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Effect of the receding ice-edge on the condition of mid-water fishes in the northwestern Weddell Sea: results from biochemical assays with notes on diet

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Abstract *Electrona antarctica* and *Bathylagus antarcticus* are dominant fishes in the Southern Ocean pelagic ecosystem with disparate life histories, making them excellent subjects for comparative studies. Twenty-one indices of nutritional condition were compared in both species by observing changes in each index as it varied spatially and temporally in association with the marginal ice-zone bloom in the northwestern Weddell Sea. *E. antarctica* and *B. antarcticus* exhibited very different patterns of response to the bloom. Nutritional condition increased in 11 of 21 measures in *E. antarctica*, suggesting that it was in poorer condition at ice-covered stations and in greatly improved condition in post-bloom, open-water stations. The data, combined with a few observations of larvae, indicate that *E. antarctica* increases feeding before the spring bloom, rapidly building stores of lipid, which are probably used for spawning. In contrast, only three measures of condition were variable in *B. antarcticus*, and lipid actually decreased from ice-covered to open water. RNA:DNA values increased in open water, coinciding with an increase in food volume present in their guts. These observations, coupled with observations of larvae in ice-covered stations, suggest that *B. antarcticus* possesses sufficient energetic stores to spawn prior to the bloom, so that larvae are able to fully utilize available resources associated with the spring bloom. Increased somatic growth, as indicated by RNA:DNA, may not begin until later, when the bloom was beginning to decline. Earlier work, which suggested that the deeper living, non-migrating species, like *B. antarcticus*, would not be affected by the bloom until after shallower, migratory species, like *E. antarctica*, is supported. Of the assays tested, RNA:DNA appeared to be the most sensitive. The combined assessment of many measures including biochemical, compositional, dietary, and age estimates from single specimens is possible if the samples are treated with sufficient care. The reliance upon a single measure to help interpret the ecology of a species, especially in nekton-sized species, is not as effective as techniques used in combination.

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Introduction

During austral spring, in the wake of the receding Antarctic ice-edge in the northwestern Weddell Sea, there is a large increase in primary productivity (Smith and Nelson 1985; Garrison and Buck 1989; Bianchi et al. 1992). The increase in primary productivity provides an immediate increase in the food supply for herbivorous species such as copepods, which increase in both abundance (Foster 1987; Atkinson and Peck 1988; Hubold et al. 1988) and quality (Falk-Peterson et al. 1987; Schnack-Schiel et al. 1991). Zooplankton biomass in the upper 1,000 m of the water column increased from 37 to 150 g per 1,000 m^3 (189–5,278 individual zooplankters per 1,000 m^3) when moving from closed pack ice to marginal ice zones and open-water stations (Siegel et al. 1992). Most of this difference was attributed to *Salpa thompsoni*, a salp, and four dominant species of copepods (*Calanoides acutus*, *Calanus propinquus*, *Rhincalanus gigas*, and *Metridia gerlachei*). The same species of copepods have been observed to have large increases in gut fullness during bloom conditions (Hopkins and Torres 1989), which they use to stockpile lipid (Schnack-Schiel et al. 1991). In some carnivorous fishes, such as *Electrona antarctica* (Pisces: Myctophidae), there is evidence for increasing energy stores from spring, through summer, and into winter, while other species, such as *Bathylagus antarcticus* (Pisces: Bathylagidae), show no such seasonal variation (Donnelly et al. 1990). The vast quantity of energy being stockpiled in secondary production is transferred to higher trophic

levels, as evidenced by the increased populations of flying seabirds, minke whales, and fur seals (Hopkins et al. 1993). Whales especially use the periods of increased food abundance to supply most of their year's energy intake (Brown and Lockyear 1984). However, the effects of the spring bloom on zooplanktivorous fishes remains poorly documented.

E. antarctica is endemic to the waters south of the Antarctic convergence and is distributed throughout the Southern Ocean with the possible exception of the waters beneath the ice shelves of the Weddell and Ross Seas (McGinnis 1982; Hulley 1990). *E. antarctica* can be found throughout the water column, from the surface to depths of at least 1,000 m. It exhibits pronounced diurnal vertical migrations from a daytime distribution of 650 m into the upper 100 m at night (Torres and Somero 1988; Lancraft et al. 1989). It reaches sizes of at least 103 mm, and may reach sexual maturity by a size of 61 mm. *E. antarctica* prey primarily upon copepods, amphipods, and other crustaceans (Miller 1993), but also take some fraction of their diet from nearly all available metazoans with little seasonal fluctuation in composition, although a decrease in quantity of food taken occurs in winter (Hopkins and Torres 1989; Lancraft et al. 1991; Hopkins et al. 1993). *B. antarcticus* is also found throughout the Southern Ocean, but two collections have been made north of the convergence as well (Gon 1990). *B. antarcticus* is found deeper in the water column than *E. antarctica*, centered at depths of 650–920 m during the daytime, with some upward migration at night to 170–470 m. It exhibits seasonal shifts in its center of distribution (Lancraft et al. 1989). Specimens have been collected at depths of up to 4,000 m (Gon 1990). Larvae reach at least 26 mm, and adults of 141 (Miller 1993) to 160 mm (present study) have been collected. In addition, we collected gravid females at lengths of 137–160 mm standard length (SL) and seemingly ripe males (histological studies have not been conducted) of 71–112 mm SL. *B. antarcticus* takes a broad prey spectrum that varies in size (Hopkins et al. 1993). Small specimens (30–57 mm SL) favor smaller items, such as copepods, while larger specimens (60–116 mm SL) also consume larger items such as euphausiids and larvaceans (Hopkins and Torres 1989). No shift in diet occurs during winter periods, although quantity is reduced (Lancraft et al. 1991). *E. antarctica* and *B. antarcticus* comprise about half of the pelagic fish biomass in the Southern Ocean and probably consume more of the secondary production than birds, seals, and whales combined (Lancraft et al. 1989). Their different life histories and importance in the ecosystem make these two species excellent study subjects.

Measurements of nutritional condition can provide estimates of the recent feeding history of the organism being studied (Koslow et al. 1985). In larvae, a large proportion of the assimilated energy is converted into growth (Kiørboe 1989; Mathers et al. 1993); therefore, individuals with better nutritional condition grow more rapidly. In adults, most of the assimilated energy will be used to produce offspring, or will be stored as energy reserves to either be used in future spawning events or simply as a safe-guard against periods of famine (Withers 1992). Successful measurements of nutritional condition can provide insights into growth patterns of individual species and populations.

Traditional measures of condition included various combinations of length and weight (as reviewed in Ferron and Leggett 1994). Traditional methods of studying condition using biochemistry include compositional analysis for protein, lipid, and carbohydrates, among others (Love 1970; Ehrlich 1974; Mayzaud 1976; Frovlov and Pankov 1992; Donnelly et al. 1994). These techniques are excellent for long-term measures of condition (Sullivan and Somero 1983). More recent measures such as metabolic enzyme activity and nucleic acid ratios can respond within days (McLaughlin et al. 1995; Segner and Verreth 1995). One widely used technique for studying condition in fishes is the ratio of ribonucleic acids to deoxyribonucleic acids (RNA:DNA) (Buckley 1982; Westerman and Holt 1988; Foster et al. 1993). Metabolic rates and their proxies, metabolic enzyme activities, provide measures of current energy expenditure (Somero and Childress 1980; Hochachka and Somero 1984; Torres and Somero 1988; Lallier and Walsh 1991) and growth rate (Brightman et al. 1997).

The objective of the present study was to examine the importance of the spring bloom to two intermediate-trophic level fishes with different lifestyles. Changes in nutritional condition of *E. antarctica* and *B. antarcticus* over the brief time during progression of the spring bloom were examined. Indices of nutritional condition (RNA:DNA, LDH, CS, %Protein, %Lipid) and diet were studied across three zones differing in ice cover: ice-covered waters (pre-bloom conditions), marginal ice-zone waters (maximal bloom conditions), and open water (post-bloom conditions).

Materials and methods

Sample collection and processing

Both species of fish, *Bathylagus antarcticus* and *Electrona antarctica*, previously shown to be zooplanktivores (Hopkins et al. 1993), were collected in sufficient numbers to address zonal differences. First, rapid transects along and perpendicular to the ice-edge were conducted. The rapid transects allowed the geographic extent of the bloom to be defined. Next, the following zones were sampled for 5 days each: ice-covered waters, assumed (cf. Burghart et al. 1999) to be in pre-bloom conditions, the marginal ice zone, where the bloom was occurring, and open water, were the phytoplankton bloom was already dissipating (Fig. 1).

A majority of the fishes were collected using a Tucker trawl, 9 m^2 in mouth area, with 4 mm mesh and a 500 μm mesh cod-end net. The trawl was deployed obliquely, usually from the surface to 1,000 m, although both shallower and deeper tows were conducted. The cod end was emptied into a large tub, diluted with an additional 2–3 vol. of seawater, and transferred to a shipboard laboratory. Those fish that were sorted from the cod-end bucket within 1 h were frozen immediately in liquid nitrogen and stored at −50 °C. Those fishes were shipped from Punta Arenas, Chile, to St. Petersburg,

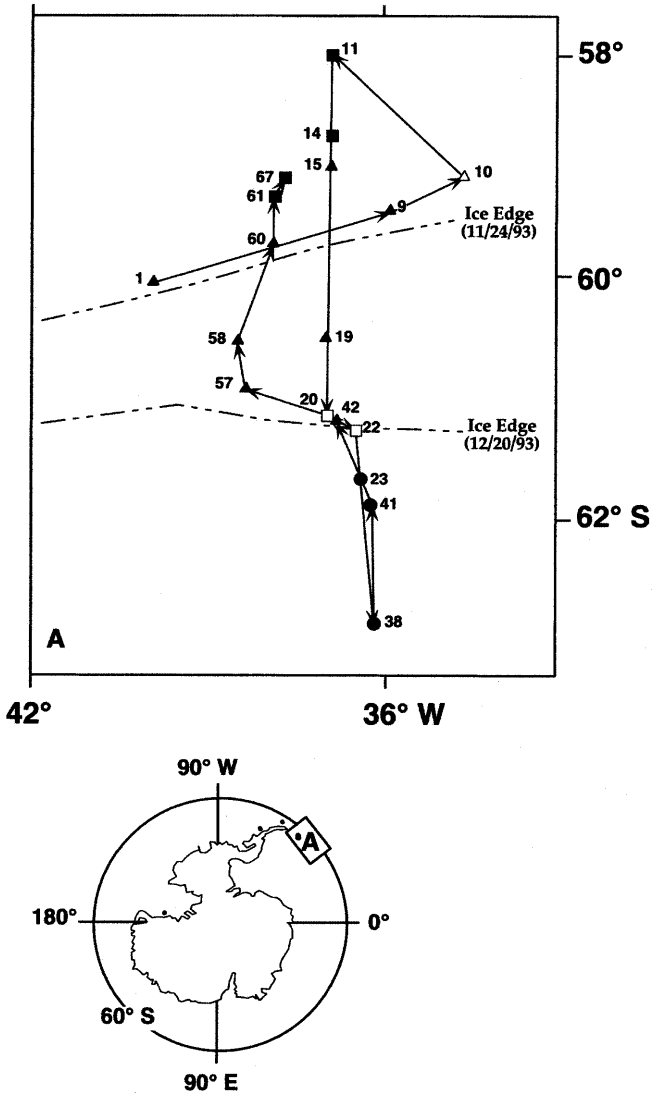

Fig. 1 Location of the sample region relative to the Antarctic Peninsula is indicated by the *inset A* (*lower right*). Ice-covered stations, 23–41; ice-edge stations, 42–58; open-water stations, 60–67; the ice-edge receded from the *upper* to the *lower dashed lines*

Florida on dry ice, where they were stored at −80 °C until assayed. The remainder of the samples was used for diet analysis; these were fixed immediately in 10% buffered formalin and were transferred to 70% isopropyl alcohol for longer term storage.

Fifteen biochemical indices of condition were quantified from frozen specimens (see Table 1). The entire animal as well as their gonads, liver, intestine, stomach, and adipose tissue were weighed to determine wet mass. Proximate chemical composition, including water, ash, protein, and lipid, all as a percentage of white muscle tissue wet mass, were determined using the methods of Donnelly et al. (1995). During dissections for diet (see below) we noted that many fish had what appeared to be fatty deposits associated with the intestine but separate from the true adipose tissue. Fishes are also known to store lipids in their livers (Pelster 1997). Therefore, lipid was also determined for liver and intestine. Adipose and gonad were assumed to be predominantly lipid and were not assayed. Three more recently developed biochemical assays were also conducted: RNA:DNA ratio, citrate synthase activity (CS; EC 4.1.3.7), and lactate dehydrogenase activity (LDH; EC 1.1.1.27).

Each specimen was partially thawed so that the SL and wet mass could be determined and the internal organs removed. A wedge of white epaxial muscle was taken from behind the head of each specimen, weighed, and homogenized in 24 vol. of ice-cold deionized water. An aliquot of 200–1000 µl was placed in a pre-weighed crucible, dried for 3 days at 65 °C to measure water levels, and then baked for 6 h at 600 °C to measure ash. Triplicate aliquots (10–50 µl) were frozen at −50 °C for protein analysis. Duplicate aliquots of 50–200 µl were frozen at −50 °C for RNA:DNA determination. The remaining homogenate was centrifuged at approximately 10,000 *g* and stored on ice for enzyme rate determinations (15–80 µl per assay). Following excision, the wet mass of each of the following organs was determined: gonad, liver, intestine, stomach, and adipose (when visible). The organs and a second wedge of white muscle were then refrozen individually at −80 °C. Muscle, liver, and intestine ($\%L_m$, $\%L_l$, $\%L_i$) were subsequently analyzed for lipid concentration. The adipose tissue in frozen *B. antarcticus* was too small to be separated from other tissues, and was not analyzed.

Percent protein was determined according to Lowry et al. (1951). Protein aliquots were dissolved in 0.1 ml of 0.1 N NaOH for 10 min at 100 °C. After cooling to room temperature, the samples were mixed with 1.2 ml of reagent B (0.5 ml of 10 mg $CuSO_4$ ml^{-1} + 0.5 ml of 20 mg Na-tartrate ml^{-1} + 50 ml of 20 mg Na_2CO_3 ml^{-1} in 0.1 N NaOH). After 10 min, 0.1 ml of 1 N Folin's phenol reagent was added to each sample and thoroughly mixed. The reaction was allowed to proceed for at least 30 min, and the absorbance was read on a spectrophotometer at 750 nm with a 1 cm pathlength. The quantity of protein in each sample was estimated from sample absorbances that were compared to a standard curve that was generated using known quantities of bovine serum albumin.

RNA:DNA ratio was determined by a sequential degradation fluorimetric technique modified from Bentle et al. (1981). Samples were placed in 2 ml of the following solution: ethidium bromide, 1.25 µg ml^{-1}; NaCl, 11 µg ml^{-1}; Tris buffer, 2.4 mg ml^{-1}; $MgCl_2$, 0.2 mg ml^{-1}, $CaCl_2$, 0.09 mg ml^{-1}; Proteinase K, 75 µg ml^{-1}; final solution pH 7.5. This solution was incubated at 37 °C for 90 min, after which the fluorescence was read on a spectrofluorometer at an excitation wavelength of 365 nm and an emission wavelength of 590 nm. Next, 25 µl of RNase (1 µg $µl^{-1}$) was added, the entire mixture incubated an additional 45 min at 37 °C, and fluorescence read a second time. The decrease in fluorescence was attributed to RNA. Finally, 25 µl of DNase was added, the entire mixture was incubated a final time for 30 min at 37 °C, and the fluorescence was read a final time. The decrease in fluorescence was attributed to DNA. A complete standard curve was generated each day using DNA from calf thymus and RNA from Baker's yeast. Sample nucleic acid concentrations were estimated by comparing the absorbance of their sample to that of the standards.

Citrate synthase activities were determined by modifications of Sudgen and Newsholme (1975) as described by Ikeda et al. (2000). Fifty microliters of homogenate was placed in 1 ml of the following assay buffer maintained in a thermostatted spectrophotometric cuvette at 10 °C: imidazole/HCl buffer, 3.4 mg ml^{-1}; DTNB (5,5′-dithio-bis[-2-nitrobenzoesaure]), 80 µg ml^{-1}; acetyl coenzyme A, 0.1 mg ml^{-1}; final pH 8.0 at 10 °C. After the homogenate was added, the assay was observed until the slope of absorbance (1 cm pathlength) at 412 nm over time was zero (2–3 min). At this point, 12.5 µl of oxaloacetate (0.053 g per 10 ml of imidazole buffer, pH 7.0) was added, and the slope was observed for an additional 2–4 min. Usually, the slope was submaximal during the first 20–60 s, after which it became linear, so only the last 1–3 min were used. Enzyme activity was calculated as the quantity of substrate converted per minute per gram wet mass.

Lactate dehydrogenase activity was determined by a modification of Walsh et al. (1989) described in Ikeda et al. (2000). From 5 to 10 µl of homogenate was added to 1 ml of the following assay buffer maintained in a thermostatted spectrophotometric cuvette at 10 °C: imidazole/HCL buffer, 5.8 mg ml^{-1}; NADH, 0.115 mg ml^{-1}; Na-pyruvate, 0.55 mg ml^{-1}; final pH 7.2. The change in absorbance (1 cm pathlength) at 340 nm was observed for 20–60 s. Enzyme activity was calculated as the quantity of substrate (NADH) converted per minute per gram wet mass.

Table 1 *Electrona antarctica* and *Bathylagus antarcticus*. Statistical analyses of biochemical variation. Columns include variable, sample size (*n*), *t*-test (*t*) (for comparison between sexes in *E. antarctica*), correlation coefficient (*r*) between the variable and standard length (*SL*), and comparisons across zones by ANOVA (*F*). Transformations (*TR*): Mann–Whitney *U*-test was performed (*Z*), Kruskal–Wallis ANOVA on ranks was performed (*H*); square root (*S*), inverse (*I*), and log (*L*) transformations were performed. Groups in any given comparison were significantly different when $P < 0.05$. %Lipid from muscle (*m*), liver (*l*) and intestine (*i*). *n.d.* No data

Variable	*Electrona antarctica*							*Bathylagus antarcticus*				
	n	Sex		SL		Zone		*n*	SL		Zone	
		TR	*t*	TR	*r*	TR	*F*		TR	*r*	TR	*F*
%Ash	68	–	0.36	–	-0.422^{**}	–	9.05^{***}	45	–	0.111	–	4.25^{*}
$\%H_2O$	68	–	4.85^{*}	–	-0.493^{***}	–	8.74^{***}	44	–	0.498^{***}	–	1.71
%Protein	72	–	1.17	–	–0.162	–	5.17^{**}	45	–	-0.520^{***}	–	3.18
%Lipid (m)	69	–	0.42	–	0.344^{**}	–	1.74	31	–	0.112	I	6.03^{*}
%Lipid (l)	29	–	2.68^{*}	–	0.714^{*}	L	5.55^{*}	n.d.	–	–	–	–
%Lipid (i)	28	–	0.15	–	–0.151		10.04^{***}	n.d.	–	–	–	–
RNA:DNA	72	–	0	–	0.162	H	19.26^{***}	49	–	0.396^{***}	L	12.29^{***}
CS	72	–	0.03	–	0	L	3.30^{*}	49	–	–0.535	–	1.94
LDH	72	–	1.05	–	0.854^{***}	S	6.24^{**}	49	–	0.673	L	2.74
Mass (g)												
Total	72	I	0.47	L	0.960^{***}	H	1.63	49	S	0.908	S	0.77
Gonad	70	I	0.09	L	0.830^{***}	S	0.16	32	L	0.709	L	0.73
Liver	70	I	0.82	L	0.920^{***}	–	1.62	32	L	0.737	L	2.04
Intestine	70	Z	1.06	L	0.840^{***}	S	31.60^{***}	32	L	0.871	L	0.19
Stomach	70	–	0.74	L	0.840^{***}	S	5.85^{**}	32	L	0.847	L	0.23
Adipose	70	Z	1.27	L	0.700^{***}	S	0.45	n.d.	–	–	–	–

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Lipid was assayed by Reisenbichler and Bailey's (1991) modification of earlier assays (Bligh and Dyer 1959; Marsh and Weinstein 1966). Tissue was homogenized in a chilled glass centrifuge tube at 9:1 (water to tissue wet mass). To an equal volume of homogenate (usually 100 µl), 2 vol. of methanol and 1 vol. of chloroform were added and mixed thoroughly, then let stand for 10 min. Next a single volume of chloroform was added, and the total volume mixed again, and let stand an additional 10 min. Finally, an additional volume of water was added, then mixed thoroughly, and let stand for 30 min. The mix was then poured into a Teflon microfilter, the original mixing vessel rinsed with 2 vol. each of methanol and chloroform, and then centrifuged at low speed (50 *g*) to increase the filtration speed. After centrifugation, the filtrate was poured into a clean glass tube (the filtrate vessel was rinsed again with chloroform). The tube containing the filtrate was placed in a heating block at 50 °C, and the chloroform and methanol were evaporated under a gentle flow of nitrogen gas, leaving any lipid as residue. Next a 200 µl volume of concentrated sulfuric acid was added, and the mixture was charred at 200 °C for 10 min. Finally, the remaining sulfuric acid/lipid mixture was diluted in known ratios (usually 9:1) to bring absorbance under one, as read in a spectrophotometer at 375 nm (1 cm pathlength). The absorbance values of samples were compared to absorbances of known samples of stearic acid, treated identically.

Diet was analyzed on preserved specimens of both *E. antarctica* and *B. antarcticus* by dissecting out the entire digestive tract. Intestine, pyloric caeca, and stomach were examined separately. Adipose tissue became readily recognizable in preserved specimens, and was estimated on a scale of zero to five, zero being none visible, and five representing an animal in which adipose tissue was abundant, surrounding each organ, and extensively intertwined with the vascular tissue. Food items were identified to major taxonomic group only (copepod, euphausiid, etc.) when possible. The state of digestion was estimated on a scale of one to five, one being no or very little digestion, and five being almost completely digested. Stomach fullness was also estimated on a scale of zero to five, zero being empty, and five being grossly distended due to fullness. The volume of each diet item was estimated by measuring length and width. Additional analyses such as comparison of percent frequency of particular diet items were conducted. Net feeding bias has been found to be minor (Hopkins and Baird 1975) and was not considered. Food in the esophagus was not counted.

Statistical analysis

One important objective of the present study was to compare the biochemical assays with more traditional methods of analyzing nutritional condition (e.g. proximate composition). In order to do this, variation in each index was determined across the three sampling zones, taking into account the effects, if any, of sex and size. Two obvious preliminary factors were considered prior to the comparison across zones. First, sex-related differences of *E. antarctica* were considered by *t*-test or appropriate non-parametric analyses if transformations could not be found. In most cases, the sex of the *B. antarcticus* could not be determined due to the undeveloped state of the gonad and lack of identifiable external characteristics (Gon 1990). If sex-related differences were detected, sex was retained as a factor for further analyses. Next, size-related differences were examined. A scatterplot of each index versus the standard length was examined for non-linear patterns. If obvious non-linear patterns were observed, data transformations were attempted and correlation analysis was performed on the transformed data, otherwise correlation analysis was performed on the raw data. If the index was found to vary with size, analysis of covariance (ANCOVA) was performed, with the index as the dependent, and zone as independent variable (as well as sex if necessary), and size as covariate. If no variation with size was found, zonal variation was compared by ANOVA, if the assumptions of normal distribution and homogeneity of variances were met. The reproductive biology of these two species remains undescribed, and neither species could be reliably separated into distinct reproductive stages other than larval (which were not used) and post-metamorphic. For this reason, analysis using SL as a continuous variable (covariate) was used. If the necessary assumptions were not met, transformations of the data in order to meet the assumptions of parametric statistics were attempted; if these were not successful, zonal variation was compared by Kruskal–Wallis test. When any test indicated that significant variation occurred, the appropriate post hoc analyses were conducted to determine where the variation occurred.

Results

The bloom associated with the receding ice-edge was extensive, with a marked increase in chlorophyll persistent along the entire ice-edge front. Chlorophyll biomass in the top 40 m was 15–17 mg chl m^{-2} in ice-covered stations (pre-bloom), peaked at 113 mg chl m^{-2} in the ice-edge bloom, and dropped to a mean of 69 mg chl m^{-2} in open water (post-bloom) (Burghart et al. 1999). In the three dominant species of copepods studied, *Calanoides acutus*, *Calanus propinquus*, and *Metridia gerlachei*, there was an increase in abundance from ice-covered to open-water stations (Burghart et al. 1999). The nutritional condition of the potential prey, especially in terms of lipid content, also increased rapidly during our sample period (Geiger 1999). The population of micronekton, likewise important in the diet of our study species, also appeared to increase during the bloom, although trends were overshadowed by high variability between samples at open-water stations. Some species, such as *Salpa thompsoni* increased in median numerical abundance from almost undetectable levels under the ice, 1×10^4 m^{-3} (range = 0–29) to 788×10^4 m^{-3} (0–2,581) in the ice-edge bloom, and declined to 234×10^4 m^{-3} (0–6,392) in open waters. *Euphausia superba* was distributed similarly (median abundance:ice-covered = 8×10^4 m^{-3}, 0–1,702; ice-edge = 102×10^4 m^{-3}, 3–447; open water = 166×10^4 m^{-3}, 25–624). Amphipods as a group increased in abundance throughout the sample region (median abundance:ice-covered = 6.4×10^4 m^{-3}, 0–23; ice-edge = 5.7×10^4 m^{-3}, 0–120; open water = 11.4×10^4 m^{-3}, 0–73) (authors' unpublished data). All available evidence indicated that the prey available to higher trophic levels increased in both quantity and quality from ice-covered to edge and open-water zones. The values indicated that our intended experimental conditions of high chlorophyll in the bloom, with lower values (spatially and temporally) on either side of the bloom, accompanied by a rise in secondary production, were realized.

Analyses of *Electrona antarctica*

A total of 302 specimens of *E. antarctica* were collected, ranging in size from 10 to 146 mm. Two of the 302 samples, both collected in open-water stations, were larvae that had not metamorphosed yet. The larvae were not used in any assay. Biochemical assays were conducted on 78 fish (all of the available frozen specimens), and 28 were

used for diet analysis. The remainder of the preserved specimens was stored for possible future studies.

Biochemical measurements

Only the percentage of water (Table 1) and liver lipid (Table 1) varied significantly between sexes. Eleven of the 15 measures varied significantly with SL (Table 1); for each of these variables SL was retained as a covariate in the statistical analyses. No variation in %Protein, $\%L_i$, RNA:DNA, and CS with changes in SL was observed.

Ten of the 15 measures varied across the ice front (Table 1). %Ash was higher at edge than open-water stations, but other comparisons were not significant (Fig. 2A). %Water was lowest in open-water stations,

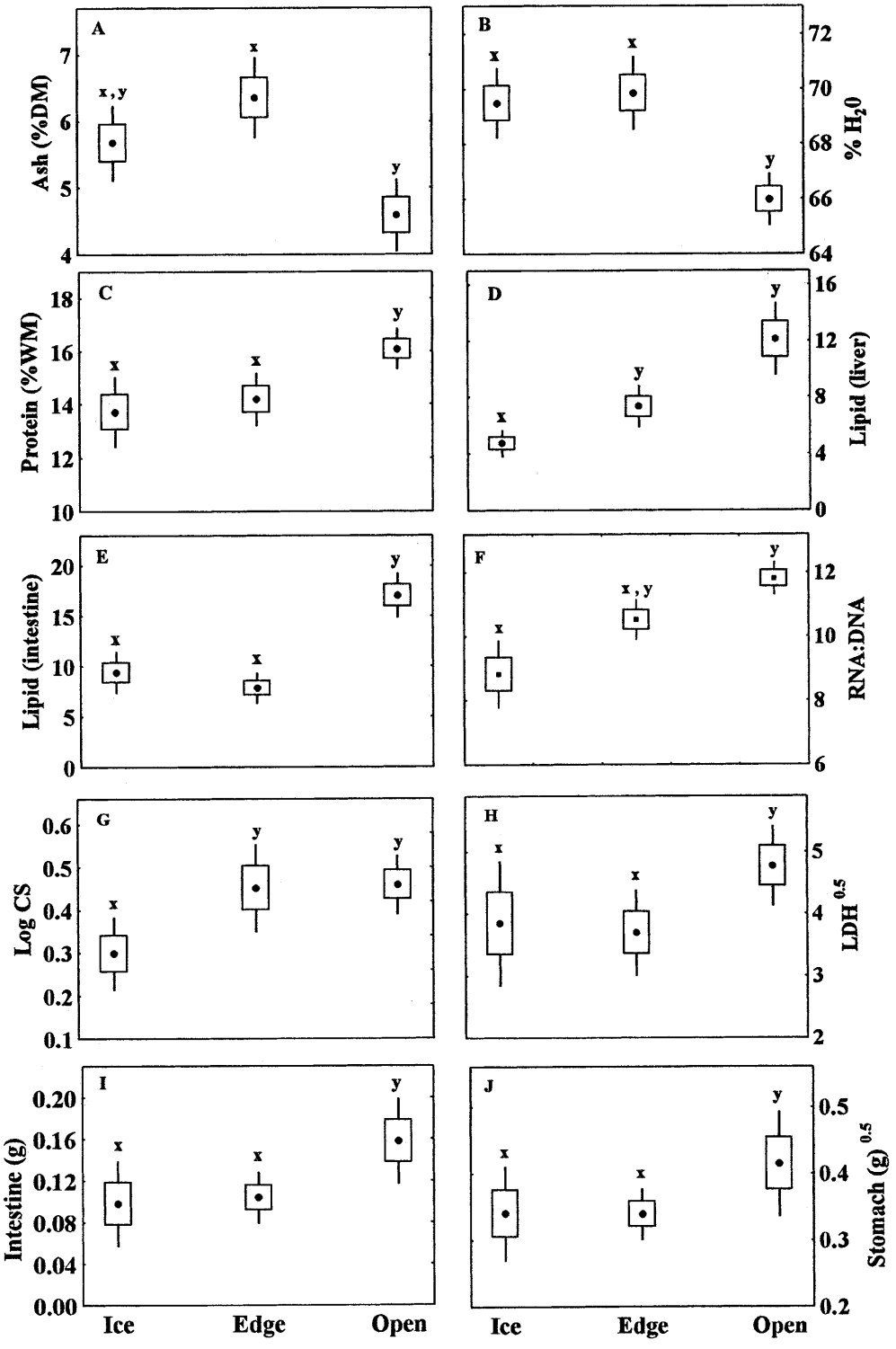

Fig. 2A–J *Electrona antarctica*. Variation in ten measures of nutritional condition. For all figures significant variation occurred between at least two groups. For all variables except RNA:DNA: *datapoint*, the mean; *box* standard error (SE); *bars* 95% confidence intervals (CI). For RNA:DNA: *datapoint*, median; *box* quartiles; *bars* non-outlier range. CS and LDH expressed as micromoles substrate converted per gram per minute

while ice and edge stations were similar (Fig. 2B). %Protein was highest at open-water stations; ice-covered and edge stations were similar (Fig. 2C). Liver lipid levels were lowest at ice-covered stations; edge and open-water stations were higher, and similar to one another (Fig. 2D). Intestinal lipid levels were highest at open-water stations; ice-covered and edge stations were lower, and similar to one another (Fig. 2E). RNA:DNA was lower at ice-covered than open-water stations, but both were similar to edge stations (Fig. 2F). ANOVA suggested that significant variation occurred in CS. However, post hoc analysis by Tukey's HSD failed to indicate that any stations differed significantly. Analysis by the less rigorous Duncan's technique suggested that ice-covered stations had the lowest CS activity, and that edge and open-water stations were similar, but these findings should be viewed cautiously (Fig. 2G). LDH was highest at open-water stations, and similar at ice-covered and marginal ice-zone stations (Fig. 2H). Intestine mass (WM_i) (Fig. 2I) and stomach mass (WM_s) (Fig. 2J) were both highest at open-water stations, and similar at ice-covered and edge stations. Wet mass, gonad mass, liver mass, adipose mass, and %Lipid in the muscle did not vary between zones. In summary, of the variables which changed across the three zones, eight increased towards open water (%Protein, L_l, L_i, RNA:DNA, CS, LDH, WM_i, WM_s), while two decreased (%H_2O, %Ash). Interactive effects between size and zone were observed in three variables (%H_2O, WM_i, and WM_s). Percent H_2O changed greatly between zones in the smallest fishes, but less so in larger fishes (Fig. 3A). In all cases water level dropped from ice-covered to open-water stations. The intestine mass (Fig. 3B) and stomach mass (Fig. 3C) increased slightly for any given size from ice-covered to open-water stations (most of the change appeared to occur between ice-edge and open water). This change was larger in larger fish.

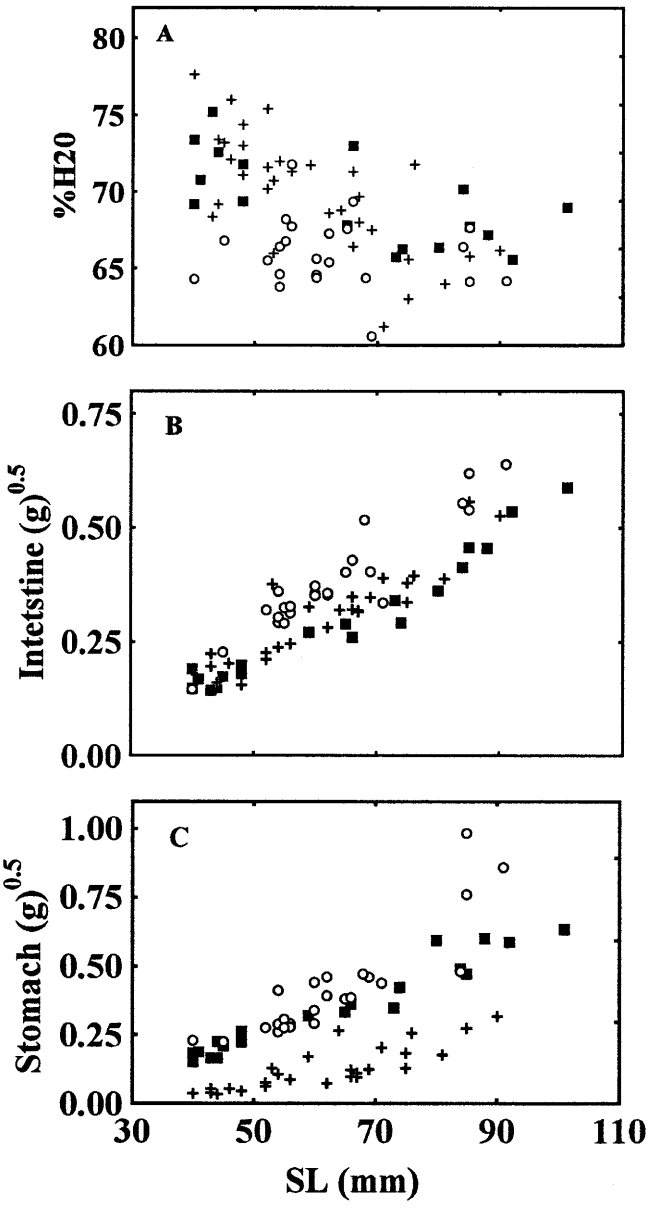

Fig. 3A–C *Electrona antarctica*. Interactive effects of: **A** size (SL in mm) on %H_2O, **B** intestine wet mass (g), and **C** stomach wet mass (g) (*open circles* open-water stations; +, ice-edge stations; *solid squares* ice-covered stations)

Diet analyses

An average of 6.2 items per fish was observed, not including fish scales which we assumed were ingested in the net. Only four fish ingested scales, but one individual consumed 25 scales. Copepods were found in 23 of the 32 *E. antarctica* dissected, and were the numerically dominant diet component, comprising 107 of the 198 diet items observed. Unidentifiable remains were observed in almost every fish; 27 items appeared to be remains of crustaceans and 24 items were either remains of gelatinous organisms or soft tissue from higher organisms. Eighteen euphausiids were observed in nine fish. Other diet items included amphipods (6 in 5 fish), ostracods (6 in 3 fish), salps (3 in 3 fish), pteropods (3 in 2 fish), eggs (2 in 2 fish), and diatoms (2 in 1 fish). No attempt was made to estimate biomass, but amphipods, euphausiids, and salps obviously have much greater mass than a similar number of copepods. Crustacea clearly dominated the diet of *E. antarctica* during this sample period.

No component of diet varied between sexes (Table 2), so all samples were pooled for further analyses. No component of diet correlated with SL (Table 2). Only adipose varied between zones. Adipose tissue was most abundant in samples collected at open-water stations, while ice-covered and edge stations were similar.

Analyses of *Bathylagus antarcticus*

A total of 175 specimens of *B. antarcticus* were collected, ranging in size from 8 to 160 mm. Biochemical assays were conducted on 51 of the samples (all available frozen samples), and diet analysis was conducted on 33. Five of

Table 2 *Electrona antarctica* and *Bathylagus antarcticus*. Statistical analyses of diet. Columns include variable, sample size (*n*), *t*-test (*t*) (for comparison between sexes in *E. antarctica*), correlation coefficient (*r*) between the variable and SL, and comparisons across zones by ANOVA (*F*). Transformations and significance values, as in Table 1. The number of diet items (*n*) for gelatinous organisms (*G.O.*), crustaceans, or other items was tested based on raw counts. Adipose and stomach fullness were based on arbitrary scales, and volume refers to the volume of diet items

Variable	*Electrona antarctica*					*Bathylagus antarcticus*			
	n	Sex	SL	Zone		*n*	SL	Zone	
		t	R	TR	*F*		*r*	TR	*F*
Adipose	28	0.059	0.044	–	8.87^{***}	33	0.017	–	7.62^{**}
G.O. (*n*)	28	1.911	0.354	–	1.06	33	0.234	–	0.72
Crustacean (*n*)	28	0.464	0.037	L	0.78	33	0.232	–	2.73
Other (*n*)	28	0.286	0.269	I	0.50	33	0.556^{**}	–	0.44
Stomach fullness	28	0.043	0.260	–	0.45	33	0.593^{***}	H	4.66
Volume	28	0.022	0.157	–	0.16	33	0.473^{**}	H	0.78

$^{**}P < 0.01$, $^{***}P < 0.001$

the *B. antarcticus* collected were still in a larval form, ranging in size from 8 to 12 mm; all were collected in the ice-covered stations. Larvae were not utilized in any assay. The remainder of the preserved specimens was retained for future studies.

Biochemical measurements

Ten of the 12 variables correlated with SL (Table 1); for these variables SL was retained as a covariate in further statistical analyses. No variation in %Ash and %Lipid in the muscle with SL was observed. Log transformations were used to improve the relation between measures of mass and SL (gonad, liver, intestine, and stomach), while total wet mass was transformed by square root.

Three of the 12 biochemical measures varied with zone (Table 1). Ash (%dry mass) was higher at ice-edge stations than ice-covered stations, other comparisons were not significant (Fig. 4A). Lipid (% wet mass of muscle) was higher at ice-covered stations than open-water stations, while other comparisons were not significant (Fig. 4B). RNA:DNA was highest at open-water stations; ice-edge and ice-covered stations were similar (Fig. 4C). Protein was not significantly different between zones, but with a *P*-value of 0.051, the data suggest that increased sample size would show that differences were present.

Interactive effects between size and zone were observed in one variable in *B. antarcticus*, RNA:DNA ratio. There was little effect of size on RNA:DNA in ice-covered or ice-edge stations, but *B. antarcticus* showed a large size dependence in open-water stations (Fig. 5). The increase in condition was more pronounced in larger individuals.

Diet analysis

An average of 12.6 items per fish was observed, not including fish scales which we assumed were ingested in the net. Only five fish ingested scales, ranging from two to six scales per individual. Copepods were the numerically dominant diet component. They were found in 27 of the 33 fish dissected, comprising 213 of the 416 diet items observed. Unidentifiable remains were observed in almost every fish; 31 items appeared to be remains of crustaceans, and 23 items were either remains of gelatinous organisms or soft tissue from higher organisms. A total of 109 unidentified gelatinous organisms and salps were observed in 25 fish. Other diet items included euphausiids (14 in 9 fish), ostracods (11 in 7 fish), amphipods (7 in 6 fish), eggs (3 in 2 fish), pteropods (2 in 2 fish), chaetognaths (2 in 1 fish), and a diatom (1). No attempt was made to estimate biomass, but the gelatinous component was often quite large, and salps have a much greater mass than a similar number of copepods. The diet of *B. antarcticus* appeared to be balanced between soft-bodied organisms and crustaceans during this sample period.

Three of the six measures of diet analysis correlated with SL (Table 2). For these variables, SL was retained in the analysis as a covariate. Two of the six variables varied significantly across the three zones. Adipose tissue was highest at ice-covered stations, with edge and open-water stations being similar (Fig. 4D) (identical to results for muscle lipid). Estimated food volume was highest at open-water stations, intermediate at edge stations, and lowest at ice-covered stations (Fig. 4E). All three stations were significantly different. Most of this difference was due to changes in the size of the gelatinous organisms consumed.

Discussion

The spring bloom associated with the receding ice-edge produced strikingly different responses in *Electrona antarctica* and *Bathylagus antarcticus*. *E. antarctica* showed marked increases in most measures of condition from ice-covered stations through the ice-edge and out

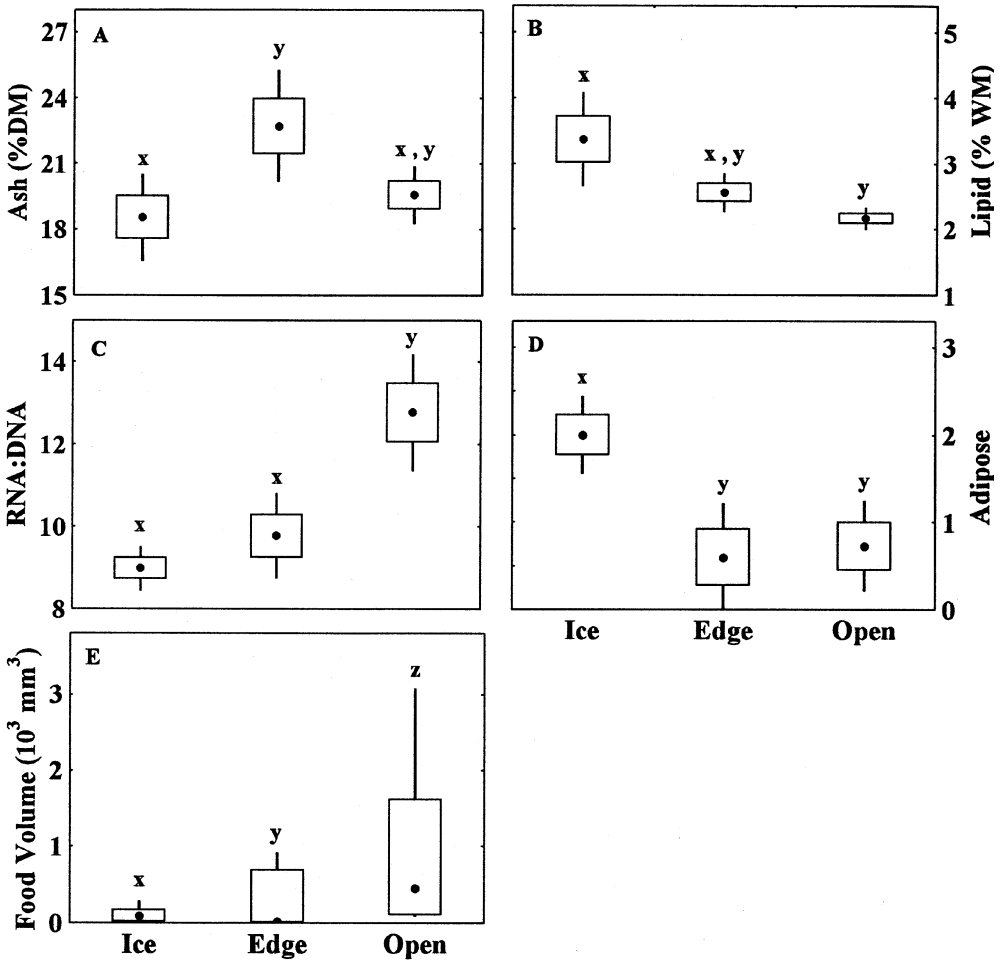

Fig. 4A–E *Bathylagus antarcticus*. Variation in five measures of condition. For all variables except food volume the symbols are as follows: *symbol* mean; *box* SE; *bars* 95% CI. For food volume: symbol, median; box, quartiles; bars, non-outlier range. Ash (**A**), lipid (**B**), and RNA:DNA (**C**) are from frozen samples. Adipose (**D**) and food volume (**E**) from preserved samples. Adipose estimated on a scale of zero to five

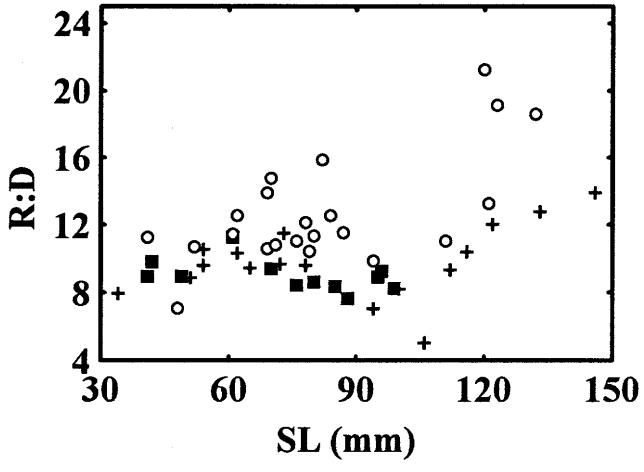

Fig. 5 *Bathylagus antarcticus*. Interactive effects of SL (mm) on RNA:DNA (*R:D*) across three zones (*open circles* open-water stations; +, ice-edge stations; *solid squares* ice-covered stations)

into open-water stations. *B. antarcticus* showed non-significant changes in most measures, with significant decreases towards open water in some measures, most notably muscle lipid.

Cofactors

The sex of the specimen affected only 2 of the 21 variables studied in *E. antarctica*. No sex determinations were made in *B. antarcticus*, and this factor was ignored. In both cases, there were no obvious asymmetries, although Greely et al. (1999) reported that female *E. antarctica* grew slightly larger than males. For the purposes of this study, the differences warranted attention, but the proportion of the variability related to sex in *E. antarctica* was easily accounted for statistically, and did not alter the more important findings about condition as it related to zonal differences.

Size, on the other hand, influenced many results. In *E. antarctica*, 10 of the 21 variables were affected, and in *B. antarcticus*, 13 of the 18 variables measured were affected. In all cases, the factor was retained in further analysis. There was little interactive effect on condition measures between zones, and the patterns observed are easily explained. In the case of *E. antarctica*, the greater magnitude of change in $\%H_2O$ in smaller fishes indicates that younger fishes are more greatly affected by the seasonal change in food supply. Larger individuals are more insulated from variation in food supply, possibly due to energy reserves in somatic lipid or adipose tissue,

both of which increased with increasing SL. The greater relative increase in the mass of the stomach and intestine in larger *E. antarctica* suggests that they respond to the increase in food supply more quickly than smaller fish (i.e. there was more food in the digestive tract). In addition, larger individuals may have been accumulating energy reserves in the abdominal cavity, especially adipose and gonadal tissues, while smaller individuals directed available energy towards somatic growth.

In *B. antarcticus*, RNA:DNA increased in all sizes, but the increase was greater in larger fishes. This may be related to the increase in gelatinous items in their diet, which are not utilized by smaller individuals (Hopkins and Torres 1989). Metabolic enzymes and nucleic acids commonly vary with size in fishes. LDH increased with size (units g^{-1} wet mass min^{-1}) in sablefish (*Anoplopoma fimbria*: Anoplopomatidae) (Sullivan and Somero 1983) and in *Sebastolobus* sp. (Scorpaenidae), while CS declined with increasing size (Siebenaller and Somero 1982). RNA:DNA commonly shows size dependence (Buckley 1982; Mathers et al. 1993; Brightman et al. 1997; Geiger 1999). In summary, the variability related to changes in size of the organism was easily accounted for statistically by treating size as a covariate when necessary. Through analysis of covariance we could focus on the more important issue of changes in condition related to the progression of the bloom through the three sampling zones.

Electrona antarctica – biochemical measures

E. antarctica has been shown to have large variations in lipid levels over the course of a year, from 9% of wet mass in the spring to 14% during mid-winter (Donnelly et al. 1990). These lipid stores are largely concentrated subcutaneously and intramuscularly (Reinhardt and Van Vleet 1986). Other body components exhibited less seasonal variation; water, ash, and protein did not change significantly (Donnelly et al. 1990). In the current study similar or greater variation than found in Donnelly et al. (1990) was observed in most variables over a period of weeks, rather than months, underscoring the importance of the spring bloom in the life of *E. antarctica*.

Some of the variation in body composition was probably related to reproduction. Summer spawning is usual in myctophids; *Electrona carlsbergi* spawned in summer and fall (Mazhirina 1991) and, of 47 species of tropical myctophids studied, most spawned in spring or early summer (Clarke 1973). *Electrona* larvae have been collected in summer (Efremenko 1985; Sinque et al. 1986), and the presence of small larvae in open-water stations during the present study shows that some spawning occurred in locations where the bloom was past its maximum and was proceeding ice-ward. This timing most likely allows the larvae to take advantage of the abundant supply of early life stages of copepods present during summer months (Schnack-Schiel and Hagen 1994; Burghart et al. 1999). However, the presence of eggs, larvae, and postlarvae in deeper waters during fall and winter (Efremenko 1985) suggests that spawning is protracted, and not completely synchronized with the spring bloom. Greely et al. (1999), using back-calculated hatching dates estimated from otoliths, made similar conclusions for *E. antarctica*. Undoubtedly, lipid reserves accumulated during the bloom play an important role in reproductive output.

The observation that greater variation in protein, water, and ash occurred over a time span of weeks during the 1–2 month spring bloom than throughout the rest of the year is very interesting. *E. antarctica* was replacing its body water with protein and lipid, an obvious sign of recovery from a period of fasting. Reinhardt and Van Vleet (1986) found that a large proportion of the lipid in *E. antarctica* was wax ester, which is commonly used for long-term storage to buffer periods of reduced food supply. It is possible that the entire population undergoes a rapid increase in nutritional condition during the spring, and then individual members of the population utilized those energy stores at more variable rates. Some individuals spawned immediately and apparently utilized a large pool of energy reserves, as evidenced from the presence of larvae. Others probably continued to build reserves throughout the year, particularly those that were not yet mature. *E. antarctica*'s mode of reproduction is undescribed. However, whether it is single batch or continuous wave oogenesis (Gartner 1990), it would greatly affect the seasonal variation in body composition. A prolonged spawning season by the population (but not necessarily individuals) might account for the reduced seasonal variation observed in previous studies (Donnelly et al. 1990).

Electrona antarctica – diet analysis

E. antarctica eat a broad range of zooplankton, especially larger copepods, euphausiids, and larvaceans (Hopkins and Torres 1989; Hopkins et al. 1993). Tropical and temperate myctophids exhibited similar diets. *Notoscopelus kroeyerii* (Myctophidae) in the North Atlantic ate mostly copepods, hyperiids, and euphausiids (Filin 1995). Of ten species of tropical myctophids, the main prey was either copepods (8 of 10), euphausiids (1), or ostracods (1); stomach fullness averaged 33% (Clarke 1978). In *Diaphus taaningi* (Myctophidae) from the Caribbean, 70% of the diet was composed of copepods and larvaceans (Baird et al. 1975). *E. antarctica* had initiated a period of intense feeding in our ice-covered stations. Seasonal variation in diet composition has not been observed in previous studies (Hopkins and Torres 1989; Lancraft et al. 1991; Hopkins et al. 1993) so there was little surprise that no variation occurred between the three sample regions described in the present study. The large volume found in some stomachs from each zone and the results from condition analysis suggest that an

increase in feeding must have been initiated at deep ice stations.

Bathylagus antarcticus – biochemical measures

The lack of change in condition in *B. antarcticus* stands in stark contrast to the marked changes observed in *E. antarctica*. Lipid and adipose exhibited decreases from high values in ice-covered stations to lower values in open-water stations. Only RNA:DNA and food volume increased according to expectations. One probable cause is that *B. antarcticus* appears to have been spawning upon our arrival in the deep ice, producing larvae coincident with the spring bloom. If the gonads were spent, that would explain the difficulty in sexing many individuals based on gross morphology of the gonads. Future study of these samples on a microscopic histological level will be conducted. Eggs from *B. antarcticus* have been collected from December through April in the region of the Weddell-Scotia confluence (Yefremenko 1982) and larvae have been collected from August through May (Efremenko 1982). Evidence from northern hemisphere bathylagids supports the hypothesis of a winter/spring spawning. Of five species of Bathylagidae from the waters off central Japan, all spawning peaked in winter (Miya 1994; Sano et al. 1995). *B. ochotensis* spawned mostly in January and February (Miya 1995). *Leuroglossus schmidti* (Bathylagidae) spawning peaked in December, but it also had a protracted season, lasting from October through December (Sobolevski and Sokolovskaya 1994). Finally, Dunn (1983) found protracted winter spawning in *Leuroglossus* spp. collected in the northern Atlantic Ocean. Thus, one might predict that the family generally spawns just prior to or in conjunction with the spring bloom in temperate and higher latitude habitats. Collections of tropical bathylagids are too poor to address seasonality in spawning (Bigelow et al. 1964).

B. antarcticus was shown to store lipid in muscle tissue (Reinhardt and Van Vleet 1986). The lipids were predominantly triglycerides and phospholipids, which are indicative of short-term energy use. We observed shifts in the abundance of lipid in both muscle and in internal adipose deposits. Surprisingly, these two values were high under the ice and decreased in open-water stations. A possible explanation is that fish were rapidly transferring calories into reproductive output. No evidence of winter dietary limitations occurred in *B. antarcticus* in a study of the winter community under the ice (Lancraft et al. 1991). If starvation were occurring, one would have expected lipid stores to already be depleted, not to undergo a rapid lipid loss during the peak of the bloom.

Bathylagus antarcticus – diet analysis

Evidence from diet analysis was possibly more telling in *B. antarcticus* than *E. antarctica*. Although the number of items in *B. antarcticus* did not change between zones, there was an astounding increase in the estimated volume of food items. This change, largely attributed to *Salpa thompsoni*, may have indicated the true onset of a seasonal pulse of food for *B. antarcticus*. Throughout the year *B. antarcticus* eat a broad range of zooplankton, especially smaller copepods, ostracods, and coelenterates, with larvaceans appearing in diets of larger animals (Hopkins and Torres 1989; Lancraft et al. 1991; Hopkins et al. 1993).

Although bathylagids have a diverse diet, soft-bodied organisms are a preferred prey (Mauchline and Gordon 1983; Caillet and Ebeling 1990). Evidence for this in *B. antarcticus* is the observation that although full guts were observed in all regions, the volume peaked in open water where salps were more abundant. Concomitantly, RNA:DNA, previously shown to be an excellent predictor of condition in fishes (Goolish et al. 1984; Black and Love 1986; Mathers et al. 1992), underwent similar peaks in open-water stations. The RNA:DNA value changes seem to indicate that the sample period coincided with the initiation of a growth phase in this species. Fish may have a several day lag time in initiation of compensatory growth after fasting (McLaughlin et al. 1995), but the parallel tracks of RNA:DNA and food volume offer compelling evidence of a very tight coupling of the indicator to diet. Lied et al. (1983) found that RNA:DNA could return to normal levels within 8 h upon refeeding after starvation in *Gadus morhua* (Gadidiae) maintained at 8 °C, lending support to this hypothesis.

Measures of condition

Each of the measures of condition chosen here has been utilized previously, but rarely in tandem. In most cases, single assays have been conducted without direct comparison between measures of condition. This is partly due to the fact that most research is focused on the early life history of fishes, when the volume of tissue available for assay is limited. Studies on adult fishes often focus on food quality in fishes raised in aquaculture facilities, and those results are often difficult to compare with studies of nutritional condition in field-caught specimens. Also, many theories of fish recruitment assume that food limitation is limited to larval stages. In addition to being an excellent environmental laboratory, the location of the current study in the Southern Ocean allowed comparison between two species that respond to increasing food supply very differently. RNA:DNA was the most sensitive indicator of condition. The differences in this ratio between zones were large in *E. antarctica*, and it was the only biochemical measure in *B. antarcticus* to respond to increased food intake. RNA:DNA values were much higher than those reported for temperate or tropical species, a finding which was anticipated from previous research indicating that RNA:DNA increases in response to decreased temperature within species (Buckley 1984; Mathers et al. 1993;

Brightman et al. 1997). More notable than absolute values, the relative changes between zones are clear indicators of rising condition in both species as the spring bloom progressed. Also, RNA:DNA has been shown to be more sensitive to feeding than aerobic enzymes (Foster et al. 1993). The theory behind this observation is that RNA levels must increase before enzyme levels increase, because RNA is used to synthesize enzymes. There was no evidence to indicate otherwise in this study, from either species. The increase in RNA:DNA in *E. antarctica* was more pronounced than that in enzyme or protein levels, and appeared to precede them. Increases in RNA:DNA clearly preceded any other indicator in *B. antarcticus*.

The time scales observed for changes in the present study agree well with previous reports. RNA:DNA was found to be a good indicator of growth integrated over about 1 week in *Pomoxis nigromaculatus* (crappies: Centrarchidae) (Haines 1980), but McLaughlin et al. (1995) found that in *Salvelinus fontinalis* (brook trout: Salmonidae) RNA:DNA response to fluctuating food levels required as long as 10–14 days. The levels of RNA:DNA responded in less than a day in larval fish (Wright and Martin 1985) and within 1–2 weeks in adult *Salvelinus alpinus* (arctic charr) (Miglavs and Jobling 1989). Metabolic enzymes respond in larvae over time scales of one to a few days in many fish larvae (Clarke et al. 1992; Segner and Verreth 1995), but trout fry required up to 65 days for differences to be detectable (Mathers et al. 1993). In saithe, *Pollachius virens* (Gadidae), held at 8–12 °C, metabolic enzymes (CS, LDH, CO) responded within 2 weeks to varying food rations (Mathers et al. 1992). Sullivan and Somero (1983) found that aerobic enzyme levels did not change at all during 24 weeks of starvation in *Anoplopoma fimbria*, but that glycolytic enzyme levels were directly related to ration. In the current study, changes in both aerobic and anaerobic enzymes occurred in *E. antarctica*, while neither changed in *B. antarcticus*. Clearly, there are differences among species, but RNA:DNA appears to be a more universal indicator of condition than metabolic enzymes in adult fishes.

Protein levels in Antarctic fishes have been shown to decline within 5 days of the initiation of starvation (Smith and Haschemayer 1980), and lipid levels are known to be mobilized more rapidly than protein (Sullivan and Somero 1983). Tissue water also increased during starvation in coldwater fishes (Moon and Johnston 1980). All three measures of proximate composition were observed to change in this study. The time scales required to induce the changes in *E. antarctica* and *B. antarcticus* were minimally 2 weeks. Measures of proximate composition, while providing important information regarding condition, are less sensitive to short-term changes than biochemical indicators.

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