Agriculture & Food Security

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The association of test diameter and gonad weight with some toxic trace metals level in black sea urchin (*Stomopneustes variolaris*)

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Abstract

Background: Quantification of toxic trace metals in biota is very important to monitor both ecosystem health and public health. *Stomopneustes variolaris* is a widespread species of sea urchin in the world that have edible gonads that are in high demand. This study attempted to examine the levels of accumulated toxic trace metals and their relationship between the test diameter and the gonad weight of the black sea urchin (*S. variolaris*) in Sri Lanka.

Results: As *S. variolaris* is highly abundant in southwest coast of Sri Lanka, three sampling sites were selected in this region (Mount-Lavinia, Beruwala and Tangalle). The concentration of toxic trace metals (Hg, Cd, Pb and As) in the gonads of *S. variolaris* was determined in randomly selected subset from the above samples. The levels of above metals in gonads did not exceed the permitted standard levels of the EU and Sri Lanka. The mean size and weight of *S. variolaris* collected from Mount-Lavinia was 5.55 ± 1.04 cm and 101.40 ± 57.76 g, respectively; for Beruwala it was 6.54 ± 0.86 cm and 147.90 ± 50.40 g, respectively, and for Tangalle it was 6.41 ± 1.05 cm and 150.50 ± 59.45 g respectively. There was a significant relationship between all the analysed trace metals and the test diameter and gonad weight of *S. variolaris* in Sri Lanka.

Conclusions: This study revealed that the trace metal levels in gonads of *S. variolaris* did not exceed the harmful level for human consumption.

Keywords: Accumulation, Echinodermata, Roe, Indian Ocean

Background

Heavy metals that are less soluble in water tend to accumulate in marine ecosystems as they do not degrade [1]. Therefore, these toxic trace metals will accumulate along the trophic levels which will affect the entire marine community. The levels of trace metal accumulation in marine organisms depend on their detoxification mechanism in metabolism. The harmfulness of these toxic trace metals is due to their role in biochemical and metabolic processes. Excessive consumption of sea food with high levels of accumulated toxic trace metals can lead to metabolic dysfunctions. Therefore, quantification of toxic trace metals in the all biota is very important to monitor both ecosystem health and public health. Currently, sea urchin gonads are a delicacy in many countries, and thus, there is a very high demand. However, nothing is known about the levels of accumulated toxic trace metals in sea urchins [1, 2].

The black sea urchin, *Stomopneustes variolaris,* is one of the edible species of sea urchin in the world. In Sri Lanka, *S. variolaris* is restricted to the western and southern coastal areas than other coastal areas [3, 4]. The black sea urchin (*S. variolaris*) is one of the warm water sea urchin species that belongs to the family Stomopneustidae. They bore into the rock, reefs and other substrates in littoral regions up to a depth of 18 m, and

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they prefer to live in the shady areas. *S. variolaris* is an omnivore species, and also they are known to mainly feed on algae and seaweeds [5–13]. *S. variolaris* species has a widespread distribution [12, 14, 15], in tropical and subtropical regions of the Indo-Pacific from the east African coast and Madagascar to New Caledonia and Samoa to the Bonin Islands to the north [12]. It is an abundant edible species in the Indian Ocean, in the geographical regions such as Lakshadweep Islands, Andaman Islands, India and Sri Lanka [16]. The growth rate of sea urchins and natural mortality mainly depend on temperature and food availability [6]. The sea urchins accumulate nutrients in the gonad, which is a highly sought-after delicacy in the world [17–19].

The trace metals are accumulated in most body parts of echinoderms including coelomic fluid, spines, gut, gonads and Aristotle's lantern. Hence, levels of trace metals in sea urchins could be used as a bio-indicator to determine the level of environmental pollution [20]. Currently, nothing is known about the levels of trace metals in the gonads of sea urchins in Sri Lanka, which has high demand in the marine fisheries export industry [18, 19]. Therefore, this study was conducted to get an idea on the levels of toxic trace metals in *S. variolaris* in Sri Lanka.

Methods

A total of 197 individuals of *S. variolaris* were collected from three selected sites in the western and southern coasts of Sri Lanka, namely Mount-Lavinia ($n=43$), Beruwala ($n=99$) and Tangalle ($n=55$) reefs (Fig. 1) from May to November in 2014. All collected individuals were transported immediately with seawater to analytical chemistry laboratory at the Institute of Post-Harvest Technology (IPHT), National Aquatic Research Agency (NARA) within 24 h. After that, total body weight to nearest 0.01 g and the horizontal test diameter was measured to the nearest 0.02 mm using a vernier caliper. Horizontal test diameter was measured twice perpendicularly to the first measurement and the averages of the two measurements were taken. Finally, gonads were removed from each individuals and weighed after removing excess water using blotting papers.

To determine the toxic trace metal levels in gonads of *S. variolaris*, individuals with test/shell diameter between 3 and 9 cm were selected and 6, 13 and 11 individuals were selected from Mount-Lavinia, Beruwala and Tangalle, respectively [21]. The levels of toxic trace metals, Hg (mercury), Pb (lead), Cd (cadmium) and As (arsenic) were assayed in the atomic absorption spectrophotometer (AAS) (Varian 240 FS, Springvale, Australia). The standard solutions of Hg, Pb, Cd and As at 1000 mg/L (ppm) were obtained from Sigma-Aldrich (Dorset, United Kingdom) which were used to calibrate. All standards and reagents were prepared using ultrapure water. One gram of homogenized sea urchin gonad samples were taken into a Teflon microwave digestion tube and all the samples were performed in duplicates. Acid digestions of gonad samples were performed by using the 10 mL of HNO_3 (65% nitric acid, AR) allowed to 10–15 min and capped the microwave digestion tubes. The sea urchin gonad samples were digested using the CEM/MARS XP-1500 + microwave oven (CEM, Matthews, USA). Microwave accelerated system and allowed to cool room temperature and pressure was released carefully by opening the valve. Then the digested gonad samples were transferred into 50 mL of volumetric flasks and volumed up using the de-ionized water was used to determine the Hg, Pb, Cd and As of digested sea urchins' gonads samples. A graphite tube atomizer (Varian GTA-120) was used to determine the Pb and Cd, for the determinations of As palladium nitrate ($Pd(NO_3)_2$) was used as a modifier. The Hg concentration of sea urchins' gonads were determined by using the cold vapour techniques (Varian VGA 77) using the $SnCl_2$ as a reduction [3, 4, 22].

The Pearson's correlation coefficient (r) and significant correlation tests were conducted to assess the relationship between gonadal trace metals levels and test diameter and gonad weight of *S. variolaris*. Data were analysed using the Minitab 16.0 version and Microsoft Excel 2010 version at the level of significance at $\alpha=0.05$ (5%).

Results

Toxic trace metals levels in gonads

Levels of toxic trace metals in gonads of *S. variolaris* were determined separately from selected sites and the average values of accumulated concentration (ppm and ppb) and the total amount of trace metals with their average test diameter and gonad weight in selected individuals' are determined.

The average concentration (ppb) of Hg in selected individuals from Mount-Lavinia reef ($n=11$) ranged from (minimum to maximum) 0.00 (±0.00) to 14.40 (±5.33) µg/kg and total concentration in gonads varied from 0.00 to 0.27 µg (Table 1). The trace metal Cd ranged from 47.81 (±19.58) to 336.10 (±21.46) µg/kg and total concentration in gonads varied from 0.08 to 6.28 µg. Pb was not detected in the selected individuals from Mount-Lavinia. However, concentration of As ranged from 0.00 (±0.00) to 1805.87 (±104.54) µg/kg, and total accumulation by gonads varied from 0.00 to 33.75 µg.

For the Beruwala reef population, accumulated toxic trace metals ($n=13$) are shown in Table 2. The average concentration of Hg in the Beruwala reef population ranged from 34.40 (±1.62) to 104.39 (±2.18) µg/kg, and the total level in the gonads varied from 0.07 to 0.44 µg. Cd levels ranged from 150.72 (±1.81) to 1149.55

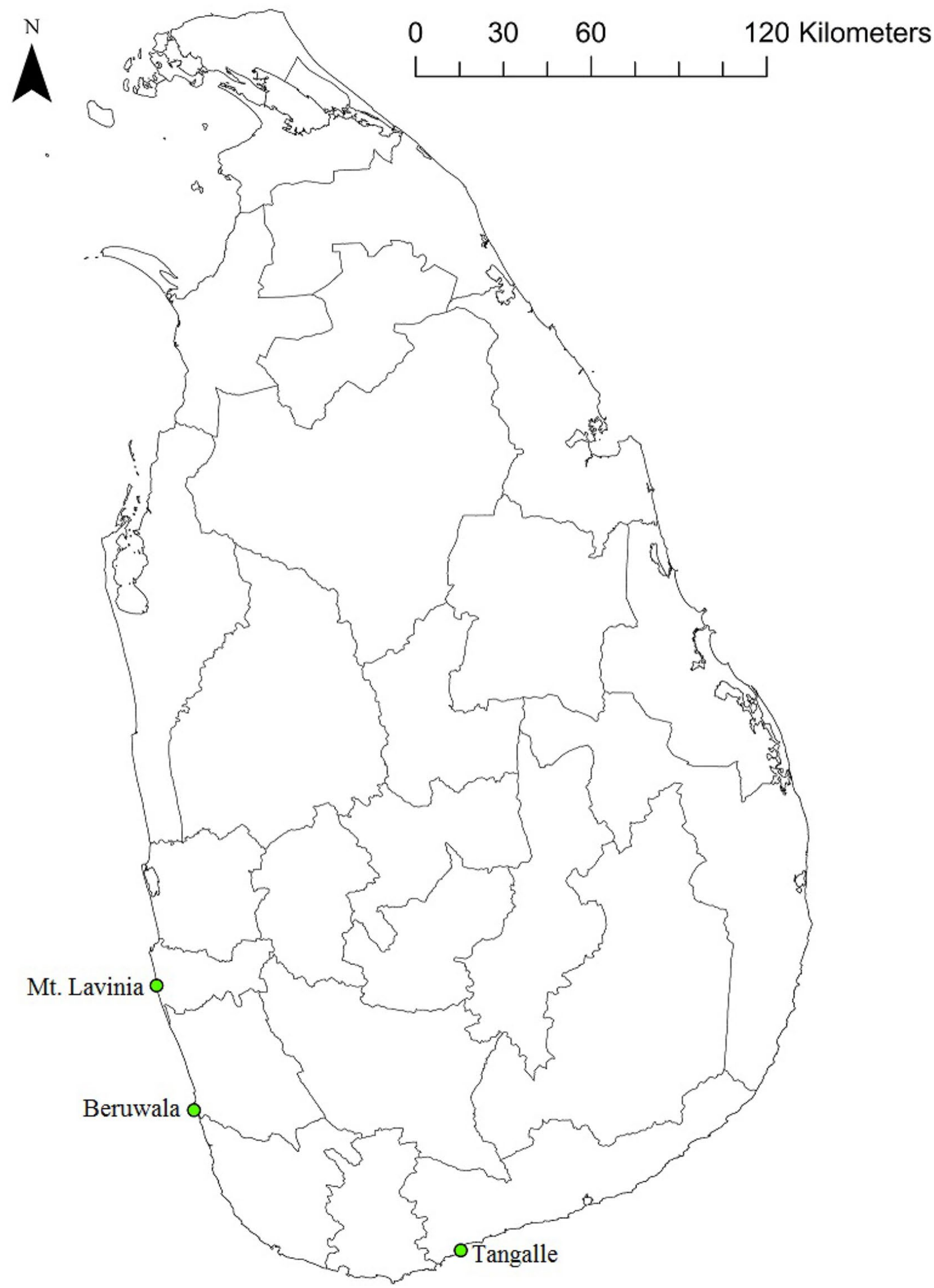


Fig. 1 Sampling sites of *S. variolaris*

(± 90.07) μg/kg and total levels in gonads varied from 0.17 to 9.86 μg. Pb ranged from 0.00 (± 0.00) to 206.27 (± 90.07) μg/kg and total level in gonads varied from 0.00 to 0.36 μg. Also As content in the selected individuals ranged from 1142.52 (± 83.51) to 4635.66 (± 90.07) μg/kg and total accumulation by gonads varied from 2.99 to 22.23 μg.

Table 1 Toxic trace metals (Hg, Cd, Pb and As) concentration and total amount of particular trace metals present in *S. variolaris* at Mount-lavinia reef ($n = 6$) with standard deviation (± SD)

Test diameter (cm)	Total gonad weight (g)	Hg		Cd		Pb		As	
		Average (Hg) (μg/kg)	Total Hg (μg)	Average (Cd) (μg/kg)	Total Cd (μg)	Average (Pb) (μg/kg)	Total Pb (μg)	Average (As) (μg/kg)	Total As (μg)
3.70	1.77	0.00 (± 0.00)	0.00	47.81 (± 19.58)	0.08	0.00 (± 0.00)	0.00	0.00 (± 0.00)	0.00
4.50	1.92	0.00 (± 0.00)	0.00	48.38 (± 9.42)	0.09	0.00 (± 0.00)	0.00	1637.16 (± 396.96)	3.14
5.10	3.10	9.68 (± 0.82)	0.03	156.69 (± 2.63)	0.49	0.00 (± 0.00)	0.00	50.27 (± 156.08)	0.16
6.20	10.07	0.00 (± 0.00)	0.00	158.03 (± 2.73)	1.59	0.00 (± 0.00)	0.00	305.31 (± 64.61)	3.07
7.10	8.63	4.74 (± 0.91)	0.04	217.00 (± 0.78)	1.87	0.00 (± 0.00)	0.00	434.28 (± 324.83)	3.75
8.90	18.69	14.40 (± 5.33)	0.27	336.10 (± 21.46)	6.28	0.00 (± 0.00)	0.00	1805.87 (± 104.54)	33.75

Table 2 Toxic trace metals (Hg, Cd, Pb and As) concentration and total amount of particular trace metals present in *S. variolaris* at Beruwala reef ($n=13$) with ± SD

Test diameter (cm)	Total gonad weight (g)	Hg		Cd		Pb		As	
		Average (Hg) (μg/kg)	Total Hg (μg)	Average (Cd) (μg/kg)	Total Cd (μg)	Average (Pb) (μg/kg)	Total Pb (μg)	Average (As) (μg/kg)	Total As (μg)
4.90	1.15	61.91 (± 7.58)	0.07	150.72 (± 1.81)	0.17	0.00 (± 0.00)	0.00	2600.20 (± 1.14)	2.99
5.30	2.13	54.38 (± 23.32)	0.12	254.07 (± 15.25)	0.54	0.00 (± 0.00)	0.00	3043.89 (± 15.87)	6.48
5.80	4.94	34.40 (± 1.62)	0.17	231.60 (± 30.46)	1.14	36.45 (± 60.40)	0.18	1142.52 (± 83.51)	5.64
5.90	2.91	102.26 (± 3.65)	0.30	858.61 (± 225.66)	2.50	11.94 (± 49.54)	0.03	4483.33 (± 272.99)	13.5
6.00	1.73	91.12 (± 23.50)	0.16	270.28 (± 25.63)	0.47	206.27 (± 5.51)	0.36	2170.50 (± 168.73)	3.75
6.00	3.59	89.19 (± 1.87)	0.32	1149.55 (± 90.07)	4.13	41.24 (± 13.39)	0.15	4480.31 (± 312.27)	16.08
6.30	3.00	85.69 (± 1.61)	0.26	885.50 (± 34.07)	2.66	91.36 (± 33.28)	0.27	1928.18 (± 187.36)	5.78
6.40	4.04	31.15 (± 1.22)	0.13	308.68 (± 31.87)	1.25	7.57 (± 34.02)	0.03	1772.28 (± 135.50)	7.16
6.50	5.59	60.56 (± 13.97)	0.34	331.47 (± 5.63)	1.85	61.45 (± 53.93)	0.34	2040.64 (± 250.12)	11.41
6.70	3.86	85.09 (± 0.09)	0.33	835.22 (± 39.26)	3.22	24.78 (± 3.28)	0.10	4193.81 (± 84.00)	16.19
7.00	8.81	44.73 (± 6.13)	0.39	1119.41 (± 21.88)	9.86	22.03 (± 19.29)	0.19	2543.45 (± 233.71)	22.23
7.30	3.48	104.39 (± 2.18)	0.36	915.82 (± 35.99)	3.19	11.92 (± 5.12)	0.04	4635.66 (± 813.92)	16.13
7.70	8.72	50.23 (± 2.55)	0.44	395.64 (± 3.12)	3.45	21.60 (± 45.87)	0.19	1691.77 (± 185.39)	14.75

Table 3 Toxic trace metals (Hg, Cd, Pb and As) concentration and total amount of particular trace metals present in *S. variolaris* at Tangalle reef ($n = 11$) with ± SD

Test diameter (cm)	Total gonad weight (g)	Hg		Cd		Pb		As	
		Average (Hg) (μg/kg)	Total Hg (μg)	Average (Cd) (μg/kg)	Total Cd (μg)	Average (Pb) (μg/kg)	Total Pb (μg)	Average (As) (μg/kg)	Total As (μg)
4.10	0.75	0.00 (± 0.00)	0.00	83.60 (± 1.61)	0.06	0.00 (± 0.00)	0.00	0.00 (± 0.00)	0.00
4.10	0.80	188.12 (± 24.29)	0.15	289.72 (± 18.01)	0.23	415.03 (± 30.92)	0.33	1866.37 (± 325.00)	1.49
4.80	1.12	164.31 (± 7.12)	0.18	201.39 (± 35.36)	0.23	772.27 (± 211.65)	0.86	3050.80 (± 145.32)	3.42
5.00	1.46	92.92 (± 6.75)	0.14	247.80 (± 33.81)	0.36	309.80 (± 18.40)	0.45	2785.16 (± 168.17)	4.07
5.70	3.31	51.51 (± 2.21)	0.17	796.99 (± 19.78)	2.64	93.70 (± 11.10)	0.31	1574.48 (± 97.97)	5.21
6.50	7.71	12.90 (± 1.80)	0.10	997.91 (± 647.59)	7.69	2.15 (± 9.97)	0.02	869.85 (± 44.62)	6.71
6.50	8.73	56.61 (± 8.09)	0.49	559.31 (± 19.37)	4.88	42.93 (± 26.77)	0.37	1621.43 (± 37.61)	14.16
7.20	11.80	6.27 (± 4.70)	0.07	379.09 (± 3.39)	4.47	0.00 (± 0.00)	0.00	3037.02 (± 34.40)	35.84
7.30	3.66	115.49 (± 1.98)	0.42	4590.83 (± 155.96)	16.80	87.68 (± 16.02)	0.32	4219.97 (± 104.78)	15.45
7.70	6.61	11.87 (± 0.13)	0.08	500.57 (± 8.29)	3.31	0.00 (± 0.00)	0.00	1696.57 (± 198.02)	11.21
8.30	10.72	65.66 (± 1.38)	0.70	1499.39 (± 25.00)	16.07	118.34 (± 35.89)	1.27	4563.32 (± 175.59)	48.92

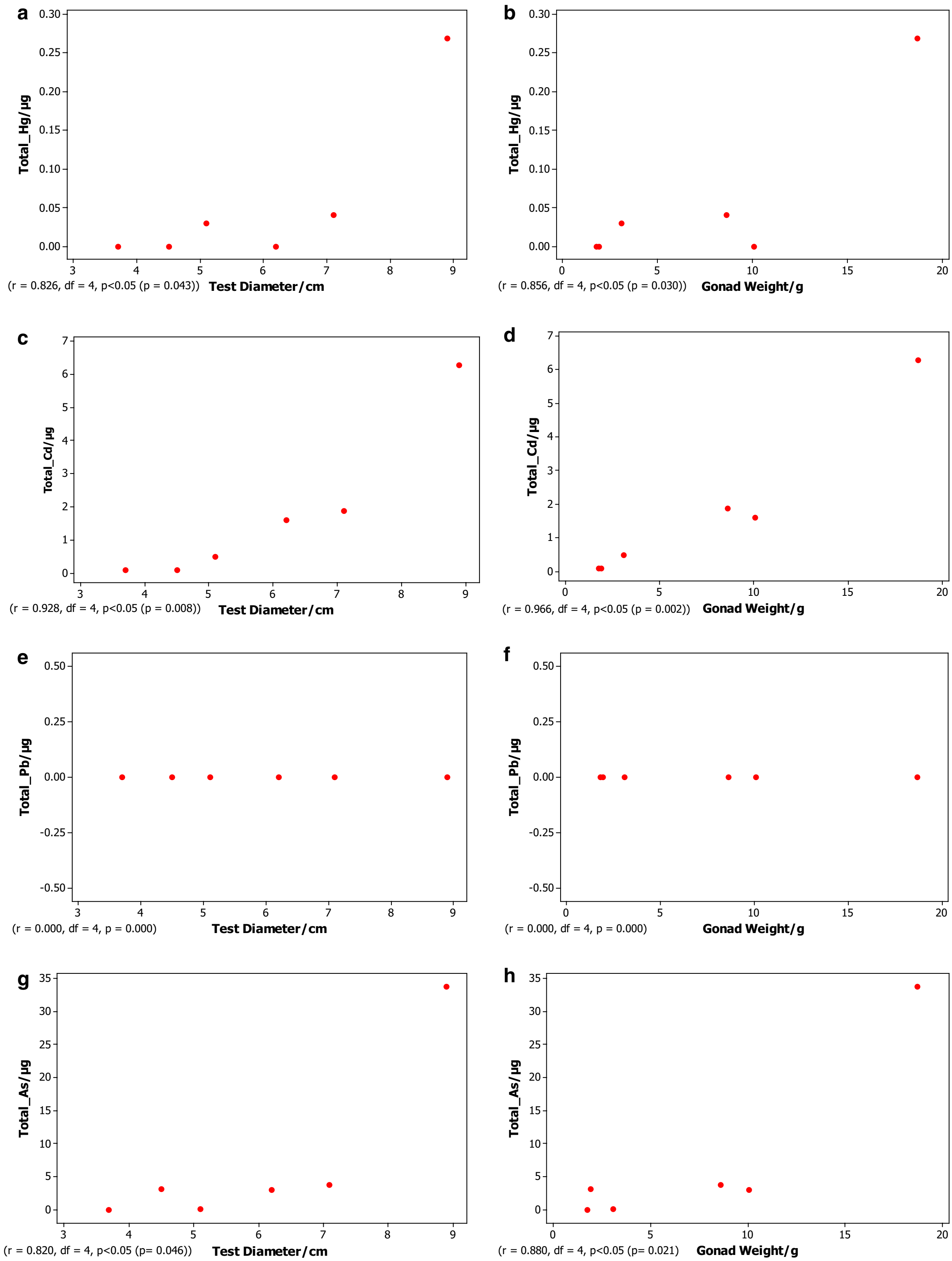
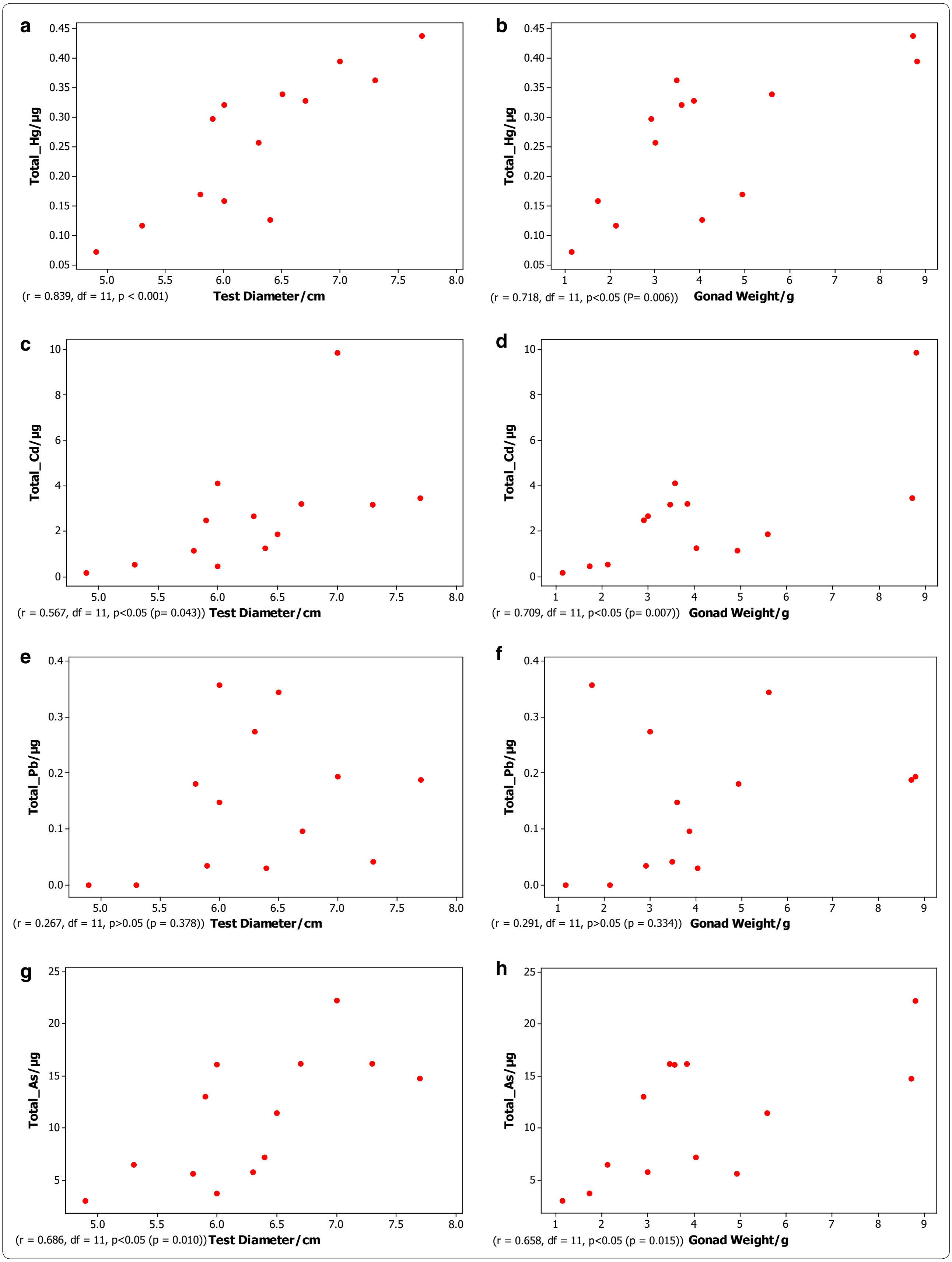


Fig. 2 Relationships of toxic heavy metal concentration in *S. variolaris* at Mount-Lavinia reef. **a** Hg versus test diameter, **b** Hg versus total gonad weight, **c** Cd versus test diameter, **d** Cd versus total gonad weight, **e** Pb versus test diameter, **f** Pb versus total gonad weight, **g** As versus test diameter, **h** As versus total gonad weight


a
Total_Hg/µg
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
5.0 5.5 6.0 6.5 7.0 7.5 8.0
(r = 0.839, df = 11, p < 0.001)
Test Diameter/cm
b
Total_Hg/µg
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
1 2 3 4 5 6 7 8 9
(r = 0.718, df = 11, p<0.05 (P= 0.006))
Gonad Weight/g
c
Total_Cd/µg
10
8
6
4
2
0
5.0 5.5 6.0 6.5 7.0 7.5 8.0
(r = 0.567, df = 11, p<0.05 (p= 0.043))
Test Diameter/cm
d
Total_Cd/µg
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9
(r = 0.709, df = 11, p<0.05 (p= 0.007))
Gonad Weight/g
e
Total_Pb/µg
0.4
0.3
0.2
0.1
0.0
5.0 5.5 6.0 6.5 7.0 7.5 8.0
(r = 0.267, df = 11, p>0.05 (p = 0.378))
Test Diameter/cm
f
Total_Pb/µg
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6 7 8 9
(r = 0.291, df = 11, p>0.05 (p = 0.334))
Gonad Weight/g
g
Total_As/µg
25
20
15
10
5
5.0 5.5 6.0 6.5 7.0 7.5 8.0
(r = 0.686, df = 11, p<0.05 (p = 0.010))
Test Diameter/cm
h
Total_As/µg
25
20
15
10
5
1 2 3 4 5 6 7 8 9
(r = 0.658, df = 11, p<0.05 (p = 0.015))
Gonad Weight/g

(See figure on previous page.)
Fig. 3 Relationships of toxic heavy metal concentration in *S. variolaris* at Beruwala reef. **a** Hg versus test diameter, **b** Hg versus total gonad weight, **c** Cd versus test diameter, **d** Cd versus total gonad weight, **e** Pb versus test diameter, **f** Pb versus total gonad weight, **g** As versus test diameter, **h** As versus total gonad weight

Hg levels in *S. variolaris* individuals from Tangalle reef ($n = 11$) ranged from 0.00 (±0.00) to 188.12 (±24.29) µg/kg and total level in gonads varied from 0.00 to 0.70 µg. Cd levels ranged from 83.60 (±1.61) to 4590.83 (±155.96) µg/kg and total level in gonads varied from 0.06 to 16.07 µg. Pb ranged from 0.00 (±0.00) to 772.27 (±211.65) µg/kg and total levels in gonads varied from 0.00 to 1.27 µg. In addition, As in selected individuals ranged from 0.00 (±0.00) to 4563.32 (±175.59) µg/kg and total levels in gonads varied from 0.00 to 48.92 µg (Table 3).

Relationships with test diameter and gonad weight for trace metal accumulation

The results revealed that there is a positive correlation with between gonadal Hg levels and the test diameter ($r = 0.826$, $df = 4$, $p = 0.043$) and also a between gonad weight ($r = 0.856$, $df = 4$, $p = 0.030$) (Fig. 2a, b) for *S. variolaris* population in Mount-Lavinia. Gonadal Cd levels also had a significant relationship with test diameter ($r = 0.928$, $df = 4$, $p = 0.008$), but strong positive r and have significant relationship with gonad weight ($r = 0.966$, $df = 4$, $p = 0.002$) (Fig. 2c, d). Total Pb levels showed no correlation with the test diameter and gonad weight (Fig. 2e, f). Arsenic levels showed a significant positive relationship with test diameter ($r = 0.820$, $df = 4$, $p = 0.046$) and also with the gonad weight ($r = 0.880$, $df = 4$, $p = 0.021$) (Fig. 2g, h).

Hg levels of the Beruwala population showed a significant positive relationship with test diameter ($r = 0.839$, $df = 11$, $p < 0.001$) and gonad weight ($r = 0.718$, $df = 11$, $p = 0.006$) (Fig. 3a, b). Similarly, Cd levels also showed a significant positive relationship with test diameter ($r = 0.567$, $df = 11$, $p = 0.043$) and gonad weight ($r = 0.709$, $df = 11$, $p = 0.007$) (Fig. 3c, d). Pb has a small positive r value and has no significant relationship with test diameter ($r = 0.267$, $df = 11$, $p = 0.378$) and gonad weight ($r = 0.291$, $df = 11$, $p = 0.334$) (Fig. 3e, f). Levels of the trace metal As also had a significant positive relationship with test the diameter ($r = 0.686$, $df = 11$, $p = 0.010$) and as well as with the gonad weight ($r = 0.658$, $df = 11$, $p = 0.015$) (Fig. 3g, h).

The analysis of the Tangalle population revealed that there is a significant positive relationship between Hg levels and the test diameter ($r = 0.542$, $df = 9$, $p = 0.085$) and with gonad weight ($r = 0.414$, $df = 9$, $p = 0.205$) (Fig. 4a, b). Similarly, cadmium levels showed a strong significant positive relationship with the test diameter ($r = 0.764$, $df = 9$, $p = 0.006$) as well as with the gonad weight ($r = 0.509$, $df = 9$, $p = 0.110$) (Fig. 4c, d). Levels of As also had a significant positive relationship with test diameter ($r = 0.779$, $df = 9$, $p = 0.005$) and gonad weight ($r = 0.825$, $df = 9$, $p = 0.002$) (Fig. 4e, f). Similarly, Pb levels also did not show a significant relationship with test diameter ($r = 0.151$, $df = 9$, $p = 0.657$) and gonad weight ($r = 0.048$, $df = 9$, $p = 0.888$) (Fig. 4g, h).

Discussion

There were low levels of trace metals in the gonads of *S. variolaris* in all the studied locations. The significant finding was that there were undetectable levels of Pb in *S. variolaris* gonads collected from the Mount-Lavinia reef. Further, at this location *S variolaris* population had accumulated As (0.00 ppm–1.81 mg/kg) at a high level (Table 4) compared to other studied metals (Hg and Cd) though this did not exceeded the European Union (EU) (Table 4) and Sri Lanka permitted level. *S. variolaris* populations in the Beruwala reef was not highly contaminated by Hg (0.03–0.10 mg/kg) and Pb (0.00–0.21 mg/kg), but highly contaminated by As (1.14–4.64 mg/kg) and Cd (0.15–1.15 mg/kg), which exceeds the permitted level of the 1.00–2.00 mg/kg (Table 4). According to the EU, there were very low levels of Hg (0.00–0.12 mg/kg) in the *S. variolaris* population of Tangalle reef. However, there were higher levels of Cd (0.08–4.60 mg/kg) and As (0.00–4.563 mg/kg) in this population though they were below the permitted levels. Accumulated Pb levels in *S. variolaris* (0.00–0.77 mg/kg) varied between permitted level (0.30–1.50 mg/kg) (Table 4). The low levels of toxic trace metals could be seen in the Mount-Lavinia

(See figure on next page.)
Fig. 4 Relationships of toxic heavy metal concentration in *S. variolaris* at Tangalle reef. **a** Hg versus test diameter, **b** Hg versus total gonad weight, **c** Cd versus test diameter, **d** Cd versus total gonad weight, **e** Pb versus test diameter, **f** Pb versus total gonad weight, **g** As versus test diameter, **h** As versus total gonad weight

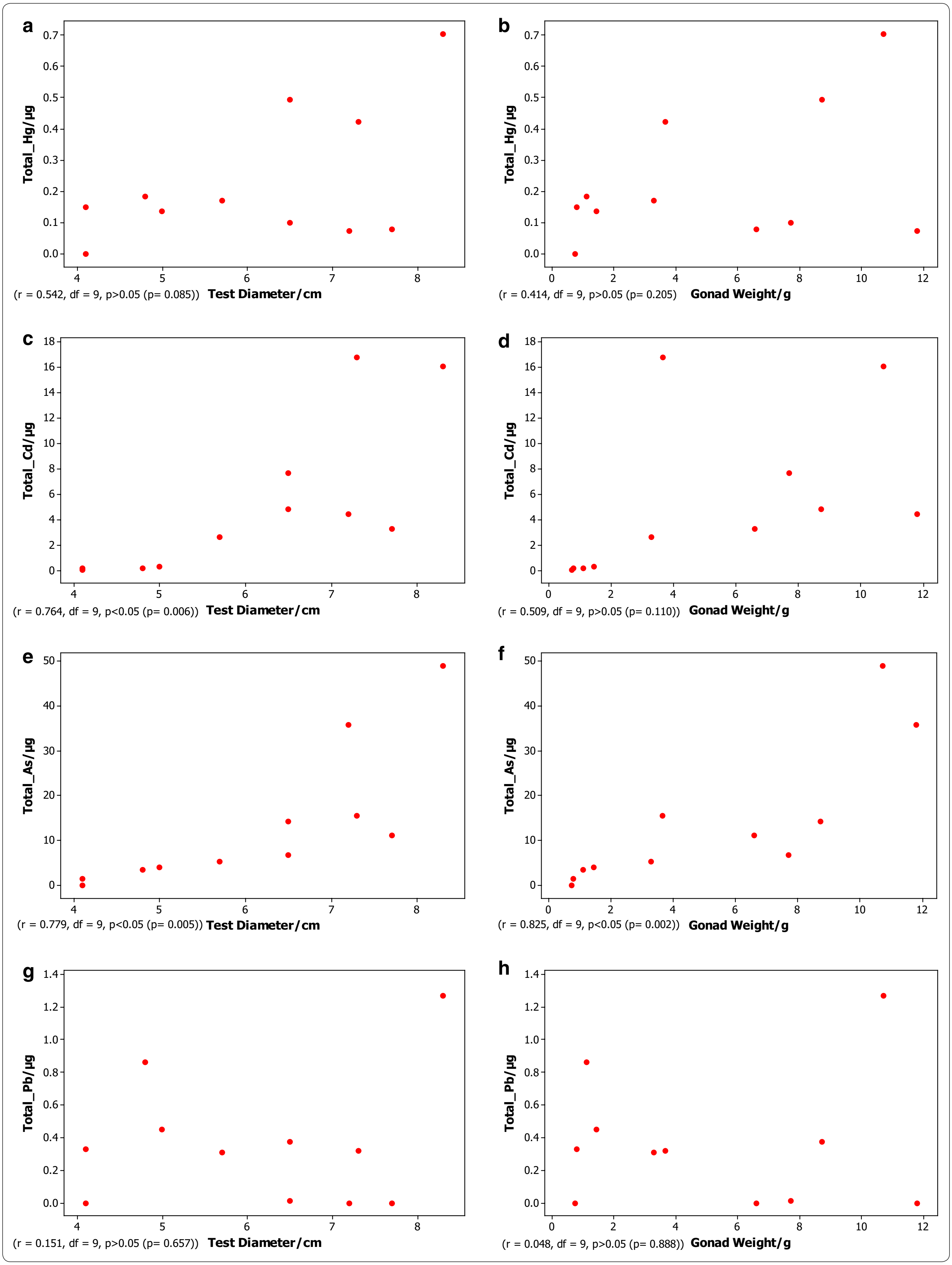

a
Total_Hg/µg
(r = 0.542, df = 9, p>0.05 (p= 0.085)) Test Diameter/cm
b
Total_Hg/µg
(r = 0.414, df = 9, p>0.05 (p= 0.205) Gonad Weight/g
c
Total_Cd/µg
(r = 0.764, df = 9, p<0.05 (p= 0.006)) Test Diameter/cm
d
Total_Cd/µg
(r = 0.509, df = 9, p>0.05 (p= 0.110)) Gonad Weight/g
e
Total_As/µg
(r = 0.779, df = 9, p<0.05 (p= 0.005)) Test Diameter/cm
f
Total_As/µg
(r = 0.825, df = 9, p<0.05 (p= 0.002)) Gonad Weight/g
g
Total_Pb/µg
(r = 0.151, df = 9, p>0.05 (p= 0.657)) Test Diameter/cm
h
Total_Pb/µg
(r = 0.048, df = 9, p>0.05 (p= 0.888)) Gonad Weight/g

Table 4 Maximum level of toxic trace metals contaminants of selected seafoods species according to EU and Sri Lanka regulations [22–24]

Contaminants	Food types	Maximum level of contaminants/(mg/kg wet weight)
Pb	Muscle meat of fish	0.30
	Crustaceans	0.50
	Bivalve, molluscs	1.50
Cd	Muscle meat of fish	0.05
	Crustaceans	0.50
	Bivalve, molluscs	1.00
Hg	Fish products and muscle meat	1.00
As	Fish, crustacea, molluscs	1.00–2.00

population when compared to the other populations. The levels of accumulated trace metals in sea urchin gonads represent the environmental condition and thus can be used as bio-indicator to determine the trace metal pollution of that particular coast. As a consequence, most sea urchin species are used as bio-indicators in the world (*Paracentrotus lividus*) [20, 23]. However, there is currently no literature available for trace metal contamination in sea urchins, specifically for Sri Lanka,.

The average levels of accumulated toxic trace metals in sea urchins were in the order from As > Cd > Pb > Hg in Beruwala population, Cd > As > Pb > Hg in Tanagalle population and As > Cd > Hg > Pb in Mount-lavinia population. According to the above order, both Beruwala and Tangalle reefs had the lowest Hg contamination than the Mount-Lavinia sample.

The accumulated levels of all of the tested toxic trace metals (Hg, Cd and As) significantly correlated with the test diameter of *S. variolaris* population in the Beruwala reef. This indicates that the accumulation of Hg, Cd and As increases with a size of the test. Nevertheless, accumulated Pb levels did not significantly correlate with average test diameter and total gonad weight. Therefore, it seems that the accumulation of Pb does not depend on the total gonad weight or the average test diameter. In the Tangalle sample, Cd and As metals showed a significant relationship between these toxic trace metal accumulations with test diameter while Hg and Pb metals did not significantly correlate with test diameter. However, total gonad weight of *S. variolaris* showed that accumulation of As significantly correlated though Hg, Cd and Pb did not. The Mount-Lavinia samples showed that there was a significant relationship between toxic trace metals accumulation with average test diameter with Hg, Cd and As metals and Pb has not significantly correlated with average test diameter. Similarly, the total gonad weight of *S. variolaris* revealed a significant relationship with Hg, Cd and As metals and Pb did not because Pb accumulation was not at detectable level. Therefore, it could not be seen any relationship between average test diameter and total gonad weight of Pb accumulation. Finally, As was the only metal which showed a clear significant relationship between both average test diameter and total gonad weight in all the selected sites.

These relationships of *S. variolaris* revealed that toxic trace metals accumulation increase with their age. This would be due to long time accumulation as a result of exposure to the polluted environment in the lifetime. In addition, accumulation of excess trace metals in gonads of sea urchins showed a relationship with their age and a similar result was also reported by Mostafa and Collins [20], because the maximum value of trace metal concentration resulted in the high average test diameter recorded individuals than the low average test diameter recorded individuals. The study conducted by Mostafa and Collins [20] revealed that accumulation of Cd and Pb showed the negative linear relationship between their wet weight and accumulated concentration in the species *P. lividus*.

The frequency distribution of shell diameter and weight of *S. variolaris* range from a minimum of 3.30 to a maximum of 8.90 cm and weight from a minimum of 30.15 g to a maximum of 346.56 g for all the population selected from Sri Lanka. But Smith and Kroh [7] recorded *S. variolaris* having maximum shell diameter of 11 cm from the Visakhapatnam Coast (India). However, 8.90 cm is the highest value recorded for the Sri Lankan *S. variolaris*.

Conclusion

The findings from this study will be helpful to the get an idea about the trace metal accumulation *S. variolaris*. Most importantly, it also indicates that the accumulated levels of trace metals did not exceed the harmful level for human consumption. Hence, due to safe levels of trace metals in *S. variolaris* gonads, there is possibility to export the gonads and gonad products from Sri Lanka, which will be a highly profitable industry in the future.

Abbreviations

IPHT: Institute of Post-Harvest Technology; NARA: National Aquatic Resources Research and Development Agency; Hg: mercury; Pb: lead; Cd: cadmium; As: arsenic; AAS: atomic absorption spectrophotometer; $Pd(NO_3)_2$: palladium nitrate; ppb: parts per billion; EU: European Union.

Authors' contributions

All authors contributed to the manuscript improvement. All authors read and approved the final manuscript.

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Acknowledgements

The authors are grateful to the National Aquatic Resources Research and Development Agency (NARA). Also, we thank to K. Ukuwela for his invaluable comments on manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data sets generated and analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

This study was supported in part by a grant from the National Aquatic Resources Research and Development Agency (NARA), Crow Island, Colombo 15, Sri Lanka.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 20 February 2018 Accepted: 7 August 2018
Published online: 16 August 2018

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