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Seasonal growth and lipid storage of the circumglobal, subantarctic copepod, *Neocalanus tonsus* (Brady)

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Abstract—*Neocalanus tonsus* (Brady) was sampled between October 1984 and September 1985 in the upper 1000 m of the water column off southeastern New Zealand. The apparent spring growth increment of copepodid stage V (CV) differed depending upon the constituent considered: dry mass increased 208 μg, carbon 162 μg, wax esters 143 μg, but nitrogen only 5 μg. Sterols and phospholipids remained relatively constant over this interval. Wax esters were consistently the dominant lipid class present in CV's, increasing seasonally from 57 to 90% of total lipids. From spring to winter, total lipid content of CV's increased from 22 to 49% of dry mass. Nitrogen declined from 10.9 to 5.4% of CV dry mass as storage compounds (wax esters) increased in importance relative to structural compounds. Egg lipids were 66% phospholipids. Upon first appearance of males and females in deep water in winter, lipid content and composition did not differ from co-occurring CV's, confirming the importance of lipids rather than particulate food as an energy source for deep winter reproduction of this species. Despite contrasting life histories, *N. tonsus* and subarctic Pacific *Neocalanus plumchrus* CV's share high lipid content, a predominance of wax esters over triacylglycerols as storage lipids, and similar wax ester fatty acid and fatty alcohol composition.

INTRODUCTION

THE calanoid copepod *Neocalanus tonsus* (Brady) is widespread and abundant within the subantarctic province of the world ocean. From BRODSKII (1964), together with subsequent work, it can be established that *N. tonsus* has a continuous, circumglobal distribution in Pacific (FLEMINGER, 1985; MARIN and ANTEZANA, 1985), Atlantic (WIBORG, 1964; KAWAMURA, 1974; VORONINA, 1975) and Indian (DE DECKER and MOMBECK, 1965; KAWAMURA, 1974; DE DECKER, 1984) sectors of the subantarctic. The species inhabits both Australasian Subantarctic water and Circumpolar Subantarctic water (*sensu* HEATH, 1981, 1985). It is broadly delimited in meridional extent by the Polar Front (Antarctic Convergence) to the south and the Subtropical Convergence to the north. Occasional transport north of the Subtropical Convergence can occur in subsurface waters (DE DECKER and MOMBECK, 1965; JILLETT, 1968).

Relatively high population densities of *N. tonsus* have been observed in subantarctic surface waters. These include regions as diverse as New Zealand shelf/slope waters, open Atlantic waters, the southern Benguela Current and the Indian Ocean (see Table 1). As noted by JILLETT (1968), such aggregations resemble those of *Neocalanus plumchrus* in

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Table 1. *Maximum abundance of* Neocalanus tonsus *reported from different oceanic regions*

Region	Location	Max abundance (no. m^{-3})	Reference
Otago, New Zealand	45°49′S, 171°06′E	534	JILLETT (1968)
Kaikoura, New Zealand	42°25′S, 173°48′E	4425	BRADFORD (1972)
S.W. Australia	37°56′S, 106°39′E	23,680	KAWAMURA (1974)
S. Atlantic	43° S, 26° W	2260	VORONINA (1975)
S.E. Tasmania	43°08′S, 147°28′E	404	TAW and RITZ (1981)
S. Benguela Current	–	"Swarms"	HUTCHINGS (1981)
Tristan da Cunha	37°03′S, 12°18′W	(See text)	DE DECKER (personal communications)

the northern, subarctic Pacific (KAWAMURA and HIRANO, 1985). Near Tristan da Cunha, in the South Atlantic, *N. tonsus* was found in such densities that ". . . their multitudes blocked the cooling water intake of the fishing boats . . ." (the late A. H. B. DE DECKER, personal comunication). Considering such abundances and the large body size of individuals (dry mass up to 600 μg; see below), *N. tonsus* may be expected to have considerable impact on the structure of subantarctic food webs through predation on microplankton (OHMAN, 1987), as a prey item for higher trophic levels (BRADFORD, 1972; PETROVA and CKEKUNOVA, 1979; KAWAMURA, 1980; HUTCHINGS, 1981), and as a mediator of vertical fluxes of lipids and other biogenic particulate material (cf. CORNER *et al.*, 1986; WAKEHAM and CANUEL, 1988)

Of particular interest in the present study is the emerging life history contrast between *N. tonsus* (Brady) from the subantarctic ocean and *N. plumchrus* (Marukawa) from the subarctic Pacific. These were thought to be the same species until TANAKA (1956) recognized *N. tonsus* as endemic to the southern hemisphere. BRADFORD and JILLETT (1974) subsequently assigned both *tonsus* and *plumchrus* to the genus *Neocalanus*. Since both the adult males and females of *N. plumchrus* have nonfunctional mouthparts and never emerge from deep waters in the open ocean [MILLER *et al.* (1984), but see SMITH and VIDAL (1986) for the southeast Bering Sea], the wax esters stored by CV's of *N. plumchrus* are an essential source of carbon and energy for adults (LEE *et al.*, 1972). *N. tonsus* shares the characteristic of deep winter reproduction with *N. plumchrus*, but it is now clear that the life history of *N. tonsus* differs from that of *N. plumchrus* in many ways. These include the occurrence of *N. tonsus* females in surface waters in spring (JILLETT, 1968; BRADFORD, 1970), active feeding by spring females, and an apparent alternation between egg production in mesopelagic depths in winter and egg production in epipelagic depths in spring (OHMAN, 1987). Because these deep-dwelling winter copepods utilize lipid during egg production (OHMAN, 1987), the seasonal dynamics of growth and lipid storage need to be understood more clearly. Wax esters are commonly stored by high latitude zooplankton species in the northern hemisphere and are likely to be a principal substrate metabolized during dormancy and other intervals of limiting food supply (LEE and HIROTA, 1973; LEE, 1974; BÅMSTEDT, 1986; SARGENT and HENDERSON, 1986; FALK-PETERSEN *et al.*, 1987). However, at present there is scant information on the storage and metabolism of lipids of copepods in higher latitudes in the southern hemisphere.

The objectives of the present study therefore were to characterize the lipid composition of *N. tonsus* in comparison with the subarctic Pacific *N. plumchrus* and to determine whether sufficient organic reserves are accumulated by copepodid stage V of

N. tonsus to account for the lipids required for reproduction by winter adults. The South Island of New Zealand was selected as a field site for this investigation because of year-round access to oceanic subantarctic zooplankton and proximity of experimental facilities at the Portobello Marine Laboratory. A narrow continental shelf (*ca* 15–20 km off the Otago Peninsula) permits ready access to continental slope depths and the larger-scale circulation maintains Subantarctic water within a few tens of kilometers of shore (JILLETT, 1969; HEATH, 1981; REID, 1986).

METHODS

Collection

Copepods were collected from the R.V. *Munida* at a continental slope station (45°55'S, 171°06'E), 32 km southeast of Taiaroa Head, Otago Peninsula, South Island of New Zealand, water depth 1200 m. Cruises were made approximately monthly between October 1984 and September 1985 and all sampling was conducted between 0900 and 1545 local time. Discrete depth strata were sampled in successive vertical hauls using a paired closing net frame (BROWN and HONEGGER, 1978) fitted with 0.7 m diameter, 202 or 75 μm mesh nets and TSK flowmeters. The standard sampling depths were 1000–500, 500–150 and 150–0 m with the following departures: the deepest stratum was modified to 900–500 m on 2 November and 800–500 m on 27 December. Only the 150–0 m level was sampled on 27 November and only the 1000–500 m stratum on 6 August. A time/depth recorder established that the true maximum depth attained and the net closing depth were within *ca* 10% of the target depths.

Immediately upon net retrieval copepods were frozen by immersion in liquid nitrogen, then later transferred to a –70°C freezer ashore. Replicate samples were preserved in 5% borate-buffered formalin in seawater. Frozen samples were not taken on 24 September.

N,C *Analysis*

Analysis of changes in body mass and organic composition with season and depth was restricted to *N. tonsus* copepodid stage V's (CV), adult males and females. Thawed copepods were sorted under a dissecting microscope, rinsed with 10 μl distilled water and dried in lots of 1–4 individuals at 55°C in precombusted aluminum boats. Dry mass was determined with a Cahn G-2 electrobalance, then nitrogen and carbon content determined by combustion at 900°C in a 10% oxygen stream in high purity helium. The resultant CO_2 and N_2 were quantified with a Carl 311 gas chromatograph (M. Downes, Division of Marine and Freshwater Science, Taupo) and peak areas recorded on a HP 3390 integrator. Rectilinear calibration curves showed $r^2 = 0.99$. Four to 20 replicates were analysed for each developmental stage and depth stratum.

Lipid class composition

Animals for analysis of lipid class composition were pooled in lots of 1–4 individuals of a single developmental stage. Copepods were then homogenized in chloroform:methanol:water (1:2:0.8, v/v/v) with a glass pestle and taken through the remainder of the BLIGH and DYER (1959) procedure. This extraction was then repeated for each sample. HPLC grade solvents were used throughout. Lipid solutions were maintained under N_2 below –10°C prior to analysis. Usually 10 replicate extractions were done for each developmental stage and depth stratum.

Lipid classes (wax esters, triacylglycerols, free fatty acids, sterols and phospholipids) were separated and quantified by thin layer chromatography/flame ionization dectection (TLC/FID) using an Iatroscan TH-10 Mark III with a HP 3392 recording integrator. Total lipid was obtained by summing these five lipid classes plus occasional trace quantities of other lipids. A new double-development, single scan procedure giving highly reproducible baseline separations was used to separate lipid classes on SII chromarods (slightly modified from OHMAN, 1988). Chromarods were developed for 20 min in hexane:diethyl ether (95:5, v/v), dried at 110°C for 4 min, developed for 20 min in hexane:diethyl ether:formic acid (82:18:0.1, v/v/v), dried, then scanned twice at 40 s scan^{-1}. [Although these solvents do not separate sterol esters from wax esters, no sterol esters were detected using plate TLC and the reaction of JATZKEWITZ and MEHL (1960).] Hydrogen flow rate for TLC/FID analyses was 160 ml min^{-1} and air flow 2000 ml min^{-1}. Three or more replicate analyses were performed per extraction. Chromarods were cleaned nightly in 50% nitric acid. Cetyl alcohol was used as an internal standard since free alcohols were either undetectable or present in only trace quantities in *N. tonsus* lipids. Palmityl oleate, purified yeast triacylglycerol, stearic acid, cholesterol and lecithin were used as standard compounds. Standard mixtures were prepared that approximated the lipid class proportions in *N. tonsus* samples. Standard curves were described by power curves ($0.93 < r^2 < 0.99$), which provided better statistical fits than rectilinear relationships. The identity of copepod lipid classes was confirmed by standard plate TLC.

The *t*-distribution is inappropriate for estimating the variance of the ratio of lipid/dry mass because the two quantities were analysed from separate lots of animals. Consequently the standard error of this ratio was approximated as follows (cf. note 1, ENRIGHT, 1967):

$$S_{x/y} \approx \left\{ \frac{1}{\bar{y}^2} \left(S_x^2 + \frac{\bar{x}^2}{\bar{y}^2} S_y^2 \right) \right\}^{\frac{1}{2}}, \tag{1}$$

where $\bar{x}$ is mean lipid content, y is mean dry mass, S_x is standard error of $\bar{x}$, S_y is standard error of $\bar{y}$ and $S_{x/y}$ is standard error of $(\bar{x}/\bar{y})$.

Eggs for lipid analysis were obtained in the laboratory from females collected in August between 1000 and 500 m. Eggs were pooled in lots of 99–410, rinsed with filtered seawater, then lipids extracted by sonication in chilled Bligh and Dyer solvents. Egg lipid class composition was analysed by Iatroscan as above.

Fatty acid/fatty alcohol composition

The fatty acid and fatty alcohol composition of *N. tonsus* CV collected in spring near the surface (2 November 1984, 150–0 m) was compared with that of CV's collected in summer at depth (28 January 1985, 1000–500 m) using gas–liquid chromatography (GLC). Pooled lots of *N. tonsus* CV were sequentially extracted twice by the Bligh and Dyer procedure. One aliquot of the extracted lipid was transesterified in 6% methanolic HCl. Fatty acid methyl esters and fatty alcohols were separated by preparative TLC on Kieselgel G (Type 60, prewashed) in hexane:diethyl ether (70:30, v/v). From the January, but not the November lipid sample, intact wax esters were separated from other lipid classes by preparative plate TLC using hexane:diethyl ether:acetic acid (70:30:1, v/v/v). Eluted wax esters were then transesterified as above.

Fatty acid methyl esters and long chain alcohols were analysed by GLC using a Pye Unicam GCV chromatograph with a 4 mm i.d., 1.5 m long glass column packed with 10% SP-2330 on 100/120 Chromosorb WA/W. Analyses were done isothermally at 185 or 200°C. Peaks were identified by cochromatography with authenthic standards, plots of log retention times vs C number and comparison of chromatograms obtained before and after hydrogenation of selected samples in the presence of PtO_2 (KATES, 1986).

Analysis of the carbon chain lengths of intact wax esters was done on a column packed with 3% OV-1 on Chromosorb WA/W, programmed between 170 and 300°C at 10°C min^{-1}, and peak area recorded on a HP 3390 integrator. Peaks were identified with reference to authentic standards as well as wax esters extracted from the orange roughy, *Hoplostethus atlanticus* (GRIGOR *et al.*, 1983).

Carotenoids

N. tonsus collected in November were starved 1–4 days to evacuate guts, frozen, then tissue pigments later extracted by either the BLIGH and DYER (1959) method or the acetone extraction of DAVIES (1976). Absorption spectra of extracted carotenoids were determined between 250 and 700 nm with a Shimadzu UV-240 scanning spectrophotometer using synthetic trans-β-carotene as a reference standard. Total carotenoid content was estimated from absorbance at 474 nm in acetone, using the specific extinction coefficient of astaxanthin ($E^{1\%}_{1cm}$ = 1600; ANDREWS *et al.*, 1976).

RESULTS

Stage structure

The developmental stage structure in October, austral spring, reflected the emergence of a developing generation of *N. tonsus* (Fig. 1A). Copepodid stages I–III, as well as later developmental stages through the adult female, occurred in surface waters during this spring period. Following progression through stage CIV in November–December the population was almost exclusively CV's after January. Nearly all the population remained submerged below 500 m depth from late January to September (Fig. 1B). Molting to the adult male, at depth, was first detected at the end of May but most males appeared in late June–July. Females did not reappear until July–August and were confined to deep water at this time of year. Females dominated the stage structure of the population from mid-August to September. *N. tonsus* eggs were identified in all three depth strata on 24 September, using the morphological criteria in BRADFORD *et al.* (1988). If ova were positively buoyant, their presence in all three depth strata would imply that some, if not all, egg release occurs in mesopelagic depths in late winter followed by ascent into shallower strata. However, the buoyancy of *N. tonsus* eggs in conditions prevailing *in situ* is not presently known. With the exception of 1 male captured between 150 and 0 m on 24 September, all males were confined to deeper strata.

Nitrogen and carbon content

Copepodid V of *N. tonsus* was the numerically dominant developmental stage from October to July. Considerable change occurred in organic composition over this interval. CV's exhibited a spring–summer increase in dry mass and carbon (C) content (Fig. 2a,c) but corresponding changes were not detected in nitrogen (N) content (Fig. 2e). Between

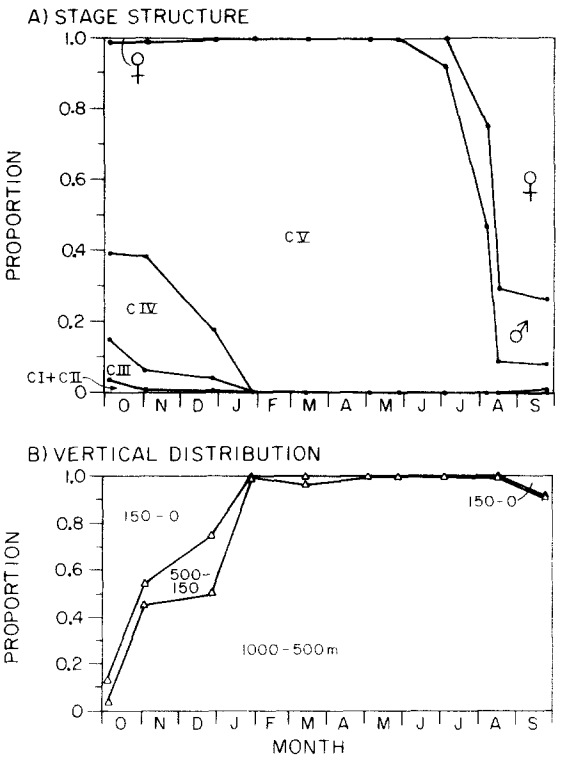

Fig. 1. Seasonal change in (A) developmental stage structure summed over the upper 1000 m and (B) vertical distribution of summed copepodid stages of *Neocalanus tonsus*.

4 October and 27 December CV's increased 208 μg in dry mass and 162 μg in body carbon, but body N changed only 5 μg (Table 2). There was a significant temporal trend in N content over this interval ($P < 0.001$, rank correlation of N content vs Julian day), but this trend explained only 8% of the variance in N content and the proportional change in N was small (16%), contrasting with C, dry mass and wax ester content (Table 2). As a consequence of the differential changes in N and C compounds during spring, the C:N ratio of surface CV's increased from 5.54 to 9.06 (Fig. 2g). Following the accretionary phase during spring–summer, dry mass, C and N content of CV's residing in deeper water declined between late summer (March) and winter (August; Fig. 2).

The dry mass (Fig. 2b) and C content (Fig. 2d) of adult females collected near the surface in spring did not differ from those of females collected between 1000 and 500 m in winter ($P > 0.05$). In contrast, surface spring females had twice the N content of winter deep females (Fig. 2f). The C:N ratio was, as a consequence, twice as high in winter females (Fig. 2h). Comparing surface-dwelling females with CV's, dry mass and C content were similar but the N content and C:N ratio of these two stages differed significantly ($P < 0.05$).

Males occurred only in deep water in winter. Their dry mass, C, N and C:N ratio did not differ from deep-dwelling CV's collected concurrently.

Changes in organic composition with developmental stage and depth are further illustrated in Fig. 3. Females and males occurring at depth in winter show a similar

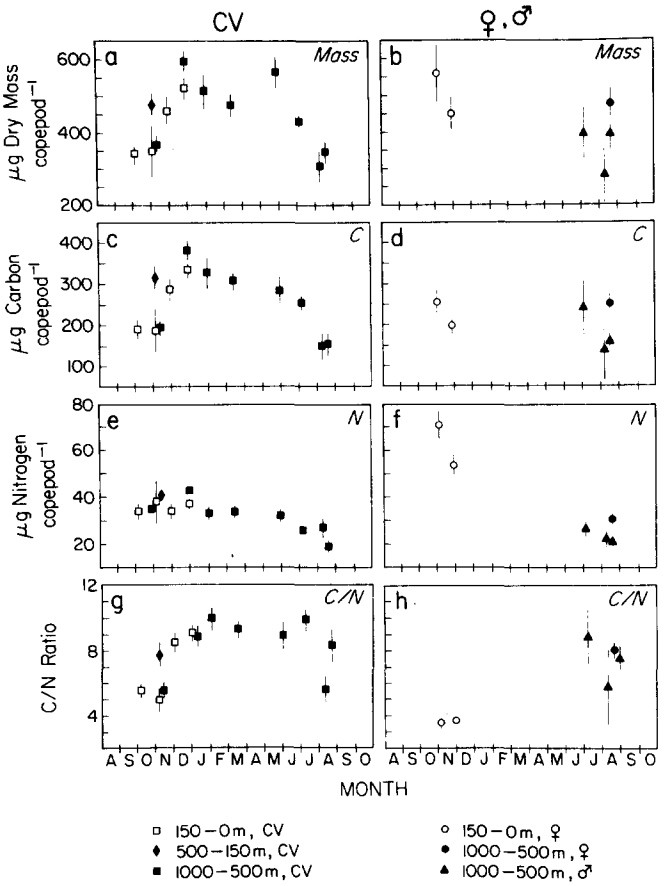

Fig. 2. Seasonal variation in (a,b) dry mass, (c,d) carbon content, (e,f) nitrogen content and (g,h) C:N mass ratio of *Neocalanus tonsus*. Left hand panels illustrate copepodid stage V and right hand panels adults. Overlapping symbols offset in time for clarity ($\bar{x} \pm 95\%$ confidence limit)

Table 2. Change in organic composition of Neocalanus tonsus *CV during spring–early summer (October–December). Spearman's rank correlation between Julian day and each constituent includes all depth strata on each date during this interval*

Constituent	4 Oct. $\bar{x}$ (μg)	27 Dec. $\bar{x}$ (μg)	Δ (μg)	Δ (%)	Correlation N	r^2	P
Nitrogen	33.9	39.3	5.4	16	85	0.08	<0.01
Carbon	190.3	352.8	162.5	85	89	0.56	<0.0001
Dry mass	342.8	550.3	207.5	60	89	0.60	<0.0001
Wax ester	38.4	181.2	142.8	372	78	0.53	<0.0001

relationship between N and C (Fig. 3b), which in turn is comparable to that for CV's collected at depth (Fig. 3a). However, for females of a given C content, spring surface individuals had a significantly higher N content than winter deep individuals, as well as a steeper relationship between the two properties (Fig. 3b). Surface-dwelling CV's smaller than ~240 μg C had a higher N content than deep-dwelling CV's of the same C content

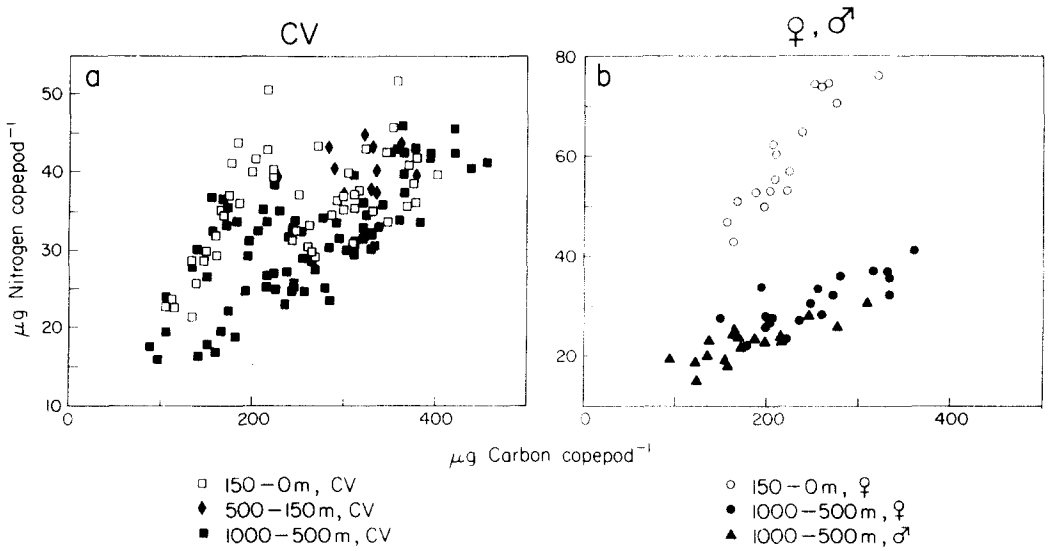

Fig. 3. Relationship between total nitrogen content and total carbon content for *Neocalanus tonsus* CV's, males and females. (Note different scales.)

($F_{1,51}$ = 10.70, $P < 0.01$, analysis of covariance), but this difference was not maintained for CV's larger than 240 μg C ($F_{1,75}$ = 1.62, $P > 0.10$; Fig. 3a).

Lipid content and composition

Total lipid content of *N. tonsus* CV's (Fig. 4a) varied seasonally in parallel with variations in C and dry mass (Fig. 2a,c). Wax esters accounted for most of the variation in total lipid (Figs 4c and 5a). The wax ester content of surface-captured CV's increased 143 μg between October and December (Table 2). The concentration of triacylglycerols, the other class of storage lipids, was 10–100 fold lower than wax esters (Fig. 4e). Triacylglycerols declined after December, prior to the decrease in wax esters. Sterols and phospholipids, usually considered structural lipids, showed little variation with season or depth (Fig. 4g,i), in contrast with the seasonal variations in CV depot lipids. The reason for the high phospholipid content on 27 December is not clear; other lipid classes did not reflect such an anomaly.

Despite the decrease in wax ester content of deep-dwelling CV's between May and August, CV's still contained considerable wax ester at the end of winter. This wax ester was also reflected in the CVI males and females captured in mesopelagic depths (Fig. 4d). The wax ester content of mesopelagic winter females was 10–41 times (mean = 24×) higher than that of epipelagic spring females (Fig. 4d). In contrast, triacylglycerols, sterols and especially phospholipids were higher in epipelagic spring females (Fig. 4f,h,j) than in either mesopelagic winter females or males.

Changes in the organic composition of *N. tonsus* are also expressed in Fig. 5. From spring to winter wax esters increased from 57 to 90% of total CV lipids (Fig. 5a), as the lipid content of CV's increased from 22 to 49% of dry mass (Fig. 5b). CV nitrogen content decreased concurrently from a maximum of 10.9% in spring surface CV's to 5.4% of dry mass in deep winter CV's (Fig. 5e). Mesopelagic adults had a significantly

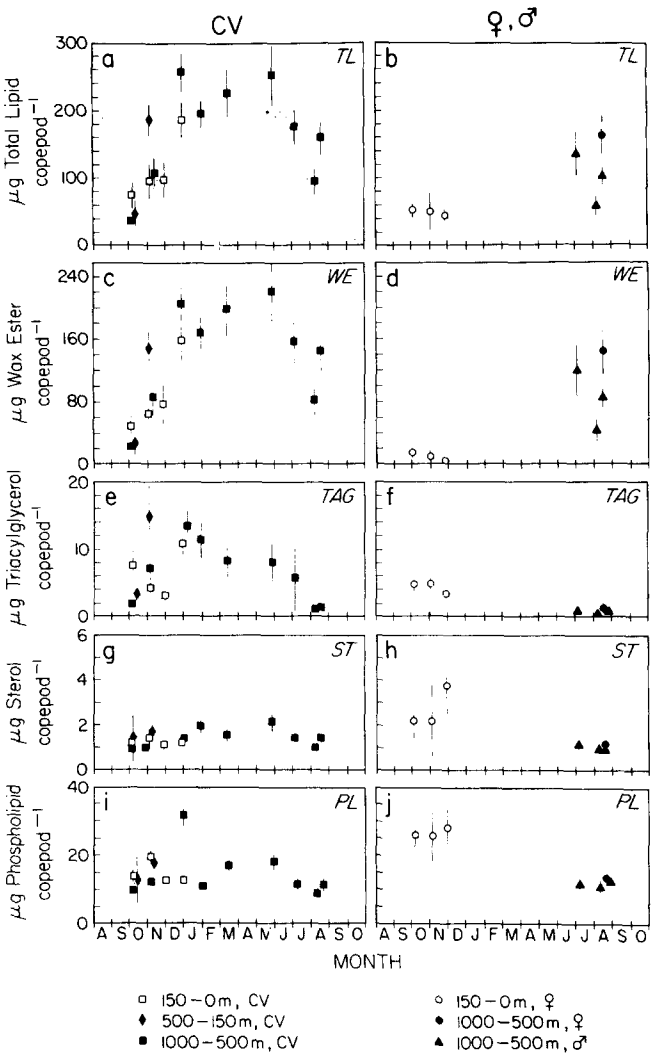

Fig. 4. Seasonal variation in (a,b) total lipid, (c,d) wax esters, (e,f) triacylglycerols, (g,h) sterols, and (i,j) phospholipids of *Neocalanus tonsus* ($\bar{x} \pm 95\%$ confidence limit).

higher proportion of dry mass in the form of lipid and lower proportion as nitrogen (Fig. 5b,f), again corresponding approximately to the composition of the CV's from which they were derived. The lipids of *N. tonsus* females were predominately wax esters at depth in winter (89% of total lipid), with only a small fraction as wax esters near the surface in spring (8–29% of total lipid).

Despite the conspicuous orange-red coloration of *N. tonsus*, carotenoid pigments were a minor component of total lipids and thus unlikely to bias estimates of other polar lipid constituents (Table 3). Pigments were identified as carotenoids by absorption spectra in hexane, carbon disulfide, petroleum ether, chloroform, acetone and pyridine. The absorption maxima of pigments corresponded closely to those reported for astaxanthin

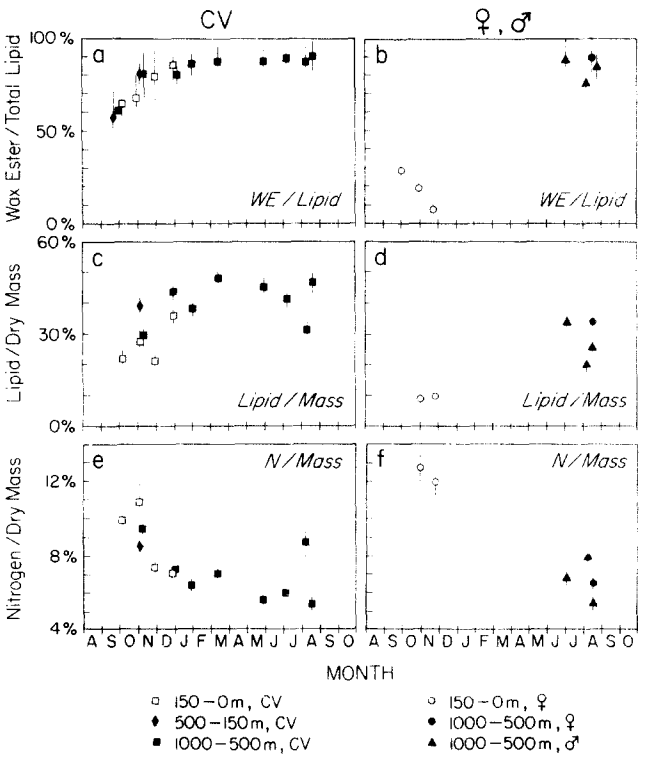

Fig. 5. *N. tonsus*. Seasonal variation in (a,b) percentage wax ester/total lipid ($\bar{x} \pm 95\%$), (c,d) percentage lipid/dry mass ($\bar{x} \pm 1$ S.E.), (e,f) percentage nitrogen/dry mass ($\bar{x} \pm 95\%$).

Table 3. *Carotenoid content of* Neocalanus tonsus *collected in November, between* 150 *and* 0 m

Development stage	Carotenoid copepod^{-1} $\bar{x} \pm 95\%$ (*N*)
Copepodid V	93 ± 21 ng (7)
Female	164 ± 97 ng (3)

(DAVIES, 1976; D'ABRAMO *et al.*, 1983) although multiple pigment bands were detected using TLC.

The lipid content of *N. tonsus* eggs released in the laboratory in August was 144 ± 48 ng total lipid ($\bar{x} \pm$ S.D., $N = 3$ extractions). Of egg lipids, depot lipids (wax esters + triacylglycerols) constituted 17.5% of the total with phospholipids the predominant constituent (65.9%; Table 4). Egg dry mass was not determined, but assuming the spherical eggs of diameter 139 μm (BRADFORD *et al.*, 1988) had specific gravity = 1.027 and that dry mass = 0.15 × wet mass, lipids constituted 66% of egg dry mass.

Lipid structural composition

The structural composition of *N. tonsus* CV lipids was analysed in two different field conditions: (1) near the surface during the spring accretionary phase, and (2) as the

Table 4. Composition of Neocalanus tonsus *egg lipids. Lipid content averaged* 144 ± 48 ng egg^{-1} ($\bar{x}$ ± s) *for three independent lots of eggs*

Lipid class	Percentage of lipids ($\bar{x}$ ± *s* (%))
Wax ester	11.2 ± 4.2
Triacylglycerol	6.3 ± 1.1
Free fatty acid	8.8 ± 1.1
Phospholipid	65.9 ± 8.4
Other	7.8 ± 3.7

population descended into deep water in summer. In both groups of CV's, wax esters, followed by phospholipids, were the principal lipid classes (Fig. 6). Wax esters were 2.6-fold higher in the summer CV's. Despite the differences in wax ester concentration, the structure of wax esters (as reflected by the distribution of carbon numbers) was similar between the two groups (Fig. 7). Slight differences included a significantly higher contribution of C_{34} and C_{40} wax esters and lower contribution of C_{44} and C_{46} wax esters in summer. Only trace quantities of odd carbon number wax esters were detected.

Constituent fatty acids and fatty alcohols isolated from the total lipid fraction also reflected an overall pattern of similarity, with some deviations, between spring and summer (Table 5). The fatty acid methyl esters of total lipids showed the same predominance of 14:0, 16:0, 18:1, 20:1, 22:1 and 22:6 fatty acids in both seasons. Fatty alcohol distributions were very similar between seasons, with the 20:1 and 22:1 alcohols contributing 82–87% of the total long-chain alcohols (Table 5). Some minor constituents (16:0, 22:0, 24:3 alcohols) were present in one season but not the other. Wax ester fatty acids and fatty alcohols were separated from those of total lipids only for the summer sample. The similarity of wax ester fatty acids and alcohols to total fatty acids and alcohols in summer reflects the fact that 87% of the total lipid was wax ester. The 22:6 fatty acids were enriched in the total lipids fraction by comparison with the wax ester

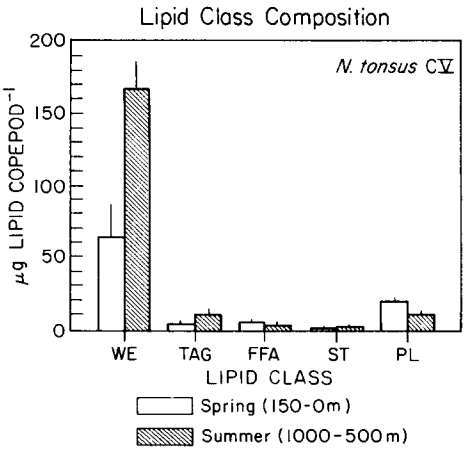

Fig. 6. Lipid class composition of *Neocalanus tonsus* copepodid V collected near the surface in spring (2 November; open) and at depth in summer (28 January; shaded). Vertical lines denote upper tail of 95% confidence limit.

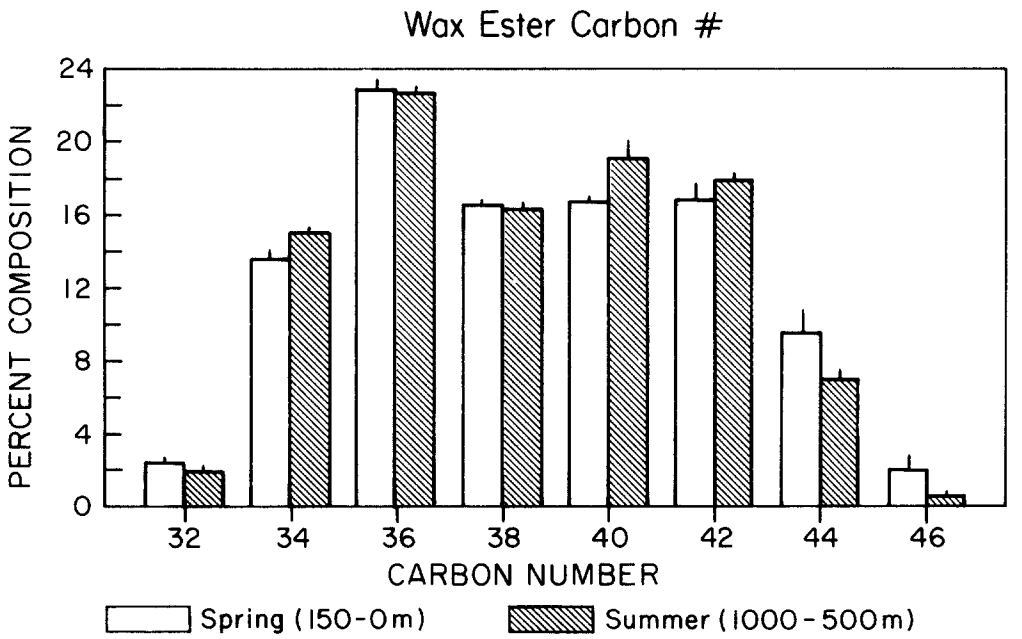

Fig. 7. Frequency distribution of carbon numbers of wax esters extracted from *Neocalanus tonsus* copepodid V near the surface in spring (2 November; open) and at depth in summer (28 January; shaded). Vertical lines denote upper tail of 95% confidence limit.

fraction. This enrichment probably reflects the importance of this long-chain polyunsaturated fatty acid in phospholipid structure.

DISCUSSION

The observed pattern of lipid accretion and mobilization in *N. tonsus* is consistent with the suggestion that lipids are the proximate carbon and energy source for deep winter reproduction of nonfeeding adults (JILLETT, 1968; OHMAN, 1987). Although the wax ester content of CV's declines during winter, considerable reserves remain at the time of first molt to adult males and females in deep water. The adult lipid content can be entirely accounted for by lipids remaining in CV's, without ingestion of particulate substrates. A limited number of other members of the Calanidae also exhibit reproduction in the absence of feeding, notably the *Neocalanus* species endemic to the subarctic Pacific and *Calanus hyperboreus* from the Arctic and North Atlantic (MILLER *et al.*, 1984; CONOVER, 1988). However, of these, only *N. tonsus* and *C. hyperboreus* subsequently appear at the surface as adults and retain the capability to ingest particulate food.

TANDE (1982) suggested that storage reserves are mobilized as overwintering CV's begin sexual maturation. The interpretation is consistent with the relatively rapid decline in storage lipids, carbon, and nitrogen of *N. tonsus* CV's during June and July, although it warrants experimental verification. It should be noted that sexual differentiation, molting and early ovarian development occur independently of spawning (TANDE, 1982). For most calanids completion of oogenesis and release of eggs requires a particulate food

Table 5. Fatty acid methyl ester and fatty alcohol composition of lipids extracted from Neocalanus tonsus *copepodid V collected in spring* (150–0 m) *and in summer* (1000–500 m). *Mean* ± 95% *confidence limits of percent composition by weight. Leftmost column indicates number of carbon atoms: number of double bonds*

	Fatty acid methyl esters			Fatty alcohols		
	Total lipids		Wax esters	Total lipids		Wax esters
Carbon Chain	Spring (150–0 m)	Summer (1000–500 m)	Summer (1000–500 m)	Spring (150–0 m)	Summer (1000–500 m)	Summer (1000–500 m)
14:0	6.4 ± 0.4	11.4 ± 0.9	13.7 ± 0.6	0.1 ± 0.2	0.8 ± 0.1	0.7 ± 0.1
14:1	0.7 ± 0.2	1.0 ± 0.1	1.3 ± 0.1			
16:0	13.8 ± 0.4	10.7 ± 0.2	11.2 ± 0.5		2.0 ± 0.2	1.8 ± 0.1
16:1	3.1 ± 0.6	5.5 ± 0.3	5.7 ± 0.3	0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
16:2		0.6 ± 0.1	0.2 ± 0.2			
17:1		0.8 ± 0.1	0.4 ± 0.2			
18:0	3.4 ± 0.3	1.0 ± 0.1	1.6 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
18:1	6.6 ± 0.3	6.7 ± 0.3	6.7 ± 0.2	2.2 ± 0.8	2.5 ± 0.3	2.4 ± 0.1
18:2	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.1	0.7 ± 0.7	0.9 ± 0.2	0.8 ± 0.3
18:3	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.1			
18:4	3.0 ± 0.6	2.5 ± 0.3	1.4 ± 0.4			
20:0	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1			
20:1	19.0 ± 1.0	17.7 ± 0.2	22.6 ± 0.4	62.6 ± 4.1	57.0 ± 1.6	59.6 ± 1.7
20:2	2.1 ± 1.0	2.5 ± 0.4	3.0 ± 0.2	2.5 ± 1.5	0.4 ± 0.2	0.4 ± 0.1
20:4	2.0 ± 0.8	1.4 ± 0.3	1.1 ± 0.4			
20:5	3.9 ± 0.3	7.1 ± 0.3	4.6 ± 0.2			
22:0	0.5 ± 0.3		0.1 ± 0.1	0.8 ± 1.8		
22:1	18.5 ± 1.2	16.2 ± 0.3	19.4 ± 0.4	24.0 ± 2.6	25.0 ± 0.9	25.6 ± 2.1
22:2				3.0 ± 0.8	2.7 ± 0.6	2.3 ± 0.6
22:3	3.4 ± 0.2					0.1 ± 0.2
22:4	0.6 ± 0.3	2.4 ± 0.4	1.5 ± 0.2	1.0 ± 1.8	0.1 ± 0.4	0.6 ± 0.7
22:6	8.5 ± 0.3	7.2 ± 0.4	1.9 ± 0.2	0.3 ± 0.8	0.6 ± 0.2	0.5 ± 0.4
24:0	0.2 ± 0.4			2.4 ± 0.4	4.5 ± 0.5	2.9 ± 0.2
24:2		1.0 ± 0.2	0.3 ± 0.3			
24:3		0.1 ± 0.2	0.1 ± 0.2		3.0 ± 0.4	1.8 ± 1.3
24:4	0.7 ± 1.0	0.4 ± 0.6				
24:5		0.3 ± 0.5	0.1 ± 0.2			
24:6	0.2 ± 0.6	0.1 ± 0.2				
Other*	1.2 ± 0.2	0.8 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Total	100%	100%	100%	100%	100%	100%

* 12:0, 15:0, unidentified.

supply (e.g. RUNGE, 1985; BÅMSTEDT and TANDE, 1988). Thus storage products should not be used as a predictor of copepod reproductive potential or total fecundity, apart from a few species for which the life history is appropriate (OHMAN, 1987).

Other physiological changes, unrelated to sexual maturation, commonly accompany changes in lipid content. The ingestion rate of CV's declines from spring to summer to winter as wax ester content builds and CV's descend into deeper waters (OHMAN, 1987). In several crustacean species, CONOVER and CORNER (1968) observed that respiration rate was inversely proportional to lipid content. Lipid-rich *Calanus pacificus* residing at depth have a reduced excretion rate and low laminarinase activity (ALLDREDGE *et al.*, 1984). Overwintering CV's of *Calanus finmarchicus* have low rates of respiration, excretion and incidence of feeding (BÅMSTEDT and TANDE, 1988). From studies in other locales *C. finmarchicus* is known to accumulate wax esters (HOPKINS *et al.*, 1985; KATTNER and KRAUSE, 1987). Other instances of relationships between metabolic activity and organic composition are discussed in LE BORGNE (1986).

The relatively high lipid content of *N. tonsus* and predominance of wax esters are in accord with higher latitude copepod species in the northern hemisphere (LEE and HIROTA, 1973; LEE, 1975; BÅMSTEDT, 1986) and much more limited evidence from southern hemisphere species (SARGENT and HENDERSON, 1986; HAGEN, 1988). However, HAGEN (1988) observed that *Calanus propinquus* and *Euchirella rostromagna* differed from four other antarctic copepods in storing primarily triacylglycerols rather than wax esters.

Comparison with *N. plumchrus* from the subarctic Pacific suggests similarities in both the content and the structural composition of lipids. Wax esters comprised up to 90% of *N. tonsus* CV lipids, comparing with 85–90% of *N. plumchrus* CV lipids (LEE *et al.*, 1971; YAYANOS *et al.*, 1978). Lipids constituted as much as 49% of dry mass of *N. tonsus* CV and a somewhat higher fraction of the dry mass of *N. plumchrus* (to 55–59%; LEE *et al.*, 1971; VIDAL and WHITLEDGE, 1982). The same monoenoic fatty alcohols (20:1, 22:1) accounted for over 3/4 of all wax ester fatty alcohols in *N. plumchrus* (LEE, 1975) and *N. tonsus*. The primary wax ester fatty acids in *N. tonsus* were 14:0, 16:0, 20:1 and 22:1, in comparison with 14:0, 16:0, 16:1, 20:1, 20:5 and 22:1 for *N. plumchrus* (LEE, 1975). SARGENT and HENDERSON (1986; see also CLARKE *et al.*, 1987; FALK-PETERSEN *et al.*, 1987) summarize data suggesting that the wax ester fatty acids and fatty alcohols of members of the family Calanidae are more similar to one another than to those of other copepod families or to euphausiids. The present analyses agree with this trend. Despite the geographical isolation of *N. tonsus* from the other species analysed, it has a calanid-like distribution of wax ester fatty acids and fatty alcohols.

N. tonsus egg lipid composition differs from egg lipids of other copepods, since 2/3 of the lipid was phospholipid rather than neutral lipids. Eggs of *C. pacificus* contain primarily triacylglycerols (60%; LEE *et al.*, 1972) as do those of *Calanus helgolandicus* (57%; GATTEN *et al.*, 1980). Ova of the predatory copepod *Euchaeta elongata* Esterly (=*japonica*) reportedly contain mostly wax esters (58%; LEE *et al.*, 1974) and those of *Euchaeta marina* 32% wax esters and 2% triacylglycerols (LEE and HIROTA, 1973; remaining composition not stated). However, ova of some other organisms have been found to contain a high proportion of phospholipid. For example, phospholipid comprised 42% of the lipid of winter eggs of the gammarid amphipod *Gammarus oceanicus* (CLARKE *et al.*, 1985). Phospholipids (primarily phosphatidylcholine) comprised 77% of the lipids of newly fertilized cod ova and were the primary lipids utilized during embryogenesis and early larval development (FRASER *et al.*, 1988).

The fraction of dry mass of *N. tonsus* eggs represented by lipids (66%) compares with 61% for eggs of *E. marina* (LEE and HIROTA, 1973) and 64% for those of *E. elongata* (LEE *et al.*, 1974). An estimate for *C. helgolandicus* eggs appears unrealistically high (GATTEN *et al.*, 1980); it may be based on an underestimate of the dry mass of *Calanus* eggs.

Our interpretation of the temporal variability in organic composition of *N. tonsus* as an underlying seasonal cycle rests on the assumption that a single population was sampled over time. This appears, to a first approximation, to be reasonable. The seasonal timing of disappearance from surface waters and subsequent reappearance of developmental stages of *N. tonsus* agree well with previous observations at this location (JILLETT, 1968; MURDOCH, 1985) and at Kaikoura, New Zealand (BRADFORD, 1972). The developmental stage compositions observed in more temporally restricted studies elsewhere (VERVOORT, 1957; KAWAMURA, 1974; VORONINA, 1975) are consistent with those observed in this

study. Also, the similarity of fatty acids, fatty alcohols and wax ester chain lengths between samples taken 3 months apart suggests that upstream elements of the population encountered rather similar food substrates to those sampled off the South Island of New Zealand. One departure may be reflected in the higher dry mass and carbon content of deep winter females compared with CV's from the same collection, an anomaly since the females do not feed at that time of year.

Other limitations of the present study include restriction of sampling to only a single year, in the upper 1000 m, by day. All were necessitated by logistical constraints. It is not clear whether an appreciable fraction of the *N. tonsus* population occurs in water deeper than 1000 m, though the maximum water depth at the sampling location was just 1200 m. Concerning possible bias due to diurnal sampling, evidence for diel vertical migration by *N. tonsus* is limited. In September BRADFORD (1970) found that most females were not present above 250 m by day, but were present between 100 and 250 m at night. In the present study daytime samples in October and November consistently detected females in the stratum between 150 and 0 m. Preliminary sampling on 12 October 1983 revealed that CIV, CV and female *N. tonsus* were present within the uppermost 50 m at 1400 h local time. Near-surface occurrence of *N. tonsus* in the daytime has also been documented by KAWAMURA (1974) and TAW and RITZ (1979). Therefore, from the evidence available it appears unlikely that diel vertical migration would significantly bias the seasonal pattern of vertical distribution on the vertical sampling scale used in the present study.

Higher latitude zooplankton species with seasonally interrupted life histories can differentially synthesize storage compounds and structural compounds during intervals of growth. The present evidence for such differential accumulation recalls results with some northern species (e.g. *C. helgolandicus*, GATTEN *et al.*, 1979; *Metridia longa*, HOPKINS *et al.*, 1984) and illustrates the importance of analytically distinguishing storage compounds (depot lipids) from structural compounds (nitrogen, non-lipid carbon). However, no inferences can be drawn regarding the relative importance of different particulate substrates in the limitation of somatic growth of zooplankton (CHECKLEY, 1980) without knowledge of the metabolic turnover of these substrates, in addition to their net accumulation in tissues over time. Combination of such approaches in the future will make it possible to determine whether the substrates optimal for growth are different for continuously reproducing species and those whose life history is punctuated by intervals of dormancy.

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