Effect of Diet and Environment on the Volatile Flavour Components of Crustaceans

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Project 92/075

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92/075 Effect of diet and environment on the volatile flavour components of crustaceans

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OBJECTIVES: To identify those volatile compounds responsible for either desirable flavours or off-flavours in wild-harvested and cultivated crustaceans and to establish the source of these compounds by:

1. analysing extracts of crustaceans by sensory gas chromatography and gas chromatography-mass spectrometry and relating the presence of particular components to sensory data obtained by taste panel analysis,
2. analysing the diets of crustaceans, and components of the environment in which they live, to establish the origin of important flavour compounds.

SUMMARY: The flavour of a seafood is one of several sensory properties that determines whether it is marketable and at what market price. Those species of crustaceans that possess characteristic flavours, such as the Endeavour prawn, the Royal Red prawn and the Balmain Bug have from time to time been unpopular in markets because of the occurrence of these natural flavours. Other species with bland or little flavour also draw critical comment from consumers paying high prices in restaurants. Although some evidence has indicated that diet and environment are major contributing factors in the determination of the flavours of crustaceans, no definite link has been established. The current study was accordingly undertaken to identify the sources of the compounds responsible for such characteristic flavours, and to provide an explanation for the occasional outbreaks of strong flavours in wild-harvested prawns, and the absence of natural flavours in cultivated animals. The technological aim of the work was to improve the flavour quality of Australian produced prawns for both domestic and overseas consumption. The ultimate return to the fishing industry from this research would be products of reliable flavour quality capable of yielding high market prices.

Evidence obtained from the chemical and sensory analyses of nine species of wild-harvested prawns and two species of cultivated prawns showed that bromophenols, particularly 2-bromophenol, 4-bromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol, enhanced the desirable seafood flavours of wild-harvested animals. Conversely, the near absence of these compounds from cultivated prawns left the flesh bland and lacking prawn-like flavours. In addition, these analyses showed that the bromophenol content of prawn heads (which includes the gut) was seven times that found in the tails for wild-harvested prawns and three times that found in cultivated animals. As an adjunct to this work 31 species of ocean fish were analysed for their bromophenol content. Results from these analyses showed that the average total bromophenol content of benthic carnivores and omnivores was 100 times greater than that of piscivorous carnivores. The analyses also showed that the bromophenol content in the animal's gut was greater than that found in the flesh. These findings supported the belief that bromophenols are derived from the diet of marine animals.

Following detailed surveys of literature pertaining to the dietary intake of prawns and fish, the likely sources of bromophenols in these animals appeared to be polychaetes and marine algae. Analyses of 16 species of polychaetes showed that these soft bodied organisms were a major source of bromophenols in the marine environment. Analyses of 50 species of marine algae showed that these plants were probably the world's major repository of bromophenols in the marine environment, because of the wide occurrence of such plants along the nations' coastlines. By comparison, the concentrations of bromophenols in commercial prawn feeds are appreciably lower than those found in natural sources, 1×10^{-5} that found in polychaetes and 1×10^{-3} that found in algae. It is therefore not surprising that prawns fed on such feeds have much lower bromophenol contents, and hence blander flavours, than those with a natural diet.

Modified prawn feeds were prepared in which bromophenols both in the free form and as their sulfate esters were added to a CSIRO formulation. The concentrations of these compounds were similar to the highest levels found in commercial feeds. Results from feeding trials showed that the prawns did not discriminate between the modified feeds and the control. Furthermore, sensory analyses carried out on these prawns showed that the modified feeds enhanced the natural flavour of prawn meat. Of equal importance, it was found prawns fed on modified feed containing free bromophenols retained more of these compounds than prawns fed on feed containing the sulfate esters.

ACHIEVEMENTS: All of the project's major objectives were achieved as well as some additional goals that were generated from the original study.

1. Four compounds, 2-bromophenol, 4-bromophenol, 2,6-dibromophenol and 2,4,6-tribromo-phenol were shown to be important in the natural flavour of wild-harvested prawns.
2. The major source of bromophenols in wild-harvested prawns was shown to be components of the animal's diet, and in particular polychaetes.
3. Prawn feeds modified by the addition of bromophenols were shown to be acceptable to cultivated prawns and that they enhanced the flavour of these animals.

CONCLUSION:

- The flavour of wild-harvested prawns is superior to that of cultivated prawns. The difference correlates with greater concentrations of bromophenols in the wild animals.
- The major repositories of bromophenols in the marine environment are polychaetes and marine algae.
- Bromophenols are present in only low concentrations in commercial prawn feeds.
- Commercial prawn feeds can be modified by the addition of bromophenols, but the form in which these compounds are added determines how well they are retained by the prawn.
- Cultivated prawns fed on a bromophenol enhanced feed have a better taste panel rating than those fed on a control feed.

NEED FOR FURTHER WORK:

- To establish the distribution of bromophenols in polychaetes and other benthic organisms collected from the natural feeding areas of wild prawns.
- To identify to family the polychaetes eaten by wild prawns.
- To establish the most efficient method for the incorporation of bromophenols into commercial prawn feeds.

- To establish the most suitable form of bromophenol for incorporation into prawn feeds so as to maximise the retention of these compounds in cultivated prawns.

ABBREVIATIONS

SDE	=	steam distillation/solvent extraction
GC	=	gas chromatograph (gas chromatography)
MS	=	mass spectrometer (mass spectrometry)
MSD	=	mass selective detector
MID	=	multiple ion detection
SD	=	standard deviation
LRI	=	linear retention index
2-BP	=	2-bromophenol
4-BP	=	4-bromophenol
2,4-DBP	=	2,4-dibromophenol
2,6-DBP	=	2,6-dibromophenol
2,4,6-TBP	=	2,4,6-tribromophenol
ND	=	not detected
ng	=	nanogram (10^{-9} grams)
g	=	gram
ml	=	millilitre (10^{-3} litre)
µl	=	microlitre (10^{-6} litre)
mm	=	millimetre (10^{-3} metre)
TBC	=	total bromophenol content

Effect of Diet and Environment on the Volatile Flavour Components of Crustaceans

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BACKGROUND

The flavour of a seafood is determined by a variety of pre- and post-harvest effects including diet, environment, handling, processing, storage and transportation. Over the past 40 years post-harvest effects on flavour have been extensively studied (Josephson, 1991), but at present little information is available on the roles played by either the animal's diet or the natural environment in which it lives. However, it was demonstrated in the early 1960s that diet can affect the flavour of seafoods, when the ingestion of certain species of invertebrates by salmon (Motohiro, 1962) or cod (Sipos and Ackman, 1964) was shown to produce recognisable flavour changes in the processed fish. Although there have been a number of reports in recent years (Whitfield, *et al*., 1988; Anthoni *et al*., 1990; Whitfield *et al*., 1995) that certain other natural seafood flavours are also related to the animal's diet, little effort has been made to demonstrate this relationship. Similarly, the impact of the natural environment on flavour has continued to receive little attention, even though some studies have shown that in lakes and ponds (both fresh and saline) environmental effects can be responsible for significant changes of flavour in cultivated fish (Yurkowski and Tabacheck, 1974) and crustaceans (Lovel and Broce, 1985). By comparison, the effect of industrial pollution on seafood flavour has been widely investigated (Whitfield, 1988).

The CSIRO Division of Food Science and Technology has been involved in the study of the flavour of crustaceans since 1980 (FIRTA grants 1980-81; 1982-83 and CSIRO funded research 1984-92). Between the years 1980 to 1984, the causes of the characteristic flavours encountered in Royal Red prawns (garlic), Endeavour prawns

(iodoform) and Balmain Bugs (garlic) were extensively investigated. These studies led to the identification of two of the compounds responsible for garlic-like flavours (bis-methylthiomethane and trimethylarsine). These identifications made possible the development of handling procedures that reduced the effect that these flavours had in the market place (Whitfield *et al*., 1981a, 1981b, 1982, 1983; Whitfield and Tindale, 1984; Freeman *et al*., 1981, 1985; Freeman and Whitfield, 1982). From 1984 to 1988 such studies concentrated on the cause of the characteristic flavour of the Endeavour prawn from Exmouth Gulf, Western Australia. This work resulted in the identification of those compounds responsible for the iodoform-like flavour (bromophenols) and led to the development of procedures aimed at reducing the impact of this flavour in the market place (Whitfield *et al*., 1988; Whitfield, 1990). Since 1988 work has continued within the CSIRO to identify the dietary components involved in the occurrence of the characteristic flavour of Endeavour prawns (Whitfield *et al*)., 1992a, 1992b). Co-incident with this work was the report that the same compounds (bromophenols) were responsible for the fresh seafood flavour of ocean fish, crustaceans and molluscs caught in the northern hemisphere (Boyle *et al*., 1992a, 1992b). As a consequence of the latter finding the need to identify the natural sources of such flavour compounds assumed far greater importance. The knowledge of the origin of such compounds could be used by the industry to modify the flavours of cultivated fish and crustaceans to satisfy the needs of specialty markets.

NEEDS

The flavour of a seafood is one of several sensory properties that determines whether it is marketable and at what market price. Those species of crustaceans that possess characteristic flavours, such as the Endeavour prawn, the Royal Red prawn and the Balmain Bug have from time to time been unpopular in markets because of the occurrence of these natural flavours. Species with bland or little flavour also draw critical comment from consumers paying high prices in restaurants. Although some evidence has indicated that diet and environment are major contributing factors in the determination of the flavours of crustaceans (Lovel and Broce, 1985; Whitfield, 1990; Whitfield *et al*., 1992a, 1992b; Anthoni *et al*., 1990), no definite link has been established. To obtain this link it will be necessary to identify the compounds

responsible for the characteristic flavours in crustaceans and to demonstrate their occurrence in the animal's diet.

Although the flavour of a seafood is to some extent an intrinsic property, it is possible to modify flavour by changing such factors as diet (for cultivated animals) and the way in which the animals are harvested, and handled after harvest. Examples of such techniques are the modification of the flavour of farmed Atlantic salmon by adjusting diet (Thrower, personal communication), and the modification of the harvesting and handling of Royal Red prawns, Balmain Bugs and Endeavour prawns to reduce the impact of their characteristic flavours (Whitfield, 1990).

The current research will provide definitive information on the origins of flavour compounds present in crustaceans and fish and this information will be used to assist the industry to improve the quality of animals, both cultivated and harvested from the wild. In turn, this information can be used to improve the quality of value-added products prepared from these materials for both domestic and export markets. The research will also assist the Australian aquaculture industry to produce crustaceans and fish to the tastes of specialty markets. The achievement of these aims will permit the expansion of both domestic and overseas markets.

The novelty of the CSIRO approach is that it will permit the use of natural resources for the improvement or modification of the flavour quality of wild-harvested and cultivated seafoods.

OBJECTIVES

To identify those volatile compounds responsible for either desirable flavours or off-flavours in wild-harvested and cultivated crustaceans and to establish the source of these compounds by:

1. analysing extracts of crustaceans by sensory gas chromatography and gas chromatography-mass spectrometry and relating the presence of particular components to sensory data obtained by taste panel analysis,

2. analysing the diets of crustaceans, and components of the environment in which they live, to establish the origin of important flavour compounds.

The CSIRO study will also provide key information for the development of value added crustacean products for export by facilitating the best methods for packaging and transportation to maximise the retention of desirable flavour components in these products.

Following reports describing the importance of bromophenols in the natural flavour of crustaceans and fish caught in the northern hemisphere (Boyle *et al*., 1992a, 1992b), the CSIRO research was directed to establish the importance of these compounds in the flavour of Australian species. As a consequence, in addition to the proposed study of the flavour of wild and cultivated Australian prawns, an extensive study of local ocean fish was also undertaken. From these studies, it was apparent that bromophenols were the most important natural flavour compounds yet identified in the flavour of seafoods. The project was therefore expanded to cover the origins of such compounds in the diets of not only crustaceans but also ocean fish. This had the effect of expanding the range of dietary components analysed for the bromophenol target compounds. Similar studies of the bromophenol content of samples of prawn feed produced both locally and overseas indicated that these materials contained much less of these compounds than the animal's natural diet. Following discussions with sections of the Australian industry, and the CSIRO Division of Fisheries, efforts were directed towards the incorporation of these compounds into manufactured feeds with the intent of increasing their levels in cultivated fish and crustaceans. As a consequence of this redirection of effort, work was not undertaken on the effects of packaging and transportation on the retention of these key flavour compounds in seafoods.

The current study achieved all of its major objectives by identifying bromophenols as key natural flavour components of Australian crustaceans and fish, and by the identification of polychaetes, macroalgae, bryozoans and sponges as the major dietary sources of these compounds in seafoods. Furthermore, as a result of the identification of a number of natural sources of bromophenols in the marine

environment it has been possible to target one of these as a future commercial source of these compounds for the manufacture of improved Australian prawn and fish feeds.

METHODS

The research project can be divided into four distinct sections: (a) collection of samples, (b) extraction, (c) analysis and (d) production of modified feed. Accordingly, the methods used in this project will be discussed under the above four sub-headings.

(a) *Collection of samples*

Samples of uncooked prawns were obtained from the following sources -

Mr Ken Horada and retail outlets of the Sydney Fish Markets, Sydney.

Mr Ken Graham, NSW Department of Fisheries, Sydney.

Mr Stephen Thrower, International Food Institute Queensland, Hamilton.

Mr Frank Roberts, TruBlu Prawn Farm, Palmers Island.

Mr Barry Heine, Clarence River Co-operative, Grafton.

Mr Bill Izzard, Cairns Live Prawns, Cairns.

Prawns supplied from outside of the Sydney area were chilled to 0^{o}C and were then air freighted to Sydney on the first available flight. On receipt they were snap frozen and stored at -20^{o}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^{o}C.

All samples of fish were supplied by Mr Ken Graham (NSW Department of Fisheries) and were caught off the coast of NSW by the research vessel MV Kapala during 1994 and 1995. As with the samples of prawns, whole fish were held in ice until they were delivered to the laboratory where they were snap frozen and stored at -20^{o}C.

Samples of prawns were identified to species by either Mr Ken Horada (Sydney Fish Marketing Authority) or Mr Ken Graham (NSW Department of Fisheries) while all samples of fish were identified by Mr Ken Graham.

Polychaetes were either collected at low tide from the Sydney region by members of the project or were purchased from commercial suppliers. Dr Jim Patterson (Wynnum, Queensland) supplied samples of *Marphysa sanguinea* and *Glycera americana*, while Mr John Park (Seal Rocks, NSW) supplied samples of *Australonuphis teres*. Collections of polychaetes were routinely sorted into species and one sample of each was preserved in 7 percent formaldehyde and was identified to species by Dr Anna Murray of the Marine Ecology Department of the Australian Museum, Sydney. The remaining material was frozen and stored at -15^{o}C.

Samples of marine algae were collected at low tide from the ocean, rocks and beaches of the central and southern coasts of NSW and the Great Barrier Reef, Queensland. These collections were routinely sorted into species and one sample of each was forwarded, unpreserved, to Dr Alan J.K. Millar of the Royal Botanic Gardens Sydney for identification. The remaining material was frozen and stored at -20^{o}C.

Bryozoans were collected from Tasmanian waters by Dr Adrian Blackmore (University of Tasmania), and sponges from Exmouth Gulf WA by the Western Australian Department of Fisheries. The samples of bryozoans were identified by the staff of the Marine Biology Department of the University of Tasmania and the sponges by Dr John N.A. Hopper of the Division of Natural Sciences, Queensland Museum. All retained samples were snap frozen and stored at -20^{o}C. Samples of sediment were either collected at low tide in the Sydney area or by Mr Ken Graham

aboard the MV Kapala. All samples were held at -20^{o}C until required. Sea water samples were collected from the shore line and were extracted upon delivery to the laboratory.

(b) ***Extraction***

The preparation of individual samples before extraction varied according to the type of material under examination. Fish were gutted and the flesh separated from the heads and tails, prawns were separated into heads and tails, while polychaetes, algae, bryozoans, sponges and commercial prawn feeds were extracted without preliminary preparation. However, independent of the material under examination, representative samples (10 to 760 g depending on availability) were homogenised in purified water (500 ml) for 5 minutes using a Panasonic Super Blender (Whitfield *et al*., 1995). The homogenates were transferred to 3-litre flasks, water (1 litre) was added, and the mixtures acidified to pH 1 with 36 N sulfuric acid. The acidified homogenates were then allowed to stand at 20^{o}C for at least 2 hours, but preferably overnight, to confirm that sufficient acid had been added to achieve pH 1. The volatile components were isolated by combined steam distillation/solvent extraction (SDE) with pentane (27 ml) and diethyl ether (3 ml) as solvent (Whitfield *et al*., 1988). After 3 hours the residues were checked to confirm that they had remained acid during the distillation/extraction. The internal standard, 2,6-dibromophenol-d_3 (100 ng in 10 µl ethanol), was added to the extracts which were then dried by cooling to -15^{o}C. The extracts were concentrated to about 100 µl by distillation using a micro-wet-wall fractionating column and the concentrates stored at -15^{o}C until required for analysis.

(c) ***Analysis***

Sensory gas chromatography of prawn extracts - A Carlo Erba HRGC 5160 Mega Series gas chromatograph (GC), fitted with a laboratory constructed sniffing port and a fused silica column (50 m x 0.33 mm i.d.) coated with methylsilicone BP1 phase (0.5 µm film thickness) was used for all sensory analyses. The carrier gas was helium (1 ml/min through the column), detection was by flame ionisation, and the response was recorded by a Spectro Physics SP 4270 chromatography integrator. The GC was fitted with a split introducer and the split ratio was at 1:10. At the

detector end of the GC column the effluent was split so that 0.8 ml/min flowed to the sniffer port and 0.2 ml/min to the detector. A make-up gas (nitrogen at 10 ml/min) was added to the effluent flowing to the sniffing port to facilitate the coincident arrival of the eluted components to both the observer's nose and the GC detector. For the sensory analyses, 1 μl of extract was injected into the column introducer held at 250^{o}C. The column temperature was initially held at 60^{o}C for 5 min and then programmed to 280^{o}C at a rate of 4^{o}C/min. During the course of the analyses the sensory observer recorded both the descriptions of the odours eluted from the sniffing port and the retention time at which the odour was detected.

Qualitative gas chromatography-mass spectrometry of prawn extracts - The extracts were analysed using a Varian 1440 gas chromatograph coupled to a Varian-MAT 311A mass spectrometer (MS) controlled by a Finnigan-MAT INCOS 2200 data system. The GC oven was fitted with a fused silica column the same as that used for the sensory analyses. The GC was fitted with a split introducer and the split ratio was set at 1:10. The carrier gas was helium (about 1 ml/min). For the qualitative analyses 1 μl of extract was injected at an introducer temperature of 250^{o}C. The column temperature was initially held at 60^{o}C for 5 min and then programmed to 280^{o}C at a rate of 4^{o}C/min. The GC-MS transfer line was heated to 280^{o}C.

The mass spectrometer was operated in the electron ionisation mode at an electron energy of 70 eV and a source temperature of 250^{o}C. The mass analyser was scanned from mass 34 to mass 340 every 2 seconds. The detector was a secondary electron multiplier held at 1.3 kV. Four thousand spectra were acquired for each GC-MS run and the data were stored and processed by the INCOS data system. Compounds were identified by comparison of their mass spectra with those in three libraries totalling 43,244 spectra.

Quantitative analyses of bromophenols in seafoods and dietary extracts - The extracts were analysed using a Hewlett-Packard HP5890 Series II gas chromatograph coupled to a Hewlett-Packard HP5971A mass selective detector (MSD). The system was controlled by an HP Vectra 386/25 computer running an HP MS-ChemStation

data system. The GC was equipped with an HP7673 automatic injector, a pre-column splitter set at a split ratio of 1:20, and fitted with a methylphenylsilicone HP5 fused silica GC column (25 m x 0.2 mm id x 0.5 μm film thickness). Because the extracts contained a high content of steam volatile fatty acids, the column was protected by a retention gap (5 m x 0.25 mm id), uncoated but deactivated. Helium flow was 0.48 ml/min and the column temperature, initially held at 60°C for 1 minute, was programmed in two stages, first from 60°C to 225°C at 15°C/min and second from 225°C to 280°C at 40°C/min before holding at this temperature for a further 37 minutes. For the quantitative analyses, 1 μl of extract was automatically injected at an introducer temperature of 280°C.

The MSD was operated in the electron ionisation mode with an electron energy of 70 eV and a source temperature of 170-180°C. Quantitation of individual target compounds was achieved by multiple ion detection (MID) performed under software control by the HP Vectra data system. For the analysis of bromophenols a total of 12 ions were monitored over three separate time intervals m/z 172, 174 (monobromophenols - start time 5.8 min, dwell time 150 ms) m/z 250, 252, 253, 254, 255, 257 (dibromophenols and internal standard - start time 9.4 min, dwell time 50 ms) and 328, 330, 332, 334 (tribromophenol - start time 11 min, dwell time 100 ms). The GC-MSD was calibrated by the analysis of three different concentrations each of 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol and 2,4,6-tribromophenol (0.5, 5 and 25 μg/ml) with a constant concentration of 2,6-dibromophenol-d_3 (1 μg/ml). Response factors for the bromophenols, with respect to the internal standard, were calculated by the data system software and were used to determine the concentrations of the target compounds in the extracts. The calibrations were performed on the day of the analysis and each analysis was carried out in duplicate. If a sample contained analytes outside of the calibration range of the mass spectrometer, more internal standard was added and a diluted sub-sample was analysed.

Extraction efficiencies for the recovery of bromophenols from prawn meat, fish flesh and marine algae were determined and these figures were used to correct the raw data obtained from the quantitative analysis of the target compounds in the volatile extracts. In the current studies the average percentage recoveries were as follows:

2-bromophenol 94% (SD=6%), 4-bromophenol 41% (SD=1%), 2,4-dibromophenol 74% (SD=0), 2,6-dibromophenol 81% (SD=0) and 2,4,6-tribromophenol 74% (SD=4%). Recoveries of bromophenols from polychaetes, bryozoans and sponges were assumed to be the same as those obtained with the other three materials.

Sensory panel assessment of prawn meat - A proportion of the prawn samples supplied to the laboratory from either commercial sources or from the CSIRO Division of Fisheries were also assessed by a consumer panel for colour, flavour, texture and acceptability. The panellists were selected from laboratory staff that regularly ate boiled prawns. The size of the panel varied between 10 and 12 persons and only these panellists were used during the assessment program. Wherever possible the sensory assessments were made on the day the prawns were received in the laboratory. When this was not possible they were frozen and stored at -20^{o}C and were then assessed at the earliest opportunity. For sensory assessment, the prawns were prepared as follows. Batches of whole prawns (about 1 kg), with shell and heads attached, were placed in a large saucepan and covered with unsalted, boiling odour-free water. The water was then boiled (about 2 minutes) until the prawns floated to the surface. The prawns were immediately removed from the water and were allowed to cool to room temperature, about 20^{o}C. Four prawns were served to each panellist together with a request to rate the colour, flavour, texture and acceptability on a 100 mm line scale covering the range of choices from unacceptable to excellent. Panellists were also requested to describe the flavour of individual samples.

(d) ***Production of modified feed***

Three prawn feeds (a control and two modified feeds) were prepared at the CSIRO Division of Fisheries Cleveland from commercially available ingredients. The dry ingredients including, fish meal, squid meal, prawn meal, soybean meal, wheat flour, vitamins, minerals and chemical binder were blended for 15 minutes in a Hobart mixer until the mixture was homogeneous. It was then divided into three batches (about 3 kg each); squid oil was added to two batches, while squid oil containing a dilute solution of five bromophenols was added to the third. This solution was prepared by dissolving in ethanol (1 ml) the following compounds, 2-bromophenol

(510 μg), 4-bromophenol (330 μg), 2,4-dibromophenol (540 μg), 2,6-dibromophenol (120 μg) and 2,4,6-tribromophenol (180 μg). The three batches were mixed separately for a few minutes and to these were added minced squid flesh. These materials were again mixed until the resultant dough became crumbly. To one of the batches not containing the bromophenols was added an aqueous solution of sodium bromophenyl sulfate containing the same quantities of the individual bromophenols as was added in the squid oil. After further mixing, the three batches were twice passed through a Hobart mincer. The products were then steam cooked for 10 minutes at 100^{o}C to complete the polymerisation and gelatination processes before being dried at 60^{o}C for 5 hours.

A small representative sample from each batch of feed was retained for bromophenol analyses, while the remaining materials were fed under carefully monitored conditions to 72 juvenile *Penaeus monodon* prawns housed in six treatment tanks sited at the Cleveland laboratory. Each feed was fed to a total of 24 prawns housed in two separate tanks. After six weeks, the prawns (about 15 g each) were harvested, snap frozen and transported to the North Ryde laboratory for sensory and chemical analysis.

RESULTS

The results obtained from this study will be discussed under the following headings: (a) ***Analysis of seafoods***, (b) ***Analyses of diets and the environment***, and (c) ***Modified prawn feed***.

(a) ***Analysis of seafoods***

Sensory GC analyses of prawn volatiles - The purpose of these assessments were to identify differences in the volatile content of wild-harvested and cultivated prawns; in particular, those compounds that could be derived either from the animal's diet or its environment. Five species of prawns, *Penaeus plebejus, P. esculentus, P. latisulcatus, P. monodon* and *P. stylirostris* were chosen for sensory GC assessment. Of these species the first three were harvested from the wild, while the last two were obtained from commercial prawn farms. At least two samples of each species were assessed.

A total of 36 odorous compounds were detected in two or more of these species and the presence or absence of these compounds in a particular species is recorded in Table 1. Of these compounds, 32 were detected in *P. plebejus*, 30 in *P. esculentus*, 25 in *P. latisulcatus*, 17 in *P. monodon* and 15 in *P. stylirostris*. A total of 12 compounds were only detected in the wild-harvested species and the majority of these (some nine in total) were described as possessing odours reminiscent of seaweed, algae, sea air and rock pools. Four of these compounds had the same Linear Retention Indexes (LRI) as 2-bromophenol (1038), 4-bromophenol (1232), 2,6-dibromophenol (1343) and 2,4,6-tribromophenol (1601). The only simple bromophenol not detected by odour was 2,4-dibromophenol (1311) and this compound has the highest odour threshold of the five bromophenols previously detected in prawns (Whitfield *et al*., 1988). The LRI values of the other five compounds with ocean-like odours could not be related to any compound previously detected in wild-harvested prawns. However, the compound with the onion-type odour (510) has an LRI value similar to that of dimethylsulfide while the compound with the odour described as fried onions (560) has an LRI value similar to trimethylarsine. Both of these compounds have previously been detected in some species of wild-harvested prawns (Whitfield *et al*., 1983). Importantly, all odorous compounds detected in the cultivated prawns were detected in the wild-harvested species. The 12 odorous compounds only detected in the wild-harvested species could be derived either from the animal's diets or from the environment in which it lived. Therefore, the identification of these compounds became the subject of further investigation.

Table 1 Volatile prawn odours assessed by sensory gas chromatography

LRI[a]	Odour Description	Species Assessed				
		[b] PP	PE	PL	PM	PS
< 500	Stench	[c] +	+	+	+	+
510 [d]	Onion/prawns	+	-	+	-	-
560 [d]	Fried onions	+	+	+	-	-
815	Cooked prawns	+	+	-	-	+
877	Geraniums	+	+	-	+	-
893	Cooked prawns	+	+	+	-	+
910	Baked potatoes	+	-	-	+	+
927	Cooked prawns	+	+	+	+	+
938	Cooked prawns	+	+	+	+	+
941	Fruity / sea air	+	-	-	+	-
973	Cooked prawns/ seaweed	+	-	+	-	-
975	Geraniums	-	+	+	+	-
985	Green / algae / seafood	+	+	-	-	+
994	Sea air / rotten algae	+	+	-	+	+
1007	Sea odour / rock pool	+	+	+	+	-
1010	Sea odour / geraniums	+	+	+	+	-
1022	Geraniums	+	+	+	-	+
1026	Green	+	+	-	-	-
1038	Salty/sweet/seaweed	+	+	+	-	-
1059	Cheesy	+	-	+	+	+
1079	Sea air / fruity	+	+	-	-	+
1111	Cooked prawns / boiled eggs	+	+	+	+	+
1172	Green / cheesy	+	+	+	+	-
1187	Sea air / algae / green	+	-	+	+	-
1209	Earthy / algae	+	+	+	-	-
1233	Sea air / algae	+	+	+	-	-
1276	Sea air / salt water	-	+	+	-	-
1326	Stock cubes	+	+	+	+	+
1342	Rock pool / salt air / medicinal	+	+	-	-	-
1363	Pineapple	+	+	-	-	-
1371	Sea air / salt air	-	+	+	-	-
1439	Sea air/rockpool/algae	+	+	+	-	-
1503	Salty / sea air	+	+	+	+	-
1579	Salty / sea air	+	+	+	+	+
1601	Seaweed	+	+	+	-	-
1658	Rockpool	-	+	-	-	+

[a] LRI = Linear Retention Index

[b] PP = *Penaeus plebejus;* PE = *Penaeus esculentus;* PL = *Penaeus latisulcatus;* PM = *Penaeus monodon*; PS = *Penaeus stylirostris*.

[c] ' + ' = Detected in sample; ' - ' = Not detected in sample

[d] Approximate values only

Qualitative analysis of prawn extracts - Three species of prawns, *P. plebejus*, *P. latisulcatus* and cultivated *P. monodon* were selected for qualitative analysis of their volatile extracts. However, the use of the data system to search the spectra generated by the mass spectrometer failed to provide any information on the identities of the 12 target compounds. Compounds identified by the data system included lipid degradation products (aliphatic ketones and aldehydes), aromatic and aliphatic hydrocarbons, and a series of monoterpenoids. Accordingly, the computer generated spectral data was manually searched in those regions of the Reconstructed Ion Chromatograms in which the target compounds would occur. With the exception of 2-bromophenol, which was identified in the extract of *P. plebejus*, no other compound at the designated LRI values (Table 2) could be identified from the available mass spectral data. Either the spectra were too weak for identification or the compounds identified did not possess the odours detected in the sensory GC analyses. For example, 2-undecanone was identified at LRI 1277, but this compound has a fruity soapy odour not an odour of sea air or salt water (Table 2).

Table 2 Target compounds identified in wild-harvested prawns

LRI	Odour Description	Compound [a]
508	Onions / prawns	Unknown
555	Fried onions	Unknown
973	Cooked prawns / seaweed	Unknown
1026	Green	Unknown
1038	Salty / sweet / seaweed	2-Bromophenol
1209	Earthy / algae	Unknown
1232	Sea air / algae	4-Bromophenol
1277	Sea air / salt water	Unknown
1343	Rock pool / sea air / medicinal	2,6-Dibromophenol
1371	Sea air / salt air	Unknown
1439	Sea air / rock pool / algae	Unknown
1601	Seaweed	2,4,6-Tribromophenol

[a] Compounds identified by multiple ion detection GC-MS.

2,4-Dibromophenol was detected at LRI 1310

As odours characteristic of bromophenols were detected during the sensory GC analyses at LRI values 1038, 1232, 1343 and 1601 (Table 2), the three prawn

extracts were reanalysed using the more sensitive and selective technique of multiple ion detection-mass spectrometry. Results from the analyses showed that all five bromophenols (including 2,4-dibromophenol not previously detected by sensory GC analysis) were present in the extracts of *P. plebejus* and *P. latisulcatus*, while 2,6-dibromophenol and 2,4,6-tribromophenol were found in the extract of *P. monodon*. It can only be assumed that the concentrations of these compounds in *P. monodon* were below their threshold levels in the extract used for sensory GC analysis.

As a result of the above multiple ion detection GC-MS analyses, four of the 12 targeted odorous compounds were identified as 2-bromophenol (LRI 1038), 4-bromophenol (LRI 1232), 2,6-dibromophenol (LRI 1343) and 2,4,6-tribromophenol (LRI 1601). However, of the remaining eight compounds little information, other than their LRI values, was forthcoming. No mass spectral data were obtained even though the spectral information acquired from these analyses was examined thoroughly.

Accordingly, a major research project, such as that required originally to identify bromophenols in prawns (Whitfield *et al*., 1988) would be required to obtain additional data on these eight compounds. Thus the remainder of this project was directed towards defining the role of bromophenols in seafoods, and towards identifying the sources of these compounds in the diets of marine animals and in the environment in which these animals live.

Quantitative analysis of prawn extracts - A total of 39 samples representing ten species of prawns were analysed for their bromophenol content. Three species received greatest attention because of their availability in the Sydney area. These were *P. plebejus* (13 samples), *P. esculentus* (9 samples) and the cultivated species *P. monodon* (8 samples). Of the remaining seven species, only one or two samples of each were subjected to analysis. Results from these bromophenol analyses are recorded in Tables 3 to 6.

Table 3 Distribution of bromophenols in uncooked *Penaeus plebejus*

Code Number	Sample	Bromophenols (ng/g)					
		2-BP [a]	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
PP 8993	heads	0.12	0.54	0.54	0.89	trace [b]	2
	tails	ND [c]	0.22	0.68	0.09	ND	1
PP 5194	heads	0.42	530	690	3	41	1264
	tails	0.13	33	103	0.42	1.6	138
PP 161193	heads	42	158	610	ND	trace	810
	tails	2	80	160	ND	0.12	242
PP 17593	heads	3.5	290	450	5.9	150	899
	tails	0.81	129	152	0.73	30	313
PP 24394	heads	13	110	430	1.5	23	578
	tails	1.1	1.5	25	ND	1.8	29
PP 11195	heads	0.14	2.5	8.5	2.6	8.2	22
	tails	0.08	2.1	3.5	0.12	5.3	11
PP 3295	heads	2.9	408	155	4.3	36	606
	tails	1.2	0.12	58	2.7	19	81
PP 7395	heads	0.66	18	4.3	1.3	4.4	29
	tails	0.67	15	1.8	0.09	0.84	18
PP 22395	heads	1.2	70	23	5.8	48	148
	tails	0.02	3.3	1.6	0.04	0.74	6
PP 23395	heads	0.24	9	20	1.1	9.8	40
	tails	0.08	2.2	7.8	ND	4.4	14
PP 28395	heads	0.21	6.5	7.4	ND	2.3	16
	tails	0.02	2	1.5	0.11	1.8	5
PP 5495	heads	0.07	0.12	3.3	7.6	4.2	15
	tails	ND	0.51	0.99	2.2	1.7	5
PP 18495	heads	0.3	56	9.8	0.93	1.8	69
	tails	0.03	15	2.8	0.07	0.64	19

[a] 2-BP = 2-Bromophenol; 4-BP = 4-Bromophenol; 2,4-DBP = 2,4-Dibromophenol
2,6-DBP = 2,6-Dibromophenol; 2,4,6-TBP = 2,4,6-Tribromophenol.
[b] trace = 0.01 ng/g; [c] ND = Not Detected at a detection limit of 0.01 ng/g

Table 4 Distribution of bromophenols in uncooked *Penaeus esculentus* and *Penaeus latisulcatus*.

Code Number	Sample	Bromophenols (ng/g)					
		2-BP [a]	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Penaeus esculentus							
PE 31593	heads	0.19	1.4	48	2.0	4.8	56
	tails	0.21	0.63	11	0.35	0.01	12
PE 1693	heads	0.15	2.4	150	1.4	1.0	155
	tails	0.07	0.9	37	ND [a]	0.05	38
PE 61293	heads	0.4	3.1	20	2.0	ND	26
	tails	0.12	0.1	5.9	0.17	ND	6
PE 131293	heads	ND	45	216	ND	ND	261
	tails	0.04	0.8	19	0.09	ND	20
PE 7394	heads	0.3	13	25	1.0	4.2	44
	tails	0.1	1.0	5.8	0.2	0.5	8
PE 24394	heads	0.7	60	289	1.2	9.1	360
	tails	ND	14	32	0.6	ND	47
PE 131294	heads	0.57	1.4	26	2.8	3.0	34
	tails	ND	0.61	7.8	0.55	0.57	10
PE 22395	heads	0.08	0.53	6.5	1.7	159	168
	tails	ND	2.0	2.0	ND	128	132
PE 23395	heads	0.14	5.2	15	0.55	82	103
	tails	0.03	0.75	2.2	0.05	6.2	9
Penaeus latisulcatus							
PL 161193[b]	heads	0.68	50	25	8.5	4.7	89
	tails	0.15	2.1	5.0	ND	0.1	7
PL 191294	heads	1.0	850	1100	ND	4.9	1956
	tails	ND	210	83	1.0	2.0	296

[a] ND = Not Detected at a detection limit of 0.01 ng/g

Table 5 Distribution of bromophenols in uncooked *Penaeus merguiensis*, *Penaeus australiensis*, *Metapenaeus macleayi, Haliporoides sibogae,* and *Plesionika martia.*

Code Number	Sample	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Penaeus merguiensis							
PMe 7694	heads	0.94	510	630	1.4	103	1245
	tails	0.14	20	100	ND	4	124
Penaeus monodon							
PM 23596	heads	trace [a]	3	5	6	460	474
	tails	trace	0.3	0.6	0.2	12	13
Penaeus australiensis							
PA 22396	heads	0.2	1	0.8	2	25	29
	tails	0.1	0.3	0.2	0.6	1	2.2
Metapenaeus macleayi							
MM 25396	heads	0.1	0.3	9	0.9	28	38
	tails	0.04	ND [b]	4	0.4	5	9.4
Haliporoides sibogae							
HS 27696	heads	2.6	0.05	37	42	50	132
	tails	0.61	1.5	12	27	14	55
Plesionika martia							
PMa 27696	heads	0.09	ND	6.6	6.3	30	43
	tails	0.13	2.6	2	3.4	8.7	17

[a] trace = 0.01 ng/g; [b] ND = Not Detected at a detection limit of 0.01 ng/g

The data presented in Tables 3,4 and 5 show the distribution of bromophenols in wild-harvested prawns. Examination of this data demonstrates that the concentration of individual bromophenols varied greatly. The dominant compounds were 2,4-dibromophenol (major component on 15 occasions), 2,4,6-tribromophenol (eight occasions), 4-bromophenol (six occasions) and 2,6-dibromophenol (two occasions). Furthermore, the concentration of 2,4-dibromophenol exceeded 100 ng/g in prawn head samples on 10 occasions (150-1100 ng/g), 2,4,6-tribromophenol on four occasions (103-460 ng/g) and 4-bromophenol on seven occasions (110-850 ng/g). In those samples where 2,4-dibromophenol was the dominant compound, 4-bromophenol had the next highest concentration on eleven occasions. Such a relationship would suggest that these two compounds are derived from a common

source. However, no similar relationship was observed when 2,4,6-tribromophenol was the dominant component. On the two occasions that 2,6-dibromophenol was the major bromophenol, the total bromophenol content was low (1.5 and 9.4 ng/g). Consequently, in these samples, the concentration of 2,6-dibromophenol was only 0.89 and 7.6 ng/g respectively. Thus with few exceptions, there is no obvious pattern to the bromophenol content of wild-harvested prawns.

By comparison, the patterns of bromophenols present in cultivated prawns (Table 6) were qualitatively and quantitatively very similar throughout the nine samples analysed. However, in these animals the total bromophenol content was extremely low (average 0.7 ng/g) in comparison with that found in wild-harvested prawns (average 168 ng/g); see Tables 7 and 8. As a consequence it is difficult to establish a trend in bromophenol composition in the cultivated prawns or to select the dominant components. A likely explanation for the uniformity of bromophenol composition of these animals as compared with that of wild-harvested prawns is the relative compositions of 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.

Examination of the data in Tables 3 to 6 shows that in all samples the total bromophenol content in the head component was greater than that in the tail. This finding strongly supports the opinion expressed in earlier publications (Whitfield *et al*., 1988; Anthoni *et al*., 1990) that the bromophenols are derived from the animal's diet. In samples of wild-harvested prawns (Table 7), the ratio of the bromophenol concentrations in the heads and tails varied between 1.3:1 (*P. esculentus* PE 22395) and 36:1 (*P. monodon* PM 23596) while in the cultivated animals (Table 8) the ratios varied between 1.1:1 (*P. stylirostris* P 181193) and 8.6:1 (*P. monodon* PM 61293). This variation in ratio may well provide an indication as to how recently the animals had fed before being harvested.

Table 6 Distribution of bromophenols in uncooked *Penaeus monodon* and *Penaeus stylirostris.*

Code Number	Sample	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Penaeus monodon							
PM 10593	head	trace [a]	ND [b]	0.18	0.42	0.22	0.82
	tail	ND	ND	0.11	0.35	0.22	0.68
PM 12593	head	ND	ND	0.11	0.13	0.92	1.2
	tail	ND	ND	0.11	0.35	0.22	0.68
PM 61293	head	ND	ND	0.16	0.7	ND	0.86
	tail	ND	ND	0.03	0.07	ND	0.1
PM 4294	head	ND	ND	0.14	0.71	ND	0.85
	tail	ND	ND	0.04	0.28	ND	0.32
PM 25894	head	ND	ND	0.16	0.4	0.5	1.1
	tail	ND	0.06	ND	0.08	ND	0.14
PM 9594	head	ND	ND	0.22	0.56	0.65	1.4
	tail	ND	ND	ND	ND	ND	ND
PM 5495	head	0.02	ND	0.12	0.13	0.17	0.42
	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
	tail	0.01	ND	0.06	0.06	0.11	0.24
(Wild Harvested)							
PM 23596	head	trace	3.0	5.0	6.0	460	474
	tail	trace	0.3	0.6	0.2	12	13.1
Penaeus stylirostris (Cultivated)							
PS 181193	head	ND	ND	0.38	1.0	ND	1.4
	tail	ND	0.56	0.59	0.11	ND	1.3

[a] trace = 0.01 ng/g; [b] ND = Not Detected at a detection limit of 0.01 ng/g

Table 7 Distribution of bromophenols in whole uncooked wild-harvested prawns

Species / Code Number	Ratio	Bromophenols (ng/g)					
	heads / tails	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Penaeus plebejus							
PP 17593	2.9	2.0	202	286	0.67	84	575
PP 8993	2.1	0.05	0.36	0.62	0.44	trace[a]	1.5
PP 161193	3.3	19	114	358	ND[b]	0.07	491
PP 5194	9.1	0.25	238	298	1.5	1.8	540
PP 24394	20	6.2	48	200	0.65	11	266
PP 11195	1.8	0.11	2.3	5.6	1.2	6.5	16
PP 3295	7.5	1.9	180	101	3.4	26	312
PP 7395	1.6	0.67	16	2.7	0.52	2.1	22
PP 22395	2.6	0.5	30	10	2.4	20	63
PP 23395	2.9	0.15	5.1	13	0.47	6.7	25
PP 28395	3.0	0.11	4.1	4.2	0.06	2.0	10
PP 5495	2.8	0.03	0.34	2.0	4.4	2.7	9.5
PP 18495	3.6	0.15	34	6.0	0.47	1.2	42
Penaeus esculentus							
PE 31593	4.7	0.2	0.85	22	0.82	1.4	25
PE 1693	4.1	0.09	1.4	72	0.44	0.35	74
PE 61293	4.1	0.22	1.2	11	0.84	ND	13
PE 131293	13	0.02	18	96	0.05	ND	114
PE 7394	5.8	0.18	5.8	14	0.52	2.0	23
PE 24394	7.7	0.27	31	129	0.83	3.5	165
PE 131294	3.6	0.24	0.93	15	1.5	0.94	19
PE 22395	1.3	0.03	1.4	3.7	0.66	140	146
PE 23395	11	0.3	2.6	7.7	0.48	34	45
Penaeus latisulcatus							
PL 161193	12	0.34	19	12	3.1	1.8	36
PL 191294	6.7	0.43	580	530	0.56	3.3	1114
Penaeus marguiensis							
PMe 7694	10	0.46	215	311	0.56	43	570
Penaeus monodon							
PM 23596	36	trace	1.3	2.2	2.3	174	180
Penaeus australiensis							
PA 22396	13	0.14	0.58	0.43	1.1	10	12
Metapenaeus macleayi							
MM 25396	4.0	0.06	0.1	5.6	0.56	12	18
Haliporoides sibogae							
HS 27696	2.4	1.5	0.83	23	34	31	90
Plesionika martia							
PMa 27696	2.5	0.11	1.6	3.9	4.6	17	27
Average Values	6.8	1.2	60.5	87.7	2.4	17.9	168

[a] trace = 0.01 ng/g; [b] ND = Not Detected at a detection limit of 0.01 ng/g

In addition to providing details of the above ratios, Tables 7 and 8 also record the concentrations of individual bromophenols in whole prawns, together with their total bromophenol content. The average total bromophenol content of wild-harvested prawns was 168 ng/g and that of cultivated prawns was 0.6 ng/g; equivalent to only 0.4 percent of that found in the wild-animals. Individual bromophenols were all present in cultivated prawns in average concentrations less than 1 percent of that found in wild-harvested animals: 2,4-dibromophenol (0.002 percent), 4-bromophenol (0.1 percent), 2-bromophenol (0.8 percent) and 2,4,6-tribromophenol (0.9 percent). Only 2,6-dibromophenol was present in the cultivated material in significantly higher concentrations, of the order of 12 percent (Tables 7 and 8). A surprising observation was the ratio of 2,4-dibromophenol and 2,6-dibromophenol in cultivated and wild-harvested prawns; in the former the ration was 1:2 whereas in the latter it was 29:1. This finding further indicates a major difference in the dietary intake of bromophenols in cultivated and wild-harvested animals.

Table 8 Distribution of bromophenols in whole uncooked cultivated prawns

Species/Code Number	Ratio	Bromophenol (ng/g)					
	heads / tails	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Penaeus monodon							
PM 10593	1.2	trace [a]	ND [b]	0.14	0.38	0.22	0.7
PM 12593	1.8	ND	ND	0.11	0.26	0.53	0.9
PM 61293	8.6	ND	ND	0.08	0.33	ND	0.4
PM 4294	2.7	ND	ND	0.07	0.42	ND	0.5
PM 9594	ND	ND	ND	0.1	0.24	0.28	0.6
PM 25894	7.6	ND	0.03	0.07	0.23	0.23	0.6
PM 5495	1.8	0.01	0.01	0.07	0.09	0.13	0.3
PM 17595	1.8	0.02	ND	0.09	0.09	0.14	0.3
Penaeus stylirostris							
PS 181193	1.1	ND	0.31	0.5	0.51	ND	1.3
Average Values	3.0	trace	0.04	0.14	0.28	0.17	0.6

[a] trace = 0.01 ng/g; [b] ND = Not Detected at a detection limit of 0.01 ng/g

Sensory taste panel analyses of cooked whole prawns - Of the eleven samples of wild-harvested prawns assessed, ten were described as possessing prawn-like flavours. By comparison, only one of the five cultivated samples was reported as

possessing this flavour (Table 9). Furthermore, six of the wild prawns were also described as having ocean-like flavours whereas none of the samples of cultivated prawn was described as having this flavour. With regard to sweetness, seven of the eleven wild prawns were described as sweet, as were four of the five cultivated samples. Only on one occasion was the term bland used to describe the flavour of a wild prawn but this term was used on four occasions to describe the flavour of the cultivated animals (Table 9). Based on the above sensory analyses, wild prawns are perceived as having sweet, ocean and prawn-like flavours while the flavour of the cultivated animal is regarded as being sweet but bland.

Studies by Boyle and co-workers (1992b) have shown that the presence of low concentrations of mono-, di- and tri-bromophenols in fish, shrimp and vegetable oil matrixes imparted flavour notes reminiscent of marine fish and seafoods. In fish and shrimp muscle tissue and in oil, 2,6-dibromophenol and 2,4,6-tribromophenol provided iodine-, shrimp- (prawn-), crab- and sea salt-like flavour attributes, while 2- and 4-bromophenols enhances sweetness and overall seafood-like characteristics. When a combination of 2-bromophenol (0.5 ng/g), 2,6-dibromophenol (0.1 ng/g) and 2,4,6-tribromophenol (0.5 ng/g) was added to bland whitefish, these authors found the product had a slightly crab-, iodine- or a full sea fish-like flavour. Thus the presence of these three compounds in a combined concentration of 1.1 ng/g could be expected to enhance the natural ocean flavour of seafoods. This concentration is greater than that found in any of the five samples of cultivated prawns subjected to sensory analysis or in any of the nine samples recorded in Table 8. By comparison, this combined concentration of 1.1 ng/g was exceeded in all eleven wild-harvested prawns reported in Table 9, although in sample *P. plebejus* PP 161193 the concentrations of 2,6-dibromophenol and 2,4,6-tribromophenol were less than that specified by Boyle and co-workers (1992b). Furthermore, only two of the 30 wild-harvested samples reported in Table 7 had values less than 1.1 ng/g. The absence of prawn- and ocean-like flavours in the samples of cultivated prawns can thus be

Table 9 Taste panel assessment of cooked wild-harvested and cultivated prawns

Species / Code Number	TBP ng / g	Flavour descriptions
Wild-Harvested		
Penaeus plebejus		
PP 17593	575	Slightly bitter, briney, ocean and prawn flavour
PP 161193	491	Slightly bitter, fishy, average prawn flavour
PP 24394	266	Sweet, briney, good prawn flavour
PP 11195	16	Sweet, salty good prawn and ocean flavour
PP 23395	25	Sweet, mild ocean and prawn flavour
PP 28395	11	Sweet, mild ocean and prawn flavour
PP 5495	9.5	Sweet, fresh ocean and prawn flavour
PP 18495	42	Sweet, slightly briney, good prawn flavour
Penaeus esculentus		
PE 7394	23	Bland, no off-flavours
PE 24394	165	Sweet, slightly briney, good prawn flavour
PE 131294	19	Fresh ocean and prawn flavour, low in sweetness
Cultivated		
Penaeus monodon		
PM 4294	0.5	Sweet, bland, absence of ocean flavour
PM 9594	0.6	Sweet, bland, absence of prawn flavour
PM 25894	0.6	Sweet, fresh prawn, absence of ocean flavour
PM 5495	0.3	Slightly sweet, bland, absence of prawn flavour
PM 17595	0.3	Sweet, bland, absence of prawn flavour.

explained, at least in part, by the observed low levels of these three bromophenols. With the exception of one sample (*P. esculentus* PE 7394), the wild prawns reported in Table 9 were consistently described as possessing prawn-, ocean- or brine-like flavours. All of these observed flavours can be attributed to the quantities of 2-bromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol present in these samples (Boyle *et al*, 1992b). Furthermore, it has been observed that 4-bromophenol can impart a bitter taste to whitefish flesh when present in concentrations greater than 20 ng/g (Boyle *et al*., 1992b). It is therefore significant that the wild-harvested prawns were described as being slightly bitter (Table 9) when 4-bromophenol was present in concentrations greater than 100 ng/g (see Table 7).

Based on the above analytical and sensory data, bromophenols, particularly 2-bromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol, enhance the desirable seafood flavours of wild-harvested prawns. Conversely, the near absence of these compounds from cultivated material leaves the flesh bland and lacking prawn-like flavours. However, high concentrations of 4-bromophenol (greater than 100 ng/g) can add a slight bitter taste to the flesh, while 2,6-dibromophenol, in similar concentrations, can produce a strong iodoform-like flavour (Whitfield *et al*., 1988). The presence of bromophenols, and their concentrations in prawns, can thus have a significant effect on the perceived flavour of these animals, which in turn can determine their acceptability in the market place.

Quantitative analysis of fish extracts - A total of 44 samples, covering 32 species of ocean fish, were analysed for their bromophenol content. The results from these analyses are recorded in Tables 10, 11 and 12. For ease of presentation, the species have been categorised according to their dietary habits, namely piscivorous and benthic carnivores, and diverse and restrictive omnivores. Of the 44 samples analysed, only six samples were found not to contain any bromophenols; three of these were piscivorous carnivores and the other three were benthic carnivores. In the remaining 38 samples the total bromophenol content varied between trace quantities (*Pterygotrigla polyommata*) and 434 ng/g (*Sillago bassensis*). Of these, 21 samples had total bromophenol concentrations in excess of 2 ng/g and nine samples were in excess of 10 ng/g. In terms of categorised species only the piscivorous carnivores contained less than 1 ng/g of these compounds (Table 10). The greatest total concentrations of bromophenols were found in the benthic carnivores: *Sillago bassensis* (434 ng/g), *Nemodactylus douglasii* (176 ng/g), *Nemadactylus macropterus* (51 ng/g) and *Paristiopterus labiosus* (48 ng/g) (Table 11). These were followed by the diverse omnivores: *Rhabdosargus sarba* (18 ng/g) and *Pseudocaranx dentex* (17 ng/g) (Table 12). In restricted omnivores, the greatest concentration was found in *Girella tricuspidata* (11 ng/g) (Table 12). In whole prawns the total concentrations of 2-bromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol exceeded 1.1 ng/g in 93 percent of the wild-harvested animals analysed. However, in the flesh of fish this value was only exceeded in eight

samples. Another two samples had values of 1.0

Table 10 Distribution of bromophenols in uncooked ocean fish (piscivorous carnivores)

Species/Code Number	Sample	Bromophenols (ng/g)						
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Whole fish
Zeus faber	gut	ND [a]	ND	ND	ND	ND	ND	ND
(ZF 94)	flesh	ND	ND	ND	ND	ND	ND	
Zeus faber	gut	ND	ND	0.28	0.15	1.7	2.1	0.78
(ZF 95)	flesh	ND	ND	ND	ND	0.22	0.22	
Pseudorhombus arsius	gut	ND	ND	ND	ND	ND	ND	ND
(PA 94)	flesh	ND	ND	ND	ND	ND	ND	
Pseudorhombus arsius	gut	ND	ND	ND	0.32	ND	0.32	0.26
(PA 95)	flesh	ND	ND	0.05	0.05	0.15	0.25	
Seriollela punctata	gut	ND	0.2	0.36	0.12	0.46	1.1	0.41
(SP 95)	flesh	ND	ND	0.05	ND	0.12	0.17	
Genypterus blacodes	gut	ND	ND	ND	ND	ND	ND	ND
(GB 95)	flesh	ND	ND	ND	ND	ND	ND	

[a] ND = Not Detected at a detection limit of 0.01 ng/g.

and 1.01 ng/g. Thus these eight or ten samples of fish would have full sea fish-like flavour. However, all samples of fish that contained measurable concentrations of bromophenols could be expected to possess some ocean-like flavour (Boyle *et al*., 1992b) whereas those devoid of these compounds would be bland by comparison.

(b) ***Analysis of diets and the environment***

Reported diets of prawns and fish - Of the nine species of wild-harvested prawns analysed for bromophenol content, only five have been extensively studied to establish the components of their natural diets (Moriarty and Barclay, 1981; Wassenberg and Hill, 1987). Basic components of these diets have been shown to be crustaceans, polychaetes, foraminifera (protozoa), molluscs and detritus (Table 13). By comparison, the diets of fish have received greater attention, mainly due to work undertaken in Botany Bay NSW (Anon, 1981). Thus of the 32 species of fish analysed, information was available on the diets of 29, from either published

Table 11 Distribution of bromophenols in uncooked ocean fish (benthic carnivores)

Species / Code Number	Sample	Bromophenols (ng/g)						
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Whole fish
Nemadactylus douglasii	gut	2.6	100	80	5.8	170	358	40
(ND 94)	flesh	ND [a]	trace [b]	0.1	trace	3.4	3.5	
Nemadactylus douglasii	gut	27	305	315	3.9	234	885	176
(ND 95)	flesh	5.2	17	23	0.6	12	58	
Nemadactylus macropterus	gut	1.0	241	14	2.6	22	281	51
(ND 95)	flesh	0.07	7.4	0.4	0.1	0.4	8.4	
Branchiostegus wardi	gut	5.2	22	78	5.7	100	211	24
(BW 94)	flesh	0.1	0.2	0.4	0.1	0.4	1.2	
Pseudorhombus jenynsii	gut	ND	ND	ND	0.4	37	37	5.0
(PJ 94)	flesh	ND	ND	ND	ND	ND	ND	
Pseudorhombus jenynsii	gut	ND	ND	0.18	0.16	0.53	0.81	0.6
(PJ 95)	flesh	0.03	ND	0.11	0.06	0.34	0.54	
Chelidonichthys kumu	gut	ND	ND	2.6	ND	2.8	5.4	0.8
(CK 94)	flesh	ND	ND	ND	ND	0.4	0.4	
Chelidonichthys kumu	gut	ND	ND	18	0.75	12	31	7.0
(CK 95)	flesh	0.03	ND	1.5	ND	1.8	3.3	
Platycephalus arenarius	gut	trace	ND	ND	3.3	ND	3.3	0.8
(PA 94)	flesh	0.1	ND	trace	0.1	ND	0.2	
Platycephalus marmoratus	gut	ND	ND	ND	ND	ND	ND	ND
(PM 94)	flesh	ND	ND	ND	ND	ND	ND	
Platycephalus marmoratus	gut	ND	1.1	0.34	10	ND	11	3.0
(PM 95)	flesh	ND	ND	0.53	ND	0.61	1.1	
Platycephalus richardsoni	gut	ND	ND	ND	ND	ND	ND	ND
(PR 94)	flesh	ND	ND	ND	ND	ND	ND	
Platycephalus richardsoni	gut	ND	0.02	0.61	ND	1.8	2.4	2.0
(PR 95)	flesh	ND	1.6	0.14	ND	0.24	2.0	
Platycephalus caeruleopunctatus	gut	ND	ND	2.3	0.21	3.5	6.0	3.0
(PC 95)	flesh	0.03	ND	1.2	0.09	0.64	2.0	
Centroberyx affinis	gut	ND	ND	ND	ND	6.1	6.1	0.9
(CA 94)	flesh	trace	trace	ND	trace	0.5	0.5	
Centroberyx affinis	gut	0.2	ND	3.31	0.79	11	15	3.0
(CA 95)	flesh	0.05	ND	0.39	0.1	0.91	1.5	

Table 11 cont.

Species / Code Number	Sample	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Whole fish
Helicolenus percoides	gut	0.2	0.2	0.5	0.8	2.6	4.3	0.8
(HP 94)	flesh	trace	ND	trace	trace	0.3	0.3	
Nelusetta ayraudi	gut	trace	ND	0.1	0.1	0.5	0.7	0.2
(NA 94)	flesh	trace	ND	trace	trace	0.1	0.1	
Nelusetta ayraudi	gut	1.1	0.5	1.2	0.6	8.5	12	5.0
(NA 95)	flesh	0.3	ND	0.5	0.1	1.8	2.7	
Pagrus auratus	gut	ND	ND	0.3	ND	ND	0.3	0.1
(PA 94)	flesh	trace	ND	0.1	trace	ND	0.1	
Pterygotrigla polyommata	gut	trace	ND	ND	ND	ND	trace	trace
(PP 94)	flesh	trace	ND	ND	ND	ND	trace	
Pterygotrigla polyommata	gut	ND	ND	0.54	ND	4.3	4.8	1.0
(PP 95)	flesh	ND	ND	ND	ND	0.34	0.34	
Sillago bassensis	gut	0.57	2279	17	4.0	57	2358	434
(SB 95)	flesh	0.04	46	0.9	0.26	2.4	50	
Paristiopterus labiosus	gut	ND	10	78	0.72	103	192	48
(PL 95)	flesh	ND	ND	3.4	0.2	3.1	6.7	
Upeneichthys lineatus	gut	ND	1.9	4.3	0.49	6.8	13	2.0
(UL 95)	flesh	ND	ND	0.16	ND	ND	0.16	
Pseudorhombus tenuirastrum	gut	ND	ND	ND	1.2	3.2	4.4	2.0
(PT 95)	flesh	0.05	ND	0.61	0.14	0.82	1.6	
Branchiostegus serratus	gut	ND	ND	2.8	ND	3.1	5.9	0.6
(BS 95)	flesh	0.03	ND	0.2	ND	ND	0.23	
Allotaius spariformes	gut	ND	ND	ND	ND	ND	ND	ND
(AS 94)	flesh	ND	ND	ND	ND	ND	ND	

[a] ND = Not Detected at a detection limit of 0.01 ng/g.

[b] trace = 0.01 ng/g

sources or museum records. As with prawns, dietary components of these fish included crustaceans, polychaetes and molluscs together with other fish (such as teleosts), algae, seagrass, bryozoans, sponges, gastropods and detritus (Tables 14 and 15). Bromophenols have been identified in five of these dietary components: polychaetes (Weber and Ernst, 1978), algae (Whitfield *et al*., 1992b), bryozoans and

sponges (Whitfield *et al*., 1992a) and molluscs (Boyle *et al*., 1992b). However, the largest concentrations of these compounds were found in the polychaetes and algae.

Examination of the data in Table 13 showed that the major difference between the diets of wild-harvested and cultivated prawns was the absence of polychaete material from the manufactured prawn feed. Prawn and squid meal provide contributions from both crustaceans and molluscs. It was thus inferred that the absence of a polychaete component in the diet was a possible reason for the low bromophenol content of the cultivated animals. Similarly, amongst the fish species analysed, the piscivorous

Table 12 Distribution of bromophenols in uncooked ocean fish (diverse and restricted omnivores)

Species / Code Number	Sample	Bromophenols (ng/g)						
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Whole fish
Diverse Omnivores								
Rhabdosargus sarba	gut	ND [a]	ND	150	ND	trace	150	18
(RS 94)	flesh	ND	ND	2.0	trace	0.1	2.1	
Acanthopagrus australis	gut	1.2	ND	2.2	18	55	76	8.0
(AA 94)	flesh	ND	ND	0.1	0.2	trace	0.3	
Pseudocaranx dentex	gut	0.6	0.5	5.8	18	7.2	32	7.0
(PD 94)	flesh	0.1	ND	ND	0.6	ND	0.7	
Pseudocaranx dentex	gut	0.17	56	7.3	1.2	26	91	17
(PD 95)	flesh	0.03	1.2	0.36	0.07	1.3	3	
Meuschenia trachylepsis	gut	0.2	0.7	1.5	1.0	5.7	9.1	3.0
(MT 94)	flesh	trace	ND	ND	ND	1.4	1.4	
Meuschenia freycineti	gut	0.65	2.2	2.4	1.3	17	24	10
(MF 95)	flesh	0.38	ND	0.26	0.32	4.3	5.3	
Parika scaber	gut	0.1	ND	0.1	0.6	1.1	1.9	0.3
(PS 94)	flesh	trace	ND	ND	ND	trace	trace	
Parika scaber	gut	ND	ND	0.24	0.44	3.31	ND	4.0
(PS 95)	flesh	ND	ND	ND	ND	ND	ND	
Restricted Omnivores								
Girella tricuspidata	gut	0.7	ND	19	5.3	32	57	11
(GT 94)	flesh	ND	ND	0.2	0.2	0.8	1.2	
Kyphosus sydneyanus	gut	0.15	0.83	2.5	2.2	7.0	13	5.0
(KS 95)	flesh	0.02	0.05	0.24	0.16	0.14	0.61	

[a] ND = Not Detected at a detection limit of 0.01 ng/g.

Table 13 Relationship between total bromophenol content (TBC), habitat and diet of individual species of prawns

Species	TBC whole prawns (ng/g)	Habitat [a]	Diet [b]
Wild			
Penaeus merguiensis	580	mud / sand	crustaceans, foraminifera, polychaetes, and molluscs
Penaeus plebejus	183[c]	sand	crustaceans, molluscs, polychaetes and foraminifera
Penaeus monodon	180	mud / sand	crustaceans, polychaetes, foraminifera, and molluscs
Haliporoides sibogae	90	mud	not identified
Penaeus esculentus	69[c]	mud / sand	foraminifera, crustaceans, polychaetes and detritus
Penaeus latisulcatus	575[c]	sand / mud	polychaetes, crustaceans, molluscs and foraminifera
Plesionika martia	27	mud	not identified
Metapenaeus macleayi	18	mud	not identified
Penaeus australiensis	12	mud	not identified
Cultivated			
Penaeus stylirostris	1.3	mud	fish meal, plant material, prawn and squid meal
Penaeus monodon	0.6[c]	mud	fish meal, plant material, prawn and squid meal

[a] Composition of ocean floor, [b] Listed in order of importance
[c] Average value

carnivores, which eat neither polychaetes nor algae, had a far lower bromophenol content than the benthic carnivores, or the diverse and restricted omnivores. As a result of these observations, a survey of polychaetes and algae from the east coast of Australia was undertaken to measure the concentrations of bromophenols present in these aquatic animals and plants. Some data have also been obtained on the levels of bromophenols in bryozoans, sponges, sediment and sea water.

Quantitative analysis of polychaete extracts - Of the 44 polychaete samples analysed (representing 16 species), all contained bromophenols, with concentrations varying greatly between and within species, and between sampling areas (Tables 16 and 17). All five compounds were present in 40 of these samples and 2,4,6-tribromophenol was the dominant compound in most species. The greatest variation in total bromophenol content within a species was observed for *Marphysa sanguinea*

(10106 to 262094 ng/g) and the least for *Scoloplos normalis* (32508 to 60427 ng/g), while the greatest average total bromophenol content was found in *Nephtys australiensis* (357772 ng/g) and the least in *Australonuphis teres* (306 ng/g).

Table 14 Relationship between total bromophenol content (TBC) and diet of individual species of carnivorous fish

Species	TBC whole fish (ng/g)	Diet[a]
Piscivorous Carnivores		
Seriollela punctata	0.41	planktonic tunicates
Zeus faber	0.39[b]	fish
Pseudorhombus arsius	0.13[b]	crustaceans and teleosts
Genypterus blacodes	ND	prawns and fish
Benthic Carnivores		
Sillago bassensis	434	crustaceans, amphipods, decapods, mysidaceans and polychaetes
Nemadactylus douglasii	108[b]	polychaetes, invertebrates and crustaceans
Nemadactylus macropterus	51	polychaetes, invertebrates and crustaceans
Paristiopterus labiosus	48	not identified
Branchiostegus wardi	24	polychaetes, gastropods, bivalves, molluscs, amphipods and fish
Chelidonichthys kumu	3.9[b]	crustaceans and polychaetes
Platycephalus caeruleopunctatus	3	crustaceans, polychaetes, teleosts and molluscs
Pseudorhombus jenynsii	2.8[b]	crustaceans, polychaetes, and teleosts.
Nelusetta ayraudi	2.6[b]	crustaceans, polychaetes and fish
Centroberyx affinis	2.0[b]	fish, crustaceans, polychaetes and zooplankton
Pseudorhombus tenuirastrum	2.0	not identified
Platycephalus marmoratus	1.5[b]	crustaceans, polychaetes and teleosts
Platycephalus richardsoni	1.0[b]	crustaceans, polychaetes and teleosts
Platycephalus arenarius	0.8	crustaceans and teleosts
Helicolenus percoides	0.8	fish, crustaceans and polychaetes
Branchiostegus serratus	0.6	fish, molluscs, polychaetes and crustaceans
Pterygotrigla polyommata	0.5[b]	polychaetes and crustaceans
Allotaius spariformes	ND	

[a] Listed in order of importance, [b] Average value

[c] ND = Not Detected at a detection limit of 0.01 ng/g.

Table 15 Relationship between total bromophenol content (TBC) and diet of individual species of omnivorous fish

Species	TBC whole fish (ng/g)	Diet [a]
Diverse Omnivores		
Rhabdosargus sarba	18	crustaceans, polychaetes, algae, molluscs and gastropods
Pseudocaranx dentex	12[b]	crustaceans, polychaetes, molluscs, algae, and detritus
Meuschenia freycineti	10	crustaceans, algae, seagrasses, bryozoans and polychaetes
Acanthopagrus australis	8.0	polychaetes, algae, crustaceans and molluscs
Meuschenia trachylepsis	3.0	algae, seagrasses, crustaceans, polychaetes, bryozoans
Parika scaber	2.2[b]	sponges, ascidians, hydroids, bryozoans, jellyfish and algae
Upeneichthys lineatus	2.0	detritus, algae, crustaceans and mud
Restricted Omnivores		
Girella tricuspidata	11	algae, zostera, crustaceans and detritus
Kyphosus sydneyanus	5.0	algae, zostera, crustaceans and detritus

[a] Listed in order of importance, [b] Average value

The greatest single concentration of bromophenols was found in a single sample of *Barantolla lepte* from Silver Beach NSW. The bromophenol concentrations in this sample varied from 59 ng/g for 2-bromophenol to 8.3 million ng/g (8.3 mg/g) for 2,4,6-tribromophenol, and the concentrations of 2,4-dibromophenol and 2,6-dibromophenol were appreciably higher (at 11000 ng/g and 32000 ng/g respectively) than in any other sample analysed. Such concentrations, particularly of the dibromophenols, are of interest with respect to the levels of these compounds found in samples of the Western Australian prawn *Metapenaeus endeavouri* (Whitfield *et al*., 1988). Furthermore, the small size of *B. lepte* (30 mm long x 1 mm diameter) may render it more likely to be consumed by prawns than some of the larger polychaete species.

Table 16 Distribution of bromophenols in polychaetes of the families ONUPHIDAE, EUNICIDAE and NEREIDIDAE.

Species	Source	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
ONUPHIDAE							
Australonuphis teres	Crowdy Bay	90	2.4	58	30	60	240
Australonuphis teres	Seal Rocks	8	2.4	35	77	140	262
Australonuphis teres	Crowdy Bay	13	6	34	61	220	334
Australonuphis teres[a]	Seal Rocks	261	0.8	51	32	150	495
Australonuphis teres	Seal Rocks	9	4	14	7	24	58
Australonuphis teres	Seal Rocks	3	3	55	34	350	445
Diopatra dentata	Bateau Bay	27	35	71	48	61	242
Diopatra dentata	Bateau Bay	10	99	169	79	690	1047
EUNICIDAE							
Marphysa sanguinea	Moreton Bay	30	26	5600	43	22000	27699
Marphysa sanguinea	Calabash Bay	25	2	38	41	10000	10106
Marphysa sanguinea	Careel Bay	5	4	283	850	78000	79142
Marphysa sanguinea	Point Clare	63	31	500	1500	260000	262094
Marphysa sanguinea (juv)	Calabash Bay	42	14	345	160	61000	61561
Marphysa macintoshi	Calabash Bay	3	ND[b]	27	64	7900	7994
Marphysa macintoshi	Calabash Bay	30	5	140	51	15000	15226
Marphysa macintoshi	Calabash Bay	47	2	99	9	2700	2857
Marphysa macintoshi	Wynnum	160	1300	1100	19	340	2919
NEREIDIDAE							
Australonereis ehlersi	Calabash Bay	2	ND	44	130	28000	28176
Australonereis ehlersi	Hen & Chicken Bay	32	71	750	2400	95000	98253
Australonereis ehlersi	Careel Bay/Bayview	5	10	410	430	8700	9555
Australonereis ehlersi	Koolewong	18	49	260	65	10000	10392
Ceratonereis aequisetis	Rodd Point	12	23	340	420	32000	32795
Ceratonereis aequisetis	Point Clare	58	ND	2800	3300	940000	946158
Ceratonereis limnetica	Koolewong	3	9	61	24	5800	5897

[a] Adult sample (kingworm) - other samples of *A. teres* were juveniles.
[b] ND - Not Detected at a detection limit of 0.01 ng/g.

The highest concentrations of 2-bromophenol and 4-bromophenol were found in *Glycera americana* (320 and 4200 ng/g respectively). In the "kingworm" *Australonuphis teres*, 2-bromophenol was the major bromophenol present (261 ng/g), as was 4-bromophenol in the sample of *Marphysa macintoshi* from Wynnum (1300 ng/g). The lowest levels of all five bromophenols were found in a sample of

juvenile *A. teres* collected from Seal Rocks NSW. Concentrations in this sample varied from 4 ng/g of 4-bromophenol to 24 ng/g of 2,4,6-tribromophenol. Of all the samples analysed, only *Glycera americana* and *Marphysa macintoshi*, both from Wynnum, had greater concentrations of 4-bromophenol and 2,4-dibromophenol than 2,4,6-tribromophenol. As other samples of *M. macintoshi* did not share this pattern of bromophenols (Table 16) it is possible that an environmental factor in the Wynnum area favours the formation of 4-bromophenol and 2,4-dibromophenol.

The concentrations of bromophenols in *B. lepte* and most other species sampled in the current study were much greater than those found by other workers in polychaetes from European or Northern American waters. Goerke and Weber (1990) found maximum concentrations of 1500 ng/g of 2,4-dibromophenol and 500 ng/g of 2,4,6-tribromophenol in *Lanice conchilega* from the North Sea, while Weber and Ernst (1978) found up to 2300 ng/g of 2,4,6-tribromophenol in the same species. In contrast, Woodin *et al*., (1987) found 6.3 million ng/g of 2,6-dibromophenol in *Arenicola cristata* from the waters of South Carolina. Interestingly, of the eight species analysed by these workers (including *A. cristata*), none were reported to contain 2,4,6-tribromophenol.

An important trend to emerge from the current study was that species collected from muddy areas had much greater concentrations of bromophenols than those collected from sandy beaches or rocky shores (Table 18). As mud would have a greater natural microbial content than sand or rock this would support the view that bromophenols serve as antimicrobial rather than antipredatory agents; the latter view has been suggested by Steward *et al*. (1992). The low levels of bromophenols in *A. teres* and *Diopatra dentata* could be a peculiarity of the family Onuphidae, but could also be related to the relatively low microbial content of the environment that they inhabit. Among the estuarine sites, there appears to be no direct relationship between sediment type and bromophenol levels. A notable exception to this, however, was observed in those polychaetes collected at Point Clare NSW. This site was characterised by very fine mud that had a strong smell of hydrogen sulfide suggesting microbial degradation. A stormwater or sewage overflow pipe also drained onto the

Table 17 Distribution of bromophenols in polychaetes of the families NEPHTYIDAE, GLYCERIDAE, ORBINIIDAE, LUMBRINERIDAE, CAPITELLIDAE, CIRRATULIDAE, and PHYLLODOCIDAE

Species	Source	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
NEPHTYIDAE							
Nephtys australiensis	Calabash Bay	3.0	36	140	240	79000	79419
Nephtys australiensis	Rodd Point	3.0	4.0	79	715	83000	83801
Nephtys australiensis	Koolewong	11	28	460	2300	860000	862799
Nephtys australiensis	Point Clare	20	19	440	2300	800000	802779
Nephtys australiensis	Careel Bay/Bayview	20	27	700	3000	340000	343747
Nephtys australiensis	Calabash Bay	5.0	8.0	160	380	220000	220553
Nephtys australiensis	Silver Beach	16	23	170	1100	110000	111309
GLYCERIDAE							
Glycera americana	Wynnum	320	4200	4600	72	650	9842
ORBINIIDAE							
Scaloplos normalis	Calabash Bay	7.0	100	190	130	60000	60427
Scaloplos normalis	Calabash Bay	10	18	380	100	32000	32508
Scaloplos normalis	Point Clare	ND [a]	ND	2000	4200	54000	60200
LUMBRINERIDAE							
Lumbrineris latreilli	Hen & Chicken Bay	44	55	1200	4900	190000	196199
Lumbrineris latreilli	Bayview	5.0	23	100	160	42000	42288
Lumbrineris latreilli	Careel Bay	6.0	18	1000	1700	250000	252724
Lumbrineris latreilli	Silver Beach	12	18	580	1700	1600000	1602310
CAPITELLIDAE							
Notomastus chrysosetus/torquatus	Calabash Bay	11	29	970	850	380000	381860
Barantolla lepte	Silver Beach	59	97	11000	32000	8300000	8343156
CIRRATULIDAE							
Cirriformia filigera	Silver Beach	42	70	1500	800	130000	132412
Cirriformia cf tentaculata	Silver Beach	15	29	322	340	23000	23706
PHYLLODOCIDAE							
Phyllodoce cf novaehollandiae	Silver Beach	32	55	510	770	160000	161367

[a] ND = Not Detected at a detection limit of 0.01 ng/g.

Table 18 Sediment type at each of the twelve polychaete sampling sites.

Site	Sediment Type
Seal Rocks NSW	Clean, sandy beach
Bateau Bay NSW	Rocky shore, amongst pebbles
Moreton Bay and Wynnum QLD	Estuarine, most likely muddy sand to sandy mud
Hawkesbury River	
Calabash Bay	Mud to sandy mud with detritus. Near mangroves, very eutrophic
Parramatta River	
Rodd Point	Sandy to muddy sand with some shells.
Hen and Chicken Bay	Muddy sand with shell. Near mangrove.
Pittwater	
Careel Bay	Sandy mud amongst seagrass (*Posidonia* and *Zostera*)
Bayview	Sandy to muddy sand, quite clean, no detritus.
Brisbane Waters	
Point Clare	Mud, near mangroves, very eutrophic
Koolewong	Sandy to muddy sand, quite clean.
Botany Bay	
Silver Beach	Muddy sand to sandy mud amongst seagrass

mud flats at the high water mark. Of the four species collected at this site (*M. sanguinea, Ceratonereis aequisetis, Nephtys australiensis* and *Scaloplos normalis*), bromophenol levels were 3-30 times greater than those found in the same species at other sites. This observation would tend to reinforce the view that these compounds are synthesised by polychaetes as antimicrobial agents.

The findings presented in Table 13 demonstrate that polychaetes are major dietary components of wild-harvested prawns. Furthermore, they are also components of many species of ocean fish, both benthic carnivores and diverse omnivores (Tables 14 and 15). Those prawns and fish that ingest polychaetes are also the species with the greatest concentrations of bromophenols and, also in the case of prawns, the species that have the best seafood flavours. Polychaetes must therefore be regarded as a major dietary source of bromophenols in those species of prawns and fish that feed on such benthic animals. However, all of the polychaetes analysed in this current study were taken from the intertidal zone, and worms from deeper waters

should be studied to complete our knowledge of the bromophenol content of Australian polychaetes.

Quantitative analysis of algal extracts - The data obtained from the analysis of 85 samples of marine algae (representing 50 species) are presented in Tables 19 to 22. For ease of presentation, the individual species of algae have been tabulated according to the division to which they belong, namely Rhodophyta, Phaeophyta and Chlorophyta. The exceptions are samples from the genus *Sargassum*, of the division Phaeophyta, which at present cannot be identified with certainty to species level. Data for these samples are reported separately in Table 22. For 36 of the species studied, data were obtained from single collections only, whereas for the remaining 24 species, two or more collections of each were analysed for the target compounds. Where more than one collection was analysed, they were either taken from different geographical locations or harvested over several years from the same site. The selection of a species for multiple collection was determined either because the species was dominant at a particular site or because it was known to be a major food source for omnivorous fish and other marine animals.

*Rhodophyta (red algae)***.** Results from the analysis of 18 species of Rhodophyta are recorded in Table 19. Bromophenols were found in all species and 2,4,6-tribromophenol was the dominant compound in all but one species. All five bromophenols were found in 12 of the species. The highest total bromophenol concentration was found in *Pterocladia capillacea*. Five samples of this species were analysed and the total concentration varied from 1064 to 2590 ng/g. By far the most abundant of these compounds was 2,4,6-tribromophenol, its concentrations varying between 1000 and 1900 ng/g. Other bromophenols present in relatively high concentrations were 2,4-dibromophenol (25 to 320 ng/g) and 2,6-dibromophenol (17 to 440 ng/g). The two monobromophenols were present in only minor concentrations (ND to 39 ng/g). Two of the five samples of *P. capillacea* taken from Bateau Bay were collected from opposite sides of the bay (Samples N91 and S91). These had total bromophenol concentrations of 1064 and 1592 ng/g and the major difference in these results were the relative

concentrations of 2,4-dibromophenol (41 and 170 ng/g) and 2,6-dibromophenol (17 and 180 ng/g). Higher concentrations were found in material from the southern (sunny) side of the bay. Higher total bromophenol concentrations were found in material collected during 1992 and 1993 (1726 and 2091 ng/g respectively) from the southern side of Bateau Bay. The major differences between the 1993 sample and those of 1991 and 1992 was an increase in concentration of 2,4,6-tribromophenol (up to 1900 ng/g) and decrease in the

Table 19 Distribution of bromophenols in eastern Australian red marine algae (Rhodophyta)

Species	Source (year)	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Amphiroa anceps	Bateau Bay (91)	1.6	5.1	26	6.4	1300	1339
Callophycus tridentifer	Bateau Bay (91)	7.6	trace [a]	0.3	1.1	2.7	12
Cheilosporum sagittatum	Bateau Bay (91)	7.1	0.8	27	23	220	278
Chondria succulenta	Batemans Bay (95)	64	2.2	12	36	150	264
Corallina berteri	Bateau Bay (91)	0.1	0.7	9.6	0.1	16	27
Corallina afficinalis	Bateau Bay (91)	0.8	0.5	5.4	0.4	6.6	14
Delisea pulchra (N) [b]	Bateau Bay (91)	10	ND[c]	1.4	68	81	160
Delisea pulchra (S) [d]	Bateau Bay (91)	2.0	ND	5.0	49	96	152
Delisea pulchra	Botany Bay (93)	1.3	ND	24	8.5	47	81
Delisea pulchra	Botany Bay (94)	9.3	ND	1.6	1.6	160	173
Delisea pulchra	Batemans Bay (95)	11	ND	21	14	230	276
Galaxaura marginata	Bateau Bay (92)	ND	ND	19	3.3	18	40
Galaxaura marginata	Bateau Bay (93)	0.02	ND	5.2	2.2	22	29
Galaxaura marginata	Batemans Bay (95)	ND	6.6	11	4.7	41	63
Galaxaura obtusata	Bateau Bay (91)	2.8	ND	18	9.0	96	126
Gracilaria edulis	Botany Bay (93)	3.1	17	18	4.2	78	120
Gracilaria edulis	Botany Bay (93)	0.6	1.4	15	13	37	67
Gracilaria edulis	Botany Bay (94)	0.5	29	65	1.1	28	124
Gracilaria edulis	Botany Bay (94)	0.3	0.1	7.3	10	55	73
Gracilaria secondata	Batemans Bay (95)	2.2	2.5	5.2	4.3	3.0	17
Haliptilon roseum	Bateau Bay (91)	7.2	35	105	26	190	363
Halymenia floresia	Barrier Reef (92)	6.3	ND	6.2	10	72	95
Lomentaria cotenata	Batemans Bay (95)	2.1	1.8	9.0	2.9	110	126
Plocanium angustatum	Batemans Bay (95)	ND	2.9	61	23	250	337
Pterocladia capillacea N [b]	Bateau Bay (91)	6	ND	41	17	1000	1064
Pterocladia capillacea S [d]	Bateau Bay (91)	15	27	170	180	1200	1592
Pterocladia capillacea	Bateau Bay (92)	7.3	39	260	120	1300	1726
Pterocladia capillacea	Bateau Bay (93)	2.3	4	25	160	1900	2091
Pterocladia capillacea	Turimetta Head (94)	ND	30.0	320	440	1800	2590
Pterocladia lucida	Bateau Bay (91)	0.2	0.2	1.2	5.9	39	47
Pterocladia lucida	Batemans Bay (95)	30	ND	29	9.6	110	179
Solieria robusta	Batemans Bay (95)	0.7	2.1	5.2	10	35	53

[a] trace < 0.01 ng/g ; [b] N = northern side ; [c] ND = Not Detected at a detection limit of 0.01 ng/g ; [d] S = southern side.

concentration of 2,4-dibromophenol (down to 25 ng/g). Of interest, the sample of *P. capillacea* from Turimetta Head, about 100 km south of Bateau Bay had the highest

total concentrations of bromophenols (2590 ng/g) and this was principally due to the presence of greater levels of 2,4-dibromophenol (320 ng/g) and 2,6-dibromophenol (440 ng/g).

Only one other species of Rhodophyta contained more than 1000 ng/g of total bromophenols; this was *Amphiroa anceps (Corallinaceae)*. This species had a total bromophenol concentration of 1339 ng/g of which 2,4,6-tribromophenol accounted for 1300 ng/g. The remaining four bromophenols were present in far smaller concentrations (1.6 to 26 ng/g).

Of the remaining 16 species of Rhodophyta, ten had total bromophenol concentrations between 95 and 340 ng/g and in the majority of these species the major bromophenol was 2,4,6-tribromophenol. An exception was one sample of *Gracilaria edulis*, in which the major compound was 2,4-dibromophenol (65 ng/g). This compound was also present in relatively high concentrations in *Haliptilon roseum* (105 ng/g). Included among these ten species was *Delisia pulchra*, one of the most common and widespread algae found along the mid NSW coastline where it grows at depths from 1 to 20 metres throughout the year. In this species the total bromophenol concentration varied from 81 to 276 ng/g. An interesting feature of this algae was the absence of 4-bromophenol from all of the samples analysed. Unlike *P. capillacea*, samples of *D. pulchra* taken from different sides of Bateau Bay gave very similar results. However, samples taken from Botany Bay, about 150 km south of the above site, showed significant differences in total bromophenol concentrations when sampled from different areas of the bay (81 compared with 173 ng/g). The concentrations of 2,4,6-tribromophenol also differed significantly (47 compared with 160 ng/g). In this species, the highest total bromophenol concentration (276 ng/g) and the highest concentration of 2,4,6-tribromophenol (230 ng/g) were found in material from Batemans Bay, about 400 km south of Bateau Bay.

Four samples of *Gracilaria edulis* were collected from two sites in Botany Bay during 1993 and 1994. This species is a known dietary component of the restricted omnivore *Girella tricuspidata*. Of the two samples collected in 1993, a significant

difference in total bromophenol content was observed between the two collection sites (120 compared with 67 ng/g). A similar difference (124 compared with 73 ng/g) was observed in the 1994 collection; however, whereas 2,4,6-tribromophenol was the dominant component in 1993, in one of the 1994 samples it was supplanted by 2,4-dibromophenol. The other 1994 sample had a bromophenol composition very similar to that of the 1993 sample from the same site.

The remaining six species of Rhodophyta had total bromophenol concentrations that varied between 12 and 63 ng/g. Only one of these species, *Galaxaura marginata*, was collected on more than one occasion. In this species the total bromophenol content varied between 29 and 63 ng/g. The bromophenol composition also varied among the three samples. The most obvious variation was the presence of 4-bromophenol (6.6 ng/g) in only one sample.

Phaeophyta (brown algae). The results from the analyses of 13 species of Phaeophyta are recorded in Table 20. All five bromophenols were found in at least one sample of each of 11 species and four bromophenols were found in each of the remaining two species. 2,4,6-Tribromophenol was the dominant compound in all species. The highest total bromophenol concentration was found in *Phyllospora comosa* (234 to 454 ng/g). In this species the concentrations of 2,4,6-tribromophenol varied between 130 and 290 ng/g while 4-bromophenol was the only bromophenol present in significant concentrations in three of the four samples analysed (40 to 140 ng/g). The three remaining bromophenols were present in relatively minor concentrations. Two of the four samples of *P. comosa* taken from the opposite sides of Bateau Bay in 1992 had similar total bromophenol concentrations (234 and 298 ng/g) but had quite different bromophenol compositions. In the sample from the northern side, the concentrations of 4-bromophenol, 2,4- and 2,6-dibromophenol (40, 35, 29 ng/g respectively) were greater than those found on the southern side (3.9, 3.5, 0.7 respectively). However, the concentrations of 2,4,6-tribromophenol were

Table 20 Distribution of bromophenols in eastern Australian brown marine algae (Phaeophyta)

Species	Source (year)	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Cladostephus spongiosus	Bateau Bay (91)	0.02	1.2	41	0.1	6.9	49
Cladostephus spongiosus	Bateau Bay (92)	0.7	8.6	12	2.0	19	42
Cladostephus spongiosus	Bateau Bay (93)	0.3	4.5	5.3	0.9	14	25
Colpomenia sinuosa	Bateau Bay (92)	0.6	6.5	22	ND [a]	16	45
Colpomenia sinuosa	Bateau Bay (93)	3.5	3.4	8.1	2.1	26	43
Cystophora intermedia (N) [b]	Bateau Bay (91)	0.2	ND	0.5	0.1	3.2	4
Cystophora intermedia (S) [c]	Bateau Bay (91)	1.6	0.7	8.8	1.2	1.0	13
Cystophora intermedia	Batemans Bay (95)	2.7	1.5	4.9	1.0	14	24
Cystophora intermedia	Bateau Bay (93)	1.0	ND	2.8	1.0	7.8	13
Cystophora moniliformis	Bateau Bay (93)	ND	trace	1.2	0.3	7.4	9
Cystoseira trinodis	Bateau Bay (93)	trace	0.2	4.7	0.1	3.1	8
Ecklonia radiata	Bateau Bay (92)	1.6	13	26	1.5	190	232
Ecklonia radiata	Bateau Bay (93)	ND	ND	0.2	0.02	13	13
Ecklonia radiata	Botany Bay (94)	ND	0.1	1.2	ND	18	19
Halopteris paniculata	Bateau Bay (91)	0.02	1.8	6.0	0.4	14	22
Halopteris paniculata	Bateau Bay (92)	0.3	3.7	62	0.2	2.8	69
Halopteris platycena	Bateau Bay (92)	1.8	8.7	17	5.5	26	59
Homoeostrichus sinclairii	Bateau Bay (91)	8.4	ND	0.8	71	22	102
Homoeostrichus sinclairii	Bateau Bay (91)	58	8.1	16	2.6	6.4	91
Homoeostrichus sinclairii	Bateau Bay (91)	16	9.0	35	29	63	152
Homoeostrichus sinclairii	Bateau Bay (93)	6.3	ND	8.1	9.1	27	51
Hormosira banksii	Bateau Bay (92)	2.9	3.2	1.8	0.3	32	40
Lobophora variegata	Bateau Bay (93)	0.4	ND	2.1	3.3	15	21
Phyllospora comosa	Bateau Bay (91)	ND	98	55	0.2	280	433
Phyllospora comosa (N)	Bateau Bay (92)	0.3	40	35	29	130	234
Phyllospora comosa (S)	Bateau Bay (92)	0.1	3.9	3.5	0.7	290	298
Phyllospora comosa	Garie Beach (93)	3.0	140	52	9.0	250	454
Sporochnus comosus	Bateau Bay (92)	1.8	2.1	8.7	1.2	18	32

[a] ND = Not Detected at a detection limit of 0.01 ng/g ; [b] N = northern side ;
[c] S = southern side; [d] trace , 0.01 ng/g.

reversed; on the southern side the value was 290 ng/g while on the northern side it was only 130 ng/g. A sample collected in 1991 from the northern side gave a similar pattern of bromophenols as that found in 1992 but the total bromophenol concentration was almost double (433 compared with 234 ng/g). The 1991 sample had greater concentrations of both 4-bromophenol (98 ng/g) and 2,4,6-tribromophenol (280 ng/g). The sample of *P. comosa* from Garie Beach gave very similar results with a total bromophenol concentration of 454 ng/g.

Only one other species of Phaeophyta, *Ecklonia radiata*, was found to have a total bromophenol content in excess of 200 ng/g. This relatively high concentration (232 ng/g) was only found in one sample of this species; the other two samples contained low concentrations of these compounds (13 and 19 ng/g). In the sample with the high bromophenol content the major compound was 2,4,6-tribromophenol (190 ng/g). *E. radiata*, like *P. comosa*, is strictly subtidal and grows at depths from 5 to 10 metres.

Of the remaining 11 species of Phaeophyta studied, only *Homoeostrichus sinclairii* had a total bromophenol content that exceeded 100 ng/g. Two of the three samples collected in 1991 were from opposite sides of the bay while the third sample was collected from a rock pool. As previously observed with *P. capillacea* and *P. comosa*, the sample from the southern side had a higher total concentration of bromophenols (152 ng/g compared with 91 ng/g). The major bromophenol in the southern sample was 2,4,6-tribromophenol (63 ng/g) and that in the northern sample was 2-bromophenol (58 ng/g). Of interest, in the sample from the rock pool the major bromophenol was 2,6-dibromophenol (71 ng/g).

Chlorophyta (green algae). Results from the analysis of 11 species of Chlorophyta are recorded in Table 21. All five bromophenols were found in nine species and four in each of the other two species. As with the other two divisions, Rhodophyta and Phaeophyta, 2,4,6-tribromophenol was the dominant compound in all species. The highest total bromophenol concentration was found in *Enteromorpha intestinalis*. Three samples of this species were analysed and the total concentration varied from 535 to 2393 ng/g. By far the most abundant compound was 2,4,6-tribromophenol, its concentrations varying between 520 and 1400 ng/g. With the exception of the sample collected from Bateau Bay in 1993 all other bromophenols were present in low concentrations (trace to 35 ng/g). However, in the Bateau Bay sample the concentrations of 4-bromophenol (260 ng/g) and 2,4-dibromophenol (640 ng/g) were significantly high. It is interesting to note that the total bromophenol content of the sample from Botany Bay, an industrial port with relatively high levels of hydrocarbon pollution, was much lower than those found in the relatively low

pollution areas of Turimetta Head and Bateau Bay. A similar result was observed for *D. pulchra* (Table 19).

Table 21 Distribution of bromophenols in eastern Australian green marine algae (Chlorophyta)

Species	Source (year)	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Caulerpa cactoides	Bateau Bay (93)	2.8	0.6	94	2.8	19	119
Chlorodesmis major	Barrier Reef (92)	0.8	1.7	150	3.0	7.0	163
Cladophoropsis herpestica	Barrier Reef (92)	0.2	6.8	5.4	1.3	32	46
Codium fragile	Bateau Bay (91)	ND [a]	0.2	0.2	0.03	0.5	0.9
Codium geleatum	Bateau Bay (92)	0.1	0.2	20	3.1	62	85
Codium geleatum	Bateau Bay (93)	2.2	1.2	12	13	39	67
Codium lucasii	Bateau Bay (91)	0.02	ND	34	0.06	1.5	36
Enteromorpha intestinalis	Turimetta Head (94)	0.5	ND	35	5.6	1300	1341
Enteromorpha intestinalis	Botany Bay (93)	trace [b]	2.7	11.0	1.4	520	535
Enteromorpha intestinalis	Bateau Bay (93)	18	260	640	75	1400	2393
Halimeda cuneata	Barrier Reef (92)	13	1.0	15	1.1	41	71
Halimeda discoida	Barrier Reef (92)	0.03	6.6	8.6	2.0	31	48
Halimeda opuntia	Barrier Reef (92)	0.04	1.7	11	3.0	17	33
Ulva lactuca	Bateau Bay (91)	1.5	6.6	130	1.1	400	539
Ulva lactuca	Bateau Bay (93)	0.1	2.2	25	1.2	1200	1229
Ulva lactuca	Turimetta Head (94)	0.2	0.4	47	3.3	390	441
Ulva lactuca	Botany Bay (93)	0.1	ND	6.2	1.2	420	428

[a] ND = Not Detected at a detection limit of 0.01 ng/g ; [b] trace < 0.01 ng/g .

Only one other species of Chlorophyta, *Ulva lactuca*, contained more than 1000 ng/g of total bromophenols. This concentration (1229 ng/g) was only found in one sample from Bateau Bay. The remaining three samples analysed had total concentrations that varied between 428 and 539 ng/g. Of interest, the level of bromophenols found in the sample from Botany Bay was comparable with that from Turimetta Head, unlike comparable samples of *E. intestinalis* and *D. pulchra* from these sites. 2,4,6-Tribromophenol was the major bromophenol found in all samples (390 to 1200 ng/g); however, in the sample from Bateau Bay collected in 1991, 2,4-dibromophenol was also present in a relatively high concentration (130 ng/g).

Of the remaining ten species of Chlorophyta, only two had total bromophenol concentrations in excess of 100 ng/g. Of interest, in both of these species, *Caulerpa cactoides* and *Chlorodesmis major*, the major bromophenol was 2,4-dibromophenol

(94 and 150 ng/g respectively). Of the other eight species, the total bromophenol content varied between 1 and 85 ng/g. *Codium fragile* was the species with the lowest bromophenol content yet found in Australian marine algae.

Sargassum (Phaeophyta). Results from the analyses of eight samples of *Sargassum* species are recorded in Table 22. All five bromophenols were found in four of these samples and four in the remaining four samples. 2,4,6-Tribromophenol was the dominant compound in most of the samples; however, in Sample BB34 the major component was 2,4-dibromophenol. The highest total bromophenol concentration was found in Sample BB14 (84 ng/g) and the lowest in Sample BB06 (4 ng/g). Thus in these eight samples of *Sargassum* species, not one exceeded 100 ng/g of total bromophenols and this is consistent with the majority of samples analysed from the division Phaeophyta (Table 20).

Marine algae are major dietary components of many species of omnivorous fish (Table 15) and at some times of the year can be their sole source of food; this is the situation with *G. tricuspidata* and *Myxus elongatus* (Anon, 1981). Other species heavily dependent on algae are *R. sarba* and *A. australis*. Algae eaten by these species include *E. intestinalis, U. lactuca, G. edulis, P. capillacea* and *D. pulchra*. Three of these algae have been shown to have total bromophenol concentrations in

Table 22 Concentration of bromophenols in eastern Australian *Sargassum* species (Phaeophyta)

Species	Source (year)	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Sargassum sp (BB03)	Bateau Bay (91)	0.2	ND[a]	0.7	0.1	18	19
Sargassum sp (BB06)	Bateau Bay (91)	0.04	ND	0.9	0.3	2.8	4
Sargassum sp (BB14)	Bateau Bay (91)	0.3	ND	1.6	0.5	82	84
Sargassum sp (BB22)	Bateau Bay (92)	1.7	1.2	7.4	0.4	22	33
Sargassum sp (BB29)	Bateau Bay (92)	2.2	9.7	14	0.5	56	82
Sargassum sp (BB34)	Bateau Bay (92)	0.1	2.0	30	0.05	0.8	33
Sargassum sp (BB36)	Bateau Bay (92)	0.6	4.4	12	0.2	18	35
Sargassum sp (BB45)	Bateau Bay (92)	0.02	ND	0.7	0.1	7.7	9

[a] ND = Not Detected at a detection limit of 0.01 ng/g .

excess of 1000 ng/g and the other two have in excess of 100 ng/g (Tables 19 and 21). In the current studies, the total bromophenol concentrations of three of the above fish, *G. tricuspidata, R. sarba* and *A. australis*, varied between 8 and 18 ng/g. At such concentrations, these fish could be expected to possess some level of ocean-like flavour. However, at those times when the fish would be feeding exclusively on algae, much higher concentrations of bromophenols could be expected. It is therefore not surprising that these species of fish are well known for their distinctive seafood flavours.

The 50 species of algae surveyed in this study all grow on the coastal fringe from the intertidal zone to depths of 25 metres. These species thus provide a reasonable selection of algae available to those fish that feed in this region of the NSW coast. Three of the algae known to be eaten regularly by fish have the highest levels of bromophenols and this tends to confirm that algae is an important source of these compounds in omnivorous species. These findings could also suggest that bromophenols can act as attractants to certain species of fish.

Quantitative analysis of bryozoan extracts - Of the 14 samples of bryozoans analysed (representing 10 species), all contained bromophenols and with the exception of one sample their total bromophenol concentrations exceeded 100 ng/g (Table 23). All five bromophenols were present in nine of the samples, four in four samples and three in the remaining one sample. Without exception the major bromophenol in all species was 2,4,6-tribromophenol, although significant concentrations of 2,4- and 2,6-dibromophenol were found in three of these species. One of these species was *Amathia wilsoni*, in which the concentrations of the major bromophenols, 2,4,6-tribromophenol, 2,4-dibromophenol and 2,6-dibromophenol, were 1100, 330 and 210 ng/g respectively. The total bromophenol concentration for this sample was 1668 ng/g. Another species with a high total bromophenol concentration was *Bugularia dissimilis* (1124 ng/g). In this species the concentrations of the above bromophenols were also relatively high, 610, 330 and 130 ng/g. Three species also had total bromophenol concentrations in excess of 500 ng/g: these were *Orthoscuticella ventricosa* (613 ng/g), *Pleurotoichus sp*. (729 ng/g) and *Pleurotoichus sp.* (837 ng/g). Of interest, *O. ventricosa* was one of the species

in which 4-bromophenol was not detected. Other species that did not contain this compound were *Bugula dentata* and *Cladostephus spongiosus*. *B. dentata* and *B. dissimilis* did not contain 2-bromophenol.

Table 23 Distribution of bromophenols in eastern Australian bryozoans

Species	Bromophenols (ng/g)					
	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Amathia cornuta	1.3	7.8	16	52	180	257
Amathia cornuta	5.9	21	15	56	110	208
Amathia wilsoni	1.0	4.1	6.1	28	70	109
Amathia wilsoni	6.0	22	330	210	1100	1668
Amathia sp	5.0	7.9	13	28	101	155
Bugula dentata	ND [a]	ND	12	130	180	322
Bugularia dissimilis	ND	54	330	130	610	1124
Cellaria pilosa	4.4	5.1	34	40	83	167
Cellaria pilosa	2.2	3.9	6.8	54	40	107
Cladostephus spongiosus	0.22	ND	17	1.4	17	36
Orthoscuticella ventricosa	1.5	ND	24	47	540	613
Orthoscuticella ventricosa	trace [b]	ND	94	37	82	213
Pleurotoichus sp	0.7	5.0	19	44	660	729
Pleurotoichus sp	1.0	10	22	74	730	837

[a] ND = Not Detected at a detection limit of 0.01 ng/g ; [b] trace < 0.01 ng/g

Of the 31 species of ocean fish studied, bryozoans are significant components of the diets of three species: *Meuschenia freycineti, Meuschenia trachylepsis* and *Parika scaber* (Table 15). However, from discussions with Dr J Paxton (Australian Museum) it is evident that these colonising animals would contribute to the diets of many other omnivorous species. Bryozoans may therefore be considered as yet another significant dietary source of bromophenols in certain species of fish. However, like the polychaetes, the bryozoans analysed in the current study represent only a small sample and additional species need to be studied to extend our knowledge of the bromophenol content of these animals.

Quantitative analysis of sponge extracts - All five bromophenols were found in the seven species of sponge analysed. The total bromophenol content varied from 12 to 425 ng/g (Table 24) and the major bromophenol in five of these species was 2,4,6-

tribromophenol. 2,4-Dibromophenol was the dominant compound in the remaining two species. The sponge with the highest total bromophenol content (425 ng/g) was an Ascidiacea. In this species the major bromophenols were 2,4,6-tribromophenol (240 ng/g) and 2,4-dibromophenol (110 ng/g). 4-Bromophenol was also present in a significant concentration in this species (62 ng/g). The only other sponge with a total bromophenol content greater than 100 ng/g was a *Niphates sp*. 2,4,6-Tribromophenol was again the major bromophenol (97 ng/g). The remaining five sponges had total bromophenol contents less than 60 ng/g.

Table 24 Concentration of bromophenols in sponges from the Exmouth Gulf.

Species	Bromophenols (ng/g)					
	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Clathria major	0.24	2.2	2.1	9.5	23	37
Niphates sp.	0.96	3.2	7.6	9.0	97	118
Echinodictum clathrioides	0.68	6.3	5.3	1.5	21	35
Ascidiacea	3.2	62	110	9.6	240	425
Ascidiacea	5.8	3.1	46	1.3	1.5	58
Dysidia sp.	2.0	2.0	4.1	2.8	1.5	12
Spongosorites sp.	0.24	0.98	4.2	0.74	5.6	12

Based on the very limited number of species analysed it would appear that sponges contain a lower concentration of bromophenols than some other colonising animals, such as bryozoans. Tables 14 and 15 show that, of the 31 species of fish recorded, only one species, the diverse omnivore *Parika scaber*, eats large quantities of sponges and ascidians. However, it is possible that, like bryozoans, sponges do on occasions form part of the diets of other species of omnivorous fish. Sponges must therefore be considered as yet another source of bromophenols in the marine food chain. As such, additional species should be analysed to extend our knowledge of their bromophenol content.

Quantitative analysis of sea water and sediment - Results from the analyses of two sea water samples and the 16 sediment samples are recorded in Table 25.

The sea water samples were collected from the shore and close to outgrowths of *G. edulis* (Botany Bay) or *P. capillacea* (North Narrabeen). However, at both of these sites the concentrations of the bromophenols can be regarded as negligible. By comparison, most of the samples of sediment were collected at sea from the Fisheries Research Vessel, MV Kapala. Three of them had total bromophenol concentrations in excess of 100 ng/g; these were the samples from East Coast NSW (216 ng/g), Moreton Bay QLD (710 ng/g) and Hen and Chicken Bay (176 ng/g). In the East Coast sample and that from Moreton Bay, the major bromophenol was 4-bromophenol (150 and 510 ng/g respectively), together with significant amounts of 2,4-dibromophenol (65 and 180 ng/g respectively). However, in the sample from Hen and Chicken Bay the dominant bromophenol was 2,4,6-tribromophenol (170 ng/g). The high level of 2,4,6-tribromophenol in the sediment from this site can be explained, as it was from here that samples of the polychaetes *Australonereis ehlersi* and *Lumbrineris latreilli*, species with very high 2,4,6-tribromophenol content, were collected (Table 17). It is known that polychaetes exude bromophenols into the sediment surrounding their burrows (Steward *et al*., 1992). A species of polychaete that has been shown to contain high concentrations of 4-bromophenol is *Notomastus lobatus* obtained from the sandflats of South Carolina USA (Steward *et al*., 1992) while 2,4-dibromophenol is the major bromophenol in *Saccoglossus kowalewskii* from Maine USA (King, 1986). Therefore, the presence of polychaetes in the sediments from the East Coast NSW or Moreton Bay could account for the high concentrations of 4-bromophenol and 2,4-dibromophenol in samples from these sites. If this were the case it would strengthen the opinion that prawns and fish feeding in these regions could contain disproportionately high concentrations of these compounds in their gut and flesh (Tables 3, 4, 5 and 11).

Of the remaining 13 samples of sediment, five had total bromophenol concentrations in excess of 10 ng/g (range 12 to 29 ng/g). Most of these samples contained a high proportion of mud. By comparison, samples with low total bromophenol content (0.1 to 3.3 ng/g) were essentially sand. Results obtained from the current study would suggest that polychaetes with high bromophenol

Table 25 Concentration of bromophenols in sea water and benthic sediment

Material	Source (year)	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Sea Water							
	Botany Bay	ND [a]	ND	ND	ND	ND	ND
	North Narrabeen	trace [b]	ND	trace	trace	trace	trace
Sea Sediment							
	East Coast NSW	trace	150	65	ND	1.0	216
	Newcastle	0.3	5.9	5.6	ND	ND	12
	Newcastle	trace	0.4	0.2	0.06	0.05	1
	Tathra	0.7	12	3.1	2.1	11	29
	Newcastle	0.2	6.2	5.7	1.8	1.9	16
	Tathra	1.0	12	2.4	2.3	2.5	20
	Moreton Bay, QLD	9.8	510	180	5.6	4.4	710
	Mossman QLD [c]	0.2	0.3	0.8	0.9	ND	2.2
	Wollongong	0.1	1.1	ND	ND	ND	1.2
	Brush Is	0.6	4.2	2.4	ND	ND	7
	Ulladulla	0.9	5.8	4.7	0.2	ND	12
	Batemans Bay	0.7	9.0	3.9	1.4	1.5	17
	Wollongong	1.1	22	4.7	0.2	ND	28
	North Tathra	0.1	0.6	1.6	0.4	0.6	3.3
	Wreck Bay	ND	0.1	ND	trace	ND	0.1
	Hen and Chicken Bay [d]	0.2	1.8	2.2	1.3	170	176

[a] ND = Not Detected at a detection limit of 0.01 ng/g ; [b] trace = 0.01 ng/g;

[c] Commercial farm sediment; [d] Polychaete burrows.

content would not be found in such sandy sediments (Table 17 and 18). The collection of polychaetes for analysis from the ocean floor is a difficult and expensive task. However, based on the above observations, it may be possible to predict the bromophenol content of prawns and fish in a particular area by the analysis of sediment samples. Further work is required to assess the plausibility of this procedure.

It is of interest that the sample of sediment from a commercial prawn farm had a total bromophenol concentration of 2.2 ng/g (Table 25). The small quantity of bromophenols found in this material probably would be derived from the prawn feed, which contains varying amounts of these compounds (see following Section).

(c) ***Modified prawn feed***

Quantitative analysis of extracts of commercial prawn feeds - Results from the analysis of 11 samples of *Penaeus monodon* feed and two samples of *Penaeus*

japonicus feed are recorded in Table 26. All five bromophenols were present in five samples of the *P. monodon* feed, four in another five samples and three in the remaining sample. By comparison, in the *P. japonicus* feed, one sample had five bromophenols, while there were four in the remaining sample. The total bromophenol concentration in the *P. monodon* feed varied from 1.4 to 40 ng/g, while the variation in *P. japonicus* feed was from 7 to 17 ng/g. The major source of bromophenols in such feeds is probably prawn meal or prawn shell meal. Therefore, the composition and concentration of the bromophenols in these prawn meals would depend on whether the feed prawns were originally cultivated or wild-harvested. If they were wild-harvested, then it would depend on the nature of the feed prawns' diet and a major variation in bromophenol composition and concentration could be expected in the processed feed. Such a variation is evident from the analytical data for the *P. monodon* feed reported in Table 26.

Table 26 Concentration of bromophenols in commercial prawn feed

Sample	Bromophenols (ng/g)					
	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Penaeus monodon feed						
A	17	10	3.4	3.5	5.8	40
B	0.9	11	18	3.5	5.7	39
C	1.9	5.8	4.2	9.1	11	32
D	1.4	ND	6.2	5.2	5.5	18
E	1.1	3.7	2.2	3.6	4.2	15
F	0.3	1.2	0.9	0.8	0.7	3.9
G	0.2	1.4	0.6	0.6	ND	2.8
H	0.3	ND	0.8	0.5	0.5	2.1
I	0.5	ND	0.8	0.6	0.3	2.2
J	0.4	ND	ND	0.7	0.5	1.6
K	0.4	ND	0.4	0.4	0.2	1.4
Penaeus japonicus feed						
L	1.3	0.4	11	1.6	2.7	17
M	0.4	ND	1.7	2.5	2.4	7

[a] Not Detected at a detection limit of 0.01 ng/g

The analytical data for cultivated *P. monodon* prawns (Table 8) show that the maximum total bromophenol concentration in these samples was less than 1 ng/g. Consequently, the final diet of these animals would in all probability have been a prawn feed with low concentrations of these compounds, such as those in feed

samples F to K (Table 26). Current evidence would suggest that bromophenols make an important contribution to the natural flavour of seafoods. Thus if the flavours of cultivated prawns are to approximate those of wild-harvested prawns the concentrations of bromophenols in most prawn feeds will need to be increased. A simple approach would be to use prawn meal with a high bromophenol content in the finisher feeds. However, as all prawn meal is imported, it could be difficult to obtain regular supplies of material of the required specification. An alternative would be to use a natural source high in bromophenols that is available locally, as an adjunct feed. The search for such materials could form the basis of another investigation.

Quantitative analysis of modified prawn feeds - To investigate the difficulties associated with the inclusion of bromophenols into prawn feed, batches of modified feed were prepared by the addition of these compounds to a CSIRO formulation, both as free bromophenols and as sulfate esters. Concentrations of the bromophenols in the modified feed and a control feed are recorded in Table 27. With the free bromophenols, the total bromophenol content in the uncooked fed was 43 ng/g out of an expected 5600 ng/g, an inclusion rate of only 0.8 percent. As the preparation of this material did not involve any process that could cause physical loss, a possible explanation is that the bromophenols had chemically reacted with other components in the mixture. Some physical loss was observed during the cooking process that further reduced the total bromophenol content to 30 ng/g in the finished feed. Similar results were obtained with the feed containing the bromophenol salt esters except that the initial loss was about one tenth of that observed for the free compounds. The total bromophenol content of the uncooked feed was 535 ng/g or 9.5 percent inclusion. However, after cooking, this value was reduced to 86 ng/g or 1.5 percent inclusion in the finished feed. Bromophenols are steam volatile; thus, during the cooking process these compounds could be removed from the feed by evaporation. An example of the volatility of the bromophenols was the contamination of the Control trial feed that was processed immediately after the processing of the modified feed containing free bromophenols (Table 27).

Table 27 Concentration of bromophenols in CSIRO trial feeds (June 1995) [a]

Sample treatment	Sample	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Free bromophenols	Uncooked	7.1	8.9	17	0.5	9.6	43
	Cooked	3.7	4.7	14	0.3	7.1	30
Bromophenol salt esters	Uncooked	117	77	283	18	40	535
	Cooked	56	ND	11	6	13	86
Control trial feed	Uncooked	0.3	0.8	1.1	0.5	0.9	3.6
	Cooked [b]	3.3	3.6	11	1.6	8.3	28
CSIRO standard feed	Cooked	0.2	ND	1.6	0.5	0.6	2.9

[a] Amount of bromophenols added to feed mixture, 2-BP = 1700 ng/g; 4-BP = 1100 ng/g; 2,4-DBP = 1800 ng/g; 2,6-DBP = 400 ng/g; 2,4,6-TBP = 600 ng/g; Total 5600 ng/g
[b] Standard feed cooked after feed treated with free bromophenols
[c] ND = Not Detected at a detection limit of 0.01 ng/g

In an effort to overcome this volatility problem, the bromophenols were converted to their salt esters in the belief that this would lead to greater retention. However, the results presented in Table 27 show that, while the salt esters were better retained in the uncooked feed, there was only a marginally improved inclusion rate for the cooked material, compared with that containing the free compounds. This unexpected result would suggest that either the salt esters themselves were steam volatile or they were hydrolysed to the free bromophenols during the cooking process. Accordingly, the reactivity and volatility of bromophenols represent two important problems that will need to be overcome before they can be successfully incorporated into prawn feeds.

Quantitative analysis of extracts of prawns fed on modified feeds - With the development of the modified feeds, trials were conducted at the CSIRO Division of Fisheries to establish whether these feeds were as acceptable to prawns as the control trial feed and to determine the effect that the modified feeds had on the flavour of the cultivated prawns. The trials, carried out in duplicate, showed that the modified feeds were readily accepted by the prawns and did not have a detrimental effect on their growth. The mature prawns were assessed for flavour by a taste panel and were analysed for their bromophenol content.

Unfortunately, the sensory data was inconclusive. Replicate taste panel sessions were conducted in the morning and in the afternoon. Results from the morning session showed that prawns fed with feed containing free bromophenols had the highest flavour rating, followed by those fed on feed containing bromophenol salt esters and, finally, those fed on the control trial feed. However, in the afternoon session no significant differences were observed among the three treatments.

Results from the analysis of the six prawn samples are recorded in Table 28. Examination of these data shows that there is reasonable agreement for the bromophenol content of prawns from replicate tanks. For example, the total bromophenol contents of prawns fed on a diet containing free bromophenols were 18 and 20 ng/g, those for prawns fed on a diet containing bromophenol salt esters were 4.5 and 4.8 ng/g and those for prawns fed on the control diet 2.5 and 1.9 ng/g. However, these results are at variance with the total bromophenol contents of the individual feeds reported in Table 27. Here, the highest bromophenol content was found in the feed containing the bromophenol salt esters (86 ng/g), while in the other two feeds the bromophenol content was almost the same (30 and 28 ng/g). However, some of the analytical data for the feed was slightly misleading. In the case of the control feed most of the bromophenol content would only be on the surface (having been adsorbed during the cooking process) and would be easily washed from the feed when it was placed in water. This material would accordingly have an effective bromophenol content similar to that of the CSIRO standard feed (2.9 ng/g). Even so the bromophenol content of the prawns fed on the control feed was more than double that normally found in commercially cultivated prawns (Table 8).

Table 28 Concentration of bromophenols in prawns fed on modified feeds during the CSIRO feeding trials (June, 1995)

Sample treatment	Sample	Bromophenols (ng/g)					
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total (whole prawn)
Free bromophenols (tank 1)	head	0.4	2	6.1	1.1	6.2	18
	tail	[a] ND	ND	2	ND	ND	
Free bromophenols (tank 2)	head	0.6	1.3	10	ND	8.2	20
	tail	ND	ND	0.2	ND	ND	
Bromophenol ester salts (tank 1)	head	ND	ND	1.1	1	1.4	4.5
	tail	0.5	ND	0.5	ND	ND	
Bromophenol ester salts (tank 2)	head	0.4	ND	1.2	0.5	2.7	4.8
	tail	ND	ND	ND	ND	ND	
Control feed (tank 1)	head	ND	ND	0.6	0.6	1.3	2.5
	tail	ND	ND	ND	ND	ND	
Control feed (tank 2)	head	ND	ND	1.6	ND	ND	1.9
	tail	ND	ND	0.3	ND	ND	

[a] ND = Not Detected at a detection limit of 0.01 ng/g

Another anomaly concerned the relative concentrations of the bromophenols in prawns fed on feed containing free bromophenols compared with those in prawns fed on feed containing bromophenol salt esters. Here the former feed had the lower total bromophenol content (30 ng/g as compared with 86 ng/g) but in the prawns this situation was reversed (19 ng/g as compared with 4.7 ng/g). Based on these results, it would appear that the free bromophenols are more readily retained in the gut of prawns than the ester salts. This finding would suggest that the form of the bromophenols in the feed (either free or bound) dictates the degree to which they are retained by the animal.

The production of the modified feeds, and the feeding trials themselves, highlighted several deficiencies in the processing and handling of these materials. However, the fact that a proportion of the added bromophenols were incorporated into the feed and that, in the case of the prawns fed on feed containing free bromophenols, the

concentrations of these compounds were increased, must be considered encouraging. Also encouraging was the acceptance by prawns of the modified feed.

BENEFITS

Two sectors of the prawning industry, namely the harvesting of wild prawns, and commercial cultivation of selected species, will benefit most from this research. In either case, the ultimate beneficiaries will be local and overseas consumers through the provision of prawns of superior quality.

The current study (by taking bromophenols as an example) has demonstrated that diet and environment have a major role in determining the natural flavour of prawns and fish. In doing so, it has provided an explanation for unexpected variations in the flavour of seafoods at different times of the year and from different locations. This information may now be used by the industry to target markets that are seeking particular natural flavours in their seafoods. As an example, Endeavour prawns from Exmouth Gulf frequently have a strong flavour that is well accepted locally but is not acceptable in the eastern states.

The study has also shown that in both prawns and fish the major concentration of bromophenols is in the gut. Thus, the flavour of prawn meat can be modified by deheading before further processing. The resulting tails could then be sold to markets requiring a more subtle flavour in their seafoods.

However, in the case of cultivated prawns it is the absence of bromophenols that contributes to their rather bland flavour. Such prawns sell for about one half of that paid for wild-harvested prawns in most Australian markets. A contributing factor to this low price is the perceived absence of natural flavour in this material. The current work has demonstrated that the bromophenol content of cultivated prawns can be increased by the addition of these compounds to the feed. Thus, although there were problems with the preparation of the modified feeds and some evidence indicated that the "right" form of bromophenols must be used, feeds with predetermined bromophenol content are a practical possibility. With an

improvement in bromophenol content, and "natural" ocean flavour, cultivated prawns should receive greater consumer acceptability and an increase in price. The extent of this increase will no doubt be dictated by existing market forces.

The current benefits and beneficiaries are the same as those identified in the original application.

INTELLECTUAL PROPERTY AND VALUABLE INFORMATION

No patents have been processed on any of the work described in this report. However, this work is essentially basic research, and as such could in the future be of value to certain sections of the Australian fishing industry.

FURTHER DEVELOPMENT

A number of issues arising from this study should be considered for future research if a more complete understanding of the role that diet and environment have on seafood flavours is to be achieved. Those projects of highest priority are detailed below.

1. Identify the six additional compounds present in wild-harvested prawns that had aromas described as seaweed, algae, rock pool and sea air (Table 2). These compounds, like the bromophenols, could contribute to the natural flavour of prawns and as such, in addition to their identity, their natural source should also be determined.
2. Determine the quantities of bromophenols that are incorporated by natural processes into prawn meat and establish the physiological function involved. Along with this study, the quantity of bromophenols that diffuse from the gut into the tail meat, when prawns are cooked whole, should also be determined.
3. Identify the form in which bromophenols (free or derivatised) that occur in the natural diets of wild prawns. This information is essential if the levels of bromophenols in cultivated prawns are to be increased.

4. Determine the bromophenol content of all benthic organisms (including polychaetes) eaten by wild prawns. These benthic animals would need to be collected from the feeding ground of individual species of prawns.
5. Identify suitable natural sources of bromophenols for their incorporation into modified prawn feeds. The bromophenols in this material would need to be in a form that prevented their loss from the feed during processing, but be readily retained by the prawns during digestion.
6. Develop, with the support of industry, a modified commercial prawn feed that could deliver predetermined concentrations of bromophenols to cultivated prawns.

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Two scientists from CSIRO Division of Fisheries (Cleveland) provided advice and support during the preparation of the modified prawn feeds and in the management of the subsequent feeding trials. These were -
Mr D Smith (Nutritional Physiologist)
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FINAL COST

Total FRDC costs	$ 97,965
Total CSIRO costs	$272,879
Total cost of project	$370,844
Difference from projected costs	$ 11,490

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