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Unusually high levels of non-saponifiable lipids in the fishes escolar and rudderfish Identification by gas and thin-layer chromatography

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Abstract

Analysis of the non-saponifiable lipids of the fishes *Lepidocybium flavobrunneum* and *Ruvettus pretiosus* (escolar), and *Centrolophus niger* and *Tubbia* spp. (rudderfish) was performed. The analyses were used to clarify the cause of recent reports of illness (diarrhoea) in Australia from consumption of purported rudderfish. Both escolar and rudderfish contained very high levels of oil (generally between 14 to 25%, as % wet mass) in the fillet and the oil compositions were different to most seafood. Escolar oil contained mainly wax ester (>90% of oil). The oil from five specimens of rudderfish contained mainly diacylglyceryl ether (DAGE, >80% of oil) or hydrocarbon (>80% of oil, predominately squalene). One rudderfish specimen contained mainly polar lipid. Major differences in oil content and composition, including fatty alcohol and glyceryl ether diols (derived from DAGE), were observed between purported individuals of the same species or related species of rudderfish, raising the possibility of geographic or seasonal differences affecting the oil composition. The oil composition of fish fillet samples associated with the health issues were consistent with the profiles for escolar, rather than rudderfish species. These findings, in particular the lipid class and fatty alcohol profiles, were supported by general protein fingerprinting results and were consistent with the samples originating from individuals of the escolar species *L. flavobrunneum*. The high wax ester content of the escolar group clarifies the reported diarrhoeal effects to consumers. Purgative properties of high wax ester containing fish oils have been reported for escolar and other species. The results highlight the potential for non-saponifiable lipid profiles to be used for identification of fish fillets and oils to at least group level.

Keywords: Fish; Food analysis; *Lepidocybium flavobrunneum*; *Ruvettus pretiosus*; *Centrolophus niger*; *Tubbia* spp.; Lipids; Squalene; Diacylglyceryl ethers; Wax ester

1. Introduction

Marine seafoods are increasingly marketed for their health benefits to consumers (e.g., Refs. [1,2]). However, consumption of a very limited number of species has also been reported to produce purgative properties in some consumers, with evidence including results of rat-feeding studies, e.g., for the escolar *Lepidocybium flavobrunneum* and *Ruvettus pretiosus* [3,4]. In general this effect is reported to be due to the presence of wax ester-rich oils in the flesh [5].

Health complaints have occurred recently in Australia associated with the consumption of fillets sold as rudderfish. The marketing group rudderfish consists of species from three trevalla (family *Cen-*

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trolophidae) genera, *Centrolophus*, *Schedophilus* and *Tubbia*, with several undescribed species and uncertain distribution in Australian waters. Consumers had purchased ''rudderfish'' fillets and, after cooking and eating, some had suffered severe diarrhoeal effects sometimes associated with the fish [6]. However, concern existed over the species identity and the cause of the health effects, particularly as both groups are caught as long-line by-catch and anedoctal evidence suggested that escolar species were being sold as rudderfish. The name ''escolar'' in Australian fisheries includes two known gemfish (family *Gempylidae*) species, *Lepidocybium flavobrunneum* and *Ruvettus pretiosus*. The latter species has also been referred to as the ''castor oil fish'' [7]. Both species have reported purgative properties [8].

The lipid composition of *R. pretiosus* obtained from a Tokyo, Japan fish market has been reported [7]. However, prior to this study, the oil content and composition of Australian escolar and rudderfish were not available. We present here a comparison of the oil content and composition profiles, with particular emphasis on the non-saponifiable lipids, of two fish fillets associated with consumer illness, and those obtained for reference samples from the escolar and rudderfish groups. The analyses, supported by general protein fingerprinting (unpublished data) of the samples, highlight the potential for non-saponifiable lipid profiles to be used for identification of selected escolar and rudderfish samples to at least group level.

2. Experimental

2.1. Samples

Two frozen fillet samples of unknown species were air freighted to Hobart, Tasmania from the South Australian Public and Environmental Health Service – unknown samples 1 and 2. Both samples were associated with cases of severe diarrhoea in consumers. Reference samples (2–3 specimens of each species, Table 1) of muscle tissue of escolar species *Ruvettus pretiosus* (Cocco, 1829) and *Lepidocybium flavobrunneum* (Smith, 1849), and

Table 1
Oil class composition and content of escolar and rudderfish (oil class determined by TLC–FID)

Sample	Oil class composition (% of oil)							Total oil[b] (% wet mass)
	HC	WE	TAG	DAGE	FFA	ST	PL	
Unknown (1)[a]	–	98.1	–	–	0.4	0.2	1.3	22.1
Unknown (2)	–	97.5	–	–	0.3	0.2	2.0	24.5
Escolar								
L. flavobrunneum (1)	–	96.1	–	–	0.7	0.4	2.8	18.9
L. flavobrunneum (2)	–	96.6	–	–	–	0.2	3.2	20.3
R. pretiosus (1)	–	96.9	–	–	0.6	0.4	2.1	21.2
R. pretiosus (2)	1.1	90.1	1.5	0.5	0.7	0.6	5.7	ND
R. pretiosus (3)	–	95.8	0.4	–	0.4	0.5	2.9	17.8
Rudderfish								
C. niger (1)	0.7	–	4.5	92.5	0.6	0.3	1.4	14.1
C. niger (2)	–	1.5	13.0	17.4	21.6	4.3	42.2	1.7
C. niger (3)	–	–	9.7	87.5	1.0	0.5	1.3	19.8
Tubbia sp. (3)	80.5	–	10.5	6.0	0.6	0.3	2.1	20.5
Tubbia sp. (4)	93.4	–	0.3	2.2	0.7	0.2	3.2	15.9
Tubbia sp. (8)	–	–	14.9	82.1	0.9	0.4	1.7	24.8

Abbreviations: HC, hydrocarbon; WE, wax ester; TAG, triglyceride; DAGE, diacylglyceryl ether; FFA, free fatty acid; ST, sterol; PL, polar lipid; ND, not determined; –, not detected.

[a] Denotes sample reference number.

[b] Gravimetric determination.

rudderfish species *Centrolophus niger* (Gmelin, 1789) and *Tubbia* spp. had been stored at −80°C at the CSIRO Marine Laboratories [6] and were used for comparison of oil content and composition. Skin, including lipid deposits, was removed from the samples analysed in this study.

2.2. Lipid extraction and fractionation

Small samples of flesh without skin were quantitatively extracted using a modified Bligh and Dyer [9] one-phase methanol–chloroform–water (2:1:0.8, v/v) extraction; the samples were extracted overnight and the phases were separated the following day by the addition of chloroform and water (final solvent ratio, methanol–chloroform–water, 1:1:0.9, v/v). The total lipid was concentrated (i.e., solvents removed in vacuo) using rotary evaporation at 40°C. Lipid class analyses were conducted within 3 days, with samples stored in a known volume of chloroform.

An aliquot of the total lipid was analysed using an Iatroscan MK V TH10 thin-layer chromatography–flame ionization detector (TLC–FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes [10]. Samples were applied in duplicate or triplicate to silica gel SIII Chromarods (5 μm particle size) using disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane–diethyl ether–acetic acid (60:17:0.2, v/v), a mobile phase resolving non-polar compounds such as wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA) and sterols (ST). A second non-polar solvent system of hexane–diethyl ether (96:4 v/v) was also used to separate hydrocarbon (HC) from WEs and TAGs from diacylglyceryl ether (DAGE). After development, the chromarods were oven-dried and analysed immediately to minimize adsorption of atmospheric contaminants. The FID system was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, wax ester (derived from fish oil), triglyceride (derived from fish oil) and DAGE (purified from shark liver oil); 0.1–10 μg range]. Peaks were quantified on an IBM compatible computer using DAPA software (Kalamunda, Australia). Iatroscan results are generally reproducible to ±10% [10].

An aliquot of the total lipid was saponified in an aqueous solution of methanolic KOH (MeOH–water–KOH, 80:20:5, v/v/w; 60°C, 3 h). The non-saponifiable lipids were extracted with hexane–chloroform (4:1, v/v, 3×) and treated with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA, 60°C, 12 h) to convert alcohols, sterols and glyceryl ether diols to their corresponding *O*-TMSi (trimethylsilyl) ethers. With the wax ester-rich species, the saponification step was not used, as the reaction was incomplete. Rather, the lipid was treated with methanol–hydrochloric acid–chloroform (10:1:1, v/v; 80°C, 2 h). The fatty acid methyl esters (FAME) and alcohol products were extracted into hexane–chloroform (4:1, v/v, 3×) and the mixture treated with BSTFA as above to convert alcohols and sterols to their corresponding *O*-TMSi ethers.

2.3. Gas chromatography and gas chromatography–mass spectrometry

Gas chromatographic (GC) analyses of non-saponifiable lipids were performed with a Hewlett-Packard 5890A GC system (Avondale, PA, USA) equipped with a HP-5 cross-linked methyl silicone fused-silica capillary column (50 m×0.32 mm I.D.), an FID system, a split/splitless injector and a HP 7673A autosampler. Hydrogen was the carrier gas. Following addition of methyl nonadecanoate and methyl tricosanoate internal standards, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C/min, then to 250°C at 2°C/min and finally to 300°C at 5°C/min. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component abundance. GC–mass spectrometric (MS) analyses were performed on a Finnigan Thermoquest GCQ GC–mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC system was fitted with a capillary column similar to that described above.

3. Results and discussion

3.1. Escolar

Oil content of reference samples of the two escolar species (*L. flavobrunneum* and *R. pretiosus*) was in the range 18% to 21% (as % wet mass, Table 1). These values represent to our knowledge one of the highest oil content values recorded for wild-caught Australian fishes (200 species, mean 1%, range 0.3 to 8%) [11,12]. Wax ester was the dominant oil class in all escolar samples (90 to 97% of oil, Table 1, Fig. 1).

With the wax ester-rich species, a saponification step was not used to obtain the non-saponifiable lipids, as we observed that the reaction was incomplete. Instead we used a direct transmethylation procedure. This procedure provides a mixture of FAMEs and fatty alcohols (Fig. 2). The main fatty alcohols, in decreasing order of abundance, in *L. flavobrunneum* were 16:0 (53%, mean value), 18:1($n-9$) (21%), 18:0 (9%), 18:1($n-7$) (3.3%), 16:1($n-7$) (3.1%), 14:0 (2.6%) and 20:1($n-9$) (2.5%) (Table 2). These seven components accounted for approximately 94% of the total fatty alcohols present. The profile for *R. pretiosus* was very similar, although relatively higher levels of 18:1($n-9$) and 16:1($n-9$), and lower levels of 18:0 were observed. A similar fatty alcohol profile was also reported for muscle wax ester of *R. pretiosus* obtained from a Japanese fish market [7].

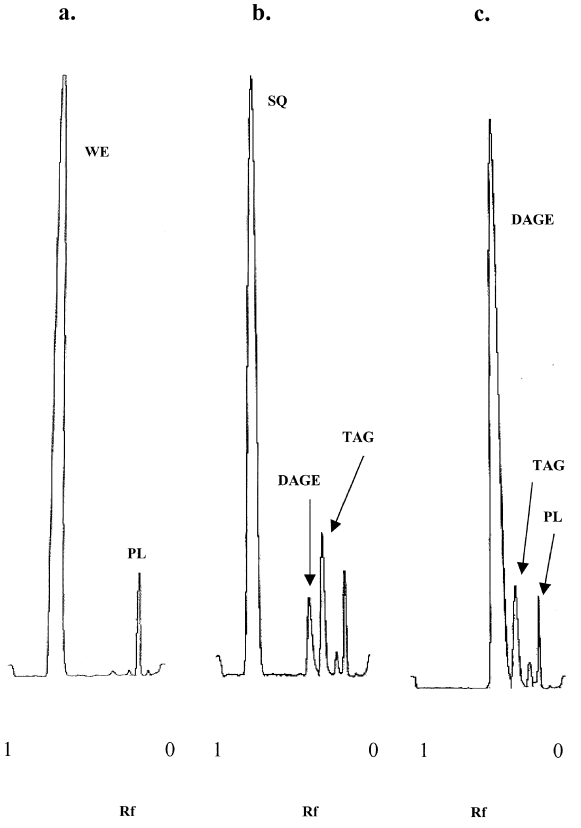

Fig. 1. TLC–FID chromatograms showing lipid class distributions for escolar: (a) *Lepidocybium flavobrunneum*, and rudderfish: (b) *Tubbia* spp. and (c) *Centrolophus niger*. Abbreviations: WE, wax ester; PL, phospholipid; SQ, squalene; DAGE, diacylglyceryl ether; TAG, triglyceride; Rf, retention factor. Solvent: hexane–diethyl ether (96:4, v/v).

Several reports document the fatty alcohol profiles of Japanese specimens of *L. flavobrunneum* (e.g., [13,14]). These earlier studies did not distinguish the different isomers of the C_{16}, C_{18}, C_{20} and C_{22} monounsaturated fatty alcohols. As noted above, we have used several of these specific components to distinguish the two escolar, *L. flavobrunneum* and *R. pretiosus*. One Japanese profile showed markedly higher levels of 20:1 and 22:1 (16%), with lower levels of 16:0 (34%) [13] than our findings. The other profile showed higher 16:0 (65%) and lower 18:1 (16%), with similar levels of 20:1 and 22:1 to our findings [14]. The reason(s) for these differences may be due to environmental factors such as catch location and/or diet of the fish, and/or analytical factors. It remains to be determined whether and how such differences in fatty alcohol profiles may influence the purgative properties of these species.

The wax ester-derived fatty alcohol profiles of *L. flavobrunneum* and *R. pretiosus* are also readily distinguished from those of other wax ester-rich fish. For example, orange roughy (*Hoplostethus atlanticus*) and six species of deep-sea oreos collected from Australian waters contained considerably higher levels of C_{20} and C_{22} monounsaturated fatty alcohols (40 to 90%) [15,16] compared to the escolar species (2 to 6%, Table 2).

3.2. Rudderfish

In contrast to the lipid class profile for escolar, wax ester was either absent or only a minor com-

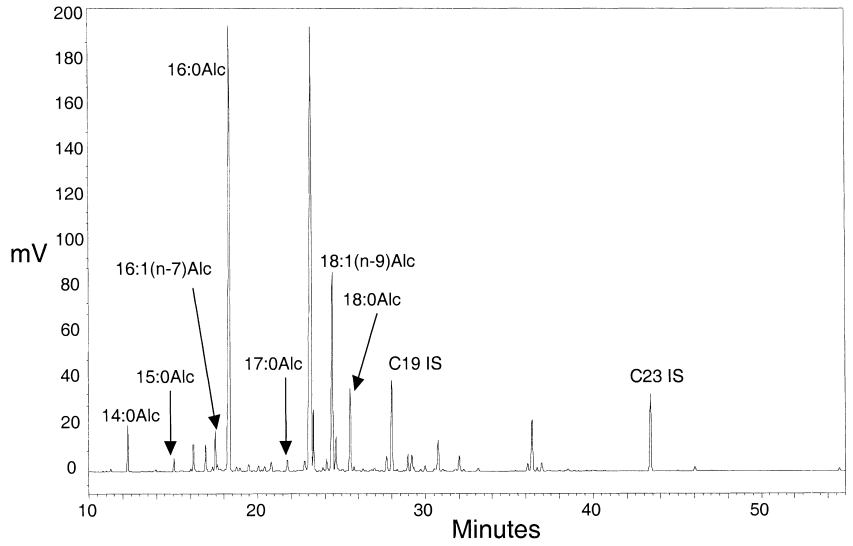

Fig. 2. Partial gas chromatogram of fatty alcohols (as OTMSi ethers) derived from the wax ester-rich escolar *Lepidocybium flavobrunneum* (fatty alcohols obtained by transmethylation of total lipid). HP5 capillary column. Abbreviations: Alc, alcohol; I.S., internal standard. Unlabelled peaks are fatty acid methyl esters or minor fatty alcohols.

ponent in the lipids of the rudderfish *C. niger* and *Tubbia* spp. (Table 1, Fig. 1). With the exception of one specimen of *C. niger*, oil content in both species was in the range 14 to 25% (% wet mass). Once again, the oil content of these fishes is considerably higher than previously reported for Australian fishes. For *C. niger*, the two oil-rich specimens contained high levels of DAGE (93%, as % of total oil). The third specimen of *C. niger* was lower in oil content (1.7%) and contained only 17% DAGE, with 42% polar lipid (PL) and 22% FFA (Table 1).

Glyceryl ether diols (GED) are formed by saponification of DAGE. GED profiles obtained for the DAGE-containing rudderfish are provided in Table 3, with a representative GC trace shown in Fig. 3. The GED profiles for the rudderfish *C. niger* are generally simpler than the fatty acid (data not shown) and fatty alcohol profiles. The main GED, in decreasing order of abundance, were 18:1($n-9$), 16:0, 18:0, 18:1($n-7$) and 16:1 (Table 3). Considerable variation was observed between the three *C. niger* samples in the relative levels of these and other minor GED. This finding will be discussed further below.

Tubbia specimens differed from both escolar and *C. niger*. HC was the main oil class (mean 87%, as % total oil) present in two specimens. The predominant component in the non-saponifiable lipids was identified by GC analysis as squalene (Fig. 4). A third specimen did not contain HC. Rather this sample was rich in DAGE (82%).

When a transmethylation procedure was used on the total lipid of the two HC-rich samples, we observed virtually complete degradation of squalene. We are aware that squalene levels in certain marine oils may sometimes be measured after the use of a transmethylation procedure (e.g., BF_3–methanol [17]); our findings indicate that care is needed should such procedures be used.

The high levels of squalene in the flesh of *Tubbia* is extremely unusual. Although squalene is common in liver oils of deep sea sharks [18], where it is metabolically inert and its only known function is in buoyancy [19,20], its presence as the predominant lipid in *Tubbia* spp. muscle is unique. One other study has reported squalene at 12% of the lipid in the head and body of the teleost eulacon *Thaleichthys pacificus* [21]; the main lipid class was triglyceride (84%). The role of squalene in *T. pacificus* was suggested to be to dilute and reduce the viscosity or melting point of the triglyceride in the marine life phase in cold ocean water. It may be speculated that

Table 2
Fatty alcohol composition of escolar and rudderfish (derived from wax esters and determined by GC analysis)

Fatty alcohol	Percentage composition							
	Escolar, *L. flavobrunneum*		Unknown		Escolar, *R. pretiosus*			Rudderfish, *C. niger*
	(1)[a]	(2)	(1)	(2)	(1)	(2)	(3)	(2)
14:0	2.9	2.4	3.6	3.7	2.6	3.4	2.8	0.0
a15:0	0.1	0.1	0.1	0.3	0.1	0.2	0.2	0.0
15:0	1.0	0.9	1.0	1.3	0.9	1.1	1.2	0.0
i16:0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
16:1($n-9$)	0.4	0.2	0.4	0.3	1.1	0.4	0.9	0.0
16:1($n-7$)	3.4	2.8	3.8	2.8	3.5	4.4	3.5	2.7
16:1($n-5$)	0.6	0.3	0.5	0.3	0.3	0.6	0.6	0.0
16:0	53.2	51.9	47.9	44.4	48.0	48.5	46.8	41.9
i17:0	0.4	0.4	0.3	0.7	0.6	0.4	0.8	0.0
a17:0	1.1	1.0	1.2	1.2	1.2	1.1	1.3	0.0
17:0	1.0	1.1	1.1	1.4	1.1	1.0	1.2	0.0
i18:0	0.2	0.5	0.4	0.5	0.5	0.3	0.4	0.0
18:1($n-9$)	21.4	21.1	22.9	20.1	26.4	26.6	25.8	28.6
18:1($n-7$)	3.4	3.2	3.4	3.3	3.7	3.7	3.5	21.6
18:1($n-5$)	0.3	0.4	0.2	0.2	0.3	0.2	0.3	0.0
18:0	8.3	9.4	8.5	10.4	6.4	6.1	7.7	5.2
20:2	0.3	0.4	0.4	0.4	0.4	0.1	0.4	0.0
20:1($n-9$)	1.7	3.3	3.8	8.0	2.5	1.5	1.6	0.0
20:1($n-7$)	0.1	0.2	0.2	0.3	0.1	0.1	0.2	0.0
20:0	0.2	0.4	0.3	0.4	0.3	0.3	0.2	0.0
22:1	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Denotes sample reference number.

Table 3
Glyceryl ether diol composition (derived from DAGE) of escolar and rudderfish (determined by GC analysis)

Glyceryl ether diol	Percentage composition						
	Escolar, *R. pretiosus* (3)[a]	Rudderfish					
		C. niger			*Tubbia* spp.		
		(1)	(3)	(2)	(3)	(8)	(4)
14:0	0.0	1.5	1.4	3.2	3.9	0.0	1.7
15:0	0.0	0.7	0.8	1.8	2.0	0.0	1.9
16:1	4.2	2.8	2.5	5.0	3.2	3.2	2.8
16:0	45.7	14.9	15.9	25.1	37.6	32.1	32.3
a17:0	0.0	1.2	1.1	1.6	1.9	0.0	1.5
17:0	0.0	0.8	0.8	1.5	1.1	0.0	1.2
18:1($n-9$)	34.0	61.7	61.2	41.3	21.6	29.5	40.1
18:1($n-7$)	4.2	3.8	3.7	5.4	2.9	3.3	4.8
18:0	11.9	11.4	11.5	15.2	25.8	31.9	12.9
20:1	0.0	1.2	1.1	0.0	0.0	0.0	0.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Denotes sample reference number.

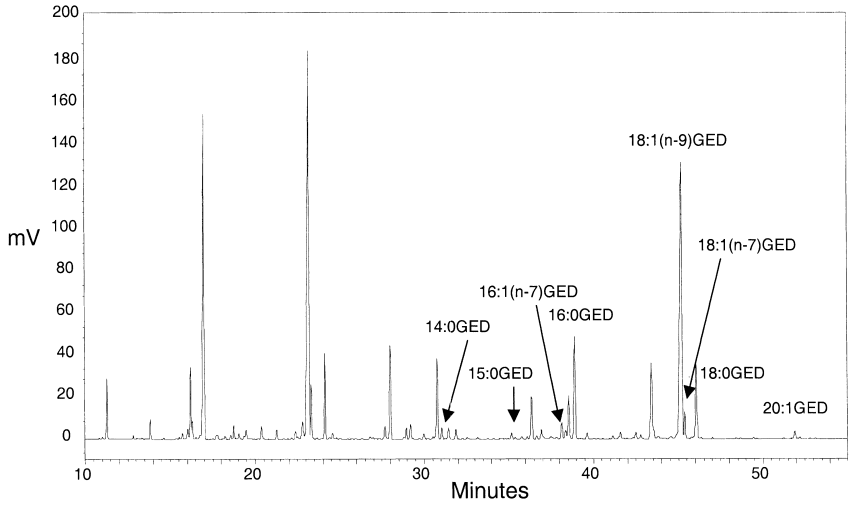

Fig. 3. Partial gas chromatogram of non-saponifiable lipids of the rudderfish *Centrolophus niger*. The glyceryl ether diols (GEDs, as OTMSi ethers) are derived from base saponification of the diacylglyceryl ethers. HP5 capillary column. Abbreviations: Alc, alcohol; GED, glyceryl ether diol. Unlabelled peaks are fatty acid methyl esters and minor GEDs.

the unusually high levels of squalene in *Tubbia* may have a similar buoyancy or other physiological role, however, at this stage the precise role remains to be determined.

The GED profile of *Tubbia* spp. was generally similar to *C. niger*, although the relative level of individual components varied between individuals. The main GED, in decreasing order of abundance, were 16:0, 18:1($n-9$), 18:0, 18:1($n-7$) and 16:1 (Table 3). The individual variation may be a reflection of species or geographic differences; additional taxonomic and lipid compositional research is required for the rudderfish.

Given the differences observed between individual rudderfish specimens of the same species (*Tubbia* spp. and *C. niger*), a larger number of authentic

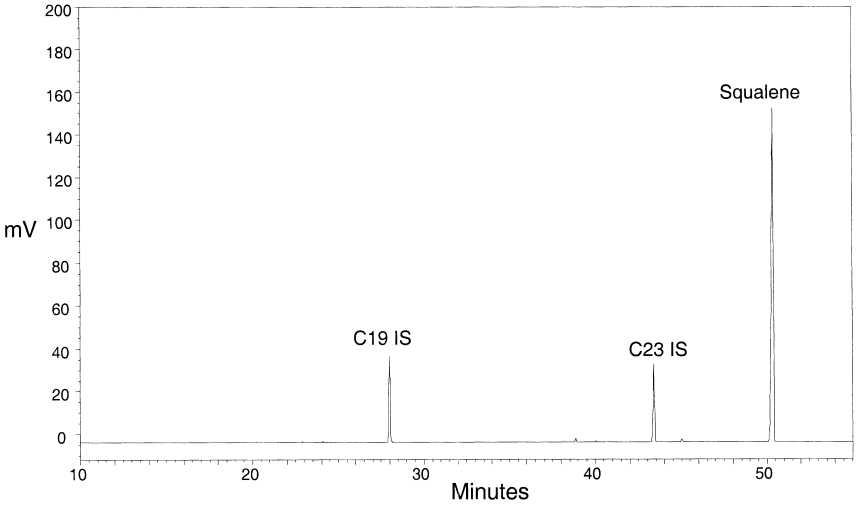

Fig. 4. Partial gas chromatogram of non-saponifiable lipids of the rudderfish *Tubbia* spp. showing squalene as the dominate component. HP5 capillary column. Abbreviation: I.S., internal standard.

samples will need to be examined to fully chemotaxonomically characterise these species.

3.3. Unknown samples

Both of the unknown fillet samples that had been associated with consumer illness had a very high oil content (>22%, as % wet mass) and the dominant lipid class was wax ester (97%, as % of total oil, Table 1). Such a high oil content and unusual composition were similar to that found in the reference collection samples for both escolar species (Table 1).

Although the lipid class and fatty alcohols profiles for *L. flavobrunneum* and *R. pretiosus* are very similar, higher levels of 18:1($n-9$) and 16:1($n-9$), and lower levels of 18:0 were observed in *R. pretiosus* as noted above. Based on comparison of the fatty alcohol profiles, the unknown samples grouped more closely with *L. flavobrunneum* than with *R. pretiosus* (Table 2). This finding is consistent with results obtained from general protein fingerprinting of the two unknown (unpublished data) and the reference samples [6].

3.4. Purgative and other properties of the oils

The first report on the purgative properties of *R. pretiosus* occurred in 1841 [22]. More recently, and based on the high wax ester content in Japanese specimens of *L. flavobrunneum* and *R. pretiosus* [7], the diarrhoea and seborrhoea-producing activity of these fishes was investigated [13]. Based on the results for feeding trials in rats, the flesh and acetone-derived oil of both species were deemed not suitable for human food. However, the flesh of sun-dried fish is believed to be less harmful, as oil easily leaks out during sun drying [13].

The high levels of wax ester-rich oil in orange roughy has been previously reported (fillets 7–10% oil; oil composition 95% wax ester) [15]. Extrapolating results obtained from feeding growing rats and pigs with orange roughy, it was proposed that ''normal consumption'' of orange roughy by humans was unlikely to cause serious health problems [23]. However, the level of wax ester oil in escolar (18–24%) is nearly three times greater than in orange roughy.

Wax ester oils derived from orange roughy and oreo dories have been incorporated into several industrial cleaning, degreasing and other products in the past decade [16,24]. Based on the findings of this study, escolar may represent an additional source of wax ester oils for consideration by industry.

Capsules of DAGE and hydrocarbon oils derived from the livers of deep-sea sharks are marketed as neutraceuticals for human consumption. Based on the findings of our study, rudderfish represent an additional source of the DAGE oils. The health benefits proposed to be associated with shark liver oils include an enhanced immune system, antibacterial activity, and possible regression of tumour growth and radiation induced damage (e.g., Refs. [25–27]). Purgative effects of DAGE and hydrocarbon (squalene) oils have not to our knowledge been reported, however, the effect of intake of such oils at levels of 14% or greater are unknown. Therapeutic doses of these oils are of the order of 1 to 5 g per day, whilst 14% of a 150 g serving would be considerably greater (approximately 20 g).

4. Conclusions

Our study provides a bench mark for the use of lipid profiles, in particular the non-saponifiable lipids, for the identification of fish fillets and oils to at least group level. Members of the escolar and rudderfish groups are unusual in containing very high levels of oil (14–25%, as % of total oil) and having oil composition profiles different to most seafood. Oils from seafood, with some exceptions, are generally rich in triacylglycerol and/or phospholipid. The unusual oil profiles of specific members of the escolar and rudderfish groups are not consistent within their families. Members of both families (*Gempylidae* and *Centrolopidae*) are known to have more conventional triglyceride or phospholipid oils [11,12]. Oil from escolar species was rich in wax ester, for which purgative properties have been previously reported. Rudderfish species in contrast contained mainly DAGE or hydrocarbon (predominantly squalene). Purgative effects of DAGE and hydrocarbon oils have not to our knowledge been reported and capsules of these oils derived from the livers of deep-sea sharks are marketed as nutraceuti-

cals for human consumption. The high wax ester oil composition results obtained for two unknown fillet samples clarify the reported diarrhoeal effects on consumers. The results and possible incorrect naming of the fillets suggest that consumers should be made aware of the oil type in these two groups and that strict use be made of recommended marketing names to avoid similar health issues. Oil composition results support genetic and taxonomic evidence (unpublished) that several undescribed species may exist in the rudderfish and escolar groups. Further specific taxonomic research on these groups is required, together with comparison of the oil profiles between and within (geographic and seasonal) species. In addition, insight into the physiological basis for the occurrence of these very different oils in the muscle of teleosts would be valuable.

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References

[1] J.E. Kinsella, Seafoods and Fish Oils in Human Health and Disease, Marcel Dekker, New York, 1987.
[2] P.R.C. Howe, World Rev. Nutr. Diet. 83 (1998) 215.
[3] Y. Ochiai, S. Watabe, K. Hashimoto, H. Narita, Y. Ukishima, M. Nara, Bull. Jpn. Soc. Sci. Fish. 50 (1984) 721.
[4] T. Kinumaki, K. Arai, K. Sugii, S. Iseki, Bull. Tokai Regional Fish. Res. Lab. 91 (1977) 73.
[5] W.M. Cox Jr., E.E. Reid, J. Am. Chem. Soc. 54 (1932) 220.
[6] G.K. Yearsley, P.R. Last, R.D. Ward, Australian Seafood Handbook, CSIRO Marine Research, Hobart, 1999.
[7] J.C. Nevenzal, W.A. Rodegker, J.F. Mead, Biochemistry 4 (1965) 1589.
[8] P. Berman, E.H. Harley, A.A. Spark, S. Afr. Med. J. 59 (1981) 791.
[9] E.G. Bligh, W.J. Dyer, Can. J. Biochem. Physiol. 37 (1959) 911.
[10] J.K. Volkman, P.D. Nichols, J. Planar Chromatogr.-Modern TLC 4 (1991) 19.
[11] P.D. Nichols, B. Mooney, P. Virtue, N. Elliott, Australian Fisheries Research and Development Corporation Report, 95/122, Australian Fisheries and Development Corporation, 1998.
[12] P.D. Nichols, P. Virtue, B.D. Mooney, N.G. Elliott, G.K. Yearsley, Seafood the Good Food. The Oil Content and Composition of Australian Commercial Fishes, Shellfishes and Crustaceans, Australian Fisheries Research and Development Corporation Guide, 95/122, Australian Fisheries and Development Corporation, 1998.
[13] M. Mori, T. Saiko, Y. Nakanishi, K. Miyazawa, Y. Hashimoto, Bull. Jpn. Soc. Sci. Fish. 32 (1966) 137.
[14] N. Kawai, Y. Nakayama, S. Matsuoka, T. Mori, Yukagaku 34 (1985) 25.
[15] M.J. Bakes, N.G. Elliott, G.J. Green, P.D. Nichols, Comp. Biochem. Physiol. 111B (1995) 633.
[16] N.G. Elliott, J. Skerratt, P. Nichols, Aust. Fish. August (1990) 32.
[17] G. Neill, personal communication.
[18] M.J. Bakes, P.D. Nichols, Comp. Biochem. Physiol. 110B (1995) 267.
[19] C.F. Phleger, in: Hochachka and Mommsen (Eds.), Biochemistry and Molecular Biology of Fishes, Vol. 1, Elsevier, Amsterdam, 1991, p. 209.
[20] C.F. Phleger, Am. Zool. 38 (1998) 321.
[21] R.G. Ackman, R.F. Addison, C.A. Eaton, Nature 220 (1968) 1033.
[22] R.T. Lowe, Trans. Zool. Soc. London 2 (1841) 180.
[23] K.A.C. James, D.R. Body, W.C. Smith, NZ J. Technol. 2 (1986) 219.
[24] P. Nichols, N. Elliott, M. Bakes, B. Mooney, Fisheries Research and Development Corporation Report, 94/115, Australian Fisheries Research and Development Corporation, 1997.
[25] A. Brohult, Nature 193 (1962) 1304.
[26] A. Brohult, J. Brohult, S. Brohult, I. Joelsson, Acta Obstet. Gynecol. Scand. 56 (1977) 441.
[27] A. Brohult, J. Brohult, S. Brohult, I. Joelsson, Acta Obstet. Gynecol. Scand. 65 (1986) 779.