Distribution of Bromophenols in Species of Marine Polychaetes and Bryozoans from Eastern Australia and the Role of Such Animals in the Flavor of Edible Ocean Fish and Prawns (Shrimp)

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Sixteen species (44 samples) of marine polychaetes and 10 species (14 samples) of bryozoans from eastern Australia were analyzed by GC/MS for the key seafood flavor components 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol, and 2,4,6-tribromophenol. All five bromophenols were found in 91% of polychaetes and 64% of bryozoans. The remaining samples all contained at least three bromophenols. 2,4,6-Tribromophenol was found in all polychaetes and bryozoans and, with few exceptions, was present in the highest concentrations. The total bromophenol content determined on a wet-weight basis varied widely between species: for polychaetes, from 58 ng/g for *Australonuphis teres* to 8.3 million ng/g for *Barantolla lepte*, and for bryozoans, from 36 ng/g for *Cladostephus spongiosus* to 1668 ng/g for *Amathia wilsoni*. Species of polychaetes with the highest concentrations of bromophenols were all collected from muddy environments. The possible effects that dietary polychaetes and bryozoans have on the ocean-, brine-, or iodine-like flavors of certain seafoods are discussed.

Keywords: *Polychaetes; bryozoans; bromophenols; GC/MS analyses; flavor; prawn/fish diet*

INTRODUCTION

The simple bromophenols, 2- and 4-bromophenol (2-BP, 4-BP), 2,4- and 2,6-dibromophenol (2,4-DBP, 2,6-DBP), and 2,4,6-tribromophenol (2,4,6-TBP), have been identified as key flavor components in seafoods (Whitfield et al., 1988; 1997a,b, 1998; Boyle et al., 1992a,b). In water, the most strongly flavored of these compounds, 2,6-DBP, 2-BP, and 2,4,6-TBP, have flavor threshold concentrations (FTC) of 5×10^{-4}, 3×10^{-2}, and 0.6 ng/g, respectively (Whitfield et al., 1988). At these concentrations, the flavors of 2,6-DBP and 2,4,6-TBP are described as iodoform-like and the flavor of 2-BP is described as phenolic/iodine-like. However, at levels below their FTC some bromophenols contribute recognizable marine- or ocean-like flavors to seafoods as well as enhance the intensities of existing seafood flavors (Boyle et al., 1992a; Whitfield et al., 1997a,b, 1998). These three bromophenols, together with 4-BP and 2,4-DBP, have been found in nanogram per gram concentrations in Australian ocean prawns (Whitfield et al., 1988, 1997b), in Australian ocean fish (Whitfield et al., 1998), in North American crustaceans, North American molluscs, and Pacific salmon (Boyle et al., 1992b), and in North Atlantic shrimp (Anthoni et al., 1990). In all of these animals, the bromophenols were believed to be derived from natural dietary sources. Polychaetes, marine worms of the phylum Annelida, are considered to be a major source of bromophenols in Australian ocean prawns (Whitfield et al., 1997b), Australian ocean fish (Whitfield et al., 1998), and North Atlantic shrimp (Anthoni et al., 1990). Bryozoans have also been implicated as a source of bromophenols in some species of Australian ocean fish (Whitfield et al., 1998). The occurrence of such bromophenols in the marine environment has been reviewed (Boyle et al., 1993).

In a survey of the dietary habits of edible Australian ocean fish, 15 families of polychaetes have been identified in the stomach contents of some 40 species of benthic carnivores and diverse omnivores (Anonymous, 1981). The most commonly found family of polychaetes (>61% of all samples) was Nereididae (formally Nereidae). Polychaetes have also been identified as a major dietary component of six species of Australian ocean prawns (Whitfield et al., 1997b). As a result of these findings, 16 species of polychaetes representative of 10 of the 15 families (including Nereididae) found in fish have been analyzed for their bromophenol content. Ten species of bryozoans have also been analyzed for these compounds. The paper will discuss the role of polychaetes and bryozoans as sources of bromophenols and of ocean-, brine-, or iodine-like flavors in edible ocean fish and crustaceans. The possible function of bromophenols in marine animals is discussed.

EXPERIMENTAL PROCEDURES

Materials. Forty-four samples of marine polychaetes were either collected at low tide from the Sydney, NSW, region by members of the project or purchased from commercial suppliers. Dr. J. Patterson (Wynnum, QLD) supplied samples of *Marphysa sanguinea*, *Marphysa macintoshi*, and *Glycera americana*, and Mr. J. Park (Seal Rocks, NSW) supplied samples of *Australonuphis teres*. The weight of individual polychaetes varied greatly across species from 0.01 g for the smallest to 29 g for the largest animal. Collections of polychaetes were routinely sorted into species, and representative samples of each were preserved in 7% formaldehyde. These preserved samples were identified to species by Ms. A. Murray of the Marine Ecology Department of the Australian Museum, Sydney, NSW. Sixteen samples of bryozoans were collected

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from Tasmanian waters by Dr. A. J. Blackman (University of Tasmania, Hobart, TAS) and were identified to species by the staff of the Marine Biology Department of that University. The size of individual bryozoan colonies varied between 20 and 30 g. All retained samples of polychaetes and bryozoans were snap frozen and stored at −20 °C.

Reference samples of the five target bromophenols were purchased from Aldrich Chemical Co. Inc., Milwaukee, WI, and 2,6-dibromophenol-d_3 was obtained from C/D/N Isotopes Inc., Pointe Claire, PQ, 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. Composite samples of individual species of polychaetes or bryozoans were allowed to thaw and then surface dried by gentle patting between cotton tea towels or filter paper. When applicable, the animals were cut into small pieces and a portion of this material (1−200 g depending on the size of the animals) was blended in purified water (1.5 L) for 5 min using a Panasonic Super Blender. The homogenates were acidified to pH 1 with 10 M sulfuric acid and were left to stand at 20 °C for 2 h 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 residual material was measured again to confirm that the homogenates had remained acid during the isolation procedure. The internal standard, 2,6-dibromophenol-d_3 (100 ng in 100 μL of iso-octane), was added to the solvent extracts, which were dried by cooling to −15 °C and decanting the solvent fraction. The extracts were concentrated by the careful removal of the solvent by fractional distillation, and the concentrates in iso-octane (~100 μL) were stored in glass autosampler vials at −15 °C until required for analysis by GC/MS.

Analysis by GC/MS. The bromophenols in the extracts of polychaetes and bryozoans were analyzed by a Hewlett-Packard HP5890 gas chromatograph interfaced to a Hewlett-Packard HP5971A 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 of 0.33 μm film thickness (Hewlett-Packard, Palo Alto, CA) and a precolumn retention gap 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 some extracts. However, it was necessary to replace the retention gap frequently when linearity of the calibration curve no longer applied; this usually occurred suddenly. 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 programmed as follows: the temperature was initially held at 60 °C for 1 min, programmed from 60 to 225 °C at 15 °C/min and 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 the electron ionization mode with an energy of 70 eV and an ion source temperature of 180 °C.

Quantitative analyses 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-BP 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 for these compounds were as follows: 2-BP, 6.60 min; 4-BP, 9.01 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 internal standard (1 μg/mL). Disappearance of the lowest calibration level and loss of peak shape indicated the need to replace the retention gap and occasionally the column. 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 carried out in duplicate. If a sample contained analytes outside the calibration range, a diluted subsample was analyzed after addition of more internal standard. The detection limit for individual bromophenols in the polychaetes and bryozoans was 0.01 ng/g based on a factor of 3 times 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 polychaetes and bryozoans were determined as follows. Samples (10 g) of a polychaete (*Australonuphis teres*) and a bryozoan (*Cladostephus spongiosus*), of low bromophenol content, were homogenized in water (1.5 L). To these homogenates was added an aliquot (1 mL) of a solution containing each of the five bromophenols (1 μg/mL in ethanol). As previously described, the mixtures were extracted by SDE after acidification to pH 1. The extractions were performed in duplicate. The percentage recoveries of the bromophenols from both the polychaetes and bryozoans were comparable, and the average values were as follows: 2-BP, 90% (RSD = 5%); 4-BP, 40% (RSD = 4%); 2,4-DBP, 75% (RSD = 5%); 2,6-DBP, 80% (RSD = 2%); and 2,4,6-TBP, 75% (RSD = 5%). The low recovery of 4-BP was attributable to the greater water solubility of this compound in water compared to the solubilities of the other bromophenols.

RESULTS AND DISCUSSION

Bromophenol Content of Australian Polychaetes. The data obtained from the bromophenol analyses of 44 samples of marine polychaetes, representing 16 species, are recorded in Table 1. Of the 16 species studied, the data for 9 species were obtained from single collections. When more than one collection was analyzed, the samples either had been taken from different geographical locations or were collected from the same site at different times of the year. The selection of a species for multiple collection was determined either by the dominant nature of that species at a particular site or because it was known to be a possible food source for carnivorous and omnivorous species of fish and prawns.

Bromophenols were found above their detection limit of 0.01 ng/g in all 44 samples analyzed. All five bromophenols were found in 40 of the 44 samples studied, four bromophenols in three samples, and three in the remaining sample. In all multisampled species, five bromophenols were found in at least one of the samples. Of the five bromophenols, 2,4-DBP, 2,6-DBP, and 2,4,6-TBP were found in all samples, 2-BP in all but one sample, and 4-BP in all but three samples. In 41 of the 44 samples, 2,4,6-TBP was present in the highest concentrations, whereas in the remaining three samples, 4-BP was the major bromophenol in two samples and 2-BP in one sample. Of interest, the two samples dominated by 4-BP, *Marphysa macintoshi* and *Glycera americana*, both came from the same site at Wynnum. When 2,4,6-TBP was the dominant bromophenol, 2,6-DBP was present in the next highest concentrations in 22 samples and 2,4-DBP in 14 samples, and in the

Table 1. Distribution of Bromophenols in Eastern Australian Polychaetes[a]

species	source	bromophenol content[b] (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total
Capitellidae[c]							
Barantolla lepte	Silver Beach, NSW	59	97	11000	32000	8300000	8343156
Notomastus chrysosetus/torquatus	Calabash Bay, NSW[d]	11	29	970	850	380000	381860
Cirratulidae							
Cirriformia filigera	Silver Beach, NSW	42	70	1500	800	130000	132412
Cirriformia cf tentaculata	Silver Beach, NSW	15	29	320	340	23000	23704
Eunicidae							
Marphysa macintoshi	Calabash Bay, NSW	47	2	99	9	2700	2857
Marphysa macintoshi	Calabash Bay, NSW	3	ND[e]	27	64	7900	7994
Marphysa macintoshi	Calabash Bay, NSW[d]	30	5	140	51	15000	15226
Marphysa macintoshi	Wynnum, QLD	160	1300	1100	19	340	2919
Marphysa sanguinea	Calabash Bay, NSW	25	2	38	41	10000	10106
Marphysa sanguinea	Calabash Bay, NSW[d]	42	14	350	160	61000	61566
Marphysa sanguinea	Careel Bay, NSW	5	4	280	850	78000	79139
Marphysa sanguinea	Moreton Bay, QLD	30	26	5600	43	22000	27699
Marphysa sanguinea	Point Clare, NSW	63	31	500	1500	260000	262094
Glyceridae							
Glycera americana	Wynnum, QLD	320	4200	4600	72	650	9842
Lumbrineridae							
Lumbrineris latreilli	Bayview, NSW	5	23	100	160	42000	42288
Lumbrineris latreilli	Careel Bay, NSW	6	18	1000	1700	250000	252724
Lumbrineris latreilli	Hen & Chicken Bay, NSW	44	55	1200	4900	190000	196199
Lumbrineris latreilli	Silver Beach, NSW	12	18	580	1700	1600000	1602310
Nephtyidae							
Nephtys australiensis	Calabash Bay, NSW	3	36	140	240	79000	79419
Nephtys australiensis	Calabash Bay, NSW[d]	5	8	160	380	220000	220553
Nephtys australiensis	Careel Bay, NSW	20	27	700	3000	340000	343747
Nephtys australiensis	Koolewong, NSW	11	28	460	2300	860000	862799
Nephtys australiensis	Point Clare, NSW	20	19	440	2300	800000	802779
Nephtys australiensis	Rodd Point, NSW	3	4	79	720	83000	83806
Nephtys australiensis	Silver Beach, NSW	16	23	170	1100	110000	111309
Nereididae							
Australonereis ehlersi	Calabash Bay, NSW	2	ND	44	130	28000	28176
Australonereis ehlersi	Careel Bay, NSW	5	10	410	430	8700	9555
Australonereis ehlersi	Hen & Chicken Bay, NSW	32	71	750	2400	95000	98253
Australonereis ehlersi	Koolewong, NSW	18	49	260	65	10000	10392
Ceratonereis aequisetis	Point Clare, NSW	58	ND	2800	3300	940000	946158
Ceratonereis aequisetis	Rodd Point, NSW	12	23	340	420	32000	32795
Ceratonereis limnetica	Koolewong, NSW	3	9	61	24	5800	5897
Onuphidae							
Australonuphis teres (J)[f]	Crowdy Bay, NSW	90	2	58	30	60	240
Australonuphis teres (J)	Crowdy Bay, NSW	13	6	34	61	220	334
Australonuphis teres (J)	Seal Rocks, NSW	8	2	35	77	140	262
Australonuphis teres (A)[g]	Seal Rocks, NSW	260	1	51	32	150	494
Australonuphis teres (J)	Seal Rocks, NSW	9	4	14	7	24	58
Australonuphis teres (J)	Seal Rocks, NSW	3	3	55	34	350	445
Diopatra dentata	Bateau Bay, NSW	27	35	71	48	61	242
Diopatra dentata	Bateau, Bay, NSW	10	99	170	79	690	1048
Orbiniidae							
Scoloplos normalis	Calabash Bay, NSW	10	18	380	100	32000	32508
Scoloplos normalis	Calabash Bay, NSW[d]	7	100	190	130	60000	60427
Scoloplos normalis	Point Clare, NSW	ND	ND	2000	4200	54000	60200
Phyllodocidae							
Phyllodoce cf novaehollandiae	Silver Beach, NSW	32	55	510	770	160000	161367

[a] Values are expressed as wet weight. [b] Average percentage recoveries for individual bromophenols are as follows: 2-BP, 90% (RSD = 5%); 4-BP, 40% (RSD = 4%); 2,4-DBP, 75% (RSD = 5%); 2,6-DBP, 80% (RSD = 2%); 2,4,6-TBP, 75% (RSD = 5%). [c] Family. [d] Collected from eutrophic mud near mangroves. [e] ND, not detected at a detection limit of 0.01 ng/g. [f] (J) = juveniles. [g] (A) = adults.

remaining 5 samples the concentrations of the two dibromophenols were about the same.

Across the 16 species of polychaetes the total bromophenol content (TBC) was found to vary greatly among species and to also show some variation within the same species. The highest TBC was found in the sample of *Barantolla lepte* from Silver Beach (8.3 million ng/g) and the lowest in juvenile samples of *Australonuphis teres* from Seal Rocks (58 ng/g). Variations within species were less dramatic but were significant in some species. The greatest variation within a species was observed for *Lumbrineris latreilli*, for which the highest TBC was in the sample from Silver Beach (1.6 million ng/g) and the lowest from Bayview (42288 ng/g). The lowest variation within a species was observed for *Scoloplos normalis*, for which two samples from Calabash Bay had TBCs of 32508 and 60427 ng/g. Five samples of polychaetes had TBCs >800000 ng/g, and these were *Barantolla lepte*, Silver Beach (8.3 million ng/g), *Lumbrineris latreilli*, Silver Beach (1.6 million ng/g), *Ceratonereis aequisetis*, Point Clare (946000 ng/g), and two samples of *Nephtys australiensis* from Koolewong and Point Clare (863000 and 800000 ng/g). Another nine samples from six different species had TBCs in excess of 100000 ng/g. Furthermore, of the eight species with TBCs >100000 ng/g, five had average body weights between 0.01 and 0.09 g (Table 2). The average body weights of the other three species with high TBCs

Table 2. Dimensions and Habitat of Australian Polychaetes

size	length[a] (mm)	av wt[a] (g)	water depth[b,c] (m)	salinity[b] (%)	environment[b]
Capitellidae					
Barantolla lepte	10−60	0.01	4−9	28−35	fine mud to mud with sand, shell and detritus
Notomastus chrysosetus/torquatus	40−60	0.09	4−9	27−35	mud to muddy sand
Cirratulidae					
Cirriformia filigera	15−50	0.03	0.5−12	38	mud to muddy sand among seagrass (*Zostera* and *Posidonia*)
Cirriformia cf tentaculata	70−100	3.3	0.5−2	38	under stones on black mud
Eunicidae					
Marphysa macintoshi	15−40	0.21	4−12	12−36	fine mud to muddy sand
Marphysa sanguinea, QLD	60−100	2.4	4−20	11−33	fine mud to medium-grained muddy sand
Marphysa sanguinea, NSW	30−60	0.81	4−20	11−33	fine mud to medium-grained muddy sand
Glyceridae					
Glycera americana	60−100	2.1	4−10	32−39	muddy sand among seagrass (*Zostera* and *Posidonia*)
Lumbrineridae					
Lumbrineris latreilli	20−50	0.39	4−12	29−35	fine mud to sandy mud with some shell
Nephtyidae					
Nephtys australiensis	10−40	0.08	4−12	12−36	fine mud to muddy sand and fine sand
Nereididae					
Australonereis ehlersi	20−60	0.40	5−6	5−37	muddy sand to fine and coarse-grained sand flats
Ceratonereis aequisetis	20−60	0.05	0.5−8	34	mud with shell and sublittoral weed holdfasts
Ceratonereis limnetica	60−80	0.40	0.5−8	0−23	fine to coarse sand
Onuphidae					
Australonuphis teres (J)[d]	60−120	2.8	littoral zone	38	clean beach sand
Australonuphis teres (A)[e]	700−1000	29	littoral zone	38	clean beach sand
Diopatra dentata	50−80	1.1	4−10	33−38	mud to sandy mud with shell
Orbiniidae					
Scoloplos normalis	20−40	0.10	6−16	3−34	mud to clay to medium-grained sand
Phyllodocidae					
Phyllodoce cf novaehollandiae	30−60	0.20	0.5−5	34	fine sand, mud among *Posidonia* seagrass

[a] Reported dimensions are from current investigations. [b] Hutchings and Murray (1984) and H. Paxton, Macquarie University, Sydney, NSW, Australia, personal communication, 1998. [c] Samples of all species can be found at low tide in the littoral zone. [d] (J) = juveniles. [e] (A) = adults.

Table 3. Sediment Type at Polychaete Sampling Sites

site	sediment type
ocean environment	
Bateau Bay, NSW	clean sand and shell grit over rock
Crowdy Bay, NSW	clean beach sand
Seal Rocks, NSW	clean beach sand
estuarine environment	
Bayview, NSW	sand to muddy sand, clean with no detritus
Calabash Bay, NSW	mud to sandy mud with detritus and near mangroves, eutrophic mud
Careel Bay, NSW	sandy mud among seagrass (*Posidonia* and *Zostera*)
Hen and Chicken Bay, NSW	muddy sand with shells, near mangroves
Koolewong, NSW	sand to muddy sand, clean with no detritus
Moreton Bay, QLD	muddy sand to sandy mud with detritus
Point Clare, NSW	fine mud near mangroves, eutrophic
Rodd Point, NSW	sand to muddy sand with shells
Silver Beach, NSW	muddy sand to sandy mud with some petrochemical contamination among seagrass (*Posidonia* and *Zostera*)
Wynnum, QLD	muddy sand to sandy mud with detritus

were between 0.2 and 0.8 g. Thus, all of these worms are relatively small and would be easily consumed by fish and crustaceans foraging on the ocean floor. In Table 2 the dimensions of all species of polychaetes studied in the current survey are reported. Some species, such as the adult *Australonuphis teres*, can grow to a very large size (29 g), but their TBC is quite low, <500 ng/g. Of interest, this species is caught commercially and is used for bait by amateur fishermen. Another species, *Marphysa sanguinea*, of much higher bromophenol content, is also caught for the same purpose.

Effect of Habitat on the Bromophenol Content of Polychaetes. Australian polychaetes are found in a diversity of habitats that varies greatly among species and also within species (Hutchings and Murray, 1984). Their livable environments range from eutrophic mud to clean beach sand, with salinities from 0 to 39% and ocean depths from the littoral zone to 20 m (Table 2). In the current survey, samples were collected from a full range of environments that included eutrophic mud, muddy-sand, sandy-mud, beach sand, and shell grit over rock. Analyses of samples from these environments showed that polychaetes from locations with a significant mud content contained far greater concentrations of bromophenols than those animals from beach sand or shell grit (Tables 1 and 3). All 14 samples, representing 10 species, with TBCs >100000 ng/g were collected from mud or muddy-sand, whereas the 8 samples, representing 2 species, with TBCs <1050 ng/g were found in beach sand or shell grit. Furthermore, when a species was collected from more than one site, some variation in bromophenol content was also observed. Thus, samples of *Lumbrineris latreilli* collected at

Table 4. Distribution of Bromophenols in Some Eastern Australian Bryozoans[a]

species	source	bromophenol content[b] (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total
Amathia cornuta	Betsey Island, TAS	1.3	7.8	16	52	180	257
Amathia cornuta	North Bruny Island, TAS	5.9	21	15	56	110	208
Amathia wilsoni	Kingston, TAS	1.0	4.1	6.1	28	70	109
Amathia wilsoni	North Bruny Island, TAS	6.0	22	330	210	1100	1668
Amathia sp.	Bateau Bay, NSW	5.0	7.9	13	28	100	154
Bugula dentata	Kettering Jetty, TAS	ND[b]	ND	12	130	180	320
Bugularia dissimilis	South Bruny Island, TAS	ND	54	330	130	610	1124
Cellaria pilosa	North Bruny Island, TAS	4.4	5.1	34	40	83	167
Cellaria pilosa	Betsey Island, TAS	2.2	3.9	6.8	54	40	107
Cladostephus spongiosus	South Bruny Island, TAS	0.2	ND	17	1.4	17	36
Orthoscuticella ventricosa	Betsey Island, TAS	1.5	ND	24	47	540	613
Orthoscuticella ventricosa	South Bruny Island, TAS	tr[c]	ND	94	37	82	213
Pleurotoichus sp.	Batemans Bay, NSW	0.7	5.0	19	44	660	729
Pleuotoichus sp.	Bateau Bay, NSW	1.0	10	22	74	730	837

[a] Values are expressed as wet weight. [b] The average percentage recoveries for individual bromophenols are as follows: 2-BP, 90% (RSD = 5%); 4-BP, 40% (RSD = 4%); 2,4-DBP, 75% (RSD = 5%); 2,6-DBP, 80% (RSD = 2%); 2,4,6-TBP (RSD = 5%). [c] ND, not detected at a detection limit of 0.01 ng/g. [d] tr, trace = 0.01−0.05 ng/g.

Bayview, a location with sand, had a lower TBC than samples taken from the muddy sites at Careel Bay and Hen and Chicken Bay (Tables 1 and 3). Similar variations between bromophenol content and site of collection were shown by samples of *Marphysa sanguinea*, *Nephtys australiensis*, *Australonereis ehlersi*, and *Scoloplos normalis*. For these species, samples collected from eutrophic mud beside mangroves in Calabash Bay and Point Clare generally had higher TBCs than samples from areas consisting of mud or sandy mud (Tables 1 and 3). Also of interest, five of the six species of polychaetes collected from Silver Beach had TBCs >100000 ng/g, including *Barantolla lepte*, the species with the highest TBC (8.3 million ng/g). The mud of Silver Beach is contaminated with petrochemical hydrocarbons as a result of the close proximity of this site to an oil terminal and to an international airport. Accordingly, there appears to be a correlation between the concentration of bromophenols in some polychaetes and the total organic content of their habitat.

Bromophenol Content of Australian Bryozoans. The data obtained from the bromophenol analyses of 14 samples of bryozoans, representing 10 species, are recorded in Table 4. Bromophenols were found above their detection limit of 0.01 ng/g in all 14 samples. All five bromophenols were found in 12 of the samples analyzed and four bromophenols in the remaining 2 samples. In 11 of the 14 samples, 2,4,6-TBP was present in the highest concentrations, whereas in the remaining three samples, 2,6-DBP and 2,4-DBP were the major bromophenol in one sample each and in the remaining sample the concentrations of 2,4-DBP and 2,4,6-TBP were the same. When 2,4,6-TBP was the dominant bromophenol, 2,6-DBP was present in the next highest concentrations in nine samples and 2,4-DBP in the other two samples.

Across the 10 species of bryozoans studied, the TBC was found to vary both among species and within the same species (Table 4). However, these variations were not as great as those previously observed for Australian polychaetes (Table 1). Of interest, all samples of bryozoans were collected from the same type of shallow sublittoral habitat, from rocky outcrops above beach sand or shell grit. The highest TBC found in the 14 samples of bryozoans was in the sample of *Amathia wilsoni* from North Bruny Island (1668 ng/g) and the lowest in the sample of *Cladostephus spongiosus* from South Bruny Island (36 ng/g). The greatest variation within species was also observed for *Amathia wilsoni* (1668 and 109 ng/g), whereas the lowest variation was for *Amathia cornuta* (257 and 208 ng/g). Of the 14 samples of bryozoans studied, 2 samples had TBCs >1000 ng/g and another 3 samples had TBCs >500 ng/g (Table 4). These concentrations are thus comparable with the highest levels of bromophenols found in some species of Australian marine algae (Whitfield et al., 1999) but are considerably less than those found in the majority of Australian polychaetes (Table 1).

Function of Bromophenols in Marine Polychaetes and Bryozoans. Biologically induced halogenation and, in particular, bromination occur as secondary metabolic processes in a wide range of marine animals and plants (Neidleman and Geigert, 1986; Butler and Walker, 1993). These reactions are catalyzed by haloperoxidases, enzymes found in all classes of marine organisms including hemichordate and polychaete worms (Ahern et al., 1980; Chen et al., 1991; Yoon et al., 1994). Such enzymes, in the presence of hydrogen peroxide, oxidize bromide to hypobromous acid, molecular bromine and tribromide ion (Neidleman and Geigert, 1986; Yang et al., 1995). These active sources of bromine can react with suitable natural organic substrates to produce bromophenols (Chen et al., 1991; Yang et al., 1995).

Many bromophenols, including 2,6-DBP and 2,4,6-TBP, are known to possess fungicidal, bacteriocidal, ascaricidal, and molluscicidal activities (Zsolnai, 1960; Jeney and Zsolnai, 1967; Hashimoto, 1978). Consequently, it has been suggested that these compounds are produced by marine worms as defensive chemicals against microorganisms and some predatory marine animals (Sheikh and Djerassi, 1975; Higa et al., 1980; Woodin et al., 1987). Such protection could be achieved by constantly discharging the bromophenols either into the mucus covering their bodies (Higa et al., 1980) or into the mucus lining the walls of their sedimentary burrows (King, 1986; Woodin et al., 1987; Steward et al., 1992). In support of these views, studies have shown that 2,4-DBP, a major metabolite of the hemichordate worm *Saccoglossus kowolewskii*, inhibits the aerobic microbial degradation of the mucus lining of the burrow-walls of these animals but has little effect on the anaerobic bacteria present in surrounding marine sediments (King, 1986, 1988). Subsequent studies have also shown that burrow microbial communities are markedly different from those of surrounding surface and sub-

surface sediments (Steward et al., 1996). These findings suggest that polychaetes use bromophenols as a means of manipulating the microbial environment in which they live (King, 1988).

Bromophenols have also been shown to exhibit allelochemical properties in marine sediments; for example, larvae of the polychaete *Nereis vexillosa* will not colonize sediments occupied by the bromophenol producing polychaete *Thelepus crispus* (Woodin et al., 1993). This negative response of larvae to sediments contaminated with these compounds is considered a novel and very important mechanism in which sediment-dwelling organisms can control the composition of their community. It has also been suggested that some polychaetes use bromophenols to discourage epifaunal predators (Yoon et al., 1994). The polychaete *Notomastus lobatus* is a head-down feeder leaving its tail exposed above the sediment. The highest concentrations of bromophenols have been found in the tail segment, which has caused some authors to speculate that these noxious compounds reduce predatory pursuit (Yoon et al., 1994).

The rationale for bromophenol production in marine worms is uncertain; however, it is possible that these compounds are used by the worm to manipulate both the microbiology and chemistry of its immediate environment so as to promote its growth, reproduction, or survival. Similar claims have been made for the role of bromophenols in marine algae (Whitfield et al., 1999).

No attempt, to date, has been made to identify the function of bromophenols in bryozoans.

Role of Polychaetes and Bryozoans in the Flavor of Edible Ocean Fish and Prawns. Recent studies have shown that many species of Australian ocean prawns and ocean fish contain relatively high concentrations (11−312 ng/g) of total bromophenols in the animal's edible flesh (Whitfield et al., 1997b, 1998). These studies also showed that these compounds were totally derived from the diets of these animals. Polychaetes are a major dietary component of many species of edible Australian prawns and fish that forage for food in the ocean's sediment. Importantly, these prawns and fish also have the highest concentrations of bromophenols, and their flesh have the strongest ocean-, brine-, or iodine-like flavors (Whitfield et al., 1997b, 1998). The current study has shown that many species of Australian polychaetes contain very high concentrations of bromophenols (101000−8.3 million ng/g). Accordingly, polychaetes should be regarded as a major source of bromophenols in those prawns and fish that feed on sedimentary worms. In addition, these worms must also be regarded as a principal source of ocean-, brine-, and iodine-like flavors in such prawns and fish. Of interest, in some Asian countries polychaetes are used as a finishing feed for prawns cultivated commercially in ponds (Dr. K. Williams, Division of Marine Research, CSIRO, Cleveland, QLD, Australia, personal communication, 1997). The purpose of this feeding procedure was not revealed; however, as manufactured prawn feeds from this region normally contain only low levels of bromophenols, between 1.4 and 40 ng/g (Whitfield et al., 1997b), the use of polychaetes could be to improve the natural ocean flavor of the cultivated animals.

Bryozoans, by comparison with polychaetes, contain relatively low concentrations of bromophenols (36−1668 ng/g). However, bryozoans are consumed by some species of fish, principally diverse omnivores, and are accordingly considered the principal source of bromophenols in these species (Whitfield et al., 1998). Marine algae and sponges that also contain these compounds (Whitfield et al., 1996, 1999) are the other important sources of bromophenols in diverse omnivores.

Conclusion. Polychaetes are a major dietary source of bromophenols in Australian ocean prawns and bottom-feeding fish. Accordingly, the intensity of flavors caused by the presence of bromophenols in such seafoods will depend on the quantity, and species, of polychaetes, consumed by individual prawns and fish. Thus, outbreaks of iodoform-like off-flavors, such as those observed in some species of prawns (Whitfield et al., 1988), probably result from the animal's feeding largely on polychaetes of high bromophenol content. On the other hand, desirable ocean-like flavors in seafoods are also known to vary greatly depending on where the animals were caught and at what time of year. A likely explanation for this variation would be the absence of suitable polychaetes as food in a particular location or at certain times of the year. An Australian survey has shown that the role of polychaetes as a major dietary component of fish does vary significantly over a two-year sampling period (Anonymous, 1981). Other marine organisms that contain large concentrations of bromophenols include marine worms of the phyla Hemichordata (Woodin et al., 1987) and Phoronida (Sheikh and Djerasi, 1975), and in some locations these organisms could be dominant contributors of bromophenols in the diets of local fish and crustaceans. Of interest, to the palates of some Australians, certain fish from the families Sparidae and Sciaenidae and crustaceans such as *Callinectes sapidus* and *Homarus americanus*, caught along the eastern coast of the United States, possess strong iodine- or iodoform-like flavors. The identification of the source of bromophenols in such animals could establish whether marine organisms, other than polychaetes, have a major dietary influence on the bromophenol content of seafoods.

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