Journal of the National Aquatic Resources Research & Development Agency of Sri Lanka

National Aquatic Resources Research
and Development Agency

Volume 51-52
2023

Journal of the National Aquatic Resources Research and Development Agency of Sri Lanka

PUBLICATION

One volume is published annually in December by the National Aquatic Resources Research and Development Agency of Sri Lanka.

SCOPE OF THE JOURNAL

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JOURNAL OF THE NATIONAL AQUATIC RESOURCES RESEARCH AND DEVELOPMENT AGENCY OF SRI LANKA

Volume 51-52 (2023)

National Aquatic Resources Research and Development Agency (NARA)

Crow Island, Colombo 15
Sri Lanka

ISSN – 1391 – 6246

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Spatial variations in the length – weight relationship and Fulton's condition factor of two commercially important fish species: *Lethrinus olivaceus* (Valenciennes, 1830) (family Lethrinidae) and *Lutjanus lutjanus* (Bloch, 1790) (family Lutijanidae), in Sri Lankan waters

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Abstract

Fulton's condition factor (*K*) and length-weight relationship (LWR) are widely used in fisheries biology for comparing the condition, fatness, well-being of fish and to determine the growth characteristics. The present study aimed to understand the spatial variation in the LWRs and the *K* of *Lethrinus olivaceus* (Valenciennes, 1830) and *Lutjanus lutjanus* (Bloch, 1790) in Sri Lankan waters. The samples of *L. olivaceus* (n=288) and *L. lutjanus* (n=181) were collected from the ecosystem survey conducted in Sri Lankan coastal waters by R/V Dr Fridtjof Nansen from 24thJune to 16thJuly, 2018. The LWRs and *K* were estimated for populations of two species in six different regions. The estimated LWRs for *L. olivaceus* populations in the Northeast, Centraleast, Southeast, South, Southwest and Northwest regions were $W=0.02L^{2.87}$, $W=0.02L^{2.88}$, $W=0.02L^{2.85}$, $W=0.01L^{3.23}$, $W=0.01L^{2.87}$ and $W=0.02L^{2.97}$ respectively. The estimated LWRs for *L. lutjanus* populations in Southeast, South, Southwest regions were $W=0.02L^{2.84}$, $W=0.02L^{2.89}$ and $W=0.02L^{2.97}$ respectively. The mean *K* values of the *L. olivaceus* populations in above regions were 1.39 ± 0.14, 1.32 ± 0.10, 1.31 ± 0.17, 1.32 ± 0.09, 1.24 ± 0.06 and 1.25 ± 0.15 respectively. The mean *K* values of the *L. lutjanus* populations in above regions were 1.57 ± 0.13, 1.43 ± 0.15 and 1.50 ± 0.18 respectively. The *L. olivaceus* populations in Northeast, Centraleast, Southeast regions and all *L. lutjanus* populations exhibited negative allometric growth. *L. olivaceus* populations in Southern region exhibited positive allometric growth while populations in Southwest and Northwest region exhibited isometric growth. The *L. olivaceus* populations in Centraleast, Northeast regions and *L. lutjanus* populations in Southeast regions likely having better condition/well-being.

Key words: Isometric growth, allometric growth, well-being, long face emperor, bigeye snapper

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Introduction

The status of the fish stocks is largely dependent on some of their biological parameters such as length and weight (Hampton, 2000; Fromentin and Fonteneau, 2001).The Length-Weight Relationship (LWR) and different condition factors are important biological parameters to assess the condition of the fish stocks (Bagenal and Tesch, 1978; Hossain, 2010).

The importance of determining LWRs in fish has been emphasized by many studies. LWRs provide information about the growth pattern, general health, habitat conditions, life history, fish fatness and condition, as well as morphological characteristics of the fish (Schneider *et al*., 2000; Froese, 2006). Length and weight measurements together with age data can give information on the stock composition, size at maturity, life span, mortality, growth, and production (Diaz *et al*.,2000; Froese,2006). Furthermore, LWRs are used for deriving the weight from length as direct weight measurements can be difficult and time-consuming in the field (Fafioye and Ayodele, 2018; Mehanna and Farouk, 2021). Apart from this, the LWRs can also be used for deriving comparisons between different stages in life history and between fish populations from regions or habitat groups (Petrakis and Stergiou, 1995; Gonçalves *et al*., 1997). LWRs are expressed in a formula, which allows the estimation of the fish weight (W) using a particular length (L). Values of the exponent '*b*' of that equation provide information on fish growth. When $b = 3$, isometric growth i.e., growth of all body parts at equal rates, is assumed. When the value of '*b*'significantly different from 3, growth is allometric (positive if $b > 3$, negative if $b < 3$) (Froese, 2006; Nehemia *et al*., 2012).

Success of a fish population in a habitat can also be estimated through different condition factors (Richter, 2007). The condition factor of fish has been shown to reflect information on the physiological state of the fish in relation to its welfare (Ighwela *et al*., 2011). It also gives information when comparing two populations living in certain feeding, density, climate, and other conditions; when determining the period of gonadal maturation and when following up the degree of feeding activity of a species to verify whether it is making good use of its feeding source (Wootton, 1990; Ndiaye *et al*., 2015). Among the various versions of the condition factor, the Fulton's condition factor (*K*) is widely used in fish biology and fisheries. It is based on the principle that individuals of a given length, exhibiting higher weight, are in a better condition. It is used for comparing the condition, fatness, or well-being of fish (De Giosa *et al*., 2014). This factor is calculated from the relationship between the weight of a fish and its length, with the intention of describing the "condition" of that individual (Froese, 2006).

The coastal fishery sector in Sri Lanka plays a significant role in the country's economy as it contributes about 48% to the total annual fish production (Ministry of Fisheries, 2020). In the coastal fishery, demersal fish are of major interest both for local consumption and for export market (Ministry of Fisheries and Aquatic Resources, 2020). Species of family Lethrinidae (emperor fish) and family Lutijanidae (snappers) are two of the dominant groups in the demersal fish catch (Maldeniya, 2011; Dalpathadu, 2020). Despite the importance in coastal fisheries, some of the fundamental biological information such as LWRs and condition factor are limited for the fish species of family Lethrinidae and family Lutijanidae in Sri Lankan waters. This study was conducted with the aim of estimating and spatial comparison of the LWRs and Fulton's condition factor (*K*) for different populations of *Lethrinus olivaceus* (Valenciennes, 1830) (family Lethrinidae) and *Lutjanus lutjanus* (Bloch, 1790) (family Lutijanidae) in Sri Lankan waters.

Materials and Methods

The samples were collected from the ecosystem survey conducted in Sri Lankan coastal waters by R/V Dr Fridtjof Nansen from 24th June to 16thJuly, 2018. The territorial waters of Sri Lankan were divided in to six regions: Northwest, Southwest, South, Southeast, Centraleast and Northeast (Figure 1). A total of 288 specimens of *L. olivaceus* and 181 specimens of *L. lutjanus* were recorded during the survey (Table 1). The biological samples for the study were obtained from the ''Super Gisund'' bottom trawl attached to the research vessel. Species identification was done on-board using Allen (1985), De Bruin *et al*. (1994) and Munro (2000). The total length (TL) (to the nearest 1.0 cm) and total weight (TW) (to the nearest 0. 5 g) of each specimen were measured on-board by respectively the electronic measuring board and the electronic balance mounted in the laboratory of the research vessel.

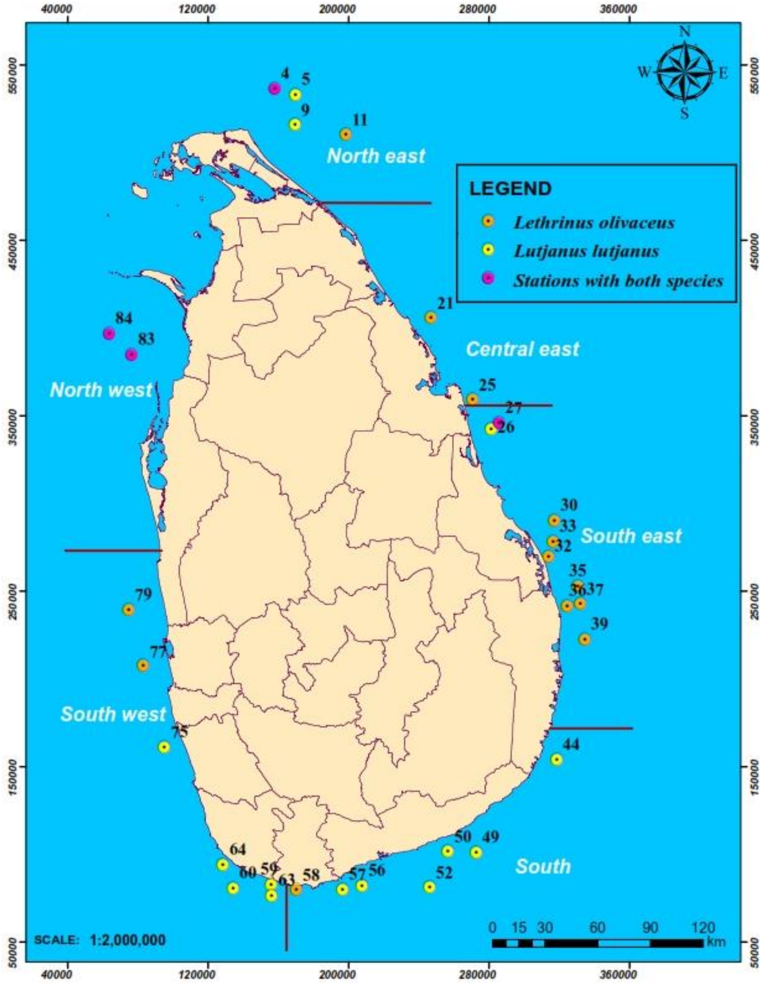

Fig.1. Six regions in Sri Lankan waters and the sampling stations where *L. olivaceus* (LO) and *L. lutjanus* (LL) recorded during the RV Dr Fridtjof Nansen Survey Programme 2018, Leg 3.1. (Regenerated after Krakstad *et al*., 2018).

Table 1. The summery of sample collection of *L. olivaceus* and *L. lutjanus* and average towing time in Sri Lankan waters during the survey.

Region		**North East**	**Central East**	**South East**	**South**	**South West**	**North West**
Species (n)	*L. olivaceus*	45	99	117	11	11	05
	L. lutjanus	_	_	62	89	30	_
Average towing time (min)± SD		30.05	39.08 ± 14.02	26.24 ± 4.89	24.00 ± 3.97	26.55 ± 3.08	22.97 ± 1.70

The LWRs were estimated from the following formula (Le Cren, 1951),

$W = aL^b$ --------------1

where W is total body weight (g), L is the total length (cm), *a* and *b* are the regression coefficients between W and L (Beckman, 1948; Ricker, 1973). The values of constants *a* and *b* were estimated by the least-square linear regression from the log-transformed values of length and weight (Zar, 1984; Fafioye and Ayodele, 2018; Mehanna and Farouk, 2021) given below.

$log\, W = log\, a + \ b \log L$ --------------2

To confirm whether the values of *b* obtained in the linear regressions were significantly different from the isometric value (i.e.,*b* = 3), the confidence interval (CI) at 95% was estimated (Bagenal and Tesch, 1978). In addition, Student's t-test (Zar, 1984) was used to see if parameter *b* is significantly different from 3 and to identify the type of growth.
The Fulton's condition factor (*K*) which shows the degree of well-being of the fish in their habitat was determined for the populations in each region by using the Equation 3 (Htun-Han, 1978);

$K = 100W/L^3$ -----------------3

where W = the weight of the fish (g) and L = the total length of the fish (cm). Non-parametric Kruskal-Wallis test (*H*) was used to determine the significance of the resulted *K* values of populations in different regions. Post-hoc comparisons were conducted using Wilcoxon rank Tests and Benjamini-Hochberg test with adjusted *p* value. In order to analyse the data and prepare the graphs and charts, Microsoft office 2013 Excel, R studio (version 4.1.3), and SPSS 20.0 software packages were used.

Results

According to the results of the LWRs (Table 2) all the *b* values were significantly different ($p < 0.01$) from the value for the isometric growth of 3, except for the *L. olivaceus* populations thrived in the South west ($t_{(9)} = -1.05$, $p = 0.32$) and North west regions ($t_{(3)} = -1.99$, $p = 0.14$). Accordingly, all the populations of *L. lutjanus*, and the populations of *L. olivaceus* in the Northeast, Centraleast and Southeast regions exhibited a negative allometric growth pattern. The *L. olivaceus* populations in the Southwest and the Northwest regions exhibited an Isometric growth pattern while *L. olivaceus* population in the South region exhibited a positive allometric growth at least during the study period.

Table 2. Biometrics, LWRs, Growth pattern and *K* of *L. olivaceus* and *L. Lutjanus* in six different regions of Sri Lankan waters.

Species	**Region**	**Max TL (cm)**	**Min TL (cm)**	**Mean TL±SD (cm)**	**Max TW (g)**	**Min TW (g)**	**Mean TW±SD (g)**	**LWRs**	**Growth pattern**	***K*±SD**
L. olivaceus	Northeast	45.0	10.0	26.81 ± 6.25	1060.0	10.0	297.56 ± 177.64	$W=0.02L^{2.87}$	-A	1.39 ± 0.14
	Centraleast	65.0	17.5	28.95 ± 9.11	3840.0	84.0	418.15 ± 640.39	$W=0.02L^{2.88}$	-A	1.32 ± 0.10
	Southeast	82.0	15.0	32.09 ± 14.68	6700.0	51.0	694.67 ± 1142.82	$W=0.02L^{2.85}$	-A	1.31 ± 0.17
	South	75.0	51.0	67.18 ± 8.00	6310.0	1710.0	4190.91 ± 1450.38	$W=0.01L^{3.23}$	+A	1.32 ± 0.09
	Southwest	58.0	34.5	46.00 ± 9.31	2500.0	530.0	1339.09 ±759.16	$W=0.01L^{2.87}$	A	1.24 ± 0.06
	Northwest	54.0	25.5	38.30 ± 12.29	1891.0	240.0	854.20 ± 709.93	$W=0.02L^{2.97}$	A	1.25 ± 0.15
L. lutjanus	Southeast	22.0	13.0	15.81 ± 1.72	170.0	40.0	64.03 ± 23.92	$W=0.02L^{2.84}$	-A	1.57 ± 0.13
	South	26.0	13.5	17.56 ± 3.00	224.0	34.0	83.43 ± 43.55	$W=0.02L^{2.89}$	-A	1.43 ± 0.15
	Southwest	30.0	22.0	26.42 ± 2.35	420.0	150.0	283.20 ± 77.17	$W=0.02L^{2.97}$	-A	1.50 ± 0.18

TL – Total Length; TW – Total Weight; SD – Standard deviation; *K* – Fulton's condition factor

The scatter plots between $\log_{10}$TW and $\log_{10}$TL of the LWRs of all populations (Figures 2, 3) showed that there was a strong positive linear relationship between the two parameters, which was confirmed with a coefficient of determination (r^2) of greater than 0.80.

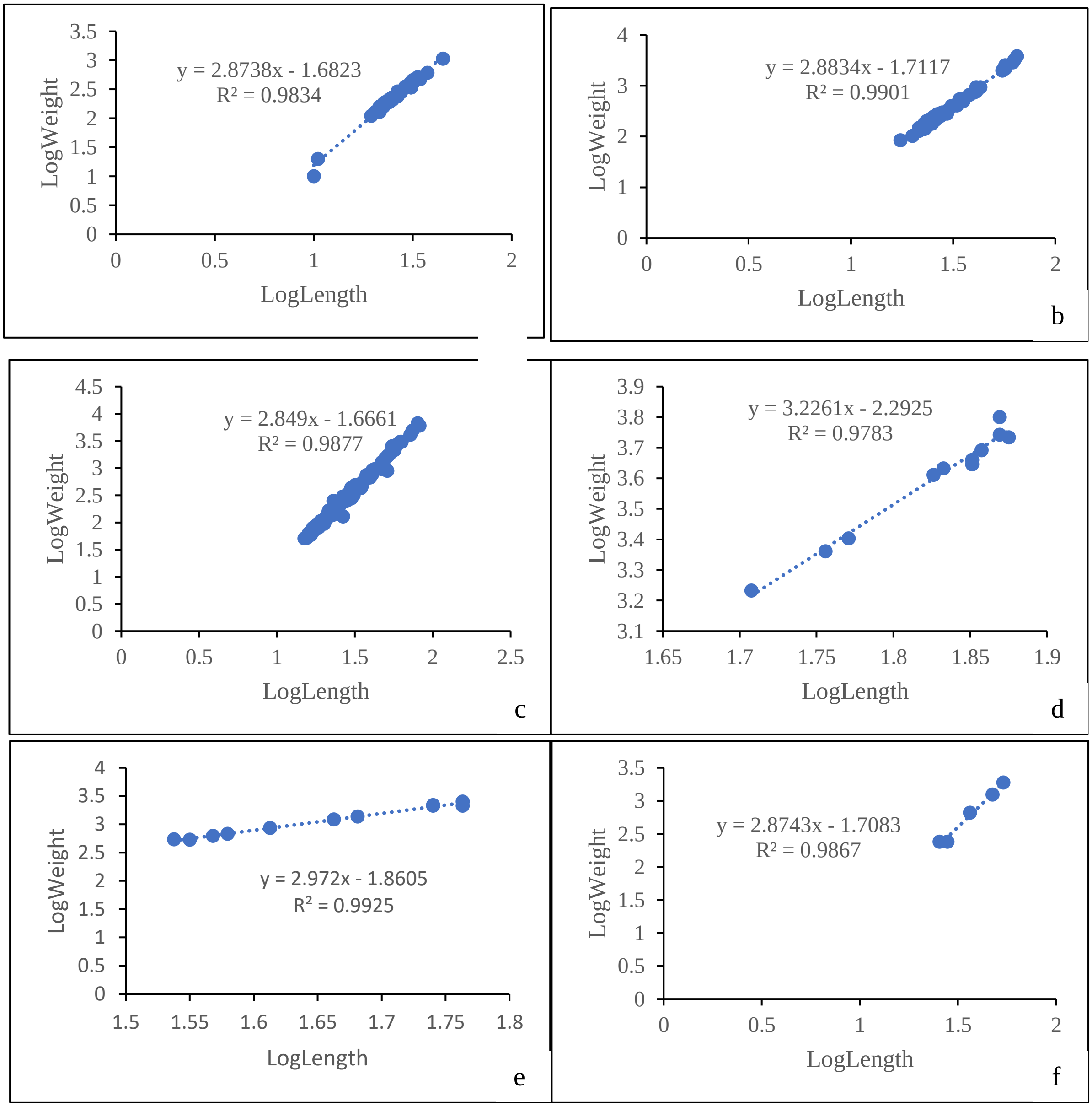

Fig. 2. The graphs of linear regressions obtained from the log- transformed values of total length and total weight for *L. olivaceus* in (a) Northeast region; (b) Central east region; (c) Southeast region; (d) South region; (e) Southwest region; (f) Northwest region of Sri Lanka

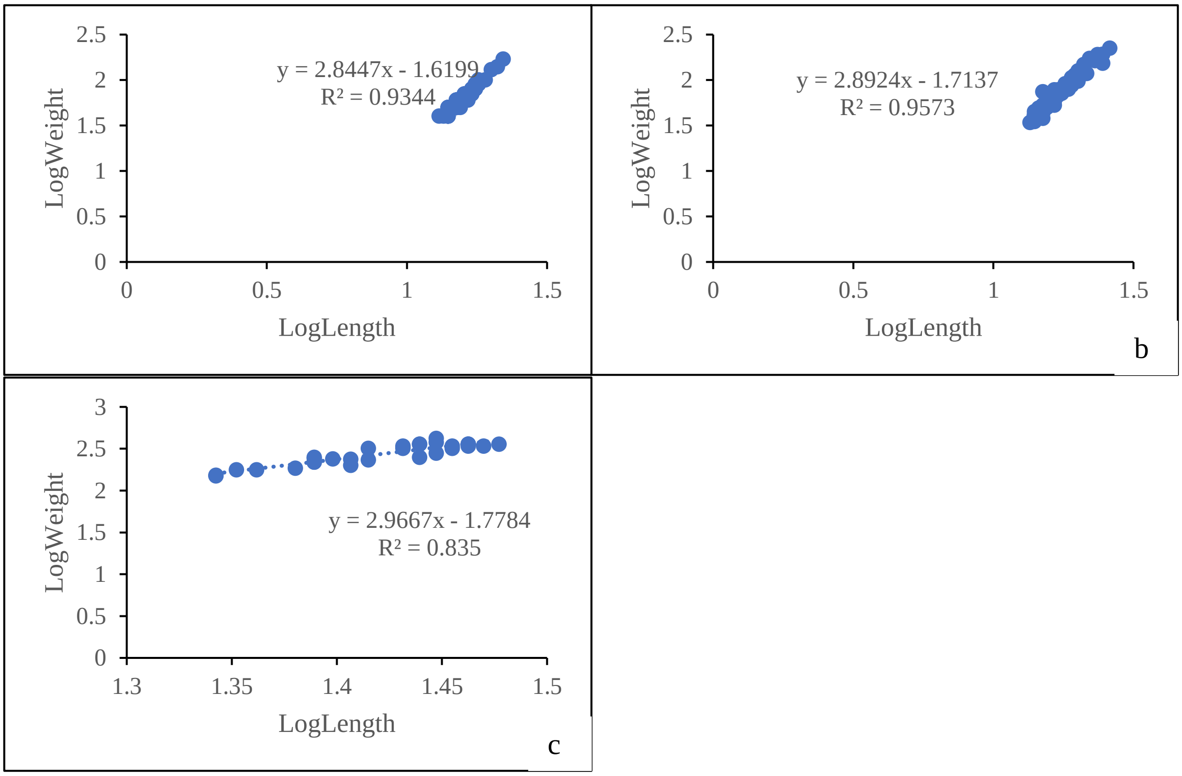

Fig. 3. The graphs of linear regression obtained from the log- transformed values of total length and total weight for *L. lutjanus* in (a) Southeast region; (b) South region; (c) Southwest region of Sri Lanka

There was a significant difference among the mean *K* values of six populations of *L. olivaceus* in Sri Lankan waters ($H_{(5)} = 25.19$, $p< 0.01$). A significant difference of mean *K* values were obtained for the *L. olivaceus* populations between the Southwest – Centraleast ($p = 0.02$), Southwest – Northeast ($p = 0.01$), Northeast – Centraleast ($p = 0.01$) and Northeast – Southeast ($p = 0.01$) regions. The mean *K* values between the populations in other region pairs were not significantly different (Table 3).

Table 3. Statistical comparison (*p* values) of the mean *K* values of six populations of *L. olivaceus* in Sri Lankan waters (significant *p* values are marked with * mark).

Region	Centraleast	Northwest	Northeast	South	Southeast
Northwest	0.3861	-	-	-	-
Northeast	0.0110*	0.1857	-	-	-
South	0.8220	0.3861	0.1247	-	-
Southeast	0.3861	0.4397	0.0073*	0.8789	-
Southwest	0.0224*	0.9130	0.0054*	0.0842	0.1247

Considering the *L. lutjanus* populations in the Sri Lankan waters, a significant difference among the mean *K* values of three populations was obtained ($H_{(2)} = 35.14$, $p < 0.01$). The mean *K* of population in the South region was significantly different than the population in the South east region ($p < 0.01$) while the differences in mean *K* was not significant for other populations (Table 4).

Table 4. Statistical comparison (*p* values) of the mean *K* values of three populations of *L. lutjanus* in Sri Lankan waters (significant *p* values are marked with * mark).

Region	South	Southeast
Southeast	<0.01*	-
Southwest	0.064	0.062

Discussion

The present study has demonstrated the spatial variations in the Fulton's condition factor (*K*), growth pattern and the length-weight relationship of the commercially important two fish species: *L. olivaceus* and *L. lutjanus* in Sri Lankan waters. The *b* values of both species in all studied regions in Sri Lankan waters were within the expected range of $2.5 < b < 3.5$, suggesting that the results of this study are valid

(Froese, 2006). The estimated *a* and *b* values of the LWR and the growth pattern for *L. olivaceus* in this study were in agreement with other studies conducted by Matthews *et al*. (2019); Kamikawa *et al*. (2015) and Ontomwa *et al*. 2018. Considering *L. lutjanus*, the estimated values were in agreement with other studies conducted by Yamagawa (1994) and Letourneur *et al*. (1998).

The *K* is an indicator of the healthy status of the fish over time and hence acts as an index reflecting interactions between biotic and abiotic factors in the physiological condition of fishes (Lizama *et al*., 2002; Tesfaye and Tadesse, 2008).The significantly higher *K* values of the populations of *L. olivaceus* in the Central east and North east regions while *L. lutjanus* population in the South east regions might attributed to the better environmental conditions which could enhanced the well-being of the populations of the two species in those regions (Le Cren, 1951; Ontomwa *et al*., 2018).Some studies have proven that the *K* could be lower in the larger/older fishes of the same species due to some health problems and increasing sensitivity to ambient surroundings (Percin and Akyol, 2009; Jin *et al*., 2015). Considering the mean TL and the mean TW of the populations with significant *K* values, relatively smaller sized fish could be identified in the samples (Table 2). Thus, the availability of high proportion of smaller/younger fish in the sample might be another possible reason for resulting a significantly higher *K* values for the population of *L. olivaceus* in the Central east and North east regions while *L. lutjanus* population in the South east regions. However, it should be noted that some other factors such as fullness of the stomach (Percin and Akyol, 2009), food supply and parasitism (Le Cren, 1951) may also affect the *K* of fish populations.

The parameters of LWRs indicate the allometric growth as well as isometric growth of the *L. olivaceus* populations while only allometric growth for the *L. lutjanus* in the studied regions (Table 2). Tesch (1971) stated that the difference in the growth behaviours of a fish species might be attributed to the observed length ranges of the specimens considered. As the mean TL of the populations of each species differed from each other, the differences in the TL of the specimens may be the reason of having different growth patterns for the *L. olivaceus* and *L. lutjanus* populations in the spatial scale. Furthermore, the variations in the '*b*' of the LWRs of a fish species within a season could be attributed to the combination of one or several factors such as fish physiology, growth phase, sex, sexual maturity, stomach fullness, sampling size, habitat, feeding rate, diet, and health (Le Cren, 1951; Wooten, 1998; Froese *et al*., 2011; Mondol *et al*., 2017).

Conclusion

The population of *L. olivaceus* in the Central east and North east regions and *L. lutjanus* populations in the South east regions likely having better condition/well-being than their counterparts who lived in other regions in Sri Lankan waters. The populations of *L. olivaceus* exhibited all three growth patterns; negative allometric, positive allometric and isometric growth while all the *L. lutjanus* populations exhibited only negative allometric growth in same sampling period in Sri Lankan waters. The study attempted to address the issues of lacking the basic parameters of *L. olivaceus* and *L. lutjanus* in different regions in Sri Lankan waters and growth phases over those regions. The findings of the study related to *L. olivaceus* and *L. lutjanus* would be beneficial for fishery biologists and conservationists to impose adequate regulations for sustainable fishery management and conservation of biodiversity in respective regions. Although basic information on LWRs and *K* values are provided, the reasons behind the variations of such parameter values require further studies; the explicit relationship between morphological characteristics and environmental changes is yet to be revealed.

Acknowledgements

This research was supported by the EAF Nansen program of the Food and Agriculture Organization (FAO), the Norwegian Agency for Development Cooperation (NORAD) and the government of Sri Lanka. We greatly appreciate the support given by the National Aquatic Resources Research and Development Agency (NARA), Sri Lanka and the Institute of Marine Research (IMR), Norway.

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An efficient invitro plant regeneration of *Cryptocoryne wendtii* through shoot tip

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Abstract
An efficient protocol was developed for the micro propagation of *Cryptocoryne wendtii* an endemic threatened aquatic plant in Sri Lanka. Shoot tips of *C. wendtii* were established in Murashige and Skoog (MS) medium with 3% sucrose with 2 mg/L 6-benzyle aminopurine (BAP) and Indole Butyric Acid (IBA). Regenerated shoots were cultured on MS medium supplemented with different combinations of BAP, Naphthalene Acetic Acid (NAA) with IBA for multiple shoot generation. The control medium without growth hormones did not show any shoot generation. Most of the combinations showed the multiple shoots. However, the highest mean number of shoots 10.00±1.15 was showed in MS medium supplemented with 4 mg/L BAP and 1.0 mg/L IBA. The mean of the root length per plant was the highest in the MS liquid medium with 0.5 mg/L IBA (2.33±0.17 cm). Hundred percent survival of the plantlets observed during hardening. Maximum mean leaf length was observed in the hydroponic medium nutritious with 0.5 g/L Albert's Solution.

Keywords: *Cryptocoryne wendtii*, growth hormones, micropropagation, shoot tip culture

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Introduction

Cryptocoryne wendtii which belongs to family Araceae, is one of the most common and widely used aquatic plants in aquaria and water gardens. *Cryptocoryne* spp. mainly occurs in the South-western lowland ever-green rain forests, central midlands, and central western lowlands in the semi-deciduous monsoon forests having a seasonal change in precipitation and few are scattered in the dry zone riverine forests. Many of these species are restricted to the Kalutara and Ratnapura Districts in the low wetlands and the banks of the Mahaweli River in Gannoruwa-Hallolluwa area, Kandy (IUCN Red List, 2012). These species mostly thrive in slow running water or seasonally inundated soils. They occur both submerged or emerged depending on the growth stage, vegetative or reproductive.

This genus consists of more than 50 species in South East Asia. In Sri Lanka, ten endemic species are recorded, all of which are listed in the IUCN Red list; five *Cryptocoryn*e species are placed under the Critically Endangered (CE) category, three species are under Endangered (E) category and two are under vulnerable category. *Cryptocoryne* plants show polyploidy. *C. wendtii* plants show two chromosome numbers (2n), which consist 28, 36 or 42 chromosome numbers. The triploid plants were showing more variations (chromosome 42) than the diploid plants (Chromosome 28) (Jacobsen, 1976). The triploid plants are sterile and could not produce seeds; hence, the only way for the propagation is vegetative propagation (Dissanayake *et al*., 2007).

C. wendtii is very popular among the fresh water aquaculture and aqua scaping industry (Dissanayake *et al*., 2007). It is one of the easiest plants to grow and cultivate; and also could survive for longer periods (Wijesundara & Shatha Siri, 2004). *C. wendtii* can tolerate low or high light, and seems to respond with longer foliage in lower light conditions. The plant only requires stable conditions and sometime to adjust after being introduced into a new setting. Most important character of these species are the range of colors in the leaves such as reddish, reddish-brown, reddish-brown marble leaf coloration to the aquarium, hence these plants are popular in aqua scaping industry (Wijesundara & Siri, 2004).

Introduction of tissue culture to produce these valuable aquatic plants holds several advantages to the industry. It will provide mass production of good quality plants without pest or disease problems at a competitive price for the export market, while conserving aquatic plants in their natural habitats (Yapabandara & Ranasinghe, 2007). Production cycles can be planned according to the demand, without the effect of climatic and environmental condition. It is another big advantage in tissue culturing these plants. Thus, development of a proper protocol for the production of *C. wendtii* will help to supply continuous and mass production of this plant to cater the demand in the industry.

Materials and Methods

Materials

Basal shoot tip explants (2-5 mm long) of *C. wendtii* were taken from mother plants grown in plant house at National Aquatic Resources Research and Development Agency (NARA). Mother plants were cultured in hydroponic system.

Sterilization of explant

Explants were first thoroughly washed with liquid soap and kept under running tap water for one day. After that they were washed with 70% ethanol for one minute. Then different concentrations of Sodium Hypochlorite solutions 20% - 4% were applied with one drop of tween twenty per 100 mL. Exposure times used ranged from 5 to 15 minutes. There were five treatments with a control as 70% Ethanol (C), 20% Clorox (T1), 15% Clorox (T2), 10% Clorox (T3), 8% Clorox (T4) and Clorox 4% (T5). Each treatment had three replicates (10 samples/replicate). After sterilization procedure, all explants were thoroughly washed with sterilized distilled water for three times for three minutes. Washed explants were cultured in solid Murashige and Skoog medium (MS) for 60 days. Numbers of survived explants were counted and the percentage survival of plants was taken as a parameter to assess its effectiveness.

Explant culture

Sterilized explants were cultured in MS Medium with 3% Sucrose with 0.8% Agar. pH of the medium ranged from 5.6 to 5.8. Different concentration of 6-benzyleaminipurine (BAP), Naphthalene Acetic Acid (NAA) and Indole Butyric Acid (IBA) used as Growth promoting Hormones. Treatments comprised of hormone concentrations: BAP (1 mg/L-10 mg/L), NAA (1 mg/L-10 mg/L) and IBA (0.5 mg/L and 1 mg/L). Growth hormones were not added to the Control. In total, 24 types of treatments were used and each treatment had five replicates (10 sample / replicate). Samples were cultured *in vitro* with 16:8 light and dark hours condition for 60 days. Initiated shoots were counted and average percentage of shoot initiation was used as a parameter to assess surface sterilization.

Initiated shoots were subculture in MS Medium with different concentrations of BAP, NAA and IBA hormones. BAP (6 mg/L-1 mg/L), NAA (4 mg/L-1 mg/L), IBA (1 mg/L-0.5 mg/L). Control was free from growth hormones. There were 24 treatments and each treatment had 30 samples. Samples were cultured *in vitro* with 16:8 light and dark hours condition for 60 days.

Rooting

After shoot multiplication, shoots were cultured in three different culture Media with 2 mg/L IBA, 1 mg/L IBA or 0.5 mg/L IBA. MS medium with either 0.8% agar, 0.4% agar without agar were used as different culture media. There were nine treatments. Controls were free from growth hormones. Each treatment had five replicates (one replicate has 10 samples). Samples were cultured *in vitro* with 16:8 light and dark hours condition for 60 days. Length of roots was measured and average length of roots was selected to assess the growth.

Acclimatization

Plantlet's roots were washed with hot water to remove agar and transferred to a plant house at NARA. Plantlets were cultured in a hydroponic system with different concentrations of Albert's solutions. Control was free from fertilizers. T1 consisted of 1.5 g/L Albert's solution, T2 1.0 g/L Albert's solution, T3 0.5 g/ L Albert's solution and T4 0.25 g/L. The number of leaves and leaf length of the selected leaves were measured for a period of one month.

Data Analysis

The cultures were arranged in a completely randomized manner and the experiments were conducted as three trials. One Way ANOVA was used to analysis of sterilization procedure, shoot initiation, multiplication and hardening treatments. Rooting treatments were analyzed by using Two Way ANOVA (SPSS 16 Software).

Results and Discussion

In this study, the treatment five (T5) showed the highest percentage of survival among the sterilization treatments (90.0±2.9% survival (Fig 1.)). Thus, it can be concluded that the best results obtained by sterilization using treatment 5 (10% Clorox for 5 minutes, 8% Clorox for 10 minutes and 4% Clorox for 15 minutes). Kane *et al.,* (1999) also mentioned that ethanol and Sodium Hypochlorite can be used for the surface sterilization of *C. wendtii*. Obtaining explants free of contamination was very difficult considering aquatic plants. Because aquatic plants grow submerged in water, and their wet surfaces highly colonized with microbes. In warm tropical environment a greater amount of microorganisms are available in the aquatic environment than in temperate conditions. The explant of this species, presence with an uneven and hairy surface, which prevents the proper contact of explant surface with the disinfectant. Therefore, microorganisms very easily could retain there and enter into the media leading to contamination.

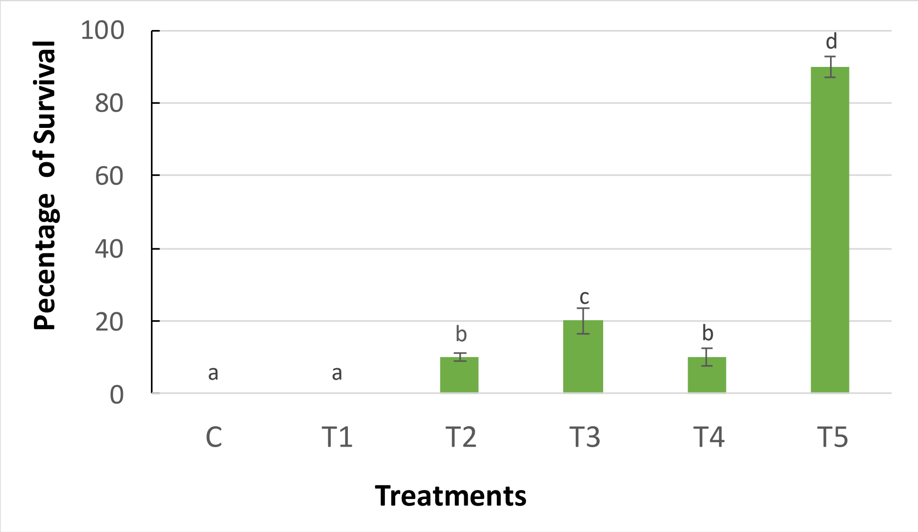

Fig. 1. The Percentage of survival of explants of *C. wendtii* by using different disinfectants for surface sterilization. Errors bars are standard error of mean.
C-Control (with 70% ethanol); T1 (20% Clorox), T2 (15% Clorox), T3 (10% Clorox), T4 (8% Clorox), T5 (4% Clorox) Different lowercase letters show significant differences between treatments.

Dissanayke *et al*., (2007) and Stanly *et al*., (2010) conducted research to determine a surface sterilization procedure for *C. wendtii*. Both of these studies showed that treating with ethanol followed by Sodium Hypochlorite was not sufficient to obtain best survival rate. Instead, they used 0.1% Mercuric Chloride ($HgCl_2$) to gain high survival rate. However, in our experiment, 90.0±2.9% survival was obtained without using $HgCl_2$. Developing a procedure without $HgCl_2$ is a good initiative as disposing this hazardous chemical into the environment is quite problematic.

Shoot initiation and multiplication in *C. wendtii*

In this study, various combinations of auxins and cytokinins were tested for better shoot initiation. The shoots were initiated with the combinations of BAP and NAA with IBA. In total, 24 combinations were tested (Fig 2.). Treatment five, containing

2 mg/L BAP with 0.5 mg/L IBA combination showed the maximum percentage (80.0±0.0%) of shoot initiation for the study species. The control did not show any shoot initiation (Fig 2.).

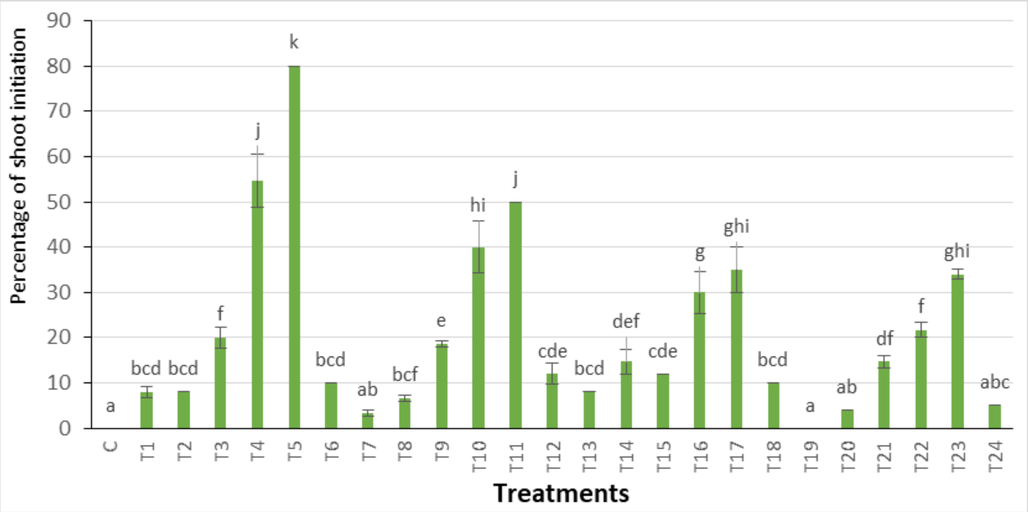

Fig. 2. Percentage shoot initiation in different treatments as different concentrations of BAP, NAA, and IBA. Error bars are standard error of mean. Different lowercase letters show significant differences between treatments.

The results of this study showed that the supplementation of growth hormones is essential in shoot initiation of *C. wendtii*. The effect of growth regulators was reported in various other studies. According to a study conducted by Herath *et al*., (2008) *C. backetii* initiated shoots without any growth regulators, but high number of shoots were obtained after the supplementation of growth hormones. The study done by Dissanayake *et al*., (2007) showed that shoot induction of *C. wendtii* occurred by supplementation of 44 µM BAP alone and the combination of 66 µM BAP with 13.4 µM NAA. Fig 3. shows the results of number of shoots per explant with the combination of growth regulators.

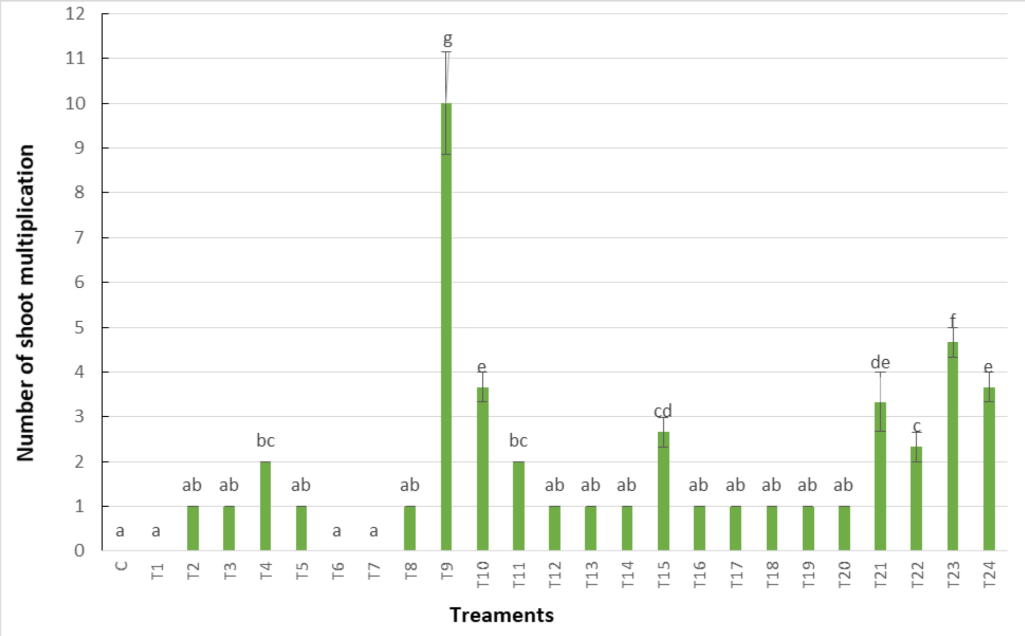

Fig. 3. The Percentage of shoot multiplication of *C.wendtii*. Errors bars are standard error of mean. Treatments are different concentrations of BAP, NAA, and IBA. Different lowercase letters show significant differences between treatments.

After 60 days of culture period the shoots were separated and sub culturing was conducted. During this study, different concentrations of BAP and NAA with IBA were tested. The number of shoots per explant was observed. In total, 24 treatments were conducted and treatment 9 (T9) showed a significantly high number of shoots with the combination of 4 mg/L BAP with 1 mg/L IBA (10.00±1.15). The study conducted by Herath *et al*., (2008) mentioned that the combination of BAP: IAA 5 mg/L: 0.1 mg/L showed the maximum number of shoots in multiplications of the same species. According to the results obtained by Dissanayake *et al*., (2007), the maximum shoot number was obtained by using only BAP for the same species.

Rooting of *C. wendtii*

After the shoot multiplication, shoots were cultured in different culture medium (MS Solid, MS Semi Solid and MS Liquid in three different IBA concentrations) to select the best medium. According to the results liquid medium was the most suitable for rooting (1.33±0.17). As agar is much expensive the cost for micro-propagation could be reduced by using a liquid medium. A gradient of IBA has been used in every medium to select the suitable IBA concentration. Treatment 9 in C3 showed the maximum average root length (2.33±0.17cm). Among all the medium 0.5 mg/L IBA showed highest average length of roots (2.33±0.17). The Fig 4. showed the average root length with different culture media and gradient of IBA.

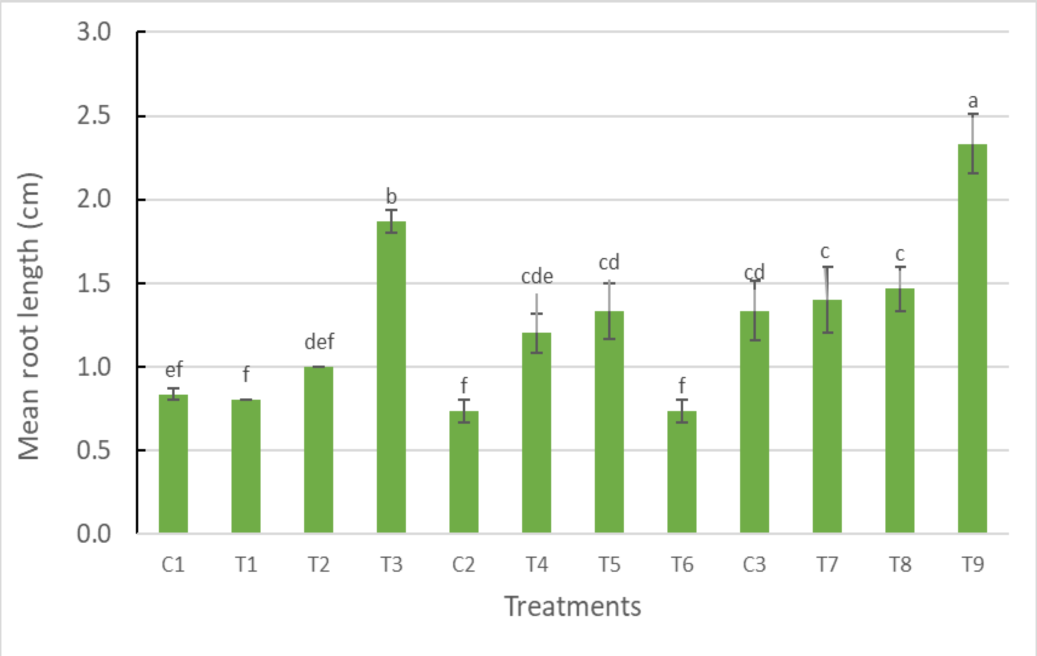

Fig. 4. The Percentage of root initiation of *C. wendtii* by using three different media as MS Solid, MS Semi Solid, and MS Liquid in three different IBA concentrations. Error bars are standard error of mean. Different lowercase letters show significant differences between treatments.

Ranasinghe *et al*., (2000) showed that the rooting could be obtained without growth regulators. Hence in our experiments, very low concentration of IBA was used for rooting. All the three controls showed rooting without any growth regulators. According to Dissanayake *et al*., (2007); control without any growth hormones has given a very less amount of rooting and the medium with IBA 0.5µM showed the rooting capacity.

Hardening

Yapabandara & Ranasinghe (2007) mentioned that hardening of tissue cultured plants are the most important aspect that determines commercial feasibility in terrestrial plants. Even though aquatic plants grow in an aquatic environment they also require good care at this stage. Rooted plantlets were transferred into hydroponic tanks in plant house at NARA. Plants were treated with different concentrations of Albert's solution (inorganic fertilizer). The number of leaves and leaf length were recorded. Fig 5. showed the plants grown in the tanks.

Fig. 5. Hardening of *C. wendtii*

Albert's solution with 0.5 g/L (T3) showed the highest number of leaves (6.7±0.7 cm) and the maximum leaf length (16.3±0.4 cm) during hardening period (Fig 6.). Considering the leaf length T3 was significantly differed from other treatments. But considering the number of leaves T3 was not significantly different from T2 (5.3±0.7 cm). Harshani *et al*., (2020) showed similar results for the hardening of *C. wendtii.*

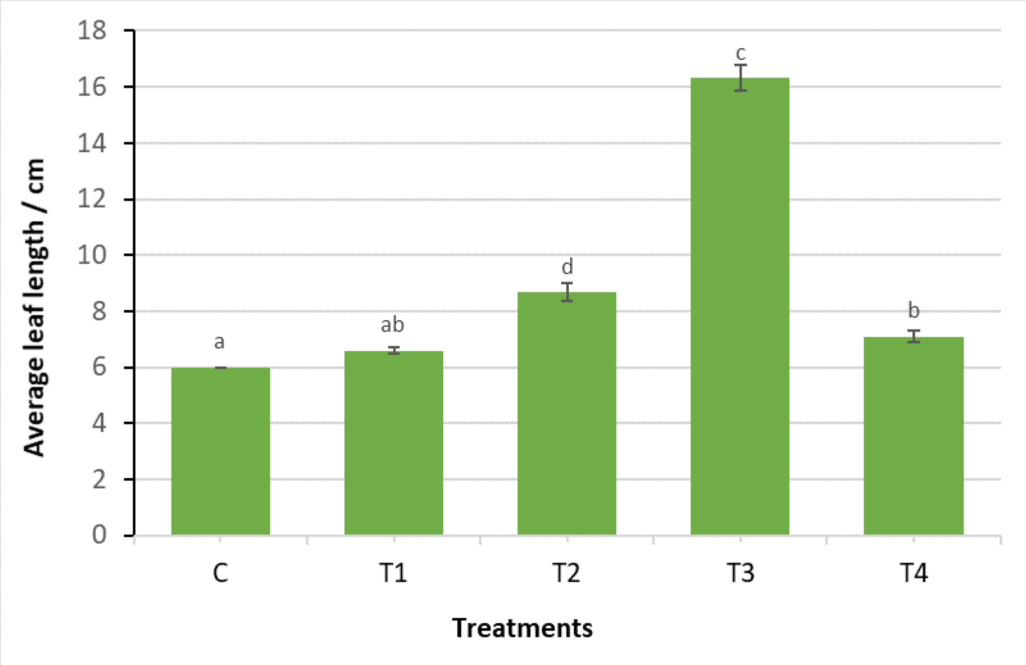

Fig. 6. The average leaf length of *C. wendtii* by using different concentrations of Albert's solution as 0 g/L (Control), 1.5 g/L (T1),1.0 g/L (T2), 0.5g/L (T3), 0.25g/L (T4). Errors bars are standard error of mean. Different lowercase letters show significant differences between treatments.

Conclusion

Most of the combinations showed the multiple shoot generations, but the highest mean number of shoots was showed in MS medium supplemented with 4 mg/L BAP and 1.0 mg/L IBA. During the acclimatization period, hundred percent survival was observed of all the plantlets and Albert's solution with 0.5 g/L showed the best results according to the assessed parameters.

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A Review on Antibiotic Usage and Antibiotic Resistance in Shrimp Culture; Asian, South Asian and Sri Lankan Scenario

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Abstract

Asian and South Asian regions contributed to aquaculture and shrimp farming with a major proportion, with a rapid increase in production during the past two decades. Although production experienced exponential growth, farming practices and production were affected by the occurrence of diseases. Antibiotic drugs in shrimp farming were practiced as an alternative to combat the diseases that have affected shrimp production to a greater extent. However, the misuse and overuse of antibiotic drugs continued to be practiced as a prophylactic or metaphylaxis measure and as a treatment option. Antibiotic resistance has become an alarming global issue owing to the overuse and misuse of therapeutic antibiotics around the world. Due to the irrational use of antibiotics in the shrimp farming, shrimp industry has become one of the most prominent industries that harness antibiotic-resistant genes to human health. Although surveillance and monitoring programmes are being implemented to monitor antibiotic usage in food animal production, the burden of resistant gene occurrence in shrimp farming is still high. The occurrence of resistant genes in food animals can easily be transferred to human bodies causing antibiotic resistant bacteria in human health. Numerous evidence from literature in Asian, South Asian and Sri Lankan studies suggest the presence of antibiotic drug residues and adverse effects of antibiotic resistance at an alarming level for shrimp farming industry, and human health.

Keywords: Antibiotic resistance; antibiotic usage; shrimp farming

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Introduction

Aquaculture is the main contributor to global fish consumption, and more than 70% of such aquaculture production is from the Asian and South Asian regions (FAO, 2019). Shrimp culture has expanded rapidly over the past two decades in South Asia due to favourable environmental conditions in the region. Shrimp culture has given rise to numerous employment opportunities as well as capital for the country; whereas negative impacts on the natural environment have also been detected within the past decade (Henriksson *et al.*, 2018). The brackish water shrimp farming systems have been categorized as traditional extensive, semi-intensive, and intensive farming systems. The major shrimp species involved in the brackish water farming system is the *Penaeus* shrimp (Thuy *et al.,* 2011). Shrimp are heavily traded commodities and represent the second main group of exported species in value terms (FAO, 2018). Shrimp is cultured worldwide in terms of international trade. The world production of farmed shrimp reached six million tons in 2018 (FAO, 2020).

Infectious diseases are a major health concern in shrimp culture due to poor water quality maintenance and the need for antibiotics and other chemical products to manage the disease emergence. Thus it has become a major issue in the culture-based shrimp industry in Asia (Heal *et al.*, 2021).Proper management aspects are needed in shrimp farming practices to mitigate the impact of diseases to the economic benefit and to protect cultured stock from diseases. Management practices to control infectious diseases in shrimp farming include; hygiene maintenance, effective prevention of common infectious diseases, enabling biosecurity measures, supplementation of dietary supplements such as vitamin C to enhance immunity in cultured animals, and probiotics and prebiotic usage to support the healthy growth of stocks (Manage, 2018). The continuous impact of disease risk involves losses of shrimp stocks; at which stage usage of antibiotic agents to treat such infections came into concern. Antibiotic usage is needed to be monitored and surveillance methods are to be established to control the overuse and misuse of recommended antibiotics. As no specific antibiotic agents are specially designed to be used for aquaculture and shrimp farming, veterinary drugs recommended for other livestock are used for shrimp culture practices. However, a few veterinary medicines that are licensed in aquatic animals are allowed for human consumption, and such medicines still need certain withdrawal periods before human consumption (Honda *et al.,* 2016). Permitted antibiotics that are recommended to be used in shrimp culture vary from different geographical areas in the world. However, from a South Asian perspective, India, Bangladesh and Bhutan have established policies and legislations to develop and monitor antibiotics for food animal production (Allcock *et al.,* 2017). These documents and legislations emphasize the procedures for the establishment of permissible Maximum Residue Limits (MRL) of pharmacologically active substances in food commodities of animal origin (Okeke *et al.,* 2005). Governments of each country are responsible for monitoring the MRL levels for each permitted antibiotic or veterinary drug to ensure food safety for human consumption.

Frequent usage of antibiotic agents as a prophylaxis measure or a treatment, in an abusive way, causes major impacts on human health. Cabello, 2006 suggests that the heavy use of antibiotics in aquaculture causes serious problems to human health and the environment. Heavy use of antibiotics in shrimp farming can cause residual effects that may be retained in the sediments and the water bodies; causing antibiotic residue accumulation in food animals and surrounding water bodies. The presence of residual antibiotics even in lower doses in food animals such as shrimp can pass through food chains and bio accumulate within human bodies in larger quantities causing toxic effects on human health (Sarmah *et al.,* 2006). The persistent usage of antibiotics in food animals can cause antibiotic resistance; which is a huge threat to human health (Allcock *et al.,* 2017). The greatest associated risk of the use of inappropriate antibiotics is the development and spread of Antibiotic Resistant Genes (ARG) and antibiotic resistant bacteria (Martínez *et al.,* 2015). Once the antibiotics are being used, the residual antibiotics can remain in shrimp farms affecting the natural microflora of the farm. ARG may either accumulate in aquatic bacteria and food animals that are farming, and they possess the ability to transfer within genes via horizontal gene transfer technique or reach human pathogens causing resistance among zoonoses (Cloeckaert *et al.,* 2017). ARG in human health can lead to significant issues, including reduced effectiveness of existing therapeutic treatments(Martínez *et al.*, 2015).Certain banned antibiotics in shrimp farming, such as chloramphenicol and nitrofurans, and banned dye residues that are used as antimicrobial compounds such as malachite green result in slowdown imports, causing huge economic losses for the producers as well as for the government (Food and Agriculture Organization, 2020).

The worldwide situation of antibiotic use in shrimp farming is worrisome. Shrimp aquaculture, heavily involved in international trade and closely linked to aquatic ecosystems, may play a role in spreading Anti-Microbial Resistance (AMR). Despite efforts to decrease antibiotic usage, significant challenges remain, particularly in major shrimp-producing nations such as China, India, Indonesia, Thailand, and Vietnam. Ongoing monitoring and action are crucial to maintaining sustainable and safe shrimp production (Thornber *et al.*, 2020; Ibarra *et al.*, 2022).

The Joint Food and Agriculture Organization (FAO)/World Organization for Animal Health (OIE)/World Health Organization (WHO) Expert Meeting on Antibiotic Use and Antibiotic Resistance in Aquaculture identified that the two hazards to be considered are antibiotic residues and antibiotic resistance (AMR) (FAO/OIE/WHO, 2006). Measuring the usage of antibiotic data in food animal production can address different objectives; to monitor Anti-Microbial Usage (AMU) over time, to settle benchmarks to promote the reduction of AMU, and to assess possible associations between AMU and AMR (Cuong *et al.*, 2018).

Antibiotic agents usually lose their effectiveness over time due to the emergence of resistant pathogens. This phenomenon, when a particular species is resistant to almost all classes of persistent antibiotics, they are known as superbugs (Krishnasamy *et al.*, 2015). They can be either extreme drug-resistant or completely drug resistant. However, regulation and monitoring of veterinary drug usage over food animals requires sensitive and selective methods to analyze the present antibiotic residues and to ensure food safety among the general public. To date, there is no globally widespread system available to monitor the usage and circulation of antibiotic agents in shrimp farming.

Antibiotic usage in Asian countries

Farmed shrimp production has always been dominated by Asia, which contributed 85% of the world's production in 2018. Shrimp production in the Asian Region is mainly concentrated in countries such as China, Indonesia, Vietnam and India in 2018, and mainly targeted the export market (FAO, 2020). Due to the elevated shrimp production in the Asian region, antibiotic agents are considered high in these regions to prevent diseases and maintain healthy brooder stocks (Tollefson and Miller, 2000). The most common route of administration of antibiotic agents in shrimp culture is via the oral route. However, they may also be administered via injection or as a bath to the species reared. Common antibiotics such as aminopenicillins (amoxicillin, ampicillin), sulphonamides, and quinolones (oxolinic acid) are usually administered via the oral route. Tetracyclines (tetracycline, oxytetracycline), and macrolides (erythromycin) are administered during an infection orally or as a bath (O'Neil, 2014; WHO, 2014).

According to the literature, a considerable fraction of antibiotic residues are found in water resources from aquaculture in several studies conducted in India, Bangladesh, Indonesia, Vietnam and Thailand (Tollefson and Miller, 2000; Sandu *et al.,* 2017). Erythromycin and tetracycline were detected in water bodies of aquaculture up to 180 ng/L from Thailand (Von Baum and Marre, 2005), and fluoroquinolones were detected from fish and shrimp samples in Vietnam (~12 ng/L) (WHO, 2014).

A study conducted in Vietnam on shrimp culture in mangrove areas suggests the presence of norfloxacin and oxolinic acid, trimethoprim and sulphamethoxazole, which are named critically important and highly important antibiotic agents for human health by WHO (Le *et al.,* 2005). Antibiotics in human medicine have been categorized into three categories; as critically important, highly important and important based on their importance to human medicine (WHO, 2018). The WHO list of medically significant antimicrobials for human health (WHO MIA list) serves as a risk management instrument in making decisions to reduce the antimicrobial usage in sectors other than human on the development of antimicrobial resistance in humans (WHO, 2024).

Another study conducted in Vietnam discusses the availability of trimethoprim (TMP), sulfamethoxazole (SMX), norfloxacin (NFXC) and oxolinic acid (OXLA) in water and sediment samples in shrimp ponds in mangrove areas. Results emphasize the availability of the above antibiotics in all samples of both shrimp ponds and surrounding water canals. The highest concentrations of TMP, SMX, NFXC and OXLA are 1.04, 2.39, 6.06, and 2.50 ppm in water samples; and 734.61, 820.49, 2615.96, 426.31 ppm (based on wet mud weight), in sediment samples respectively (Le and Munekage, 2004) as shown in Table 1. Among the above antibiotic agents, TMP and SMX are known as highly important and NFXC and OXLA are quinolones, and critically important antibiotics to human health, according to WHO standards, they are not recommended for non-human use such as food, fish and aquaculture production (WHO, 2005).

Table 1. Range of antibiotics concentration (ppm) (Le and Munekage, 2004)

Specimen	Type of antibiotic	Minimum level (ppm)	Maximum level (ppm)
Water from the surface layer	TMP	0.08	1.04
	SMX	0.04	2.39
	NFXC	0.06	6.06
	OXLA	0.01	2.50
Water from the bottom layer	TMP	0.08	2.03
	SMX	0.04	5.57
	NFXC	0.08	4.04
	OXLA	0.01	2.31
Wet bottom layer mud (5cm depth in the upper layer)	TMP	9.02	734.61
	SMX	4.77	820.49
	NFXC	6.51	2615.96
	OXLA	1.81	426.31

Another study conducted in Vietnam by Tran *et al.*, (2017) discusses the use of up to 20 different antibiotics in shrimp farming and fish culture in Vietnam, including tetracycline, oxytetracycline, ampicillin, rifampin, chloramphenicol, enrofloxacin, linomycin, ciprofloxacin and sulfamethoxazole/trimethoprim. However, the authors declare that only one shrimp farmer was reported to be using antibiotics; and the reported antibiotic was oxytetracycline. Authors also states that tetracyclines are antibiotics that farmers often use in shrimp culture for disease prevention and treatment, and is a highly important antibiotic for human health according to WHO antibiotic classification (WHO, 2018). Tetracycline has been used extensively in aquaculture for chemotherapy against fin rot and skin ulcers (Mortazavi, 2014).

A study conducted based on shrimp and fish-related aquatic environments in rural areas of Vietnam shows that 53 of the total 362 samples were positive for antibiotic residues. Thirty-nine out of total 362 (10.8%) samples tested, for antibiotic residues showed positive results for enrofloxacin. Enrofloxacin is considered to be banned in Vietnam shrimp culture since 2012, and authors describe the presence of enrofloxacin as well as ciprofloxacin; often considered as a metabolite of enrofloxacin (Nguyen *et al.,* 2015). Authors further described that trimethoprim and sulfamethazine or sulfamethoxazole are normally mixed in commercial aquatic feed, and are often detected as combinations (Uchida *et al.,* 2016).

Another study associated with antibiotic residues in retail shrimp purchases in Vietnam states that 22.5% (9/40) of total samples were positive for antibiotic usage. Antibiotics; tetracyclines (7.5%), fluoroquinolones (7.5%), sulfonamides (2.5%), and macrolides (2.5%) were detected and the shrimp purchased at supermarkets showed a higher AMU (50%) compared to shrimp purchased at street markets (13.3%) (Yen *et al.,* 2020).

Thuy *et al.*, 2011 describe the usage of antibiotics in several shrimp farming sites in Vietnam. Authors express that although oxytetracycline is the most widely used antibiotic for many years, however, quinolones and the combinations of sulfadiazine and trimethoprim have become more popular in Asian shrimp farming (Thuy *et al.*, 2011; Holmström *et al.,* 2003). For Vietnam, enrofloxacin was the most used antibiotic (43%), and norfloxacin (25%) of the farmers in the Mekong delta. In addition, they have also used sulfamethoxazole, Co-trimoxazole and trimethoprim (Thuy *et al.*, 2011; Nga, 2004). Authors divide the most common antibiotics that are used in Vietnam into five categories; (1) Fluoroquinolones (enrofloxacin, norfloxacin, ciprofloxacin, and oxolinic acid), (2) sulfonamides (sulphamethoxazole, sulfadiazine), (3) tetracyclines (oxytetracycline), (4) diaminopyramidines (trimethoprim, ormethoprim), and (5) unclassified (griseofulvin and rifampicin) (Thuy *et al.*, 2011).

Data from the Can Duoc District show the most commonly used antibiotics are sulfonamides in combination with trimethoprim. 73% of the interviewed farmers were using trimethoprim and sulfadiazine. The next most commonly used antibiotics are composed of sulfadimethoxine and ormetoprim as well as trimethoprim and sulfamethoxazole. In the Can Duoc District, the most commonly associated disease for shrimp is white spots and white faeces; norfloxacin and trimethoprim have been used to treat such diseases. More seriously, in the Can Duoc District, besides ciprofloxacin, rifampicin and griseofulvin are used for shrimp larvae; which are considered antibiotics for human use (Thuy *et al.*, 2011); Table 2.

A study conducted by Holmström *et al.*, 2003 on shrimp culture in Thailand shows that shrimp farmers use antibiotics as both therapeutic as well as prophylactic means. Authors also emphasize that the antibiotics are available in powder form, and the farmers either mix them with feed or throw them into water. The most common antibiotics the farmers used in shrimp ponds are; norfloxacin, oxytetracycline, enrofloxacin and different sulphonamides (Holmström *et al.*, 2003). Norfloxacin and enrofloxacin are quinolones and fluoroquinolones, and tetracyclines and sulphonamides are named critically important and highly important antibiotics respectively by WHO (WHO, 2005). The same study reveals farmers use up to 0.5-6 g/Kg of antibiotics, feeding three times a day per week (Holmström *et al.*, 2003).

Table 2. The most commonly used antibiotics in Vietnamese shrimp farming (Thuy *et al.,* 2011)

S/N	Commercial Name	Composition	Percentage of farmers	Usage
1	Ciprofloxacin 500mg	Ciprofloxacin	100%	Larvae
2	Cotrim	Sulfamethoxazole	8.7%	Post larvae- adult shrimp
3	Cotrim-La	Sulfamethoxazole, trimethoprim	N/A	Post larvae- adult shrimp
4	Daitrim	Sulfamethoxazole 10%, trimethoprim 2%	N/A	Post larvae- adult shrimp
5	Griseofulvin 500mg	Griseofulvin	100%	Larvae
6	N300	Norfloxacin, hydrochloride 30%	N/A	Post larvae- adult shrimp
7	Osamet	Sulfadimethoxine 25%, ormetoprim 5%	11.2%	Post larvae- adult shrimp
8	Prawnox	Oxolinic acid 25%	N/A	Post larvae- adult shrimp
9	Rifampicin 300mg	Rifampicin	100%	larvae
10	Romet 30	Sulfadimethoxin 25%	N/A	Post larvae- adult shrimp
11	Silva 54	Sulfadiazine, trimethoprim	N/A	Post larvae- adult shrimp
12	Sulfa-prim	Sulfadiazine, trimethoprim	21.74%	Post larvae- adult shrimp
13	TA-1 oxytetracycline	oxytetracycline	100%	Larvae
14	TMT	Sulfadiazine, trimethoprim	15.9%	Post larvae- adult shrimp

Antibiotic usage in Chinese fish and shrimp farming is studied by Liu *et al.,* 2017 and the authors describe 13 antibiotics are authorized to be used in China; doxycycline, enrofloxacin, florfenicol, flumequine, neomycin, norfloxacin, oxolinic acid, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, thiamphenicol, and trimethoprim. Authors have identified that 12 antibiotics that are used in fish and shrimp farming are not authorized; amoxicillin, chloramphenicol, chlortetracycline, ciprofloxacin, erythromycin, furazolidone, gentamycin S, oxytetracycline, penicillin G, streptomycin, sulfamerazine S, and sulfisoxazole(Liu *et al.*, 2017). Some antibiotics are used occasionally although they have been banned for usage over the years. For example, erythromycin was banned in 2002, but its usage was reported in 2012 (Bondad-Reantaso *et al.*, 2012).

However, compared to the antibiotic usage in other food animals, antibiotic usage in shrimp farming is considerably lower even in developed countries. In Vietnam, antibiotic usage was 1.44 g/ton of production, and it was 1.67 and 4.53 g/tons in China and Thailand respectively (FAO, 2020).

Antibiotic usage in South Asia

A study conducted based on shrimp culture in Kerala, Tamil Nadu, Karnataka and Andhra Pradesh of India describes the presence of antibiotic residues in collected samples. Residual Levels of antibiotics viz: chloramphenicol, sulphonamide, tetracycline, erythromycin, streptomycin and β-Lactams were determined, and authors were able to identify sulfonamides (35.017 to 97.81 ppb with an average of 56.91 ppb) and erythromycin (49.46 to 77.49 ppb and an average value of 61.12 ppb) in levels. The presence of chloramphenicol was detected at <1 ppb levels. The reported chloramphenicol residue concentrations were ranging from 0.1134 to 0.2398 ppb with an average of 0.1761 ppb. Streptomycin, tetracycline and β- lactam antibiotics were not detected in any of the collected samples (Swapna *et al.*, 2012).

Chloramphenicol was detected as an antibiotic agent in fish farms in Bangladesh (~5ng/L), and in shrimp farms in India (~32ng/L) and Indonesia (~45ng/L) (Allcock *et al.,* 2017, Von Baum and Marre, 2005, WHO, 2014) .

Another study conducted in India shows oxytetracycline and erythromycin residues up to 49 µg/L and 1.6 µg/L concentrations respectively from fish and shrimp farms. The same study reveals oxytetracycline was frequently detected in sediments in shrimp farms with concentrations up to 6908 µg/Kg (Manage, 2018;Koeypudsa *et al.,* 2010).

Antibiotic usage in Sri Lanka

According to the authors' knowledge, there is limited data available on antibiotic usage in aquatic food animal farming in Sri Lanka. This may be due to the unavailability of proper monitoring and documentation system to track the usage levels of antibiotic agents in fish and shrimp culture.

The higher usage of tetracycline and Oxy-Tetra-Cycline (OTC) in a study conducted using 16 selected aquaculture farms including shrimp farming. The effluent water in all farming sites except Dambulla, Muthupanthiya and Udappuwa was positive for the usage of tetracycline and oxytetracycline. Detected OTC levels in shrimp hatcheries were 0.056±0.001 µg/mL - 0.234 ± 0.014 µg/mL (Manage, 2018). Authors suggest that high tetracycline levels were detected in shrimp hatcheries (0.012±0.019 µg/mL - 0.112± 0.017 µg/mL). WHO recommendation for tetracycline in aquatic environments is less than 0.001 µg/mL and less than 0.1 µg/mL in soil (Liyanage and Manage, 2019; O'Connor and Aga, 2007). The study found lower levels of erythromycin concentrations (~0.001µg/mL) whereas penicillin and sulfonamides were not detected (Liyanage and Manage 2016). Another study conducted by Munasinghe *et al*., (2012) describes the usage of oxytetracycline in 15% of the total shrimp farms (92/603) in Puttlam District, Sri Lanka.

Being a country that exports cultured fish, shrimp and fishery products to the international market, especially to the EU, the Ministry of Fisheries and Aquatic Resources Development has a gazette of the Aquaculture (Monitoring of Residues) Regulations 2002" under the Fisheries and Aquatic Resources Act No 2 of 1996. Since then, an "Annual Residual Monitoring Plan" for exports of cultured fish, shrimp and fishery products has been under implementation by the Department of Fisheries and Aquatic Resources which is the competent authority for the export of fishery products from Sri Lanka.

Development of antibiotic resistance

Shrimp farms once operated in intensive farming systems with elevated stocking densities, extended farming periods, poor sanitation and poor biosecurity measures enhance the ability of a farming system to acquire diseases and spread rapidly over populations (Walsh, 2000). Rapid disease spread causes the entire shrimp farm to contaminate, and once disease outbreaks happen, the requirement for antibiotic agents to treat the prevailing disease condition and prevent the spread of the current outbreak arises. Antibiotic agents are used in shrimp farming and aquaculture both as a treatment method, as well as a prophylaxis measure. Not all the administered antibiotics can be absorbed and utilized by shrimps and fish in the farm. Studies suggest that up to 80% of applied antibiotic agents are excreted through the urine and faecal matter of such animals without complete decomposition (Blair *et al*., 2014). Accumulation of such excretory matter, sometimes along with feed components if they were fortified with antibiotic agents as a therapeutic or prophylactic measure, can give rise to aquatic microorganisms which are resistant to the exposed antibiotics over an extended period (Allcock *et al*., 2017). AMR in microorganisms has primarily developed due to the selective pressure exerted on aquatic microbial populations with the presence of antibiotic agents. Antibiotic agents act on microbes in several ways; they interrupt the cell wall biosynthesis of microorganisms, and they either interfere with bacterial protein synthesis, DNA replication and repair or other cellular mechanism parameters (Walsh, 2000). However, microorganisms develop mechanisms to withstand the selective pressure of antibiotics and evolve into a more resistant community to antibiotic agents when they are utilized frequently. These developed mechanisms are; enzyme production to inactivate antibiotics, alter the target site of action for antibiotics, alteration of metabolic pathways, change of permeability of antibiotic to outer cell membrane and presence of effluent pumps (Walsh, 2000). Resistant microorganisms may exhibit one or more mechanisms to overcome antibiotic activity on them and may be resistant to one or more classes of antibiotics (Blair *et al*., 2014). Once the AMR gene is acquainted within the bacterium with a single mutation, the mutant gene starts to withstand the selective pressure by antibiotics, by surviving while all the sensitive bacteria are killed; which makes the mutant gene survive successfully and replicate throughout the population and become dominant. This replication causes the bacterial strains to evolve gradually and emerge as AMR bacteria with time (Blair *et al*., 2014).

Microorganisms in a shrimp farming system act as an enormous pool of genes in ecosystems where they can acquire resistant genes with prolonged antibiotic exposure. This acquisition of resistant genes is occurred mainly via mobile platforms such as plasmids, transposons and integrons and can spread over different ecosystems (Davies, 1994; Courvalin and Arthur, 1993). Further, certain genes can be located on plasmids that can segregate within transposons. Such genes can cut their genes from one strand of DNA and transfer and fit into another locus of a DNA strand. This mechanism can transfer the AMR genes within a gene pool (Courvalin and Arthur, 1993). It takes a period from months to years for the AMR genes to emerge and clinically significant resistance to appear (Allcock *et al*., 2017).

The most important impact that occurred due to multiple antibiotic resistance genes is the reduction of effectiveness of existing antibiotic agents. Reduced effectiveness to the infectious disease-causing pathogens makes it brings the requirement of new effective antibiotics that would meet the ready effectiveness; however, the main problem is that there are no new classes of antibiotics reported in the recent past. What is currently being done is making alterations to existing classes of antibiotics (Silver, 2011), which may also continue to use their effectiveness unless they were used deliberately.

AMR in Asian and South Asian scenarios

Although AMR is a globally recognized concern, the burden of the problem in Asian and South Asian regions shows an upward trend with the excessive and irrational use of antibiotic agents in food animal production. Countries such as China, India, Thailand, Vietnam, the Philippines and Sri Lanka have demonstrated increasing trends in AMR problems (Bhatia, 2019). When a single bacterial strain become resistant to more than one antibiotic agent, it is called multiple antibiotic resistance (MAR). Numerous studies prove the presence of MAR in common bacteria associated with shrimp farming in Asia indicating the usage of antibiotic agents as prophylactic, metalactic and therapeutic agents. However, the alarming concern with antibiotic usage is that discovering the evidence to prove that the drugs that are not generally permissible for food animal production, such as chloramphenicol and trimethoprim have also been used in shrimp culture in Asia (Changkaew *et al.,* 2014). According to literature, numerous evidence has been found that MAR in the shrimp industry in Asian and South Asian scenarios. Examples of the evidence for antibiotic resistance in shrimp culture in Asian and South Asian regions are categorized in Table 3.

Table 3. Presence of multiple antibiotic resistance in Asian and South Asian Regions

Year	Country/ Origin of AMR	Species associated	Description	Reference
1973	Japan	*Vibrio anguillarum*	Sulfonamides,streptomycin, chloramphenicol, tetracycline resistance	Aoki *et al.*, 1974
1994	India	*Vibrio harveyi*	Hatchery strains were resistant to streptomycin, chloramphenicol, co-trimaxazole	Karunasagar *et al.*, 1994
1995	Thailand	*Streptococcus* and *Vibrio*	All vibrio strains were susceptible to sulfadimethoxine/ormetoprim and moderately susceptible to sulfadiazine/trimethoprim and oxytetracycline. Resistant to amoxicillin.	Maisak *et al.*, 1995
1999-2002	India	*Vibrio spp.* and *Aeromonas spp*	100% of the samples resistant to ampicillin. Lower resistance was detected in chlortetracycline and erythromycin. Vibriosshowed high resistance to the antibiotics compared to *Aeromonas* isolated from the same sample.	Vaseeharan *et al.*, 2005

2001	Philippines	*Vibrio harveyi*	Oxytetracycline, oxolinic acid, chloramphenicol and furazolidone resistance in pond cultured shrimps	Tendencia and Pena, 2001
2004	Thailand and India	*E.coli*, *Salmonella*, *Bacillus* spp and *Vibrio* spp	Samples from Thailand and India shows the presence of multiple drug resistance (MDR) associated with erythromycin, tetracycline, chloramphenicol, nalidixic acid and trimethoprim	Duran and Marshall, 2005
2005	India	*Vibrio* spp	30.3% of isolates were MDR; Out of total MDR isolates, resistance to 4–10 antibiotics in 55.5% of isolates; resistance to >10 antibiotics was observed in 14.14% of isolates	Manjusha *et al.*, 2005
2006-2007	India	*Vibrio* spp	100% resistant of all the *Vibrio* samples to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin,penicillin G and furazolidone	Srinivasan and Ramasamy, 2009
2007-2008	Thailand	*E. coli*	Presence of 29.1% of MAR in shrimp farms, with tetracycline (14.4%), ampicillin (8%) and trimethoprim (6.7%)	Changkaew *et al.*, 2014
2010-2011	India	*Vibrio* spp	The highest incidence of antibiotic resistance was evident against ampicillin and colistin followed by amoxicillin, carbenicillin, ceftazidime andcephalothin	Sudha *et al.*, 2014
2011	Malaysia	*Salmonella* and *Vibrio*	Presence of MAR for ampicillin and tetracycline, followed by doxycycline in *Salmonella* and *Vibio spp*	Banerjee *et al.*, 2012

2011	China	*E.coli* and *S. aureus*	Resistance to antibiotics such as ampicillin, trimethoprim, tetracycline, chloramphenicol and cefazolin	Zhang *et al.*, 2011
2011	India	*Vibrio* spp.	78% of isolates were MDR. The highest incidence of antibiotic resistance against amoxicillin (94%), followed by Ampicillin and carbenicillin (90%); cefuroxime and streptomycin (65%) followed by neomycin and amikacin (59.57%); rifampicin (58.00%); furazolidone (42%) and meropenem (35%)	Manjusha & Sarita, 2011
2011-2013	Malaysia	*Vibrio parahaemolyticus*	High resistance to ampicillin, cefalexin and ciprofloxacin	Al-Othrubi *et al.*, 2014
2012	China	*Vibrio vulnificus*	Resistance or intermediate resistance to cefepime (3.03%), tetracycline (6.06%), aztreonam (24.24%), streptomycin (45.45%), gentamicin (93.94%), tobramycin (100%), and cefazolin(100%).	Pan *et al.*, 2013
2013-2014	India	*Vibrio harveyi*	Resistance to ciprofloxacin, penicillin, rifampicin, and vancomycin out of 15 antibiotics tested	Stalin and Srinivasan, 2016
2014-	Thailand	*Aeromonas veronii* and *A. aquariorum*	Resistance to cefotaxime and imipenem in two *A. aquaritheorum* and in three *A. veronii* isolates; the resistance of tetracycline and ampicillin	Yano *et al.*,2015
2014	China	*Vibrio parahaemolyticus*	High level of resistance to the antibiotics ampicillin (94.2%), rifampin (93.3%), and streptomycin (77.9%)	Hu and Chen, 2016

2014	Parangipettai, India	*Bacillus pumilus* and *Bacillus flexus*	Presence of novobiocin, ciprofloxacin and vancomycin resistance	Sundaramanickam *et al.*, 2015
2015-2016	India	*Vibro* spp.	Highest resistance (50.4%) against ampicillin. Very high intermediate resistance (87.4%) against erythromycin. 20% of *Vibrio* isolates were resistant to two or more antibiotic classes with MAR index value of ≥0.28.	Singh *et al.*, 2018
2016-2018	China	*Vibrio parahaemolyticus*	High resistance to chloramphenicol, sulfamethoxazole-trimethoprim,trimethoprim, rifampicin, ampicillin, spectinomycin, kanamycin,	He *et al.*, 2019
2020	India	*Vibrio parahaemolyticus*	One pathogenic isolate was identified as MDR and 59% exhibited a MAR index of 0.2 or above. 100% resistance to ampicillin, 74.1% to cefotaxime, 48.1% to cefoxitin, 44.4% to cefepime, 29.6% to ceftazidime, 7.4% to amoxicillin/clavulanic acid, 3.7% to ciprofloxacin, gentamicin and meropenem	Narayanan *et al.*, 2020

Numerous studies suggest the presence of multidrug resistance associated with shrimp culture in the Asian and South Asian regions. The most common reason for such high resistance to persist within shrimp farms can be suggested as the irrational use of antibiotic drugs in shrimp farming practices.

In Asian and South Asian cuisine, farmed shrimp constitutes a significant portion of the human diet. However, consuming these shrimps can lead to the transfer of antibiotic-resistant genes from shrimp to humans. These acquired genes alter the natural microflora in humans, reducing the effectiveness of existing therapeutic drugs (Palaniappan and Holley, 2010). Unfortunately, new antibiotics are not emerging rapidly enough to keep pace with the generation of resistant genes and bacteria (Laws *et al.*, 2019). Critically important antibiotics, as outlined by the WHO's 2018 guidelines, are the only treatment options for certain medical conditions (WHO, 2018). If resistance develops to these critical antibiotics in human medicine, patients may face a lack of effective treatment options, potentially resulting in fatalities. While highly important and less critical antibiotics exist, frequent exposure to them through food animals, antibiotic misuse, and overuse in food animal farming can lead to resistance even to these less critical antibiotics. Over time, this could render all therapeutic options ineffective, pushing humanity toward a pre-antibiotic era. To address this critical situation, better controls on antibiotic usage in food animal production, such as shrimp farming, are essential.

AMR in Sri Lankan Scenario

Although Sri Lanka has plenty of natural and brackish water resources for fish and shrimp farming, Sri Lanka did not have a traditional aquaculture system until the beginning of 1980 (Heenatigala and Fernando, 2016). Since aquaculture and marine shrimp culture have reached their maximum commercial dimensions, brackish water ponds for shrimp culture and cage culture have gradually emerged (NAQDA, 2015). The major limiting factor to Sri Lankan shrimp farming was the incidence of infectious diseases such as white spot syndrome virus (WSSV) and Vibriosis. *Vibrio* spp. is a part of the natural microflora in wild and cultured shrimp (Heenatigala and Fernando, 2016), and the members of the family Vibrionaceae contribute up to 60% of the total bacterial population in shrimp aquaculture (Heenatigala and Fernando, 2016; Simidu and Tsukamoto, 1985). *Vibrio* spp. are one of the major pathogenic bacterial organisms which cause high mortality in shrimp culture and act as a primary pathogen spreading WSSV (Priya *et al.*, 2009). Maintaining proper water quality is an important aspect of shrimp culture as it considerably reduces the pathogenic *Vibrio* spp. population in the water system (Ganesh et al., 2010).

Management of infectious diseases in the Sri Lankan shrimp farming is mainly based on the management of aquatic microflora with the aid of probiotics and antibiotics (Heenatigala and Fernando, 2016; Havenaar *et al*.,1992). Misuse and overuse of antibiotics for disease management in shrimp farms have caused multiple antibiotic resistance among the bacterial population in water bodies and bacteria in sediments of shrimp culture systems; there they can act as a vehicle that delivers MAR aquatic pathogens to humans from one country to another (Zanetti *et al*., 2001).

According to research conducted by Heenatigala, 2013, 96 pathogenic bacterial strains such as *Vibrio alginolyticus, V. fluvialis* and *Pseudomonas aeruginosa* were isolated and subjected to antibiotic sensitivity test. None of the pathogenic bacterial strains were highly resistant to erythromycin, and only 29% showed intermediate sensitivity; while only 20% of the total isolates were sensitive to oxytetracycline, and 80% showed intermediate sensitivity to oxytetracycline (Heenatigala, 2013).

Athurupana *et al*., 2020 conducted a study to test the antibiotic resistance of *Escherichia coli* isolated from pond water, bottom sediments and shrimps (*Penaeus monodon*) from shrimp farms, in Puttalam District, Sri Lanka. In this study, antibiogram against *E. coli* to antibiotics belonging to different families, ß-Lactams: amoxicillin (30 µg); tetracycline: tetracycline (30 µg) oxytetracycline (30 µg); macrolides: erythromycin (15 µg) and chloramphenicol (30 µg) was studied. A total of 67 *E. coli* bacteria were isolated and 48 (71.64%) were resistant to at least one drug out of the total number. A high index of resistance to erythromycin (15 µg) 70.15% was reported. In contrast, none of the *E. coli* strains was resistant to chloramphenicol (30 µg). Multidrug resistance to two or more antibiotics was observed in 24 isolates. Multiple Antibiotic Resistance Index varied within the range of 0 to 0.8 for the antibiotics used.

Gallage *et al*., 2019 has reported a study consisting of 146 isolates belonging to the family Vibrionaceae were recorded and identified as, *Aeromonas hydrophila*, *Vibrio metschnikovii*, *V. anguillarum*, *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, *V.*

damsela, *V. mimicus* and *V. fluvialis*. *Vibrio* isolates were found to be resistant to amoxicillin (68.5%), nitrofurantoin (25.2%), nalidixic acid (21%), tetracycline (5.18%) and chloramphenicol (4.48%). This study showed that *Vibrio* species are more resistant to amoxicillin in comparison with other antibiotics used in this study. Results also indicated that the application of antibiotics for the control of vibriosis in shrimp farms has limited effectiveness due to the development of resistant bacterial strains.

Another study conducted by Heenatigala and Fernando, 2016 discusses the alterations of the antibiotic sensitivity of Vibrio spp. isolated from disease-infected P. monodon. Ariyawansa *et al*., 1999 reported that *Vibrio* spp. from *P. monodon* is highly sensitive to chloramphenicol (10µg and 30µg), and tetracycline (30µg); and moderately sensitive to streptomycin (25µg); when compared to furazolidone (50µg), erythromycin (15µg) and sulphafurazole (30µg). However, the study by Heenatigala and Fernando, 2016 reveals the bacterial sensitivity of chloramphenicol has changed from sensitive to moderately sensitive and/ or resistant, and oxytetracycline has changed from moderately sensitive to resistant; and the study suggests the alteration may be due to the continuous use of the antibiotics in shrimp farming.

A study conducted by Liyanage and Manage, 2016 shows *Bacillus* sp., *Acinetobacter* sp., *Achromabacter* sp. *Staphylococcus* sp., and *Micrococcus* sp., are the most abundant resistant genera for tetracycline and oxytetracycline in Sri Lankan aquaculture farms (Liyanage and Manage 2016). Maximum Inhibitory Concentrations (MIC) values for OTC-resistant bacteria ranged from 360-840 µg/mL and the highest MIC was recorded for *Pseudomonas aeroginosa* and the lowest MIC was detected for *Bacillus* sp. MIC values for tetracycline resistance varied from 320-780 µg/mL, and the highest MIC was obtained by *S. haemolyticus* and the lowest MIC was reported for *B. pumilus* (Manage, 2018; Liyanage and Manage 2016).

AMR surveillance systems and its importance

Although monitoring of AMR is required to understand the burden of AMR and to find solutions to the problem of potential gaps in the distribution of AMR within populations and individuals, the factors that contribute to AMR are needed to be understood. Large-scale epidemiological surveys are required to determine the relationships between AMR and antibiotic prescription and consumption (Allcock *et al*., 2017). This sort of surveillance system needs to access the geographical regions, different communities, and potential zoonotic transmission pathways (Van *et al*., 2012). Enabling real-time monitoring of resistance patterns and the spread of AMR genes within populations is required to monitor AMR successfully (Van *et al*., 2012). Due to the continuous usage of banned antibiotics in shrimp culture, tightened guidelines and national regulations to monitor antibiotic usage and residual level monitoring are currently available in many countries. Due to the rejections in exports and due to the reduction of profits from seafood marketing as a result of residual antibiotics, Maximum Residual Levels (MRL) accepted by Codex Alimentarius Commission is currently available for a limited number of antibiotics. However, these MRLs are not consistent among different countries. Therefore, the need for Codex regulations for all the drugs acceptable to be used in food animal production persists around the world (FAO, 2020).

Sri Lanka is in combating AMR with multisectoral programs. The development of the National Strategic Plan (NSP) 2017-2022 provides the roadmap to combat AMR. The NSP is developed under five key strategies which are aligned with a global action plan. These strategies cover all aspects of combating AMR involving human, animal, agriculture, fisheries and environment sectors. The five strategies are expressed with specific objectives and short and long term (2 years and 5 years) including improving awareness and understanding of antibiotic resistance through effective communication, strengthening the knowledge and evidence base through surveillance and research, reducing the incidence of infection through effective sanitation, hygiene and infection prevention measures, optimize the use of antibiotic medicines in human and animal health and prepare the economic case for sustainable investment and increase investment in new medicines, diagnostic tools, vaccines and other interventions.

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Study on sanitary condition and the microbiological quality of water and ice in ice plants in Sri Lanka

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Abstract
Ice is the most versatile, abundant and economic chilling agent in fish supply chains from point of harvesting to final stages of processing of fish, globally. Fish and fishery products destined as food for human and also as feed for certain animals with commercial and social significance such as seal (Pinnipeds) reared in Zoological Gardens, have been reported as contaminated with pathogenic bacteria. This study, therefore, aimed at investigating the microbial quality of ice using in fishery industry in Sri Lanka. Quality of water used for making ice and ice produced were investigated in 73 ice plants located 16 districts. Source of water used for making ice (disinfected water from municipal water supplies, well or tube-well water); ice stored in cold store; and crushed block-ice which represented three main stages of an ice production line, were sampled from each ice plant, transported to NARA, and analyzed for faecal coliforms, *Escherichia coli,* and *Salmonella* using methods of Sri Lanka Standard (SLS) and International Standards Organization (ISO). The *Salmonella* isolated from samples were characterized into serotyping within Kauffmann-White scheme. In six ice plants, all three types of samples in ice production lines found with acceptable microbiological quality complying to the potable water quality (Sri Lanka Standard 614:2013). In 67 ice plants, all or at least one type of samples showed contaminations with faecal coliforms (1 - 1800+ MPN/100 mL) and *E. coli* (1 to 920 MPN/100 mL). At least ten different serotypes of genus *Salmonella* including *Salmonella* Brunei, *Salmonella* Tananarive, *Salmonella* Edinburg, *Salmonella* Kentucky, *Salmonella* Wilmington, *Salmonella* Hvittingfoss, *Salmonella* Sekondi, *Salmonella* Graba vi, *Salmonella* Braenderup and *Salmonella* Agona were detected among sixteen comprising all three types of samples collected from 67 ice plants. This study demonstrated a trend of gradual increase in both number of samples and/or level of contaminants down the three stages of ice production lines indicating a need of improving the infrastructure facilities and handling practices of local ice plants. It is suggested introducing a code of practice for ice plants; providing regular training programs for ice handling staff and operators on implementing of good manufacturing and sanitary practices; conducting regular monitoring programs on quality of water and ice produced in ice plant by competent authorities in order to mitigate presently occurring microbiological contaminations at high levels in ice plants.

Keywords: Ice plant, water, faecal coliform, *E. coli*, *Salmonella* serotypes, fish contamination

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Introduction

Ice plays a vital role in fisheries industry of Sri Lanka and similarly in other countries. Ice is used as the most abundant, economic and primary chilling agent of fish during handling dressing or processing of fish in fish processing plants. Also, fresh edible fish is stored in ice until the destination is reached since fish is a highly perishable commodity compared to other sources of animal meat. Fish is consumed as one of the main protein sources by local consumers and also part of high value fish is exported in return of significant amount of foreign revenue to Sri Lanka. Significant amount of ice is used by local fishery industry since present fleet size of local fishing boats is about 5590 including about 4800 of multi-day fishing boats which are in the range of 36 to 59 feet in length and making fishing trips for 14 days with about 20 days variation (Anon., 2020c, Internal Report of NARA). The total annual production of fish produced locally is about 505,830 mt in 2019 and the production of off shore or deep-sea fish by multi-day fishing boats was about 172,910 mt in 2019 (Anon., 2020c). A significant amount of foreign exchange is earned by local fish export industries and quantity of fish and fishery products exported from Sri Lanka in 2019 was about 28,771 mt in return of about LKR 53,483 Mn (Anon., 2020c).

About 3580 Mt of ice is produced daily by about 90 ice plants in Sri Lanka (Anon., 2020c). Nevertheless, demand for ice by users are in the rise due to continued expansion of local fishery industries with the concern of high-quality finished products. Block ice is produced in many of the local ice plants and are large quantity of block ice, in the form of crushed ice, is provided to fishing boats including multi-day boats that are harvesting large quantity of high value fish such as yellowfin tuna targeting for export market. Large amount of ice is used in fish holds of multi-day boats to maintain freshness of high value fish that has an export potential, during its fishing trips with long durations in the offshore.

Ice dispatched from Ice plants are also used for fish transport systems connected from fishery harbors and fish landing sites in remote areas to wholesale markets in Colombo and other town areas such as Kurunegala, Kandy and Balangoda and fish selling places in all part of Sri Lanka. Part of block ice produced is channeled to cement based construction industry in Sri Lanka (Personal communication) while some amount of block ice and flake ice is also supplied to other food industries. Present quantity of ice produced in ice plant is not sufficient to keep the total fish catch reported locally if such fish is required to be chilled practically between 0-4 °C under tropical ambient temperatures around 25 °C. Therefore, there is a shortage in production of ice for fishery industry from local ice plants in Sri Lanka.

Microbiological quality of fish is directly associated with the level of degradation of fish and fishery products. Therefore, making a better understanding on microbial contamination of fishery products and associated main utilities such as water and ice through scientific assessments could support to optimize the product quality as well as optimum utilization of the aquatic resources (Geethalakshmi *et al*., 2021; Svanevik, 2015; Tantrakarnapa, 2010). Quality of fish could be optimized by handling of fish under high level of hygienic conditions at early phase of the production line (Svanevik, 2015).

Faecal coliforms are naturally existing profusely in intestinal tracts of humans and other warm-blooded animals and eventually released to the external environment through their faeces as well.

E. coli is found widely in the intestinal tracts of warm- blooded animals and is generally reported as non-pathogenic. However, gastrointestinal diseases caused by *E. coli* strains containing toxigenic genes including Entero Toxigenic *E. coli* (ETEC), Entero Pathogenic *E.coli* (EPEC), Entero Invasive *E. coli* (EIEC), Entero Hemorrhagic *E. coli* (EHEC), Siga Toxin-Producing *E. coli* (STEC), Entero Aggregative *E. coli* (EAEC or EAggEc), and Diffusely Adherent *E. coli* (DAEC) are reported contaminating food materials including seafood (Barbosa *et al*., 2016; Costa, 2013). Some *E. coli* strains are reported capable of forming histamine in harvested fish. Therefore, using of ice contaminated with such *E. coli*, for chilling fish could make fish unsafe for consumption due to its direct pathogenicity and potential as histamine former in fish tissues (Refai *et al*., 2020).

Salmonella is reported to be naturally present in the environment and even in intestinal tract of some humans and more than 2,500 serotypes of *salmonellae* have been identified to date. Some of the routes of the initiation and spread of *Salmonella* infection in human are reported as consumption of food and beverages that were contaminated directly or cross-contaminated with animal faeces/ and sewage or indirectly through contaminated equipment and infected personnel as carriers in food-processing facilities. *Salmonella* is a clinically significant pathogen that bring about critical illness and even fatal to individuals (Shin, Chang, and Kang, 2004). As one of most versatile indicator organisms for faecal contamination in seafood, *E. coli* is enumerated in water and ice in relevant samples worldwide (Kumar *et al*., 2005).

Animal effluent born bacteria groups are more often found in high numbers in nutrient-rich effluents systems such as septic and animal sewage tanks; and run-off from farmlands. There may be opportunities to enter and contaminate sources of ground water that are used for manufacturing the ice in ice plants.

With the concern of microbiological contaminations found in commercially producing ice, scientific evidence based advanced technologies has been suggested for producing safe ice such as potential usability of activated ice containing bactericidal agents through cold atmospheric plasma and acidic electrolysis (Katsaros *et al*., 2021). Use of antibacterial ice which contains chlorine dioxide (ClO_2) at levels of 40 - 50 ppm is permitted in seafood by Food and Drug Administration (FDA), USA. Shin Chang, and Kang, (2004) reported the efficacy of reducing foodborne pathogenic bacteria including *Escherichia coli* O157: H7, *Salmonella typhimurium*, *Listeria monocytogenes* on fish surface during storage of fish in ClO_2 containing ice.

It is essential and mandatory to use water and ice of acceptable microbiological quality standards as specified for potable water in all stages of handling of fish after the harvesting such as cleaning and preprocessing; chilling; transporting and storing of fish and fishery products intended for human consumption in order to prevent the cross contamination of fish and fishery with fecal origin and other pathogenic microflora (Anon., 2007; Anon., 2020a; Anon. 2020b; Samakupa, 2003). Many of the studies conducted previously reported that ice has a high potential to be the main source of microbiological contaminant in fish since some of ice used as utility for chilling agent in food and also water used in fishing boats and fish landing harbors. Ice used in fish sales places are also contaminated with faecal contaminant bacteria and pathogenic bacteria including *Salmonella* and faecal streptococci (Ganegama Arachchi *et al*., 2000; Kariyawasam *et al*., 2007; Tantrakarnapa, 2010; Teixeira *et al*., 2019; Mako *et al*., 2014, Noor Izani *et al*., 2011; Vieira, De Souza, and Patel, 1997). In a study conducted across 13 ice plants from seven districts in Sri Lanka in 2007, high level of fecal contaminations in ice were found while *Salmonella* was not detected in water used for making ice and ice produced in those ice plants (Kariyawasam *et al*., 2007).

Fish exporters and competent authorities emphasize the need of the availability of acceptable quality and microbiologically safe ice for use in chill chain of fish distribution channel especially the supply chains destined at export-oriented fish processing industry in complying with Good Manufacturing Practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) as specified in regulation for fish products export in 1998 (Anon. 2013). Therefore, potable water quality ice should be available for using in all stages of fish handling starting from fishing boats up to storage of processed products including packaged chilled fresh fish, Ready- To-Eat (RTE) fish products such sashimi or other minimally processed fish products in processing plant, and consequent stages of transportation of finished products.

Present study planned to investigate microbiological quality of ice produced together with survey on infrastructure facilities and handing operations in ice production lines in ice plants located in different districts of Sri Lanka.To our knowledge this is the first study conducted covering more number of ice plants located in 16 Districts in order to evaluate sanitary condition and microbiological quality of water and ice along production line of ice plants which are commercially producing ice mainly for fishery sector in Sri Lanka.

Materials and Methods

Ice plants which produced ice mainly for the fisheries sector, were investigated in 16 districts from March, 2018 to May, 2019. Present status of ice producing line including condition of infrastructure of facility, handling operations, and sanitary practices were studied by making one field visit to each ice plant. Three types of samples required for microbiological examination were obtained from ice producing line of each ice plant.

Sample types obtained were the source of water used to produce ice, the block or flake ice stored in cold room, and the crushed-block ice made by using ice-crushing machine before dispatching the block ice from ice plant. About one liter of water or one kg of ice samples were collected in to sterile plastic bottles and pouches by following aseptic techniques as per ISO 19458:2006; samples were packed among clean ice in sample storing insulated boxes to maintain temperature at about 2 – 4 °C; and these boxes were transported under chill condition to Quality Control Laboratory in NARA. Samples were then analyzed within 24 hours after sampling for faecal coliform and *E.coli* according to methods given in SLS 1461 Part 1/Sec 3:2013. *Salmonella* was examined following methods given in ISO 19250:2010 (E) and isolated *Salmonella* cultures were characterized by serotyping according to Kauffmann-White classification method in Food and Water Microbiology Laboratory of Medical Research Institute (MRI), Colombo. Recommendations required for upgrading the status of ice plants based on the findings of present study were made by conducting consultative workshop with key stakeholders of ice manufacturing industry and fisheries sector.

Results and Discussion

Handling practices of ice in ice plants

Study was conducted on 73 ice plants located in 16 Districts (Trincomalee, Batticaloa, Jaffna, Mullaithivu, Anuradhapura, Dambulla, Polonnaruwa, Puttalam, Chillaw, Kalpitiya, Mannar, Amapara, Ratnapura, Moneragala, Kurunegala, Galle, Matara, Hambantota, and Kaluthara) (Table 1).The four main steps of a ice production line are inflow of water to ice making molds; freezing of water in ice mold (50 kg) dipping in brine solution of freezing tank; removing from molds and transferring of ice blocks to cold store (−5 to 10°C); and moving ice blocks to the ice crusher and crushing the block ice. Generally, the block ice is crushed in an ice crusher (ice crushing machine) and crushed ice is instantly packed in to polypropylene bags at the time of dispatching upon sales. Present survey found that many of ice plants operated under poorly maintained infrastructure facilities and with untrained or apprentice staff. Improper handling of ice by such staff could be linked with the cross contamination of ice with harmful microflora and extraneous material existed along ice production line in those ice plants.

Water supplied by municipalities was used as source of water manufacturing ice in 21 ice plants while ground water obtained from well or tube well water was used by 30 and 22 ice plants, respectively (Table 1). Block ice was produced in 66 ice plants while flake ice was produced in remaining eight ice plants (Table 1).

Microbiological quality of water used and ice produced in ice plants

Out of 73 ice plants, in six ice plants produced ice with acceptable quality complying with potable water quality standards. In all these six ice plants municipal water disinfected with chlorine was used for making ice and therefore, water used for making ice; ice stored in cold store; and ice crushed in the ice crusher were found not contaminated with faecal coliform, *E. coli* or *Salmonella* (Table 2).

Table 1. Source of water used for making ice and type of ice produced in 73 ice plants located in 16 Districts

District	Number of ice plants	Number of ice plants				
		Source of water for ice making			Type of ice produced	
		Municipal water	Well	Tube-well	Block ice	Flake ice
Trincomalee	5	1	3	1	5	-
Batticaloa	4	-	4	-	4	-
Jaffna	4	1	2	1	4	-
Mullaithivu	2	-	2	-	2	-
Anuradhapura	2	-	-	2	2	1
	1	-	-	1	1	-
Polonnaruwa	1	1	-	-	1	-
Puttalam	1	-	-	1	1	-
	2	-	-	2	2	-
Mannar	7	1	2	4	7	-
	5	‘	1	4	5	-
Ampara	3	-	-	3	3	-
Ratnapura	1	1			1	
Monaragala	1	-	1	-	1	-
Kurunagala	1	-	1	-	1	-
Galle	8	4	2	2	7	1
Matara	15	2	12	1	15	-
Hambantota	5	5	-	-	2	3
Kalutara	5	5	-	-	2	3
	73	21	30	22	66	8

Infrastructure facilities of ice plants have developed to maintain clean and sanitized condition in ice producing line such as inlet water lines, ice freezing cans, cold rooms and ice crushing machine made with stainless steel, protective floor areas for moving ice from cold store to ice crushing machine. Ice handling staff of these six ice plants also explicated the understanding on good sanitary practices and were wearing attire which could prevent contamination of ice during storing and dispatching of crushed ice to the buyers. Sixty-seven (67) out of 73 ice plants produced and dispatched unacceptable quality ice due to non-compliances occurred in at least one of the stages of ice production line including microbiologically contaminated water for making the ice, post contamination of ice blocks during storing in cold store and/or in the ice crusher. (Table 2).

Microbiological contamination of source of water used for making of ice:

Water supplied for manufacturing of ice in ice molds was found contaminated with faecal coliform and *E. coli* in 51 and 42 ice plants, respectively, and both contaminants were detected in the range of 1 to 1600 MPN/100 mL (Table 2). Therefore, such ice is unacceptable to be used in food industry.

Ice should be made with potable quality water that is devoid of faecal contaminant bacteria including faecal coliforms and *E. coli* bacteria (Anon., 2007; Anon., 2013; Anon., 2020a; Anon., 2020b). Municipal water supplies that were disinfected by chlorination were used to make ice in 21 ice plants which were located in Trincomalee, Jaffna, Polonnaruwa, Kalpitiya, Ratnapura, Galle, Matatra, Hambantota, and Kaluthara (Table1).

Salmonella were detected in water used for making of ice in four ice plants located in Trincomalee, Kalpitiya, Mannar, and Kalutara areas (Table 2 and Table 3). Serotypes of three isolates have been identified as *S.* Brunei, *S.* Kentucky, and *S.* Hvittingfoss (Table 3). Contamination of inlet water could be linked with the corroded and rusty water lines directed for water freezing chambers, lack of and not implementing regular cleaning and sanitation schedules and inadequate sanitary measures of working staff in ice plants.

Microbiological contamination of ice stored in cold room of ice plants

Ice manufactured in the form of blocks or flakes by freezing water in ice plants were then transferred to cold room operated at temperature between -5 to -10 °C at the ice plant. Ice stored in cold room was contaminated with faecal coliforms in the range of 1 to 1600 MPN/100mL in 61 ice plants and *E. coli* in the range of 1 to 920 MPN/100 mL in 58 ice plants (Table 2). *Salmonella* was isolated in ice obtained from cold store in 5 ice plants situated in Trincomalee, Mullaithivu, Kalpitiya and Tangalle. *Salmonella enterica* isolates were characterized as five different serovars including Kentucky, Edinburg, Wilmington, Agona, and Sekondi (Table 2 and Table 3). Ice blocks frozen in freezing molds (cans) are separated mechanically from molds, then transferred and stacked in walked-in cold store by ice plant operators.

Table 2. Level of contaminations of faecal coliform, *E. coli* and occurrence of *Salmonella* in ice making water, ice stored in cold store and crushed ice using ice crusher in ice plants before selling of ice

Total No. of ice plants	No. of ice plants (Quality of ice)	Sample types	Number of ice plants		
			Faecal coliforms (MPN/100 mL)	*E.coli* (MPN/100 mL)	*Salmonella* (100 mL)
73	6 (Acceptable quality)	Source of water*	ND	ND	ND
		Ice in cold store	ND	ND	ND
		Ice from crusher	ND	ND	ND
	67 (Unacceptable quality)	Source of water§	51 (1 to 1600)	42 (1 to 1600)	4
		Ice in cold store	61 (1 to 1600)	58 (1 to 920)	5
		Ice from Crusher	62 (1 to 1800+)	57 (1 to 1800+)	7

ND, Not Detected; *Municipal water; § Municipal water, well or tube well

Sanitary measures were found inadequate in ice plants that contained contaminated ice in cold store since staff working in area of cold store, lacks proper sanitary outfits specific for the task such as gloves, shoes or gum boots; and movements of all staff is not controlled across the floor areas of the ice production line. Therefore, it is more likely to cross contaminate the passage of floor on which ice blocks are moved from ice molding tanks to cold store and interior of cold store itself.

Microbiological contamination of crushed ice dispatching from ice plants

In 62 ice plants, faecal coliforms were detected in the range of 1 to 1800+ MPN/100mL in crushed ice (Table 2). *E. coli* was detected in crushed ice in the range of 1 to 1800+ MPN/100 mL 57 ice plants (Table 2). Seven isolates of *Salmonella* were found from crushed ice that were prepared instantly by moving the ice blocks on a passage of floor (corridor) from the adjacent cold store to the ice crusher and then, crushing the ice blocks loaded in to the ice crusher.

It was observed that there is a possibility to contaminate ice at site of preparation of crushed ice as the final stage of ice production line since floor area of ice moving path which is not demarcated for restricting the movement of the unauthorized staff for between cold store and corridor near ice crushing machine. Ice handling staff were not in appropriate clothing, and outfits required for maintain good sanitary conditions. Tantrakarnapa *et al*., (2010) have suggested the need of establishing and implementing proper sanitary programs in ice plants as they have found the presence of coliform bacteria in ice plants where workers are wearing net caps, aprons and boots. In the present study, *Salmonella* isolated from crushed ice belonged to three serovars including *S*.Tananarive, *S*.Graba vi, and *S*.Braenderup. Ice crushing machines used at many of ice plants were with corroded and rusty interior surfaces making those difficult to clean and sanitize properly. Harbouring of stray animals and birds in ice handling area which is not covered with protective barrier fence or cover for keeping stray animals, and bird droppings could be identified as other sources of contamination of crushing ice with faecal origin bacteria and *Salmonella*.

A trend of gradual increase in number of incidences and level of faecal contaminant bacteria (faecal coliforms and *E. coli*) was observed along ice production line (Table 2). Similarly, gradual increase in incidences of *Salmonella* was detected as 4, 5 and 7 in samples of ice making water, ice in cold store and crushed ice, respectively, in the present study (Table 2).

Salmonella is a significant food and seafood born pathogen in concern of public health that could enter seafood supply chain from fish contaminated with *Salmonella* naturally present in the aquatic environment, or fish, water, ice and handling surfaces contaminated with sewage and faecal matter originated from human and animals (Olgunoglu, 2012). Additionally, ice contaminated with *Salmonella* also has the potential for spreading infections through other chill products such as beverages prepared with and or cross contaminate other foods in food processing or cooking facilities. Centers for Disease Control and Prevention (CDC) in USA has reported about 62 people infected due to the consumption of frozen raw tuna with *Salmonella* across 11 states of USA in 2016 (Sanjee and Karim, 2016). Local and international regulations and standards specify that fecal coliform, *E. coli* and *Salmonella* should not be contained in water and ice used in food industry (Anon., 2007; Anon., 2013, Anon., 2020a; Anon., 2020b). Information on *Salmonella* infections or outbreaks in Sri Lanka is not abundant due to the common practice of not consuming raw or mildly cooked fish products by local consumers and poor recording of such clinical data. Sanjee and Karim (2016) have reported a similar situation in Bangaldesh as foodborne illness related to fresh or frozen seafood consumption has not been traced and reported or data on cases are still lacking and they have suggested that the scientific information on analysis for pathogenic bacteria related to seafood industry would help in preventing and controlling future outbreaks related to seafood consumption.

As effective mitigatory measures for controlling *Salmonella* in seafood, implementing integrated programs including the monitoring of water quality; disease surveillance; consumer education; harvesting, processing, and marketing seafood industry by competent authorities and experts from sector of public health, veterinary and food safety who are responsible for seafood industry have been suggested (Amagliani *et al*., 2012). Present investigation also emphasized a need of taking appropriate measures for improving manufacturing lines of local ice plants to combat high levels faecal coliform counts and *Salmonella* in water and ice from ice plants ensuring food safety of local consumers as well as local fish export industry that brings considerable amount of foreign exchange to Sri Lanka.

Preparation of recommendations for upgrading ice plants for producing microbiologically acceptable quality ice

A consultative workshop organized and held in NARA, was attended by 73 stakeholders representing owners of ice plants, buyers or users of ice, traders or distributors of ice, personnel from Department of Fisheries as Competent Authority, officials from Food Control Unit of Ministry Health, Sri Lanka Standards Institute, and Consumer Affairs Authority.

Results of microbiological analysis of water and ice from 73 ice plants were communicated individually with relevant ice producers in view of poor sanitary conditions of ice plants by posting the test reports on water and ice in advance. Further discussions were extended at the workshop as a common forum on the mitigatory measures and practical solutions on how to upgrade infrastructure facilities and unit operations of ice manufacturing line of their ice plants to a level of producing acceptable quality ice under good sanitary and GMPs by providing them with a printed copy of hand book which contained information on way of producing good quality ice. Chlorination of water for making potable quality water is given in detail by World Health Organization (Anon., 2017).

Table 3. Serotypes of *Salmonella* identified in 15 samples (ice making water, ice from cold store and crushed ice) obtained from ice plants

Sample type	Plant located area	Serotype of *Salmonella enterica* sub species *enterica*
Ice making water	Trincomalee	Brunei
	Kalpitiya	Kentucky
	Mannar	NC
	Kaluthara	Hvittingfoss
Ice from Cold Store	Trincomalee	Kentucky
	Mullaithivu	Edinburg
	Kalpitiya	Wilmington
	Kalpitiya	Agona
	Tangalle	Sekondi
Ice from Ice Crusher	Trincomalee	Tananarive
	Moneragala	NC
	Galle	Graba vi
	Galle	Graba vi
	Tangalle	Braenderup
	Tangalle	NC

NC: Not Characterized

Hampikyan and his team (2017) have suggested the importance of complying with important prerequisites such as the maintenance, cleaning and disinfecting of ice machines which should be carried out effectively and periodically; and the availability of adequately sanitized water with sufficient amounts of chlorine or other proper methods such as UV and ozone treatments to ensure the production of acceptable quality ice in ice plants.

For manufacturing good quality ice, it is also required to have well trained technical staff for operating the ice producing line hygienically and attending the routine maintenance of ice manufacturing infrastructure facilities; the periodic checking of microbiological quality of ice and the hygienic conditions of ice machines; and ensuring the hygiene of staff working in ice plants as per the requirements of HACCP and ISO 22000 food safety management systems etc. (Hampikyan *et al.*, 2017, Mako *et al*., 2016).

Participants of many of ice plants at the consultative forum admitted that many of ice plants are in need of upgrading to suit producing high quality ice as infrastructure of some ice plants were old more than 20 years without any refurbishment. Nevertheless, they also stated that it is difficult to allocate funds required for upgrading their ice making facilities over the presently incurring high electricity costs and taxes. Representatives of six ice plants shared their protocols and experiences voluntarily on how they managed to produce good sanitary quality ice using their existing basic infrastructure facilities that were well maintained, despite the prevailing high utility costs such as electricity power and taxes incurred in manufacture of ice in their ice plants.

At an interactive session in consultative workshop, it was ratified nearly all challenges faced by ice manufacturing industry against producing good quality ice at a fare selling price affordable for fisheries industry, and accordingly, a set of recommendations to be implemented as practical solutions for preventing the presently existing high level of bacterial contaminations during then ice manufacturing processes. Matters discussed and recommendations required for initiation of implementing GMPs in local ice plants are mentioned in Table 4.

Conclusion

In conclusion, this study found the presence of high level of fecal coliforms, *E. coli* and 15 isolates of *Salmonella* belong to at least 10 serovars in water used for ice making, ice stored cold stores, and crushed ice in ice crushers of 67 ice plants located in 16 districts. All ice plants which used ground water from well or tube wells directly without performing disinfection treatments, found producing unacceptable quality ice. Whereas six ice plants were producing acceptable quality ice by using disinfected water obtained from municipal water supplies and therefore, it is suggested the importance of commencing the chlorination of water before using in ice manufacturing line as one of the immediate practical solutions to mitigate microbiological contamination of ice.

At the consultative forum, recommendations made in view to mitigate high level microbiological contamination of ice produced in ice plants, were the improvement and proper maintenance of basic infrastructure facilities of ice production line; conducting regular training programs for working/handling staff and machine operators; introduction of code of practice for production of ice; and implementation of sanitary programs and GMPs together with regular surveillance and monitoring of quality of water and in compliance with food safety regulations (Table 4).

Table 4. Matters discussed and recommendations made by stakeholders based on the findings of the study at consultative workshop

No.	Issue/Constraint	Reason/Root cause	Recommendations/Remedial measures for upgrading ice plant
1.	Ice plant facility: Rust-decay of ice cans (metal container used to freeze water for making ice bock) are used in many of ice plants	• Commonly, ice cans are made using galvanized sheets. These galvanized – ice cans cannot be used for longer period due to rust-decay. Production cost for one ice can is about Rs. 15,000.00. High costs for stainless steel is not affordable. • Ice crushing machine: Ice holding passage of machine made with galvanize sheets, in some ice plant, are not in suitable condition due to rust-decay. • Cold room (Ice storing): Floor of cold room is made of wood; therefore, it is difficult to clean the floor. • Poor designing of ice plant lay out has led to cross contamination.	Provide funds for developing/Improving infrastructure facility of ice plant: • Government intervention is required for obtaining funds. • Loan schemes with low interest. • Provide grants for all ice plant. • Tax concession for ice industry. • Project on Enterprise Sri Lanka (Funding opportunities under Enterprise Sri Lanka) should provide loan facilities. Provide Technical support: • Marine grade stainless steel (316 stainless steel or suitable grade) material should be introduced to make ice cans. • Crusher: Should be made of stainless steel, and be Movable/portable type. • Permanent cover should be made over the ice crusher. • Use of fibreglass cover/ pellet for interior floor/fibber glass pellets of cold room is suggested. • Other alternate material with higher strength for floor of cold room is to be introduced. • Re- building of ice factories for good hygienic practices. • Establish safety equipment in ice plant.
2.	Source of Water: Unacceptable quality water is used for manufacture of ice	• Municipal water or other clean water sources that disinfected adequately to meet microbiological standards of potable quality, is not available in some areas of Districts	Guidance/Technical support: • Identify service providers/companies for water filtration and chlorination and dosing pump facilities etc. should be identified for ice manufacturing industry.

3.	Quality assurance of production process and environment of Ice Plant: *Production process*	• Lack of monitoring of ice production process.	• Establishment of regular programs for inspection/auditing/monitoring ice plant by Competent Authority. • Establish Official sample testing program for ice and water from Ice plant by Competent Authority, Health Ministry. • Implementation of Good manufacturing practices (GMPs) in ice plant. • Introduction and implementation of monthly maintenance schedule for Ice Plant. • Introduction of Code of Practice, Sri Lanka Standard Institute (SLSI)/International Standard Organization (ISO) Standards for ice production facility. • Health issues of workers: Schedules to be introduced for regular medical check- up work force (regular compiling) and obtaining medical reports by competent authorities. • Motivation program for ice producers such as rewarding of best Ice factory annually.
	Environment	• Lack of knowledge on sanitation of ice plants. • No action has taken to reduce the environment pollution of surroundings of ice plants.	• Onsite-awareness programme for staff of ice plant and ice users on sanitation and environmental pollution by competent authorities/Public Health Inspectors (PHI) and/or Medical Officers of Health (MOH). • Consumer awareness programs. Note: Many of participants of this meeting indicted that pollution of surrounding of ice plant at Galle Fishery Harbour. They emphasized need of cleaning/removing obsolete boats and other rubbish.
4.	Trainings and awareness programs for ice plant staff (Operators /Managers) and ice users	• Lack of trainings for ice plant staff members.	• Providing technical trainings/ Technology transfer programs/Awareness programs/ Occupational safety programs by competent authority/NARA/MOH/PHI and/or Food and Drug Inspectors. • Onsite tailor-made trainings on Ice plant sanitation and personal health and safety for staff of each Ice Plant. • Trainings on Good manufacturing practices (GMP) for Ice Plant, Sanitation, Personal hygiene, occupational safety etc. • Regular refresher trainings for staff.

			• Training materials: Manuals, leaflets, booklets, posters, pictures/images for creative of awareness effectively. For example, Posters explaining the cross contaminations, health risks due to presence of pathogenic bacteria in Ice Plant.
5.	Price increase for ice at Ice Plant	• Ice quality should be improved by upgrading facilities and process therefore; price of ice block is to be increased.	• Price of ice should be increased in order to produce high quality ice while improving and developing existing infrastructure facilities and operations in ice plant (to maintain best manufacturing practice, invest in better sanitary quality and setting of safety equipment and labour training etc.)
6.	Shortage of labour force	• Shortage of labour /lack of apprentice/ difficulty retain ice plant operators for several years continuously.	• Take action to retain ice plant staff by offering them with a good salary, training, good work environment etc.
7.	Contamination of ice during distribution	• Ice blocks and crushed ice purchased at ice plant are highly contaminated due to use of contaminated plastic bags, bins boxes etc for moving after the purchase .	• Establishment of procedures to avoid cross contamination of ice during the dispatch from ice plant. • Introduce clean packaging systems packaging materials of ice for buyers. • Awareness programme for buyers about the potential for cross contamination of ice during subsequent handling, transportation and storage. • Education programs should be conducted to MOHs and PHIs on present sanitary status of ice plants.

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Status of shrimp trawl fisheries in the seas off Kalpitiya and Mannar on the Northwestern coastal waters of Sri Lanka

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Abstract

A study was carried out to find out variations of Catch Per Unit Effort (CPUE) and total shrimp production in Kalpitiya and Mannar trawl fisheries. Monthly catch and effort data were collected from January to December 2021, and monthly average CPUE (shrimp weight per fishing trip) and monthly total shrimp production were estimated. Generally, 23, 11 ton trawlers and 180, 3.5 ton boats were operated in Kalpitiya and Mannar respectively without any closer seasons. Being opposite to Kalpitiya (06 fishing days per trip per week), in Mannar, fishing was conducted in the night (03 single fishing days per week). *Penaeus semisulcatus* was the most abundant targeted shrimp species and pony fishes were dominant in the by-catch. The CPUE (kg of shrimps 06 days $trip^{-1}$ $boat^{-1}$) of Kalpitiya fishery ranged from 94 kg (in February) to 189.7 kg (in August). Also, the estimated annual total shrimp production in Kalpitiya was 142 tons which mostly comprised medium sized (30 – 49 g) shrimps. CPUE (kg of shrimps 01day $trip^{-1}$ $boat^{-1}$) of Mannar exhibited the lowest (12.2 kg) in October and the highest (38.7 kg) in February and an annual total shrimp production of 415 tonnes mainly with small sized (13 – 29 g) shrimps. In addition, higher mean CPUE of Kalpitiya was recorded during the Southwest monsoon season (around 48% of total annual shrimp production) while, for Mannar it was coincided with the Northeast season (about 30% of total production). However, statistical analysis revealed that in the Mannar shrimp fishery only, there is a significant difference ($P< 0.05$) in mean CPUE between four monsoonal seasons. Net income per trawl trip was LKR 180,000 and 15,000 while operational costs were LKR 140,000 and 9,000 in Kalpitiya and Mannar respectively.

Keywords. Fisheries, monsoon, shrimp, sustainability, trawling.

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Introduction

Shrimp fishery provides greatest contribution in Sri Lankan fisheries sector via aquaculture production as well as wild capture (Jayasinghe *et al*., 2019). Bottom trawling is widely used to capture the wild shrimp around the world and in Sri Lanka too, trawling is widely practiced especially in the Northwest and Northern coastal sites (Wijesundara and Amunugama, 2017). In early days, Sri Lankan fishermen were engaged in trawling in some offshore trawling grounds that included Wadge Bank, Pedro Bank, Palk Bay and Gulf of Mannar. However, after signing the maritime agreements in 1974 and 1976, Sri Lanka faced the restrictions and challenges to access these fishing grounds (Kularatne, 2019). As a considerable portion of the continental shelf around Sri Lanka has rocky bottoms, shrimp trawling is restricted to muddy areas (Jayawardane and Dayaratne, 1998).

In 2017, bottom trawling was banned in Sri Lanka through an amendment made to the Fisheries and Aquatic Resources Act of 1996. Although there are alternative fishing gears and methods that may be economically viable in certain situations, capturing of benthic target species such as penaeid shrimps would pose challenges without some form of bottom trawling (Suuronen *et al*., 2012). Due to the lack of efficient fishing gear for wild shrimp harvesting in Sri Lanka, trawling is still continued within the demarcated fishing grounds, concerning the socioeconomic aspects of fishers. Trawl fisheries are consequently confined to limited areas in the West coast; North of Colombo (Hendala), North of Negombo, Portugal Bay off Kalpitiya, Mannar off Pesalei and Jaffna peninsula by ensured with the absence of eco-sensitive habitats (NARA, 2019).

The trawling activities conducted in particular regions almost throughout the year are expected to cause heavy resource exploitation in an unsustainable manner. Hence, proper scientific management is much needed to ensure the sustainability of fishery resources in defined coastal waters. In the present study, a survey was conducted in Kalpitiya and Mannar to assess seasonal variation of the shrimp trawl fisheries using Catch Per Unit Effort (CPUE) and total production. Also, based on the biological sampling of the landings, monsoonal changes in length frequency, weight frequency, sex and maturity stage of major shrimp species exploited were investigated.

Materials and Methods

The study was carried out from January to December 2021. About 14.9 km^2 and 427.9 km^2 of fishing ground area were permitted for the trawling activities in Kalpitiya and Mannar respectively (Fig 1). In the present study, samplings were conducted at the landing sites and shrimp landing huts in Kalpitiya and Mannar/ Pesalei. Throughout the year, monthly visits were conducted to each study site to collect catch data from the active trawls. Subsequently, shrimp and major bycatch species composition from trawl catches were identified using standard identification guides (De Bruin *et al*., 1995; Dore, 2012). Additionally, detailed information regarding vessel operation, gear type, and fishing periods were gathered by interviewing fishermen. Furthermore, the logbook was utilized to gather catch and effort data from trawl fishers in each region.

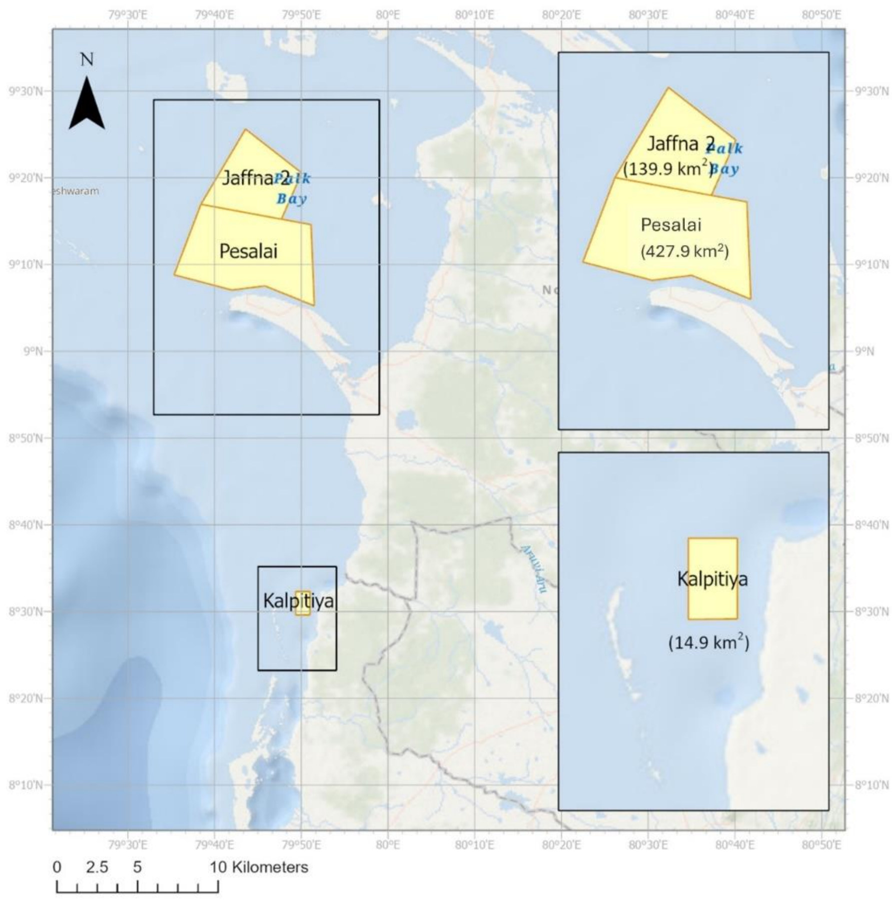

Fig. 1. Shrimp trawl fishing grounds in Mannar (Peaslai) and Kalpitiya

Catch and effort (CPUE) data collection and analyses:

Data collections were conducted on a monthly basis, and the logbook's monthly fishing trips and catch data were collected and standardized. All the relevant data sets were digitalized and estimates were made for CPUE and total shrimp production for each month at each site, considering different size categories (Large-L, Medium-M, Small-S, and Very small-VS). CPUE was expressed as kilograms of shrimp caught per boat per fishing trip. Monthly total shrimp production (P) was estimated as follows:

$P = CPUE \times N \times T$

where N is the mean number of fishing crafts operated per trip and T is the mean number of fishing trips per month.

Monthly variations of CPUE and total catch of shrimp were assessed separately for Kalpitiya and Mannar. To investigate the influence of monsoonal patterns on shrimp landings, CPUE data were grouped into four monsoonal phases i.e., Northeast monsoon (December-February), First inter-monsoon (March-April), Southwest monsoon (May-September) and Second inter-monsoon (October-November).

CPUE of four monsoonal phases were statistically investigated and to better satisfy the assumptions of normality required for the one-way analysis of variance, log-normal distribution data converted into normal distribution data *(Ln (CPUE+1))* as prescribed by Gulland (1983). Subsequently one-way ANOVA was carried out in order to reveal whether the mean values in different seasons are significantly different ($\alpha = 0.05$) using Minitab® 21.2 statistical software. In presence of significant difference, post-hoc test (Tukey test) also carried out for pair-wise comparison of monsoonal phases.

Biological sample collection and analyses:

The shrimps were categorized into four size groups by fishermen for sorting: large size (L) for specimens above 50 g, medium size (M) for specimens weighing between 30 g and 49 g, small size (S) for specimens weighing between 13 g and 29 g, and very small size (VS) for specimens weighing between 08 g and 12 g. As these size categorizations allowed for assessing the quantity of shrimp populations overall, regardless of specific species.

On each sampling day at each site, a standardized amount of (2 kg) pre-sorted shrimp was extracted from the trawl catch. These sub-samples were assumed to represent the composition and distribution of trawling sites during the study period. All samples were properly labelled, kept in styrofoam boxes with ice and transported to the NARA laboratory at Kalpitiya Regional Research Center for further analyses. From these samples, total length, carapace length, individual weight, sex and maturation level were examined for each specimen. Seasonal variations of length frequency and sex distribution were also investigated.

Results and Discussion

Catch and effort information

The demarcated trawling ground in Kalpitiya is 14.9 km^2 in extent at the site of Portugal Bay. There are 23 registered 11ton trawlers with 95 HP inboard engines. On average, 20 boats are engaged in fishing in a day. The number of fishing trips per month is not consistent and as such, the actual number of trawler fishing trips was verified through harbour records. Bottom trawl nets with typical size (body mesh size: 3.8 cm, cod-end mesh size: 2.5 cm and mouth width: 9.4 m) are used by all trawler boats. Trawl fishing in Kalpitiya is a year-round activity without any off-season. The number of fishing days per trip is generally about 06 days (Monday to Saturday) with four fishing trips per month. About 16 number of hauls are carried out by single boat per trip and the trawling process strictly restricted to daytime only.

For Mannar trawl fishery, the designated area is approximately 428 km^2 in the vicinity of Palk Bay and Gulf of Mannar. About 180 number of 3.5ton trawler boats with 30 HP inboard engines are operated from the landing site of Pesalei. Bottom trawl nets with typical size (body mesh size: 3.8 cm, cod-end mesh size: 2.5 cm and mouth width: 3.6 m) are used by all trawler boats. Fishing is conducted on a single-day basis and average number of boats operated per day is 150. There are three fishing trips (Monday, Wednesday and Saturday) per week and about 12 fishing days per month. In Mannar too, trawling is a year-round activity. Unlike Kalpitiya trawl fishery, the trawling activity in Mannar is performed during night-time.

In both trawl fishing grounds of Kalpitiya and Mannar, *Penaeus semisulcatus* (green tiger shrimp) is the major target shrimp species. Other shrimp species that contribute to the landings are *Penaeus merguiensis* (banana shrimp), *Penaeus indicus* (Indian white shrimp) and *Metapenaeus moyebi* (moyebi shrimp). *M. moyebi* has relatively less commercial value, while prices of other species both in export and local markets are based on different size categories. *Leiognathus* sp. (pony fish), ranks as the primary bycatch species in both locations followed by *Gerres abbreviates* (Deep-bodied mojarra), *Selaroides leptolepis* (yellowstripe scad), *Portunus pelagicus* (blue swimming crab) and *Sepia* sp. (cuttlefish) also documented as bycatch, making a noteworthy contribution to the total catch.

By referring to catch composition, the average shrimp caught contribution to total trawl catch is about 30% and 45% in Kalpitiya and Mannar respectively. In Kalpitiya, *P. semisulcatus* accounts for ca. 80% of the total shrimp production and ca. 25% of the total trawl catch, meantime *Leiognathus* sp constitutes ca.70% of bycatch and ca.50% of total trawl catch. In Mannar, *P. semisulcatus* responsible for a sole contribution of ca. 88% to the total shrimp production and ca. 40% of total trawl catch. Simultaneously, *Leiognathus* sp comprises ca. 57% of bycatch and ca. 32% of total trawl catch.

Catch per unit effort (CPUE) and total shrimp production

Kalpitiya trawl fishery:

The CPUE of Kalpitiya trawl fishery ranged between 94 kg. $boat^{-1}trip^{-1}$ and 189 kg. $boat^{-1}trip^{-1}$ throughout the study period. The lowest CPUE was recorded in the February period (94 kg. $boat^{-1}trip^{-1}$) and the highest was in August (189.7 kg. $boat^{-1}trip^{-1}$). The results show there was a gradual rise in CPUE from February to May and a gradual decline from August to December having a distinct decline during the period of June to July (Fig. 2).

The highest value of CPUE representing small-sized/ juvenile shrimps mostly observed during April possibly representing the recruitment season of shrimp to particular trawl ground. However, during June – October, small-sized shrimp catch was very low. Also, when compared to other sizes, catch of medium-sized (30g - 49g) shrimp dominated throughout the year.

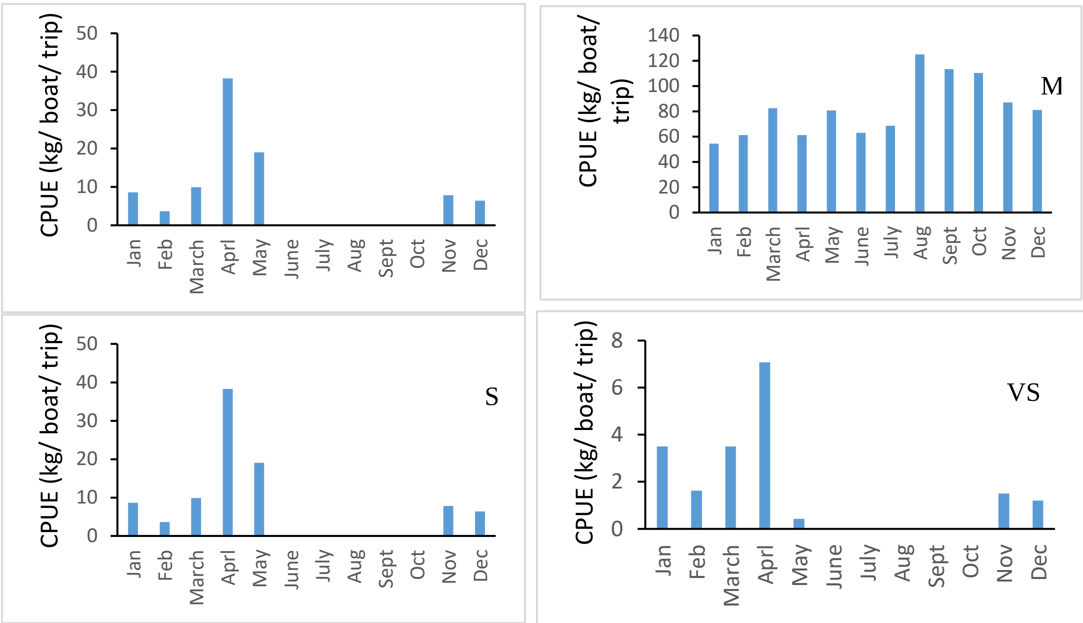

Fig. 2. Monthly variation of CPUE for different sized shrimp caught in Kalpitiya. L: Large size; M: Medium size; S: Small size; VS: Very small size

The total shrimp production of Kalpitiya trawl fishery in 2021 was estimated at 142 tonnes. Monthly total shrimp production (Fig. 3) indicates that the highest shrimp production occurred from August to November, having a contribution of about 47% of the annual shrimp production. In comparison, in the period between January and July, shrimp production was the lowest in Kalpitiya.

Variations in mean CPUE of 4 categories of shrimp in the trawl landings in Kalpitiya (Fig. 4) during four seasons indicate that medium-sized shrimp dominated during all four seasons followed by large-sized shrimps. The pattern of variation of mean CPUE of medium-sized and large-sized shrimp was similar registering the peak values during the southwest monsoon period and second inter-monsoonal period. The mean CPUE of small and very small shrimp was much lower in all four seasons. As the CPUE of small-sized shrimp was the highest during the north-east monsoonal period, this season might represent recruitment of shrimp to the fishing area.

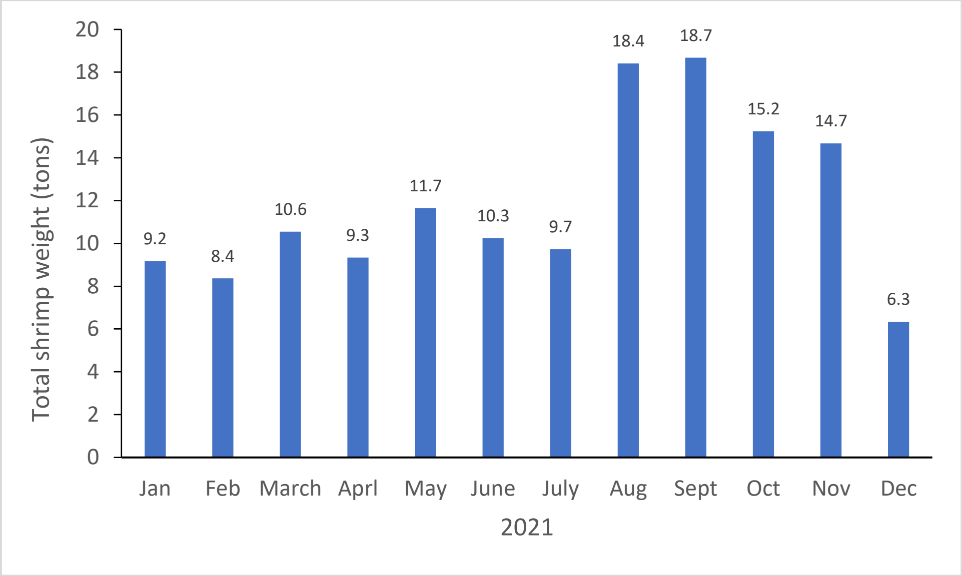

Fig. 3. Total monthly shrimp production in Kalpitiya trawl fishery in year 2021

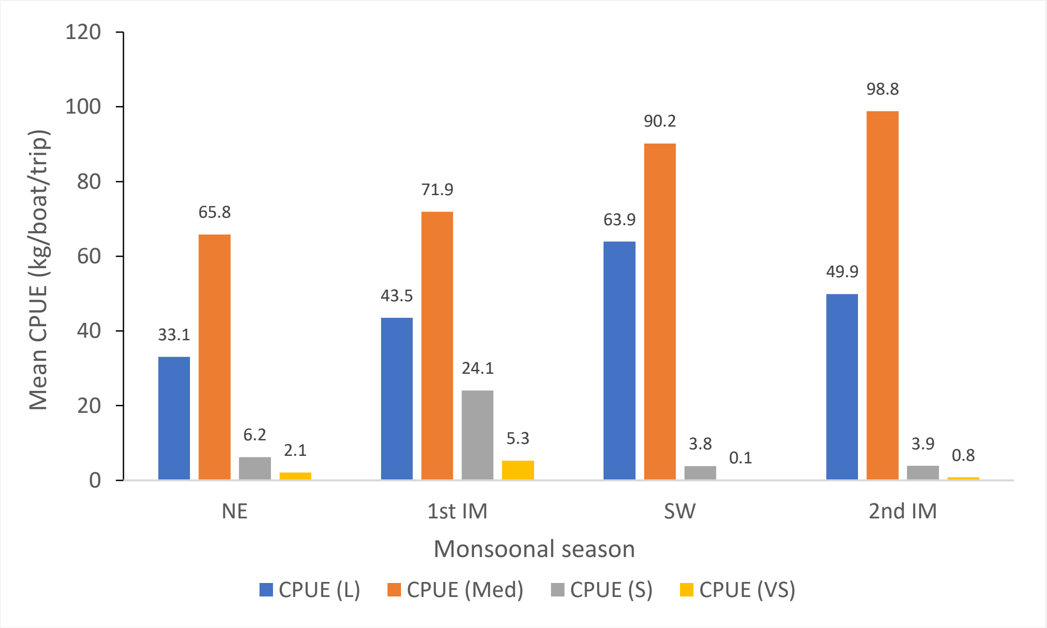

Fig. 4. Seasonal variation of mean CPUE represent Kalpitiya shrimp production in 2021. NE: North-east monsoon; 1st IM: First inter-monsoon; SW: South-west monsoon; 2nd IM: Second inter-monsoon

Statistical analysis on CPUE reveals, that no significant differences ($P > 0.05$) exhibited in overall shrimp production among four monsoonal phases irrespective to size variations. In concern of four size classes, small-sized and very small sized shrimps only show significant differences ($P < 0.01$) among four monsoons. Post-hoc analyses (Tukey pairwise comparison test between monsoons) reveals the significance of shrimp catch among different monsoonal phases (Fig. 5). For small-sized shrimps, differences exist between northeast–southwest and 1^{st} inter-monsoon – southwest, while for VS sized shrimps, differences are observed among northeast–southwest, northeast – 2^{nd} inter-monsoon, 1^{st} inter-monsoon – southwest, and 1^{st} inter-monsoon – 2^{nd} inter-monsoon.

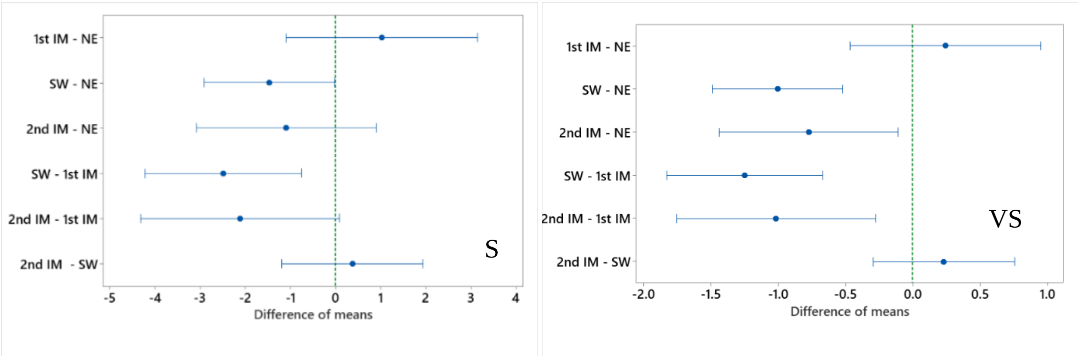

Fig. 5. Interval plots of Tukey simultaneous test (at individual confidential level of 95%) for differences of the means of CPUE represents Kalpitiya with pairwise monsoonal phases (S: Small sized shrimp; VS: Very small sized shrimp). An interval does not intercept the zero-reference line of mean difference (green dotted line), the corresponding means are significantly different. NE: North-east monsoon; 1st IM: First inter-monsoon; SW: South-west monsoon; 2nd IM: Second inter-monsoon

Mannar trawl fishery:

The CPUE of Mannar trawl fishery ranged between 12 kg $boat^{-1}$ $trip^{-1}$ and 38 kg $boat^{-1}$ $trip^{-1}$ throughout the study period. The lowest CPUE was recorded in the October period (12.2 kg $boat^{-1}$ $trip^{-1}$) and the highest was in February (38.7 kg $boat^{-1}$ $trip^{-1}$). The results show there was a firm rise in CPUE from January to February and a gradual decline from March to May, having a distinct decline during the period of June to October. The highest value of CPUE represents small and very small-sized/juvenile shrimps mostly observed during January to February, possibly representing the recruitment season of shrimp to particular trawl ground. However, from June to October, small and very small-sized shrimp catch was very low. In contrast, the CPUE of large-sized shrimp shows a gradual rise from March to June and has a distinct peak up to the period of September. Also, when compared to other sizes, the catch of small-sized (13 g – 29 g) shrimp dominated throughout the year (Fig. 6). The total shrimp production of Mannar trawl fishery in 2021 was estimated at 415 tonnes. Monthly total shrimp production (Fig. 7) indicates that the highest shrimp production occurred from January to April, having a contribution of about 42% of the annual shrimp production. In comparison, the period between July to October, shrimp production was the lowest in Mannar.

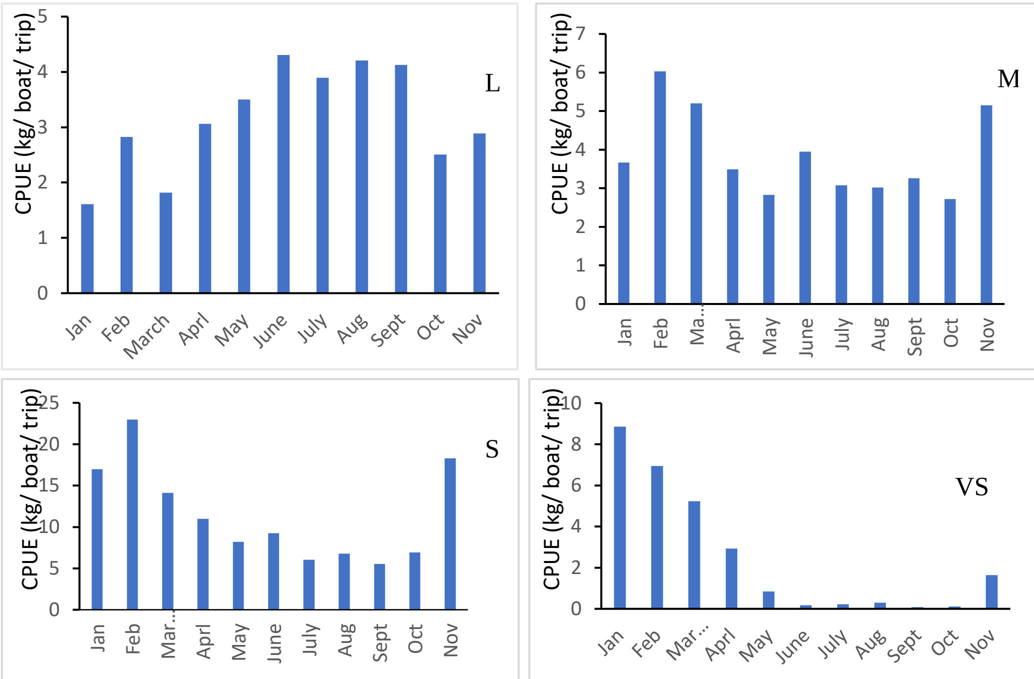

Fig. 6. Monthly variation of CPUE for different sized shrimp caught in Mannar. L: Large; M: Medium: S: Small; VS: Very small

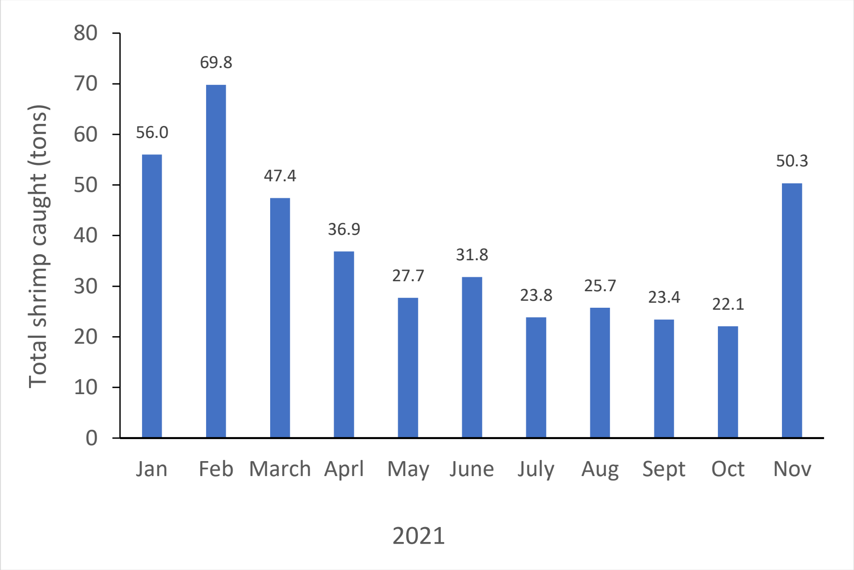

Fig. 7. Total monthly shrimp production in Mannar trawl fishery in 2021

Variations in the mean CPUE of 4 categories of shrimp in the trawl landings in Mannar (Fig. 8) during four seasons indicate that small-sized shrimp dominated during all four seasons followed by medium-sized shrimps. The pattern of variation of mean CPUE of small-sized and medium-sized shrimp was similar registering the peak values during the Northeast monsoon period. Moreover, small-sized shrimp, solely represent about 57% of caught shrimp in the Northeast season. The mean CPUE of large-sized shrimp was much lower in all four seasons. As the CPUE of small and very small-sized shrimp was highest during the Northeast monsoonal period, this season might represent the recruitment of shrimp to the fishing area.

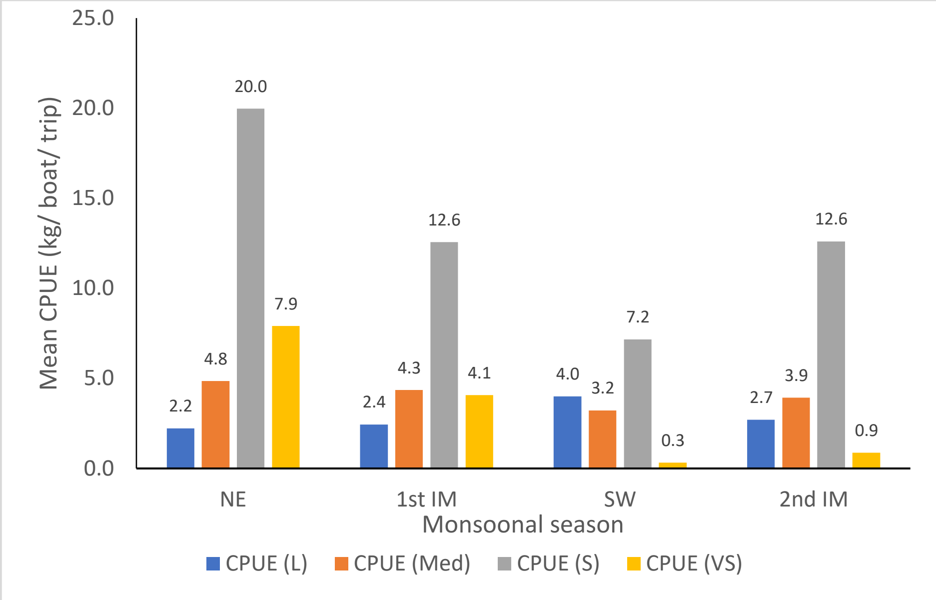

Fig. 8. Seasonal variation of mean CPUE represents Mannar shrimp production in 2021. NE: North-east monsoon; 1st IM: First inter-monsoon; SW: South-west monsoon; 2nd IM: Second inter-monsoon

Statistical analysis on CPUE reveals that a significant difference ($P < 0.01$) exists in overall shrimp production among four monsoonal phases irrespective of size variations. In concern of size categories, all four sizes show significant differences (large: $P < 0.05$; medium, small and very small: $P < 0.01$) among the four monsoons. Tukey pairwise comparison test reveals the specific significance of shrimp caught in between different monsoonal phases. For all four sizes, differences exist between northeast–southwest and 1st inter-monsoon – southwest. Moreover, small and very small-sized categories expressed the mean difference between northeast – 1st inter-monsoon, northeast – 2nd inter-monsoon, 1st inter-monsoon – 2nd inter-monsoon phases also (Fig. 9).

Seasonal size variation of main target shrimp species

In both Kalpitiya and Mannar trawl fisheries, *Penaeus semisulcatus* was the most targeted and abundant shrimp species. Seasonal variation of length classes of *P. semisulcatus* in the two fishing areas was investigated. In Kalpitiya, trawl fishery, size class of 14 -16cm (total length) was abundant in the landings during the northeast and 1st inter-monsoonal period (Fig. 10). Throughout the southwest and 2nd inter-monsoonal period, 12 to 14 cm sized shrimp were prominently caught, meanwhile, 16 to18 cm sized shrimp were also high (above 20%) all around the four seasons. Largest sized (18 to 20 cm) shrimp were caught only during 1st inter-monsoon and southwest monsoon periods.

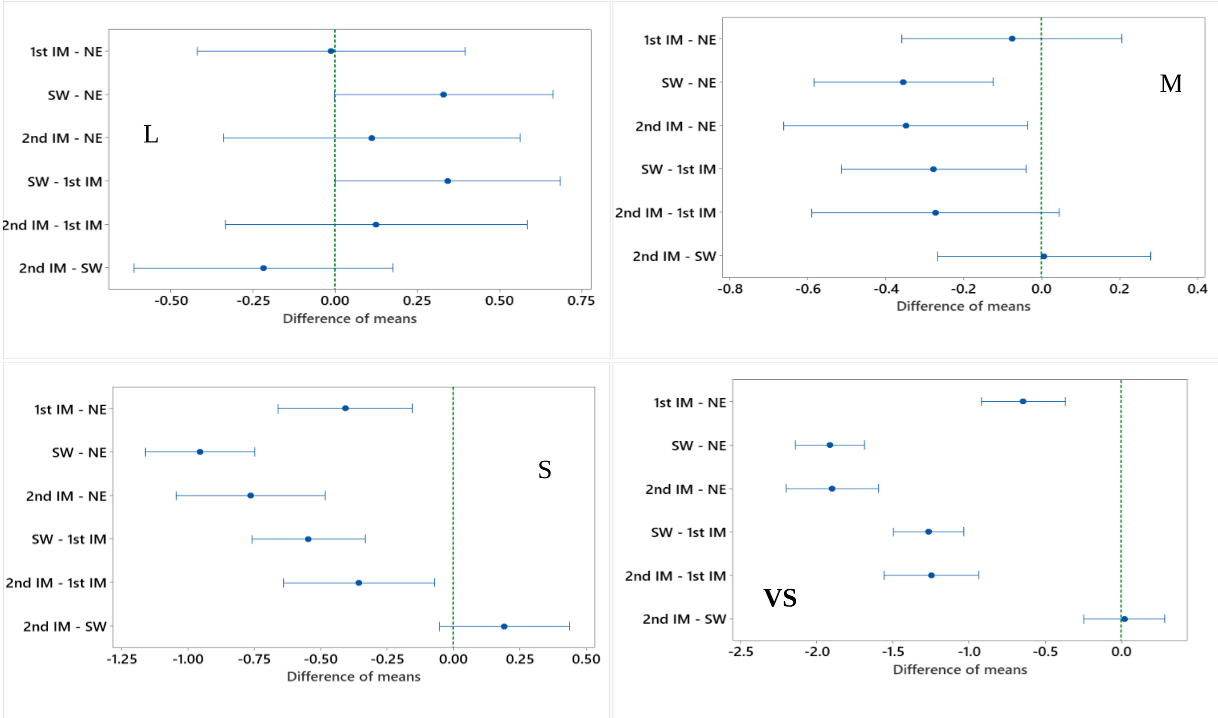

Fig. 9. Interval plots of Tukey simultaneous test (at the individual confidential level of 95%) for differences of the means of CPUE represents Mannar with pairwise monsoonal phases (L: Large sized shrimp; M: Medium-sized shrimp; S: Small- sized shrimp; VS: Very small sized shrimp). An interval does not intercept the zero-reference line of mean difference (green dotted line), the corresponding means are significantly different. NE: North-east monsoon; 1st IM: First inter-monsoon; SW: South-west monsoon; 2nd IM: Second inter-monsoon

In Mannar trawl fishery, 12 to 14 cm sized *P. semisulcatus* were caught throughout the year representing above 35 % of total landings in all four monsoonal phases (Fig. 11).

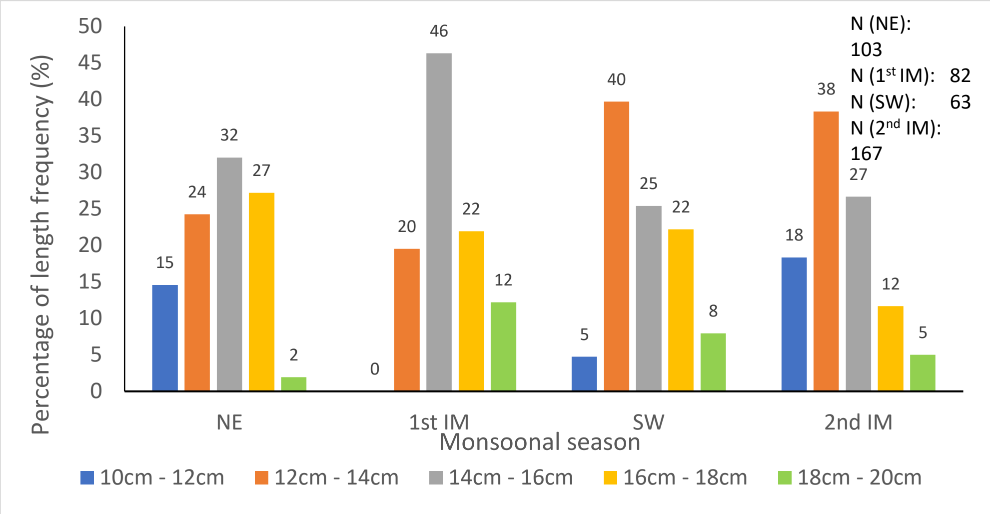

Fig. 10. Seasonal variation of length frequency for *P. semisulcatus* (Kalpitiya)

Length classes of 10 to 12 cm and 14 to 16 cm also contributed to reasonable percentage (mostly above 20%) of shrimp caught throughout the year. Smallest-sized (8 to 10 cm) *P. semisulcatus* were caught only during the northeast monsoonal period perhaps reflecting their recruitment season to the fishing ground.

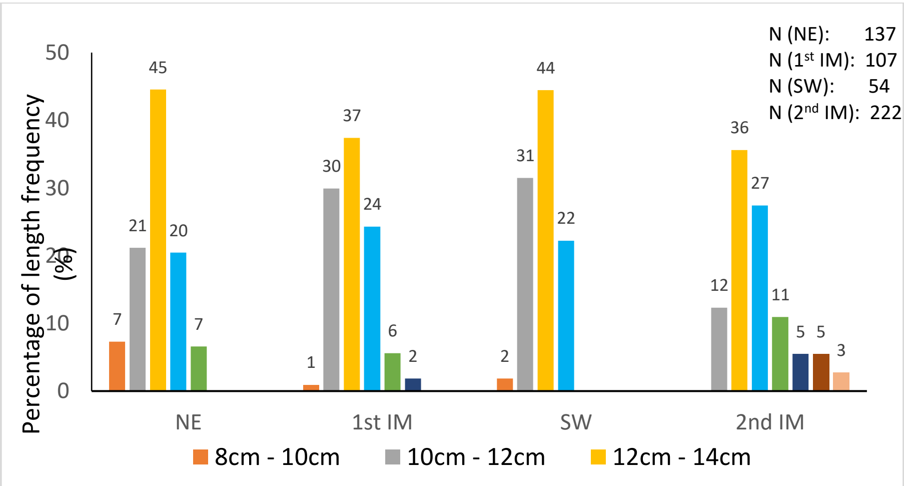

Fig. 11. Seasonal variation of length frequency for *P. semisulcatus* (Mannar)

Seasonal variation in the sex ratio of P. semisulcatus

The sex composition of *P. semisulcatus* in both trawl grounds did not show any prominent changes between different monsoonal phases, but there was a contrasting difference in the sex ratio between the two sites (Fig. 12). In Kalpitiya, female shrimp dominance was evident whereas in Mannar, male shrimps were dominant throughout the year except 1st inter-monsoonal phase. Further studies are needed to understand these site-specific variations of sex ratio.

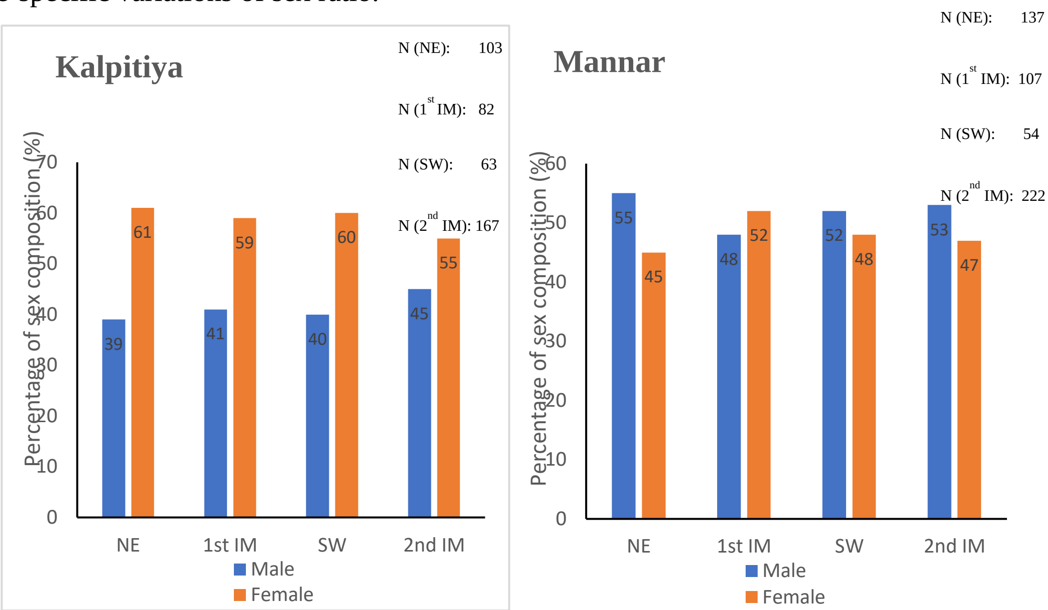

Fig. 12. Seasonal variation of sex composition for *P. semisulcatus* (Kalpitiya & Mannar)

Effect of monsoonal wind patterns on shrimp production

Shrimp catch rates of trawl fisheries were reported to be considerably influenced by monsoonal wind patterns and wind velocity (Jayawardane *et al.*, 2004). The seasonal wind pattern of the northwest coast off Kalpitiya coincides with the southwest monsoonal phase (May to October) and during this period, the average wind velocity is also high. From the present study, it is evident that in Kalpitiya, shrimp catch was the highest during the period of the Southwest monsoonal phase, registering around 48% of total annual shrimp production during this season. In addition, a peak value of mean CPUE (about 155 kg boat^{-1}trip^{-1}) also was recorded during the Southwest monsoon. Lower total monthly shrimp production and relatively lower mean CPUE (about 107 kg boat^{-1}trip^{-1}) of shrimp caught in Kalpitiya were observed during the period of the Northeast phase when there was low wind with calm sea.

In contrast, on the Northern coast off the Gulf of Mannar during the Northeast monsoonal period (December to February), the average wind velocity was also relatively high. Shrimp catch rates in the Mannar trawl fishery were higher (about 30% of total production) during the northeast monsoon. Furthermore, the highest mean CPUE (about 35 kg $boat^{-1}trip^{-1}$) was also recorded during the Northeast monsoon. Lower total monthly shrimp production and lower mean CPUE (about 15 kg $boat^{-1}trip^{-1}$) were noted during the period of the Southwest monsoonal season having low wind and calm sea off Mannar. Similar studies conducted earlier in west coast trawl grounds also evidently prove that the high shrimp catch recorded within the period of high average wind velocity over the west coast (Southwest monsoon) and relatively low shrimp catches were observed during the northeast and the inter-monsoon periods with calm seas (Haputhantri and Jayawardena, 2006). Further, the earlier study was conducted during 2008/2009 in Kalpitiya trawl fishery by NARA (Sanders and Jayasinghe 2009) reported that total annual shrimp production was about 109 tons and *P. semisulcatus* was by far the most abundant shrimp species. Accordingly, the present annual shrimp production in Kalpitiya area (142 tons in 2021) was around 30% increase over the past decade.

Fishery management generally encompasses technical measures concerning gear and operations, spatial controls, impact quotas, and fishing effort controls. The effectiveness and feasibility of these measures, whether implemented individually or in combination. It also varies based on fishery characteristics, management capabilities, environmental impacts, food security, income, and employment (McConnaughey *et al*., 2020).

Addressing trawling concerns becomes much more manageable through the adoption of current technical gear and management practices, such as altering gear design and implementing spatial controls. For instance, the reduction of bycatch and discards in trawl fisheries in Europe, North America, and Australia can be attributed to improved gear selectivity and decreased fishing effort, as noted by Kennelly and Broadhurst in 2021. Additionally, a significant change in Southeast Asia involves the increased utilization of all species in trawl fisheries such as for local markets and aquaculture feed, ultimately resulting in a decline in discarding (Suuronen *et al*., 2020). If stakeholders and regulatory bodies adopt these measures and eliminate the rush to fish, it seems that bottom trawling could have a lesser environmental impact. This is important, as these alternatives might replace trawl-caught fish in the event of a trawling ban.

Acknowledgments

The authors would like to thank the trawl fishers, shrimp collectors, and sellers in Kalpitiya and Mannar. Financial support provided by the National Aquatic Resources Research and Development Agency (NARA) is greatly acknowledged. The staff of the NARA's Kalpitiya Regional Research Centre helped in many ways with this research. The critical comments of two reviewers helped to improve the scientific contents of the manuscript.

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Investment opportunities of small-scale *Penaeus vannamei* (Vannamei) shrimp farming in the Northwestern Province of Sri Lanka

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Abstract

There has been an increasing interest in sustainable management of *Penaeus vannamei* shrimp farming in Sri Lanka since many years, yet little attention has been paid to the economic viability of farming systems. This study aims to assess the economic viability of small-scale *P. vannamei* shrimp farming in the Northwestern Province of Sri Lanka. The study examined 45 farms in year 2021 and revealed that it was a male-dominated small-scale industry and one hectare of land with 4047m2 of the pond incurred Rs. 5,720,360 (28,891 US$) of an initial capital investment and annual variable cost of Rs. 5,355,387 (27,047 US$). Further, an economic analysis revealed that the Net Present Value (NPV) of the capital invested by the end of nine years was Rs. 1,465,552 (7,402 US$) with a pay-back period of approximately 4 years while the Internal Rate of Return (IRR) and Benefit Cost Ratio (BCR) were 16% and 1.04 respectively. All those factors indicated that farming systems were economically viable in the study area.

Keywords: Economic analysis, white-leg shrimp, *Penaeus vannamei*, shrimp farming

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Introduction

Shrimp farming is one of the world's fastest-growing production systems (Emerenciano *et al*., 2022) and it contributes to the food security and livelihoods of the people in many regions of the word (Hossain *et al.*, 2013) as it fetches high value in the global market and emerges as one of highly profitable export products (Engle *et al*., 2017). Although traditional shrimp farming has a long history in Asia for centuries farming of penaeid shrimp species (Kungvankij, 1985) in commercial level has begun in the 1970s resulted in shrimp production has been growing steeply to cater the demand gaps (Biao and Kaijin, 2007). The global shrimp production in 2021 is set to be at least 8.9 % higher than 2020, and over 5 % growth is forecasted for the 2022 (FAO, 2021) and after 2007, the sector was dominated by farmed shrimp over the wild capture which accounts for 55 % of total shrimp produced globally (Stentiford *et al*., 2012). Among farmed shrimp, two warm - water species; Asian black or giant tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Penaeus vannamei*) are dominated in the global context (*Glenn et al*., 2005; Christie, 2014; Liao and Chien, 2011) and account for roughly 80% to the total farmed shrimp production in the world (Rosenberry, 2001).

Sri Lanka initiated shrimp farming in the 1980's in Northern and Eastern parts of the country and it has been emerged as the second largest export species, in value terms, in exports of fish and fishery products of the country in recent years (Siriwardena 1999; and Gammanpila, 2014). At the very beginning farmers confined to culture *P. Monodon*, as a brackish-water monoculture, (Gammanpila, 2014) until an exotic and genetically improved (Alday-Sanz, 2020) Specific Pathogen Free (SPF) Pacific white shrimp (*Penaeus vannamei*) were introduced in 2018, as a solution for frequent crop failures of *P. monodon* due to the white spot disease (Munasinghe, 2010) and since then the annual production has been increasing and has contributed about 20 % to the total farmed shrimp production of the country in 2020.

Farming of *P. vannamei* has been rapidly expanding (FAO, 2009) around the world mainly due to many factors; availability of selectively bred SPF seeds with an increase in tolerance to a wide range of salinities (0 to 45 ppt), amenable for high stocking densities, rapid growth, column feeding habit and feeding on natural bio-flock, low dietary protein requirement (30-35%) and higher meat yield (65-70%) (Mathew *et al*., 2020; Ravichandran *et al*., 2009) were some of them. Despite these factors, small-scale farmers in the Northwestern Province often claimed that productivity and profitability of *P. vannamei* farming system has been decreasing partly due socio-economic factors; lack of technical know-how in systematic farming and disease controlling, lack of institutional supports in the entire production process and high input costs were some of them. There were 1,012 farm systems with 4,347 ponds extended to 2,180 hectare of lands in the areas of Chilaw, Ambakandawila, Thoduwawa, Iranwila, and Muthupanthiya in the Northwestern Province by the end of 2019 and have produced 543 Mt of vannamei shrimp or contributed 8% percent to the total farmed shrimp produced in the country (MFARD, 2020) and therefore, this paper aims to examine the economic viability of small-scale *P. vannamei* farming systems in the Northwestern Province for the sustainability of shrimp farming industry of Sri Lanka.

Materials and Methods

Farming of *P. vannamei* is abundant in Ambakandawila, Thoduwawa, Iranwila and, Muthupanthiya areas in the Northwestern Province of Sri Lanka and therefore selected as the study area. All farms less than two hectares in size were considered as the study population according to the farm classification maintained by the National Aquaculture Development Authority (NAQDA) for its administrative purposes. In total 45 farms were randomly selected for the study and pre-tested structured questionnaires were administered to collect data on socio-economic and demographic factors. The respondents were farm owners. In addition to that one focus group discussion was conducted in each area with the participation of key informants and field observations were undertaken to verify the data given by respondents. Apart from that farm record books were used whenever required. All data were collected during the time period of January to July in 2021 and an integrated methodology was applied to analyse data and SPSS and Microsoft excel software packages were used whenever appropriate. The costs and benefits were calculated on one acre ($4047m^{2)}$ pond area. Results are presented using tables and charts whenever relevant. A model was developed for simulation of production, finance, cash flow, capital replacement, depreciation, and profitability with the annual values of the farms. In the analysis values related to the land, buildings, fencing and equipment were considered as initial investment of a farm while NPV was used to analyse the profitability;

$$NPV = \sum_{i=1}^{n} \frac{Cash\ Flow_i}{(1+r)^i} - Initial\ Investment$$

Where: r – discount or interest rate, n – the number of time periods, i – the cash flow period,

The following assumptions were also made; the financing of a farm was done through both the owner equity and an outside loan with a 10% interest and nine years pay-back period. All depreciation were estimated using the straight-line method and the percentages for buildings, equipment and fencing were 4, 10 and 20 percent respectively. A 10% discount rate was considered. All the inputs and output related to current study are based on price at the 2021 and US dollars (US$) exchange rate is 198 Rs/US$. The culture period of one cycle was considered as 4 months and only two cycles were operated per year. Secondary data of the study were collected from the Ministry of Fisheries and Aquatic Resources Development (MFARD), National Aquatic Resources Research and Development Agency (NARA), NAQDA, Sri Lanka Seafood Exporters' Associations and Shrimp Farmers' Associations in the respective areas.

Results and Discussion

Socio- economic characteristics of *P. vannamei* farmers

All farmers were male with a majority (42%) from the age group of 40-49 years old, while 56 % have engaged in shrimp farming over 10 years indicating that though most of farmers were middle aged but have long term experience. The four decades old shrimp farming industry supports almost all dwellers for their livelihood. Farmers used three major sources to get water into farming systems and of them lagoon (49%) was the major followed by Dutch Canal (40%) and tube wells (4%) but some farmers used both lagoon and tube wells (7%) (Table 1).

Table 1: Source of water for shrimp farms

Type of water source	Number of farms	Percentage (%)
Lagoon	22	49
Tube well	2	4
Dutch Canal	18	40
Both lagoon & tube well	3	7

Main reasons for the selection of water source were convenience and availability but farmers understood that using the lagoon and Dutch Canal waters may be a possible cause of infections of white spot disease and other waterborne pathogens.

Farm management settings

Present study revealed that a community-based management system was prominent in managing the farming activities in the areas. Farms were grouped into several zones and each zone was managed by small-scale farmers associations which were called *Samithiya*. All those community level associations collectively made decisions, involved in resolving issues and members met on monthly basis. Special meetings were also called whenever necessary in urgent matters. This management setting seemed successful in resolving issues related to the farming activities in the area and resulting in sharing a common pool of ecological resources in a meaningful way. Further, it enabled farmers to ensure better management of their resources. A zonal crop calendar system existed to manage and implement zonal and sub zonal level cycles, pond renovation, post-larvae stocking, and harvesting of resources. This management system gained momentum after the farming industry collapsed due to the white spot disease outbreak and resulted in several legal actions, as well as management practices being implemented specially with the introduction of SPF *P. vannamei* to the farming industry. NAQDA has introduced a set of better management practices and involved in the implementation of regulations and management specifications but community associations were responsible for frequent reporting on adherence to these Best Management Practices (BMP). BMP have been practiced on a basis of three categories of farms such as Grade A, Grade B, and grade C and were provided with specifications on stocking density, farm size, aeration, post-larval transport, deposit, food management, water management, harvesting, record keeping, water management and treatment of the farm category.

Initial capital investment of farming systems

Shrimp farming required a higher level of initial capital investment and the result found that a total of Rs.5, 720,360 (28,891 US$) have been invested by a small-scale farmer and of them, the highest proportion was invested in land purchased and construction of earthen ponds (61%), fencing (12%) and permanent building (6%). Higher cost of fencing was recorded due to the specifically designed nylon nets to cover the culture ponds to protect from predators (Table 2).

Table 2: Investment cost for *P. vannamei* shrimp farm

Description	Value (Rs)
Cost of Land, buildings and Fencing	**4,520,000**
Land and ponds	3,500,000
Building	350,000
Fencing and nets	670,000
Cost of equipment	**1,200,360**
Refrigerator	130,000
Generator	250,360
Water pump	250,000
Paddle wheel	570,000

Variable costs of farming systems

Variable costs were production related short-term expenses, incurred in farming and varied based on scale of production which comprised mainly of seeds, feeds, chemicals, electricity and labour. Result found that feed had contributed to more than half (55%) of total variable cost. Chemicals and electricity cost are 15% and 9% respectively. The labour cost has contributed 9 % to the total variable cost of a farming cycle. The total variable cost of a farming cycle was estimated to Rs 2,677,693 (13,523 US$) and the annual variable cost of a farming system was Rs. 5,355,387 (27,047 US$) due to two cycles were conducted per year. It was estimated that variable cost per kg of shrimp was Rs. 744 (3.76 US$) per cycle (Table 3).

Table 3: Variable cost of one culture cycle for one acre pond of *P. vannamei* farm

	Number of units	Unit cost (Rs.)	Total cost (Rs)
Labor (2×4 months)	8	30,420	243,360
Post Larvae	150,000	1.1	165,000
Feed	4,428	335	1,483,380
Electricity	23	10,500	241,500
Chemicals			400,000
Transport cost			25,000
Fuel			62,453
Harvesting charges			39,000
Other			18,000
Total Variable Cost			**2,677,693**

Production and Revenue

Results revealed that the total production of one acre (4047 m^2) pond area was 3,600 kg per one cycle which span for a period of 4 months and the total annual production was approximately 7,200 kg. The production varied on stocking density and rate of natural mortality of shrimp during the farm cycle. It was observed that maximum stocking density was 40 PL/m^2 and the average survival rate was 63 %. The initial average weight of a post larvae was 0.01 g and at the end of the culture cycle, it was on average 27 to 35 g (Table 4). The weight of a shrimp mainly depended on feeding and the status of hygiene management of the farm. The feeding amount and frequency varied through the growth dynamic of shrimp and result revealed that on average the feed conversation ratio was (FCR) 1.3 during the farm cycle.

Table 4: Price and quantity produced of one culture cycle

Description	Number of unit (kg)	Sales price (Rs)	Revenue (Rs)
Production	3,600	975	3,510,000

Financial Analysis of farming systems

Financial analysis of one farming cycle of small-scale farming system indicates in Table 5 and it depicts that the total cash flow was negative during the first year of farming cycle manly due to higher initial capital investment. However, after that the cash flow showed a positive value and at the end of 9 years it was Rs. 1,248,478 (6,305 US$) for a small-scale *P. vannamei* farm.

Table 5: Economic indicators for one culture cycle

Cost Item	**Value**
NPV (Rs.)	1,465,552
Pay Back (years)	4.6
Discounted Payback Period (years)	7.4
IRR (%)	16.13%
Benefit Cost Ratio	1.04

The financial viability of the farming system was estimated to Rs. 1,465,552 (7,402 US$) for the total capital invested in the farming system. The higher NPV value indicated that investing in *P. vannamei* shrimp farming was financially better off and financially viable. It could be said that small-scale *P. vannamei* farming was financially viable. It served as an incentive for new entries to the farming industry in the area. The Payback Period for total capital invested was estimated to be approximately four years which means that within a four year period invested money could be recovered. This value was highly positive, thus would attract new farmers. The Payback Period of an investment is the approximate return period of capital or business activities (Weingartner, 1969; Nguyen, 2012) and it indicates the number of years required to recover the initial capital investment from the net cash flow generating from the investment. Engle, (2017) explained that aquaculture investments are preferred with the shortest Payback Period while other factors being equal because of risk considerations. In addition to that the discounted Payback Period was also calculated to assess the number of years after which the cumulative discounted cash inflow covers the initial investment in *P. vannamei* farming and found that it was 7.4 years.

Positive NPV value together with relatively a shorter Payback Period indicated that the investment in small scale *P. vannamei* farming in the area was highly motivated. Further, the Internal Rate of Return (IRR) was estimated to 16.13 % which was above the Minimum Attractive Rate of Return (MARR) of 10% at the end of 2021. It was obvious that invested in *P. vannamei* farming is more beneficial than invested in alternative business or saving in a bank. The IRR was calculated to analyse the interest rate at which capital could be borrowed for the farm or the interest that could be earned on capital invested (Tisdell, *et al*., 1993; Sureshwaran *et al*., 1994). The Benefit Cost Ratio (BCR) was estimated and it summarizes the overall relationship between the relative costs and benefits of the investment and if the ratio is greater than 1.0, the investment receives a positive Net Present Value while if the value is lower than 1.0, costs of investment outweigh the benefits (Boyd, *et al.,* 2017; Islam *et al.*, 2005) and the result of the study found that the BCR was 1.04 which indicated that investment in *P. vannamei* farming delivered a positive net present value and farmers were benefited from the investment. The BCR compares the present value of all benefits with that of the cost and investments of a project (Kusumastanto, 1996).

Conclusion

Small-scale shrimp farming in the Northwestern Province of Sri Lanka is a male dominant industry engaged by middle-aged people in the area with long term farming experiences. Although the initial capital investment of farming systems is high, operating of one hectare of a farm with a 0.4047 hectare sized pond generates a NPV which reveals the revenue of a farm system is greater than cash outflows resulting in net profits of the *P. vannamei* farming. Further, shorter the Payback Period of capital investment, gives rise an economic incentive which persuades new investment into the industry which may lead further intensification of *P. vannamei* farming in the area. In practicing BMP and strictly confined to the SPF strains of *P. vannamei* together with appropriate hygienic practices in farming, the farmers can overcome the vulnerability of cash flow in the farming system by ensuring economic as well as environmental sustainability of *P. vannamei* farming in the Northwestern Province of Sri Lanka.

Acknowledgments

The authors wish to thank scientists and research assistants of the Socio-economic and Marketing division of NARA for their support in data collection of the study.

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Short Communication

Optimum utilization of brewers' yeast (*Saccharomyces cerevisiae*) to replace local fish meal in juvenile Sea cucumber (*Holothuria scabra*) diets

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Abstract
The juvenile sea cucumber, *Holothuria scabra* was reared for 55 days in a re-circulatory tank system consisting of filtered seawater flowing at 2.5-3 L/min. One hundred and forty-four juveniles of an average weight of 0.88±0.03 g were randomly distributed in 12 tanks of 250 L. Sea cucumber were fed four diets containing different dietary concentrations of brewer's yeast (BY), *Saccharomyces cerevisiae* (BY_0: 0%; BY_{10}: 10%; BY_{30}: 30%; BY_{50}: 50 %) to replace the local fish meal (CM fish meal®). At the end of the experiment, the specific growth rate (SGR), weight gain (WG) and final body weight (FW) of juvenile sea cucumber fed with BY_{10} was significantly higher than those of juveniles fed BY_0 and BY_{50} ($p<0.05$) though, not significantly higher than those fed BY_{30} ($p>0.05$). The optimum replacement level of CM fish meal® with brewers' yeast was 13.75% for juvenile *H. scabra* by polynomial regression analysis of WG.

Keywords: Brewer's yeast, *Saccharomyces cerevisiae*, local fishmeal, juvenile sea cucumber, *Holothuria scabra*

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Introduction

Sea cucumber became a high-demanded commodity among all seafood varieties in the world after the new trade economy of China started in the 1980's (Hamel *et al.*, 2001). Many coastal nations lost their wild sea cucumber population due to overexploitation (Ajith Kumara *et al*., 2013; Battaglene *et al*., 1999; Hamel *et al*., 2001; Purcell *et al*., 2012). Hence, the larval production of *Holothuria scabra* increased gradually in commercial hatcheries and it cost a high market value. The species is naturally distributed in shallow coastal areas across the Indo-Pacific region. As the pioneer in the South Asian region, India started to produce *H. scabra* seeds while the wild stock was declining (James, 2004). The production of *H. scabra* juveniles in Sri Lankan hatcheries was initiated in 2011 (Kumara and Dissanayake, 2017). The processed sea cucumber production collected from wild and pen cultured in Sri Lanka ranged from 258 tons in 2005 to 248 tons in 2018, with several annual fluctuations (DOF, 2019). The highest export value was reported as 10.33 USD million in 2013, while the second-highest value reported was 7.65 USD million in 2018 (DOF, 2019).

In general, dried and powdered seaweed are used as feeds in sea cucumber hatcheries. Seasonality issues in the availability of seaweed, drying issues, and nutrient imbalances occur in feeding seaweed for sea cucumber juveniles. The predominant protein source in aqua feeds is still fishmeal (FM). The fish meal provides adequate levels of amino acids for aquaculture species. The negative impacts, such as imbalances in amino acid profile, reduced palatability, and availability of anti-nutritional factors, have been reported in using plant protein sources as alternative sources (NRC, 2011). Therefore, higher levels of plant protein sources to replace the fish meal are also impractical. However, soybean meal replaced 40% of fishmeal in sea cucumber, *Apostichopus japonicus* juvenile diets without affecting growth (Fan *et al*., 2010). In contrary, 60% FM replacement by soybean meal did not affect growth in sea cucumber, *Apostichopus japonicus* juveniles (Liao *et al*.,2015). Other alternative fish meal replacers in sea cucumber diets such as silkworm caterpillar meal (Sun *et al*., 2014) and alga *Spirulina* meal *(Tan et al*., 2009) were suggested to be good fish meal replacers in sea cucumber, *Apostichopus japonicus* juvenile diets without affecting growth.

Saccharomyces species are used in the brewing industry to produce beer from wort (Bourdichon *et al*., 2012). The fermented product in beer production called brewers' yeast (BY) has been studied as a dietary supplement and growth promoter for hybrid striped bass, *Morone chrysops*× *M. saxatilis* (Li and Gatlin, 2003) and hybrid of African catfish, *Clarias gariepinus* (Essa *et al*., 2011), as an immune stimulant for hybrid striped bass (Li & Gatlin, 2004), and as an FM replacer for sea bass, *Dicentrarchus labrax* juveniles (Oliva-Teles & Gonçalves, 2001), goldfish, *Carassius auratus* (Gumus, Aydin, & Kanyilmaz, 2016) and pacu, *Piaractus mesopotamicus* (Ozório *et al*., 2010). The studies on the utilization of brewers' yeast products suited for aqua feeds have been expanded up to compounds contained in brewers' yeast, *Saccharomyces cerevisiae*. For instance, glucan derived from *S. cerevisiae* (bakers' yeast) was injected to channel catfish, *Ictalurus punctatus* and improved disease tolerance against *Edwardsiella ictaluri* (Chen & Ainsworth,1992). However, partial substitution of dietary baker's yeast *S. cerevisiae* did not affect trout fish growth (Rumsey, Hughes, & Kinsella, 1990). Dietary addition of *S. cerevisiae in* rainbow trout, *Oncorhynchus mykiss* diets enhanced disease resistance against *Aeromonas salmonicida* (Siwicki, Anderson, & Rumsey, 1994) and improved growth and immunity with disease resistance against *Yersinia ruckeri* (Tukmechi *et al*., 2011).

Brewers' yeast grouped into the single-cell protein (SCP) category gives many advantages to fish feeds (Couttau & Lavens, 1989). Single-cell proteins like microalgae, yeast and bacteria are utilized in fish feeds considering their nutrients like proteins, pigments, B-vitamins, and carbohydrates (especially glucans) (Sanderson & Jolly, 1994;Tacon, 1995). B-glucan consisted in the yeast cell wall is an immunostimulant/ feed additive for aquaculture organisms (Sakai, 1999; Thanardkit *et al.*, 2002). Generally, microbial products or fractions like barley and brewers'yeast are utilized for the β-glucan extraction (NRC, 2011). Likewise, sea cucumber, *A. japonicus* juveniles fed diets containing dietary β-glucan showed an improved immune response and balance of intestinal microbiota (Yang *et al*., 2015).

Therefore, we aimed to find the optimum supplement level of brewers' yeast, *S. cerevisiae* by replacing a local FM in juvenile sea cucumber (*H. scabra*) diet to maximize their growth.

Materials and Methods

Experimental site

The experiment was carried out in a re-circulatory tank system of 12 fiberglass tanks of 250 L at the hatchery of the regional research center, National Aquatic Resources Research and Development Agency (NARA), Kalpitiya, Sri Lanka.

Experimental feeds

The main ingredients, a local fish meal/ CM fish meal® (CMFM) procured from Cool man fish meal factory, Pesalei and brewers' yeast provided by Lion Brewery (Ceylon) PLC, Biyagama were used with all other ingredients that purchased from the retail market, Pettah. Soybean meal, meat and bone meal, maize, poonac, rice polish and brewers' yeast were ground separately by a hammer mill and sieved with a 0.5 mm sieve. Four diets (BY_0: 0% brewers' yeast (BY); BY_{10}: 10% CMFM replaced by BY; BY_{30}: 30% CMFM replaced by BY; BY_{50}: 50 % CMFM replaced by BY) were formulated and prepared practical diets (Table 2). Then, prepared feeds were stored at – 20 ^{o}C until the commencement of the feed trial.

Table1. Proximate composition of dry brewers'yeast (*Saccharomyces cerevisiae*)

	Crude Protein%	Moisture%	Ash%	Fiber%	Fat%
CMFM	42.37	2.9	33.9	1.4	5.92
BY	39.3	6.0	6.5	4.3	0.7

Experimental Design

One hundred and forty-four sea cucumber juveniles produced in SL Aquatech International (Pvt.) Ltd., Chilaw averaging 0.88 ± 0.04 g size were placed in 12 fiberglass tanks of 250 L capacity at a stocking density of 12 juveniles per tank. The four experimental diets were randomly assigned in triplicated tanks.

Feeding trial

Prior to the commencement of the trial *H.scabra* juveniles were acclimated to the tank system and feeds for a week. The culture system was operated with filtered seawater for 55 days of the feeding trial. The re-circulatory system was operated 2 hrs per day at a 2.5-3 L/ min flow rate and water was exchanged three times per week. Salinity, dissolved oxygen (DO) and ionized ammonia NH_3+ were checked daily while the values were maintained 25-28ppt, 5.5-6 mg/ L and 0.07-0.10 mg/ L respectively. Feeding proceeded once a day at 4% of the body weight. Growth data were collected biweekly to adjust the feed amount.

Table 2. Composition of experimental feeds and their proximate composition (means ± SE)

Ingredient	BY_0	BY_{10}	BY_{30}	BY_{50}
CMFM®[1]	20	18	14	10
Brewers'yeast[2]	0	2	7	10
Soybean meal[3]	20	20	20	20
Meat and bone meal[3]	5	5	5	5
Maize[3]	15	15	15	15
Poonac[3]	5	5	5	5
Rice polish[3]	5	5	5	5
Wheat flour[3]	26	26	25	26
Fish oil[3]	0.5	0.5	0.5	0.5
Vitamin mineral premix[3,4]	3	3	3	3
DL-Methionine[3]	0.3	0.3	0.3	0.3
L-lysine[3]	0.2	0.2	0.2	0.2
Proximate composition (DM basis)				
Moisture	9.32±0.35	9.21±0.79	8.82±0.01	9.01±0.11
CP	29.89±1.01	29.67±0.97	29.94±0.25	29.12±0.24
Ash	16.53±0.51	14.57±0.29	13.52±0.16	11.92±0.02
Crude Fat	3.17±0.24	2.97±0.27	2.64±0.32	2.31±0.15
Crude Fiber	2.75±0.14	2.86±0.11	3.08±0.10	3.30±0.09
NFE	38.34±0.23	40.72±0.49	42.6±0.20	44.34±0.13

[1] Cool Man fish meal® (CMFM) was procured from the cool man fish meal factory, Thaleimannar road, Pesalei, Sri Lanka.

[2] Brewers' yeast (BY) was provided by Lion Brewery (Ceylon) PLC, Colombo road, Biyagama, Sri Lanka.

[3] All the other ingredients were purchased from the retail market, Pettah, Sri Lanka.

[4] Contains (as mg/kg in diets): Vitamin A, 9000IU; Vit K, 2 mg; Vit E, 5 mg; Vit B1, 2 mg; Vit B2, 3.6 mg; Vit B6, 1 mg; Vit B12, 10 mg; Vit D3, 2000 IU; Chlorine chloride,150 mg; Mn,60 mg; Zn, 50 mg; Fe, 25 mg; Niacinamide, 16 mg; I,5.5 mg; Cu,5 mg; Calcium pantothenate,4 mg; Folic acid,0.5 mg; CO,0.1 mg.

Statistical Analysis

Growth performance data were subjected to one-way analysis of variance (ANOVA) tests at $p<0.05$ level of significance. If there is a significant difference, the treatments were compared by Tukey's test at $p<0.05$ using SAS 9.4 program.

Results and Discussion

During feeding trial, *H. scabra* juveniles accepted all the experimental feeds well. At the end of the experiment, a significantly higher specific growth rate (SGR), weight gain (WG) and final body weight (FW) showed by the juvenile sea cucumber fed BY_{10} followed by BY_{30} compared to juveniles fed BY_0 and BY_{50} ($p<0.05$). However, there were no significant differences in the growth performances of juveniles fed BY_{30} and BY_{10} ($p>0.05$) (Table 3).

For the last decades, the attention for brewers' yeast to be used in aquafeeds has increased. As it is a byproduct in the beer industry (Bourdichon *et al*., 2012) which consisting of protein, vitamin B complex and β-glucan (Sanderson & Jolly, 1994;Tacon, 1995), brewers' yeast has been suggested utilizing in aquafeeds as a feed additive and fish meal replacer. In our experiment, the dietary addition of brewers' yeast improved the growth of *H. scabra* juveniles. So far, the utilization of dietary brewers' yeast, *S.cerevisiae* in sea cucumber *H. scabra* juvenile diets has not been reported. Benefits of using brewers' yeast in the diets of other cultured species like freshwater prawn

Macrobrachium rosenbergii post larvae (Prasad *et al*.,2013) and finfish like sea bass *Dicentrarchus labrax* juveniles (Oliva-Teles & Gonçalves, 2001), hybrid striped bass *Morone chrysops* × *M. saxatilis* (Li & Gatlin, 2003),(Li & Gatlin, 2004), hybrid of African catfish *Clarias gariepinus* (Essa *et al*.,2011), pacu *Piaractus mesopotamicus* (Ozório *et al.*, 2010) and goldfish *Carassius auratus* (Gumus *et al*., 2016) were reported. In the study, 10% dietary addition of brewers' yeast enhanced growth performance results; though not significantly better than 30% brewers' yeast. Ozório *et al*. (2010) stated that brewers' yeast could replace 50% FM in pacu *Piaractus mesopotamicus* diets without affecting growth.

Table 3. Growth performances of *H. scabra* juveniles fed four different feeds (means ± SE) (n=3)[1]

	Diets			
	BY_0	BY_{10}	BY_{30}	BY_{50}
Initial weight (g)	0.86±0.00	0.86±0.00	0.91±0.01	0.89±0.00
Final weight (g)	1.63±0.15^b	2.39±0.12^a	1.97±0.09^{ab}	1.73±0.15^b
WG (%)[2]	81.03±16.82^b	165.61±12.88^a	119.08±9.71^{ab}	92.30±17.14^b
SGR(%day^{-1})[3]	1.06±0.17^b	1.77±0.09^a	1.42±0.08^{ab}	1.17±0.17^b
Survival (%)[4]	58.33±16.67	52.78±2.78	63.89±7.35	55.56±10.02

[1]Values in each column with different superscripts are significantly different (p<0.05).

[2]Weight gain $= \left[\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}}\right] \text{x}100$

[3]Specific growth rate (SGR) = $\frac{\text{(Ln)final weight} - \text{(Ln)initial weight)}}{\text{days}} \text{x } 100$

[4]Survival = $\frac{\text{No.of juveniles at the start} - \text{No.of juveniles at the end}}{\text{No.of juveniles at the start}} \text{x } 100$

The optimum replacement level of CMFM with brewers' yeast for juvenile sea cucumber, *H. scabra* was 13.5% by the polynomial analysis of WG (Fig. 1).

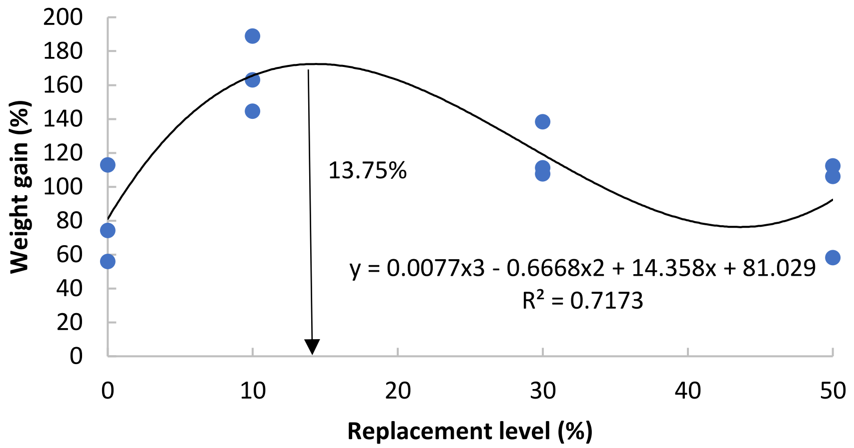

Fig. 1. Optimum replacement level of CMFM by brewers' yeast on weight gain of sea cucumber, *Holothuria scabra* juveniles fed 55 days.

Moreover, in goldfish *Carassius auratus* diets, brewers' yeast was able to replace 35% FM without adverse effects on growth (Gumus *et al*., 2016). Oliva-Teles & Gonçalves (2001) mentioned that brewers' yeast could replace 50% of FM in *Dicentrarchus labrax* juvenile diets with no effect on growth performances. Furthermore, 30% of dietary brewers' yeast in *Dicentrarchus labrax* diets enhanced protein utilization and feed efficiency. Likewise, the total nitrogen in trout diets can replace over 50% with bakers' yeast *S. cerevisiae* nitrogen without changing the growth (Rumsey *et al*., 1990). However, FM replacement with brewers' yeast at low levels in fish feeds showed best performances while no significant effect up to 45-50% replacement levels. In this study, 50% brewers' yeast replacement level significantly affected growth. Rumsey *et al*.(1990) mentioned that fish would not perform well, if brewers' yeast is the only protein source, because brewers' yeast is deficient in essential amino acids, vitamins, minerals, and nucleic acids. So high supplement levels of brewers' yeast in fish diets reduces growth and feed intake of fish (Gumus *et al*., 2016; Ozório *et al*., 2010).

The fish meal provides essential amino acids adequately in aquafeeds (NRC, 2011). Therefore, while partial replacement of fish meal by brewers' yeast increases growth performances, excessive levels could decrease their performance. For instance, alga Spirulina meal replaced 100% fish meal in sea cucumber, *Apostichopus japonicus* juvenile diets without affecting growth. Even though, sea cucumber juveniles showed better growth performances at 25% fish meal replacement level with alga Spirulina meal (Tan *et al*., 2009).

Conclusion

The optimum replacement percentage of CMFM® with brewers' yeast was 13.5% in juvenile *H. scabra* diets by the polynomial regression analysis of weight gain**.** Brewer's yeast could be a partial dietary replacer for high-value fishmeal in juvenile sea cucumber diets for maximizing growth and reducing production cost of sea cucumber feed in future.

Acknowledgements

The authors would like to thank Lion brewery (Ceylon) PLC, Colombo road, Biyagama, Sri Lanka for providing dry brewers' yeast.

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