Marine Biology 98, 169–180 (1988)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes

J.J. Torres[1] and G.N. Somero[2]

[1] **Department of Marine Science, University of South Florida, 140 Seventh Avenue South, St. Petersburg, Florida 33701, USA**
[2] **Marine Biology Research Division, A-002, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA**

Abstract

Several species of Antarctic mesopelagic fishes that have different minimal depths of occurrence but the same environmental temperature were collected in November–December 1983 and in March 1986 between 0 and 1 000 m in the open water near the marginal ice zone in the vicinity of 60°S 40°W (1983) and 65°S 46°W (1986), and oxygen consumption rate (V_{O_2}) and the activity of two metabolic enzymes, lactate dehydrogenase (LDH, an indicator of the anaerobic potential of locomotory muscle) and citrate synthase (CS, an indicator of citric acid cycle activity or aerobic potential), were determined. In four dominant species, whole-individual oxygen-consumption rate (y, ml O_2 individual^{-1} h^{-1}) varied with weight (X, g) according to the equation $y=aX^b$, with b values falling between 0.889 and 1.029. The relation of weight-specific LDH activity (y, U g^{-1} wet wt) with weight (x, g) was also described by the equation $y=aX^b$, with b values varying between 0.229 and 1.025. Weight-specific CS activity declined with weight, with b values from −0.031 to −0.369. V_{O_2}, LDH activity and CS activity all declined markedly with increased species' minimum depth of occurrence (the depth below which 90% of a species' population lives). Comparisons with previous studies on ecologically equivalent species of the California Borderland indicate that depth-related decreases in metabolism are the result of adapted traits of deeper-living species, not declining temperature within the water column. The metabolic rate of Antarctic mesopelagic fishes is approximately twice that of California species at equivalent temperatures; similar rates were found at the normal habitat temperatures of the two groups. Thus, a well-developed compensation for temperature is present in the Antarctic fishes: cold adaptation. Differences in enzymic activity among species, and among different sized individuals of a species are related to differences in metabolic rate and locomotory capacity. Enzymic indices can be used to estimate metabolic rates and evaluate ecological parameters such as predatory strategies and niche separation.

Introduction

The Antarctic pelagial has several important characteristics. Sea-surface temperatures are uniformly cold, varying seasonally from −1.87 °C to 0 °C near the continent, and −1° to 4 °C nearer the Antarctic Convergence (Gordon et al. 1982). The remainder of the water column is nearly isothermal; temperatures change less than 2 C° between 100 and 1 000 m. Within the area bounded on the north by the Antarctic Convergence and on the south by the Antarctic continent, ice expands to cover 50 to 60% of the sea surface in winter, contracting to less than 10% cover in summer (Zwally et al. 1983). Daylength differs widely through the year, varying from 0 to 24 h. The Circum-Antarctic current and Antarctic convergence isolate the Antarctic pelagic system, producing a high degree of endemism among its pelagic biota (McGinnis 1977).

Despite these characters, the mesopelagic fishes of the Antarctic are all members of widely distributed midwater-fish families (Andriashev 1965, DeWitt 1970). They occupy similar niches to their more northern counterparts, and are usually in the same family and sometimes in the same genus. Thus, zoogeographic comparisons do not cut across wide phylogenetic boundaries. Antarctic midwater fishes provide a system for investigating adaptation to cold temperature and to the deep sea in general.

Metabolic rate decreases with increasing depth of occurrence in temperate-water pelagic species (Smith and Hessler 1974, Childress 1975, Childress and Somero 1979, Torres et al. 1979, Smith and Laver 1981). However, it has been difficult to distinguish the effects of decreasing temperature with increasing depth from other depth-related effects which select for lower metabolic rates. The nearly isothermal water column of the Antarctic allows

direct comparison of shallow- and deep-living species that live at the same temperature, i.e., the effects of increasing depth of occurrence can be distinguished from any depth-related effects of temperature.

We examined the oxygen consumption rates of several Antarctic mesopelagic fishes and the activities of two metabolic enzymes: lactate dehydrogenase (LDH), an indicator of the capacity of locomotory muscle for generating ATP via anaerobic glycolysis, and citrate synthase (CS), an indicator of citric acid cycle (aerobic) activity. The enzymic activity measurements were performed to complement the whole-organism respiratory study by providing additional information on the fishes' capacities for ATP production and for powerful locomotory activity. The purpose of this study was to determine if the level of organismal and sub-cellular metabolism of mesopelagic fish from the Antarctic is an adaptation to low temperatures or to other characteristics of the fish and their environment.

Materials and methods

Collection of fishes

All pelagic fishes were collected using mouth-closing Tucker trawls equipped with either blind or thermal-turbulence protecting cod-ends (Childress et al. 1978) in November–December 1983 and in March of 1986. Fishes were taken between 0 and 1 000 m in the open water near the marginal ice zone, in the vicinity of 60°S 40°W in 1983 and 65°S 46°W in 1986.

The entire catch from each haul was transferred into seawater at approximately 0 °C, and the most active fishes were selected for determination of oxygen consumption rate. Those individuals were placed in cylindrical tanks (28 liters) and held in a temperature-controlled van (0° to 1 °C) for 1 to 2 h before use. All had suffered some mechanical damage during capture; only those that were lively and pristine in appearance were selected for study.

Fishes used for analysis of enzyme activity were frozen in a conventional (–20 °C) deep-freeze for the first three months after capture and for four months thereafter in a cryogenic (–80 °C) freezer.

Coastal species were captured in McMurdo Sound using either hook and line or baited traps (Wohlschlag 1964), frozen in a conventional deep freeze, and then held on dry ice. Activity losses over the period of storage are virtually non-existent (Childress and Somero 1979).

Respiratory measurements

Oxygen-consumption rates were determined by allowing individuals to deplete the oxygen in a sealed water-jacketed chamber filled with filtered (0.45 μm pore-size) seawater. Temperature was maintained at 0.5 °C ($\pm$ 0.1 C°) using a circulating refrigerated water-bath. Oxygen partial-pressure was continuously monitored using a Clark polarographic oxygen electrode (Clark 1956), as an individual reduced oxygen levels to intermediate ($\sim$ 80 mm Hg) partial-pressures. Electrodes were calibrated using air- and nitrogen-saturated seawater at the experimental temperature (Childress 1971). The time required for consumption of oxygen to intermediate levels varied from 4 to 8 h. Streptomycin and Neomycin (each 25 mg l^{-1}) were added to the seawater to minimize microbial growth. To control for possible oxygen consumption by microorganisms, an individual was removed after selected runs, its volume was replaced with fresh seawater, and oxygen consumption was again measured for 2 to 4 h. In all cases microbial oxygen consumption was negligibly low.

The rectangular chambers were constructed of lucite and contained a perforated lucite false-bottom that isolated the fish from a stirring bar. A low stirring speed was used to minimize disturbance. All experiments took place in the dark, with brief periods of observation in low light.

Data were recorded using a computer-controlled digital data-logging system. Each oxygen probe was scanned once per minute, its signal averaged over a period of 1 s, and then recorded. Data were reduced by first averaging the 30 recorded values in each 30 min increment of an entire 4 to 8 h experiment, producing between 8 and 16 30-min points per run. Data obtained during the first hour were discarded due to the high activity of the fish after its introduction into the respirometer. All 30-min points thereafter, down to an oxygen partial-pressure (P_{O_2}) of 80 mm Hg, were averaged to produce a routine rate for each individual. Maximum rates were the maximum 30 min rate and minimum rates the minimum 30 min rate recorded for each individual after the first hour, but above a P_{O_2} of 80 mm Hg. Usually, maximum rates were associated with the beginning of an experiment and minimum rates during middle portions of a run.

Homogenate preparation

Fishes were dissected whilst frozen. The tissue remained frozen until it was placed into the homogenizing medium: ice-cold Tris/HCl buffer (10 m*M*, pH 7.5 at 10 °C). Muscle-tissue homogenates of all species except *Cyclothone microdon* were prepared by excising sections of white muscle from immediately behind the skull and above the midline of the body. The overlying skin and red muscle, if present, were removed before excision. For *C. microdon* the entire muscle mass was removed with otherwise identical treatment.

Tissues were homogenized by hand at 0° to 4 °C in conical glass homogenizers having ground-glass contact surfaces (Duall, Kontes Glass Corporation, Vineland, New Jersey). The homogenates were centrifuged at 2 500 $\times g$ for 10 min; the supernatant fractions were saved for the enzyme activity assays. The solution was drawn from beneath lipid layers when they were present on top of the centrifuged samples.

Table 1. Oxygen-consumption rates of Antarctic mesopelagic fishes. To use conversion factors multiply wet weight-specific rate by indicated value to yield dry weight- or ash-free dry weight (AFDW)-specific rates. Information on habit is from unpublished data on diet and vertical distribution. All rates reported as $\bar{x} \pm 95\%$ confidence intervals, CI

Species and (habit)	Min. depth occurrence (m)	(*n*)	Wet wt range (g)	Mean wt ±95% CI (g)	Oxygen-consumption rate at 0.5 °C (μl O_2 mg^{-1} wet wt h^{-1})			Conversion factors (μl O_2 mg^{-1} h^{-1})	
					routine	min.	max.	dr. wt	AFDW
Bathylagidae									
Bathylagus antarcticus (deep mesopelagic, lethargic)	400	(26)	1.3–53.0	10.4±4.9	0.018±0.003	0.007±0.002	0.028±0.008	7.776	9.840
Gonostomatidae									
Cyclothone microdon (deep mesopelagic, lethargic)	500	(3)	0.53–1.03	0.80±0.62	0.016±0.002	0.011±0.003	0.027±0.027	2.751	2.989
Macrouridae									
Cyanomacrurus piriei (benthopelagic as adults)	ND	(1)	1.8		0.016	0.012	0.020		
Melamphaidae									
Poromitra crassiceps (zooplanktivorous, mesopelagic)	ND	(1)	4.5		0.028	0.014	0.046		
Myctophidae									
Electrona antarctica (zooplanktivorous, mesopelagic migrator)	50	(47)	1.0–13.8	4.6±0.8	0.042±0.003	0.022±0.004	0.069±0.006	3.006	3.310
Gymnoscopelus braueri (zooplanktivorous mesopelagic migrator)	150	(18)	1.5–21.3	12.2±2.7	0.026±0.003	0.016±0.002	0.043±0.006	2.957	3.215
Gymnoscopelus opisthopterus (zooplanktivorous, mesopelagic migrator)	150	(15)	7.1–40.0	19.2±5.8	0.022±0.003	0.013±0.007	0.034±0.013	3.224	3.514
Scopelarchidae									
Benthalbella elongata (piscivorous, mesopelagic predator)	ND	(1)	35.3		0.037	0.023	0.052		
Zoarcidae									
Melanostigma gelatinosum (zooplanktivorous, mesopelagic, lethargic)	ND	(1)	47.2		0.021	0.012	0.055		

Enzyme activity assays

All enzymes were assayed in freshly-prepared supernatant fractions. Activity was measured at 10 °C ± 0.2 C° in a thermostated Varian-Techtron 635 spectrophotometer. Substrate and cofactor concentrations yielding maximum reaction velocities were used in all assays. Our values thus indicate the highest potential activity possible in the tissues (exclusive of the effects of enzyme modulators). All activities are expressed in units (μmol substrate converted to product min^{-1}) per gram wet weight of tissue.

L-lactate dehydrogenase (LDH, EC 1.1.1.27; oxidoreductase)

LDH activity was assayed in the pyruvate reductase direction, in a medium containing 80 m*M* Tris/HCl buffer (pH 7.5 at 10 °C), 150 μM NADH, 5 m*M* sodium pyruvate, and 100 m*M* KCl. Following the addition of a small aliquot of supernatant (generally 10 μl), the reaction was followed by recording the decrease in absorbance at 340 nm resulting from oxidation of NADH. The slope of the initial portion of the tracing was used as the reaction rate.

Citrate synthase (CS, EC 4.1.3.7; citrate: oxaloacetate-lyase (CoA-acetylating))

CS activity was assayed in a medium containing 50 m*M* imidazole/HCl buffer (pH 8.0 at 10 °C), 0.5 m*M* 5,5-dithiobis (2 nitrobenzoic acid) (DTNB) and 1.5 m*M* $MgCl_2$. The reaction was followed by recording the increase in absorbance at 412 nm due to the reaction of the reduced coenzyme A (liberated from the enzymic reaction)

Table 2. Activities of lactate dehydrogenase and citrate synthase in white skeletal muscle of Antarctic mesopelagic and coastal fishes. All determinations at 10 °C. Units are μmol substrate converted to product min^{-1}. Information on habit and depth distribution of Antarctic coastal species from DeVries and Lin (1977), DeVries and Eastman (1981) and Targett (1981)

Species and (habit)	Min. depth occurrence (m)	(*n*)	Wet wt range (g)	Mean wt ±95% CI (g)	LDH activity (U g^{-1} wet wt)		CS activity (U g^{-1} wet wt)	
					range	$\bar{x}$±95% CI	range	$\bar{x}$±95% CI
Bathydraconidae								
Gymnodraco acuticeps (shallow water, benthic)	30	(1)		58.7		57.0		0.53
Bathylagidae								
Bathylagus antarcticus	400	(20)	0.5 – 45.5	14.0±6.4	1.2 – 9.9	4.9±1.2	0.16 – 1.25	0.36±0.12
Gonostomatidae								
Cyclothone microdon	500	(8)	0.63 – 1.15	1.04±0.15	287 – 499	374±53.5	0.24 – 0.59	0.36±0.09
Myctophidae								
Electrona antarctica	50	(28)	0.4 – 14.2	4.5±1.5	4.8 – 187	50.1±20.6	1.25 – 3.52	2.21±0.22
Gymnoscopelus braueri	150	(19)	1.3 – 19.2	8.2±2.5	4.2 – 46.4	14.0±6.1	0.85±2.06	1.22±0.17
Gymnoscopelus opisthopterus	150	(16)	5.7 – 41.6	17.8±5.8	5.8 – 81.4	23.1±10.1	0.59 – 1.35	0.90±0.1
Nototheniidae								
Pagothenia borchgrevinki	0.6	(5)	27.1 – 122.1	61.0±51.0	87.0 – 98.0	93.6±10.3	1.84 – 2.57	2.26±0.37
Trematomus bernacchii (shallow and deep water, benthic)	30	(3)	103.0 – 197.5	147.5±118.0	81.0 – 136.0	106.3±68.3	1.32 – 1.79	1.60±0.62
Trematomus centronotus (shallow water, benthic)	30	(1)		106.0		80.0		1.44
Trematomus loenbergii (deep water, benthopelagic)	350	(1)		106.0		75.0		1.65
Paralepididae								
Notolepis coatsi (mesopelagic migrator)	100	(3)	0.27 – 0.38	0.32±0.15	9.0 – 31.1	21.4±28.1	0.91 – 1.29	1.10±0.14
Notolepis coatsi	100	(1)		23.2		325		0.94
Zoarcidae								
Rhigophila dearborni (deep water, benthic)	400	(2)	29.4 – 35.8	32.6±4.5 ($\bar{x}$±SD)	185.0 – 229.0	207.0±31.1 ($\bar{x}$±SD)	0.91 – 2.06	1.49±0.81 ($\bar{x}$±SD)

with DTNB. The rate of absorbance increase in the presence of enzyme (but in the absence of oxaloacetate) was first recorded, and the full reaction was then initiated by addition of oxaloacetate. The blank (no oxaloacetate) activity was less than 10% of CS activity, and was substracted from the total activity to compute true CS activity.

Wet, dry and ash-free dry weights

After the termination of each respiratory run, fishes were blotted dry, wrapped in heavy duty aluminum foil, and frozen. Fishes were subsequently thawed, weighed and then dried in a 60 °C oven to constant weight, and reweighed. Dried specimens were ashed in a muffle furnace at 450 °C to a constant weight to obtain the ash-free dry weight.

Statistical analysis

All regressions were calculated using the least-squares method on a programmable calculator. Analysis of covariance was computed as in Snedecor and Cochran (1967). Means or regression coefficients and 95% confidence limits (Student's *t*-test) are given unless stated otherwise.

Results

Routine oxygen-consumption rates ranged from 0.016± 0.002 μl O_2 mg^{-1} wet wt h^{-1} in *Cyclothone microdon*, to 0.042 ± 0.003 μl O_2 mg^{-1} wet wt h^{-1} in *Electrona antarctica*, (Table 1). Within species, oxygen consumption rate (V_{O_2}) varied by a factor of 2 to 4 between minimum and maximum rates. Four of the nine species shown in Table 1 were very rare in the study area, (e.g. only single individuals of *Poromitra crassiceps* and *Melanostigma gelatinosum* were captured), precluding replicate determinations of V_{O_2}. Their rates are reported for general comparison only.

Mean LDH activities ranged from a low of 4.9 ± 1.2 U g^{-1} wet wt in *Bathylagus antarcticus*, to a very high 374±

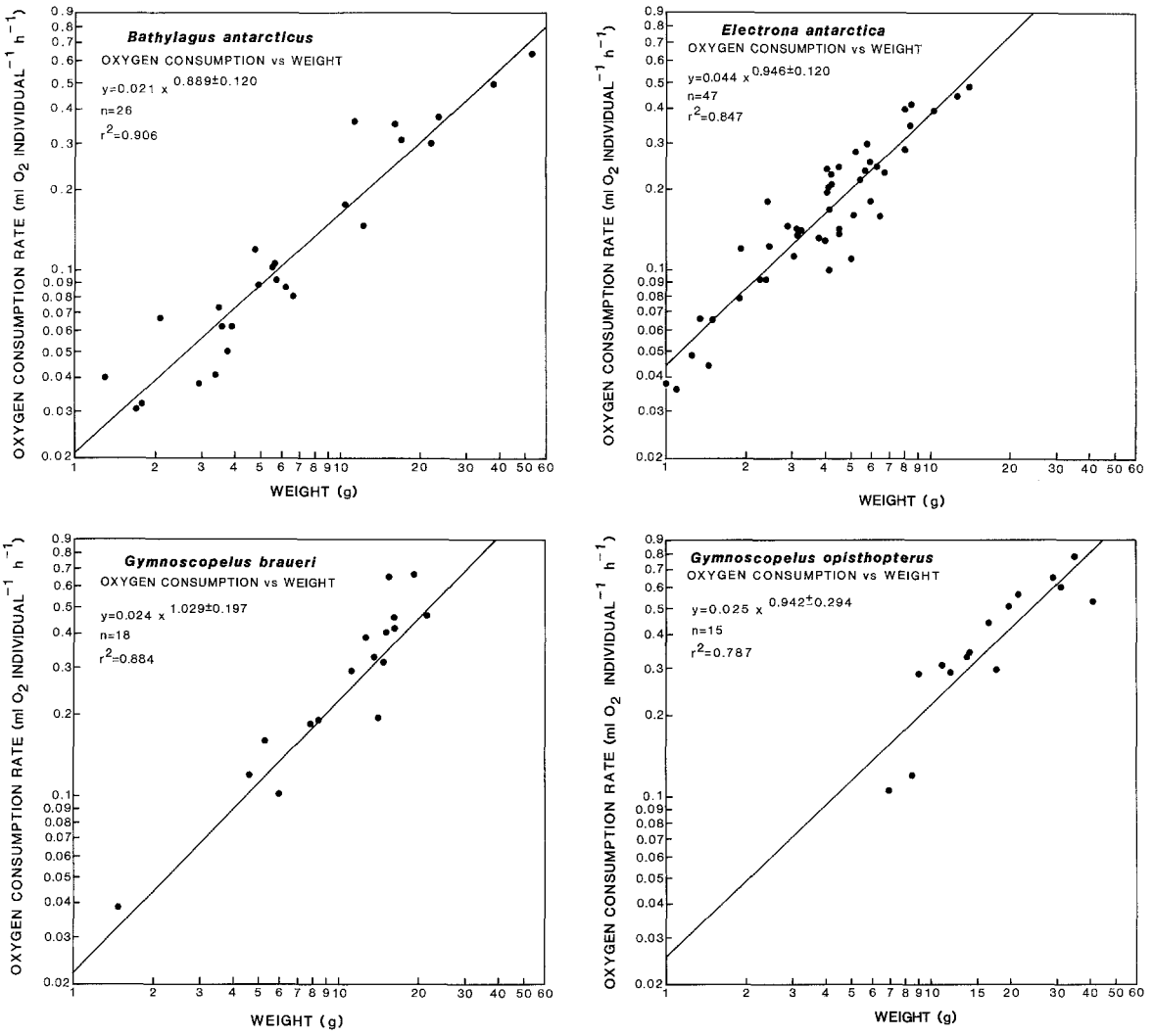

Fig. 1. *Bathylagus antarcticus, Electrona antarctica, Gymnoscopelus braueri* and *G. opisthopterus.* Whole-individual oxygen-consumption rate as function of total wet body weight in four species of Antarctic mesopelagic fishes. Each data point represents a routine-rate determination on an individual fish. Slopes reported as $b \pm 95\%$ confidence intervals, CI

53.5 U g^{-1} wet wt in *Cyclothone microdon* (Table 2). Except for *C. microdon,* LDH activities among species varied regularly with reported activity level; the most active fishes had the highest LDH activity (Table 2). Within species, LDH activity increased with increasing weight.

Mean CS activities varied from a low of 0.36 U g^{-1} wet wt observed in both *Bathylagus antarcticus* and *Cyclothone microdon* to a high of 2.26 ± 0.37 U g^{-1} wet wt in *Pagothenia borchgrevinki* (Table 2). Among species, CS activity varied with reported activity levels; within species, CS activity decreased with increasing weight.

Weight relationships

Oxygen consumption (M, ml O_2 individual^{-1} h^{-1}) varied with weight (W, g) according to the allometric equation $M = aW^b$ in those four species with sufficiently high n to examine the relation (Fig. 1, Table 3). Values for the slope, b, ranged from a low of 0.889 ± 0.120 in *Bathylagus antarcticus* to a high of 1.029 ± 0.197 in *Gymnoscopelus braueri,* indicating that metabolism scales in nearly direct proportion to weight. Weight-specific metabolism decreased slightly with increasing weight (Table 3) in three of the four species. In the fourth, *G. braueri,* weight-specific metabolism was nearly independent of weight (Table 3).

Weight-specific LDH activity (y, U g^{-1} wet wt) increased with increasing weight (X, g) in all four species examined, according to the equation $y = aX^b$ (Fig. 2, Table 3). Slopes (b values) ranged from 0.229 ± 0.221 in *Bathylagus antarcticus* to 1.025 ± 0.280 in *Gymnoscopelus opisthopterus.*

Weight-specific CS activity (y, U g^{-1} wet wt) decreased with increasing weight in the four species examined, according to the equation $y = aX^b$ (Fig. 3, Table 3). In this case, b values were negative and ranged from -0.031 ± 0.100 in *Electrona antarctica* to -0.369 ± 0.103 in *Bathylagus antarcticus.*

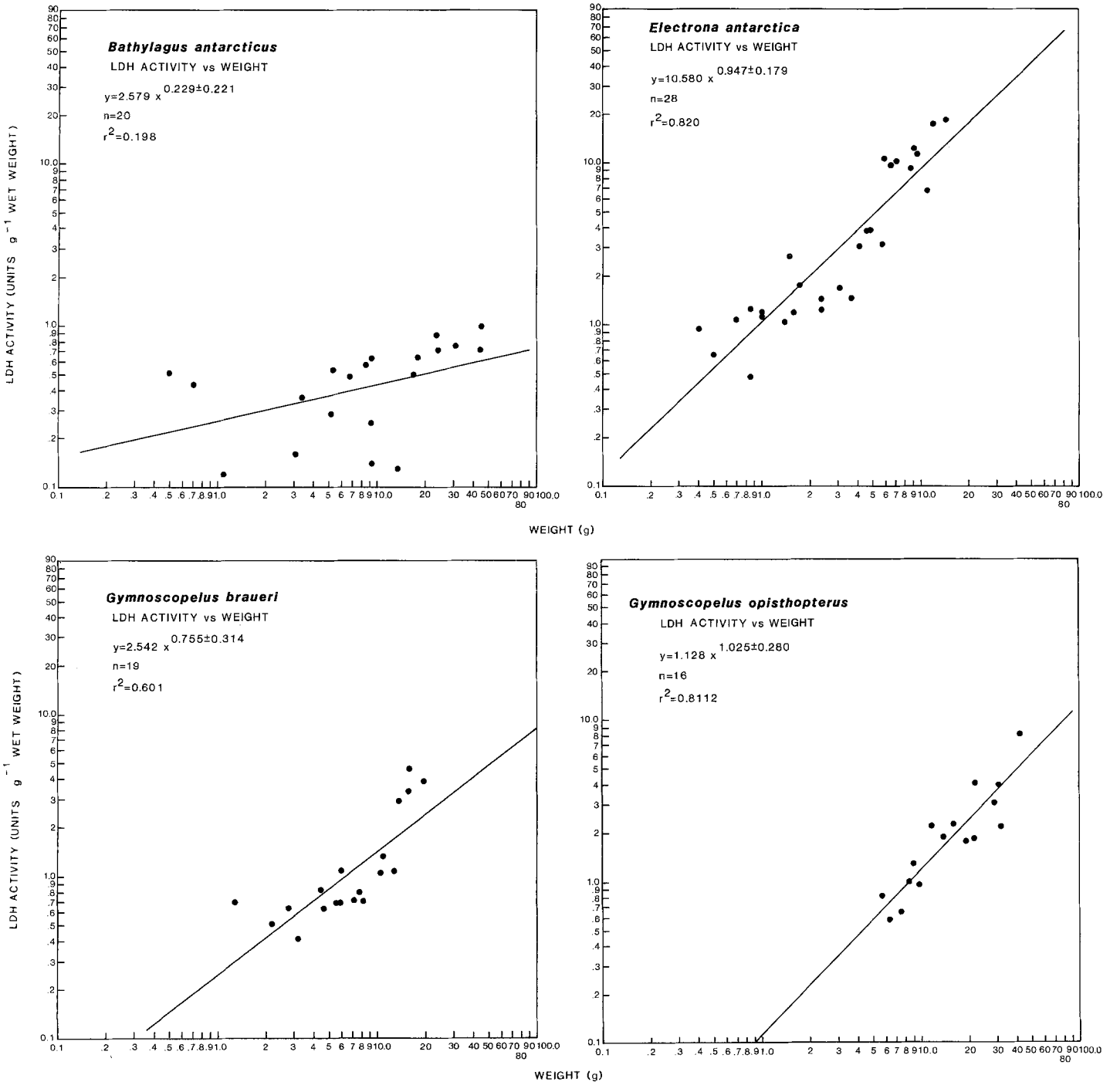

Fig. 2. *Bathylagus antarcticus, Electrona antarctica, Gymnoscopelus braueri* and *G. opisthopterus.* Lactate dehydrogenase activity in white skeletal muscle as function of total wet body weight in four species of Antarctic mesopelagic fishes. Each data point represents mean of two replicate determinations on an individual fish. Units of enzyme activity are in μmol substrate converted to product min^{-1}. Slopes reported as $b \pm 95\%$ CI

Table 3. Weight-scaling in VO_2, LDH and CS activity. Values are for equation $y = aX^b$, where x = wt (g) and y = ml O_2 individual h^{-1} (oxygen-consumption rate), μl O_2 mg^{-1} wet wt h^{-1} (weight-specific oxygen-consumption rate), or U g^{-1} wet wt (LDH, CS activity). Slopes reported as $b \pm 95\%$ CI

Species	Oxygen-consumption rate		Weight-specific oxygen-consumption rate		LDH activity		CS activity	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Bathylagus antarcticus	0.889±0.120	0.021	−0.111±0.120	0.021	0.229±0.221	2.579	−0.369±0.103	0.662
Electrona antarctica	0.946±0.120	0.044	−0.054±0.120	0.044	0.947±0.179	10.580	−0.031±0.100	2.126
Gymnoscopelus brauerí	1.029±0.197	0.024	0.029±0.197	0.024	0.755±0.314	2.542	−0.317±0.089	2.138
Gymnoscopelus opisthopterus	0.942±0.294	0.025	−0.058±0.294	0.025	1.025±0.280	1.128	−0.061±0.177	1.040

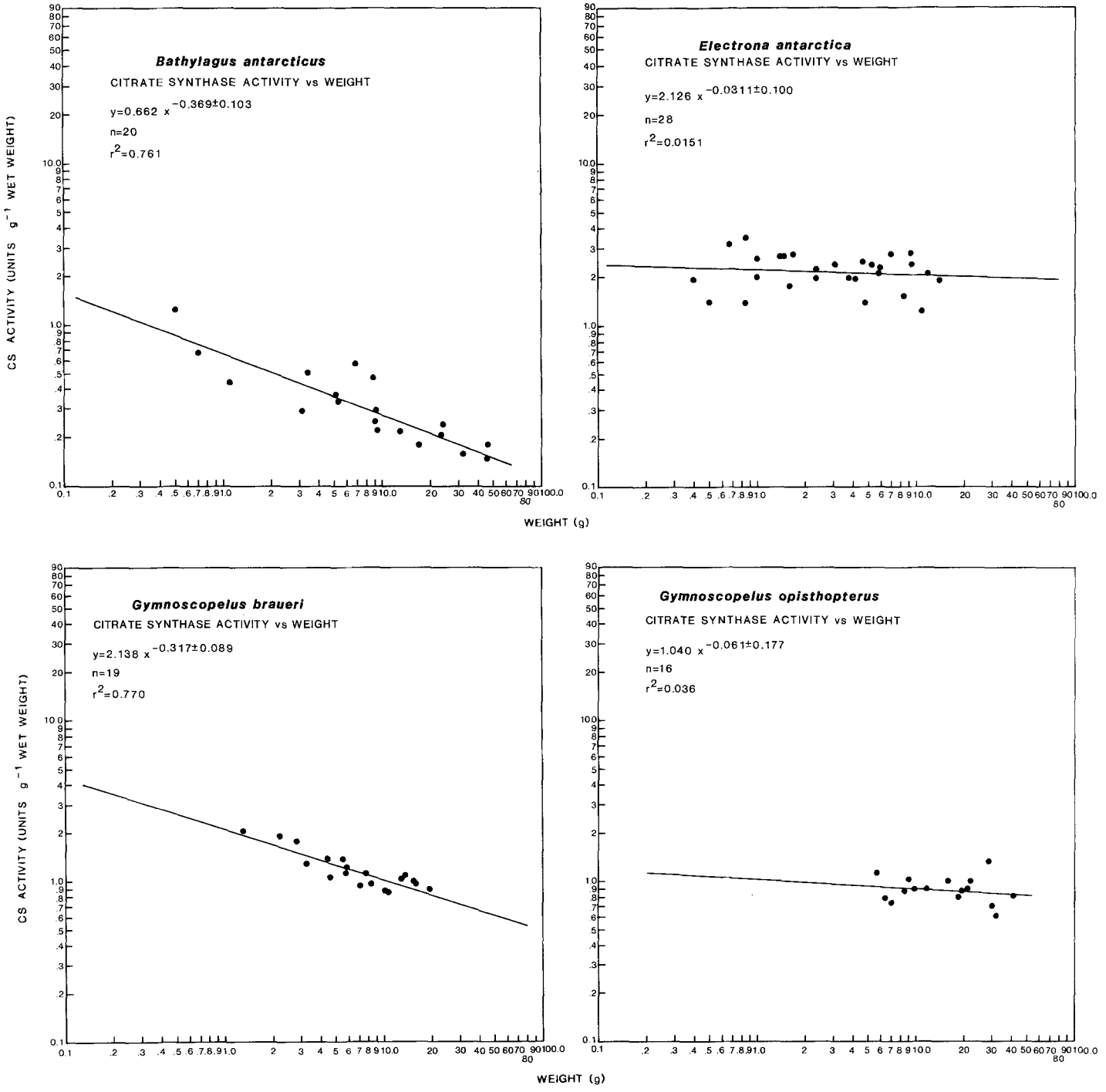

Fig. 3. *Bathylagus antarcticus, Electrona antarctica, Gymnoscopelus braueri* and *G. opisthopterus.* Citrate synthase activity in white skeletal muscle as function of total wet body weight in four species of Antarctic mesopelagic fishes. Each data point represents activity determination on an individual fish. Units of enzyme activity are in μmol substrate converted to product min^{-1}. Slopes reported as $b \pm 95\%$ CI

Depth relationships

Routine oxygen-consumption rate (y, μl O_2 mg^{-1} wet wt h^{-1}) declined with increasing species' minimum depth of occurrence (x, m) according to the relation $y = 0.162\ x^{-0.371 \pm 0.044}$ (Fig. 4). The curve for routine rate vs depth was bracketed by the curves for minimum rate ($y = 0.142\ x^{-0.445 \pm 0.414}$) and maximum rate ($y = 0.308\ x^{-0.402 \pm 0.219}$). Activities of LDH and CS declined with minimum depth of occurrence (Fig. 5).

Discussion

Weight relationships

Slopes (b values) relating oxygen consumption to weight in the four mesopelagic species examined fall within the range of b values reported for fishes ($b \cong 0.80$ for a wide range of freshwater fishes, Winberg 1956; $0.67 < b < 1.00$ for sockeye salmon, Brett 1965), Antarctic fishes (Wohlschlag 1960, 1963, 1964, Ralph and Everson 1968, Hem-

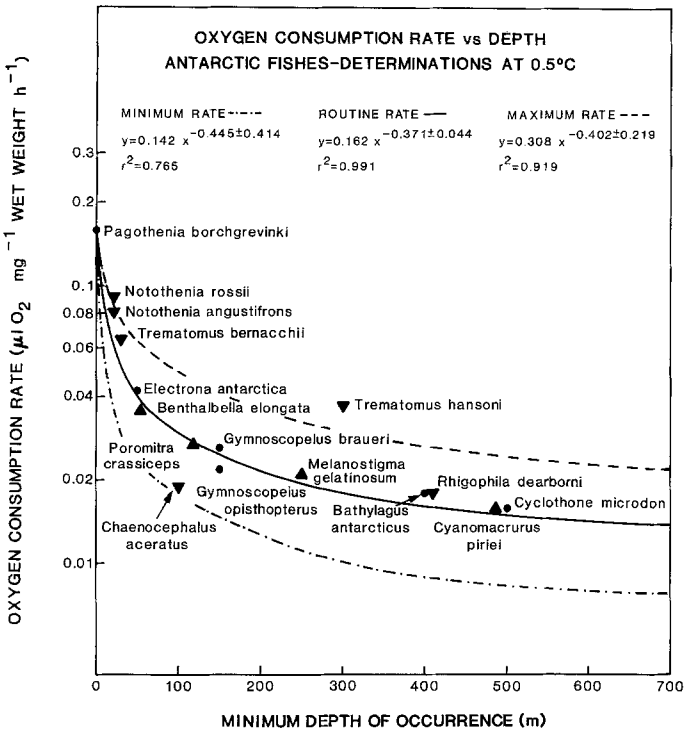

Fig. 4. Oxygen consumption rate of Antarctic mesopelagic fishes as function of minimum depth of occurrence. Regression line describing relation of routine rate with depth was computed using only those values shown on graph as circles. Triangles represent values from mesopelagic fishes with singleton-rate determinations, inverted triangles values obtained from literature. Both groups are placed on graph for comparison. Values are from following sources: *Notothenia rossii, N. angustifrons, Trematomus hansoni,* Morris and North 1984; *Chaenocephalus aceratus,* Hemmingsen and Douglas 1970; *Rhigophila dearborni,* Wohlschlag 1963; *T. bernacchii,* Wohlschlag 1964; *Pagothenia borchgrevinki,* Wohlschlag 1964 for a 30 g fish swimming at 2 m min^{-1} (his Eq. 35). Minimum and maximum rate lines calculated from values in Table 1 of present study

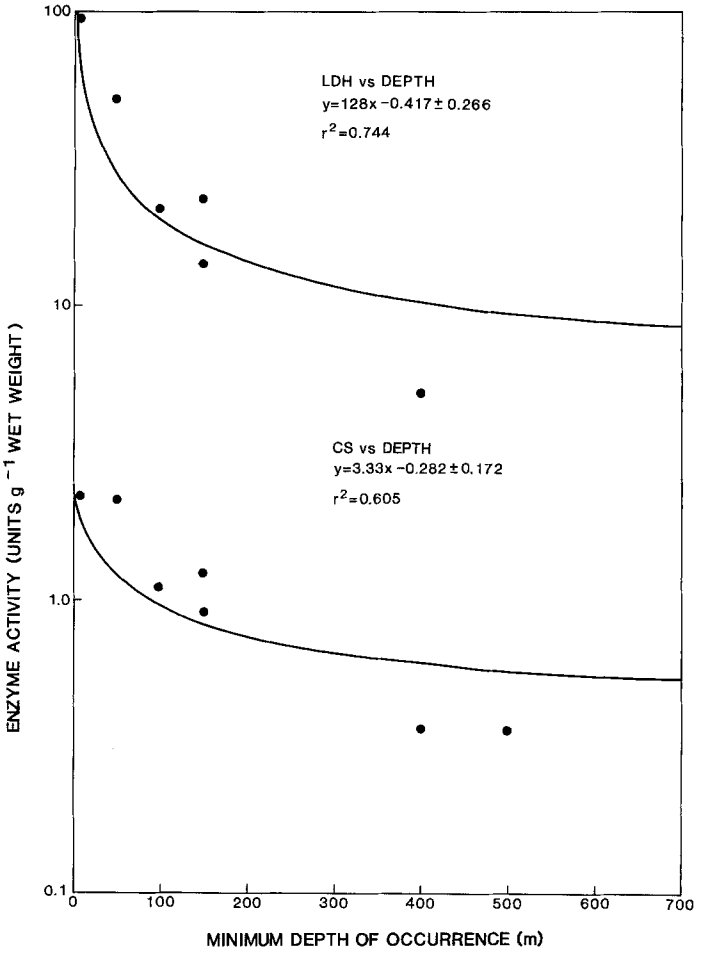

Fig. 5. Activity of lactate dehydrogenase and citrate synthase of white skeletal muscle in Antarctic mesopelagic fishes as function of minimum depth of occurrence. All determinations made at 10 °C, units of activity are μmol substrate converted to product min^{-1}. Regressions were calculated using mean values for Antarctic mesopelagic species shown in Table 2 for which n was greater than 1 and the cryopelagic *Pagothenia borchgrevinki.* These data points are represented on graphs as circles. *Cyclothone microdon* was excluded from LDH vs depth curve since this species displayed extremely high LDH activity

mingsen and Douglas 1970, Holeton 1970, Morris and North 1984) and animals in general ($b \cong 0.75$, Hemmingsen 1960). As a group, the mesopelagic fishes showed b values that were closer to 1.0 than 0.67, indicating that V_{O_2} scales with weight rather than surface area. For two species (*Bathylagus antarcticus* and *Electrona antarctica*) the curves of V_{O_2} vs weight included an even representation of the entire adult size-range of the species (Fig. 1), while in the two *Gymnoscopelus* species the curves favored intermediate and large-sized individuals. The modest size range of the four species and the range of our data within each species indicate that any error associated with size-bias in the curves is minimal and that all four curves provide a reasonable estimate of V_{O_2} vs weight for adults of the four species.

For three of the four species studied here, the weight-specific LDH activity of white muscle increased with increasing weight (Fig. 2). In the three myctophid species, LDH activity per gram of white muscle varied by at least an order of magnitude over the weight range examined. Since white muscle represents approximately half of the body mass in fishes, larger individuals of a species have a vastly higher potential for anaerobic glycolysis. This phenomenon occurs in several other marine fishes, and has been interpreted on the basis of the observed size-independence of burst-swimming performance in fishes (Somero and Childress 1980, 1985, Siebenaller et al. 1982). Burst-swimming is powered by anaerobic glycolysis in white muscle (cf. Somero and Childress 1980, 1985). Larger fishes require proportionally more anaerobic metabolic power to perform at an equivalent level to smaller fishes in terms of body lengths s^{-1} swum during burst-swimming (Wu 1977, Somero and Childress 1980, 1985).

The magnitude of the scaling coefficient, b, relating LDH activity to body size may reflect the locomotory mode and predatory behavior of a species. Among the four species studied here, scaling of LDH activity was

lowest in *Bathylagus antarcticus.* The difference between *B. antarcticus* and the three myctophid species is consistent with their way of life. The three myctophids are robust, strongly migrating, zooplanktivorous fishes that feed on the krill *Euphausia superba* when it is available within their depth range (Rowedder 1979, T. L. Hopkins personal communication). In contrast, *B. antarcticus* is a sluggish, watery fish that feeds largely on gelatinous zooplankton (T. L. Hopkins personal communication), a life habit that does not require a well-developed burst-swimming capability. In comparisons of enzymic activity levels and scaling parameters in five deep-sea macrourid fishes, only in the most active swimmer, *Coryphaenoides armatus,* did LDH and CS activity scale significantly with body size (Siebenaller et al. 1982). LDH activities in *C. armatus* were up to an order of magnitude higher than those of similar-sized confamilial species. Therefore, both the LDH activity per gram white muscle and the strength of scaling of that activity (size of the *b* exponent) may correlate with the capacities of the species for robust swimming.

The high LDH activity of the bristlemouth *Cyclothone microdon* is not readily explained. A congener from the California current, *C. acclinidens,* showed an LDH activity over an order of magnitude lower (13.6 ± 5.9 U g^{-1} wet wt, Somero, unpublished observations) yet both live almost exclusively below 500 m, and are considered sluggish fishes that feed primarily on small crustacean zooplankton (Fitch and Lavenberg 1968, T. L. Hopkins, personal communication). The oxygen consumption rates of both species are quite similar (0.011 to 0.027 μl O_2 mg^{-1} wet wt h^{-1} for *C. microdon;* 0.017 to 0.035 μl O_2 mg^{-1} wet wt h^{-1} for *C. acclinidens,* from Smith and Laver 1981) as are the CS activities (0.36 ± 0.09 U g^{-1} wet wt for *C. microdon;* 0.63 ± 0.12 U g^{-1} wet wt for *C. acclinidens,* Somero unpublished observations). Both species are widely distributed in the world ocean (Kobayashi 1973) and may co-occur. The large interspecific difference in LDH activity could reflect differences in predatory behavior, with *C. microdon* having a much higher capacity for short bursts of rapid swimming. Where these species co-occur, *C. microdon* and *C. acclinidens* may prey on organisms which themselves differ in capacities for rapid swimming.

The general decline in weight-specific CS activity with increasing weight is similar to that observed in the weight-specific oxygen-consumption rate for fishes (Winberg 1956). Comparisons of the relation between CS vs weight and oxygen consumption vs weight within species show that in *Electrona antarctica* and *Gymnoscopelus opisthopterus* the slopes of the two curves are very similar, while in *Bathylagus antarcticus* and *G. braueri* they are significantly different. Thus, while the trend is for both aerobic metabolism and CS activity to scale with weight in the same general fashion, the correlation between the two within a species is imperfect.

The data describing the relation of CS activity and weight for several pelagic species living in the California Borderland (Somero and Childress 1980), and for the deep-sea macrourid *Coryphaenoides armatus* (Siebenaller et al. 1982) are similar to those reported here. Slopes of the CS vs weight relations reported by Somero and Childress (1980) range from -0.05 ± 0.27 ($b \pm 95\%$ confidence interval; $n = 14$) in the Pacific anchovy *Engraulis mordax* to -0.23 ± 0.10 ($n = 6$) in the bathypelagic alepocephalid *Bajacalifornia burragei.* For *C. armatus,* the *b* value for CS vs weight was -0.59 ± 0.01 ($n = 13$). The activity of citrate synthase seems to follow the same trends with weight as does oxygen consumption.

Comparison of metabolism in other Antarctic fishes

Oxygen consumption rates of Antarctic mesopelagic fishes fall in the low to intermediate end of the range of values reported for Antarctic fishes (Wohlschlag 1960, 1963, 1964, Ralph and Everson 1968, Hemmingsen and Douglas 1970, Holeton 1970, Morris and North 1984). Morris and North found similar, if somewhat higher, metabolic rates for notothenioid fishes in a weight range similar to our mesopelagic species (1 to 50 g). If the V_{O_2} for all species is normalized to a common weight of 10 g using reported equations when available, or assuming a *b* value of 0.75 when not, our rates and those of Morris and North are at the low end of the metabolic-rate spectrum for Antarctic fishes. Because 10 g is outside the range of weights for many of the species used by the investigators in determining the relation of weight and V_{O_2}, direct comparisons are questionable.

The deepest-living Antarctic fish for which a published V_{O_2} value is available is *Rhigophila dearborni,* a benthic zoarcid found at depths greater than 400 m in McMurdo Sound (Wohlschlag 1963). Wohlschlag attributed the low metabolic rate of *R. dearborni* to an absence or limited degree of cold adaptation. We believe that the low metabolic rate exhibited by *R. dearborni* is most likely a consequence of its depth of occurrence, a factor that must be considered an important part of a species' life habit.

Depth relationships

The oxygen consumption of deep-living fishes (Smith and Hessler 1974, Torres et al. 1979, Smith and Laver 1981) and crustaceans (Childress 1975) in the California Borderland decline with increased depth of occurrence when measured at temperatures approximating those at a species' minimum depth of occurrence. The effects of temperature on metabolism can be separated from the effects of other depth-related variables by examining the metabolism of congeneric species with similar ways of life living in an isothermal, or nearly isothermal water column. The Antarctic pelagial varies by less than 4 C° between 0 and 1 000 m (−1.87° to 2.0 °C) (Gordon et al. 1982). The oxygen-consumption rate declined with increasing minimum depth of occurrence in Antarctic fishes, even though experimental temperature was constant at 0.5 °C for all

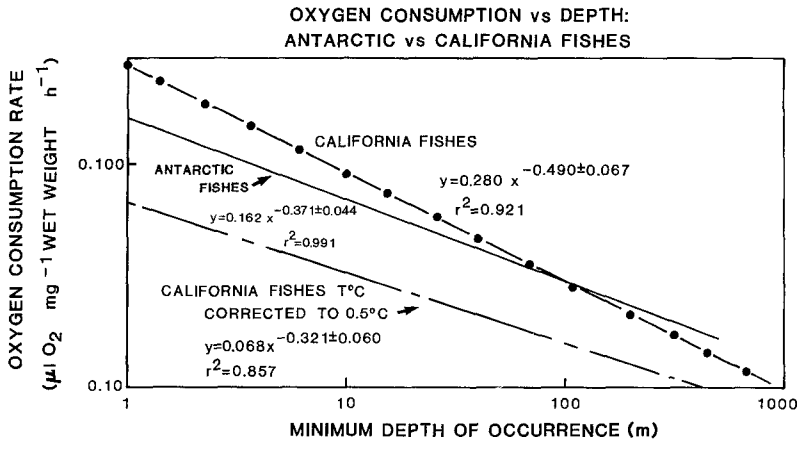

Fig. 6. Comparison of oxygen consumption rate versus minimum depth of occurrence in Antarctic and California mesopelagic fishes. Regression line for Antarctic fishes is from Fig. 4, that for California fishes is from Torres et al. 1979. Q_{10} of 2.0 was used to correct values for California fishes to temperature of 0.5 °C

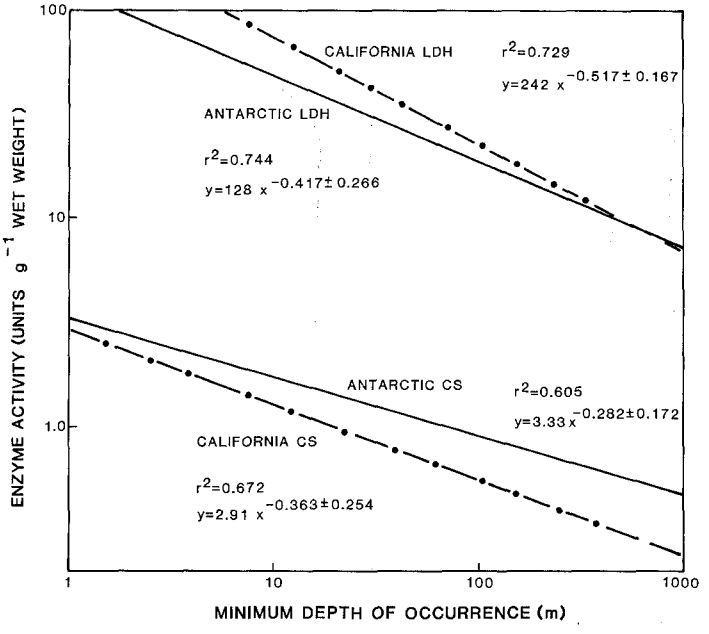

Fig. 7. Activity of lactate dehydrogenase and citrate synthase as function of minimum depth of occurrence in white skeletal muscle of Antarctic and California mesopelagic fish species. Regression for Antarctic species calculated as in Fig. 5, for California species regressions were calculated from data in Childress and Somero (1979) and values for *Cyclothone acclinidens* from of present study. All activity determinations were made at 10 °C. Units are μmol substrate converted to product min^{-1}

species, and total variability in temperature in the top 1 000 m of the capture area was 3.1 C° (−1.6° to 1.5 °C). Low metabolic rate is a predictable adaptation of deeper living pelagic fishes in a manner similar to other characteristics such as reproductive strategy (Stearns 1976) or von Bertalanffy growth parameters (Beverton and Holt 1959).

The decline in V_{O_2} with depth is paralleled by similar decreases in activity of LDH and CS of white locomotory muscle. These decreases in white muscle enzymic activity with increasing minimum depth of occurrence are proposed to be causally related to the depth-related changes in V_{O_2}, and to be the result of selection for reduced energy expenditure for locomotory activity in deep-living pelagic fishes. The combination of a relatively low and stochastically distributed food supply (Vinogradov 1970) with the attenuation and eventual absence of sunlight may select for a reduction in vigorous predatory behavior powered by white muscle.

The hypothesis that the low enzymic activities of white skeletal muscle in mesopelagic fishes are a reflection of a selection for reduced locomotory function is supported by the finding that depth-related decreases in enzymic activity do not occur in the brain (Sullivan and Somero 1980, Siebenaller et al. 1982) or in the heart (Childress and Somero 1979). Furthermore, in white muscle the concentration of actin, the major protein of thin filaments, does not decrease with depth of occurrence (Swezey and Somero 1982, Siebenaller and Yancey 1984). Therefore, the concentration of contractile proteins which comprise the bulk of the muscle remains similar among fishes, while the activities of enzyme providing ATP to power contraction vary by up to three orders of magnitude among species (Hand and Somero 1983), depending on the fishes' capacities for high-speed propulsion.

Interspecific variation in V_{O_2} and enzymic activity at any single minimum depth of occurrence probably reflects interspecific differences in feeding and locomotory strategies (cf. Sullivan and Somero 1980, Siebenaller et al. 1982). Intraspecific variations in V_{O_2} and muscle enzymic activity may be influenced by nutritional state as well as by size (Sullivan and Somero 1983, Lowery et al. 1987).

Comparison of metabolism in Antarctic and California mesopelagic fishes

The oxygen-consumption rates in Antarctic and California mesopelagic fishes as a function of depth differ (Fig. 6). The general decline in V_{O_2} with depth is similar in both systems, with the slope for California species (−0.490 ± 0.067, Torres et al. 1979) being somewhat steeper than that for Antarctic species (−0.371 ± 0.044). Over most of the curve, the V_{O_2}s in the two systems are quite similar despite the higher environmental temperature of the Cali-

fornia fishes (5° to 10 °C). If values of the California fishes are normalized to 0.5 °C using a Q_{10} of 2.0, the Antarctic species' metabolic rates are elevated by an approximate factor of two over their California counterparts. Thus, Antarctic mesopelagic species are cold-adapted in the sense of Scholander et al. (1953) and Wohlschlag (1964). Cold adaptation in Antarctic fishes occurs in the rates of oxygen consumption at both the tissue and whole-organism level (Somero et al. 1968). This indicates that cold adaptation is not an artefact of differing activity levels during $\dot{V}_{O_2}$ measurements (cf. Holeton 1974).

The relation between activities of LDH and CS and depth in Antarctic and California fishes (Fig. 7) are directly comparable because they were determined at the same temperature (10 °C). Anaerobic potential, as indicated by LDH activity, is similar in both systems. Analysis of covariance (Snedecor and Cochran 1967) shows that the curves are not significantly different ($P > 0.05$) in residual mean-square, slope or elevation.

CS activity in Antarctic fishes is uniformly above that observed in California fishes, but the apparent cold adaptation is less than that in $\dot{V}_{O_2}$. An analysis of covariance shows that they are not significantly different in residual variance ($P > 0.40$) or slope ($P > 0.50$). The curves would most likely differ in elevation, but the difference is not significant at the 0.05 level ($0.20 > P > 0.10$). The CS data are consistent with an enzymatic basis of metabolic cold adaptation, although the difference between fishes of the two systems is less than for $\dot{V}_{O_2}$.

Acknowledgements. This research was conducted as part of the AMERIEZ (Antarctic Marine Ecosystem Research at the Ice Edge Zone) program to study ice-edge biology. The authors gratefully acknowledge the help of the following people in the laboratory and at sea: B. Weigle, J. Donnelly, T. Lancraft, I. Quintero, T. Hopkins, J. Stepien, B. Wilson, B. Robison, A. Rodriguez, K. Daly, the captain and crew of the R. V. "Melville", and the captain and crew of the USCGC "Glacier". Thanks to D. Bombard for typing the manuscript and D. Walton and D. Williams for preparing the figures. The research was supported by NSF Grants DPP 82-10437 and 84-20562 to J. J. Torres and NSF PCM83-00983 to G. N. Somero.

Literature cited

Andriashev, A. P. (1965). A general review of the Antarctic fish fauna. In: Van Mieghem, J. and van Oye, P. (eds). Biogeography and ecology in antarctica. Junk, The Hague, p. 491–550

Beverton, R. H. J., Holt, S. J. (1959). A review of the life spans and mortality rates of fish in nature, and their relation to growth and other physiological characteristics. Ciba Fdn Colloq. Ageing 5: 142–180

Brett, J. R. (1965). The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd Can. 22: 1491–1501

Childress, J. J. (1971). Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. Biol. Bull. mar. biol. Lab., Woods Hole 141: 109–121

Childress, J. J. (1975). The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off Southern California. Comp. Biochem. Physiol. 50A: 787–799

Childress, J. J., Barnes, A. T., Quetin, L. B., Robison, B. H. (1978). Thermally protecting cod ends for the recovery of living deep-sea animals. Deep-Sea Res. 25: 419–422

Childress, J. J., Somero, G. N. (1979). Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Mar. Biol. 52: 273–283

Clark, L. C. (1956). Monitor and control of blood and tissue oxygen tensions. Trans. Am. Soc. artif. internal Organs 2: 41–48

DeVries, A. L., Eastman, J. T. (1981). Physiology and ecology of notothenioid fishes of the Ross Sea. Jl R. Soc. N.Z. 11: 329–340

DeVries, A. L., Lin, Y. (1977). The role of glycoprotein antifreezes in the survival of Antarctic fishes. In: Llano, G. A. (ed.). Adaptations within antarctic ecosystems. Smithsonian Institution, Washington, D.C., p. 439–458

DeWitt, H. H. (1970). The character of the midwater fish fauna of the Ross Sea, Antarctica. In: Holgate, M. W. (ed.). Antarctic ecology. Vol. 1. Academic Press, London, p. 305–314

Fitch, J. E., Lavenberg, R. J. (1968). Deep-water fishes of California. University of California Press, Berkeley and Los Angeles

Gordon, A. L., Molinelli, E. J., Baker, T. N. (1982). Southern Ocean atlas. Columbia University Press, New York

Hand, S. C., Somero, G. N. (1983). Energy metabolism pathways of hydrothermal vent animals: adaptations to a food-rich and sulfide-rich deep-sea environment. Biol. Bull. mar. biol. Lab., Woods Hole 165: 167–181

Hemmingsen, A. M. (1960). Energy metabolism as related to body size and respiratory surfaces, and its evolution. Rep. Steno meml Hosp. 9: 7–110

Hemmingsen, E. A., Douglas, E. L. (1970). Respiratory characteristics of the hemoglobin-free fish *Chaenocephalus aceratus*. Comp. Biochem. Physiol. 33: 733–744

Holeton, G. F. (1970). Oxygen uptake and circulation by a hemoglobinless Antarctic fish (*Chaenocephalus aceratus* Lonnberg) compared with three red-blooded Antarctic fish. Comp. Biochem. Physiol. 34: 457–471

Holeton, G. F. (1974). Metabolic cold adaptation of polar fish: fact or artefact. Physiol. Zoöl. 47: 137–152

Kobayashi, B. N. (1973). Systematics, zoogeography and aspects of the biology of the bathypelagic fish genus *Cyclothone* in the Pacific ocean. Ph.D. dissertation, University of California, San Diego

Lowery, M. S., Roberts, S. J., Somero, G. N. (1987). Effects of starvation on the activities and localization of glycolytic enzymes in white muscle of the barred sand bass, *Paralabrax nebulifer*. Physiol. Zoöl. 60: 538–549

McGinnis, R. F. (1977). Evolution within pelagic ecosystems: aspects of the distribution and evolution of the family Myctophidae. In: Llano, G. A. (ed.) Adaptations with antarctic ecosystems. Smithsonian Institution, Washington, D.C., p. 547–556

Morris, D. J., North, A. W. (1984). Oxygen consumption of five species of fish from South Georgia. J. exp. mar. Biol. Ecol. 78: 75–86

Ralph, R., Everson, I. (1968). The respiratory metabolism of some Antarctic fish. Comp. Biochem. Physiol. 27: 299–307

Rowedder, U. (1979). Feeding ecology of the myctophid *Electrona antarctica* (Günther 1878) (Teleostei). Meeresforsch. Rep. mar. Res. 27 (Sdbd): 252–263. (Ber. dt. wiss. Kommn Meeresforsch.)

Scholander, P. F., Flagg, W., Walter, V., Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. Physiol. Zoöl. 26: 67–92

Siebenaller, J. F., Somero, G. N., Haedrich, R. L. (1982). Biochemical characteristics of macrourid fishes differing in

their depths of distribution. Biol. Bull. mar. biol. Lab., Woods Hole 163: 230–239

Siebenaller, J. F., Yancey, P. H. (1984). Protein composition of white skeletal muscle from mesopelagic fishes having different water and protein contents. Mar. Biol. 78: 129–137

Smith, K. L., Jr. Hessler, R. R. (1974). Respiration of benthopelagic fishes: *in situ* measurements at 1 230 meters. Science, N.Y. 184: 72–73

Smith, K. L. Jr., Laver, M. B. (1981). Respiration of the bathypelagic fish *Cyclothone acclinidens.* Mar. Biol. 61: 261–266

Snedecor, G. W., Cochran, W. G. (1967). Statistical methods. 6th ed. Iowa State University Press, Ames, Iowa

Somero, G. N., Childress, J. J. (1980). A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. Physiol. Zoöl. 53: 322–337

Somero, G. N., Childress, J. J. (1985). Scaling of oxidative and glycolytic enzyme activities in fish muscle. In: Gilles, R. (ed.) Circulation, reproduction and metabolism. Springer-Verlag, Berlin, p. 250–262

Somero, G. N., Giese, A. C., Wohlschlag, D. E. (1968). Cold adaptation of the Antarctic fish *Trematomus bernacchii.* Comp. Biochem. Physiol. 26: 223–233

Stearns, S. (1976). Life-history tactics: a review of the ideas. Q. Rev. Biol. 51: 3–47

Sullivan, K. M., Somero, G. N. (1980). Enzyme activities of fish skeletal muscle and brain as influenced by depth of occurrence and habits of feeding and locomotion. Mar. Biol. 60: 91–99

Sullivan, K. M., Somero, G. N. (1983). Size- and diet-related variation in enzymic activity and tissue composition in sablefish *Anoplopoma fimbria.* Biol. Bull. mar. biol. Lab., Woods Hole 164: 315–326

Swezey, R. R., Somero, G. N. (1982). Skeletal muscle actin content is strongly conserved in fishes having different depths of distribution and capacities of locomotion. Mar. Biol. Lett. 3: 307–315

Targett, T. E. (1981). Trophic ecology and structure of coastal Antarctic fish communities. Mar. Ecol. Prog. Ser. 4: 243–263

Torres, J. J., Belman, B. W., Childress, J. J. (1979). Oxygen consumption rates of midwater fishes as a function of depth of occurrence. Deep-Sea Res. 26: 185–197

Vinogradov, M. E. (1970). Vertical distribution of the oceanic zooplankton. Israel Program for Scientific Translations, Jerusalem

Winberg, G. G. (1956). The relation of the metabolic rate of fish to temperature. Transl. Ser. Fish. Res. Bd Can. 194: 21–38

Wohlschlag, D. E. (1960). Metabolism of an Antarctic fish and the phenomenon of cold adaptation. Ecology 41: 287–292

Wohlschlag, D. E. (1963). An Antarctic fish with unusually low metabolism. Ecology 44: 557–564

Wohlschlag, D. E. (1964). Respiratory metabolism and ecological characteristics of some fishes in McMurdo Sound, Antarctica. In: Lee, M. D. (ed.). Biology of the antarctic seas, Vol. I. American Geophysical Union, Washington, D.C., p. 33–62

Wu, T. Y. (1977). Introduction to the scaling of aquatic animal locomotion. In: Pedley, T. J. (ed.). Scale effects in animal locomotion. Academic Press, New York, p. 203–232

Zwally, J. H., Comiso, J. C., Parkinson, C. L., Campbell, W. J., Carsey, F. D., Gloerson, P. (1983). Antarctic sea ice, 1973–1976: satellite passive-microwave observations. NASA, Washington, D.C.

Date of final manuscript acceptance January 13, 1988.
Communicated by J. M. Lawrence, Tampa