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High levels of the endocrine disruptors bisphenol-A and 17β-estradiol detected in populations of green mussel, *Perna viridis*, cultured in the Gulf of Thailand

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ABSTRACT

The occurrence of the endocrine disruptors bisphenol-A (BPA) and 17β-estradiol (E_2) in cultured populations of green mussel (*Perna viridis*) and water samples collected from selected fresh and marine coastal environments along the eastern coast of Thailand were investigated. Analysis found high levels of BPA in mussel tissues that correlated with levels found in coastal (maximum 37.13 ng/L) and freshwater (50.7 ng/L) sites situated near industrial and densely populated areas. By comparison, high levels of E_2 (62.99 ± 5.03 ng/L) were found in freshwater sites near to urban areas. Higher concentrations of BPA and E_2 were found in mature green mussels (> 6 mo. old; 6.40 ± 0.52 cm shell length) than levels determined in juveniles (< 2–3 mo. old; 2.29 ± 0.65 cm shell length). To evaluate the potential risks associated with the consumption of green mussels, the bio-concentration factor (BCF) for BPA was determined to be 1650 for adult bivalves and 283 for juveniles. As *P. viridis* can accumulate BPA from the environment, this raises concerns regarding the risks posed by consuming seafood sourced from zones near to major conurbations. Whether the high levels of E2 found in green mussels is due to accumulation or to de novo synthesis as seen in other molluscs, requires further investigation. While industrial and domestic wastewater may be important sources of BPA, E_2 contamination within the eastern part of the Gulf of Thailand has been linked to domestic waste. The study highlights the importance of the temporal and spatial monitoring of sentinel species, such green mussels, for environmental contaminants, the results of which can lead to the construction of regional risk maps helping to inform national strategies regarding aquaculture zoning and aquatic food safety.

1. Introduction

Global concern regarding human exposure to endocrine disrupters (EDCs) such as 17β-estradiol (E_2) and bisphenol-A (BPA), which mimic natural endogenous estrogens, has increased because of the health risks associated with their long term exposure, resulting in suggested impacts on reproduction (Li et al., 2010; Sharpe, 2010a) and increased incidences of conditions such as diabetes (Provvisiero et al., 2016) and certain cancer types in humans (Yang et al., 2009; Erler and Novak, 2010; Hilakivi-Clarke et al., 2013; Seachrist et al., 2015). E_2 is a potent natural estrogen produced, primarily, within the ovaries of vertebrates. It is also widely used in a range of pharmaceutical drugs as part of hormone therapy for menopause (Adeel et al., 2017). BPA ($((CH_3)_2C(C_6H_4OH)_2$) is an organic synthetic compound that is widely used in the manufacture of polycarbonate plastics, epoxy resins and in the protective lining of food and drink containers (Vandenberg et al., 2009). Although, BPA is a xenoestrogen with properties able to mimic natural estrogen, there is much controversy regarding its use and whether it exerts adverse effects on humans (Vandenberg et al., 2007; Diamanti-Kandarakis et al., 2009; Ryan et al., 2009; Sharpe, 2010b; Kelley et al., 2015; Ejaredar et al., 2017). In 2008, the U.S. Food and Drug Administration (FDA) set a No-Observed-Adverse-Effect Level (NOAEL) for BPA at 5000 μg/kg bdy wt/day (U.S. FDA, 2008; EFSA, 2010). According to the London School of Hygiene & Tropical Medicine data on average adult body weights, this suggests levels of between 247,000–437,000 μg/adult/day (av. 62 kg adult; range 49.59–87.4 kg;

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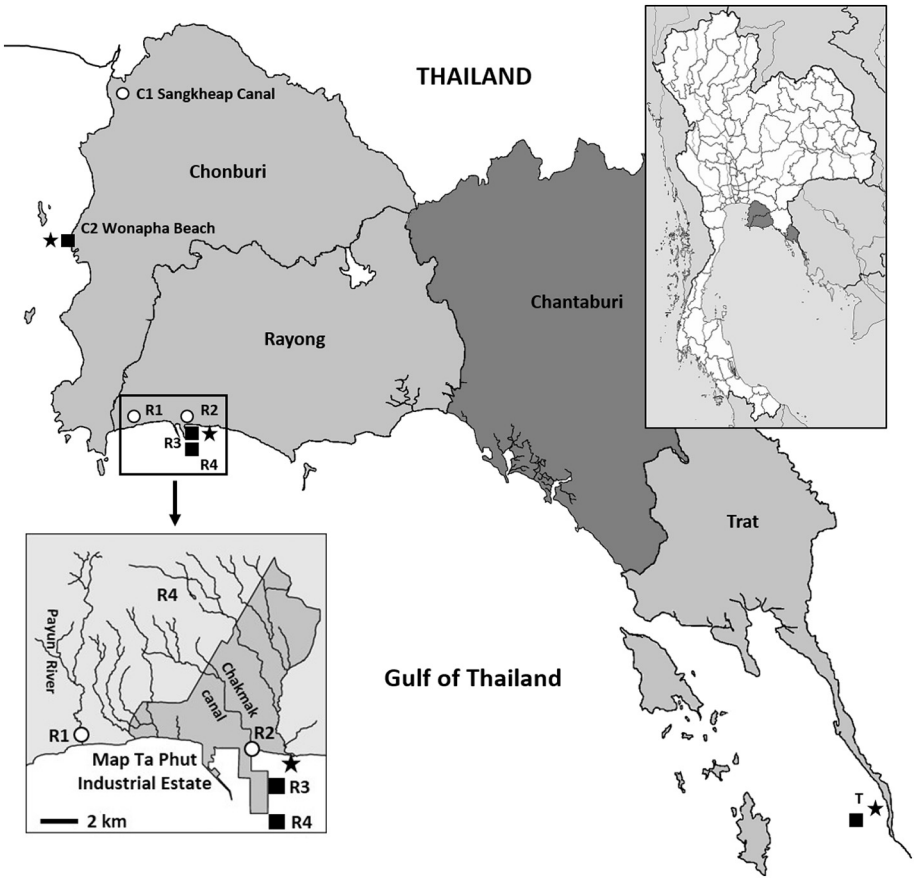

Fig. 1. Map of the study areas and sampling stations along the eastern seaboard of the Gulf of Thailand. Open circles (○) represent freshwater sample sites; solid squares (■) represent marine sample sites; and, solid stars (★) represent green mussel farms. Abbreviations: C = Chonburi Province station; R = Rayong Province station; and, T = Trat Province station.

Quilty-Harper, 2012). Subsequently, in 2010, the U.S. Environmental Protection Agency (USEPA, 2010) set the chronic oral reference dose at 50 μg/kg bdy wt/day; while the European Union's Tolerable Daily Intake (TDI), was lowered further by the European Food Standards Agency (2018) to 4 μg/kg bdy wt/day. BPA and E_2 based-studies, however, suggest that doses below these levels may have adverse effects especially if exposure occurs in young infants or to a foetus in utero. As BPA is regarded a substance that pose a risk to human health, several countries including the USA and Canada, put a ban in place regarding the use of BPA in plastic products for infants (European Commission, 2011; Vom Saal et al., 2012; Vandenberg, 2014).

The predicted no effect concentration (PNEC) for BPA for aquatic organisms, however, was derived from a series of studies coordinated within the European Union and was set at 1.5 μg/L (European Union, 2008), which included a five-fold uncertainty factor (cited in USEPA, 2010). However, following a re-evaluation of the aquatic ecological risks for BPA using aquatic toxicity data garnered for many species, Wang et al. (2018) proposed a revised PNEC of 4.01 μg/L. For E_2, Caldwell et al. (2012) set a PNEC of 2 ng/L following an assessment of the risks it posed to aquatic organisms, however, a more recent study conducted by Wu et al. (2014), proposed a lower PNEC value of 0.73 ng/L.

The release of E_2 into the environment results from agricultural run-off from livestock and from human waste. Human activity in densely populated areas results in an increase of contaminants in urban run-off released into aquatic environments (Adeel et al., 2017). Consequentially, the residual BPA from plastic manufacturing activity in urban discharge released into aquatic environments, is accumulated by living organisms (Vethaak et al., 2005; Canesi and Fabbri, 2015). BPA and E_2 pollutants are accumulated either by diffusion through the skin and gills (bioconcentration) or by the ingestion of adulterated food (biomagnification). Organisms can accumulate these pollutants via food chains at successively higher levels than the environment (Franke et al., 1994; Lu et al., 2011; Staniszewska et al., 2014). As sessile organisms that feed by the filtration of particulate matter in the water column, mussels are frequently used in biomonitoring for marine pollution in coastal areas (Yap et al., 2002; Nicholson and Lam, 2005). One of these is the green mussel, *Perna viridis* (see Monirith et al., 2003; Isobe et al., 2007), which are extensively commercially marketed in most Asia-Pacific countries, notably Thailand. In 2015, for which latest comprehensive global figures from FAO are available, a total of 147,503 tons of green mussels were produced from aquaculture activities in six Asian states; Thailand produced 118,775 tons, i.e. 80.52% of global production (FAO FishStatJ, 2017). Given the Thai nation's predilection for eating green mussels, if levels of either EDC are found to be high, then it may limit aquaculture production in zones where levels are found to be high. Additionally, in the absence of surveillance, if levels go ignored, then the consumption of this seafood item may pose a risk to human health.

The semi-enclosed system such as the Eastern Gulf of Thailand and its bays, are very sensitive to contamination from mainland human activities. The accumulation of a range of chemicals within the Gulf have been demonstrated and include studies on, for example, heavy metals (Ruangwises and Ruangwises, 1998), organochlorines (Cheevaporn et al., 2005; Wattayakorn and Rungsupa, 2010), polychlorinated biphenyls (Jaikanlaya et al., 2009; Wattayakorn and Rungsupa, 2010), and, microplastics (Thushari et al., 2017). There are, however, very few studies that have focused on BPA or E_2 as pollutants within the Gulf of Thailand. This study set out to investigate the levels of BPA and E_2 contamination in coastal waters along the eastern coast and to identify some of the possible routes of contamination. The levels of both pollutants were determined at sites near to densely populated areas and sites of industrial activity from relevant samples collected from freshwater, coastal waters and from farmed green mussels, *P.*

Table 1
Description of the sites sampled along the eastern seaboard of the Gulf of Thailand.

Sampling site					Water parameters			
Site	Location	Category	North	East	pH	Temperature	Salinity	DO
			Latitude	Longitude		(°C)	(‰)	(mg/L)
Chonburi								
C1	Sangkhep Canal	Municipality wastewater	13.22.28.8	100.59.10.0	7.8	29.8	0.5	9.72
C2	Wonapha Beach	Coastal water, green mussels	13.16.07.9	100.55.21.8	8.0	26.9	28.9	NA
Rayong								
R1	Payun River	Freshwater	12.40.32.5	101.09.26.9	7.0	32.1	0.1	6
R2	Chak-mak canal	Freshwater, green mussels	12.40.35.6	101.04.12.6	7.0	32.9	0.3	5.5
R3	MTP1	Coastal water	12.38.54.0	101.10.06.4	7.8	25.7	29.4	6.05
R4	MTP2	Coastal water	12.38.07.8	101.10.06.6	8.1	25.9	29.6	6.59
Trat								
T	Reference	Coastal water, green mussels	11.43.05.8	102.53.07.0	8.0	29.6	29.0	5.78

NA = not available; MTP1, MTP2 = Map Ta Phut industrial estate.

viridis, located along the coastline of the Eastern Gulf of Thailand. In addition to determining levels, the study set out to evaluate the health risks associated with the consumption of green mussels harvested from polluted zones.

2. Materials and methods

2.1. Study area

Seven sampling sites within three provinces bordering the Gulf of Thailand were selected: two Chonburi (C1-2), four in Rayong (R1-4), and one in Trat Province (T), each of which are exposed to industrial wastewater (Fig. 1; Table 1). In Chonburi, C1 was located on the Sangkheap Canal, while the C2 sample was taken from Wonapha beach. Both sites receive urban runoff at high levels. In Rayong, R1 was based on the Payun River, which also receives high volumes of urban runoff, however, the levels were anticipated to be lower because of the lower population density in this area. Collection site R2, was on the Chak-mak canal, which receives petrochemical industrial wastewater runoff from the Map Ta Phut industrial estate, which is the world's eighth-largest petrochemical industrial hub. R3 and R4 were sites located on the coast that were exposed to high levels of petrochemical industrial wastewater runoff but taken at points some distance from the source (ca. 2.5 and 4 km respectively from Chak-mak Canal). It was theorised, however, that the two sites might have low concentrations of the EDCs because of their distance from the source of chemical inputs. The reference site located in Trat Bay, Trat Province (T, see Fig. 1) to the far south eastern extreme of Thailand, was expected to have the lowest concentrations of BPA and E_2 among the sites selected from the current study. Green mussels are the principal mollusc species cultured in Thailand and are grown extensively throughout the Gulf of Thailand; green mussels serve as an appropriate species to determine BPA and E_2 contamination in the coastal waters of the eastern seaboard of the Gulf of Thailand and whether their consumption poses a risk to human health.

2.2. Water samplings and preparation

Sampling was conducted throughout December 2016 to January 2017. At each site, three × ten litre samples of water were taken 0.5 m below the water surface using a Kitahara-type water sampler. For the measurement of BPA and E_2, 15 L polyethylene bottles were rinsed with ultrapure water and methanol (HPLC grade) at least three times to ensure that the containers were free from residual contamination before they were rinsed and then filled with the water sample. Water parameters (pH, dissolved oxygen, salinity and temperature) were measured in situ using a hand-held meter (YSI 85, YSI Inc., Yellow Springs, USA) which had been calibrated according to the manufacturer's instruction before use. All water and green mussel samples were chilled on ice immediately following their collection and then transferred directly to the laboratory (transit time < 6 h), where they were stored in a −20 °C freezer until they were analysed.

For analysis, each 10 L water sample was initially filtered through a glass microfiber filter (Whatman, GF/F, 0.7 μm) and then subjected to solid phase extraction (SPE) using a C18 Sep-Pak cartridge (#WAT020515, Waters Corp., Milford, MA) that was activated before use with 6 mL of methanol and 4 mL of ultrapure water. The flow rate of the water sample was adjusted to 5 mL/min and then washed with 5 mL of 5% methanol at 5 min intervals. Subsequently, the cartridge was dried under a nitrogen gas stream (Friesen et al., 2012). To concentrate the BPA and E_2 compounds, the cartridge was eluted with 3 mL of methanol and then injected into a HPLC for BPA and E_2 analysis.

2.3. Mussel sample collection and preparation

The green mussel samples were collected from the hanging ropes used for their culture at each farm site, with both mature and juvenile mussels being randomly selected from at least 3 ropes within each production unit (i.e. 18 mussels per rope; 9 mature and 9 juvenile). The collected bivalves were then categorised on their shell length as either as “juvenile”, i.e. with a shell length < 30 mm; green mussels typically reach sexual maturity at 15–30 mm shell length which approximates to 2–3 months of age (Global Invasive Species Database, 2018), or as “mature” – specimens with a shell length exceeding 50 mm. Mature specimens measuring between 50 and 70 mm were collected for this study; all specimens were measured using digital Vernier calipers (Fuzhou Guanghui, China). In the study, the average shell lengths of the two mussel classes were 2.29 ± 0.65 cm for the “juveniles”, and 6.40 ± 0.52 cm for the “mature” mussels (across all sites). The mature mussels were estimated to be 7–8 months of age based on charts provided by Chatterji et al. (1984). Digestive tissue from each mussel was excised using a sterile scalpel blade. One gram (wet weight) of green mussel digestive tissue was then processed using an Omni TH homogenizer (OMNI International, USA) and the resultant homogenate subjected to an extraction with an 8 mL mixture of 5 mL methanol and 3 mL ultrapure water and then incubated at 50 °C for 30 min. Thereafter, the samples were centrifuged at 14,000*g* for 10 min., the resultant supernatant was then transferred to a new vessel and diluted with ultrapure water to make a final volume of 100 mL (Gatidou et al., 2010).

2.4. BPA and E_2 analysis

All the chemical reagents used in this study were HPLC grade

(Merck, Germany) and used without further purification (> 98% purity). Analytical standards for BPA and E_2 were purchased from Sigma-Aldrich (Switzerland).

The measurement of BPA and E_2 in the samples was performed using a Waters Alliance® e2695 Separations Module (Milford, MA, USA) equipped with degasser, quaternary solvent organizer pump, auto sampler and a Waters 2489 UV/Visible Detector. Separations were performed using Sunfire C18 reverse phase columns (4.6 mm × 250 mm, 5 µm) (Waters Corporation, Ireland). Data were performed using an Empower 2.0® software package. The method followed the modified protocol of Card et al. (2012) and Zhou et al. (2014), the column temperature was held at 30 °C and UV detection wavelength was set at 280 nm. The mobile phase was a mixture of methanol and ultrapure water (75:25 v/v) with the constant flow rate of 1 mL/min.

2.5. Determination of bioconcentration factors (BCF)

The BCFs were calculated using the following formula (Staniszewska et al., 2014):

BCF (L/kg) = C_{mussel}/C_{water} where C_{mussel} is the BPA concentration in the green mussel tissue (ng/kg) and C_{water} is the concentration of BPA in the ambient water (ng/L).

3. Results

3.1. HPLC analysis

Calibration curves for BPA and E_2 were determined using a series of nine concentrations, i.e. 0.5, 1, 5, 10, 50, 100, 150, 200 and 250 ng/mL prepared from the analytical standards. The subsequent regression coefficients (R^2) for both EDCs were > 0.99 (i.e. $y = 0.0008x + 2.1916$; $R^2 = 0.9980$ for BPA; $y = 0.0019x + 0.8244$; $R^2 = 0.9984$ for E_2). Performance of the instrumentation and methodology were cross-validated by spiking authentic BPA and E_2 (100 ng/L) into the sample matrices and then passing these through the entire analytical procedure to evaluate recovery efficiency (triple repeats) with resultant efficiencies of 98.38 ± 3.35% and 103.54% ± 2.22% for BPA and E_2, respectively. Analysis of an EDC standard consisting of a mixture of 40 ng/mL of BPA and 40 ng/mL E_2 found that the retention time (RT) was 2.935 min for BPA and 3.933 min for E_2, with a detection limit of 1.10 and 1.30 ng/mL, respectively (Fig. 2).

3.2. Occurrence of BPA and E_2 in freshwater

In the current study, a total of 10 samples were analysed; three from freshwater, four from seawater, and three collections of green mussels in three different provinces. The physical parameters of the water sampled at each site are presented in Table 1. The freshwater results are shown in Table 2 along with levels recorded in other studies conducted on urban, industrialised and notably polluted waters. Table 2 shows that the samples taken in the Sankheap Canal in Chonburi Province (C1) and from the Payun River in Rayong (R1) to the west of the Map Ta Phut Industrial Estate were similar in their BPA concentrations at 1.1–1.2 ng/g. The sample taken from Chak-mak canal (R2) within the Map Ta Phut Industrial Estate, however, was the highest recorded in the study 50.56 ng/L. In a marked contrast to this, the E_2 levels within Chak-mak canal were the lowest of the three freshwater samples at < 1.30 ng/L. The samples taken in Rayong and Chonburi, near to the main provincial cities were 35.53 ± 2.99 and 62.99 ± 5.03 ng/L, respectively.

3.3. Occurrence of BPA and E_2 in coastal water

The concentrations of BPA and E_2 determined in the seawater samples are shown in Table 3. Of the four samples, that collected from Wonapha Beach in Chonburi (C2; Fig. 1) had the highest levels of BPA at 37.13 ± 2.70 ng/g; the remaining three samples had values < 4 ng/g. The E_2 concentrations were also low in all four samples, only that taken from R3, the seawater collection point closest to the Map Ta Phut Industrial Estate had a value of 3.52 ± 0.79 ng/L. These levels declined at greater distances from the coast and the industrial estate as demonstrated by the lower levels of E_2 obtained for sample R4 with < 1.30 ng/L.

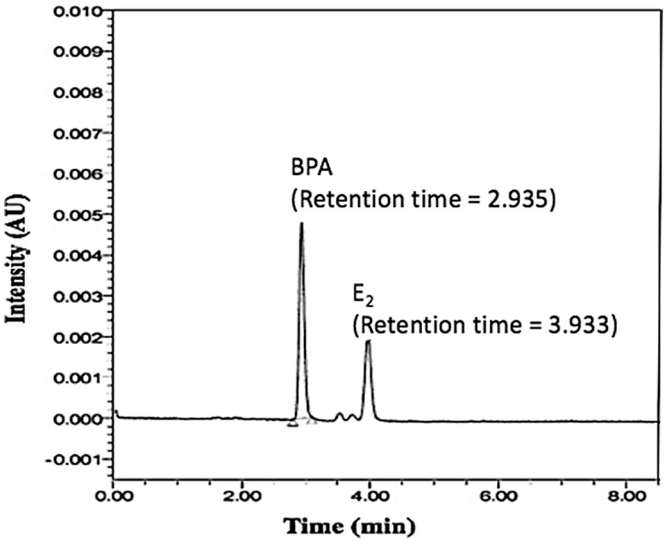

Fig. 2. Representative high-performance liquid chromatography (HPLC) chromatogram of the standard BPA and E_2 mixture solutions (40 ng/mL of each compound) using UV detection at 280 nm; Column SunFire C18; 3.5 µm; 3.0 × 150 mm. Mobile phase methanol/water (75, 25 v/v), constant flow rate of 1 mL/ min, column temperature of 30 °C and an injection volume of 10 µL.

3.4. Occurrence of BPA and E_2 in green mussels

Green mussels were collected at sites near to the cities of Chonburi, Rayong and Trat (Fig. 1) and BPA was detected within each sample (Fig. 3). The mature bivalves collected from the farms near to site R2 receiving the effluent from Chak-mak canal, showed the highest value of BPA concentration (109.97 ± 14.80 ng/g). This result agrees with BPA contamination in Chak-mak canal compared to Trat Province, a reference in that mussels contained the lowest concentration of BPA (15.30 ± 0.62 ng/g). The levels of BPA found within the juvenile mussels were low not only from this site (13.092 ± 2.180 ng/g), but also from all sampling stations. The sample of juvenile mussels collected within Trat Province, for example, had levels of BPA that were below the limits of detection by the HPLC instrumentation (i.e. < 1.1 ng/g). The occurrence of BPA contaminants demonstrated the exposure of mature bivalves to contaminants in the Eastern Gulf of Thailand and suggested the ability of these animals to accumulate BPA.

E_2 levels in adult mussels collected from Rayong and Chonburi Province were found at high concentrations (152.80 ± 18.00 and 138.04 ± 15.33 ng/g, respectively) compared to those collected from Trat Province (12.96 ± 0.69 ng/g). The levels of E_2 were also very low in the juvenile mussels collected from all sampling sites (below limit of detection < 1.3 ng/g for E_2; Table 4).

3.5. Bioconcentration of BPA in green mussels

The US EPA uses the following BCF values to categorise the bioaccumulation potential of a species: < 1000 = bioaccumulative ranking 1; ≥ 1000 = bioaccumulative ranking 2; and, > 5000 = bioaccumulative ranking 3 (US EPA, 2012). The average BCF values determined from the analysis of green mussels from the current study

Table 2
Concentration levels of BPA and E_2 in different freshwaters.

Country	Surface water	BPA (ng/L)	E_2 (ng/L)	Reference
Brazil	Atibaia River	25–84	< 0.6–7.3	Sodré et al. (2010)
China	Chaobai River, Beijing	12.0–120.8	NA	Li et al. (2013)
China	Haihe River, Tianjin	19.1–8300	NA	Jin et al. (2004)
China	Jiulongjiang River	< 0.50–4687	< 2.50–74.4	Zhang et al. (2012)
China	Pearl River Delta	< LOD-376.6	NA	Yang et al.
China	Rivers in S. Jiangsu	48.24–725.94	0–52.71	Yuan et al. (2014)
China	Taihu Lake	9.15–34.42	< 0.13–10.75	Lu et al. (2011)
China	The Pearl River	2.2–1030	< 1.0–7.5	Zhao et al. (2009)
Egypt	Surface water in Cairo	NA	265,130-7,988,200	Khattab et al. (2016)
India	R. Kaveri, Tamiraparani & Vellar, S. India	2.8–136	NA	Selvaraj et al. (2014)
Japan	Nagara River	60–330	NA	Funakoshi and Kasuya (2009)
Japan	River samples	ND-230	NA	Suzuki et al. (2004)
Japan	Tama River	16.5–150.2	2.6–14.7	Furuichi et al. (2004)
Korea	Hyeongsan River	< LOD-636.9	NA	Kim et al. (2014)
Malaysia	Labu river	14.3–203	NA	Santhi et al. (2012)
Malaysia	Langat River	NA	0–4	Praveena et al. (2016)
Netherlands	Surface water	8.8–1000	0.8–1	Vethaak et al. (2005)
Portugal	Mondego River	< 6.6–880	< 3.0	Ribeiro et al. (2009a, 2009b)
Thailand	Chaophraya River	NA	1380–2010	Ruchiraset and Chinwetkitvanich (2014)
Thailand	Khong River	2.4 ± 0.5	6.1–7.5	Duong et al. (2010)
Thailand	Nan River	9.04–51.86	NA	Deemoon et al. (2016)
Thailand	Sankheap Canal (Chonburi Province; C1)	< 1.10[a]	62.98 ± 5.03	This study
Thailand	Payun Canal (Rayong Province; R1)	< 1.10[a]	35.53 ± 2.99	This study
Thailand	Chak-mak Canal (Rayong Province; R2)	50.67 ± 4.19	< 1.30[a]	This study

LOD = Limit of detection.
NA = Not available. No data provided in the original report.
[a] Not detectable (i.e. below the limits of detection by the HPLC method used in the current study.

were 283 for juveniles and 1650 for mature mussels (see Table 5), indicating abilities of this species to bioaccumulate BPA.

4. Discussion

Juvenile and mature green mussels were analysed from three sites along the eastern coastline of the Gulf Thailand, two of which were near to large cities and regions of industrial activity. All the mature mussel samples, including those collected from the reference site in Trat Province, were found to contain high levels of BPA (15.3–109.97 ng/g) and E_2 (12.96–152.8 ng/g) and appear to represent among the highest levels recorded in green mussels in SE Asia (see Table 4). The juvenile mussels collected near to the Map Ta Phut Industrial Estate in Rayong were also high for BPA (i.e. 13.09 ng/g; Table 4). The study also found that BPA and E_2 in Thai aquatic environment have increased in the 20 years since the expansion of industrialization and urbanization (Isobe et al., 2007; Duong et al., 2010; Pookpoosa et al., 2014; Deemoon et al., 2016). BPA levels, for example, have increased in the

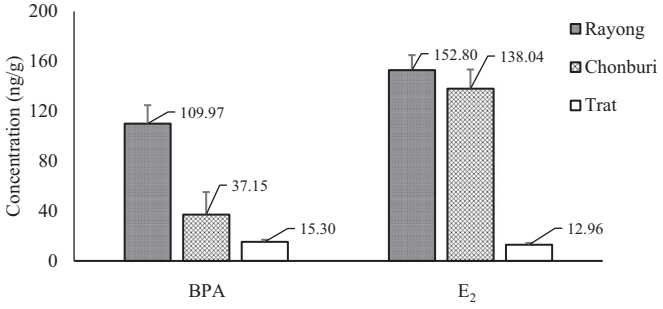

Fig. 3. BPA and E_2 concentrations in mature green mussels, *Perna viridis*, (i.e. > 50 mm shell length) from three different sampling sites from three neighbouring provinces along the eastern seaboard of the Gulf of Thailand.

Table 3
Concentration levels of BPA and E_2 in different marine environments.

Countries	Marine water	BPA (ng/L)	E_2 (ng/L)	References
Brazil	Santos Bay	< 3.30–76.8	< 3.00–18.2	Lisboa et al. (2013)
Canada	Halifax harbor	< 2.60–32.4	< 0.57–5.3	Robinson et al. (2009)
China	South China Sea	12–138	NA	Xu et al. (2014)
Egypt	Suez Canal Region	NA	284,000–1,029,000	Elnwishy et al. (2012)
Greece	Thermaikos Gulf	10.6–52.3	< 5	Arditsoglou and Voutsa (2012)
Italy	Venice Lagoon	2.6–136	< 1–175	Pojana et al. (2007)
Kuwait	Arabian Gulf	NA	0–6.6	Saeed et al. (2017)
Singapore	Singapore Coastal	< 96–964	NA	Bayen et al. (2013)
Singapore	Singapore Coastal	40–190	NA	Basheer and Lee (2004)
Thailand	Map Ta Phut1, Rayong (R3)	3.23 ± 0.08	3.52 ± 0.79	This study
Thailand	Map Ta Phut2, Rayong (R4)	2.49 ± 0.01	1.31 ± 0.96	This study
Thailand	Trat (T; reference site)	1.21 ± 0.01	< 1.30[a]	This study
Thailand	Wonapha Beach, Chonburi (C2)	37.13 ± 2.70	< 1.30[a]	This study
USA	Florida	4.4–190	0–1.8	Singh et al. (2010)

NA = Not available.
[a] Not detectable (i.e. below the limits of detection by the HPLC method used in the current study).

Table 4
The occurrence of BPA and E_2 (ng/g) documented in various tissues of aquatic animals.

Country	Species	BPA (ng/g)	E_2 (ng/g)	Reference
Cambodia	*Perna viridis*	1.1–1.9[c]	NA	Isobe et al. (2007)
China	*Anabarilius alburnops*	83.5	< 11.3	Liu et al. (2011)
	Ruditapes phillipinarum	181.32[a]	3.62[a]	Zhang et al. (2011)
France	*Dreissena polymorpha*	NA	7.4–316.2	Peck et al. (2007)
Greece	*Madiola barbatus*	209.2–515.2	NA	Gatidou et al. (2010)
	Mytilus galloprovincialis	342.8–611.9	NA	
	Venus gallina	298.1–626.3	NA	
India	*Perna viridis*	1.1–13.7[c]	NA	Isobe et al. (2007)
Indonesia	*Perna viridis*	0.32–1.8[c]	NA	Isobe et al. (2007)
Japan	*Semisulcospira libertina liberta*	11 ± 3.4	NA	Kang and Kondo (2006)
Malaysia	*Perna viridis*	0.51–4.2[c]	NA	Isobe et al. (2007)
Malaysia	*Perna viridis*	0.51–4.2	NA	Yap (2013)
Philippines	*Perna viridis*	1.1–4.7[c]	NA	Isobe et al. (2007)
Poland	*Clupea harengus*	19.7–440.1	NA	Staniszewska et al. (2014)
	Mytilus edulis trossulus	68–197.2	NA	
	Platichthys flesus	98.3–755.7	NA	
Poland	*Mytilus trossulus*	< LOD-273.6[c]	NA	Staniszewska et al. (2017)
Singapore	*Perna viridis*	3.3[c]	NA	Isobe et al. (2007)
Spain	*Mytilus galloprovincialis*	< 3.3–714	NA	Salgueiro-González et al. (2016)
Thailand	Wild fish, including:	9.04–44.12	NA	Deemoon et al. (2016)
	Barbonymus schwanenfeldii			
	Henicorhynchus siamensis			
	Kryptopterus cryptopterus			
	Oxygaster anomalura			
Thailand	*Perna viridis*	0.96–4.54	NA	Isobe et al. (2007)
Thailand (C2)	*Perna viridis* (mature)	37.15 ± 12.08	138 ± 15.33	This study
	Perna viridis (juvenile)	< 1.3[b]	< 1.1[b]	This study
Thailand (R2)	*Perna viridis* (mature)	109.97 ± 14.80	152.8 ± 18.00	This study
	Perna viridis (juvenile)	13.09 ± 2.18	< 1.1[b]	This study
Thailand (T)	*Perna viridis* (mature)	15.30 ± 0.61	12.96 ± 0.69	This study
	Perna viridis (juvenile)	< 1.3[b]	< 1.1[b]	This study
Vietnam	*Perna viridis*	0.58–1.3[c]	NA	Isobe et al. (2007)

NA = Not available.
[a] ng/g (lipid).
[b] Not detectable (i.e. below the limits of detection by the HPLC method used in the current study).
[c] ng/g-dry tissue.

Table 5
Bioconcentration factors of BPA found in green mussels, *Perna viridis*, collected from the eastern Gulf of Thailand.

Stage of mussel	EDC	Bioconcentration factor (BCF) (wet weight)		Categorizations (US EPA, 2012)
		average	maximum	
Juvenile	BPA	283	392	Bioaccumulative (1)
Mature	BPA	1650	2689	Bioaccumulative (2)

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Gulf of Thailand since 1994 when Isobe et al. (2007) collected green mussels from Sichang Island, Chonburi (see Isobe et al., 2007), a site which is close to the C2 sampling station at Wonapha, Chonburi.

Of the water samples, the freshwater collected from the Chak-mak Canal (R2; this is connected 1 km away to a treatment reservoir that receives petrochemical industrial wastewater runoff from the entire Map Ta Phut industrial estate; the R2 sampling point was approx. 100 m from the coast), which runs through the heart of the Map Ta Phut Industrial Estate in Rayong Province had the highest concentration of BPA (i.e. 50.67 ± 4.19 ng/L). This finding is perhaps not surprising given that ca. 150,000 tons of BPA product is manufactured annually within the industrial estate (PTT Global, 2016). By comparison, BPA levels in a neighbouring river running outside the estate (i.e. sample R1), had levels of BPA that were below the limits of detection (see Table 2). The finding concurs with that of Flint et al. (2012), who suggested that the main sources of BPA are usually from industrial and urban areas. The levels found at R2, then drop offshore, i.e. at sample stations R3 (3.23 ng/L) and R4 (2.49 ng/L), which were taken at distances of approximately 2.5 and 4 km from the R2 sample site. The levels, however, were > ×2 that measured at the reference site in Trat Province, a rural area, with the lowest concentration of BPA found within the seawater samples analysed for the current study (i.e. 1.21 ± 0.01 ng/L).

The sample collected from the C2 station located near Wonapha beach in Chonburi Province (see Fig. 1), had the highest concentration of BPA among the seawater samples collected in the current study. This area is located to the south of Chonburi city, and is close to the districts of Bangsaen and Angsila, two popular tourist destinations and important centres of commercial capture fishing activity. There are 43 communities, including the fishing villages, with large numbers of visitors to the area generating large volumes of waste (Kaewmanee and Wijaya, 2014). Samples of periwinkles, *Littoraria* sp., rock oysters, *Saccostrea forskalii*, and striped barnacles, *Balanus amphitrite*, collected from the shoreline along both districts by Thushari et al. (2017), found that all three-species contained high levels of microplastics (i.e. 0.17–0.57 counts/g).

High levels of E_2 were also detected within the two freshwater samples taken from C1 (Chonburi Province; 62.98 ± 5.03 ng/L) and R1 (Rayong Province; 35.53 ± 2.99 ng/L) and in all samples of mature green mussels. The city of Chonburi is approximately three times the size of Rayong in terms of its population (ca. 200,000 versus ca. 65,000) and the E_2 values may correlate with the volume of pharmaceutical drugs containing E_2 used by the local populus and, consequentially, the seepage into water courses from sewage and waste disposal sites. Other factors that may contribute to the inputs are that real GDP has increased by 3.2% over the period 1995–2015 (SBC, 2016); per capita disposable

income has increased from US$ 797 in 1990 to US$ 3607 in 2016 (Trading Economics, 2018; Worldometers, 2018); tourism has increased by 13% year-on-year with Chonburi attracting 13% of the 29.9 M tourists visiting Thailand in 2015. Increased affluence may translate to increases in the purchase of health care products, pharmaceutical drugs and in BPA-associated commodities. The Sankheap Canal (sample site C1), for example, passes through residential areas and the centre of Chonburi city. This finding is supported by the study of Ruchiraset and Chinwetkitvanich (2014) who recorded markedly higher levels within the Chaophraya River which flows through the centre of Bangkok and empties into the Gulf of Thailand. The observed levels are also not too dissimilar from those seen within selected studies conducted within China (see Zhang et al., 2012; Yuan et al., 2014), where the highest concentrations were recorded in the dry season – a consequence of the reduced water flow allowing levels to build up (Ying et al., 2009; Minh et al., 2016). Regardless, the current levels of E_2 recorded at the C1, R1 and R3 sites all exceed the PNEC of 2 ng/L proposed by Caldwell et al. (2012) as a level beyond that which poses a risk to aquatic organisms. The limits of detection on the instrumentation used for the current study for the measurement of E_2 was 1.30 ng/L, although three of the water samples in this study were below this, what is not known is if they were higher than the amended PNEC value of 0.73 ng/L proposed by Wu et al. (2014).

It is, however, the levels of BPA and E_2 seen within the green mussels, a popular, commonly consumed seafood item in Thailand, that raises questions regarding its food safety. While the distribution and bioaccumulation of contaminants within an organism will depend on a variety of interplaying factors such as species, age at the time of sampling, period of exposure, metabolism and diet (Jaikanlaya et al., 2009; Beltran-Pedreros et al., 2011; Yap et al., 2016), so will the local hydrodynamic features of the organism's environment, the migratory movements of the host, and, its relative proximity to point sources of EDC input. The water exchange rates within the Gulf of Thailand (13, 161 km^3) are the lowest for the Sunda Shelf Seas with an average flush rate of 80–170 days and a particle flush rate of only 17% in > 730 days (Mayer et al., 2015), highlighting that environmental pollutants such as microplastics and EDCs have the potential to build up over time. These flush rates agree with the earlier work of Boonphakdee and Fujiwara (2008) who calculated a rate of 80 days for the Bangphakong River Estuary, which empties into the Gulf of Thailand, during the dry season and also of that of Sutthasom and Boonphakdee (2013) who determined residence times for the inner Gulf of Thailand to be 87 ± 15.6 and 166 ± 1 days for the wet and dry seasons respectively. Green mussels have a high growth rate (i.e. 8–10 mm/month location dependent; Cheung, 1991), they can reach up to 120 mm in a year and are typically harvested when they are between 60 and 120 mm shell length (ICAR, 2003). The potential of green mussels to bioaccumulate BPA is shown Table 5, with US EPA (2012) bioaccumulation scores of 1 and 2 for juveniles and mature green mussels respectively. Bisphenol A has a moderate solubility of 120–300 mg/L and a half life of 38 days in water (Corrales et al., 2015). As the half life of BPA is < 2 months, this is classified as a category 1 persistent chemical by the US EPA (2012); category 2 chemicals have half-lives exceeding 2 months, while category 3 contain those exceeding 6 months. The combined, normalised, persistence and bioaccumulation scores for BPA in juvenile and mature mussels are “low” and “moderate” respectively.

E_2 was found at low levels in the seawater samples analysed within the current study but in high levels within the mature mussels harvested at all three farm sites. A study conducted by Zhu et al. (2003) exploring the estradiol content of gonadal tissue in *Mytilus edulis*, found that the mussels can synthesize E_2 within the body but this is not released into the immediate environment. This highlights that some caution is needed in interpreting E_2 results from mature green mussels and calls for further studies to investigate their potential to produce E_2. The levels of E_2 in mature samples taken from the two mussel collection sites near to the cities of Chonburi (i.e. 138 ng/g) and Rayong (i.e. 152.8 ng/g), however, were a magnitude higher those collected from the reference site in Trat Province (i.e. 12.96 ng/g) and so the relative contributions from de novo production and bioaccumulation from the environment require unpicking. The sex ratio in each of these collections was biased towards females, i.e. 74.01% among the two Chonburi samples and, 88.88% in the Rayong sample but 55.56% female in those collected from the reference site in Trat. No BCF values, therefore, were calculated for E_2.

The demonstrably high levels of both BPA and E2 from collection points and cultured green mussels around the Map Ta Phut Industrial Estate at the near apex of the Gulf of Thailand would suggest that this would serve as a key site for future biomonitoring in the Gulf to assess the performance of remediation strategies. Further work is now needed to monitor levels of EDCs in cultured mussels and other seafood commodities grown in the coastal zone around the Gulf of Thailand so that a seafood risk map may be established, in addition to providing baseline data for the future improvement of water quality and aquaculture sites within the region.

5. Conclusions

The occurrence of two EDCs, BPA and E_2, were detected at all sample sites along the eastern coast of Thailand. The highest levels were found in water samples taken near to areas of intense industrial and domestic activity, suggesting that the sources of the observed EDC originated from the municipal and industrial wastewaters where there are deficiencies in wastewater management. Levels of both EDCs were high in mature green mussels collected at points within Chonburi and Rayong Province and within the neighbouring province of Trat which served as the reference site. The recorded levels of BPA appear to be among the highest in Asia for the species and, temporally, levels at the Chonburi collection site have risen dramatically in over the last two decades. Increases over this period can be attributed to increases in urbanization and industrialization in the coastal zone, increases in tourism, changes to the economy, affluence and disposal income. The demonstration that many Thai sites along the eastern coast are contaminated with residues of BPA and E_2 highlights the need for a review of wastewater treatment processes, the management systems and the technologies in place, with the objective of reducing current EDC levels. The levels of EDCs that were found are such that they may pose a risk to human health, to those regularly consuming green mussels, and to aquatic ecosystems and while the findings from this study may be regarded as preliminary, they do serve as a baseline for future studies and for programmes of restoration.

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Statement of relevance

Green mussel culture represents the principal molluscan industry in Thailand; the high EDC content of mussels reared in the Gulf of Thailand raises food safety concerns.

Conflict of interest statement

We declare that we have no conflicts of interest to disclose.

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