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Biochemical composition of *Nyctiphanes australis* and its possible use as an aquaculture feed source: lipids, pigments and fluoride content

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Abstract *Nyctiphanes australis* contained, on a dry weight basis, an average of 52% crude protein and 5.0 to 9.5% lipid. The fatty acid profile of *N. australis* was markedly unsaturated, with a mean total ω3 fatty acid content of 48.6±2.4% of total fatty acids. *N. australis* contained high levels of the essential long-chain polyunsaturated fatty acids eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3), ranging from 16.6 to 36.5% and 11.1 to 24.8%, respectively. The concentration of total carotenoids ranged from 137 to 302 μg g^{-1} dry wt, with no significant differences in concentrations found with season or life stage. The carotenoids were comprised of 79.5% astaxanthin and 20.5% canthaxanthin. The lipid and pigment compositions of *N. australis* suggest that the species could serve as a suitable feed source for cultured salmonids. Like other euphausiids, *N. australis* contained high levels of fluoride, with a seasonal range between 277 and 3507 μg g^{-1} dry wt. The high fluoride levels found in *N. australis* would not detract from its potential as a feed source for salmonids because ingested fluoride is largely absorbed by the skeleton.

Introduction

Nyctiphanes australis is the principal euphausiid in continental shelf waters off south eastern Australia (Ritz and Hosie 1982), and is the most important single food item for a variety of abundant fishes in Tasmanian waters (O'Brien 1988). It was found, for example, to constitute 99.9% of the stomach contents of jack mackerel, *Trachurus declivis*, seined during a 19 mo study (Webb 1976). These fish, in turn, support a commercial fishery of several tens of thousands of tonnes per year (Williams et al. 1987). Simple calculations suggest that this predator alone must therefore consume several tens of thousands of tonnes of krill per year.

Nyctiphanes australis is also the principal food of a number of seabird species, including the short-tailed shearwater *Puffinus tenuirostris*, whose population numbers approximately 19 million adults plus immature individuals (Skira 1986). Taken together, these observations suggest that the annual production of this euphausiid may exceed 100 000 tonnes in these waters.

Krill have been shown to be of high nutritional value as components of aquaculture feed (Storebakken 1988, Shimizu et al. 1990), and are used extensively in feed for farmed salmonids and other fishes in Japan (Odate 1979) and Canada (Sloan and Fulton 1987). An important biochemical characteristic in this connection is the typical high concentration of the polyunsaturated fatty acids (PUFAs) in krill. PUFAs are essential nutritional requirements of various commercially raised fish and shellfish, including salmonids (Lall 1991); however, quantitative levels required by fish are not known. The fatty acid composition of fish is largely a reflection of that of their diet (Cowey and Sargent 1972). High PUFA content in fish is commercially desirable, as these fatty acids, in particular eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), have been implicated in the prevention of human circulatory disorders (Bremner et al. 1989)

The carotenoid pigment astaxanthin is also typically present in high concentrations in krill (Funk and Hobson 1991). Astaxanthin and canthaxanthin are responsible for imparting the red colour to the flesh of salmonids and many other marine fish and shellfish species. Fish and other animals are unable to perform de novo synthesis of carotenoids, hence commercially farmed marine animals must obtain astaxanthin from their diets.

Tasmania supports a substantial salmonid farming industry which constitutes 2% of the world production (A.

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Smithies, Salmonid Growers Association, personal communication). Due to the high local abundance of *Nyctiphanes australis*, the potential of this species as a constituent of fish feed in terms of lipid and astaxanthin content was investigated. Other comparable lipid studies of *N. australis* have been restricted to their analysis as stomach contents of predator species (Cheah and Hansen 1970; Bishop et al. 1976).

Fluoride analysis of *Nyctiphanes australis* was also undertake, since other krill are known to have unusually high concentrations of fluoride in their exoskeletons (Nicol and Stolp 1989) which makes them unacceptable for direct human consumption. This has raised the issue of their appropriateness as food for animals reared for human consumption (Grave 1981).

Materials and methods

Sample collection

Between November 1989 and January 1991, *Nyctiphanes australis* was sampled monthly with a 500 µm-mesh plankton net at various locations along the 80 m contour in Riedle Bay, east of Maria Island off the Tasmanian east coast. Additional sampling details are provided in Young et al. (1993). Individual krill for chemical analyses were picked from the krill haul immediately after emptying the net. About 2 to 10 mg (dry weight) of krill were used for each analysis (water content of krill ranged between 75 and 83%, n=12; and a mean of 79% was used to convert wet weights to dry weights). Within minutes of capture, krill samples were placed in vials and stored in liquid nitrogen. On return to the laboratory the samples were transferred to a storage dewar containing liquid nitrogen, and remained there until analysis.

Individual net hauls were invariably dominated by *Nyctiphanes australis* within a narrow size range. However, mean krill size varied considerably between net hauls, and has been classified as adults >10 mm, subadults 5 to 10 mm; and juveniles < 5 mm. These size classes correspond approximately to adult, post-larval and adolescent, calyptopis and furcilia stages (Sheard 1953; Young et al. 1993). Measurements were based on Mauchline's (1980) Standard 1.

Lipid extraction and analysis

Nyctiphanes australis were ground in chloroform and extracted quantitatively by the one-phase chloroform/methanol/water method of Bligh and Dyer (1959), as modified by White et al. (1979). After phase-separation, the lipids were recovered in the lower chloroform layer (solvents were removed in vacuo) and stored at –20°C in 1.5 ml vials fitted with teflon-lined caps. Fatty acid methyl esters (FAME) and free alcohols and sterols were formed by direct transesterification of an aliquot of the total lipids with methanol/chloroform/hydrochloric acid (10:1:1; 100 °C; 60 min). After cooling, 1 ml milli-Q-water was added and the products were extracted into hexane/chloroform (4:1). Solvents were removed under a stream of nitrogen and the alcohols and sterols were converted to their corresponding O-trimethylsilylethers by treatment with N,Obis(trimethylsilyl)trifluoroacetamide.

A portion of the total lipid extract was analysed for lipid composition with an Iatroscan MK III TH10 thin-layer chromatography/flame ionization detection (TLC/FID) analyser (Iatron Laboratories, Japan) (Volkman et al. 1986; Volkman and Nichols 1991). Gas chromatographic analysis of fatty acid methyl esters and sterols were performed on a Hewlett Packard 5890 GC equipped with a methyl silicone fused-silica capillary column and a flame-ionization detector. Operating instruction have been described in detail by Nichols et al. (1988). Fatty acids and sterols were identified by comparing mass spectral and retention-time data with that obtained for authentic and laboratory standards (see Nichols et al. 1989). Gas chromatography–mass spectroscopy (GC–MS) analysis of selected samples was performed on an HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and a split/splitless injector.

Protein analysis

A sample of mixed krill (0.61 g wet wt) using individuals of different stages collected at various times of the year was analysed for crude protein. Total nitrogen was assayed by the Kjeldahl method (Association of Analytical Chemists 1984). Wet weight was converted to dry weight using a mean water content of 79.33%.

Pigment analysis

Total carotenoid content was estimated spectrophotometrically; a portion of the total lipid extract in 90% acetone was analysed for pigment composition using a Shimadzu UV-240 spectrophotometer (300 to 700 nm) (Ookubo and Matsuno 1985).

A representative sample of mixed krill using individuals of different stages collected at various times of the year was analysed using high-performance liquid chromatography, HPLC (Wright et al. 1991). Two peaks were resolved and identified by co-chromatography. One peak matched both the maximum wavelength (473 nm) and retention time (12 min) of the astaxanthin standard. The other peak co-chromatographed with known canthaxanthin from the blue green algae *Anabaena flos-aquae* with a maximum wave length of 467 nm and a retention time of 8 min (Wright et al. 1991). High-resolution MS data of the astaxanthin standard was acquired using a Kratos Concept ISQ with a direct probe inlet. Accurate mass data was acquired at 10000 resolution at a scan speed of 1 s per decade using perfluorokerosene as an internal reference. The standard was determined to contain pure astaxanthin.

Fluoride analysis

Fluoride was liberated from freeze-dried krill samples in a petri dish by acid digestion using perchloric acid saturated with Ag_2SO_4 (Lewis et al. 1987). The fluoride from this digest diffused into the petri dish lid which was coated with ethanolic sodium hydroxide solution (4% wt/vol. NaOH in 90% EtOH) while being heated at 50°C for 16 h. The alkaline mixture was dissolved in a 1:1 solution of water and TISAB II buffer consisting of NaCl and CDTA (cyclohexylenedinitrilo tetraacetic acid) in glacial acetic acid and water. The pH was adjusted to between 5.0 and 5.5 with 20% NaOH (Lewis et al. 1987). Fluoride concentration was measured with an Orion Model 96–09 combination fluoride electrode and a Radiometer Ion 85 pH/mV meter (resolution 0.1 mM).

Statistical analyses were performed by analysis of variance (ANOVA) using the "statistical analysis system" (Version 6.03, SAS Institute Inc 1988). Arcsine-transformation was done on all percentage data used for statistical analysis. Data are expressed as means ± standard deviation.

Results

The average crude protein content of *Nyctiphanes australis* was 52% of the total dry weight. The lipid content of *N. australis* ranged from 5.1 to 9.6% of the total dry weight, and no statistical difference was found between season or life stage. The lipid composition was dominated by polar lipids (including phospholipids and glycolipids) ranging from 68 to 86%. Triacylglycerols and sterols ranged between 5 to 21 and 5 to 8%, respectively (Table 1). No sig-

Table 1 *Nyctiphanes australis*. Dry weight, lipid class, total lipid, lipid-class ratios, cholesterol, carotenoid and fluoride levels as a function of season and life stage (*A* adult; *SA* subadult; *J* juvenile)

	1989				1990											Mean (SD)
	Nov	Dec			Feb	Mar					Apr	May	June	Sep	Nov	
	A	J	A	A	J	J	SA	A	A	A	SA	A	A	J	A	
Krill sample (g dry wt)	0.05	0.11	0.02	0.02	0.05	0.02	0.03	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.04	0.04 (0.03)
Lipid class (mg g^{-1} dry wt)																
triacylglycerol	12.2	5.3	9.8	4.8	6.2	9.9	8.8	14.9	11.4	14.0	10.9	16.1	4.1	14.0	14.6	11.2 (4.1)
free fatty acid	0.0	2.2	2.7	2.4	4.3	2.8	2.6	3.7	2.1	0.0	2.7	4.0	3.4	2.3	3.1	2.8 (0.8)
polar lipid	39.5	39.5	66.2	58.1	52.6	52.1	48.4	69.9	58.0	70.1	55.8	61.1	60.8	63.2	48.0	64.1 (10.0)
sterol	4.5	4.0	4.3	3.9	5.0	4.7	4.4	6.1	5.2	5.8	5.4	5.6	4.1	5.9	4.7	5.5 (0.7)
Total lipid (mg g^{-1} dry wt)	56.2	50.9	83.0	69.3	68.1	69.6	64.1	94.6	76.7	89.9	74.8	86.8	82.4	85.4	70.4	84.7 (11.4)
Cholesterol (mg g^{-1} dry wt)	1.1	0.4	3.9	2.0	1.1	0.3	1.9	2.4	2.4	2.4	2.4	2.4	0.8	2.6	1.9	1.9 (1.0)
Percentage lipid composition																
triacylglycerol	15.7	10.3	11.8	7.0	9.1	14.3	13.7	15.8	14.9	15.6	14.5	18.5	5.0	16.4	20.7	14.7 (4.3)
free fatty acid	0.0	4.3	3.3	3.5	6.4	4.1	4.0	3.9	2.7	0.0	3.6	4.6	4.1	2.7	4.4	3.8 (1.1)
polar lipid	78.5	77.6	79.7	83.9	77.3	74.8	75.4	73.9	75.7	77.9	74.5	70.4	86.0	74.0	68.1	87.1 (4.5)
sterol	5.8	7.8	5.2	5.6	7.3	6.8	6.9	6.4	6.8	6.5	7.3	6.5	5.0	6.8	6.7	7.5 (0.8)
Total lipid (% dry wt)	7.8	5.1	8.3	6.9	6.8	7.0	6.4	9.5	7.7	9.0	7.5	8.7	8.2	8.5	7.0	8.5 (1.1)
Triacylglycerol:polar lipid	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.3	0.2 (0.1)
Free fatty acid:polar lipid	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.5 (0.1)
Total carotenoid[a]																
μg g^{-1} dry wt	161	151	196	169	178	174	137	303	186	140	263	252	206	200	146	215 (46)
μg g^{-1} lipid dry wt	2080	2959	2361	2432	2616	2500	2130	3202	2420	1555	3517	2904	2494	2342	2068	2895 (489)
Fluoride (μg g^{-1} dry wt)	531	548	1079	1084	684	3506.5	277	1198	665	2521.3	3212	1077	3500	1930	883	1556 (1129)

[a] Carotenoid included both astaxanthin and canthaxanthin at ≃79.5 and 20.5%, respectively

nificant seasonal differences were detected in the lipid class composition of the samples.

Cholesterol was the major sterol with an average concentration over season and life stage of 1.9±1.0 mg g^{-1} dry wt. Only traces of desmosterol (cholesta-5, 24-dien-3β-ol) and brassicasterol (24-methylcholesta-5,22E-dien-3β-ol) were detected. Non-esterified (free) fatty acids constituted only minor components of the total lipid (3.8%±1.1, Table 1). This relatively low percentage of free fatty acid indicates adequate storage of the samples prior to extraction (Saether et al. 1986).

The saturated fatty acid 16:0 and the long-chain polyunsaturated fatty acids eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) dominated the fatty acid composition of *Nyctiphanes australis* (Table 2). The two major polyenoic acids, EPA and DHA, ranged from 16.6 to 36.5 and 11.1 to 24.8% of the total fatty acids, respectively. The fatty acid profile was markedly unsaturated, with the mean total ω3 fatty acid being 48.6±2.4%. Significant differences were found in the levels of EPA and DHA between life stages. Both adult and subadult life stages differed significantly from juvenile krill in levels of these two PUFAs. Juveniles were found to have a higher mean percent EPA (23.6%) than the adults (17.7%) and subadults (19.5%). Juveniles had lower mean percent DHA (18.1%) compared with adults (22.3%) and subadults (23.7%). These differences were probably due to the influence of one sample (September 1990) which was significantly different from the total data set. No significant differences were found however in the total ω3 fatty acids between season and life stages.

Both astaxanthin and canthaxanthin were detected in *Nyctiphanes australis*, with total carotenoid levels ranging from 137 to 303 μg g^{-1} dry wt. Levels of astaxanthin and canthaxanthin were 79.5 and 20.5%, respectively. Fluoride concentrations ranged between 277 and 3507μg g^{-1} dry wt. No significant differences in either carotenoid or fluoride levels were evident between season or life stage (Table 1).

Discussion

Marine zooplankton tend to have lower lipid concentrations at lower latitudes (Sheard 1953), and *Nyctiphanes australis* is no exception. The total lipid in *N. australis* (8.5% dry wt) was less than values which characterize high-latitude krill species. *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis*, for example, all of which are found in northern polar regions, attain seasonal peak lipid contents of about 40, 40 and 50% dry wt, respectively (Falk-Petersen et al. 1981), and concentrations vary seasonally by roughly threefold, fourfold, and fourfold respectively (Saether et al. 1986). Lipid levels of the Antarctic euphausiid *Euphausia crystallorophias* range between 10% dry weight in November and 35% dry weight in May (Littlepage 1964). All these polar species depend on lipid stores to survive periods of low plankton production in the winter. Lipid levels of the Antarctic krill *E. superba* collected between spring and autumn are also highly variable, and range from 3 to 36% dry weight (Clarke 1980; Ellingsen and Mohr 1981; Saether et al. 1986; Hagen 1988; Virtue et al. 1993). It has been proposed that this latter species may not rely solely on lipid sources for overwintering (Ikeda and Dixon 1982). Unlike high-latitude species, little seasonal variation in lipid content was detected in *N. australis* in the present study.

All samples of *Nyctiphanes australis* analysed in this study had relatively low levels of the storage lipid, triacylglycerols (11%), compared to other euphausiids such as *Meganyctiphanes norvegica* (53%), *Thysanoessa raschii* (44%) and *Euphausia superba* (37%) (Sargent and Falk-Petersen 1981; Virtue et al. 1993). The majority of lipid in *N. australis* was in the form of structural components. Phospholipids and cholesterol are structural elements of the plasma membrane, whereas storage lipids such as triacylglycerols and wax esters are used as energy stores (Lee et al. 1970; Sargent 1976).

The similarity in lipid content and composition of these samples would imply a relatively constant available food supply throughout our sampling period. Harris et al. (1987), however, reported season fluctuations in phytoplankton in this region, with bloom conditions during spring. *Nyctiphanes australis* is omnivorous (Mauchline 1980), and Ritz et al. (1990) concluded from a study of stomach contents analysed quantitatively throughout the year, that *N. australis* was an opportunistic omnivore/detritivore. Young et al. (1993) reported no significant relationship between length and weight of *N. australis* with season, using samples collected from the same stations as those of the present study. Due to their ability to exploit a variety of food sources, *N. australis* is able to maintain physiological status in terms of lipid content and composition under conditions of reduced phytoplankton.

Although physiological condition appears to be maintained in *Nyctiphanes australis* throughout the year, a response to environmental conditions may be evident in a more dynamic sense. Young et al. (1993) reported significant variation both seasonally and interannually in density, biomass and population structure of *N. australis*. This variation was attributed to regional oceanographic conditions driving phytoplankton production. Biomass estimates may also be influenced by both seasonal predation upon *N. australis* and the fact that this species may live at depths out of plankton-tow range during the winter months (Young et al. 1993).

In terms of mariculture feed, the total lipid level of *Nyctiphanes australis* is near the optimum range used for salmonids. Fish and crustaceans fed diets high in total lipid accumulated excess lipid which was deposited as visceral fat. This excess is discarded as waste during processing. The weight of the mesenteric adipose tissue in salmonid fish has been found to increase with increasing dietary lipid (Lin et al. 1977; Takeuchi et al. 1978).

A number of samples analysed which were not included in this data set contained wax esters that ranged from 2 to 7% of the total lipid. All these samples consisted of very

Table 2 *Nyctiphanes australis*. Percentage fatty acid composition as a function of season and life stage (*A* adult; *SA* subadult; *J* juvenile; *other* includes all categories (<0.5%)

Fatty acid	1989				1990											Mean (SD)
	Nov	Dec			Feb	Mar					Apr	May	June	Sep	Nov	
	A	J	A	A	J	J	SA	A	A	A	SA	A	A	J	A	
14:0	5.5	4.9	5.3	4.2	4.4	6.1	5.6	5.9	5.4	5.8	5.8	6.2	3.5	3.5	6.0	5.2 (0.9)
15:1ω8	0.1	0.1	0.2	0.0	0.2	0.2	0.3	0.0	0.3	0.4	0.0	0.0	0.7	0.0	0.0	0.2 (0.2)
15:0	1.1	1.2	0.9	0.5	1.3	1.2	1.0	1.0	1.0	1.7	1.0	1.5	1.2	0.3	0.9	1.1 (0.3)
16:4ω1	0.6	0.4	0.6	0.5	0.4	0.5	0.3	0.4	0.3	0.5	0.5	0.8	0.5	1.3	0.4	0.5 (0.2)
16:3ω4	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.4	0.0	0.1	0.2	0.6	0.1	0.2(0.1)
16:1ω7	2.6	4.3	2.7	2.6	2.6	2.9	1.6	1.7	1.7	2.3	2.2	2.2	2.1	7.0	2.5	2.7 (1.4)
16:1ω5	0.7	0.4	0.7	0.3	0.4	0.4	0.3	0.3	0.2	0.6	0.3	0.1	0.4	0.5	1.0	0.4 (0.2)
16:0	25.2	24.8	22.5	21.3	23.0	23.1	21.6	21.2	20.9	23.4	21.4	20.9	23.5	20.2	21.4	22.3 (1.5)
17:0	0.9	0.9	0.8	0.5	1.0	1.0	0.9	0.9	0.9	1.2	0.8	1.1	1.1	0.4	0.9	0.9 (0.2)
18:4ω3	5.1	2.7	5.5	3.1	2.9	4.3	2.9	4.7	3.5	2.6	4.2	5.3	4.0	1.8	7.3	4.0 (1.4)
18:2ω6	3.6	3.0	3.5	2.5	3.2	3.5	3.5	4.1	3.5	3.5	4.0	3.2	3.0	1.2	2.8	3.2 (0.7)
18:3ω3	3.8	2.3	3.2	2.0	3.4	3.1	2.5	3.7	2.6	3.2	3.5	3.4	3.2	0.5	3.0	2.9 (0.8)
18:1ω9	6.5	6.2	6.8	6.8	7.9	7.0	8.6	8.2	8.2	7.9	8.5	7.3	7.8	5.3	5.6	7.2 (1.0)
18:1ω7	3.2	3.9	3.3	3.8	3.2	3.3	2.6	2.8	2.7	3.8	3.1	3.6	3.3	5.8	2.9	3.4 (0.8)
18:0	2.3	2.3	2.2	1.6	2.9	2.7	2.2	2.2	2.3	2.3	2.1	2.1	2.7	2.2	1.6	2.2 (0.4)
20:4ω6	0.0	0.0	0.0	2.5	0.0	0.0	0.0	1.8	2.3	1.9	1.8	0.0	1.9	0.0	0.0	0.8 (0.3)
20:5ω3	17.1	20.9	17.7	21.3	19.3	18.8	20.6	16.6	18.4	16.6	17.1	17.5	16.7	36.5	17.9	19.5 (4.9)
20:4ω3	0.5	0.4	0.5	0.3	0.5	0.3	0.2	0.4	0.2	0.4	0.3	0.3	0.4	0.5	0.6	0.4 (0.1)
20:2ω6	0.3	0.2	0.2	0.1	0.3	0.2	0.2	0.6	0.1	0.3	0.0	0.2	0.0	0.0	0.2	0.2 (0.1)
20:1ω9	0.5	0.7	0.4	0.5	0.6	0.4	0.3	0.2	0.1	0.4	0.3	0.1	0.7	0.5	0.9	0.5 (0.2)
22:6ω3	18.9	19.2	21.8	24.8	21.3	20.6	24.4	22.4	24.4	20.5	22.9	23.0	22.0	11.1	22.6	21.3 (3.3)
22:5ω3	0.4	0.4	0.5	0.3	0.4	0.3	0.4	0.5	0.3	0.4	0.2	0.5	0.6	0.8	0.5	0.4 (0.1)
other	0.5	0.4	0.3	0.0	0.5	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.2	0.0	0.4	0.2 (0.1)
16:1ω7/16:0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1 (0.1)
18:1ω7/18:1ω9	0.5	0.6	0.5	0.6	0.4	0.5	0.3	0.3	0.3	0.5	0.4	0.5	0.4	1.1	0.5	0.5 (0.2)
total ω7	5.8	8.3	5.9	6.4	5.8	6.1	4.2	4.5	4.3	6.1	5.2	5.7	5.4	12.8	5.4	6.1 (2.1)
total ω3	45.8	45.8	49.3	51.8	47.9	47.4	51.0	48.2	49.5	43.7	48.2	50.0	46.9	51.2	52.0	48.6 (2.4)

small individuals, and microscopic examination revealed that they also contained significant numbers of copepods, unlike samples consisting of large euphausiids which could be readily sorted. Analyses of samples containing copepods are not presented here. These observations however, may explain previous conflicting reports concerning the presence or absence of wax esters in *Nyctiphanes australis*: Bishop et al. (1983) reported significant concentrations in this species, whereas Cheah and Hansen (1970) found none.

Total ω3 fatty acids constituted almost half the total fatty acids found in *Nyctiphanes australis*, with the two essential polyenoic acids, EPA and DHA dominating. These levels compared well with those found in partially digested krill from stomachs of slender tuna (Bishop et al. 1976). Levels of both EPA and DHA were 25 to 30% higher than those reported for polar species such as *Meganyctiphanes norvegica, Thysanoessa raschii, T. inermis* (Sargent and Falk-Petersen 1981), and *Euphausia superba* (Virtue et al. 1993). The food source of *N. australis* may contain substantial quantities of both eicosapentaenoic and docosahexaenoic acids. Alternatively/in addition, *N. australis* may possess some ability to elongate and desaturate dietary fatty acids.

The superiority of long-chain over short-chain C_{18} PUFAs in maintaining growth has been reported in nutritional studies for marine fish (reviewed by Sargent and Whittle 1981). It has been suggested that carnivorous marine fish, which have natural diets high in EPA and DHA, may not have evolved or may perhaps have lost the enzymatic pathways for the elongation and desaturation of shorter-chain PUFAs to these longer ones (Cowey and Sargent 1977; Sargent and Whittle 1981). ω3 fatty acids are essential for salmonid development (Ashton et al. 1993). Species such as rainbow trout (*Oncorhynchus mykiss*) require ω-3 rather than ω-6 fatty acids (Castell et al. 1972). Dietary deficiencies in ω-3 fatty acids in salmonid fish cause physiological dysfunction of developing fish and early embryonic mortality (Watanabe 1982; Leray et al. 1985). Because *Nyctiphanes australis* contains very high levels of long-chain ω3 PUFAs, it would therefore be an attractive food source for mariculture species.

The crude protein content of *Nyctiphanes australis* (52%) was typical of that found in euphausiids (reviewed by Mauchline 1980). Crude protein is not a measure of metabolizable energy. Krill carapace, which is a nitrogenous polysaccharide, represents a portion of crude protein. Amino acid protein constitutes 78% of the crude protein in whole *Euphausia superba* (Pierce et al. 1969; Siebert et al. 1980). Amino acid content was not analysed in *N. australis* in the present study; however, assuming levels similar to those found in *E. superba*, protein in terms of metabolizable energy would be ~40%. A survey of the quality of various fish meals available to the Tasmanian salmonid industry reported percentage protein in the range of 60 to 70% (Foster 1991). The protein content of *N. australis* would be considered adequate in terms of a mariculture feed, although supplemental protein might be required.

Although, as in other euphausiids, the fluoride concentration in whole *Nyctiphanes australis* would be considered too high for both human consumption and stock feed, this species would be suitable as mariculture feed. The United States Food and Drug Administration (USFDA) allowance for human consumption is 100 $\mu g\ g^{-1}$ of fluoride per day (Budzinski et al. 1985). In both vertebrates and invertebrates, fluoride is accumulated in the skeletal structures. Grave (1981) reported that the elevated fluoride concentration in salmonids fed a pure krill diet was restricted largely to skeletal tissue. Fluoride content of muscle tissue increased slightly, but did not exceed concentrations reported in various wild salmonid species and was < 2% of the USFDA limit for human consumption. Oehlenschlager and Manthey (1982) investigated the fluoride content of Antarctic fish, and found no differences in fish feeding on krill and fish feeding predominantly on other fish. They found fish muscle to contain 2 $\mu g\ g^{-1}$ fluoride, which is similar to that of fishes in other waters; however, the fluoride level found in the bone tissue of these fish was in the order of 600 to 1200 $\mu g\ g^{-1}$. The flesh of mariculture species such as salmonid fed on a krill-based diet would not, therefore, be expected to contain high levels of fluoride. Rather, if accumulated, fluoride might be expected to be found in the skeletal material.

A large variation in fluoride levels in *Nyctiphanes australis* was found in this study. Adelung et al. (1987) reported >99% of fluoride to be concentrated in the cuticle of euphausiids (2600 $\mu g\ g^{-1}$ dry wt in *Euphausia superba* and 3300 $\mu g\ g^{-1}$ in *Meganyctiphanes norvegica)*. As fluoride accumulates mainly in the chitinous exoskeleton of crustaceans and is found in very low concentrations in the muscle and soft tissue (50 to 100 $\mu g\ g^{-1}$; Szewielow 1981), a substantial fluctuation within the moult cycle would be expected in *N. australis*.

Astaxanthin is the major carotenoid in many crustaceans and was found to be the dominant carotenoid in *Nyctiphanes australis* in this study. *N. australis* is somewhat richer in this pigment than *Euphausia superba*, which was found to have a mean astaxanthin content of 94 $\mu g\ g^{-1}$ dry wt (converted from wet weight using 77% tissue water content for this species; Clarke 1980). Carotenoids such as astaxanthin and canthaxanthin are used as pigmenting agents by the mariculture industry. Carotenoids cannot be synthesised de novo by salmonids, and hence dietary supplements are required in net-pen reared fish (Storebakken and No 1992).

Euphausia superba, which is rich in carotenoids and contains mainly (3R,3'R)-astaxanthin diester, was used successfully to enhance integument pigmentation of cultured yellow tail and sea bream (Fujita et al. 1983; Maoka et al. 1985; Miki et al. 1985). Shigeru et al. (1987) fed oil extracted from *Euphausia superba* to 180 g coho salmon (*Oncorhynchus kisutch*) for 8 wk, and reported marked flesh pigmentation which was found to consist mainly of astaxanthin.

Physiological conditions vary within and between species, and the need for size- and sex-specific research is necessary to determine the rate of deposition of carotenoids in

fish flesh. Based on our results, *Nyctiphanes australis* is a potential astaxanthin source for use in salmonid culture. Feeding studies are warranted to confirm the commercial use of this species as a pigmentation agent. Other factors relevant to the species' potential commercial value will be discussed elsewhere (Johannes in preparation).

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