Distribution of Bromophenols in Species of Ocean Fish from Eastern Australia

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Thirty-two species of ocean fish from eastern Australia 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. With the exception of one species, bromophenols were found in all species of benthic carnivores, diverse omnivores, and restricted omnivores but were not detected in five species of pelagic carnivores. The total bromophenol content in whole fish varied greatly between species from not detected to 438 ng/g. Separate analysis of the gut and flesh showed that the gut contained the bulk of the bromophenols, supporting opinions that these compounds were derived from components of the fishes' natural diets. The paper discusses likely dietary sources of bromophenols in Australian fish, arguing that polychaetes are major sources of bromophenols in benthic carnivores and diverse omnivores, and marine algae are sources of bromophenols in restricted omnivores. The importance of bromophenols in the flavor of Australian ocean fish is also discussed.

Keywords: *Fish; bromophenols; GC/MS analysis; dietary origins; polychaetes; marine algae; flavor*

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 natural flavor components of seafood (Whitfield et al., 1988, 1997a,b; 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 descibed as phenolic/iodine-like. To reproduce such flavors in prawn meat, these concentrations need to be increased ~100-fold (Whitfield et al., 1996). At such concentrations bromophenols can impart an undesirable iodoform- or iodine-like flavor to the flesh of prawns, shrimp (Whitfield et al., 1988; Anthoni et al., 1990), and fish (Whitfield et al., 1994). However, at levels below their FTC some bromophenols are reported to contribute recognizable marine or ocean flavors to seafoods as well as enhancing the intensity of existing seafood flavors (Boyle et al., 1992a,b; Whitfield et al., 1997b). Thus, in bland marinated whitefish the flavor of 2,6-DBP at 0.1 ng/g is crab- or shrimp-like, that of 2,4,6-TBP at 10 ng/g is sea salt- or sea fish-like, and that of 2-BP at 10 ng/g is rich, full-flavored, and sea-like (Boyle et al., 1992b).

The desirable effect of bromophenols on the flavor of fish has been extensively investigated in North America by Boyle et al. (1992a). These authors studied the bromophenol content of four species of Pacific salmon (*Oncorhynchus* spp.) obtained from both salt- and freshwater environments; they showed that, wheras the flesh of saltwater fish contained between 6.1 and 34.8 ng/g of total bromophenols, that of freshwater fish contained virtually none. Importantly, freshwater fish lacked the characteristic brine- or sea-like flavor of saltwater salmon (Boyle et al., 1992a). Consequently, it was suggested that the bromophenols had been purged from the saltwater salmon after they had entered the brackish water and had ceased feeding. The same authors similarly showed that the flesh of cultivated Atlantic salmon (*Salmo salar*) contained <2 ng/g of bromophenols. They concluded that fish grown in marine aquaculture systems did not contain these compounds as their manufactured diet contained little or no bromophenols (Boyle et al., 1992a). However, the natural sources of the bromophenols in North American saltwater fish were not identified.

Recently, Whitfield et al. (1997b) have shown that the total bromophenol content of whole wild-harvested Australian prawns varied between 9.5 and 1114 ng/g, whereas in commercially pond raised animals this value was <1 ng/g. They also examined the natural diets of six species of wild prawns and one species of cultivated prawn and concluded that marine polychaetes were a major source of bromophenols in wild-harvested prawns. However, the only source of these compounds in cultivated prawns was from the addition of 5−7% of shrimp shell meal to the manufactured feed (Whitfield et al., 1997a,b).

The flavor of Australian saltwater fish, particularly those species caught by amateur fishermen, is highly prized for its characteristic ocean-like flavors. The current study was therefore undertaken to measure the concentrations of bromophenols across a wide range of species and to relate these concentrations to the perceived flavor of each species. In addition, as the feeding habits of most of the selected species are well documented, this information has been used to link the presence of bromophenols in individual species to particular components of the diet of each species. However, to simplify the presentation of these data, individual

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species have been categorized as pelagic carnivores, benthic carnivores, diverse omnivores, and restricted omnivores. As no true piscivorous carnivore, such as the Spanish mackerel (*Scomberomorus commerson*) or barracuda (*Thyrsites atun*), were included in this study, the term pelagic carnivore has been used for demersal and midstream fish that feed on a variety of swimming animals including fish, crustaceans, and planktonic invertebrates. By comparison, fish categorized as benthic carnivores typically forage for their food in the silt, sand, and debris of the ocean bottom. Similarly, the category called restricted omnivore was included to differentiate species that are predominantly herbivorous from those omnivores that are totally diverse in their feeding habits.

EXPERIMENTAL PROCEDURES

Materials. Samples of ocean fish were supplied by the state department of New South Wales (NSW) Fisheries and were caught off the coast of NSW by the research vessel Kapala during August and September 1994 and 1995. After the catch was sorted, the fish were stored in crushed ice until delivery to the laboratory, about 12 h after they were caught. They were then snap frozen and stored at −20 °C until required for analysis. The individual species were identified by Mr. K. Graham (NSW Fisheries Research Institute).

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 analytical reagent grade (>98% pure). The solvents were further purified by distillation through a packed fractionating column.

Isolation of Bromophenols. Samples of frozen raw fish were allowed to thaw, the carcasses were immediately gutted, and the flesh was separated from the heads, tails, and backbone. Representative samples of the guts (stomach contents and alimentary canals) and flesh (100−760 g depending on availability) from between two and six carcasses were separately homogenized in purified water (500 mL) for 5 min in a Panasonic Super Blender. Additional water (1 L) was added, and the homogenates were acidified to pH 1 with 10 M sulfuric acid. They 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 of isooctane), 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 isooctane (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 fish gut and flesh extracts 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 (0.33 μm film thickness) and a 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 of the extracts. The retention gap was replaced when the calibration curve was no longer linear; this usually occurred suddenly because of excessive contamination from particular samples. 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, 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, 174; for 2,4-DBP and 2,6-DBP at *m*/*z* 250, 252; for 2,4,6-TBP at 330, 332; and for the internal standard 2,6-dibromophenol-d_3 at *m*/*z* 255, 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 isooctane) with a constant concentration of the 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 performed in duplicate at the beginning and end of each day's run. 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 fish gut and flesh 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 fish flesh were determined as follows. Flesh (100 g) from samples of the pelagic carnivore *Zeus faber* (ZF 94) 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 the same as those previously reported for the recovery of these compounds from prawn meat: 2-BP, 94% (RSD = 6%); 4-BP, 41% (RSD = 1%); 2,4-DBP, 74% (RSD = 0%); 2,6-DBP, 81% (RSD = 0%); and 2,4,6-TBP, 74% (RSD = 4%) (Whitfield et al., 1997b). The low recovery of 4-BP was attributed to the greater solubility of this compound in water compared to the other bromophenols.

RESULTS AND DISCUSSION

Bromophenol Content of Australian Ocean Fish. The data obtained from the bromophenol analyses of 50 samples of ocean fish, representing 32 species, are recorded in Table 1. Species have been grouped according to their dietary habits: pelagic carnivores, benthic carnivores, diverse omnivores, and restricted omnivores. Of the 32 species studied, the data for 14 of these species

Table 1. Distribution of Bromophenols in the Gut and Flesh of Species of Australian Ocean Fish[a]

species (code no.)	sample	wt ratio, gut/flesh	bromophenol content[b] (ng/g) 2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total	whole fish[c]	bromophenol ratio, gut/flesh
pelagic carnivores										
Platycephalus caeruleopunctatus (PC94)	gut	0.37	ND[d]	ND	2.3	0.2	3.5	6.0	3	3
	flesh		tr[e]	ND	1.2	0.1	0.6	1.9		
Platycephalus caeruleopunctatus (PC95)	gut	0.12	ND	ND	1.4	0.5	4.4	6.3	1	7
	flesh		ND	ND	0.3	0.1	0.5	0.9		
Centroberyx affinis (CA94)	gut	0.07	ND	ND	ND	ND	6.1	6.1	0.9	12
	flesh		tr	tr	ND	tr	0.5	0.5		
Centroberyx affinis (CA95)	gut	0.13	0.2	ND	3.3	0.8	11	15	3	10
	flesh		0.1	ND	0.4	0.1	0.9	1.5		
Platycephalus marmoratus (PM94)	gut	0.11	ND	ND	ND	ND	ND	ND	ND	
	flesh		ND	ND	ND	ND	ND			
Platycephalus marmoratus (PM95)	gut	0.20	ND	1.1	0.3	10	ND	11	3	10
	flesh		ND	ND	0.5	ND	0.6	1.1		
Neoplatycephalus richardsoni (PR94)	gut	0.28	ND	ND	ND	ND	ND	ND	ND	
	flesh		ND	ND	ND	ND	ND	ND		
Neoplatycephalus richardsoni (PR95)	gut	0.29	ND	tr	0.6	ND	1.8	2.4	2	1
	flesh		ND	1.6	0.1	ND	0.2	1.9		
Helicolenus percoides (HP95)	gut	0.15	0.2	0.2	0.5	0.8	2.6	4.3	0.8	14
	flesh		tr	ND	tr	tr	0.3	0.3		
Zeus faber (ZF94)	gut	0.47	ND	ND	ND	ND	ND	ND	ND	
	flesh		ND	ND	ND	ND	ND	ND		
Zeus faber (ZF95)	gut	0.41	ND	ND	0.3	0.2	1.7	2.2	0.8	11
	flesh		ND	ND	ND	ND	0.2	0.2		
Platycephalus arenarius (PA95)	gut	0.15	tr	ND	ND	3.3	ND	3.3	0.7	17
	flesh		0.1	ND	tr	0.1	ND	0.2		
Seriolella punctata (SP95)	gut	0.33	ND	0.2	0.3	0.1	0.5	1.1	0.4	6
	flesh		ND	ND	0.1	ND	0.1	0.2		
Pseudorhombus arsius (PA94)	gut	0.11	ND	ND	ND	ND	ND	ND	ND	
	flesh		ND	ND	ND	ND	ND	ND		
Pseudorhombus arsius (PA95)	gut	0.11	ND	ND	ND	0.3	ND	0.3	0.3	1
	flesh		ND	ND	0.1	tr	0.2	0.3		
Genypterus blacodes (GB95)	gut	0.09	ND	ND	ND	ND	ND	ND	ND	
	flesh		ND	ND	ND	ND	ND	ND		
benthic carnivores										
Sillago flindersi (SF94)	gut	0.20	0.6	2300	17	4.0	54	2376	438	48
	flesh		tr	46	0.9	0.3	2.4	50		
Sillago flindersi (SF95)	gut	0.20	0.2	46	45	1.2	48	140	27	20
	flesh		tr	1.1	1.4	0.3	4.1	6.9		
Nemadactylus macropterus (NM95)	gut	0.19	1.0	240	14	2.6	22	280	52	33
	flesh		0.1	7.4	0.4	0.1	0.5	8.5		
Nemadactylus douglasii (ND94)	gut	0.11	2.6	100	80	5.8	170	358	39	97
	flesh		tr	0.1	0.1	0.1	3.4	3.7		
Nemadactylus douglasii (ND95)	gut	0.17	27	310	320	3.9	230	891	17	15
	flesh		5.2	17	23	0.6	12	58		
Paristiopterus labiosus (PL94)	gut	0.20	0.7	2.2	22	0.4	21	46	9	27
	flesh		0.1	ND	0.2	0.1	1.3	1.7		
Paristiopterus labiosus (PL95)	gut	0.19	ND	10	78	0.7	100	189	36	28
	flesh		ND	ND	3.4	0.2	3.1	6.7		
Branchiostegus wardi (BW94)	gut	0.12	5.2	22	78	5.7	100	211	24	176
	flesh		0.1	0.2	0.4	0.1	0.4	1.2		
Chelidonichthys kumu (CK94)	gut	0.12	ND	ND	2.6	ND	2.8	5.4	0.9	14
	flesh		ND	ND	ND	ND	0.4	0.4		
Chelidonichthys kumu (CK95)	gut	0.20	ND	ND	18	0.8	12	31	8	9
	flesh		tr	ND	1.5	ND	1.8	3.3		
Pseudorhombus jenynsii (PJ94)	gut	0.15	ND	ND	ND	0.4	37	37	5	
	flesh		ND	ND	ND	ND	ND	ND		
Pseudorhombus jenynsii (PJ95)	gut	0.20	ND	ND	0.2	0.2	0.5	0.9	0.6	2
	flesh		tr	ND	0.1	0.1	0.3	0.5		
Nelusetta ayraudi (NA94)	gut	0.12	tr	ND	0.1	0.1	0.5	0.7	0.2	7
	flesh		tr	ND	tr	tr	0.1	0.1		
Nelusetta ayraudi (NA95)	gut	0.29	1.1	0.5	1.2	0.6	8.5	12	5	4
	flesh		0.3	ND	0.5	0.1	1.8	2.7		
Pseudorhombus tenuirastrum (PT95)	gut	0.09	ND	ND	ND	1.2	3.2	4.4	2	3
	flesh		0.1	ND	0.6	0.1	0.8	1.6		
Upeneichthys lineatus (UL94)	gut	0.20	ND	ND	3.0	ND	ND	3	0.5	
	flesh		ND	ND	ND	ND	ND	ND		
Upeneichthys lineatus (UL95)	gut	0.20	ND	1.9	4.3	0.5	6.8	14	3	70
	flesh		ND	ND	0.2	ND	ND	0.2		
Pterygotrigla polyommata (PP94)	gut	0.17	ND	ND	0.1	ND	0.4	0.5	0.1	
	flesh		tr	ND	ND	ND	ND	tr		
Pterygotrigla polyommata (PP95)	gut	0.19	ND	ND	0.5	ND	4.3	4.8	1	16
	flesh		ND	ND	ND	ND	0.3	0.3		
Branchiostegus serratus (BS95)	gut	0.07	ND	ND	2.8	ND	3.1	5.9	0.6	30
	flesh		tr	ND	0.2	ND	ND	0.2		

Table 1. (Continued)

species (code no.)	sample	wt ratio, gut/flesh	bromophenol content[b] (ng/g) 2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total	whole fish[c]	bromophenol ratio, gut/flesh
Pagrus auratus (PA94)	gut	0.14	ND	ND	0.3	ND	ND	0.3	0.1	3
	flesh		tr	ND	0.1	tr	ND	0.1		
Allotaius spariformes (AS94)	gut	0.40	ND	ND	ND	ND	ND	ND	ND	
	flesh		ND	ND	ND	ND	ND	ND		
diverse omnivores										
Rhabdosargus sarba (RS94)	gut	0.12	ND	ND	150	ND	tr	150	18	71
	flesh		ND	ND	2.0	tr	0.1	2.1		
Pseudocaranx dentex (PD94)	gut	0.27	0.6	0.5	5.8	18	7.2	32	7	46
	flesh		0.1	ND	ND	0.6	ND	0.7		
Pseudocaranx dentex (PD95)	gut	0.20	0.2	56	7.3	1.2	26	91	18	30
	flesh		tr	1.2	0.4	0.1	1.3	3.0		
Meuschenia freycineti (MF94)	gut	0.38	0.7	2.2	2.4	1.3	17	24	10	5
	flesh		0.4	ND	0.3	0.3	4.3	5.3		
Meuschenia freycineti (MF95)	gut	0.33	ND	ND	0.6	1.0	1.8	3.4	3	1.4
	flesh		0.2	ND	0.2	0.3	1.7	2.4		
Acanthopagrus australis (AA94)	gut	0.11	1.2	ND	2.2	18	55	76	9	58
	flesh		ND	ND	0.1	0.2	1.0	1.3		
Meuschenia trachylepis (MT94)	gut	0.24	0.2	0.7	1.5	1.0	5.7	9.1	3	6
	flesh		tr	ND	ND	ND	1.4	1.4		
Parika scaber (PS94)	gut	0.20	0.1	ND	0.1	0.6	1.1	1.9	0.3	
	flesh		tr	ND	ND	ND	tr	tr		
Parika scaber (PS95)	gut	0.20	ND	ND	0.2	0.4	3.3	3.9	0.7	
	flesh		ND	ND	ND	ND	ND	ND		
restricted omnivores										
Girella tricuspidata (GT94)	gut		0.7	ND	19	5.3	32	57	11	48
	flesh		ND	ND	0.2	0.2	0.8	1.2		
Girella tricuspidata (GT95)	gut	0.20	0.7	ND	26	5	45	78	15	34
	flesh		0.1	ND	0.5	0.3	1.4	2.3		
Kyphosus sydneyanus (KS95)	gut	0.64	0.2	0.8	2.5	2.2	7.0	13	5	22
	flesh		tr	0.1	0.2	0.2	0.1	0.6		

[a] Values are expressed as wet weight. [b] The average percentage recoveries for individual bromophenols are as follows: 2-BP, 94% (RSD = 6%); 4-BP, 41% (RSD = 1%); 2,4-DBP, 74% (RSD = 0); 2,6-DBP, 81% (RSD = 0); and 2,4,6-TBP, 74% (RSD = 4%). [c] Calculated from primary data and from the weight of individual batches of gut, flesh, and total fish materials extracted. [d] ND, not detected at a detection limit of 0.01 ng/g. [e] tr, trace = 0.01−0.04 ng/g.

were obtained from single collections made in either 1994 or 1995, whereas the data for the remaining 18 species were obtained from two separate collections over the same two years. Bromophenols were found at a detection limit of 0.01 ng/g in both the gut and flesh of 41 of the 50 samples analyzed, and in 39 of these samples the total bromophenol content (TBC) was greater in the gut than in the flesh. In the other two samples the TBC values in the gut and flesh were the same. For these samples, the ratio of the TBC in the gut to that in the flesh varied between 1:1 and 176:1; in 25 samples this ratio was greater than 10:1. Another three samples had bromophenols only in the gut. These results strongly support the previously expressed opinion that in marine animals such as fish and prawns, bromophenols are derived from the animal's natural diet (Whitfield et al., 1995, 1997b).

Bromophenols were found in 11 of the 16 samples of pelagic carnivores, in 21 of the 22 samples of benthic carnivores, in all 9 samples of diverse omnivores, and in the 3 samples of restricted omnivores. For those pelagic carnivores that contained bromophenols, the highest TBC for the whole fish was 3 ng/g, and this concentration was found in only 3 of the 16 samples. By comparison, this concentration was exceeded in 11 of the 22 samples of benthic carnivores (highest value = 438 ng/g), in 5 of the 9 samples of diverse omnivores (highest value = 18 ng/g), and in all 3 samples of restricted omnivores (highest value = 15 ng/g). These results suggest that, with regard to bromophenols, the dietary intake of pelagic carnivores is less than that of benthic carnivores, diverse omnivores, and restricted omnivores. A similar pattern, though less dramatic, was observed for the TBC of the flesh of fish from these four dietary categories. Thus, the highest TBC for flesh in pelagic carnivores was 1.9 ng/g and was found in 2 samples. This value was exceeded in 8 samples of benthic carnivores, 4 samples of diverse omnivores, and 1 sample of restricted omnivore. Of interest, Boyle et al. (1992a) found that TBCs in the flesh of sexually immature saltwater Pacific salmon (*Oncorhynchus* spp.) varied between 6.1 and 34.8 ng/g. In the current study a concentration of 6.1 ng/g was exceeded only in 5 samples of benthic carnivores; these were *Nemadactylus macropterus* (NM95), *Nemadactylus douglasii* (ND95), *Paristiopterus labiosus* (PL95), and the 2 samples of *Sillago flindersi*. However, in 2 of these samples, *Sillago flindersi* (SF94) and *Nemadactylus douglasii* (ND95), the TBC in the flesh equalled or exceeded 50 ng/g.

All five bromophenols were found in 14 of the 50 samples studied, four bromophenols in 18 samples, and between one and three bromophenols in the remaining 12 samples that contained these compounds. The bromophenols most frequently found were 2,4,6-TBP and 2,4-DBP (41 samples each) and 2,6-DBP (38 samples). The two monobromophenols were found in 30 samples (2-BP) and 20 samples (4-BP). 2,4,6-Tribromophenol was present in highest concentrations in 33 samples, followed by 2,4- and 2,6-DBP in 4 samples each and 4-BP in 3 samples. However, the concentration of 4-BP (2300 ng/g) in the gut sample of *S. flindersi* (SF94) was the highest found in any of the samples analyzed. Of interest, where 2,4,6-TBP was the major bromophenol, 2,4-DBP was present in the next highest concentrations on 20 occasions and 2,6-DBP on

7 occasions. Similar relationships between the concentrations of 4-BP and 2,4-DBP, and between 2,6-DBP and 2,4,6-TBP, have been observed in wild-harvested prawns (Whitfield et al., 1997b). Such relationships suggest that these pairs of compounds could be derived from common dietary sources. By comparison, Boyle and et al. (1992a) found no more than two bromophenols in the flesh of each of the samples of Pacific salmon studied. In all of these samples 2,4,6-TBP was present in the highest concentration. Accordingly, it would appear from the current survey that the diets of Australian ocean fish contain a wider variety of bromophenols than those of Pacific salmon.

Role of Bromophenols in the Natural Flavor of Ocean Fish. On the eastern coast of Australia it is generally recognized by consumers that different species of ocean fish have dissimilar flavors. Anecdotal evidence provided by connoisseurs of seafoods, and amateur fishermen, indicate that some species have sweet but bland flavored flesh, whereas others are described as having sea salt-, iodine-, or ocean-like flavors. However, there is little documented sensory data to support these claims. Similar descriptors have been used by Boyle et al. (1992b) to describe the flavors produced in whitefish flesh by the addition of bromophenols. Accordingly, it is likely that some of the flavors observed in Australian ocean fish are attributable to the presence of such compounds.

Of the five bromophenols found in Australian ocean fish, those with the lowest flavor threshold concentrations in prawn meat are 2,6-DBP (0.06 ng/g), 2-BP (2 ng/g) (Whitfield et al., 1988), and 2,4,6-TBP (50 ng/g) (Whitfield et al., 1996). At such concentrations these compounds can impart iodoform- and phenolic-like flavors to seafoods (Whitfield et al., 1988). However, sensory studies by Boyle et al. (1992b) found that at subthreshold concentrations and in combination, 2-BP (0.5 ng/g), 2,6-DBP (0.1 ng/g), and 2,4,6-TBP (0.5 ng/g) imparted to marinated whitefish, crab-, iodine-, and sea fish-like flavors. Accordingly, characteristic "marine-like" and "ocean-like" flavors could be produced in seafoods by the presence of these bromophenols in quantities equal to or greater than these "flavor impact" concentrations. In our recent study of the effect of bromophenols on the flavor of wild-harvested and cultivated prawns, it was found that at least two of the three nominated compounds (2-BP, 2,6-DBP, and 2,4,6-TBP) needed to be present in the prawn meat to produce natural ocean- or prawn-like flavors (Whitfield et al., 1997b).

Of the 50 samples of fish flesh analyzed for bromophenols, only 1 sample, the benthic carnivore *Nemadactylus douglasii*, had concentrations of 2-BP, 2,6-DBP, and 2,4,6-TBP that were in excess of the flavor impact values of each of these compounds. However, in another 19 samples, 2,6-DBP and 2,4,6-TBP were present at their flavor impact concentrations, whereas 2-BP was also present in 14 of these samples but at significantly lower concentrations (Tables 1). On the basis of previous experience with the flavor of wild-harvested prawns (Whitfield et al., 1997b), it could be expected that the flesh of these 20 fish samples would have marine- or ocean-like flavors. These 20 samples represent 12 species of fish including two pelagic carnivores (*Platycephalus caeruleopunctatus* and *Centroberyx affinis*), six benthic carnivores (*Sillago flindersi*, *Nemadactylus macropterus*, *Nemadactylus douglasii*, *Paristiopterus labiosus*, *Nelusetta ayraudi*, and *Pseudorhombus tenuirastrum*), three diverse omnivores (*Pseudocaranax dentex*, *Meuschenia freycineti*, and *Acanthopagrus australis*), and one restricted omnivore (*Girella tricuspidata*). These species are recognized by connoisseurs of seafoods as the most likely to possess sea-salt-, iodine-, or ocean-like flavors. By comparison, the pelagic carnivores *Zeus faber* and *Genypterus blacodes* rarely have such flavors and are best known for the sweetness but blandness of their flesh. However, to confirm the proposed relationship between the perceived flavor of Australian ocean fish and their bromophenol content, additional analyses will need to be undertaken, including sensory and bromophenol analyses of comparable samples of flesh.

Origin of Bromophenols in the Natural Diets of Ocean Fish. Of the 11 species of pelagic carnivores studied, all feed in the water column, and, with the exception of *Seriolella punctata*, the principal components of their diets are fish and swimming crustaceans (Table 2). *Seriolella punctata* feeds predominantly on planktonic invertebrates and jellyfish. Significantly, none of the major dietary components of the pelagic carnivores are known producers of bromophenols, although some crustaceans will contain small quantities of these compounds from their own dietary intake (Whitfield et al., 1997b). Accordingly, the low average levels of total bromophenols, between 0.2 and 2 ng/g (Table 2), found in some species would appear to be principally derived from secondary sources of these compounds. However, four species (*Platycephalus caeruleopunctatus*, *Centroberyx affinis*, *Platycephalus marmoratus*, and *Platycephalus arenarius*) also occasionally feed on polychaetes, benthic animals that are known to biosynthesize bromophenols (Chen et al., 1991). The higher levels of total bromophenols found in three of these species could be due to the polychaetes in their diets (Table 2).

By comparison with the pelagic carnivores, benthic carnivores are true bottom feeders and forage for their food in the mud and silt of the ocean bottoms. Thus, their diets are dominated by such benthic animals as polychaetes, crustaceans, molluscs, gastropods, amphipods, brittlestars, and sea urchins (Table 2). Significantly, polychaetes were major dietary components of 11 of the 13 species of benthic carnivores studied; the exceptions were *Nelusetta ayraudi* and *Pseudorhombus tenuirastrum*. Furthermore, of the 5 species with the highest average TBC in whole fish (24−233 ng/g), polychaetes were the most favored diet of 3 of these species, *Nemadactylus macropterus*, *Nemadactylus douglasii*, and *Branchiostegus wardi* (Table 2). In the other 2 species, *Sillago flindersi* and *Paristiopterus labiosus*, polychaetes rated second and third, respectively. Accordingly, polychaetes appear to be the most likely source of bromophenols in all 5 species.

In the six species of diverse omnivores studied, crustaceans, polychaetes, molluscs, and gastropods again feature prominently as major dietary components. However, unlike benthic carnivores, these fish also feed extensively on algae and seagrasses and on colonizing animals such as sponges, ascidians, hydroids, and bryozoans (Table 3). Thus, in addition to polychaetes, these fish have alternative sources of bromophenols, including algae (Whitfield et al., 1992a), sponges, hydroids, and bryozoans (Whitfield et al., 1992b, 1997a). Even so, polychaetes would still appear to be the major

Table 2. Relationship between Total Bromophenol Content (TBC) in Whole Fish and the Diet of Individual Species of Carnivorous Fish

species	TBC (ng/g)	diet[a]
pelagic carnivores		
P. caeruleopunctatus	2[b]	small fish, swimming crustaceans, octopus, squid, and occasionally polychaetes[c,d]
C. affinis	2[b]	small fish, swimming crustaceans, and molluscs, and occasionally polychaetes[c,d]
P. marmoratus	1.5[b]	small fish, swimming crustaceans, octopus, squid, and occasionally polychaetes[c,d]
N. richardsoni	1[b]	small fish and swimming crustaceans[d]
H. percoides	0.8	squid, swimming prawns, and small fish[d]
Z. faber	0.4[b]	fish, swimming crustaceans, and molluscs[c,d]
P. arenarius	0.7	small fish, swimming crustaceans, octopus, squid, and occasionally polychaetes[c,d]
S. punctata	0.4	planktonic invertebrates, and jellyfish[d]
P. arsius	0.2	swimming crustaceans, and teleosts[c]
G. blacodes	ND	fish and swimming crustaceans[d,e]
benthic carnivores		
S. flindersi	233[b]	crustaceans and polychaetes[c,d]
N. macropterus	52	polychaetes, crustaceans, molluscs, and other echinoderms[d,f]
N. douglasii	28[b]	polychaetes, brittlestars, sea urchins, and crustaceans[d,f]
P. labiosus	23[b]	crustaceans, molluscs, polychaetes, brittlestars, and sea cucumbers[e,f]
B. wardi	24	polychaetes, gastropods, bivalves, molluscs, amphipods, and small fish[g]
C. kumu	4.5[b]	crustaceans, polychaetes, and small fish[c,f]
P. jenynsii	3[b]	crustaceans, polychaetes, and teleosts[c]
N. ayraudi	3[b]	salps, molluscs, crustaceans, and fish[d]
P. tenuirastrum	2	not identified
U. lineatus	2[b]	molluscs, polychaetes, and crustaceans[f]
P. polyommata	1[b]	polychaetes and crustaceans[g]
B. serratus	1	fish, molluscs, polychaetes, and crustaceans[h]
P. auratus	0.1	crustaceans, polychaetes, and molluscs[d,g]
A. spariformes	ND	believed to be the same as *Pagrus auratus*

[a] Listed in order of importance. [b] Average value. [c] Anonymous (1981). [d] Kailola et al. (1993). [e] Paul and Moreland (1993). [f] Francis (1996). [g] M. Gomon, Museum of Victoria, Melbourne, VIC, Australia, personal communication, 1996. [h] J. R. Paxton, Australian Museum, Sydney, NSW, Australia, personal communication, 1996.

Table 3. Relationship between Total Bromophenol Content (TBC) in Whole Fish and the Diet of Individual Species of Omnivorous Fish

species	TBC (ng/g)	diet[a]
diverse omnivores		
R. sarba	18	crustaceans, polychaetes, algae, molluscs, and gastropods[c]
P. dentex	13[b]	crustaceans, polychaetes, molluscs, algae, and detritus[c,d]
M. freycineti	7[b]	crustaceans, algae, seagrasses, bryozoans, and polychaetes[c]
A. australis	9	polychaetes, crustaceans, molluscs, algae, and teleosts[c,d]
M. trachylepsis	3	algae, seagrasses, crustaceans, polychaetes, and bryozoans[c]
P. scaber	0.5[b]	sponges, ascidians, hydroids, bryozoans, jellyfish, and algae[e]
restricted omnivores		
G. tricuspidata	13[b]	algae, small crustaceans, molluscs, and detritus[c,d,f]
K. sydneyanus	5	algae, small crustaceans, and molluscs[f]

[a] Listed in order of importance. [b] Average value. [c] Anonymous (1981). [d] Kailola et al. (1993). [e] J. B. Hutchins, Museum of Western Australia, Perth, WA, Australia, personal communication, 1996. [f] Frances (1996).

source of bromophenols in some species of diverse omnivores. The highest bromophenol contents were found in *Rhabdosargus sarba*, *Pseudocaranx dentex*, and *Acanthopagrus australis*, species that feed extensively on these benthic animals (Table 3). However, the inclusion of algae and colonizing animals in the diets of these species increases the bromophenol content. Of the diverse omnivores studied, only *Parika scaber* does not feed on polychaetes. This species also has the lowest bromophenol content of the diverse omnivores. The small levels of bromophenols present in *P. scaber* are presumably derived from its diet of colonizing animals (Table 3).

Only two species of restricted omnivores were studied, and plant material (algae and seagrasses) accounted for >85% of their dietary intake; small crustaceans and other animals living on the plants were additional sources of food (Anonymous, 1981). Of these items only marine algae are known to biosynthesize bromophenols (Whitfield et al., 1992a). Accordingly, the levels of bromophenols found in *Girella tricuspidata* and *Kyphosus sydneyanus* would appear to be totally derived from the algal component of their diets (Table 3).

Polychaetes and Marine Algae as Natural Sources of Bromophenols in Ocean Fish. During a survey of the dietary habits of Australian ocean fish, 15 families of polychaetes were identified in the stomach contents of some 40 species of benthic carnivores and diverse omnivores (Anonymous, 1981). The most commonly found family (>61% of all samples) was Nereidae. As a result of these findings, representative species of 9 of the 15 families (including Nereidae) were analyzed for their bromophenol content (Whitfield et al., 1996, 1997a). With the exception of 1 family, Onuphidae, all were shown to contain high concentrations, between 0.01 and 8.3 mg/g, of these compounds. The major bromophenol present in the majority of species was 2,4,6-TBP; the one exception was *Glycera americana* (Whitfield et al., 1997a). Bromophenols present in the next highest concentrations were 2,6-DBP and 2,4-DBP. Similar relationships between the concentrations of these three compounds were previously observed in 27 samples of ocean fish (Table 1). In *G. americana*, the major bromophenols present were 2,4-DBP and 4-BP. These two compounds were also the major bromophenols in one sample of *Marphysa macintoshi* (Whitfield

et al., 1997a). Of interest, these two compounds were found to be the major bromophenols in one sample of the benthic carnivore *Nemadactylus douglasii* (Table 1).

During a recent survey of the distribution of bromophenols in Australian polychaetes, it was found that species living in mud contained far greater concentrations of these compounds than species living on rock or in sand (Whitfield et al., 1996, 1997a). Accordingly, polychaetes living in muddy habitats were found to have TBCs that varied between 0.88 and 8.3 mg/g, whereas species from rocky or sandy environments had between 0.0005 and 0.001 mg/g. It is therefore not surprising that species of fish that feed on polychaetes in a muddy environment contain much higher concentrations of bromophenols than those feeding over rock or sand.

In a survey of the dietary habits of Australian ocean fish, at least seven genera of red, brown, and green algae were identified in the stomach contents of some 15 species of diverse omnivores and restricted omnivores (Anonymous, 1981). The most commonly identified genera were *Enteromorpha*, *Gracilaria*, and *Ulva*, but in most gut samples it was not possible to identify algal material beyond division. However, anecdotal information supplied by amateur fishermen and spear fishermen indicate that omnivorous fish feed on a wide variety of algae and particularly on those algae that have a "soft" texture. As a consequence of these observations, some 41 species of algae, including *Enteromorpha*, *Gracilaria*, and *Ulva*, were analyzed for their bromophenol content (Whitfield et al., 1992a, 1997a). However, the levels of bromophenols found in the algae were far lower than those found in polychaetes from muddy environments. The highest concentrations found in the algae were between 0.001 and 0.003 mg/g. Of interest, such levels were found in the green algae, *Enteromorpha intestinalis* and *Ulva lactuca*, and the red algae, *Pterocladia capillacea* and *Amphiroa anceps* (Whitfield et al., 1992a, 1997a). All of these species, including the Corallinaceae *Amphiroa anceps*, are known to be consumed by many species of omnivores. Once again, the bromophenol present in highest concentration in most algae studied was 2,4,6-TBP, with 2,4-DBP and 2,6-DBP the next most dominant compounds (Whitfield et al., 1992a, 1997a). In the two species of restricted omnivore studied, *Girella tricuspidata* and *Kyphosus sydneyanus*, the major bromophenols identified were, in order, 2,4,6-TBP, 2,4-DBP, and 2,6-DBP (Table 1). As these species of restricted omnivore are known to feed extensively on *Enteromorpha intestinalis* and *Ulva lactuca* (Kailola et al., 1993), the similarity in bromophenol composition of the fish and algae would be expected.

On the basis of the current study, it would therefore appear that polychaetes are a major source of bromophenols in benthic carnivores and diverse omnivores caught on the eastern coast of Australia, whereas marine algae are the major source of these compounds in restricted omnivores. Marine algae and, to a lesser extent, colonizing animals known to contain bromophenols would also contribute to the total bromophenol content of some diverse omnivores. Significantly, the bromophenol present in highest concentration in most species of Australian polychaetes and marine algae is 2,4,6-TBP. This could account for the dominance of this compound in the gut and flesh of many species of Australian fish.

Conclusion. Connoisseurs of Australian ocean fish are aware that the flavor of their favorite species can vary depending on where the fish was caught and at what time of the year. The current study has shown that the bromophenol content, and associated natural ocean-, brine-, or iodine-like flavors, of some species of fish can be related to their diet. It has also been shown elsewhere (Anonymous, 1981) that the diet of a particular species of fish can vary extensively depending on the availability of individual food components. Such availability will depend on location and on seasonal variations. As an example, the intake of polychaetes by the diverse omnivore *Acanthopagrus australis* was shown to vary from 7% to 40% over a two year study period as assessed at bimonthly intervals (Anonymous, 1981). Over the same period this species' intake of algae varied from 70% to 10%. A high intake of algae was coincidental with a low intake of polychaetes. Accordingly, samples of *A. australis* caught while feeding on a high intake of polychaetes could be expected to have a stronger ocean-like flavor than those with a high intake of algae. By comparison, the restricted omnivore, *Girella tricuspidata*, was shown to feed extensively on algae throughout the whole two year study period (Anonymous, 1981). Consequently, little variation in flavor would be expected for this species. Because the major dietary sources of bromophenols in Australian ocean fish have probably been identified, further studies should now be directed to establish the mechanisms by which these compounds are transferred from the animal's gut to the edible flesh.

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