LIPID COMPOSITION OF TWO SPECIES OF *SEROLIS* (CRUSTACEA, ISOPODA) FROM ANTARCTICA

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ABSTRACT. The lipid content and composition of *Serolis pagenstecheri* Pfeffer and *Serolis cornuta* Studer have been studied. The strikingly flat shape of serolids means that much of the animal is cuticle; as a result, over 50% of the dry weight is ash and 17% is chitin. The lipid content of males is about 1% fresh weight, individual variation being due mainly to alterations in the amount of stored triacylglycerol; phospholipid remains constant at about 4–5μmol g^{-1} fresh weight. Females with maturing ovaries accumulate both triacylglycerol and phospholipid, increasing total lipid content to almost 4%. The fatty acid composition is typical of marine benthic crustaceans, being dominated by 16:0, 16:1ω7, 18:1ω9, 18:1ω7, 20:4ω6, 20:5ω3 and 22:6ω3. Triacylglycerol fatty acids are generally more variable, less unsaturated and of shorter chain length than those from phospholipids. These differences are associated largely with variations in the proportion of 20:5ω3 and 22:6ω3. Several isomers of 20:1 and 22:1 are present in the triacylglycerols. Egg lipids are rich in monoenoic acids, and contain about 30μg polyenoic fatty acids per egg. The pigments in adults serolids are mostly xanthophylls and both sexes contain astaxanthin.

INTRODUCTION

Isopods of the family Serolidae are confined almost exclusively to the southern hemisphere and about 25 species are found in the waters surrounding the Antarctic continent (Sheppard, 1933; Kusakin, 1968). They are found mostly in areas of soft substrate into which they frequently burrow to a shallow depth. Serolid isopods feed upon a range of smaller invertebrates and are important members of the shallow water benthic community in the Southern Ocean. The biology of two Antarctic species, *Serolis polita* and *S. cornuta*, has been studied in detail at Signy Island (Luxmoore, 1981, 1982*a, b*, 1984, in press). Most other work on the Serolidae has been taxonomic, although the biology of several South American species has been described (Bastida and Torti, 1970; Moreira, 1973*a*, *b*, 1974).

In this paper the lipid content and composition of two species, *Serolis pagenstecheri* Pfeffer 1887 from South Georgia and *S. cornuta* Studer 1879 from Signy Island, are reported. A previous study of the benthic shrimp *Chorismus antarcticus* at South Georgia revealed a similar lipid content to the temperate species *Pandalus montagui* (Clarke, 1977*a, b*, 1979). In both species the major factor influencing lipid storage was vitellogenesis in females. In this study, individual variation in the lipid content and composition of *Serolis* has been examined in relation to reproductive condition and ecology. The aims of the study were to see whether the lipid content was similar to other benthic crustaceans, to determine the extent of lipid storage during vitellogenesis, and to examine the influence of egg production on fatty acid composition.

MATERIALS AND METHODS

Sampling

Serolis pagenstecheri was collected from sandy mud in shallow water (5–15 m) in King Edward Cove, South Georgia (54°17′ S, 36°30′ W) by 0.5-m Agassiz trawl, or SCUBA divers. Collection dates were 9 and 28 January, 3 February 1978, 8 and 14

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February 1980. South Georgia is the type locality for this species and the individuals examined in this study conform to the original description by Pfeffer (1887). A smaller, paler, form (var. *albida*) was described by Nordenstam (1933) from Shag Rocks, and this is very similar to *S. aspera*. Luxmoore (1981) has suggested that *S. pagenstecheri* is a very variable species that tends to be large in shallow waters (as in the individuals analysed in this study) but smaller in deeper water or further south. Specimens were either analysed immediately or gently blotted free of excess water and frozen to −40°C for analysis in UK.

Serolis cornuta was collected from 30–50 m depth in Borge Bay, Signy Island, South Orkney Islands (60°43′S, 45°38′W), by 0.75-m rectangular dredge. Collection dates were 8 March 1978 and 28 January 1980. All specimens were frozen immediately to −40°C and analysed in UK. All subsequent storage was at −40°C or below.

Proximate analyses

Fresh or frozen weight was measured following removal of excess water. Dry weight was measured after 48 h at 60°C, and ash weight after ignition at 475°C for 24 h. Care was taken not to allow ignition temperature to rise above 500°C in order to prevent loss of K^+, Na^+ (Grove and others, 1961; Isaac and Jones, 1972) or $CaCO_3$ (Hirota and Szyper, 1975) as volatiles.

For protein determination, individual serolids were weighed and digested in 50 ml 0.1 M NaOH at 50°C for 12 h. The digest was then diluted with water to a concentration of approximately 0.6 mg ml^{-1} protein, and $5 \times 100\,\mu l$ aliquots removed for assay. These were diluted to 1 ml with water and assayed for protein according to Lowry and others (1951), as modified by Hartree (1972). Fresh calibration curves were constructed for each assay with bovine serum albumin (Sigma).

Chitin was estimated as tissue stable to digestion in 1 M NaOH at 50°C for 12 h. Insoluble material was filtered on to dry, pre-weighed glassfibre filter papers and washed several times with dilute HCl, followed by deionized water. The filter papers were then dried and weighed.

Lipid and pigment content

Lipid was extracted by homogenizing individuals in ice-cold de-gassed methanol/chloroform (2:1 v/v) and filtering the homogenate rapidly through a GF/C glassfibre filter paper. Calculated volumes of chloroform and water were added and the phases allowed to separate overnight at 2°C, in the dark (Bligh and Dyer, 1959). The lower chloroform layer was then removed, 5 ml dichloromethane added to aid azeotropic drying and the solvents removed under nitrogen at 50°C in a rotary evaporator. The pure lipid was weighed and immediately re-dissolved in 5 ml chloroform. Wherever possible, all procedures were carried out in subdued light and under a blanket of nitrogen to prevent oxidation of lipids or pigments.

To determine total pigment content, 0.5 ml of the extract was blown free of solvent with a stream of dry nitrogen and the lipid immediately re-dissolved in between 4 and 10 ml *n*-hexane. The absorption spectra of *n*-hexane extracts usually showed a peak at 450 nm and thin-layer chromatography (TLC) showed that most components were more polar than astaxanthin. Chlorophyll was also present in some samples. Absorbence was therefore measured at 450 nm and total xanthophyll calculated assuming an $E^{1\%}_{1\,cm}$ of 2500 (Herring, 1969).

Lipid composition

Triplicate aliquots of lipid extract containing approximately 0.1 μmol P were

blown free of solvent and digested with 800 μl 72% perchloric acid at 180° for 30 min. The resultant orthophosphate was then assayed according to Bartlett (1959). Reagent blanks contained 800 ml perchloric acid alone and fresh calibration curves over the range 0–0.18 μmol KH_2PO_4 were constructed for each assay. Total phospholipid was calculated assuming an average molecular weight of 796.

Lipid class composition was determined by TLC-scanning densitometry. Neutral lipids were separated on 20 × 20 cm plates of silica gel H (no organic binder or fluorescent indicator, Merck 5721, BDH Ltd) in the solvent system petroleum spirit (40–60°C)/peroxide-free diethyl ether/acetic acid, 80/20/1 v/v, and polar lipids in chloroform/methanol/water, 65/25/4 (v/v). After separation, lipids were charred with 3% cupric acetate in 8% aqueous orthophosphoric acid at 180°C (Fewster and others, 1969). Plates were scanned with a Camag TLC scanner (1 cm slit, 540 nm) and peak areas were measured electronically. Correction factors were applied to the phospholipid, free sterol and sterol ester components, based on calibration with marine invertebrate lipid. Any unknown component was assumed to have a response similar to that of triacylglycerol. The method is described in detail by Clarke and Wickins (1980).

Pigment composition

Tentative identifications of carotenoid pigments and porphyrins were obtained by TLC on silica gel H. The solvent system was 30% acetone in petroleum spirit (40–60°C). Samples were run alongside chlorophyll-*a* and β-carotene (Sigma) and extracts of pigments from the caridean shrimp *Chorismus antarcticus* as a secondary standard for astaxanthin. Spectra were measured in *n*-hexane with a Pye Unicam SP8–100 scanning spectrophotometer.

Fatty acid composition

Triacylglycerol and phospholipid fractions were isolated by TLC and fatty acid methyl esters prepared by mild alkaline deacylation. This avoids contamination of the sample with aldehyde dimethylacetals from plasmalogen phospholipids, as occurs with hydrolysis under acid conditions (Dawson, 1960; Brockerhoff, 1963). The fatty acid methyl esters were analysed on a 25-m WCOT SP1000 glass capillary. Samples were introduced by Grob splitless injection (Carlo Erba 2150) and chromatographic performance was monitored with a secondary standard of methyl esters prepared from cod liver oil (Ackman and Burgher, 1965).

Tentative identifications were obtained by precision isothermal GC at 180°C, co-injection with standards, semi-log plots and hydrogenation. Peak areas were measured electronically and unsaturation parameters calculated by computer program.

A few specimens were also analysed on a 2 m × 6 mm i.d. column of 20% DEGS on 80/100 mesh AW DMCS Chromosorb W (Clarke and Prince, 1976).

Data handling

All calibration curves contained at least eight duplicate points. Regression analyses of lipid composition data were performed using the GENSTAT statistical package (Rothamsted Experimental Station).

Results

Proximate composition

Serolids are strikingly flattened in shape, the exoskeleton extending laterally well

away from the bulk of the body; this shape influences chemical composition by increasing the proportion of cuticle. The protein, mineral ash and total dry matter content of a range of sizes of male and female *Serolis pagenstecheri* are shown in Fig. 1. At this level of resolution, no difference could be detected between males and females ($P>0.05$), and so the composition of a representative serolid of 2 g fresh weight was calculated (Table I). The relatively large amount of cuticle associated with the flattened shape means that there is an unusually high percentage of mineral ash and chitin. Applying the normally accepted calorific equivalents (Petrusewicz and McFadyen, 1970) to this composition gives a representative calorific value for *Serolis* of $3.49\,kJ\,g^{-1}$ (fresh weight). This is equivalent to about $11.2\,kJ\,g^{-1}$ (dry weight), similar to the value of $11.99\,kJ\,g^{-1}$ (dry weight) found by Luxmoore (in press) for *Serolis polita*. Individual calorific values will be lower in males and higher in mature females.

Lipid content

The total lipid contents of male and female *Serolis pagenstecheri* and *S. cornuta* are shown in Table II. No significant differences were apparent between isopods sampled on different dates (all $P>0.05$) and so the data for each species were pooled by sex.

In both species the lipid content of males was low with an approximately normal distribution of individual values (Fig. 2). The variation in male lipid content is probably associated with the moult cycle and the period of time since the last meal. The few data for ovigerous female *S. pagenstecheri* (analysed without eggs) suggest a similar lipid content to males.

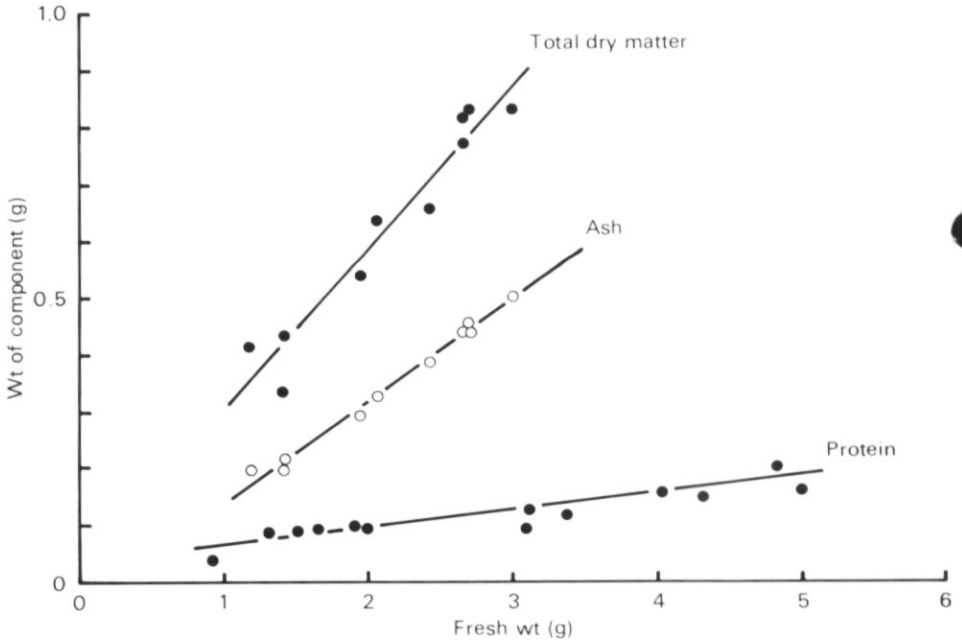

Fig. 1. *Serolis pagenstecheri*. Total dry matter, mineral ash, and total protein as a function of fresh weight; South Georgia, February 1980.

Table I. Proximate composition of a representative *Serolis pagenstecheri* of 2g fresh weight and intermediate lipid content.

	mg	*% fresh wt*	*% dry wt*
Water	1414.3	70.7	
Ash	316.6	15.8	54.1
Protein	90.4	4.5	15.4
Chitin	99.6	5.0*	17.0
Lipid, carbohydrate and other minor components (by subtraction from total dry matter)	79.1	4.0	13.5
Total dry matter	585.7	29.3	

*Mean of four determinations = 4.98% fresh wt (standard error = 0.47).

Female isopods generally had higher lipid contents than males, although in both species there was a wide spread of individual values (Fig. 2). In *Serolis cornuta* female lipid content was on average only slightly greater than in males (Table II). Females sampled in late summer close to spawning (March) tended to be richer in lipid than those sampled earlier in the summer (January: see Fig. 2), although this difference was not quite statistically significant ($0.10 > P > 0.05$). There was, however, a strong correlation between female size and total lipid content ($P > 0.01$). *Serolis cornuta* probably breeds only once (Luxmoore, 1982*a*) and in the sympatric *S. polita* ovarian development takes over two years, with 75% of the total yolk accumulation occurring in the final summer before spawning. Together, these observations suggest that the female *S. cornuta* analysed included both young animals with relatively small ovaries and mature females close to spawning. The maximum lipid content achieved by a mature female is thus about 2.5–3% fresh weight.

The mean brood size for *S. cornuta* is about 100 eggs for a female of width 4.5 cm (Luxmoore, 1982*a*), equivalent to a fresh weight of about 3.5 g. Brood size may vary from 75 to 225 eggs. Assuming an egg lipid content of 700 μg (data from Table III for *S. pagenstecheri*, scaled up to the dry weight of a single *S. cornuta* egg from data in Luxmoore, 1982*a*) and taking the lipid content of a female minus her ovary to be

Table II. Total lipid content of *Serolis pagenstecheri* (South Georgia) and *S. cornuta* (Signy Island). Data presented as mean ± standard error, with number of individuals in parentheses. nd, no data. Ovigerous females were analysed without their eggs.

	Total lipid (% fresh wt)	
	S. pagenstecheri	*S. cornuta*
Males	1.08 ± 0.07 (33)	1.08 ± 0.11 (15)
Females	2.08 ± 0.20 (24) (0.48 to 3.60)*	1.49 ± 0.11 (24) (0.62 to 2.92)*
Ovigerous females	1.20 ± 0.35 (3)	nd

*Range of values observed in all females except those brooding eggs, including both free-swimming individuals and those captured paired with males.

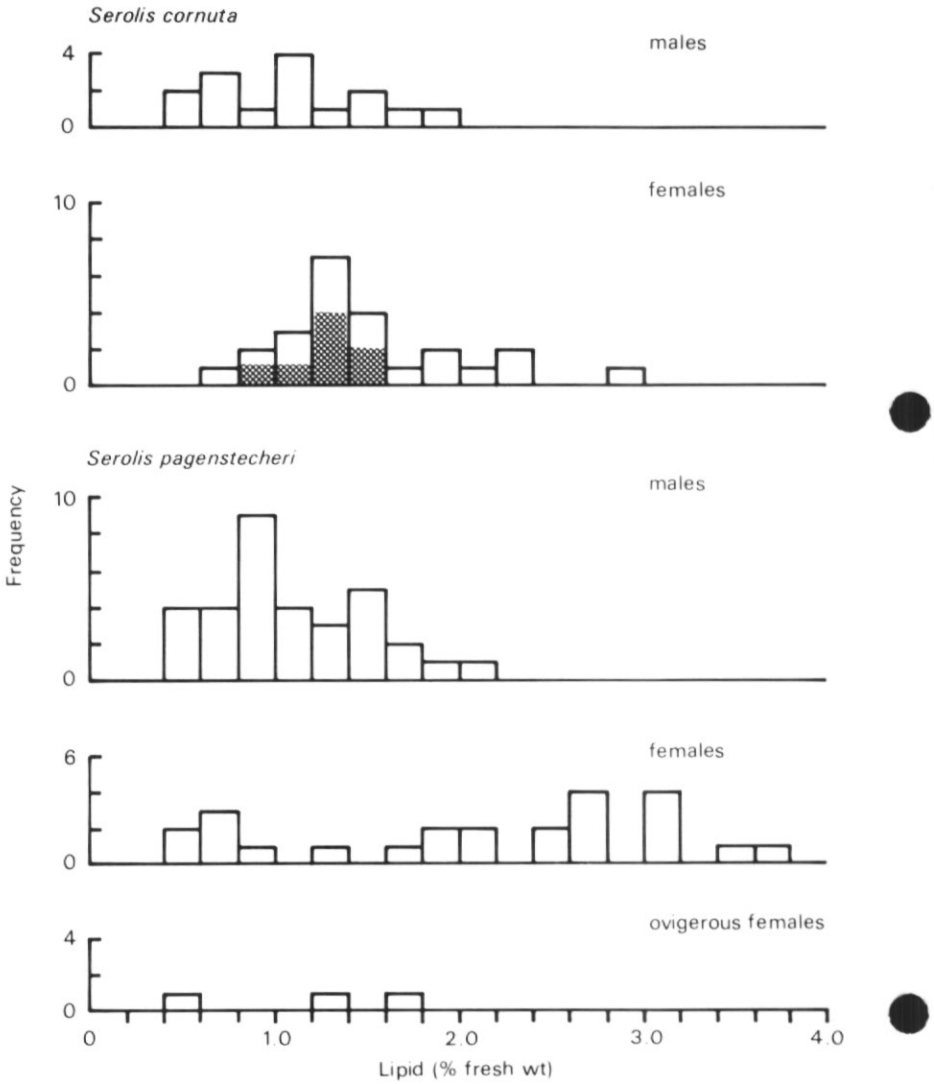

Fig. 2. Frequency histograms of lipid content (% fresh weight) of male and female *Serolis cornuta* and *S. pagenstecheri*. In histogram for female *S. cornuta* shaded areas represent females caught in January, clear areas those caught in March.

equivalent to that of a male, these brood sizes would suggest a mean female lipid content of about 3% fresh weight, with a range of 2.6 to over 5%. The greater lipid content of female *S. cornuta* compared with males can thus be explained solely by the accumulation of ovarian lipid.

The variation in lipid content is very much wider in female *Serolis pagenstecheri*, from 0.48 to 3.6% fresh weight. In this species the mean brood size is 232 for a female of width 3.7 cm (Luxmoore, 1982*a*). taking the lipid content of a newly spawned egg to be 190 μg (Table III), a calculation similar to that above indicates that mature female *S. pagenstecheri* should average 2.1% lipid, with an upper limit of about 4%.

Table III. *Serolis pagenstecheri*. Lipid content of eggs; South Georgia, February 1980. Development stages are those described by Luxmoore (1982*a*); nd, no data.

Fresh weight of ovigerous female (minus eggs) (g)	*Brood size*	*Fresh wt per egg (mg)*	*Egg lipid content*		*Remarks*
			μg	*% Fresh wt*	
2.9137	210	1.684	190	11.28	Undifferentiated, stage A
1.9803	123	1.873	173	9.24	Undifferentiated, stage A
3.2419	290	4.496	120	2.67	Eggs well differentiated; eyes, body segmentation, movement, pigment spots; late stage C
4.8084	4	12.55	nd	nd	Very small juveniles hatched, but still within the marsupium; stage E

In this species, too, the wide spread of individual lipid values suggests that the samples included females at all stages of ovarian development. The relationship between lipid content and female size, however, was significantly negative ($b = -0.76$, $F = 12.32$, $n = 24$) because of the presence of six females of between 2.7 and 4.2 g fresh weight with very low lipid contents (< 1.0%). These were probably females that had released broods of juveniles but had not regenerated ovaries, suggesting that (like *S. cornuta* at Signy) *S. pagenstecheri* at South Georgia breeds only once.

Egg lipid content

In common with many polar marine invertebrates, *Serolis pagenstecheri* produces small numbers of relatively large eggs. When newly spawned, the eggs weigh about 1.7 mg, and contain 190 μg of lipid (Table III). During the course of embryonic development, egg fresh weight increases and lipid content decreases; the lower lipid content of the second batch of undifferentiated eggs was assumed to be the result of the start of development. The increase in egg weight is partly related to an uptake of mineral salts for cuticle formation (Luxmoore, 1982*a*).

The data in Luxmoore (1982*a*) suggest that the eggs of *Serolis cornuta* are larger than those of *S. pagenstecheri* but no ovigerous females were obtained in this study.

Pigment content

Serolis pagenstecheri is largely greyish in overall colour, although a fine scatter of red and purple chromatophores is visible on close inspection of the cuticle. *S. cornuta* is pale yellow, again with a scattering of dark chromatophores. Serolids are eaten by a wide range of predators, including fish and the isopod *Glyptonotus antarcticus*, and this colouration is undoubtedly cryptic (Moreira, 1974).

The presence of astaxanthin and a number of more polar xanthophylls was indicated by TLC. There were also traces of β-carotene and several samples contained both chlorophyll and some very polar porphyrins. The latter were presumably chlorophyllides or similar degradation products. In both species, lipid extracts in chloroform fell clearly into two types: yellowish green and red–brown. Only the former contained chlorophyll but there was no correlation of lipid extract colour with species, sex or age. The eggs of *S. pagenstecheri* contained solely unesterified astaxanthin. Total pigment was estimated as xanthophyll, although this

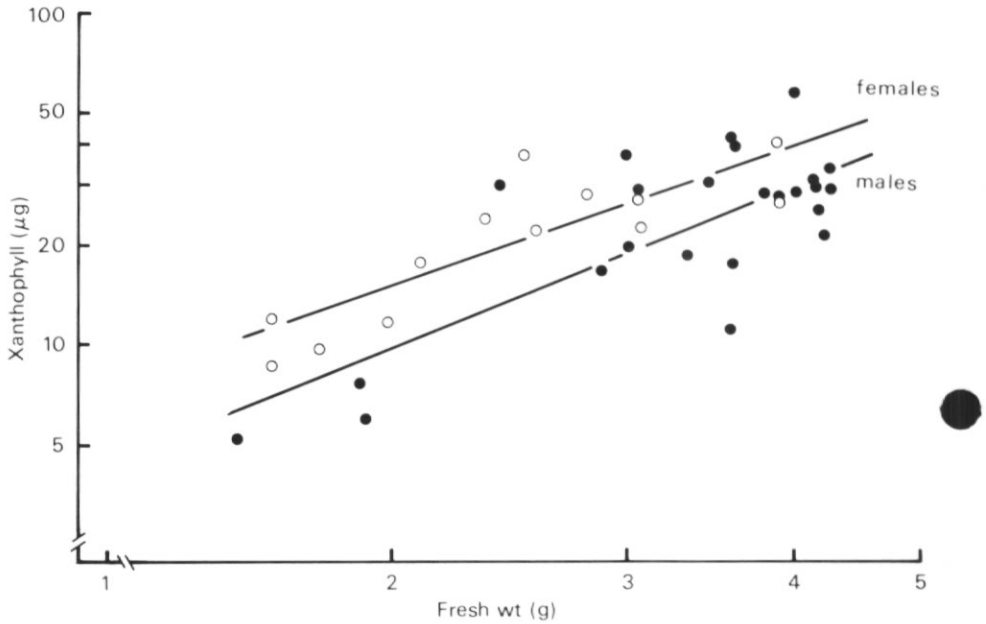

Fig. 3. *Serolis pagenstecheri*. Relationship between total pigment content (as xanthophyll, μg) and fresh weight (g) for all males (•) and all females (○) sampled; South Georgia, 1978 and 1980. Note logarithmic scales. Lines fitted by least squares to logarithmically transformed variables. Males: $\log_e y_i = 1.576 \pm 0.2092 (\log_e x_i) + 1.235$, $r^2 = 0.55$, $n = 24$. Females: $\log_e y_i = 1.399 \pm 0.258 (\log_e x_i) + 1.750$, $r^2 = 0.70$, $n = 13$.

will have underestimated the astaxanthin present; however, the presence of chlorophyll in some samples will have meant that in these total pigment was overestimated.

In both *Serolis cornuta* and *S. pagenstecheri* total pigment content increased with fresh weight, although there was a considerable amount of variance about the regression line (Fig. 3). Part of this variance was undoubtedly the usual biological variation to be found in total pigment content but some will have been caused by the presence of chlorophyll. In *S. pagenstecheri* females contained significantly more pigment than males ($P > 0.05$); this is probably due to the accumulation of astaxanthin in the maturing ovary.

Lipid class composition

Both *Serolis pagenstecheri* and *S. cornuta* had lipid compositions typical of benthic crustaceans (Table IV). The dominant lipid classes were phospholipid, free sterol and triacylglycerol; other storage lipids such as alkyldiacylglycerol or wax ester were present only in small amounts, usually $< 1\%$ total lipid. The levels of free fatty acid were higher than expected (7–9% total lipid). Free fatty acids are rarely present in cells in significant amounts and these data might imply that some autolysis had occurred during preservation. The free fatty acid content of eggs preserved by the same techniques were, however, very low, suggesting that the adult free fatty acid contents were real.

In both species the amount of phospholipid and triacylglycerol varied from individual to individual. This variability was probably related to the accumulation of

Table IV. Lipid class composition *Serolis pagenstecheri* and *Serolis cornuta*. Data presented as either mean ± standard error or range, with no. of determinations in parentheses; nd, not detected.

	Males	*Females*	*Newly spawned eggs*
Serolis pagenstecheri			
Lipid (% fresh wt)	1.08 ± 0.07 (33) (0.50 to 2.03)	2.08 ± 0.20 (24) (0.48 to 3.60)	11.28
Lipid phosphorus (μmol g^{-1}, fresh wt)	4.84 ± 0.15 (25	3.24 to 13.50 (14)	
Lipid class composition (% total lipid)			
Hydrocarbon	nd	nd	nd
Sterol ester	<0.10 (4)	nd	0.13
Wax ester	0.37 ± 0.13 (4)	<0.10 (4)	nd
Alkyldiacylglycerol	0.32 ± 0.12 (4)	1.48 ± 0.79 (4)	1.20
Triacylglycerol	13.22 to 26.27 (4)	11.51 to 42.86 (4)	46.63
Diacylglycerol (both isomers)	1.26 ± 0.42 (4)	1.00 to 0.35 (4)	0.43
Free sterol	22.64 ± 3.60 (4)	19.88 ± 2.37 (4)	19.75
Free fatty acid	9.23 ± 2.43 (4)	8.06 ± 1.79 (4)	0.06
Monoacylglycerol	nd	nd	0.02
Polar lipids	19.09 to 55.88 (33)	15.99 to 75.24 (23)	31.58
Unknowns (three or four components)	1.88 ± 0.49 (4)	1.82 ± 0.33 (4)	0.20
Serolis cornuta			
Lipid (% fresh wt)	1.09 ± 0.11 (15) (0.41 to 1.70)	1.49 ± 0.11 (24) (0.62 to 2.92)	
Lipid phosphorus (μmol g^{-1}, fresh wt)	4.24 ± 0.26 (9)	3.30 to 8.30 (16)	
Lipid class composition (% total lipid)			
Hydrocarbon	nd	nd	
Sterol ester	nd	nd	
Wax ester	2.54 ± 1.04 (10)	2.08 ± 0.87 (15)	
Alkyldiacylglycerol	0.75 ± 0.30 (10)	1.42 ± 0.36 (15)	
Triacylglycerol	8.99 to 38.16 (10)	16.30 to 45.33 (15)	
Diacylglycerol (both isomers)	1.48 ± 0.64 (10)	0.71 ± 0.22 (15)	
Free sterol	28.11 ± 1.53 (10)	22.11 ± 1.19 (15)	
Free fatty acid	7.44 ± 1.42 (10)	7.49 ± 1.06 (15)	
Monoacylglycerol	0.81 ± 0.32 (10)	0.44 ± 0.18 (15)	
Polar lipids	23.45 to 58.15 (12)	21.09 to 48.65 (20)	
Unknowns (three or four components)	2.68 ± 0.50 (10)	2.86 ± 0.20 (15)	

depot lipid but too few individuals were analysed by TLC to determine the relationship between lipid content and lipid class composition. The relationship between total lipid phosphorus (μmol g^{-1}, fresh weight) and total lipid content, however, indicated that the physiology of lipid storage was different in male and female serolids.

In male *Serolis pagenstecheri* the concentration of lipid phosphorus was independent of lipid content ($F = 1.39$, $P > 0.10$) and averaged about 4.8 μmol g^{-1} over the range 0.5–2.03% lipid. This indicated that, in males, variations in lipid content were due almost entirely to storage and depletion of triacylglycerol; such variations were probably related to feeding and the state of the moult cycle. The proportion of phospholipid in the total lipid of males would therefore be expected to vary inversely with lipid content and this was found to be the case ($F = 69.06$, $P < 0.01$; Fig. 4).

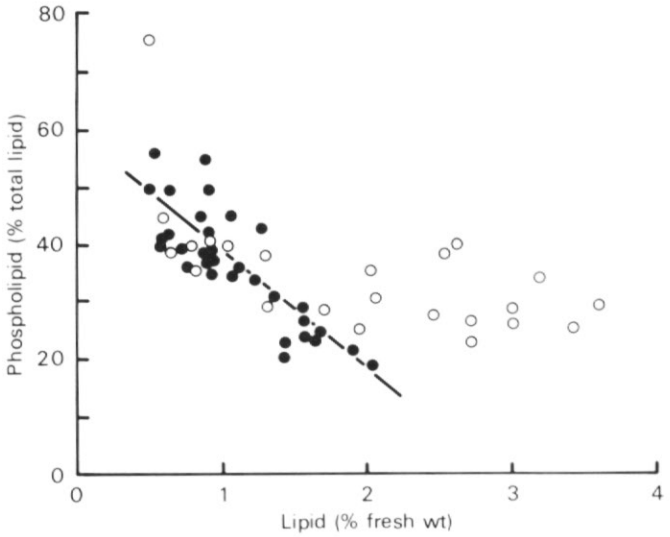

Fig. 4. *Serolis pagenstecheri*. Relationship between phospholipid (% total lipid) and total lipid content (% fresh weight) for all males (●) and all females (○) sampled; South Georgia, 1978 and 1980. Line fitted by least squares to male data only: $y_i = -20.072 \pm 2.414\ (x_i) + 58.340$, $r^2 = 0.68$, $n = 33$.

A different pattern was found in female *Serolis pagenstecheri* with maturing ovaries. Here, lipid phosphorus increased significantly with lipid content ($F = 45.16$, $P < 0.01$), indicating that both phospholipid and triacylglycerol were being stored. A single analysis of newly spawned eggs showed that these were rich in both phospholipid and triacylglycerol (Table IV). In the range of female lipid contents 2–4% fresh weight, phospholipid consistently formed about 30% of the total lipid (the concentration in egg lipid); at lower lipid contents, phospholipid rose to very high levels (up to 75% total lipid). It is clear that the production of eggs involves a significant switch in the physiology of lipid storage from that in males based on the storage and utilization of triacylglycerol alone. Similar patterns were found in *Serolis cornuta*.

The polar lipids were examined by TLC but not quantified. In both species the major phospholipid was choline phosphoglyceride, followed by ethanolamine phosphoglyceride. There were small amounts of cardiolipin, phophatidic acid, serine phosphoglyceride and inositol phosphoglyceride. This composition is similar to that reported for *Pandalus montagui* (Clarke, 1979) and *Penaeus merguiensis* (Clarke and Wickins, 1980).

Fatty acid composition

Analyses of triacylglycerol and phospholipid fatty acids on capillary columns showed that very many fatty acids were present. Over 65 different components were frequently resolved, although few of these were present in significant amounts. The major features of the fatty acid composition have been summarized in Tables V and VI.

The most important fatty acids in *Serolis pagenstecheri* were 16:0, 16:1ω7, 18:1ω9, 18:1ω7, 20:4ω6, 20:5ω3 and 22:6ω3, which together accounted for 80% of the total. These fatty acids are typical of marine crustaceans and it was variations in these major components that dictated the overall character of the various compositions.

Table V. Major features of the fatty acid composition of *Serolis pagenstecheri*. Capillary column analyses (25m SP1000 WCOT). Data are percentage total integrator response in range C_{12}–C_{24}, presented as mean ± standard deviation (when $n > 2$). PL = phospholipids; TAG = triacylglycerol; n_{id} = number of components identified; n_{unid} = maximum number of components resolved but not identified; db/mol = mean number of double bonds per molecule of fatty acid; Σ polyenoics = sum of all fatty acids with 3, 4, 5 or 6 double bonds.

	Males		*Ovigerous female*		*Eggs*		*Mature females*	
	PL ($n = 4$)	*TAG* ($n = 4$)	*PL* ($n = 1$)	*TAG* ($n = 1$)	*PL* ($n = 1$)	*TAG* ($n = 2$)	*PL* ($n = 2$)	*TAG* ($n = 2$)
16:0	16.22 ± 1.42	19.57 ± 3.41	32.82	23.35	12.92	22.49	16.01	22.93
16:1ω7	4.35 ± 0.61	7.61 ± 0.92	5.19	9.73	8.03	7.26	5.69	5.37
18:1ω9	21.53 ± 2.54	11.38 ± 2.08	14.37	8.37	25.09	26.13	20.40	22.58
18:1ω7	8.93 ± 0.79	7.02 ± 0.76	6.61	5.07	7.30	8.42	7.06	6.76
Σ 20:1 + Σ22:1	2.23 ± 0.90	7.12 ± 2.05	1.04	8.22	4.65	5.34	4.59	7.06
20:4ω6	4.66 ± 0.35	1.24 ± 0.20	3.06	1.01	3.25	1.54	3.81	1.16
20:5ω3	20.37 ± 1.47	14.57 ± 2.35	14.71	17.73	18.78	10.37	21.96	12.61
22:6ω3	5.89 ± 1.31	3.38 ± 1.69	4.02	3.19	3.31	3.17	5.41	5.80
db/mol	2.05 ± 0.13	1.66 ± 0.15	1.46	1.68	1.89	1.41	2.10	1.60
mean mol. wt	281.90	276.73	273.58	275.76	279.53	275.91	282.10	278.94
Σ saturated	22.16	26.54	42.91	32.50	16.71	26.63	20.93	27.39
Σ monoenoic	37.98	35.86	28.43	33.66	47.70	49.08	38.81	44.58
Σ polyenoic	34.19	24.27	25.72	28.68	28.60	19.36	19.09	23.94
n_{id}	42	45	43	47	42	45	42	46
$\Sigma_{unidentified}$ (n_{unid})	3.90 (14)	10.37 (23)	1.26 (13)	3.03 (21)	4.97 (14)	2.49 (22)	2.81 (15)	2.31 (21)

Table VI. Major features of the fatty acid composition of *Serolis cornuta*. Analysis by capillary (25 m SP1000 WCOT) or packed (20% DEGS) columns. Data presented as percentage total integrator response in range C_{12}–C_{24}. Abbreviations as in Table V.

	Capillary column analyses				*Packed column analyses (sexes not separated)*	
	Males		*Mature females*			
	PL ($n = 2$)	*TAG* ($n = 1$)	*PL* ($n = 1$)	*TAG* ($n = 3$)	*PL* ($n = 8$)	*TAG* ($n = 5$)
16:0	23.10	18.35	26.72	16.13 ± 3.51	16.59 ± 0.72	11.59 ± 1.71
16:1ω7	4.29	22.50	6.97	14.01 ± 2.18	3.73 ± 0.53	11.29 ± 1.76
18:1ω9	19.05	10.06	19.08	10.60 ± 2.36	22.56 ± 1.53	18.90 ± 2.42
18:1ω7	8.33	7.12	10.24	10.13 ± 1.53		
Σ 20:1 + Σ 22:1	1.61	5.57	1.50	6.22 ± 2.02	1.62 ± 0.78	8.35 ± 2.1
20:4ω6	1.81	1.13	1.41	1.22 ± 0.43	3.70 ± 0.78	3.05 ± 1.14
20:5ω3	17.98	7.02	17.47	15.46 ± 3.81	22.92 ± 0.87	18.30 ± 3.88
22:6ω3	8.19	1.72	6.54	3.15 ± 0.64	17.56 ± 1.71	10.16 ± 2.50
db/mol	1.94	1.22	1.72	1.75 ± 0.14	2.61 ± 0.09	2.18 ± 0.23
mean mol. wt	279.94	268.44	276.91	275.05	288.13	283.89
Σ saturated	27.77	28.91	30.96	22.41	21.88	17.90
Σ monoenoic	35.04	47.95	38.15	43.14	29.29	41.65
Σ polyenoic	32.44	14.54	28.20	28.36	46.31	35.57
n_{id}	42	47	41	45	23	24
$\Sigma_{unidentified}$ (n_{unid})	2.42 (15)	2.26 (22)	1.77 (14)	4.34 (19)	0.74 (2)	0.96 (2)

In male *Serolis pagenstecheri*, the phospholipid fatty acids were relatively unsaturated due to the high levels of 20:4ω6, 20:5ω3 and 22:6ω3 (Table V). In contrast, the triacylglycerol fatty acids were relatively saturated and of shorter chain length, due mainly to lower proportions of 20:5ω3 and 22:6ω3. Triacylglycerol fatty acids were also notable for the relatively high levels of 20:1 and 22:1, the latter sometimes resolved into five isomers. Overall, the triacylglycerol fatty acids contained a greater variety of fatty acids and were more variable than phospholipids; this undoubtedly reflects different strategies of storage. The range of fatty acids present in triacylglycerol results from the varied fatty acid compositions of the items eaten (Clarke and Wickins, 1980), whereas phospholipids have a fatty acid composition that is much more tightly regulated.

As an example, the exact position of the double bond in 18:1 determines the physical behaviour of the phospholipid to which it is attached and hence the biophysical properties of the membrane containing that phospholipid. In particular, the melting behaviour is strongly dependent upon double bond position (Barton and Gunstone, 1975). In *Serolis pagenstecheri* phospholipid fatty acids, the ratio 18:1ω9/18:1ω7 was tightly regulated (mean 2.44, coefficient of variation 0.10) whereas in triacylglycerol this ratio was more variable (range 1.34–2.44, coefficient of variation 0.31).

The single ovigerous female analysed was different from both males and maturing females, the phospholipids being notable for the very high level of 16:0 and relatively low levels of 20:5ω3 and 22:6ω3 (Table V). This may be because stores of polyenoic fatty acids had been depleted during the final stages of vitellogenesis.

The major fatty acids of the eggs were 16:0, 16:1ω7, 18:1ω9, 18:1ω7, 20:5ω3 and

22:6ω3. Egg lipids were particularly rich in monoenoic fatty acids, as is also the case in decapods (Morris, 1973; Clarke, 1979).

Fewer data are available for *Serolis cornuta* (Table VI) but the capillary analyses show trends similar to those of *S. pagenstecheri* in that triacylglycerol fatty acids were less unsaturated and more variable than those from phospholipids. However, an early batch of samples of *S. cornuta* analysed by packed column GC were very different. Again, triacylglycerol fatty acids were less unsaturated and more variable than phospholipid fatty acids but both compositions were far more unsaturated than the later samples analysed by capillary GC (Table VI). Most notable were the much higher levels of 20:5ω6 and 22:6ω3. The differences do not appear to be an artefact of analysis, since with both sets of analyses column behaviour and detector response were routinely monitored with a secondary standard of fatty acids prepared from cod liver oil (Ackman and Burgher, 1965). Why two samples of the same species from the same sampling area should be so different is not clear.

Discussion

The influence of shape

Both the lipid content and the pigment content of serolid isopods are influenced by their unusually flattened shape. The lateral extensions of the cuticle mean that over half of the total dry weight is mineral ash (largely skeletal $CaCO_3$) and that much of the organic matter is chitin (Table I). This of itself means that lipid content will be low and male serolids contain only about 1% lipid. Even in mature females, lipid rises to only about 3.6% fresh weight, and calorific contents are generally lower than in euphausiids or caridean shrimps.

In many decapods and euphausiids total pigment increases with weight (Herring, 1972, 1973), and in carideans and *Euphausia superba* pigment content is a function of surface area, increasing approximately as weight to the power two-thirds (Clarke, 1979, 1980). The colouration of serolids is most likely cryptic (Moreira, 1974), and so pigment content would also be expected to be a function of surface area. In male *Serolis cornuta* the weight exponent was 0.67 ± 0.30 but in females it was significantly greater (1.08 ± 0.15, $P < 0.05$). In *S. pagenstecheri* the weight exponents for the two sexes were not significantly different; weight for weight, however, females contained between one-third and half as much pigment again as males (Fig. 3). This difference in elevation was significant ($P < 0.05$). Thus, in both species, females contained more pigment, weight for weight, than males; this difference is probably due to the accumulation of astaxanthin in the developing ovary.

Carotenoid pigments and diet in Serolis

Animals are believed to be incapable of synthesizing the chemical skeleton of carotenoid pigments (which must therefore originate in plants) but they are able to modify carotenoids obtained from the diet. In many pelagic marine organisms, and especially crustaceans, the major carotenoid is astaxanthin.

The widespread occurrence of astaxanthin as a major pigment in marine crustaceans may be because its polar tautomeric structure allows it to bind readily to proteins (Cheesman and others, 1967) and also because its wavelength of maximum absorbence is longer than most other ring-containing carotenoids (Davies, 1965). This means that it is the potential metabolite of phytoplankton carotenoids best suited to absorb the wavelengths of maximum penetration of daylight into seawater, and the emission maxima of the bioluminescence of marine animals (Herring, 1972). The ease of binding to protein means that astaxanthin can be transported and

deposited within tissues without previous solution in lipid. The binding to protein can also modify the absorption spectrum and a wide range of colouration is therefore possible. For example, when crustacean eggs are first spawned they usually contain astaxanthin as the sole pigment; this is frequently bound to protein and crustacean eggs exhibit a wide range of colour. In *Serolis pagenstecheri* the newly spawned eggs are orange and free astaxanthin was the only pigment detected by TLC.

In female *Serolis pagenstecheri* astaxanthin was the major pigment visible by TLC. Much of this astaxanthin was doubtless located in the ovary but astaxanthin was also detected in males. Both sexes contained a variety of other, more polar, xanthophylls.

Previous detailed studies of the pigments of benthic marine isopods have revealed complex mixtures of xanthophylls. *Idotea resecata* lives in kelp beds or eelgrass where it feeds exclusively on plant material; the littoral species *I. granulosa* and *I. montereyensis* also take mostly plant material. In all three species lutein was the dominant component and astaxanthin was absent (Lee, 1966*a*, *b*; Lee and Gilchrist, 1972). The oceanic *I. metallica*, however, does contain astaxanthin (Herring, 1969); this species is omnivorous, as are serolids which also contain astaxanthin. For carnivorous or omnivorous marine invertebrates astaxanthin will be freely available from the diet, for the littoral herbivorous species it will be absent. Since several xanthophylls can be complexed to protein and produce a variety of pigmentation, these observations suggest that the major factor influencing the pigment composition of marine isopods is the mixture of xanthophylls available from the diet.

Lipid in benthic isopods

There have been several studies of the lipids of benthic decapods (Lawrence, 1976; Clarke, 1977*a*, *b*, 1979) but little appears to be known of isopod lipid biochemistry. Serolids are similar to other benthic crustaceans in that the lipid content of males is generally low with variations due mostly to changes in the amount of stored triacylglycerol. The most likely biological factors influencing the level of triacylglycerol are the stage of the moult cycle and the period since the last meal. There can thus be no definitive lipid composition for *Serolis* (or any other marine invertebrate) since the lipid content and composition will both vary from individual to individual. Furthermore, since triacylglycerol fatty acid composition is affected by dietary input (Brockerhoff and Hoyle, 1967; Clarke and Wickins, 1980), then fatty acid composition will also vary between individuals. It is possible that this is the reason for the difference between the two samples of *Serolis cornuta* (Table VI), although the differences observed were rather large.

In both *Serolis cornuta* and *S. pagenstecheri*, the pattern of lipid storage in females is very different from that in males. Lipid is accumulated for the eggs, often over a period of up to 18 months (Luxmoore, 1982*a*) and this egg lipid includes both triacylglycerol and phospholipid. In *S. cornuta* this can be seen as an increase in lipid content with fresh weight (and hence age) and a parallel increase in total lipid phosphorus content. In *S. pagenstecheri* the pattern is less clear; lipid phosphorus increases with lipid content, but the relationship between lipid content and female size is complex. In neither sex does lipid storage become very great and there are no signs of the large wax ester stores so characteristic of the polar zooplankton (Clarke, 1984). This suggests that although food is scarce in winter (for growth is limited to summer: Luxmoore, 1982*b*), energetic demands in the benthos are sufficiently low that what food there is can meet requirements.

As has been found in caridean decapods, egg lipids are rich in saturated and monoenoic fatty acids (Table V). These are the major end products of fatty acid biosynthesis in most marine organisms. However, polenoic fatty acids are required

Table VII. Polyenoic fatty acid (PUFA) content of eggs of four marine crustaceans. Data from Clarke (1977, 1979, 1980).

Species	*Time from spawning to first feeding stage*	*Lipid per egg (μg)*	*Total egg PUFA*	
			% of total fatty acids	*(μg)*
Isopoda				
Serolis pagenstecheri	At least 12 months	190	22.2	28.7
Decapoda				
Chorismus antarcticus	9–10 months	126	47.5	48.9
Pandalus montagui	4–5 months	23.2	34.3	6.8
Euphausiacea				
Euphausia superba	20–30 days	9.4	32.2	1.8

for membrane phospholipids and crustaceans are believed to be unable to synthesise these fatty acids *de novo* (Sargent, 1976). Polyenoic fatty acids for the eggs must therefore be accumulated from the diet. In *Serolis pagenstecheri* they total 22% of the total egg fatty acids (Table VII). Since the provision of lipid for yolk takes place over a long period of time, the accumulation of these polyenoic acids from the food is unlikely to place any physiological strain on the maturing female. Equally, the accumulation of monoenoic and saturated fatty acids can occur at the same time since these will be present in the same food (Clarke, 1982). The distinctive fatty acid composition of eggs, which in turn influences the composition of maturing females, is likely therefore to be more of a function of the metabolic demands of the developing embryo than physiological constraints upon the maturing female.

Any polyenoic fatty acids required during embryo development must be supplied with the yolk and it has been suggested that there is a direct relationship between the period of development before a feeding stage is reached and the amount of polyenoic fatty acid in the egg (Clarke and Morris, 1983). The brooding period of *Serolis pagenstecheri* at South Georgia is unknown. The eggs are large and hence incubation is likely to last at least over winter. In February 1980 females were collected containing newly spawned eggs, very well developed eggs, and newly hatched young (Table III); this suggests a brooding period of at least one year. The egg contains about 30μg of polyenoic fatty acid (Table VII). Although this is less than in the shrimp *Chorismus antarcticus*, whose brooding period at South Georgia is little under a year, it is considerably more than in the temperate water shrimp *Pandalus montagui*.

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