Distribution of Bromophenols in Australian Wild-Harvested and Cultivated Prawns (Shrimp)

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Thirty samples of 9 species of prawns (shrimp) harvested from sites along the eastern coast of Australia and 10 samples of two cultivated species were analyzed by GC/MS for the key flavor components, 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol, and 2,4,6-tribromophenol. In the commercially important wild-harvested species, *Penaeus plebejus*, *P. esculentus*, and *P. latisulcatus*, the total bromophenol content was found to vary between 9.5 and 1114 ng/g, while in the major cultivated species, *P. monodon*, the total bromophenol content was <1 ng/g. Sensory analyses of 10 samples of wild-harvested prawns showed that the meat of these animals had briny, ocean-like, and prawn-like flavors, whereas all five samples of cultivated prawns were described as bland. Furthermore, analysis by GC/MS of the heads (including the gut) and tails of these animals showed that in wild-harvested prawns the average total bromophenol content in the heads was 6.8 times greater than that in the tails; in cultivated animals it was only 3 times greater. These observations support the opinion that bromophenols are derived from components of the diets of these animals. The paper discusses the likely dietary sources of these compounds in Australian prawns and a possible controlled dietary procedure to improve the flavor of cultivated animals.

Keywords: *Prawns; bromophenols; GC/MS analysis; flavor; sensory analysis; dietary origins*

INTRODUCTION

Australia is a major producer of wild-harvested prawns (shrimp) for both international and domestic markets. However, in recent years export demand has exceeded supply and as a consequence the domestic market has become increasingly dependent on the availability of local aquaculture-produced animals. As in other Pacific rim countries, the prawn most favored for cultivation in Australia is the giant tiger prawn, *Penaeus monodon*. This animal is readily grown in salt water ponds in tropical and subtropical Australia, where it is fed on a manufactured diet consisting of fishmeal and starch with vitamin and mineral supplements. Current production stands at 1500 tonnes/year, and this quantity is expected to increase to 4000 tonnes/year by the year 2000. This prawn has become popular with local consumers because of its size and the sweetness of its flesh; however, discerning customers also find that these prawns lack the natural flavor of wild-harvested animals.

In recent years bromophenols have been identified as key flavor compounds in prawns (Whitfield et al., 1988; Anthoni et al., 1990) and in ocean fish, molluscs, and crustaceans (Boyle et al., 1992a). Of these compounds, the most potent, 2,6-dibromophenol (2,6-DBP), imparts an iodine- or iodoform-like flavor to prawn meat (Whitfield et al., 1988), while 2-bromophenol (2-BP) and 2,4,6-tribromophenol (2,4,6-TBP) produce full or intense shrimp-like flavors (Boyle et al., 1992b). Alone or in combination, these three compounds are reported to enhance the flavor of seafoods (Boyle et al., 1992b). Two other bromophenols commonly found in fish and crustaceans, 4-bromophenol (4-BP) and 2,4-dibromophenol (2,4-DBP), both possess weak phenolic-like flavors (Whitfield et al., 1988); however, their presence can still produce minor effects on the perceived flavors of some seafoods (Boyle et al., 1992b). As a consequence, the current survey was undertaken to determine the variations in concentration of bromophenols that occur in wild-harvested and cultivated prawns and to relate these variations to the perceived flavor of the meat of these animals.

In the Sydney Fish Market, the dominant species of wild-harvested prawns are *Penaeus plebejus*, *P. esculentus*, and, to a lesser extent, *P. latisulcatus*, while the principal cultivated prawn is *P. monodon*. In addition, three other species of wild-harvested prawns, *Haliporoides sibogae*, *Metapenaeus macleayi*, and *Penaeus merguiensis*, fill small but well-defined niches in the domestic market. All of these species are found in the temperate and tropical waters of eastern Australia and, together with two other wild-harvested species included in this study, *Parapenaeus australiensis* and *Plesionika martia*, are representative of a wide range of habitats and variations in dietary sources. Accordingly, these nine species of ocean prawns, including a sample of wild-harvested *P. monodon*, together with two species of locally cultivated prawns, were selected for the current investigation of the distribution of bromophenols in Australian prawns.

MATERIALS AND METHODS

Materials. Samples of wild-harvested prawns were provided by the State Department of New South Wales Fisheries and by commercial suppliers based in New South Wales and Queensland. Additional samples were purchased from the Sydney Fish Marketing Authority. Samples of cultivated prawns were purchased from growers in New South Wales and Queensland or from the Sydney Fish Marketing Authority. All samples were obtained raw and were collected over a 3 year period (May 1993−May 1996). The prawns used throughout the study weighed between 25 and 35 g each.

Prawns supplied from outside the Sydney area were chilled to 0 °C and were then air freighted to Sydney within 6 h. On

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receipt they were snap frozen and stored at −20 °C until required for sensory and chemical analysis. Samples from the Sydney area were held in ice until delivered to the laboratory, where they were also snap frozen and stored at −20 °C. Species of all prawn samples were identified by either Mr. K. Graham (State Department of NSW Fisheries) or Mr. K. Horada (Sydney Fish Marketing Authority).

Reference samples of the five target bromophenols were purchased from Aldrich Chemical Co. Inc., Milwaukee, WI, and 2,6-dibromophenol-d_3 was purchased from C/D/N Isotopes Inc., Quebec, Canada. The purity of each compound (>98%) was confirmed by gas chromatography/mass spectrometry (GC/MS) analysis. Distilled water was purified through a Milli-Q purification system (Millipore Corp., Bedford, MA). All inorganic chemicals and organic solvents were of analytical reagent grade (>98% pure). The solvents were further purified by distillation through a packed fractionating column.

Isolation of Bromophenols. Samples of frozen uncooked prawns (about 500 g) were carefully dissected into heads and tails, the intestine was removed from the muscle, and the weight of each section was recorded. The heads and tails were separately placed in water (1.5 L) and homogenized for 5 min in a Panasonic Super Blender. The homogenates were acidified to pH 1 with 10 M sulfuric acid and were then left to stand at 20 °C overnight to confirm that sufficient acid had been added to achieve the required pH. The volatile components were isolated by combined steam distillation−solvent extraction (SDE) with 30 mL of pentane/diethyl ether (9:1) as solvent (Whitfield et al., 1988). After 3 h, the pH of the residues was measured again to confirm that the homogenates had remained acidic during the isolation procedure. The internal standard, 2,6-dibromophenol-d_3 (100 ng in 100 μL iso-octane), was added to the solvent extracts, which were then dried by cooling to −15 °C and decanting the solvent fraction. The extracts were concentrated by the careful removal of the pentane/diethyl ether by fractional distillation, and the concentrates in iso-octane (about 100 μL) were stored in 2-mL glass autosampler vials at −15 °C until required for analysis by GC/MS.

Analysis by GC/MS. The bromophenols in the prawn extracts were analyzed by a Hewlett-Packard HP5890 II gas chromatograph interfaced to a Hewlett-Packard HP5971 A mass selective detector, operated in the multiple ion detection (MID) mode. The GC oven was fitted with a 25 m × 0.25 mm i.d. fused silica column coated with methyl phenylsilicone HP5 (0.33 μm film thickness) and a retention gap of 5 m × 0.25 mm i.d. uncoated but deactivated. The retention gap was necessary to protect the column from the large quantity of steam-volatile fatty acids present in the extracts. Aliquots (1 μL) of the sample extracts or calibration solutions were injected automatically by a Hewlett-Packard HP7673 autosampler. For all analyses the injections were split 1:20. The GC oven was temperature-programmed as follows: the temperature was initially held at 60 °C for 1 min, programmed from 60 to 225 °C at 15 °C/min, then from 225 to 280 °C at 40 °C/min, and finally held at 280 °C for 37 min. The helium flow was 0.48 mL/min, the injector temperature was 280 °C, and the GC/MS transfer line was 300 °C. The MS was operated in electron ionization mode with an energy of 70 eV and an ion source temperature of 180 °C.

Quantitative analysis by MID was performed under software control by a Hewlett-Packard Vectra 386/25 computer running a Hewlett-Packard MS ChemStation data system. In the analysis, ions were monitored for 2- and 4-BP at m/z 172 and 174, for 2,4-DBP and 2,6-DBP at m/z 250 and 252, for 2,4,6-TBP at m/z 330 and 332, and for the internal standard 2,6-dibromophenol-d_3 at m/z 255 and 257. The retention times of these compounds were as follows: 2-BP, 6.60 min; 4-BP, 9.02 min; 2,4-DBP, 9.67 min; 2,6-DBP, 9.99 min; 2,4,6-TBP, 12.45 min; and 2,6-dibromophenol-d_3, 9.97 min. The GC/MS was calibrated by the analysis of three different concentrations of each of the five bromophenols (0.5, 5, and 25 μg/mL in iso-octane) with a constant concentration of the internal standard (1 μg/mL). Response factors for each compound, with respect to the internal standard, were calculated by the data system software, and these were used to determine the concentration of the target compounds in the extracts. The calibrations were performed on the day of analysis, and each analysis was performed in duplicate. If a sample contained analytes outside the calibration range of the MS, a diluted subsample was analyzed after addition of more internal standard. Reported data have been corrected for losses during extraction and concentration (see below). The detection limit for individual bromophenols in prawn meat was 0.01 ng/g based on a factor of 3 times the background noise.

During the GC/MS analyses the presence of individual bromophenols was confirmed by the appearance of a single peak in the total ion chromatogram at the appropriate retention time, by the presence of the two characteristic ions listed above, and by the appearance of the correct isotopic ratios for these ions.

Extraction Efficiencies. The extraction efficiencies of the SDE technique for the recovery of individual bromophenols from prawn meat were determined as follows. Meat (100 g) from samples of cultivated *P. monodon* (PM 17595) of low bromophenol content was homogenized in water (1.5 L). To this mixture was added an aliquot (1 mL) of a solution containing each of the five bromophenols (1 μg/mL in ethanol). As previously described, the mixture was extracted by SDE after acidification to pH 1. The extractions were performed in duplicate. The average percentage recoveries were as follows: 2-BP 94% (SD = 6%), 4-BP 41% (SD = 1%), 2,4-DBP 74% (SD = 0), 2,6-DBP 81% (SD = 0), and 2,4,6-TBP 74% (SD = 4%).

Sensory Panel Assessment of Prawn Meat. Eight samples of *P. plebejus*, two samples of *P. esculentus*, and five samples of cultivated *P. monodon* were assessed by a consumer panel for appearance and acceptability. The panelists were selected from laboratory staff who regularly ate boiled prawns. The size of the panel varied between 10 and 12 persons, and the same panelists were used during the assessment program. Whenever possible, the sensory assessments were made on the day the prawns were received in the laboratory. When this was not possible, they were frozen at −20 °C and were assessed within 24 h of harvest. For sensory assessment, batches of whole prawns (about 1 kg), with shell and head attached, were placed in a large saucepan, covered with unsalted, boiling, odor-free water, and boiled until they floated to the surface (about 2 min). They were immediately removed and allowed to cool to room temperature, and four prawns were served to each panelist for assessment. The panel was requested to rate the prawns for color and the tail muscle for flavor, texture, and acceptability on 100 mm line scales covering a range of choices from unacceptable to excellent. Panelists were also asked to rate on separate 100 mm line scales the flavor of the tail muscle according to five descriptors (sweet; bland; prawn flavor; ocean- or iodine-like flavor; and brine-like flavor); the scales covered the options from absent to pronounced. The panelists were also asked to describe any other flavors present in the prawns but not covered by the above descriptors.

RESULTS AND DISCUSSION

Bromophenol Content of Wild-Harvested Prawns. Table 1 records the results of the analyses of uncooked heads (which includes the gut) and tails of 30 samples of wild-harvested prawns. Bromophenols were found in the heads and tails of all nine species examined, while in each of the samples the total bromophenol content (TBC) was greater in the heads than in the tails. The ratios of these concentrations varied from 1.3:1 in *P. esculentus* (PE 22395) to 36:1 in *P. monodon* (PM 23596). The very high value obtained with the sample of *P. monodon* probably indicates that these animals had fed just before they were caught. The average ratio (heads/tails) for the 30 samples of 9 species of wild-harvested prawns was 7.7:1.

The highest TBC in prawn heads (1956 ng/g) was found in a sample of *P. latisulcatus* (PL 191294), which also showed a high TBC in the tails (296 ng/g). A

Table 1. Distribution of Bromophenols in the Heads and Tails of Uncooked Wild-Harvested Prawns

species/ code no.	sample	bromophenol content (ng/g)						bromophenol ratio heads/tails
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total	
P. plebejus								
PP 17593	heads	3.5	290	450	5.9	150	899	2.9
	tails	0.81	130	150	0.73	30	312	
PP 161193	heads	42	160	610	ND[a]	tr[b]	812	3.4
	tails	2.0	80	160	ND	0.12	242	
PP 5194	heads	0.42	530	690	3.0	41	1264	9.4
	tails	0.13	33	100	0.42	1.6	135	
PP 24394	heads	13	110	430	1.5	23	578	20
	tails	1.1	1.5	25	ND	1.8	29	
PP 11195	heads	0.14	2.5	8.5	2.6	8.2	22	2.0
	tails	0.08	2.1	3.5	0.12	5.3	11	
PP 3295	heads	2.9	410	160	4.3	36	613	7.6
	tails	1.2	0.12	58	2.7	19	81	
PP 7395	heads	0.66	18	4.3	1.3	4.4	29	1.6
	tails	0.67	15	1.8	0.09	0.84	18	
PP 22395	heads	1.2	70	23	5.8	48	148	25
	tails	0.02	3.3	1.6	0.04	0.74	6	
PP 23395	heads	0.24	9.0	20	1.1	9.8	40	2.9
	tails	0.08	2.2	7.8	ND	4.4	14	
PP 28395	heads	0.21	6.5	7.4	ND	2.3	16	3.0
	tails	0.02	2.0	1.5	0.11	1.8	5.4	
PP 5495	heads	0.07	0.12	3.3	7.6	4.2	15	2.8
	tails	ND	0.51	0.99	2.2	1.7	5.4	
PP 18495	heads	0.30	56	9.8	0.93	1.8	69	3.6
	tails	0.03	15	2.8	0.07	0.64	19	
P. esculentus								
PE 31593	heads	0.19	1.4	48	2.0	4.8	56	4.7
	tails	0.21	0.63	11	0.35	0.01	12	
PE 1693	heads	0.15	2.4	150	1.4	1.0	155	4.1
	tails	0.07	0.90	37	ND	0.05	38	
PE 61293	heads	0.40	3.1	20	2.0	ND	26	4.1
	tails	0.12	0.10	5.9	0.17	ND	6.3	
PE 131293	heads	ND	45	216	ND	ND	261	13
	tails	0.04	0.80	19	0.09	ND	20	
PE 7394	heads	0.30	13	25	1.0	4.2	44	5.8
	tails	0.10	1.0	5.8	0.20	0.50	7.6	
PE 14394	heads	0.28	14	88	1.4	29	133	4.2
	tails	0.06	2.3	14	0.22	15	32	
PE 24394	heads	0.70	60	289	1.2	9.1	360	7.7
	tails	ND	14	32	0.60	ND	47	
PE 131294	heads	0.57	1.4	26	2.8	3.0	34	3.6
	tails	ND	0.61	7.8	0.55	0.57	9.5	
PE 22395	heads	0.08	0.53	6.5	1.7	159	168	1.3
	tails	ND	2.0	2.0	ND	128	132	
PE 23395	heads	0.14	5.2	15	0.55	82	103	11
	tails	0.03	0.75	2.2	0.05	6.2	9.2	
P. latisulcatus								
PL 161193	heads	0.68	50	25	8.5	4.7	89	12
	tails	0.15	2.1	5.0	ND	0.10	7.4	
PL 191294	heads	1.0	850	1100	ND	4.9	1956	6.6
	tails	ND	210	83	1.0	2.0	296	
H. sibogae								
HS 27696	heads	2.6	0.05	37	42	50	132	2.4
	tails	0.61	1.5	12	27	14	55	
M. macleayi								
MM 25396	heads	0.10	0.30	9.0	0.90	28	38	4.0
	tails	0.04	ND	4.0	0.40	5.0	9.4	
P. merguiensis								
PMe 7694	heads	0.94	510	630	1.4	103	1245	10
	tails	0.14	20	100	ND	4.0	124	
P. monodon								
PM 23596	heads	tr	3.0	5.0	6.0	460	474	36
	tails	tr	0.30	0.60	0.20	12	13	
Pa. australiensis								
PA 22396	heads	0.20	1.0	0.80	2.0	25	29	13
	tails	0.10	0.30	0.20	0.60	1.0	2.2	
Pl. martia								
PMa 27696	heads	0.09	ND	6.6	6.3	30	43	2.5
	tails	0.13	2.6	2.0	3.4	8.7	17	

[a] ND, not detected at a detection level of 0.01 ng/g. [b] tr, trace = 0.01 ng/g.

slightly higher TBC (312 ng/g) was found in the tails of *P. plebejus* (PP 17593). These prawns also had a high TBC in the heads (899 ng/g). The lowest TBC in prawn heads (15 ng/g) and tails (2.2 ng/g) were found in samples of *P. plebejus* (PP 5495) and *Pa. australiensis* (PA 22396), respectively.

All five target bromophenols were found in 24 head and 17 tail samples and, with the exception of only four

Table 2. Distribution of Bromophenols in Whole Uncooked Wild-Harvested Prawns[a]

species/ code no.	weight ratio heads/tails	bromophenol content (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total
P. plebejus							
PP 17593	0.82	2.0	200	290	0.67	84	577
PP 161193	0.79	19	110	360	ND[b]	0.07	490
PP 5194	0.70	0.25	240	300	1.5	1.8	543
PP 24394	0.76	6.2	48	200	0.65	11	266
PP 11195	0.75	0.11	2.3	5.6	1.2	6.5	16
PP 3295	0.79	1.9	180	100	3.4	26	311
PP 7395	0.56	0.67	16	2.7	0.52	2.1	22
PP 22395	0.69	0.5	30	10	2.4	20	63
PP 23395	0.87	0.15	5.1	13	0.47	6.7	25
PP 28395	0.85	0.11	4.1	4.2	0.06	2.0	10
PP 5495	0.72	0.03	0.34	2.0	4.4	2.7	9.5
PP 18495	0.85	0.15	34	6.0	0.47	1.2	42
P. esculentus							
PE 31593	0.41	0.20	0.85	22	0.82	1.4	25
PE 1693	0.46	0.09	1.4	72	0.44	0.35	74
PE 61293	0.50	0.22	1.2	11	0.84	ND	13
PE 131293	0.64	0.02	18	96	0.05	ND	114
PE 7394	0.67	0.18	5.8	14	0.52	2.0	23
PE 14394	0.62	0.14	6.8	42	0.67	20	70
PE 24394	0.62	0.27	31	130	0.83	3.5	166
PE 131294	0.71	0.24	0.93	15	1.5	0.94	19
PE 22395	0.63	0.03	1.4	3.7	0.66	140	146
PE 23395	0.74	0.30	2.6	7.7	0.48	34	45
P. latisulcatus							
PL 161193	0.56	0.34	19	12	3.1	1.8	36
PL 191294	0.77	0.43	580	530	0.56	3.3	1114
H. sibogae							
HS 27696	0.86	1.5	0.83	23	34	31	90
M. macleayi							
MM 25396	0.47	0.06	0.10	5.6	0.56	12	18
P. merguiensis							
PMe 7694	0.66	0.46	220	310	0.56	43	574
P. monodon							
PM 23596	0.57	tr[c]	1.3	2.2	2.3	170	176
Pa. australiensis							
PA 22396	0.62	0.14	0.58	0.43	1.1	10	12
Pl. martia							
PMa 27696	0.68	0.11	1.6	3.9	4.6	17	27

[a] Calculated from data in Table 1 and from the weight of individual batches of heads, tails, and total prawns extracted. [b] ND, not detected at a detection limit of 0.01 ng/g. [c] tr, trace = 0.01 ng/g.

samples, if a bromophenol was found in the tails, it was also present in the heads. In those cases where a bromophenol was not found in the heads, the concentration of that compound in the tails was not greater than 2.6 ng/g (Table 1). Furthermore, with only one exception, *P. plebejus* (PP 3295), when an individual bromophenol was present in high concentrations in the heads, it was also present in relatively high concentration in the tails. The dominance of bromophenols in the heads of these prawns, as a consequence of the presence of food particles and food residues retained in the gut, thus strongly supports earlier expressed views that such compounds are derived from the animal's natural diet (Whitfield et al., 1988; Anthoni et al., 1990). Furthermore, the variation in ratio of bromophenol content in the heads and tails within species could well provide an indication as to how recently the animals had fed before harvesting. Across species it could also indicate what they had been eating (Table 1).

The distribution of bromophenols in samples of whole uncooked wild-harvested prawns and the weight ratio (heads/tails) of individual samples are recorded in Table 2. In all samples the weight of the heads was less than that of the tails, with the weight ratio ranging from 0.41:1 for *P. esculentus* (PE 31593) to 0.87:1 for *P. plebejus* (PP 23395). All five target bromophenols were present in 27 samples of wild-harvested whole prawns; the remaining three samples each contained four of these compounds. Of the latter, 2,6-DBP was not detected in one sample of *P. plebejus* (PP 161193), while 2,4,6-TBP was not detected in two samples of *P. esculentus* (PE 61293 and PE 131293). The dominant bromophenols in all samples were 2,4-DBP (major component on 15 occasions), 2,4,6-TBP (7 occasions), 4-BP (6 occasions), and 2,6-DBP (2 occasions). In the 15 samples having 2,4-DBP as the dominant compound, 4-BP was found at the next highest concentration in 11 of them. Similarly, in the 6 samples for which 4-BP was the dominant component, 2,4-DBP had the next highest concentration on 5 occasions. Such a relationship could suggest that these two compounds were derived from a common dietary source. A similar relationship was also observed between 2,6-DBP and 2,4,6-TBP when 2,6-DBP was the dominant component. However, no relationship appeared to exist when 2,4,6-TBP was dominant.

The data in Table 2 show that the TBC of whole wild-harvested prawns varied considerably within species and probably also across species. Thus, in *P. plebejus*, the TBC varied from 9.5 to 577 ng/g, in *P. esculentus* from 13 to 166 ng/g, and in *P. latisulcatus* from 36 to 1114 ng/g, while those of the remaining six species all fell between these limits. The cause of such wide variations in TBCs within species is unknown. However, it has previously been suggested (Whitfield, 1990; Whitfield et al., 1992b) that such variations could be

Table 3. Distribution of Bromophenols in Uncooked Cultivated Prawns

code no.	sample	bromophenol content (ng/g) 2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total	bromophenol ratio heads/tails
P. monodon								
PM 10593	head	tr[a]	ND[b]	0.18	0.42	0.22	0.82	1.2
	tail	ND	ND	0.11	0.35	0.22	0.68	
PM 12593	head	ND	ND	0.11	0.13	0.92	1.2	1.8
	tail	ND	ND	0.11	0.35	0.22	0.68	
PM 61293	head	ND	ND	0.16	0.70	ND	0.86	8.6
	tail	ND	ND	0.03	0.07	ND	0.1	
PM 4294	head	ND	ND	0.14	0.71	ND	0.85	2.7
	tail	ND	ND	0.04	0.28	ND	0.32	
PM 9594	head	ND	ND	0.22	0.56	0.65	1.4	na[c]
	tail	ND	ND	ND	ND	ND	ND	
PM 25894	head	ND	ND	0.16	0.40	0.50	1.1	7.9
	tail	ND	0.06	ND	0.08	ND	0.14	
PM 5495	head	0.02	ND	0.12	0.13	0.17	0.44	1.8
	tail	0.01	0.02	0.04	0.06	0.11	0.24	
PM 17595	head	0.02	ND	0.12	0.13	0.17	0.44	1.8
	tail	0.01	ND	0.06	0.06	0.11	0.24	
P. stylirostris								
PS 181193	head	ND	ND	0.38	1.0	ND	1.4	1.1
	tail	ND	0.56	0.59	0.11	ND	1.3	

[a] tr, trace = 0.01 ng/g. [b] ND, not detected at a detection limit of 0.01 ng/g. [c] na, not applicable.

Table 4. Distribution of Bromophenols in Whole Uncooked Cultivated Prawns[a]

species/ code no.	weight ratio heads/tails	bromophenol content (ng/g) 2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total
P. monodon							
PM 10593	0.78	tr[b]	ND[c]	0.14	0.38	0.22	0.74
PM 12593	0.80	ND	ND	0.11	0.26	0.53	0.90
PM 61293	0.70	ND	ND	0.08	0.33	ND	0.41
PM 4294	0.52	ND	ND	0.07	0.42	ND	0.49
PM 9594	0.76	ND	ND	0.08	0.21	0.25	0.54
PM 25894	0.88	ND	0.03	0.07	0.23	0.23	0.56
PM 5495	0.62	0.01	0.01	0.07	0.09	0.13	0.31
PM 17595	0.50	0.02	ND	0.09	0.09	0.14	0.34
P. stylirostris							
PS 181193	0.80	ND	0.31	0.49	0.51	ND	1.3

[a] Calculated from data in Table 3 and from the weight of individual batches of heads, tails, and whole prawns extracted. [b] tr, trace = 0.01 ng/g. [c] ND, not detected at a detection limit of 0.01 ng/g.

due to one or more of three factors: (i) the feeding pattern of the prawns during the days before harvest, (ii) the rate of bioaccumulation, and (iii) the rate of elimination of the ingested materials. Thus, the concentration of the bromophenols in each catch of prawns would depend largely on the levels of these compounds that were present in their most recent feeds. Of interest, results recorded in Table 2 for the deep-water prawns, *H. sibogae* (90 ng/g) and *Pl. martia* (27 ng/g), were very similar to those obtained in earlier studies, for which the TBCs in these species were 52 and 25 ng/g, respectively (Whitfield et al., 1988). It is therefore possible that species taken from deep water (400−700 m) have a more consistent and perhaps lower dietary intake of bromophenols.

Bromophenol Content of Cultivated Prawns. Results from the analyses of uncooked heads and tails of nine samples of cultivated prawns are recorded in Table 3. These were made up of eight samples of *P. monodon* and one of *P. stylirostris*; *P. monodon* is the dominant species in the Australian cultivated prawn industry. At least two bromophenols were found in each of the nine samples of heads and in eight samples of tails. However, in one sample of tails (*P. monodon* PM 9594) these compounds could not be found at a detection limit of 0.01 ng/g. As with the wild-harvested prawns, the TBC was greater in the heads than in the tails, with the ratios of the concentrations varying from 1.1:1 in *P. stylirostris* (PS 181193) to 8.6:1 in *P. monodon* (PM 61293). The average ratio (heads/tails) for the nine samples of two species of cultivated prawns was 3:1. The highest TBCs in prawn heads (1.4 ng/g) were found in a sample of *P. monodon* (PM 9594) and the sample of *P. stylirostris* (PS 181193), while the highest TBC in the tails (1.3 ng/g) was also found in the sample of *P. stylirostris*. The lowest TBCs in prawn heads (0.44 ng/g) were found in two samples of *P. monodon*, PM 5495 and PM 9594, while the lowest TBC in the tails (not detected) was also found in sample PM 9594.

All five bromophenols were found in only one sample of *P. monodon* tails (PM 5495), while the heads from these prawns had four of these compounds (Table 3). In the remaining eight samples, the number of bromophenols present varied between two and four compounds. A rather different situation was found in the wild-harvested prawns, for which all five bromophenols were found in 24 of the head and 17 of the tail samples (Table 1).

The distributions of bromophenols in samples of whole uncooked cultivated prawns, together with the weight ratio (heads/tails) of individual samples, are recorded in Table 4. As with the wild-harvested animals, the heads weighed less than the tails, with the weight ratio varying from 0.50:1 (PM 17595) to 0.88:1 (PM 25894). The five bromophenols were present in only one sample of whole prawns examined, four of these compounds were found in three samples, three in three samples, and two in the remaining two samples. However, with

Table 5. Relationship between Natural Prawn Flavors and the Distribution of Certain Bromophenols

species/ code no.	flavor descriptions	bromophenol content (ng/g)					
		prawn tails[a]			whole prawns[b]		
		2-BP	2,6-DBP	2,4,6-TBP	2-BP	2,6-DBP	2,4,6-TBP
	Wild-Harvested						
P. plebejus							
PP 17593	briny, ocean, and prawn flavor, slightly bitter	0.81	0.73	30	2.0	0.67	84
PP 161193	fishy, average prawn flavor, slightly bitter	2.0	ND[c]	0.12	19	ND	0.07
PP 24394	briny, good prawn flavor, sweet	1.1	ND	1.8	6.2	0.65	11
PP 11195	briny, good prawn and ocean flavor, sweet	0.08	0.12	5.3	0.11	1.2	6.5
PP 23395	mild ocean and prawn flavor, sweet	0.08	ND	4.4	0.15	0.47	6.7
PP 28395	mild ocean and prawn flavor, sweet	0.02	0.11	1.8	0.11	0.06	2.0
PP 5495	ocean and prawn flavor, sweet	ND	2.2	1.7	0.03	4.4	2.7
PP 18495	slightly briny, good prawn flavor, sweet	0.03	0.07	0.64	0.15	0.47	1.2
P. esculentus							
PE 24394	slightly briny, good prawn flavor, sweet	ND	0.60	ND	0.27	0.83	3.5
PE 131294	ocean and prawn flavor	ND	0.55	0.57	0.24	1.5	0.94
	Cultivated						
P. monodon							
PM 4294	bland, no ocean flavor, sweet	ND	0.28	ND	ND	0.42	ND
PM 9594	bland, absence of prawn flavor, sweet	ND	ND	ND	ND	0.21	0.25
PM 25894	some prawn flavor, no ocean flavor, sweet	ND	0.08	ND	ND	0.23	0.23
PM 5495	bland, absence of prawn flavor, slightly sweet	0.01	0.06	0.11	0.01	0.09	0.13
PM 17595	bland, absence of prawn flavor, sweet	0.01	0.06	0.11	0.02	0.09	0.14

[a] Data transferred from Tables 1 and 3. [b] Data transferred from Tables 2 and 4. [c] ND, not detected.

such low levels of bromophenols in the cultivated prawns (maximum concentration of 2,4,6-TBP = 0.53 ng/g), no one compound could be claimed to be present in a dominant quantity. However, two of the five bromophenols, 2,4-DBP and 2,6-DBP, were found in all nine samples, though in concentrations no greater than 0.49 and 0.51 ng/g respectively (PS 181193). By comparison, 2,4,6-TBP was found in six samples, and 4-BP and 2-BP were found in three samples each. As all of the cultivated prawns would have been fed on similar commercial prawn feeds, the observed distribution of bromophenols probably indicates variations in the distribution of these compounds in such feeds over the 3 year period of this study.

The data in Table 4 show that the TBC of whole cultivated prawns was relatively consistent across all samples with only a 4-fold variation from 0.31 to 1.3 ng/g. This consistency contrasts greatly with the variation observed with wild-harvested animals in which the TBCs had a 117-fold range from 9.5 to 1114 ng/g (Table 2). The different patterns observed for TBCs in cultivated and wild-harvested prawns can probably be explained by their dissimilar feeding habits. The uniformity of bromophenol composition of cultivated animals as compared with that of wild-harvested prawns can be explained by their diets; the diet of the cultivated animal is regimented, whereas the diet of the wild animal is a product of a free-ranging existence.

Sensory Analysis of Cooked Whole Prawns. Of the 10 samples of wild-harvested prawns assessed, all were described as having "prawn-like" flavors (Table 5). By comparison, only one of the five samples of cultivated prawns had a similar flavor. Furthermore, six of the samples of wild-harvested prawns also had "ocean-like" flavors, whereas this flavor was not detected in samples of the cultivated animals. The term "sweet" was used on seven occasions to describe the flavor of wild-harvested prawns, and on four occasions it was applied to samples of the cultivated animals. On the basis of these flavor assessments, the wild-harvested prawns were perceived as having sweet, ocean-like, and prawn-like flavors, while the flavor of the cultivated animal was regarded as sweet but bland.

Role of Bromophenols in the Natural Flavor of Prawns. Studies by Boyle et al. (1992b) have shown that the presence of low (nanograms per gram) concentrations of the target bromophenols in fish, shrimp, and vegetable oil matrices impart flavor notes reminiscent of marine fish and crustaceans. Sea-, iodine-, sea salt-, and sea fish-like flavors were readily apparent when 2-BP, 2,6-DBP, or 2,4,6-TBP was incorporated by these workers into marinated whitefish at concentrations ranging from 0.25 ng/g for 2,6-DBP through 10 ng/g for 2,4,6-TBP. Of the other two bromophenols, 4-BP at 40 ng/g gave a green, bitter taste, while 2,4-DBP at 50 ng/g was described as slightly iodine-like and medicinal. In combination, 2-BP (0.5 ng/g), 2,6-DBP (0.1 ng/g), and 2,4,6-TBP (0.5 ng/g) produced in marinated whitefish crab-, iodine-, and sea fish-like flavors. Accordingly, characteristic marine- and ocean-like flavors could be produced in seafoods by the presence of these bromophenols in quantities equal to or greater than these concentrations. In our study those prawns containing high concentrations of 4-BP in the tails (PP 17593 and PP 161193) were assessed as possessing a slightly bitter taste (Table 5), conforming to the report of Boyle et al. (1992b).

From the analytical and sensory data presented in Table 5 there are major differences both in the concentrations of bromophenols in wild-harvested and cultivated prawns and in the perceived flavors of animals living under natural and artificial conditions. The wild-harvested animals have more bromophenols and stronger flavors.

However, from data in Table 5, there does not appear to be a clear relationship between the bromophenol content in the tail meat and its flavor, which suggests that prawn heads are the source of natural prawn flavor. Only one sample of wild-harvested prawn tails (PP 17593) had 2-BP, 2,6-DBP, and 2,4,6-TBP in concentrations in excess of the target values of Boyle et al. (1992b) (0.5, 0.1, and 0.5 ng/g, respectively). In another five samples, two of the compounds exceeded these concentrations and in the remaining four samples one compound only. By comparison, of the five samples of cultivated prawns, only one sample (PM 4294) had a single compound (2,6-DBP) present at a concentration in excess of its target value (Table 5).

Table 6. Relationship between Total Bromophenol Content (TBC), Habitat, and Diet of Wild-Harvested and Cultivated Prawns

species	TBC whole prawns (ng/g)	habitat[a,b]	diet[c]
		Wild-Harvested	
P. plebejus	198[d]	mud/sand	crustaceans, molluscs, polychaetes, and foraminiferanse[e]
P. esculentus	70[d]	mud/sand	foraminiferans, crustaceans, polychaetes, and detritus[f]
P. latisulcatus	575[d]	sand/mud	polychaetes, crustaceans, molluscs, and foraminiferans[f]
H. sibogae	90	mud	crustaceans, fish, foraminiferans, sponges, and polychaetes[g]
M. macleayi	18	mud	not identified
P. merguiensis	574	mud/sand	crustaceans, foraminiferans, polychaetes, and molluscs[f]
P. monodon	176	mud/sand	crustaceans, polychaetes, foraminiferans, and molluscs[f]
Pa. australiensis	12	mud	not identified
Pl. martia	27	mud	not identified
		Cultivated	
P. monodon	0.54[d]	mud	fish meal, plant material, prawn and squid meal
P. stylirostris	1.3	mud	fish meal, plant material, prawn and squid meal

[a] Composition of ocean floor. [b] Grey et al. (1983). [c] Listed in order of importance. [d] Average value. [e] T. J. Wassenberg. Division of Fisheries, CSIRO, Cleveland, QLD, Australia, personal communication, 1996. [f] Moriarty and Barclay (1981). [g] Rainer (1992).

In Australia the majority of prawns consumed are cooked whole, either in boiling water or on a barbecue. Under these conditions, it is possible that a proportion of the bromophenols in the heads, and possibly also the alimentary canals, will diffuse into the tail muscle. Examination of the bromophenol contents of uncooked whole wild-harvested prawns (Table 5) shows that in two samples (PP 17593 and PP 24394) the target concentrations for 2-BP, 2,6-DBP, and 2,4,6-TBP were all exceeded, while in another seven samples they were exceeded for two of these compounds and in the remaining sample (PP 161193) by only one compound. Prawns from this sample had the weakest flavor of the 10 wild-harvested samples assessed, although the terms "fishy" and "prawn-like flavor" were still used by the panel.

By comparison, in uncooked whole cultivated prawns the target concentrations were only exceeded for one compound, and this in just three samples (Table 5); in the remaining two samples, none were exceeded. Of the five samples assessed for flavor, only one was described by the panel as possessing some prawn flavor; the remaining four were called bland (Table 5). These results suggest that at least two of the target bromophenols need to be present in prawns to produce natural ocean- or prawn-like flavor.

Furthermore, studies have shown that some flavor components can be either substantially bound to proteins (Kinsella and Srinivasan, 1981) or partitioned into lipids (Persson, 1984; Trujillo et al., 1982), thereby suppressing the flavor effect of the concentrations present. In the case of bromophenols naturally acquired by prawns, the concentrations necessary to produce recognizable marine flavors might need to be greater than those indicated by the sensory studies of Boyle et al. (1992b). Such retention of bromophenols could explain the absence of marine flavors in the majority of samples of cultivated prawns (Table 5). Accordingly, to establish the operative flavor concentrations of bromophenols in prawns, future studies should include the sensory assessment of prawns fed artificial diets containing controlled concentrations of these compounds.

Origins of Bromophenols in Natural and Processed Prawn Diets. All mature prawns are benthic carnivores, and as such their diets consist principally of animals and organisms that inhabit the ocean floor. Table 6 records the major dietary components of six of the nine species of wild-harvested prawns currently under study and the habitats from which they were caught. The principal dietary components were the same for all of these species; only the order of preference was different. Crustaceans, polychaetes (marine worms), molluscs, and foraminifera (marine protozoans) were the major identifiable components in the foregut of these species, with small quantities of ophiuroids (brittle stars), nematodes, and plant material (algae and sea grass seeds). Of these, only polychaetes and algae are known to synthesize bromophenols. Whitfield et al. (1996, 1997) have shown that some marine polychaetes contain milligrams per gram concentrations of these compounds, which confirms the findings of Goerke and Weber (1990), King (1986), and Woodin et al. (1987) in other parts of the world. By contrast, the bromophenols in marine algae are present in only micrograms per gram concentrations (Whitfield et al., 1992a).

The highest TBCs found in the Australian study were in those polychaetes obtained from muddy habitats. For example, the TBC for the polychaete *Barantolla lepte* was 8.3 mg/g, and those for *Nephtys australiensis* and *Lumbrineris latrelli* were 0.88 and 1.6 mg/g, respectively (Whitfield et al., 1996, 1997). All three species were found in muddy sand among sea grasses. 2,4,6-TBP was the major bromophenol found in all 16 species, with the exception of *Glycera americana*, in which both 2,4-DBP and 4-BP were the most abundant. Species of polychaetes from sandy or rocky habitats have much lower (micrograms per gram) concentrations of these compounds (Whitfield et al., 1996, 1997). As reported above, 2,4-DBP, 2,4,6-TBP, and 4-BP are the major bromophenols in wild-harvested prawns. Significantly, there also appears to be a relationship between the appearances of 2,4-DBP and 4-BP in these animals, as was found in *G. americana*. The three bromophenols together with 2,6-DBP have all been identified as major compounds in a variety of marine polychaetes (Boyle et al., 1993). Since only 16 of a potential 1000 Australian species of these animals have been analyzed for bromophenols, further studies will probably lead to the identification of other local species in which 4-BP, 2,4-DBP, and 2,6-DBP are present in dominant concentrations.

Although it is not possible to identify the species from the residues of polychaetes found in the foregut of prawns, families can in some cases be identified. Of the 10 families of polychaetes covered by our survey (Whitfield et al., 1996, 1997), 3, Nereididae, Nephtyidae, and Lumbrineridae, have been shown to be dietary components of some species of Australian prawns (Rainer, 1992). It is probable that future studies could broaden this range of families to include others known to produce large quantities of bromophenols and even more not yet

known to produce these compounds. Current evidence makes it likely that polychaetes provide a major dietary source of bromophenols in Australian wild-harvested prawns.

In contrast to the natural prawn diet, commercial prawn feeds used by the Australian aquaculture industry are very low in bromophenols (Whitfield et al., 1997). In some 10 samples of various *P. monodon* feeds, the TBC varied from 1.4 to 40 ng/g. These studies also showed that the major source of bromophenols in such products was either prawn meal or shrimp head meal, which made up 4−7% of the final mixture; bromophenol contents were extremely variable, generally significantly <2 μg/g. The low bromophenol content of Australian cultivated prawns can accordingly be explained by the low levels of these compounds in commercial feeds.

Conclusion. In toxicological studies Sweet (1987) and Sax and Lewis (1989) have shown that bromophenols are irritants to skin, eyes, and mucous membranes and moderately toxic by ingestion. However, Boyle et al. (1993) have estimated that 8 million to 600 million prawns would need to be consumed to produce concentrations of bromophenols comparable to the respective LD_{50} doses in rodents. It was concluded that the natural occurrence or incorporation of simple bromophenols into food or feedstuff in concentrations adequate for flavor activity was unlikely to create acute toxicological problems. Evidence therefore suggests that the flavor of cultivated prawns could be safely modified by the simple addition of these compounds, in appropriate quantities, to the animal's diet. However, attempts to produce a prawn feed with a high bromophenol content by direct addition of these compounds to the unprocessed feed have not been successful (Whitfield et al., 1997). The bromophenols were lost during the preparation of the feed. Consequently, it would appear that the bromophenols need to be encapsulated, as in some natural dietary component (dehydrated polychaete or algae), to ensure that they are retained in the final extruded or pelletized feed. Whichever encapsulation approach is employed, the bromophenols should be present in the gut in the free form if they are to impact on the flavor of the cooked prawn (Whitfield et al., 1997). A prawn feed that will improve the flavor quality of aquacultured animals is currently under investigation.

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