documented. Not much is known of the feeding mechanisms of *C. nilotica* except of one key study (Fryer, 1960). *C. nilotica* is caught as a bycatch when fishing for *Rastrineobola argentea* (Kubiriza et al., 2018). *Caridina nilotica* makes up approximately 10% of the *R. argentea* that is landed. *C. nilotica* is typically sun dried alongside *R. argentea* on tarps before being separated. Farmers then mix the dried shrimp with rice bran or maize bran and

sometimes *R. argentea* meal (Ngugi et al., 2007). This shrimp is an important feed component for fish and other animals for small-scale and commercial farming in East Africa (Bundi et al. 2013; Mwamburi 2013). The current dependence on the freshwater shrimp *C. nilotica* for use in animal feed has not only resulted in a high cost for fish farmers in Kenya but also threatened the natural population of *C. nilotica* in Lake Victoria. *Caridina nilotica* is the most common source of protein in fish feed and is also used as a protein source in other livestock feeds. In general, the cost of fish feed accounts for approximately over 50% of the total cost of production (FAO 2009b). This expense is the greatest limiting factor for increasing profit and improving the livelihoods of fish farmers in Kenya. In East Africa, *C. nilotica* is only sourced from Lake Victoria. Without a sustainable alternative source of *C. nilotica*, the cost of fish feed will remain high and the natural population in Lake Victoria will be vulnerable. The population, diet, and behaviors of *C. nilotica* in Lake Victoria are largely understudied. The feeding habits and mechanisms have been described in detail by Fryer (1960), but even in this case, the diet of *C. nilotica* is described mainly as "an amorphous mass of grey-green material." It has been more than six decades since Fryer (1960) published his work, with climate change, lake dynamics, urbanization, catchment use changes reported, fish species, and population changes, a revisit of the diet composition of *C. nilotica* is necessary. There is yet to be a detailed study on the diet of *C. nilotica* in Lake Victoria since these changes.

Materials and Methods

Study area

Lake Victoria is the largest tropical lake in the world, and is shared between Tanzania, Uganda and Kenya. Only 6% of the total lake area is in the Republic of Kenya. The lake lies in a shallow continental sag between the two arms of the Great Rift Valley, 1170 m above sea level. The lake has a maximum depth of 84 m, a volume of 2760 km^3, an average depth of 40 meters and a surface area of 68,800 km^2 (Bootsma and Hecky, 1993; Crul, 1995). The mean surface temperature is about 25ºC, while the temperature of deeper layers is about 1 to 2 degrees lower (Witte and Van Densen, 1995). The primary inflows into the lake basin originate from the slopes of the western ridge of the East African Rift Valley including; Sio, Nzoia, Yala, Nyando, Sondu Miriu, Kuja, Kibos and Kisat rivers. The present study was conducted in Usoma beach next to Kisumu International Airport (0.0819° S, 34.7295° E) (Fig 1).The surface water temperatures range between 23.5º C and 29.0 ºC. Wind induced currents influence water mixing in the gulf. The Secchi transparency ranges from 35 to 155 cm.

Sampling

Samples of the *C. nilotica* were collected from the Winam Gulf (Kenyan side) of Lake Victoria. Offshore waters were accessed with a boat, and samples were collected using a seine net. Collection occurred at night when the study species exhibited diel vertical migration (Lehman et al., 1996). After collection, samples were stored in plastic bags containing water from Lake Victoria and then transported to Egerton University. The Egerton Njoro campus is located in Nakuru County, Kenya, approximately 140 km northwest of Nairobi, and 190 km east of Lake Victoria, approximately 4 hours.

Acclimatization of the samples

Once the live samples arrived at Egerton Njoro campus, they were acclimatized to their new environment. The samples while within the polythene bags were gently placed into the 50 L aquarium and left for about 30-45 min, to attain the ambient water temperature. The polythene bags were then opened, and the shrimps were allowed to freely swim into the tank.

Sample analysis

Freshly dead samples were examined for their gut contents. Before dissection, each sample was measured from the rostrum to the end of the telson in centimeters to determine the total length. Using a scalpel and forceps, under a dissecting microscope, the specimens were cut transversely along the midsagittal plane. Then, the gut contents were located based on their color and consistency and removed from the specimen. Using a pipette, the gut contents were transferred to a slide to then be examined under a light microscope. Under 40x and 100x magnification, photos were taken of the gut contents for later identification based on the

size, morphology, pattern, and color of the contents. The dominant species-most frequent was noted, as well as the lack of gut contents in individual samples. The gut contents were identified using identification keys (Komarek and Anagnostidis, 1998; Prescott, 1970) and online resources such as AlgaeBase. The sex of each individual was also noted based on the presence of eggs on the abdomen and shape (Females have a wider, more rounded abdomen, while males have a narrower, more triangular abdomen).

Results

Caridina nilotica size and sex ratio

The mean total body length was 2.12 ± 0.29 cm (Mean ± SD, n=100). The shrimps were grouped into two groups based on their sizes as follows; small with a size of <1cm and large >1cm body lengths. Nineteen specimens were females, while there were 81 males.

Gut content analysis

Thirteen genera were identified from 6 divisions (Figure 1). The divisions represented by the samples include: Cyanobacteria, Chlorophyta, Gyrista, Bacillariophyta, Charophyta, and Archaeplastida. Approximately 34 species remain unidentified. Some of the algae identified included: *Microcystis sp, Surirella sp., Staurostrum sp., Synechococcus sp., Pediastrium sp., Synedra sp., Oocystis sp., Hantzschia sp., Oscillatoria sp., Fragilaria sp. and Glaucocystis sp.* Three algae were most commonly found: *Merismodpedia* (Cyanobacteria), *Botryococcus sp* (Chlorophyta) and unidentified species.

There were three most frequent food items in the gut of *C. nilotica* as shown (Figure 2).

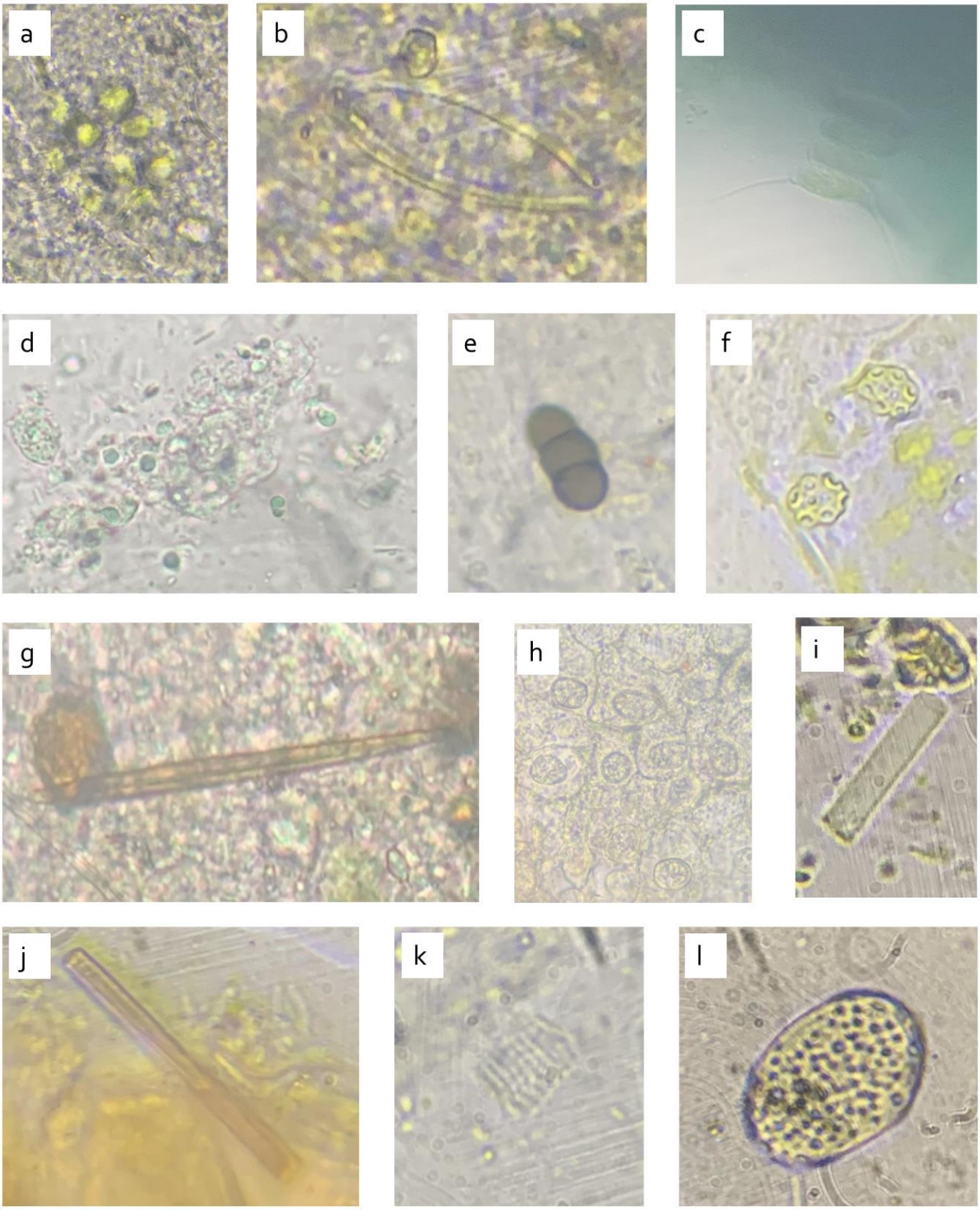

Figure 1. a, *Microcystis sp*. b, *Surirella sp.*, c, *Staurostrum sp*, d, *Synechococcus sp*, e, *Unidentified sp.* ,f, *Pediastrum*, *g*, *Synedra sp.*, h, *Oocystis sp.*, i, *Hantzschia sp.*, j, *Oscillatoria sp.*, k, *Fragilaria sp.*, l, *Glaucocystis sp.*

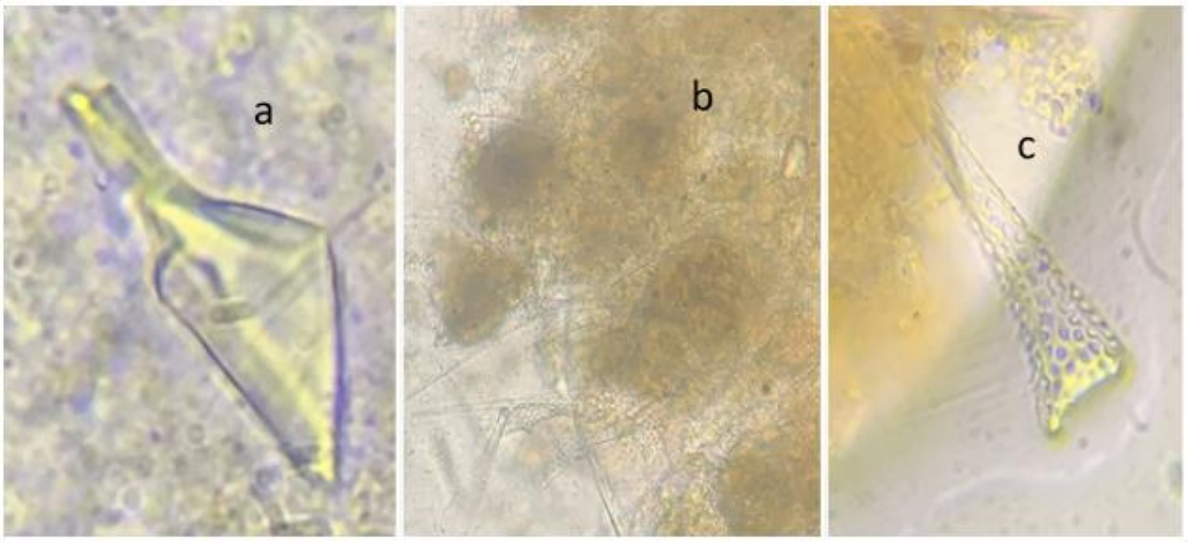

Figure 2. Three most frequently occurring species in order of the most abundant (unidentified>Botryococcus sp.>*Merismopedia sp.*)

Food distribution in Small and Large Caridina nilotica

Samples were noted as having a dominant genus when possible. The percentage of the samples with each dominant genus were compared between the small and large groups (Figure 3a). The percentage of the samples were also compared between the two groups based on the divisions that were represented (Figure 3b). The results clearly showed that smaller *C. nilotica* prefer Cyanobacteria whereas larger ones prefer Chlorophyta (Figure 3b).

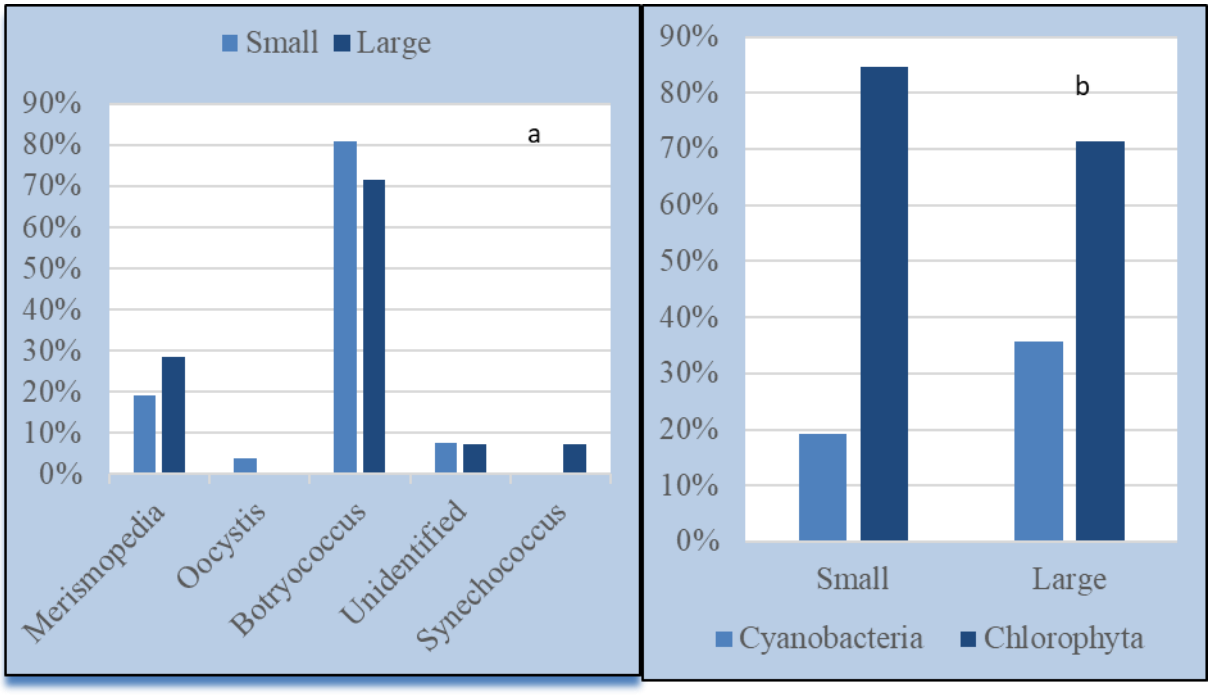

Figure 3. a, Comparison between the major algal species abundances in the small and large *C. nilotica*, and b showing a comparison of the two major algal divisions in the small and large *C. nilotica*

Discussion

Caridina nilotica is the only shrimp species known in Lake Victoria (Fryer 1960). In general, there are very few studies on the freshwater shrimp *Caridina nilotica* in Lake Victoria such as Fryer (1960), Lehman et al (1996), Bundi et al (2013), Mwamburi (2013) and Outa et al (2020).

Most of the studies on this species are mainly those focusing on its potential as a substitute for fishmeal in fish feeds in aquaculture.

The findings of this study have shed some light and useful insights into *C. nilotica*'s ecology in Lake Victoria. Firstly, the results showed that *C. nilotica* prefer feeding on algae. This finding while agreeing with the earliest study by Frier (1960), reveals further details of the "mass of greenish diet" that Fryer (1960) wrote about, but also, slightly differs on them being detritivores. It does appear that *C. nilotica* is highly selective in its diet. This study showed that *C. nilotica* feeds on algae mainly from the division Chlorophyta, Bacillariophyceae, and Cyanobacteria. Further, the study showed that there could be a potential ontogenetic shift in diet for *C. nilotica* as smaller *C. nilotica* preferred Cyanobacteria while the larger ones preferred Bacillariophyceae and Chlorophyta. There could be some possible reasons, one of them being a possibility of an ecological niche partitioning, with the smaller ones possibly feeding in surface waters, where Cyanophyta dominate due to their floating adaptive potential, and the larger ones, being benthic feeders, where the bacillariophyceae are known to be periphytic, attaching to substrates in water. In agreement with our findings, a study by Lehman et al (1996), while focusing on the abundance, biomass and diel migration of C. nilotica in Lake Victoria, it was reported that only about 9% (night) to 14% (day) of the population appeared to be epibenthic. They also suggested that the behavior of the animal is consistent with the hypothesis that it is not a strict detritivore as previously reported; rather it may engage in facultative planktivory, especially at night.

Conclusions

From the findings of this study, we make the following conclusions: first, *C. nilotica* mainly feed on algae from the division Chlorophyta, with diatoms and Cyanobacteria also being common. Secondly, there was a difference in diet between the small and large individuals. Thirdly, the data produced from this study can be used to develop *C. nilotica* cultivation and feeding techniques. This study further recommends the possibility of Genomic sequencing of gut contents to confirm identification and future research examining the ecological role of *C. nilotica* in Lake Victoria (Community structure, preferred habitat, predator-prey interactions).

Acknowledgments

We acknowledge the useful help by Ms. Jazmine Compton (Portland State University and REEU fellow), Mr. Alex Mecha and Mr. Eric Owino in sampling and identification of some algal specimen

Ethical approval

The authors declare that this study complies with research and publication ethics.

Informed consent

Not available.

Conflicts of interest

There is no conflict of interests for publishing this study

Data availability statement

The authors declare that data are available from authors upon reasonable request

Funding organizations

We thank the United States Department of Agricuture National Institute for Food and Agricuture (USDA-NIFA) for funding this study.

Author contribution

Elick Otachi: Writing original draft, Conceptualization, Data curation, Formal analysis

Anne Osano: Investigation, Methodology, Software, Funding acquisition, Resources, Writing original draft,

Joshua Ogendo: Supervision, Validation, Visualization, Project administration, Review, Editing.

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DOI: 10.5281/zenodo.14559469
e-ISSN: 2822-4140

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RESEARCH PAPER

Investigation of The Presence of *Escherichia Coli* and *Salmonella* spp. in Seafood Sold in Izmir

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Citation

Gündüz, H., Öztürk, F., Adıgüzel, H. (2024). Investigation of The Presence of *Escherichia Coli* and *Salmonella* spp. in Seafood Sold in Izmir. *Sustainable Aquatic Research*, *3*(3), 161-168.

Article History

Received: 14 October 2024
Received in revised form:25 November2024
Accepted: 28 November 2024
Available online: 30 December 2024

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Keywords

Seafood
Fish
Salmonella
Escherichia coli
Food control.

Abstract

Seafood is a valuable source of animal protein that is consumed raw, partially cooked, or fully cooked in various cultures. Due to their high water and protein content, along with a near-neutral pH, they are conducive to the growth of various microorganisms. Microorganisms can be found in freshly caught seafood and can be transmitted by cross-contamination from humans during transport, gutting, sale at fish stalls or from substances added during seafood processing. *Salmonella* is not part of the natural microbiota of aquatic organisms. The presence of *Salmonella* in seafood indicates fecal contamination or cross-contamination during transport and storage. *Escherichia coli* is usually found in faecally contaminated water or food and is an indicator of poor hygiene. This study investigated the presence of *E. coli* and *Salmonella* spp. in 50 seafood products sold in Izmir. The samples subjected to analysis included trout, red mullet, sea bream, sole, frozen haddock, bogue, sea bass, seabream, saddled seabream, red porgy, stuffed mussel, striped catfish, sardine, bluefish, salema fish, salmon, mackerel, comber, anchovy, white grouper, annular sea bream, horse mackerel, oyster, picarel, squid, shrimp, chub mackerel. As a result, *E. coli* was detected in 21 of the samples analysed, while *Salmonella* spp. was detected in only 1 sample. The total coliform count was found to vary between 1.30 and 5.85 log cfu/g. These results showed that a significant amount of *E. coli* was present in seafood products and that the level of faecal contamination, and hence the potential hazard, was high. Therefore, it is necessary to increase efforts to prevent cross-contamination during the production, distribution and sale of seafood products, pay more attention to hygiene and sanitation practices, and pay special attention to personnel hygiene.

Introduction

Seafood products are widely consumed globally due to their high nutritional value and potential health benefits (Yang et al., 2015). Consumer preferences range from raw or minimally processed fresh products to those prepared in various forms (salted, smoked, cured, canned) and

ready-to-eat (RTE) products. In addition, seafood is an important food category in international trade and is often transported over very long distances. All these factors expose seafood to various contaminants, including those of microbiological origin (Amagliani et al., 2012).

Salmonella is one of the most frequently reported causes of disease and outbreaks associated with seafood around the world (Yang et al., 2015). Worldwide, there are approximately 200 million to more than 1 billion cases of *Salmonella* each year, 93 million of which result in gastroenteritis and 155,000 deaths, 85% of which are linked to food consumption (Zhou et al., 2024). The occurrence of *Salmonella* infections transmitted by fish or shellfish was documented in eight out of 160 outbreaks, representing 7.42% of the total cases (Heinitz et al., 2000).

Salmonella are facultative anaerobic, non-spore-forming, Gram-negative bacteria. The majority of strains are motile by means of flagella. They are mesophilic, with an optimum growth temperature of 35 to 37 °C and a growth range of 5 to 46 °C. They are sensitive to low pH (4.5 or below) and do not grow at Aw 0.94, particularly at pH 5.5 and below (Amagliani et al., 2012). The natural habitat of *Salmonella* is the gastro-intestinal tract of animals, including birds and humans (Shabarinath et al., 2007).

However, *Salmonella* are not part of the normal flora of aquatic animals and the presence of these bacteria in aquatic food is a result of faecal contamination from contaminated water or cross contamination during transport or storage (Amagliani et al., 2012; Sanath Kumar et al., 2003; Yang et al., 2015). A significant corpus of epidemiological data exists concerning the presence of *Salmonella* in seafood and the diseases associated with it (Amagliani et al., 2012).

Escherichia coli is a Gram-negative, catalase-positive, oxidase-negative, facultative anaerobic, short rod-shaped bacterium belonging to the genus *Escherichia* of the Enterobacteriaceae family. It typically exhibits mobility with peritrichous flagella, although some strains are immobile (Değirmenci, 2017). *Escherichia coli* is a bacterium that is commonly employed for the detection of faecal contamination in water and food due to its association with the gastrointestinal tract of warm-blooded animals. However, certain *E. coli* serovars have undergone a process of evolution, acquiring genes that confer virulence, and thus have become highly pathogenic to humans. These pathogenic *E. coli* strains can cause infection of the gastrointestinal and urinary tracts as well as the central nervous system (Prakasan et al., 2022). In developing countries, the contamination of coastal ecosystems with human and animal waste through the discharge of untreated sewage is the primary source of *Escherichia coli* in seafood (Prakasan et al., 2022).

The importance of seafood products in human nutrition is underscored by their high nutritional value. However, these products are also susceptible to a significant risk of contamination by pathogens, both existing in the product itself and subsequently transmitted to consumers. In addition to pathogens originating from the flora of the aquatic environment in which seafood is caught or cultivated, contamination can also occur from a variety of sources during the processing, storage, and transportation phases. Of particular significance are faecal contaminations. In this context, the objective of this study was to investigate the presence of *Salmonella* spp. and *Escherichia coli* in seafood products available for purchase in Izmir. The objective of this study is to contribute to the existing research on the prevalence of these bacteria in seafood products in the Izmir region. Furthermore, the data obtained aims to contribute to the existing literature on the potential risks to public health posed by aquatic products by quantifying the microbiological risks associated with their transportation, processing and storage.

Materials and Methods

Material

In this study, a total of 50 water products obtained from markets, bazaars and restaurants in Izmir were used for the isolation of *Escherichia coli* and *Salmonella* spp. Following the collection of samples under sterile conditions, they were transported to the laboratory under cold chain conditions (+4 ºC) and subjected to microbiological analysis. The samples subjected to analysis included rainbow trout (*Oncorhynchus*

mykiss), red mullet (*Mullus barbatus*), sea bream (*Sparus aurata*), sole (*Solea solea*), frozenhHaddock (*Gadus merlangus euxinus)*, bogue (*Boops boops*), sea bass (*Dicentrarchus labrax*), saddled seabream (*Oblada melanura*), red porgy (*Pagrus pagrus*), stuffed mussel (*Mytilus galloprovincialis)*, striped catfish (*Pangasianodon hypophthalmus*), sardine (*Sardina pilchardus*), bluefish (*Pomatomus saltatrix*), salema (*Sarpa salpa*), Atlantic salmon (Salmo salar), mackerel (*Scomber scombrus*), comber (*Serranus cabrilla*), anchovy (*Engraulis encrasicolus*), white grouper (*Epinephelus aeneus*), annular sea bream (*Diplodus annularis*), horse mackerel (*Trachurus trachurus*), oyster (*Ostrea edulis*), picarel *(Spicara flexuosa*), squid (*Loligo vulgaris)*, shrimp (*Penaeus semisulcatus*) and chub mackerel (*Scomber japonicus*).

Method

The head and internal organs of fish were removed, before the muscle was cut into small pieces, and then completely homogenised.

Escherichia coli analysis

Ten grams of the seafood sample was transferred into 90 millilitres of sterile physiological saline and homogenised. The solution was diluted to 10^{-6}, ensuring adherence to the 1/10 dilution ratio. 0.1 ml of the appropriate dilutions were spread over the surface of Fluoracult Violet Red Bile Agar (FVRBA, Merck) with incubation at 37°C for 18 to 24 hours. Following incubation, red colonies surrounded by a reddish precipitate zone with a diameter of 1-2 mm were identified as coliforms. The coliform group bacteria that exhibited fluorescence under a long-wavelength UV hand lamp (Merck 1.13203) were identified as suspected *E. coli* colonies. *E. coli* ATCC (ATCC® 25922™) was used as a control during identification.

Salmonella analysis

In order to ascertain the presence of *Salmonella* spp., 25 grams of the seafood samples were homogenised in 225 millilitres of buffered peptone water for pre-enrichment and incubated at 37°C for 24 hours. A quantity of 0.1 mL of the pre-enrichment culture was transferred to a test tube containing 10 mL of Rappaport-Vassiliadis Soy Broth (RVS, Merck), and incubated at 41.5 ºC for 24 hours. Following incubation, XLT4 agar was inoculated in triplicate. The inoculated Petri dishes were incubated at 37°C for 24 hours. Following incubation, colonies with black centres were deemed to be indicative of the presence of *Salmonella* spp. (Halkman, 2005).

Biochemical identification of the bacteria

Biochemical tests were employed to identify any suspect colonies that developed following incubation. The initial step involved the evaluation of Gram reactions and microscopic morphology of the isolates.

IMViC tests (I: indole, M: methyl red, V: Voges-Proskauer, C: citrate) were employed to identify putative *E. coli* colonies (Halkman, 2005). Colonies exhibiting IMViC test results (+ + - - -) were identified as *E. coli*.

In the case of colonies suspected of being *Salmonella*, an oxidase test, urea test, and a series of tests utilising Triple Sugar Iron Agar medium, lysine decarboxylase, Voges-Proskauer reaction, and indole production were conducted. The results were evaluated in accordance with the criteria set forth in Bergey's Manual of Systematic Bacteriology (LeMinor, 1984).

Results and Discussion

As a result of the study, 50 seafood samples were tested for the presence of *E. coli* and *Salmonella*, and the total coliform count of the samples was determined (Table 1).

While *E. coli* was detected in 21 of the samples, *Salmonella* spp. was detected in only 1 sample. Total coliforms were found to vary between 1.30 and 5.85 log cfu/g in the samples. Biological agents introduced into seafood consist of bacteria, viruses and parasites that can cause serious life-threatening diseases, particularly gastroenteritis. Some of these pathogens are naturally present in the aquatic environment, while others are transmitted through contact with human or animal faeces or through sewage (Amagliani et al., 2012). The main source of transmission of *Salmonella* and other bacteria to seafood is cross-contamination during processing, transport and storage (Amagliani et al., 2012).

Table 1. Coliform counts, *E. coli* and *Salmonella* spp. contents of seafood samples

	Sample	Coliform (log kob/g)	*E. coli*	*Salmonella* spp.		Sample	Coliform (log Cfu/g)	*E. coli*	*Salmonella* spp.
1	Trout (Fresh-Culture)	3,58	+	-	**26**	Bogue 2 (Fresh)	3,04	-	-
2	Red mullet 1 (Fresh)	2,42	-	-	**27**	Bogue 3 (Fresh)	2,77	+	-
3	Red mullet 2 (Fresh)	5,56	-	-	**28**	Bogue 4 (Fresh)	2,59	-	-
4	Sea bream 1 (Fresh)	3,60	-	-	**29**	Sea bass 1 (Fresh)	4,07	+	-
5	Sole fish (Fresh)	2,97	+	-	**30**	Sea bass 2 (Fresh)	2,45	-	-
6	Frozen Haddock (Processed)	2,65	+	-	**31**	Seabream 3 (Fresh)	2,30	+	-
7	Comber (Fresh)	5,17	-	-	**32**	Saddled seabream 1 (Fresh)	3,40	-	-
8	Anchovy 1 (Fresh)	3,66	-	-	**33**	Saddled seabream 1 (Fresh)	3,11	-	-
9	Anchovy 2 (Fresh)	4,43	+	-	**34**	Red porgy (Fresh)	2,28	-	-
10	White grouper fish 1 (Fresh)	5,85	+	-	**35**	Stuffed mussel 1 (Processed)	2,94	-	-
11	White grouper fish 2 (Fresh)	2,60	-	-	**36**	Stuffed mussel 2 (Processed)	1,40	-	-
12	Annular sea bream (Fresh)	2,87	-	-	**37**	Striped catfish (Fresh)	3,56	+	-
13	Horse mackerel 1 (Fresh)	3,86	-	-	**38**	Sardine 1 (Fresh)	3,51	+	-
14	Horse mackerel 2 (Fresh)	2,64	-	-	**39**	Sardine 2 (Fresh)	2,47	+	-
15	Oyster (Fresh)	2,70	-	-	**40**	Sardine 3 (Fresh)	4,27	-	-
16	Picarel (Fresh)	2,35	+	-	**41**	Sardine 4	3,82	+	-
17	Sea bream 2 (Fresh)	4,32	-	-	**42**	Sardine 5 (Fresh)	3,12	+	-
18	Squid 1 (Fresh)	2,80	+	-	**43**	Sardine 6 (Fresh)	2,95	+	-
19	Shrimp (Fresh)	2,78	-	-	**44**	Sardine 7 (Fresh)	2,20	-	-
20	Squid 2 (Fresh)	2,45	-	-	**45**	Bluefish (Fresh)	3,81	-	-
21	Chub mackerel 1 (Fresh)	3,60	+	-	**46**	Salema porgy (Fresh)	2,56	+	-
22	Chub mackerel 2 (Fresh)	1,61	-	-	**47**	Salmon 1 (Fresh)	3,19	+	-
23	Chub mackerel 3 (Fresh)	2,56	-	-	**48**	Salmon 2 (Fresh)	3,67	-	-
24	Chub mackerel 4 (Fresh)	3,03	+	-	**49**	Salmon 3 (Fresh)	2,62	+	-
25	Bogue 1	2,30	-	+	**50**	Mackerel (Fresh)	1,30	-	-

Minimum Coliform count (log cfu/g)	**Maximum Coliform count (log cfu/g)**	**Average Coliform count (log cfu/g)**	**Total number of *E. coli* samples**	**Percentage of *E. coli* samples**	**Total number of *Salmonella* spp. samples**	**Percentage of *Salmonella* spp. samples**
1,30	**5,85**	**3,12**	**21**	**42 %**	**1**	**2 %**

In our study, *Salmonella* spp. was detected in only one (2%) of the samples analysed. Similarly, Lee (2006) reported that *Salmonella* spp. was not detected in their study of 177 seafood samples. This rate is relatively low compared to the findings in the literature. For example, Brands et al. (2005) investigated the presence of *Salmonella* spp. in oysters from the United States coast, and reported that 7.4% of the oysters analysed contained *Salmonella* spp. Similarly Shabarinath et al. (2007) reported that *Salmonella* spp. were detected in 20 of 100 seafood products analysed. In a study by Bakr et al. (2011) involving 150 samples, it was reported that *Salmonella* spp. were isolated in 10% of the samples. Furthermore, *Salmonella* spp. were isolated from shrimps, oysters and mussels. Vural and Emin Erkan (2006) identified the presence of *Salmonella* spp. in 15.69% of the 51 fish samples collected from three distinct locations along the Dicle River. Sanath Kumar et al. (2003) reported that 6 out of 20 finned fish (30%), 4 out of 20 oysters (20%) and 1 out of 20 shrimps (5%) were found to be positive for *Salmonella* spp. In a study conducted by Adesiji et al. (2014) 30 samples (6 poultry, 8 seafood and 16 clinical samples) were found to be positive for *Salmonella* spp. out of a total of 120 samples tested. Akpınar Bayizit et al. (2003) reported that 58% of the samples of frozen mussel examined were found to contain *Salmonella* spp. Atwill & Jeamsripong (2021) reported that 47% of the shrimp samples, 46% of the sea bass samples and 14-38% of the oyster samples tested positive for the presence of *Salmonella* spp. The study involved the analysis of 335 seafood products offered for sale in Thailand. Moreover, Nguyen et al. (2016), high rates of *Salmonella* contamination were detected in a variety of food products, including pork (69.7%), poultry (65.3%), beef (58.3%), shrimp (49.1%) and freshwater fish (36.6). The study involved the analysis of 409 samples. The 2% *Salmonella* spp. rate obtained in our study is relatively low in comparison to the rates reported in the existing literature (Bakr et al., 2011; Brands et al., 2005; Sanath Kumar et al., 2003; Shabarinath et al., 2007). It is hypothesised that these discrepancies are attributable to variations in hygiene standards pertaining to fishing activities, processing and storage procedures in the sampled regions.

Salmonella serovars are ubiquitous in the natural environment. These bacteria can gain access to the aquatic environment via wild and domestic animals, inadequate sanitation, and the improper disposal of human and animal waste (Amagliani et al., 2012). The source of *Salmonella* spp. in fish and other aquatic products has been identified as contamination of the water source or contamination occurring during storage and processing (Amagliani et al., 2012). *Salmonella* spp. are among the most common food-associated pathogens and are responsible for a significant proportion of deaths resulting from foodborne diseases (Brands et al., 2005).

The analysis of the samples revealed the presence of *Salmonell*a spp. only in fresh bogue fish. The low prevalence of *Salmonella* spp. in seafood products sourced from the Izmir region suggests that the processing and storage of seafood products in this region adhere to more rigorous hygienic standards. In a study conducted in Thailand, Atwill & Jeamsripong (2021) detected a prevalence of 47% for *Salmonella* spp. in shrimps and 46% in sea bass. It is presumed that this difference between the studies is a consequence of more rigorous hygiene protocols and processing procedures.

The results of our study indicated that 21 (42%) of the samples analysed were positive for *E. coli*. Moreover Akpınar Bayizit et al. (2003) reported that *E. coli* was not detected in frozen mussel samples. This finding is significantly at odds with the results of several studies previously reported in the literature. Prakasan et al. (2022) reported that *E. coli* was detected in 75 (96.2%) of the 78 seafood samples included in their study on finned fish and shellfish. Similarly, Atwill & Jeamsripong (2021) reported that 335 seafood products offered for sale in Thailand were positive for faecal coliform, with 85% of these samples also containing *E. coli*. The rates of *E. coli* detected in these studies are considerably higher than those observed in the study conducted by our research group. It is hypothesised that these discrepancies are attributable to variations in geographical factors, hygiene standards and practices employed in the catching, processing and storage of seafood products.

The study conducted by Matyar et al. (2008) in Iskenderun Bay revealed the absence of *E. coli* in 97 shrimp samples. This finding suggests that the environmental conditions in Iskenderun Bay may be more favourable than those observed in the Izmir region. Furthermore, the study by Matyar et al. (2008) revealed the presence of *E. coli* in water and sediment samples collected from the same areas where fishing took place. This finding indicates that seafood products may be susceptible to contamination from polluted water sources, underscoring the importance of water quality during the fishing process. The presence of *E. coli* in seafood may serve as an indicator of faecal contamination and inadequate processing methods (Chakravarty et al., 2015). Furthermore, contamination may be associated with additional factors, including water quality, fishing methodology, storage conditions, and processing procedures. The *E. coli* rate in the Izmir region may indicate that the risk of faecal contamination is low; however, it also suggests the necessity for improvements in regional hygiene standards. These findings are corroborated by the supposition that fishing operations in the Izmir region are conducted in accordance with more rigorous regulatory standards. However, in comparison to the elevated *E. coli* levels documented by Prakasan et al. (2022) and Atwill & Jeamsripong (2021), the diminished *E. coli* concentrations identified in the Izmir region may be attributable to regional disparities in environmental conditions or hygiene standards in seafood processing. Nevertheless, further comprehensive research across diverse seasons and regions is essential to ascertain the underlying reasons for the observed discrepancy in *E. coli* rates between the Izmir region and other areas.

Consequently, the prevalence of *Escherichia coli* in seafood originating from the Izmir region was found to be lower than that reported in numerous studies published in the scientific literature. This represents a novel contribution to the field of seafood safety in Turkey, underscoring the beneficial influence of local hygiene standards on food safety.

In the course of our study, *E. coli* was identified in 42% of the 50 seafood products subjected to analysis, while *Salmonella* spp. was detected in only 2%. The discrepancy in the detection rates of these two bacteria may suggest that high faecal coliforms do not necessarily indicate the presence of *Salmonella*. A comparable result was observed in the literature by Brands et al. (2005). The researchers observed no significant correlation between faecal coliform counts in oysters and the isolation of *Salmonella*. Furthermore, they posited that high faecal contamination does not invariably increase the risk of *Salmonella* infection, and that different bacterial contamination sources may be present. The low prevalence of *Salmonella* spp. despite the high *E. coli* rates observed in the present study suggests the existence of diverse faecal contamination sources and differences in the transmission routes of these pathogens. This result indicates that the responses of *E. coli* and *Salmonella* to environmental factors and hygiene conditions may be disparate.

A multitude of factors, including insufficient access to clean water, inadequate hygiene practices, and the absence of effective food safety measures, have been identified as key contributors to the increasing incidence of foodborne salmonellosis cases (Shabarinath et al., 2007). The most common factors contributing to salmonellosis outbreaks are inadequate cooking, improper storage, cross-contamination and the use of raw ingredients in the preparation of seafood (Amagliani et al., 2012). Although the prevalence of *Salmonella* spp. was low in our study, it should be noted that this may not be the case in unhygienic processing conditions. Given the susceptibility of seafood products to bacterial contamination and the potential for such contamination to pose health risks to consumers when appropriate conditions are not created Bakr et al. (2011), It is therefore of great importance to minimise microbial risks in order to ensure the safety of seafood products. The elevated detection of *E. coli*, an indicator of faecal contamination in seafood, highlights the potential health risks associated with its presence. The mere presence of non-pathogenic bacteria such as *E. coli* is of critical importance to public health, as it may indicate the presence of other enteric pathogens of faecal origin (Costa, 2013).

In order to safeguard the microbiological quality and safety of seafood products, it is essential to implement the following basic measures.

1. Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) are fundamental to ensuring the quality and safety of seafood products. It is imperative that rigorous adherence to hygiene standards be observed throughout the entirety of the processing procedures.

2. Sanitation Standard Operating Procedures (SSOPs) and Hazard Analysis at Critical Control Points (HACCP) are essential for ensuring the safety and quality of seafood products. These systems must guarantee that microbiological safety is monitored at all stages of the production process, from the initial stages of seafood production until the final product reaches the consumer (Amagliani et al., 2012).

3. Post-harvest care is a crucial aspect of the seafood production process. It is of paramount importance that seafood is stored and processed under appropriate conditions following its capture in order to minimise the risks of contamination.

4. It is recommended that raw or undercooked seafood be avoided. It is important to reiterate to consumers that the consumption of raw or undercooked seafood carries an inherent health risk (Costa, 2013).

The implementation of enhanced regional hygiene standards and the establishment of a continuous monitoring programme for microbiological quality will serve to enhance the safety of seafood products for human consumption.

Conclusions

The present study aimed to determine the presence of *Salmonella* spp. and *E. coli* in seafood products available for purchase in Izmir. The findings indicate that these bacteria pose a significant microbiological risk to public health. The particularly elevated *E. coli* levels suggest that the sampled seafood products are susceptible to faecal contamination. While the low prevalence of *Salmonella* spp. in the Izmir region indicates that regional hygiene standards may be effective, the value of the results is limited due to the limited sample size and data collection from a single region. Further studies conducted across different seasons and geographical regions will help generate more consistent and generalized findings on seafood safety.

Ethical approval

The author declares that this study complies with research and publication ethics.

Informed consent

Not available

Conflicts of interest

There is no conflict of interests for publishing of this study.

Funding organizations

No funding available.

Author contribution

All authors contributed equally to the writing of the manuscript, read and approved the final version.

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Sustainable Aquatic Research (2024) 3(3):169-174
DOI: 10.5281/zenodo.14563196
e-ISSN: 2822-4140

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SHORT COMMUNICATION

Ecotoxicity of Aquaculture Chemotherapy-A Case Study in Chile

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Citation

De Los Rios-Escalante, P., Barra, V., Kretschmer, C., Lozano-Muñoz, I. (2024). Ecotoxicity of Aquaculture Chemotherapy-A Case Study in Chile. *Sustainable Aquatic Research, 3*(3), 169-174.

Article History

Received: 06 July 2024
Received in revised form:18 December2024
Accepted: 18 November 2024
Available online: 30 December 2024

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Keywords

Ecotoxicity
Aquaculture Chemotherapy
Florfenicol

Abstract

Aquaculture has experienced rapid growth in Chile over the past three decades, currently ranking first in America in terms of production. The use of chemotherapy, involving chemical drugs to prevent and treat disease outbreaks, has been widespread in salmon aquaculture for managing fish population diseases. To mitigate the negative environmental impacts of aquaculture chemotherapy, an analysis of the current legal framework governing Environmental Impact Assessments (EIAs) for veterinary medicinal products was conducted, referencing the Chilean regulatory framework and international guidelines. The analysis revealed a need to enhance the Chilean legal framework to align with international standards, thereby facilitating sustainable aquatic production. The study identified a legal framework that permits high ecotoxicity acceptance parameters, resulting in elevated environmental risk due to the use of Florfenicol in seawater-phase aquaculture, as indicated by a risk quotient (RQ) value exceeding 100.

Introduction

Aquaculture is a significant economic activity in Chile, ranking as one of the largest producers and exporters of aquaculture products globally. In 2022, the country produced 758,953 tons of Atlantic salmon, 241,904 tons of Coho salmon, and 73,315 tons of Rainbow Trout (SERNAPESCA 2022). Chile leads aquaculture production in the Americas, contributing 34.21% of the total production, with a total output of 1,505.5 thousand tons of live weight, as reported by the Food and Agriculture Organization (FAO 2024). Although Chilean aquaculture has successfully generated economic growth, concerns persist regarding its environmental impact. In 2023, the industry used 5,840.45 tons of hydrogen peroxide and 5.47 tons of other antiparasitics for 1,076,627.69 tons of harvested biomass, resulting in 51,438.24 tons of dead biomass. This translates to a usage rate of 5,150.84 g/t for hydrogen peroxide and 4.85 g/t for other antiparasitics.

Sixteen antibiotics are used in animal treatments in Chile, compared to three in the United States (US) and four in Norway (Cabello 2003). In the case of aquaculture in Chile, antibiotics have been mainly used in sea water (Lozano et al. 2018). The excessive use of antibiotics in the salmon industry remains a significant issue, with 98% of the total antibiotics used in salmon farming in Chile being applied during the seawater phase. Florfenicol accounted for 98.22% of this amount. In 2023, Chile's total antibiotic usage was 338.9 tons for a total biomass production of 1,107,109 tons (SERNAPESCA 2024).

Chemotherapy has been widely used to prevent and treat disease outbreaks, resulting in multiple adverse effects on the environment and human health (Reverter et al. 2014). The presence of chemotherapy drugs in aquatic ecosystems can have ecotoxicological effects at various biological levels (Li et al. 2021). Ecotoxicological risks refer to the harmful effects that a compound or physical agent can have on both the environment and organisms, including fish, microorganisms, wildlife, and plants. The study of a pharmacological or immunological product's ecotoxicity aims to assess the harmful effects associated with the administration of the product on the environment, estimate the risk, and define the necessary measures to reduce this risk (SAG 2011; Tihulca 2013; CENMA 2014). The VICH guidelines, developed through international cooperation, provide guidance on environmental impact assessments for veterinary products. These guidelines serve as a reference for countries, including Chile, in the regulation of veterinary products.

For the registration of drugs in Chile, an analysis of environmental risk information must be presented to the Chilean Ministry of the Environment of the Agricultural and Livestock Service (SAG), based on the methodology "International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products" (VICH) (SAG 2010; Tihulca 2013; Ministerio de Medio Ambiente, Gobierno de Chile 2015). The aim of this study is to compare the Chilean regulatory framework for environmental impact assessments (EIAs) for veterinary chemical products with the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products guidelines in order to make a contribution to sustainable aquaculture production.

Methods

A comparison was performed between the Chilean regulatory framework for Environmental Impact Assessments (EIAs) of veterinary medicinal products (SAG 2011), and international guidelines (VICH 2000, 2004; EMA 2016). An environmental risk assessment was conducted to evaluate the potential risks associated with the use of Florfenicol in the marine environment, in accordance with VICH 2000 and 2004 guidelines (VICH 2000, 2004).

Results

Environmental Risk Assessment For Florfenicol 50% Oral Powder For Aquatic Use In Chile, In Accordance With Vich (Vich 2000, 2004).

Phase I Environmental Impact Assessment (VICH 2000)

Legislation requires an environmental impact assessment for this drug, as it is not a natural substance and its use does not alter the concentration or distribution in the environment. Florfenicol, an antibiotic of the Phenicoles family, derived from chloramphenicol, acts as a bacteriostatic agent by inhibiting bacterial protein synthesis through reversible binding to the 50S subunit of the ribosome, preventing polypeptide chain elongation (Horsberg et al. 1996). This product will be used in fish intended for human consumption. As there are no environmental impact studies for smaller species treated and raised similarly to larger species, this assessment focuses on the latter. The antibiotic will be used to control disease outbreaks in 100% of susceptible animals. Since the product is not completely metabolized in treated animals, it is a neutral, liposoluble compound that is widely distributed throughout the animal organism, reaching significant concentrations within cells and transcellular fluids 12 hours after treatment initiation. The product is primarily excreted through the feces and urine of treated animals (Horsberg et al. 1996). As the product will be used to treat aquatic species, releasing it into the aquatic environment, and there is no waste

disposal matrix in place, the calculation of the risk coefficient (QR) must be performed. The initial environmental concentration (EIC Aquatic) of the product released from aquaculture facilities is not less than 1 mcg/l, and there is no method or technology to reduce its environmental release.

Phase II Environmental Impact Assessment (VICH 2004)

Florfenicol, a widely utilized antibiotic in aquaculture, exhibits bacteriostatic activity against both gram-positive and gram-negative pathogens, including *Aeromonas*, *Vibrio*, *Yersinia*, *Flavobacterium*, and *Photobacterium* (Horsberg et al. 1996). Following intestinal absorption, Florfenicol is distributed to tissues, with peak concentrations detected in muscle tissue at 12 hours post-treatment, corresponding to the Minimum Inhibitory Concentrations (MIC). The antibiotic is subsequently excreted in urine and feces. Both Florfenicol and its metabolites can enter marine sediment and water columns through leaching of unconsumed food and excreta from treated animals. With a molecular weight of 358.21 Daltons, a water solubility of 1.32 g/L at pH 7, and a partition coefficient of 0.37, Florfenicol has a low bioaccumulation potential and a half-life of 4.5 days in marine sediment (Hektoen et al. 1995).

To evaluate the potential environmental risks associated with this product, an experimental model is employed, utilizing various species across different trophic levels. However, this model does not incorporate data from marine environment species in southern Chile (No data from marine environment species in southern Chile were found).

Formulas and abbreviations (VICH 2004)

Predicted Environmental Concentration (PEC) = (Total Florfenicol / mg /day) / total liters per farm
Predicted No Effect Concentration (PNEC)= LC50 o EC50 o NOEC / AF
Risk Quotient (RQ) = PEC/PNEC
LC50 = represents the Lethal Concentration 50, a standardized measure of the toxicity of a surrounding medium that is expected to cause the death of 50 percent of a sample population of a specific test animal within a specified exposure period.
EC50 = denotes the concentration of an agonist necessary to elicit a response halfway between the baseline and maximum response.
NOEC= The No Observed Effect Concentration, is the concentration at which no adverse effect is observed.
AF= Assessment Factors.

PEC Calculation involves the determination of the Predicted Environmental Concentration (Table 1), using average data for a salmon farming center in Chile (SERNAPESCA 2012) for further calculation of Predicted No Effect Concentration (PNEC) and risk quotient (RQ) (Table 2.)

Table 1. PEC Calculation (VICH 2004)

Average data for a salmon farming center in Chile (SERNAPESCA 2012)	**Values**
Standard cage dimensions (m^2)	30 x 30
Depth (m)	15
Cage volume (m^3)	13.500
Nº cages per center	20
Total volume per center (m^3)	270.000
Liters per (m3)	1.000
Total liters per center	270.000.000
Atlantic salmon density (kg/m3)	17
Average harvest weight (kg)	4
Kg of salmon per center	4.590.000
Florfenicol dose (mg/kg/day) (Horsberg et al. 1996)	10
Days of treatment (Horsberg et al. 1996)	10
Florfenicol total amount /mg/day	45.900.000
PEC=	1.67

Table 2. Predicted No Effect Concentration (PNEC) and Risk Quotient (RQ) Calculation

Type	Species	LC50 (mg/l)	Exposition time	AF	PNEC	PEC	RQ	Reference LC50
Fish	*Hyphessobrycon eques*	>100	48 h	100	>1	0,17	**<0,17**	(Carraschi et al. 2015)
	Piaractus mesopotamicus	>100	48 h	100	>1	0,17	**<0,17**	(Carraschi et al. 2015)
Snail	*Pomacea canaliculata*	>100	48 h	100	>1	0,17	**<0,17**	(Carraschi et al. 2015)
Aquatic plant	*Lemna minor*	97,03	7 days	100	>1	0,17	**<0,17**	(Carraschi et al. 2015)
Crustacean planktonic	*Daphnia magna*	>100	48 h	100	0,97	0,17	**0,18**	(Carraschi et al. 2015)
Crustacean planktonic	*Daphnia magna*	1,9	21 days	100	0,02	0,17	**8,95**	(Martins et al. 2013)
Phytoplankton (Unicelular algae)	*Skeletonema costatum*	5,93	96 h	100	0,06	0,17	**2,87**	(Liu et al. 2012)
Phytoplankton (Unicellular algae)	*Tetraselmis chui*	1,03	96 h	100	0,01	0,17	**13,08**	(Lai et al. 2009)

The Risk Quotient (RQ) is evaluated against a value of one. If the value is less than one, further testing is not recommended. However, an RQ of less than one may not necessarily imply that the risk is acceptable, as this determination must be made on a scientific basis. Metabolites excreted in quantities of 10% or more of the administered dose, which do not participate in biochemical pathways, should be added to the active substance to permit recalculation of the Predicted Concentration (PC), as stated by the European Medicines Agency in 2016 (EMA 2016). In contrast, Chile's regulations for RQ classification diverge from international standards. According to the Servicio Agrícola y Ganadero (SAG) 2010 regulation (SAG 2010), pharmaceutical products for veterinary use are considered environmentally safe when the national ecotoxicity evaluation indicates an RQ Risk Coefficient of 100 or less. If the RQ falls between 100 and 1,000, the drug registrant must propose environmental monitoring.

The Risk Quotient results from the analysis (Table 2) are exceeded for crustacean planktonic and phytoplankton (Unicelular algae) evaluated against a value less than one , when evaluating against a value of 100 (SAG 2010) the values are not exceeded .

Conclusions

Differences exist between Chile's regulatory framework for Environmental Impact Assessments of veterinary medicinal products (SAG 2011) and international guidelines, and these discrepancies should be addressed. In Chile, pharmaceutical products exclusively for veterinary use are deemed environmentally safe when the ecotoxicity assessment result under national conditions indicates a Risk Quotient (RQ) of 100 or less (Exempt Resolution No. 665, SAG), whereas the Environmental Impact Assessment for Veterinary Medicinal Products Phase II VICH Guidance compares the RQ to a value of one, and values less than one indicate no further testing is required.

The ecotoxicity analyses approved by the SAG for registered products must be based on scientific evidence. It is essential that Chile conducts these studies incorporating habitat species from national salmon farming areas, preferably endemic species associated with sea farming, and considers chronic toxicity studies in invertebrates of Chile's salmon farming marine sediment, incorporating NOEC parameters (SAG 2011; Tihulca 2013), given that the Risk Quotient results from the analysis exceed one (*Tetraselmis chui*, *Skeletonema costatum*, and *Daphnia magna*).

Ethical approval

The authors declare that this study complies with research and publication ethics.

Informed Consent

Not Applicable

Conflict of interest

There is no conflict of interests for publishing this study.

Data availability statement

The authors declare that the data from this study are available upon request.

Funding organizations

No funding available

Author contributions

Patricio de Los Rios-Escalante: Conceptualization, Editing. Veronica Barra: Editing. Cristina Kretschmer: Conceptualization, Analyses, Writing, Editing. Ivonne Lozano-Muñoz: Conceptualization, Analyses, Editing.

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