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Seasonal Distribution of Bromophenols in Selected Hong Kong Seafood

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Selected seafood including rabbitfish (*Siganus canaliculatus*), brown-spotted grouper (*Epinephelus areolatus*), clam (*Tapes philippinarum*), oyster (*Ostrea rivularis*), shrimp (*Penaeus japonicus*), and crab (*Charybdis feriatus*), commonly found in the Hong Kong wet market, was monitored for their distribution and seasonal variations of their bromophenol contents. Specifically, 2-bromophenol (**1**), 4-bromophenol (**2**), 2,4-dibromophenol (**3**), 2,6-dibromophenol (**4**), and 2,4,6-tribromophenol (**5**) were monitored due to their flavor impact to seafood. All samples of marine origin contained bromophenols throughout a year. Crab had the highest concentration of total bromophenol content throughout the season. Concentrations of compounds **1**, **4**, and **5** in the local seafood were generally higher than that found in the literature values to provide characteristic flavor, but lower than that required to cause off-flavor. Variations of the flavor impact of bromophenols in seafood during a season could be better shown by their flavor values. Distribution and seasonal variations of bromophenol content in seafood coincided well with the seasonal growth cycle of the bromophenol synthesizing seaweeds, e.g. brown algae, in the region suggesting the abundant source of bromophenols in the environment might have a high impact on the quantity of bromophenols found in seafood.

KEYWORDS: Bromophenols; seafood; Asia; seasons

INTRODUCTION

Seafood is an important food source in Hong Kong. Many consumers appreciate its delicate flavor. Previous studies focused mainly on the characteristic flavor of seafood contributed by microbial degradation, lipid derived or thermally generated compounds (*1*−*4*). Recently, bromophenols including 2-bromophenol (**1**), 4-bromophenol (**2**), 2,4-dibromophenol (**3**), 2,6-dibromophenol (**4**), and 2,4,6-tribromophenol (**5**) were considered as another important group of key flavor compounds found in seafood (*5*−*9*). Among them, compounds **1** and **4** generally have lower threshold values and more potency due to their higher concentrations (*6*, *10*). These compounds produce desirable marine- or ocean-like flavor and enhance the intensity of existing flavor in seafood at suitable concentrations (*5*, *6*, *8*). Previous studies were carried out on the distributions of these compounds in different seafoods such as prawns, salmon, and fish at different locations and times (*5*, *8*, *9*). Bromophenols were widely distributed in seafood and were virtually absent in freshwater fish (*5*). Little information is reported on the seasonal changes of the bromophenols among common seafood.

Similarly, research focusing on seafood flavor has been scarce in Hong Kong even though the consumption of seafood is high. Studies on the volatile components among Hong Kong seafood are limited to both crab and dried scallops (*11*−*13*). Specific information on the seasonal variations and levels of bromophenols in the local seafood is unavailable. Therefore, the objectives of this study were to monitor the distribution and seasonal variations of bromophenols in selected marine seafood.

MATERIALS AND METHODS

Sample Collection and Preparation. Seafoods including marine fish, mollusks, and crustaceans were studied. Live samples were bought from the local market and were obtained from the same shop throughout the study. Marine fishes including rabbitfish (*Siganus canaliculatus*) (15 fish each time) and brown-spotted groupers (*Epinephelus areolatus*) (3 fish each time) were purchased. For each species, each fish was divided into two parts including the gut and the flesh (separated from the head, body, and backbone). Both gut and flesh of each fish were collected whereas the head, body, and backbone were discarded. For subsequent extraction, 190 g of flesh and 40 g of gut (average) of rabbitfish were used. Similarly, 155 g of flesh and 20 g of gut (average) of grouper were used.

Live mollusks including clam (*Tapes philippinarum*) and oyster (*Ostrea rivularis*), and crustaceans including shrimp (*Penaeus japonicus*) and crab (*Charybdis feriatus*) were purchased. Both clams and crabs were steamed for 15 min within 1 h of purchase to facilitate manual meat picking. About 540, 220, and 470 g of clam meat, crab

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meat, and oyster meat were used for the extraction. Each shrimp was carefully divided into cephalothorax (head) and tail (body). On average, 150 and 230 g of cephalothorax and tail, respectively, were used for each extraction. Seafood samples were collected every two months from December 1999 through December 2000. Freshwater fish, grass carp (*Ctenopharyngodon idellus*), was obtained from the local market in November 1999 and prepared by the same method as for the live marine fish.

Organic solvents, pentane and diethyl ether, were purchased from Lab-scan Ltd. (Ireland) with a purity of 99% and 99.5%, respectively. Standards **1**, **3**, and **4** were bought from Aldrich Chemical Co. (Milwaukee, WI), and **2** and **5** were purchased from Acros Organics (Belgium). The purities of the five bromophenols ranged from 97% to 99%.

Simultaneous Steam Distillation and Solvent Extraction (SDE). The extraction method was adapted from Whitfield et al. (*8*, *9*). Each sample was homogenized in a blender (Model MX-T2GM, National Brand, Matsushita Electric Co. Ltd., Taipei, Taiwan) for 4 min. Homogenized samples, 1 mL of internal standard (1,3,5-trimethylbenzene, concentration at 84.7 μg/mL), and 500 mL of boiled double distilled water were transferred to a 5-L round-bottom flask. The sample was then acidified to pH 1 with 96% sulfuric acid. Extraction with 40 mL of pentane/diethyl ether (9:1 v/v) for 2.5 h in a Likens and Nickerson type SDE apparatus (Cat. No. K-523010-0000, Kontes, Vineland, NJ) was carried out. The pH of the residue after extraction was measured to ensure that the acidity was maintained throughout the process. Triplicate extractions of each sample were carried out. Each extract collected was dried with 2.85 g of anhydrous sodium sulfate and concentrated to 0.25 mL with a stream of ultrahigh purity (99.999%) nitrogen. The concentrated extract was temporarily stored in a 15-mL conical tube at −80 °C until further analyses were carried out.

Gas Chromatography−Mass Spectrometry (GC−MS). A GC−MS system consisting of a Hewlett-Packard 6890 GC coupled with a HP 5973 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA) was used for qualitative and quantitative analyses. Five microliters of each extract was injected, at splitless mode with an injector temperature of 200 °C, into a fused silica open tubular column (Supelcowax-10, 60 m length × 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA). Helium gas (ultrahigh purity grade, 99.999%) was used as the carrier gas with a constant linear velocity of 30 cm/s. The oven temperature was programmed from 100 to 200 °C at a ramp rate of 10 °C/min. The initial and final hold times were 5 and 75 min, respectively. MS interface, ion source, and MS quadrupole temperatures were set at 250, 230, and 106 °C, respectively. Ionization voltage was 70 eV and electron multiplier voltage was 1200 V. The selected ion monitoring (SIM) mode of the GC−MS was used. Ions monitored for compounds **1** and **2**; **3** and **4**; **5**; and internal standard were at mass/charge (*m*/*z*) 172 and 174; 250 and 252; 330 and 332; and 105 and 120, respectively (*8*, *11*, *14*).

Compound Identification and Quantification. The presence of each bromophenol was confirmed by the detection of a single peak at a corresponding retention time and the presence of their characteristic ions in the selected ion chromatogram (*8*).

For quantification, a three-point standard curve was established for each bromophenol. Solutions (5 mL) containing 5 mg of each of the five bromophenols were prepared. Serial dilutions at ratios of 1:5 and 1:25 were made. A constant amount of internal standard, 1,3,5-trimethylbenzene (5 mg), was added to each of the above solutions prepared. Ions chosen for the calculation of response factors were with *m*/*z* of 172 (monobromophenols), 252 (dibromophenols), 330 (tribromophenol), and 105 (internal standard). The concentration of a bromophenol in a sample was calculated by obtaining the ratio of the amount of bromophenol to internal standard in the sample with use of the response factor (*15*).

The total bromophenol content (TBC) was calculated by the summation of the concentrations (ng/g) of all five bromophenols.

Extraction Efficiency. The extraction efficiencies of the SDE technique on various bromophenols were determined by their recovery from a model system. Briefly, it was determined by extracting 10 μg of each bromophenol and 100 g of bromophenol-free freshwater grass carp flesh with the SDE method and quantifying the extract and the original standard with the same GC-MS system under the same experimental conditions. The extraction efficiency for each compound was calculated according to the ratio of concentration of bromophenol recovered to the concentration of bromophenol standard added (*15*). The average percentage recoveries were 99.7 ± 0.4%, 38.0 ± 3.2%, 99.3 ± 0.9%, 93.1 ± 0.3%, and 62.5 ± 7.8% for compounds **1**, **2**, **3**, **4**, and **5**, respectively.

Flavor Value (FV). FV of a bromophenol was calculated by dividing the concentration of a bromophenol by its corresponding evaluation flavor concentration, which was adapted from Guadagni et al. (*16*). Total FV was the summation of the individual FVs from all five bromophenols. The concentrations (i.e. evaluation flavor concentration) of bromophenols determined in the marinated whitefish were used for the calculations (*6*).

Moisture Determination. The percentage moisture of each sample was determined according to the instructions in the operation manual of the Mettler LJ16 moisture analyzer (Mettler-Toledo, Switzerland). The concentration of each bromophenol in a sample was expressed on a dry weight basis.

Statistical Analysis. The total bromophenol content (TBC) between the gut and the flesh in the fish samples, and between the cephalothorax and the tail in the shrimp was compared by Student *t*-test, using statistical software (SPSS, Version 10.0, Chicago, IL) at the $p = 0.05$ level of significance. The variations of TBCs at different months within each type of seafood were analyzed by one-way ANOVA (SPSS, Version 10.0, Chicago, IL) at the $p = 0.05$ level of significance. The Pearson product moment correlation coefficient (r), its corresponding p-value with H_o, $\rho = 0$ (i.e. Null hypothesis: the relationship between the two variables is not correlated), and linear regression coefficients (intercept and slope) between two sets of parameters in the samples were determined by SigmaStat (SPSS, Version 2.03, Chicago, IL).

RESULTS AND DISCUSSION

Distribution of Bromophenols in Seafood. Distributions of the five bromophenols and their total bromophenol contents (TBCs) in each species in a year are shown in **Tables 1−6** and **Figure 1**, respectively. The sample moisture contents of each sample are also shown in **Tables 1−6**. All these species were of marine origin and found to contain a notable amount of bromophenols. These results compared well with those of Boyle et al. (*5*) that bromophenols were widely distributed in seafood.

The magnitude of TBC has often been used in the literature to reflect the overall flavor impact produced by all bromophenols present in a food (*7*−*9*). Therefore, the current discussion would emphasize more the TBC rather than individual compounds. Among the marine species investigated, TBCs varied widely. The highest and lowest TBCs of 2420 and 2.72 ng/g were found in the crab meat and the flesh of brown-spotted grouper collected in February 2000 and August 2000, respectively. TBCs of most species were observed to fluctuate throughout the seasons. Such observations were mainly due to variations in the bromophenol composition in the species. In fact, among all the samples of the six species of seafood collected throughout a year, only 12, 37, and 10 samples contain all five, any four, and any three combinations of bromophenols out of 62 samples, respectively, in their composition (**Tables 1−6**).

Among the five bromophenol compounds studied, compound **5** was present in all seafood samples. Compounds **1**, **3**, and **4** were detected at the percentages of 90.3, 96.8, and 87.1 among all samples, respectively. Compound **2** has the lowest frequency of occurrence with a percentage of 19.4. Quantitatively, 46 samples (74.2%) collected throughout a year contained the highest concentration of compound **5** among all the bromophenols within a sample. In 12 samples (19.4%), compound **3** possessed the highest concentration. For compounds **1** and **2**,

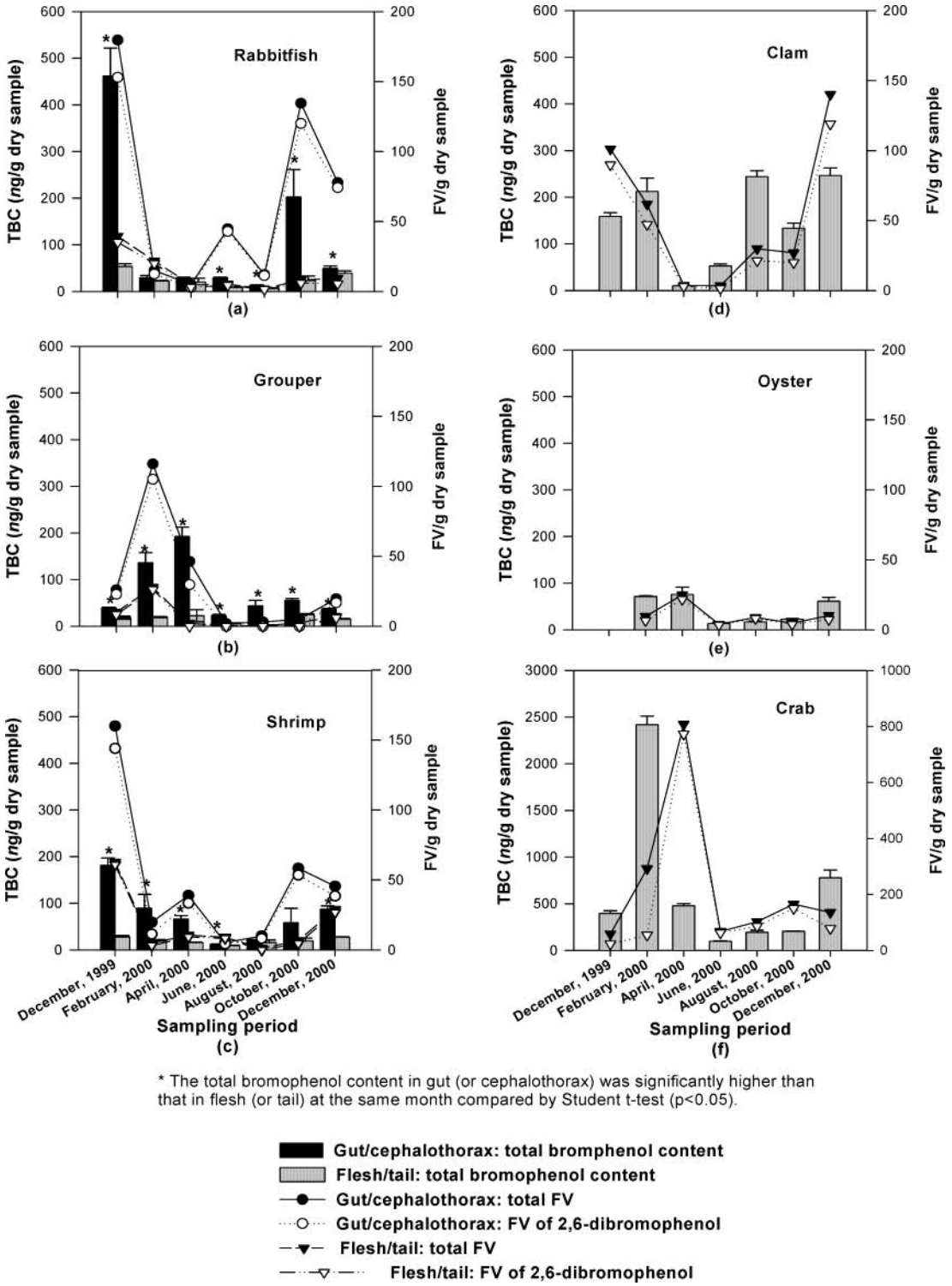

Figure 1. Mean total bromophenol contents (TBC) (*n* g/g) and flavor values (FV) in a year in (a) rabbitfish (*Siganus canaliculatus*), (b) brown-spotted grouper (*Epinephelus areolatus*), (c) shrimp (*Penaeus japonicus*), (d) clam (*Tapes philippinarum*), (e) oyster (*Ostrea rivularis*), and (f) crab (*Charybdis feriatus*).

only three and one samples had the highest concentrations, respectively.

Wide variations in the concentration of individual bromophenol appeared among the species. For example, the concentration of compound **5** ranged from the lowest of 2.18 ng/g in the gut of brown spotted groupers (*Epinephelus areolatus*) in June 2000 to the highest of 2360 ng/g in crab meat (*Charybdis feriatus*) in February 2000. Similarly, the concentrations of compounds **1**, **2**, **3**, and **4** varied from the lowest of 0 ng/g (i.e. not detected (ND)) to the highest of 34.4, 206, 214, and 77.3 ng/g, respectively, among all species. Besides such variations among species, the same species collected at different periods also fluctuated in their bromophenol content.

Bromophenols in Marine Fish. Two kinds of marine fish, rabbitfish (*Siganus canaliculatus*) and brown-spotted grouper (*Epinephelus areolatus*), commonly consumed in Hong Kong, were studied (**Figure 1a**,**b**; **Tables 1** and **2**). Bromophenols were detected in both gut and flesh of all samples. In the gut and the

Table 1. Seasonal Distribution of Bromophenols in Marine Fish *Siganus canaliculatus* (Rabbitfish)

			bromophenol concentration (ng/g dry wt.)				
month	sample	moisture, %	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
December 1999	gut	58.10	30.8[b] ± 10.3[c]	206 ± 14	97.1 ± 21.2	15.3 ± 2.4	113 ± 15
	flesh	71.00	1.47 ± 0.27	ND	9.47 ± 1.05	3.52 ± 0.99	39.2 ± 4.2
February 2000	gut	48.33	ND[d]	ND	3.41 ± 0.99	1.25 ± 0.23	24.8 ± 4.4
	flesh	68.70	ND	ND	1.97 ± 0.41	1.99 ± 0.13	18.6 ± 0.6
April 2000	gut	46.47	0.532 ± 0.054	ND	9.09 ± 0.28	0.293 ± 0.004	18.8 ± 2.0
	flesh	69.80	1.39 ± 0.44	ND	2.82 ± 1.00	0.301 ± 0.102	15.8 ± 6.7
June 2000	gut	45.70	7.15 ± 0.31	ND	8.14 ± 1.11	4.29 ± 0.78	9.39 ± 1.03
	flesh	73.20	0.646 ± 0.163	ND	0.788 ± 0.051	0.446 ± 0.032	6.99 ± 0.56
August 2000	gut	43.00	1.03 ± 0.13	ND	7.11 ± 0.69	1.14 ± 0.08	3.85 ± 0.52
	flesh	69.17	0.334 ± 0.024	ND	1.22 ± 0.28	0.136 ± 0.007	4.42 ± 0.92
October 2000	gut	45.87	4.24 ± 0.43	ND	57.0 ± 28.8	12.0 ± 0.1	129 ± 36
	flesh	67.60	0.194 ± 0.137	ND	2.14 ± 1.17	0.605 ± 0.244	20.5 ± 8.7
December 2000	gut	38.57	7.82 ± 0.35	ND	7.62 ± 1.55	7.42 ± 1.67	26.9 ± 2.0
	flesh	66.80	10.7 ± 2.9	ND	0.727 ± 0.192	0.555 ± 0.050	27.4 ± 3.3

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Average bromophenol concentration (ng/g dry wt) from the 3 replicates. [c] Standard deviation of the bromophenol concentration (ng/g dry wt). [d] ND = not detected.

Table 2. Seasonal Distribution of Bromophenols in Marine Fish *Epinepheus areolatus* (Brown-spotted grouper)

			bromophenol concentration (ng/g dry wt.)				
month	sample	moisture, %	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
December 1999	gut	47.13	8.01[b] ± 0.58[c]	ND	6.19 ± 0.11	2.28 ± 0.16	23.0 ± 1.0
	flesh	71.93	2.90 ± 0.29	ND	0.914 ± 0.052	0.835 ± 0.382	13.9 ± 2.6
February 2000	gut	52.90	ND[d]	ND	19.1 ± 2.5	10.5 ± 3.3	107 ± 16
	flesh	74.43	ND	ND	1.33 ± 0.05	2.62 ± 0.16	15.7 ± 1.1
April 2000	gut	70.33	3.49 ± 0.55	ND	31.4 ± 3.2	2.98 ± 0.40	155 ± 16
	flesh	75.00	ND	ND	2.97 ± 0.11	ND	19.7 ± 12.7
June 2000	gut	40.40	15.7 ± 1.6	ND	4.63 ± 1.19	ND	2.18 ± 0.51
	flesh	70.90	3.26 ± 0.51	ND	0.578 ± 0.06	ND	2.78 ± 0.23
August 2000	gut	52.87	2.57 ± 0.33	ND	16.5 ± 3.8	ND	24.9 ± 7.6
	flesh	74.23	ND	ND	0.286 ± 0.044	ND	2.43 ± 0.23
October 2000	gut	29.50	17.5 ± 1.1	ND	7.08 ± 0.90	ND	30.7 ± 3.3
	flesh	70.47	4.18 ± 0.38	ND	ND	ND	21.8 ± 0.7
December 2000	gut	31.33	8.28 ± 0.54	ND	6.78 ± 0.47	1.68 ± 0.14	19.4 ± 1.4
	flesh	72.53	1.31 ± 0.61	ND	5.36 ± 0.07	0.598 ± 0.342	8.51 ± 0.33

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Average bromophenol concentration (ng/g dry wt) from 3 replicates. [c] Standard deviation of the bromophenol concentration (ng/g dry wt.). [d] ND = not detected.

Table 3. Seasonal Distribution of Bromophenols in Mollusk *Tapes philippinarum* (Clam)

		bromophenol concentration (ng/g dry wt)				
month	moisture, %	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
December 1999	79.86	17.2[b] ± 0.1	ND[d]	46.7 ± 0.5	8.99 ± 0.80	85.9 ± 7.4
February 2000	86.57	14.1 ± 1.1	55.6 ± 8.9	44.5 ± 9.9	4.72 ± 0.23	93.7 ± 10.3
April 2000	84.43	0.170 ± 0.003	ND	2.54 ± 0.26	0.289 ± 0.023	7.28 ± 0.67
June 2000	82.87	1.85 ± 0.09	ND	39.5 ± 3.5	0.146 ± 0.016	11.1 ± 0.8
August 2000	87.63	4.32 ± 0.20	ND	195 ± 16	2.12 ± 0.22	43.2 ± 3.5
October 2000	88.13	5.99 ± 0.41	17.5 ± 0.9	64.6 ± 6.8	1.99 ± 0.14	43.4 ± 3.1
December 2000	88.23	4.34 ± 0.11	4.62 ± 0.35	27.4 ± 2.8	11.9 ± 1.2	198 ± 15

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Average bromophenol concentration (ng/g dry wt) from 3 replicates. [c] Standard deviation of the bromophenol concentration (ng/g dry wt). [d] ND = not detected.

flesh, the TBCs detected during the season in the rabbitfish ranged from 13.1 to 462 ng/g and from 6.12 to 53.7 ng/g whereas in the brown-spotted grouper the range was from 22.5 to 193 ng/g and from 2.72 to 26.0 ng/g, respectively. Apparently, TBCs fluctuated more widely in the gut than in the flesh. In all 14 fish samples (seven gut and seven meat samples) examined, the TBCs in the gut were higher than those in the flesh in the same month. The most often detected bromophenol was compound **5** among the fish samples whereas the least detected one was compound **2**, which was found only in one of the gut samples.

Bromophenols in Mollusks. Both clam (*Tapes philippinarum*) and oyster (*Ostrea rivularis*) were investigated for their bromophenol contents (**Figure 1d**,**e**; **Tables 3** and **4**). TBCs in clams were generally high when compared to other species currently studied except crabs (**Figure 1**). Bromophenols were detected in all the samples ranging from 10.3 to 246 ng/g in clam and 13.6 to 75.8 ng/g in oyster. In August 2000, the TBC in clams was the highest among the samples examined in the same period. Four out of five bromophenols including compounds **1**, **3**, **4**, and **5** were detected in all 13 samples (seven clams and six oysters). Among them, both compounds **3** and **5**

Table 4. Seasonal Distribution of Bromophenols in Mollusk *Ostrea rivularis* (Oyster)

		bromophenol concentration (ng/g dry wt)				
month	moisture, %	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
December 1999		NA	NA[e]	NA	NA	NA
February 2000	89.37	6.10[b] ± 0.26[c]	ND[d]	51.6 ± 0.5	0.667 ± 0.014	13.3 ± 0.5
April 2000	89.23	2.54 ± 0.58	ND[d]	52.9 ± 11.5	2.18 ± 0.48	18.1 ± 3.0
June 2000	88.07	2.07 ± 0.15	ND[d]	9.80 ± 1.13	0.345 ± 0.038	1.37 ± 0.15
August 2000	83.10	1.85 ± 0.16	ND[d]	11.0 ± 1.6	0.815 ± 0.086	3.30 ± 0.27
October 2000	89.57	2.58 ± 0.34	ND[d]	17.3 ± 0.9	0.395 ± 0.024	2.33 ± 0.55
December 2000	88.37	3.96 ± 0.44	ND[d]	41.2 ± 7.9	0.744 ± 0.021	15.5 ± 0.4

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Average bromophenol concentration (ng/g dry wt) from 3 replicates. [c] Standard deviation of the bromophenol concentration (ng/g dry wt.). [d] ND = not detected. [e] NA = sample not available.

Table 5. Seasonal Distribution of Bromophenols in Crustacean *Penaeus japonicus* (Shrimp)

			bromophenol concentration (ng/g dry wt)				
month	sample	moisture, %	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
December 1999	cephalothorax	71.90	3.85[b] ± 0.48[c]	ND[d]	9.39 ± 1.33	14.4 ± 1.4	154 ± 13
	tail	69.47	3.62 ± 0.46	ND	5.57 ± 0.84	6.09 ± 0.40	12.3 ± 2.0
February 2000	cephalothorax	75.97	5.70 ± 1.53	ND	4.90 ± 2.45	1.13 ± 0.15	77.7 ± 26.4
	tail	72.60	0.421 ± 0.029	ND	0.613 ± 0.153	0.319 ± 0.066	17.4 ± 3.0
April 2000	cephalothorax	77.07	3.84 ± 0.14	ND	9.64 ± 1.00	3.34 ± 0.24	49.5 ± 6.4
	tail	74.10	0.617 ± 0.129	ND	3.80 ± 0.52	0.926 ± 0.070	10.2 ± 0.4
June 2000	cephalothorax	74.00	1.27 ± 0.21	ND	1.78 ± 0.18	0.431 ± 0.068	8.30 ± 0.35
	tail	73.33	0.446 ± 0.066	ND	1.08 ± 0.08	0.824 ± 0.061	7.32 ± 0.08
August 2000	cephalothorax	77.90	7.72 ± 0.86	3.34 ± 0.09	1.81 ± 0.02	0.818 ± 0.048	7.65 ± 0.21
	tail	72.97	2.71 ± 0.28	ND	6.78 ± 4.97	ND	6.38 ± 0.52
October 2000	cephalothorax	75.57	6.26 ± 0.57	ND	5.98 ± 0.18	5.35 ± 0.26	40.8 ± 30.0
	tail	71.57	5.87 ± 0.73	ND	ND ± 0.18	0.465 ± 0.037	12.9 ± 5.3
December 2000	cephalothorax	74.60	1.53 ± 0.04	9.47 ± 0.15	10.4 ± 0.7	3.85 ± 0.20	62.0 ± 6.5
	tail	75.57	0.841 ± 0.106	5.39 ± 0.39	2.64 ± 0.32	2.68 ± 0.42	15.8 ± 0.6

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Average bromophenol concentration (ng/g dry wt) from 3 replicates. [c] Standard deviation of the bromophenol concentration (ng/g dry wt). [d] ND = not detected.

Table 6. Seasonal Distribution of Bromophenols in Crustacean *Charybdis feriatus* (Crab)

		bromophenol concentration (ng/g dry wt)				
month	moisture, %	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
December 1999	83.27	2.94[b] ± 0.16[c]	1.74 ± 0.04	54.2 ± 4.8	2.31 ± 0.14	336 ± 28
February 2000	82.73	5.11 ± 0.12	ND[d]	43.8 ± 5.2	5.47 ± 0.64	2360 ± 91
April 2000	80.00	34.4 ± 1.8	3.88 ± 0.13	68.1 ± 0.5	77.3 ± 11.2	295 ± 11
June 2000	81.90	0.991 ± 0.060	ND	53.0 ± 2.8	6.29 ± 0.25	38.6 ± 2.1
August 2000	79.50	2.76 ± 0.24	4.16 ± 0.10	27.6 ± 1.1	8.63 ± 0.93	151 ± 22
October 2000	79.47	3.40 ± 0.09	22.6 ± 2.5	32.0 ± 0.4	15.0 ± 1.3	132 ± 2
December 2000	78.73	8.54 ± 0.69	47.9 ± 4.6	214 ± 20	7.81 ± 0.98	501 ± 57

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Average bromophenol concentration (ng/g dry wt) from 3 replicates. [c] Standard deviation of the bromophenol concentration (ng/g dry wt). [d] ND = not detected.

were more abundant quantitatively than the rest of the bromophenols. In oysters, only compound **3** was the most abundant bromophenol detected. Such observation was different from the other samples investigated.

Bromophenols in Crustacean. Two types of crustaceans, shrimp (*Penaeus japonicus*) and crab (*Charybdis feriatus*), were studied (**Figure 1c**,**f**; **Tables 5** and **6**). Compound **5** had the highest amount among the bromophenols investigated in both species. In shrimp, bromophenol contents in the cephalothorax and in the tail were separately analyzed. Results showed that the cephalothorax (11.8 to 181 ng/g) contained much higher TBC than the tail (9.67 to 27.5 ng/g) (**Figure 1c**). In crabs, TBCs detected were often the highest among all marine species investigated during the year except for a short period in December 1999 and in August 2000. The amount of TBC in crabs ranged from the lowest of 98.8 ng/g in June 2000 to the highest of 2410 ng/g in Feb 2000 (**Figure 1f**). All bromophenols except compound **2** were detected in all the crabs throughout this study (**Table 6**). Compound **2** was detected only in five samples. According to Boyle et al. (*5*), crabs containing an abundant amount of bromophenols could contribute to the crab flavor. The high concentrations of bromophenols in crabs appeared to relate to the distinct briny flavor in crab meat (*5*).

Bromophenol Contents in Freshwater Fish. Boyle et al. (*5*) investigated the bromophenol content in freshwater fish, including Cisco herring, whitefish, rainbow trout, northern pike, and walleye pike, and did not detect the presence of bromophenols. Similarly, a popular edible freshwater fish known as grass carps, *Ctenopharyngodon idellus*, did not contain any of the five bromophenols investigated (*17*). Apparently, their diet composed of higher plants and detritus did not contain any flora or fauna responsible for the biosynthesis of bromophenols (*18*).

Seasonal Variations of the TBCs. Seasonal variations of the TBCs in different species throughout a year are also shown

Table 7. Ratios of the Total Bromophenol Content of Gut to Flesh or Cephalothorax to Tail in Selected Seafood

	Siganus canaliculatus (rabbitfish) gut/flesh	*Epinephelus areolatus* (brown-spotted grouper) gut/flesh	*Penaeus japonicus* (shrimp) cephalothorax/tail
December 1999	8.61	2.13	6.59
February 2000	1.31	6.94	4.76
April 2000	1.41	8.50	4.26
June 2000	3.27	3.40	1.22
August 2000	2.15	16.2	1.34
October 2000	8.65	2.12	3.04
December 2000	1.26	2.29	3.19
average	3.81 ± 3.37	5.94 ± 5.19	3.49 ± 1.91

in **Figure 1a**−**f**. TBCs varied widely for all seafood. As described previously, the lowest TBC detected was 2.72 ng/g in the flesh of brown-spotted grouper analyzed in August 1999. The highest TBC detected was 2420 ng/g in crab meat analyzed in February 2000. In both marine fishes, rabbitfish, and brown-spotted grouper, the TBCs did not fluctuate widely in their flesh, but did so in their guts (**Figure 1a**,**b**). In crustaceans such as shrimp, the TBCs in their tail remained relatively constant when compared to that of their cephalothorax (**Figure 1c**). However, for the other crustacean, crab, its TBCs varied greatly at high concentrations (**Figure 1f**). Some fluctuations were shown in the TBCs of the clam but for the oyster the TBCs were reasonably constant (**Figures 1d**,**e**).

In the fish, shrimp, crab, and oyster, the TBCs were high in those months with relatively low temperatures (i.e. from October to April), but significantly low ($p < 0.05$) in the hot summer months particularly from June to August. The clam showed the lowest values in April and June. These seasonal fluctuations in the animals generally coincided with the normal growth cycle of the bromophenol-synthesizing seaweeds in the region (*15*). For example, the brown algae such as *Padina arborescens*, *Sargassum siliquastrum*, and *Lobophora variegata* found in the local waters showed a similar pattern of seasonal variations as in the marine animals. TBCs of the algae were significantly lower during the hot summer ($p < 0.05$) than in the rest of the year (*15*). The bromophenol contents in the marine animals and in the algae appeared to be closely related. It was likely that the environmental factors, such as temperature in different seasons, influenced the biosynthesis of bromophenols of the producers (i.e. marine algae), and subsequently affected the bromophenol content in the predators (i.e. marine animals) as marine alga was a natural food source of many marine animals (*15*, *19*). However, the direct relationship between the bromophenol content in marine algae and animals requires further investigation.

Bromophenols in Live Animals. The bromophenol contents in the gut or the cephalothorax of the corresponding species might reflect the bromophenol contents in their diets (*8*). Often the same types of bromophenols were found in both the flesh and the gut of the fish samples at the same time. Similar observations were found in the shrimp. Previous studies on Australian prawns and fish have demonstrated this relationship (*8*, *9*).

In rabbitfish, brown-spotted grouper, and shrimp, their mean TBCs in their gut/cephalothorax were always higher than that in their flesh/tail throughout a year. Using the Student *t*-test to evaluate at each sampling period, five, seven, and five out of seven sampling periods of rabbitfish, brown-spotted grouper, and shrimp, respectively, had their mean TBCs in gut/cephalothorax significantly higher than that in their flesh/tail at $p < 0.05$ (**Figures 1a**−**c**). Higher values of TBC found in the gut than in the flesh or in the cephalothorax than in the tail of a species were very consistent most of the time.

The ratios between the TBCs of gut and flesh or between cephalothorax and tail were calculated for several samples as shown in **Table 7**. All of the TBC ratios were larger than one and in the range of 1.26−8.65, 2.12−16.2, and 1.22−6.59, respectively for rabbitfish, shrimp, and brown-spotted grouper. Their corresponding mean values were 3.81, 5.94, and 3.49, respectively. In studies of Australian animals, Whitfield et al. (*8*) showed that the ratio of TBCs in the cephalothorax and tail of prawns ranged from 1.3 to 25 (*8*) and in the gut and flesh of fish from 1 to 176 (*9*). Higher bromophenol concentrations in the gut/ cephalothorax than in the flesh/tail in the current samples appeared to be in line with the findings of Whitfield et al. (*8*, *9*). Studies on marine fish and prawns suggested that bromophenols were obtained from their natural diets (*8*, *9*).

Bromophenol Contents in Seafood and Living Habitats. As the bromophenols present in the marine animals primarily come from the animals' diet (*8*, *9*), the bromophenol contents in the animals would be affected by both their dwelling habitats and their feeding habits. It is possible to reflect the condition of the habitat of an animal based on the amount and the types of bromophenols in its flesh. For example, the marine rabbitfish, which dwells in algal seabed and feeds mainly on marine algae (*19*), contained a significant amount of bromophenols in both the gut and flesh. On the other hand, the brown-spotted grouper (*Epinephelus areolatus*) is found in either the seagrass beds or the fine sediment bottom (*19*) and fed mainly on crustacean. Mollusks such as clam and oyster are filter feeders (*20*, *21*). Clams living in the planktonic and benthic habitats in different developmental stages (*22*) survive on soft-bottom fauna by filter feeding. Oysters living on rock surfaces consume both organic substrate and organisms suspended in the ocean (*20*). The high concentration of compound **3** detected only in oyster during the whole period compared with that of compounds **4** and **5** in other seafood might suggest its habitat containing food sources with a high concentration of compound **3**.

Both shrimp and crab are benthic animals. Shrimp (*Penaeus spp*.) are omnivorous, consuming both phytoplankton and other benthic animals such as crustaceans and polycheates (*23*, *24*). The habitat of the shrimp apparently contained sources of bromophenols, e.g. polycheates (*8*), such that the TBC was comparable to those of the fish in this study (**Figure 1**). However, the TBCs of shrimp and other animals in this study were still much less than that of the crab. Crab (*Charybdis feriatus*) is a carnivorous animal. Its food sources are mainly benthic animals such as bivalves, gastropods, and polychaetes (*2*). Its exceptionally high concentrations of bromophenols in this study (highest concentration at 2420 ng/g) were likely due to both its high intake and the presence of the bromophenol-rich polychaetes available in its habitat (*26*). Overall, the

Table 8. Flavor Threshold and Evaluation Flavor Concentrations of Various Bromophenols

	water[b]		prawn meat[b]		marinated whitefish[c]	
compd[a]	threshold (ng/g)	flavor description	threshold (ng/g)	flavor description	evaluation flavor concn (ng/g)	flavor description
1	3×10^{-2}	phenolic/iodine	2	phenolic	10	rich, full flavored sea-like
2	23	phenolic	ND[d]	ND	20	mild fish-like, slightly bitter, green
3	4	phenolic	ND	ND	50	slight iodine-like
4	5×10^{-4}	iodoform	6×10^{-2}	iodoform	0.1	fishy, crabby, shrimp-like
5	6×10^{-1}	iodoform	ND	ND	10	sea salt-, fish-like

[a] **1**: 2-bromophenol. **2**: 4-bromophenol. **3**: 2,4-dibromophenol. **4**: 2,6-dibromophenol. **5**: 2,4,6-tribromophenol. [b] Reference *10*. [c] Reference *6*. [d] ND = not determined.

bromophenol contents in the marine animals have a close relationship with the habitat where the animals live (*8*, *9*).

Flavor Value (FV) of Bromophenols in Seafood. By using evaluation flavor concentrations (EFCs) (**Table 8**) determined by Boyle et al. (*6*), both the individual FV and the total FVs of bromophenols in seafood were calculated (*16*) and are shown in **Figure 1a**−**f**. Comparing both the total FV and the corresponding TBC of flesh/tail in rabbitfish, shrimp, and oyster, the maximum values of these seafood fell in the same month (**Figure 1a**,**c**,**e**). But for grouper, clam, and crab, their maximum values fell in different months (**Figures 1b**,**d**,**f**). For example, crab and grouper had the highest TBC in February and October, but they had the highest total FV in April and February, respectively. It appears that the total FV of bromphenols might be a suitable parameter to show the importance of the presence of bromophenols in the marine animals. It was observed that different marine animals might have their highest total FV fall in different months in a year, but the highest ones were more often found during the cold months. In **Figure 1**, rabbitfish, clam, and shrimp had the highest total FVs of bromophenols in their flesh/tail in December whereas both oyster and crab had theirs in April, and grouper in February.

The correlation and the linear regression between the total FV of the bromophenols and the FV of compound **4** for the whole period were calculated for each marine animal. **Figure 2** shows high correlation and significance for the parameters in each marine animal. Significance in the regression lines between the two parameters in the animals was also found ($p < 0.05$). These results may suggest the month when the total FV reaching the maximum magnitude in a marine sample can be easily identified based on the high magnitude of FV of compound **4**. Besides, fluctuations in the total FV of a sample during a year may be estimated by the FV of compound **4.**

Relationships between the bromophenol(s) of gut and its flesh in a fish, or between that of cephalothorax and its tail in shrimp, were evaluated by correlation. With the critical *p*-value set at 0.05, significant correlations between gut and flesh were found in rabbitfish, and between the cephalothorax and tail in shrimp when their TBCs were evaluated. Besides, significant correlations were found in groupers and in shrimp calculated on the basis of either the total FV or the FV of compound **4** at a more conservative level of $p = 0.01$. These results suggested that a much better correlation between the gut and flesh or between the cephalothorax and tail was found by using the FV as the basis of calculation for correlation, particularly for the evaluation of grouper and shrimp.

Finally, the linear relationships between the total FV and its corresponding TBC (ng/g), and between the FV and its corresponding content (ng/g) of compound **4** in the marine animals were tested. Most of the results showed low correlation except for rabbitfish, shrimp, and oyster. In general, there were no simple linear relationships between the TBC and its corresponding total FV, nor between the content and its FV of compound **4** for most samples.

Bromophenols as Flavor Compounds in Seafood. Bromophenols are a group of key flavor compounds in seafood with both low threshold and low EFCs (**Table 8**) (*6*, *10*). The flavor value of an individual bromophenol in the seafood samples studied was discussed in the last section. According to Boyle et al. (*6*), 10 ng/g of compound **1** in marinated whitefish would produce a rich, full-flavored sea-like flavor; 0.1 ng/g of compound **4** would produce a crabby, shrimp-like flavor; and 10 ng/g of compound **5** would produce a sea salt-, fish-like flavor. Since both compounds **2** and **3** have much larger EFCs than the other three, their flavor impacts were expected to be much lower. By using these values as references, the concentration values of each bromophenol determined for the local seafood were generally high enough to produce the desirable sea-like flavor (*5*, *6*, *8*, *9*). In fact, many bromophenols had concentrations much higher than that reported in the literature to produce the desirable sensation (*5*, *6*, *8*, *9*). In combination, 0.5 ng/g of compound **1**, 0.1 ng/g of compound **4**, and 0.5 ng/g of compound **5** produced slightly crabby, iodine-like, and full sea fish-like flavor (*6*). In the current study, both clam and crab would possess such flavor character. The detection of bromophenols, with concentrations higher than the required threshold and EFCs, in various types of seafood from different locations in the world suggested that they were important flavor compounds in seafood to produce a desirable sea-like flavor (*5*, *6*, *8*, *9*).

Compound **4**, the most potent bromophenol with the lowest flavor threshold value of 5×10^{-4} ng/g in water, was the cause of iodine-like off-flavor at high concentration in the Australian prawns, and the compound was detected in many of the current samples (87.1%) (*10*). The concentration of this compound in the current samples on a dry weight basis was in the range of 0.136−77.3 ng/g. With compound **4** in marinated whitefish, a concentration of 2.5 ng/g would produce a strong iodoform flavor (*10*). In this investigation, the highest concentration of compound **4** was detected in crab meat (April 2000) with a concentration of 77.3 ng/g on a dry weight basis (**Table 6**). After recalculation at 80% moisture content, the concentration of the crab meat was 15.5 ng/g, which would likely produce the iodine-like off-flavor. However, other seafood samples in the present study were generally free from the highly concentrated iodine-like off-flavor.

Conclusion. Bromophenols were widely distributed in selected Hong Kong seafood of marine origin in a year. The bromophenol contents varied among and within species at different periods of time. Generally, the concentrations of bromophenols in the majority of seafood investigated were high enough to contribute to the distinct sea-like or brine-like flavor.

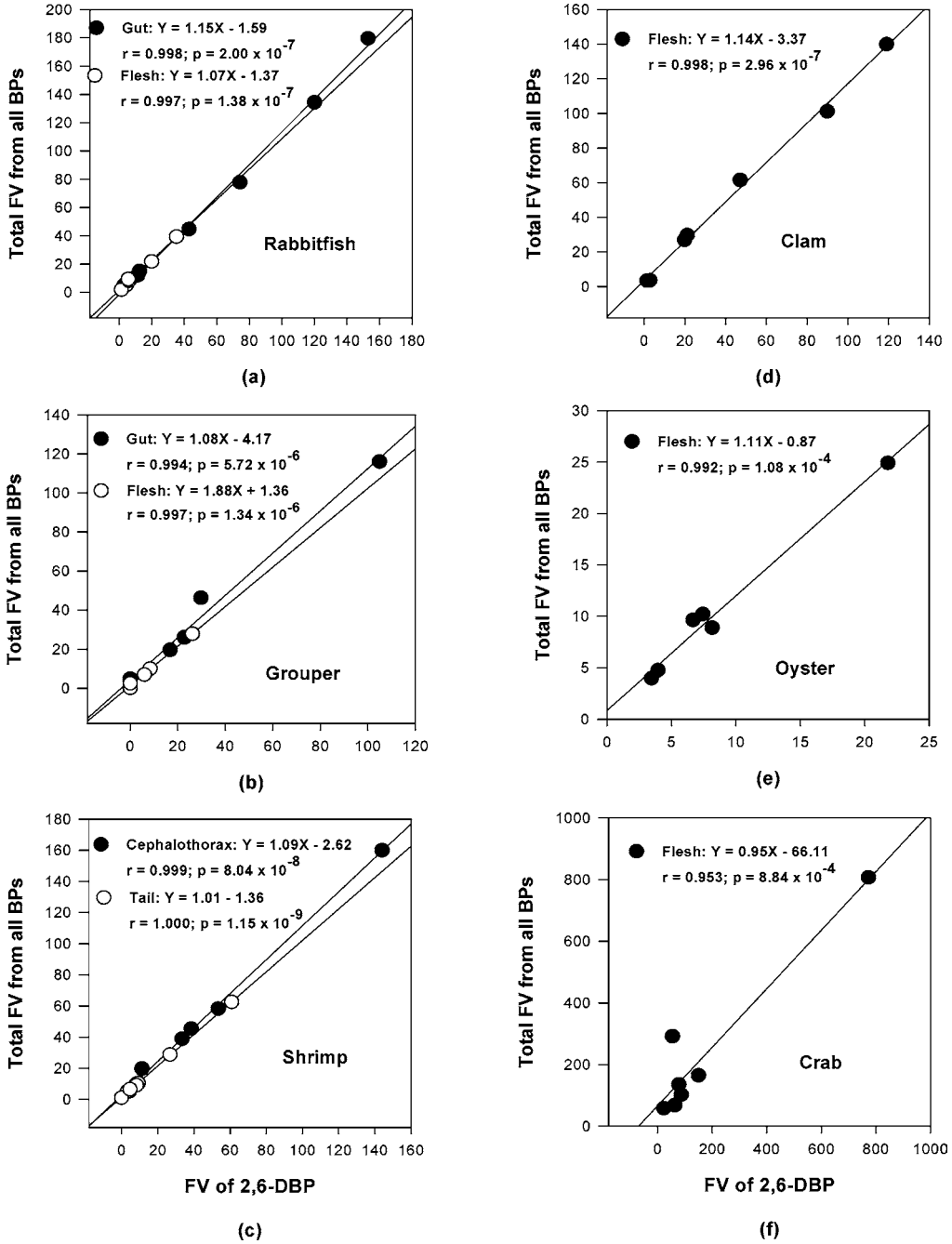

Figure 2. Correlation and linear regression between the flavor value (FV) of 2,6-dibromphenol and the total FV of all bromophenols in (a) rabbitfish (*Siganus canaliculatus*), (b) brown-spotted grouper (*Epinephelus areolatus*), (c) shrimp (*Penaeus japonicus*), (d) clam (*Tapes philippinarum*), (e) oyster (*Ostrea rivularis*), and (f) crab (*Charybdis feriatus*). r = correlation coefficient; p = calculated p-value based on Pearson product moment correlation with H_o, $\rho = 0$ (i.e. Null hypothesis: the relationship between the two variables is not correlated); X and Y = the variables of the regression line.

Both gut and flesh or cephalothorax and tail of the animals contained bromophenols, and the TBC in gut or cephalothorax was much higher than that in the flesh or tail of the animals monitored. These results agreed well with those of others (*8*, *9*) suggesting bromophenols were obtained from the diets of the animals. Data also showed that bromophenols were detected only in the animals of marine origin but not of freshwater origin. Using the total FV in place of the TBC to evaluate the impact of bromophenols might be a better way to observe the differences in the flavor impacts during a season and among different species. Overall, this study showed the distribution and seasonal variations in the quantities of bromophenols in selected seafood commonly consumed in Hong Kong and provided important information on the natural ranges of the quantities of bromophenols in the seafood for further development of aquacultured products of suitable flavor quality.

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