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Na^+-K^+-adenosine triphosphatase activities in gills of marine teleost fishes: changes with depth, size and locomotory activity level

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Abstract. Activities of the primary enzyme responsible for monovalent ion regulation, Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase), were measured in gills of marine teleost fishes with different depths of occurrence (0 to 4800 m), body weights (a range of five orders of magnitude), and locomotory capacities. Specimens were collected off the coasts of California and Oregon in 1983–1989, and at the Galápagos Spreading Center and 13 °N East Pacific Rise hydrothermal vent sites in 1987 and 1988, respectively. Except for two hydrothermal vent fishes, deep-sea species had much lower Na^+-K^+-ATPase activities g^{-1} gill filament than shallow-living species, indicating that osmoregulatory costs, like total metabolic rate, are greatly reduced in most deep-living fishes. Within a species, the total branchial Na^+-K^+-ATPase activity per individual was dependent on size; the average allometric scaling exponent was 0.83. Using published values for oxygen consumption rates, and the total branchial Na^+-K^+-ATPase activities as an index of osmoregulatory costs, we estimated the maximal cost (as percent of ATP turnover) for osmoregulation in ten teleosts. Osmoregulatory costs averaged about 10% of total ATP turnover among these species, and maximal costs were no greater than about 20%. The percent costs of osmoregulation did not differ between shallow- and deep-living fishes. The reduced total ATP expenditure for osmoregulation in deep-living fishes is proposed to result from the sluggish locomotory habits of these fishes, not from selection for reduced osmotic coast *per se*. Thus, the reduced swimming abilities of these fishes lead to lower rates of water flow over the gills and less blood flow through the gills due to reduced demands for oxygen. Consequently, passive flux of water and ions through the gills is much lower than in more active fishes, and osmotic costs are thereby minimized. The relatively high activities of Na^+-K^+-ATPase in gills of the two hydrothermal vent fishes suggest that these fishes may be more active and have higher metabolic rates than other deep-sea fishes.

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Introduction

Total numbers, biomass, caloric contents, enzymatic activities, and metabolic rates of fishes decrease rapidly with depth in the marine water column (reviewed in Siebenaller and Somero 1989). Metabolic rates of deep-sea fishes may be over an order of magnitude lower than those of similar-sized shallow-living, cold-adapted fishes (Smith and Hessler 1974, Smith 1978, Torres et al. 1979, Torres and Somero 1988a, b). These depth-related decreases in metabolic rate are not due solely to decreases in temperature with depth, because similar depth-related decreases in metabolic rates have been found in isothermal (Antarctic) and thermally-stratified (Southern California) water columns (Torres and Somero 1988a). Instead, these decreases are thought to result largely from significant reductions in locomotory energy expenditure in deep-living fishes, which typically have very low capacities for high-speed swimming, relative to shallow-living fishes (Childress and Somero 1979, Sullivan and Somero 1980, Siebenaller et al. 1982, Torres and Somero 1988a).

Although locomotory activity may account for the largest single share of ATP turnover in fishes, osmoregulation also represents a significant fraction of total metabolism. Rao (1968) and Febry and Lutz (1987) estimated that as much as one-fourth of metabolism may be expended in osmoregulation. Thus, the 10- to 100-fold reduction in metabolic rate in deep-sea fishes implies that the amount of energy devoted to osmoregulation in these species must be greatly reduced. Deep-sea fishes maintain intracellular and plasma ionic compositions similar to those of shallow-living fishes (Blaxter et al. 1971, Shelton et al. 1985), suggesting that reduction in osmoregulatory energy costs via reduction of osmotic gradients has not occurred in deep-sea fishes.

One might predict that osmoregulatory costs would be correlated with the locomotory habits of a fish. Reduced locomotory activities of sluggish fishes like most deep-sea species lead to reduced water flow over the gills and reduced perfusion of the gill lamellae, due to reduced demands for oxygen. Thus, in sluggish species, passive

fluxes of water and ions will be lower, allowing a reduction in the energy cost of osmoregulation.

We have investigated the costs of osmoregulation in marine fishes by quantifying the activity of the enzyme Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase; EC 3.6.1.3) in gill tissue. Na^+-K^+-ATPase of gills is the primary enzyme responsible for monovalent ion regulation in marine teleosts (Epstein et al. 1980), and its activity varies with the osmotic load to which a fish is subjected (Philpott 1980, Perry and Walsh 1989). Our analysis of shallow-living fishes, fishes from typical deep-sea environments, and two endemic hydrothermal vent fishes addresses the questions of depth-, size- and activity-related changes in total osmoregulatory cost and in the fraction of metabolism (ATP turnover) expended in osmoregulation in these different species.

Materials and methods

Collection and storage of fishes and tissues

Most shallow-living fishes were collected by nets near San Diego, California. Northern anchovy (*Engraulis mordax*) were purchased from bait dealers and held for one week in the laboratory until used. *Hypsoblennius jenkinsi* were collected from the flowing seawater system of the old Scripps pier during its demolition. Benthopelagic and demersal fishes were collected off the coasts of Oregon and California, USA, using otter trawls. Mesopelagic fishes were caught by midwater trawl in the San Clemente Basin off southern California. Collection of all species except the hydrothermal vent fishes were made at numerous times between August 1983 and August 1989. The hydrothermal vent zoarcid (*Thermarces andersoni*) was captured at the 13 °N site on the East Pacific Rise in 1987, and the vent bythitid [*Bythites hollisi*; initial description by Cohen et al. (1990)] was caught at the Galápagos vent site in 1988.

For most species, gills were dissected from freshly caught fish, wrapped in aluminum foil, and frozen immediately on dry ice. Fish used in the scaling studies were frozen whole on dry ice, and gills were dissected later from freshly thawed specimens. *Sebastolobus altivelis* and *Coryphaenoides acrolepis* were measured for length at sea, gills were removed and frozen, and masses (weights) of the individuals were estimated from mass vs length relationships determined for these species by Stein and Pearcy (1982) and W. W. Wakefield (personal communication). No differences in Na^+-K^+-ATPase activity were observed between fresh and frozen gills, and no loss of activity was observed during storage for several months.

Preparation of homogenates

Gill filaments were trimmed from thawed gills and homogenized in 5 to 10 vol of homogenization buffer: 250 m*M* sucrose, 50 m*M* imidazole-HCl (pH 7.5 at 20 °C), 1 m*M* EDTA, and 5 m*M* 2-mercaptoethanol. For measurement of the total branchial Na^+-K^+-ATPase activity in an individual, the entire gill mass was dissected from a fish. For large fish, as much of the gill arch as possible was cut off so as not to lose any gill filament tissue. For very small specimens ($\leq \sim 500$ mg), gills were homogenized in 450 μl of ATPase salts [200 m*M* NaCl, 40 m*M* KCl, 10 m*M* $MgCl_2$, 60 m*M* Tris-HCl (pH 7.5 at 10 °C)] and 200 μl of this homogenate were used to initiate the ATPase activity assay without further treatment.

The primary homogenates were treated in one of two ways. For assays in which the total Na^+-K^+-ATPase activity of the branchial apparatus was estimated (Table 3) the crude homogenates were used without further treatment to avoid any possible loss of enzymatic activity. The use of crude homogenates led to greater variability (up to 15 to 20% in replicate assays of the same homogenate) due to the uneven distribution of particulate fractions in different small (10 to 20 μl) aliquots of homogenate used to initiate the assays.

A second method (yielding "membrane fractions") was used to reduce variability in studies where activity g^{-1} gill filament was estimated (Table 2), and for the studies of pressure effects on Na^+-K^+-ATPase activity (Gibbs and Somero 1989). The crude homogenate was centrifuged for 15 min at $2\,000 \times g$, and the supernatant was carefully removed and spun for 90 min at $19\,000 \times g$. The pellet was resuspended in homogenization buffer and stored at −80 °C, without loss of activity. The two centrifugation steps led to the loss of approximately 20 to 40% of the total Na^+-K^+-ATPase activity.

Enzyme activity measurements

ATPase activities were measured at 10 °C using the coupled pyruvate kinase/lactate dehydrogenase assay system described in Gibbs and Somero (1989). The assay medium contained 100 m*M* NaCl, 20 m*M* KCl, 5 m*M* $MgCl_2$, 3 m*M* disodium ATP (vanadium-free), 0.5 m*M* phosphoenolpyruvate (tricyclohexylammonium salt), 0.05 m*M* fructose-1,6-bisphosphate, 0.2 m*M* β-nicotinamide adenine dinucleotide (reduced), 30 m*M* Tris-HCl (pH 7.5 at 10 °C), and excess coupling enzymes. The concentrations of Na^+, K^+, and ATP used in this assay system yield a maximal velocity for the reaction (Gibbs and Somero 1989).

To determine the fraction of total ATPase activity that was due to the Na^+-K^+-ATPase, each homogenate was divided into two fractions. To one fraction, ouabain, a specific inhibitor of Na^+-K^+-ATPase, was added to a final concentration of 5 m*M*, and the homogenate was incubated for 30 to 60 min to achieve complete inhibition. Na^+-K^+-ATPase activity was calculated as the difference between the total and ouabain-inhibited ATPase activities. Na^+-K^+-ATPase comprised between approximately one-third and one-half of the total ATPase activity. Two or more replicates of each preparation (with and without ouabain) were run, except for very small samples, for which only one determination under each condition was possible. No loss of total ATPase activity occurred during the time courses of the experiments.

Results

Large differences in Na^+-K^+-ATPase activity g^{-1} gill filament were found between shallow-living and deep-sea fishes in both crude (uncentrifuged) homogenates (Table 1) and membrane preparations (Table 2). In the crude homogenate comparisons (Table 1) mean Na^+-K^+-ATPase activities of deep-sea species were about one-half those of shallow-living species (means differed significantly; $P<0.01$; Mann-Whitney *U*-test). In studies employing membrane fractions (Table 2), distinct differences again were apparent between the two shallow-living species and the deep-sea fishes. The exceptions to this relationship were the two hydrothermal vent fishes, which had much higher enzymatic activity levels than any other deep-sea fishes (Table 2).

Individuals of the sablefish *Anoplopoma fimbria* were trawled over a wide depth range (85 to 1500 m). Na^+-K^+-ATPase activities of gill membrane fractions decreased significantly with depth of capture (Fig. 1; $P<0.001$). A significant inverse relationship between capture depth and Na^+-K^+-ATPase activity was also observed for *Sebastolobus altivelis* (captured at 600 to 1400 m), but not for *S. alascanus* (1000 to 1400 m) or *Coryphaenoides armatus* (700 to 2 000 m).

Table 1. Depth of occurrence and Na^+-K^+-ATPase activity of crude homogenates of gills (measured at 10 °C), for shallow- and deep-living marine teleost fishes. Depths of occurrence are for adults of all species (some deep-living species have epipelagic larval and juvenile stages); depth ranges for species are from Miller and Lea (1972), Childress and Nygaard (1973), Torres et al. (1979), and from references in Gibbs and Somero (1989); only the minimal depth of occurrence (cf. Childress and Nygaard, 1973) is given where maximal depth of occurrence is not known. Shallow-living species are defined as species with minimal depths of occurrence of 0 m; all other species are considered deep-living. Differences between means for shallow- and deep-living species are significantly different ($P<0.01$; Mann-Whitney U-test)

Species (*n*)	Adult depth range (m) (*T* range, °C)	Mean (range) of Na^+-K^+-ATPase activity (μmol ATP min^{-1} g^1 gill filament)
Shallow-living		
Sphyraena argentea (2) (California barracuda)	0–20 (14–18)	2.00 (1.75–2.26)
Scomber japonicus (3) (Pacific mackerel)	0–20 (14–18)	0.92 (0.64–1.30)
Engraulis mordax (5) (northern anchovy)	0–20 (14–18)	0.85 (0.23–1.87)
Anoplopoma fimbria (7) (sablefish)	0–1 500 (4–10)	1.13 (0.54–1.54)
Scorpaena guttata (1) (California scorpionfish)	0–180 (8–18)	1.00
Scorpaenichthys marmoratus (3) (cabezon)	0–75 (8–18)	1.26 (0.72–1.98)
Mean (SD) shallow:		1.19±0.42
Deep-living		
Sebastolobus altivelis (2) (longspine thornyhead)	550–1 500 (4–10)	0.72 (0.66–0.78)
Antimora microlepis (5) (Pacific flatnose)	360–3 000 (2–4)	0.23 (0.10–0.38)
Albatrossia pectoralis (2) (giant grenadier)	700–2 000[a] (2–4)	0.35 (0.33–0.37)
Leuroglossus stilbius (3)	25 (8–18)	0.42 (0.15–0.74)
Scopelogadus mizolepis bispinosus (3)	450 (8–12)	0.48 (0.42–0.53)
Mean (SD) deep:		0.44 (0.18)

[a] No published depth range data could be found for this species, but we collected individuals over a depth range of 700 to 2 000 m

Because size-related ("scaling") differences in metabolic rates and enzymatic activity levels are so prevalent (Somero and Childress 1980, 1990, Schmidt-Nielsen 1984, Childress and Somero 1990), it is necessary to analyze trends such as those presented above in the context of body size. Thus, we measured the total Na^+-K^+-ATPase activity in the gills of different sized specimens of 11 species for which we had a wide size range of individuals. To minimize possible intraspecific variation related to depth or local food availability (Sullivan and Somero

Table 2. Depth of occurrence and Na^+-K^+-ATPase activity of membrane fractions of gills for which 60 to 80% of total activity was recovered (measured at 10 °C), for shallow- and deep-living marine teleost fishes. Depth ranges for species are from Miller and Lea (1972) and from references in Gibbs and Somero (1989). For comparison with data in Table 1, Na^+-K^+-ATPase activities should be increased by one-third to correct for typical loss of enzyme during isolation of membrane fraction. Further details as in legend to Table 1

Species (*n*)	Adult depth range (m) (*T* range, °C)	Mean (range) of Na^+-K^+-ATPase activity (μmol ATP min^{-1} g^{-1} gill filament)
Shallow-living		
Anoplopoma fimbria (17) (sablefish)	0–1 500 (4–10)	1.35 (0.26–3.05)
Porichthys notatus (5) (plainfin midshipman)	0–300 (6–15)	0.83 (0.57–1.06)
Deep-living		
Sebastolobus alascanus (7) (shortspine thornyhead)	25–1 500 (4–10)	0.27 (0.15–0.56)
Sebastolobus altivelis (13) (longspine thornyhead)	550–1 500 (4–10)	0.29 (0.09–0.73,
Antimora microlepis (6) (Pacific flatnose)	360–3 000 (2–4)	0.11 (0.03–0.29)
Albatrossia pectoralis (3) (giant grenadier)	700–2 000[a] (2–4)	0.17 (0.14–0.21)
Coryphaenoides acrolepis (28) (Pacific grenadier)	700–1 820 (2–4)	0.28 (0.05–0.70)
Coryphaenoides armatus (12)	1 885–4 815 (2–4)	0.36 (0.20–0.49)
Coryphaenoides filifer (10)	2 850[a] (2–4)	0.28 (0.19–0.45)
Coryphaenoides leptolepis (5)	2 288–4 639 (2–4)	0.34 (0.11–0.53)
Hydrothermal vent		
Thermarces andersoni (1)	2 500 (2–?)	0.60
Bythites hollisi (1)	2 600 (2–?)	1.06

[a] Depth of capture is given; full depth ranges are not known

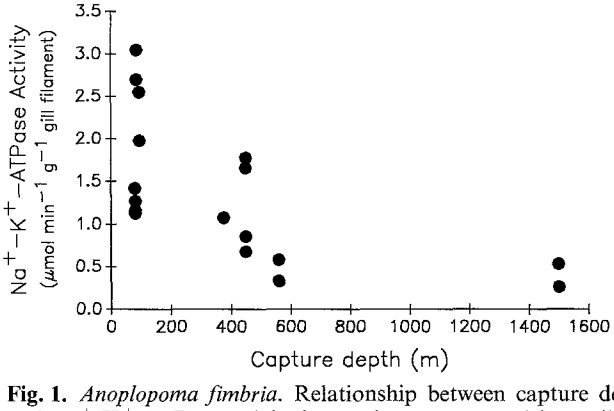

Fig. 1. *Anoplopoma fimbria*. Relationship between capture depth and Na^+-K^+-ATPase activity in membranes prepared from gills of sablefish

Table 3. Scaling relationships of Na^+-K^+-ATPase activity in shallow and deep-living marine teleost fishes. Total gill Na^+-K^+-ATPase activities (nmol ATP min^{-1} $fish^{-1}$) vs mass (M) data were fit to allometric equation: total Na^+-K^+-ATPase activity $= aM^b$, where a constant, M is body mass (g), and b is the regression scaling coefficient. Species followed by asterisk spanned an order of magnitude in mass

Species (n)	b (SE)	log a (SE)	r^2	Size range (g)
Shallow-living				
Engraulis mordax (*18*)	1.30 (0.46)	1.03 (0.30)	0.33	6.26 – 20.8
Hypsoblennius jenkinsi (*12*)	0.77 (0.23)	1.27 (0.15)	0.52	1.48 – 4.70
Deep-living				
Leuroglossus stilbius * (*23*)	0.56 (0.15)[a]	0.96 (0.47)	0.40	0.124 – 12.7
*Scopelogadus mizolepis bispinosus** (*26*)	1.17 (0.12)	0.89 (0.27)	0.80	0.319 – 12.5
*Poromitra crassiceps** (*7*)	0.74 (0.16)	0.99 (0.38)	0.81	0.16 – 29.8
Cyclothone signatus (*26*)	0.71 (0.32)	0.93 (0.32)	0.17	0.029 – 0.20
Cyclothone acclinidens (*24*)	0.58 (0.23)	0.46 (0.29)	0.22	0.137 – 1.071
Scopelengys tristis (*23*)	1.01 (0.22)	1.24 (0.15)	0.50	17.0 – 57.0
Sebastolobus altivelis (*12*)	0.66 (0.15)[a]	1.60 (0.15)	0.65	41 – 232
*Sebastolobus alascanus** (*15*)	0.56 (0.21)[a]	1.47 (0.25)	0.33	16 – 348
Coryphaenoides acrolepis (*14*)	1.07 (0.35)	0.47 (0.23)	0.43	392 – 2228
b average (all species)	0.83			

[a] Scaling coefficient (b) is significantly different from 1 ($P<0.05$)

Table 4. Scaling relationships for gill mass in marine teleost fishes. For each species, data for gill mass (including gill arches and filaments) and total body mass (M) were fit to allometric equation: gill mass $= aM^b$. Scaling exponent (b) values < 1 indicate that gill mass as a fraction of body mass decreased in larger individuals

Species (n)	b (SE)	log a (SE)	r^2
Engraulis mordax (*19*)	1.00 (0.07)	−1.21 (0.05)	0.92
Hysoblennius jenkinsi (*12*)	0.79 (0.15)	−1.64 (0.1)	0.74
Leuroglossus stilbius (*31*)	0.89 (0.03)[a]	−1.44 (0.13)	0.96
Scopelogadus mizolepis bispinosus (*27*)	0.92 (0.03)[a]	−1.23 (0.06)	0.98
Poromitra crassiceps (*7*)	0.87 (0.04)[a]	−1.23 (0.09)	0.99
Scopelengys tristis (*26*)	0.71 (0.11)[a]	−0.91 (0.08)	0.65
b average	0.86		

[a] Scaling exponent (b) is significantly different from 1 ($P<0.05$)

1983), all individuals of a species were taken from the same trawl.

Table 3 presents the size ranges and allometric equations relating total branchial Na^+-K^+-ATPase activity in a fish to body mass for 11 species. A high degree of intraspecific variation was present. Some of the variation is probably due to the particulate nature of the unfractionated homogenate preparation used to determine total branchial activity of the enzyme (see "Materials and methods – Preparation of homogenates"). Despite high intraspecific variation, the general influences of body size on total branchial Na^+-K^+-ATPase activity can be discerned. The scaling exponents (b values) of the allometric equations were usually <1, and for three species b was significantly ($P<0.05$) <1. In no case was b significantly >1. The average b value was 0.83.

Scaling of total branchial Na^+-K^+-ATPase activity could have two underlying causes. Larger individuals could have relatively smaller gills (as a fraction of body weight), or gills in larger individuals could have lower specific activities of the enzyme (activity g^{-1} gill filament). No consistent size-related differences in Na^+-K^+-ATPase specific activity were observed for six species (data not shown). However, gill mass scaled significantly ($P<0.05$) in four of the six species (Table 4). The average b value for the six species was 0.86.

The total Na^+-K^+-ATPase activity in the branchial mass of a fish was used as an approximation of the total osmoregulatory cost expressed as a fraction of overall ATP turnover. We assumed that the costs of regulating Na^+ and K^+ concentrations dominate the total osmoregulatory costs of a marine teleost, and that the activity of branchial Na^+-K^+-ATPase is a quantitative index of these costs.

For ten of the species studied, published oxygen consumption data were used as a basis for estimating total ATP turnover. We assumed (cf. Stryer 1988, p. 420) that 6 mol of ATP are generated for each mole of O_2 consumed. The oxygen consumption measurements with the ten species were made under a variety of pressure and temperature conditions. We adjusted the total Na^+-K^+-ATPase values for these species (Table 3) for size, pressure, and temperature. First, using the regression equations given in Table 3, we estimated the total branchial Na^+-K^+-ATPase activity in an individual fish of the same size as the specimen used in the respiration studies. Second, to correct for pressure effects when necessary, i.e., for the species studied by Smith and colleagues (see Footnotes c–e in Table 5), we reduced the Na^+-K^+-ATPase activities using the average pressure inhibition for the enzyme found for cold-adapted fishes (Gibbs and Somero 1989). Finally, because the respiration studies used lower measurement temperatures than the temperature used in the Na^+-K^+-ATPase assays (10 °C), we decreased the enzymatic activity values using an apparent activation energy of 20 kcal mol^{-1} (Gibbs and Somero 1989). Thus, for each species for which oxygen consumption data were available, we calculated the ATP production rate and compared it with the total branchial Na^+-K^+-ATPase activity for a fish of that size, at the pressures

Table 5. Gill Na^+-K^+-ATPase activities (corrected for size, pressure and temperature), ATP production rates (estimated from literature values for oxyen consumption), and estimated cost of osmoregulation in marine teleost fishes. ATP production was calculated assuming that 6 mol of ATP are produced for 1 mol O_2 consumed

Species (mass, g)	Na^+-K^+-ATPase activity (μmol ATP g^{-1} h^{-1})	ATP production (μmol g^{-1} h^{-1})	Gill Na^+-K^+-ATPase activity: ATP production (%)
Engraulis mordax (8.7)	2.57	15.80[a]	16.3
Leuroglossus stilbius (8.3)	0.114	6.85[a]	1.7
Poromitra crassiceps (14.1)	0.156	3.15[a]	5.0
Scopelengys tristis (37.4)	0.572	2.58[a]	22.1
Scopelogadus mizolepis bispinosis (3.6)	0.307	6.60[a]	4.6
Sebastolobus alascanus (48)	0.235	7.34[b]	3.2
Sebastolobus altivelis (155)	0.150	0.81[c]	17.3
Cyclothone acclinidens (0.55)	0.072	7.17[d]	1.0
Coryphaenoides acrolepis (1830)	0.097	0.69[e]	15.2
Anoplopoma fimbria (702)	1.40[g]	16.0[f]	8.8
Average (SD)			9.5 (7.6)

[a] Torres et al. (1979)
[b] Siebenaller (1984)
[c] Smith and Brown (1983)
[d] Smith and Laver (1981)
[e] Smith and Hessler (1974)
[f] Estimated from data in Fig. 3 of Sullivan and Smith (1982)
[g] Mean of total gill Na^+-K^+-ATPase actvities for two individuals weighing 678 and 726 g

and temperatures at which the respiration measurements were conducted.

Using these estimates of ATP production and ATP expenditure in Na^+-K^+-ATPase activity, we calculated the maximal fraction of ATP turnover that would be required to support osmoregulation (Table 5). Our analysis indicates that branchial monovalent ion-regulation costs no more than about one-fifth of total ATP production. The average maximal cost estimated for these ten species is near 10%.

Discussion

The low metabolic rates of deep-living pelagic fishes have been explained in terms of locomotory strategies that appear to reflect the darkness, the sparse and sporadically distributed food supply, and the predator-prey interactions of the pelagic deep-sea environment (Childress and Somero 1979, Siebenaller et al. 1982, Siebenaller and Somero 1989). These environmental characteristics are hypothesized to result in reductions in locomotory energy expenditure, especially for high intensity ("burst") swimming. The striking reductions in the activities of glycolytic enzymes responsible for generating ATP during high-intensity locomotion in the locomotory muscles of deep-sea fishes support this hypothesis (Childress and Somero 1979, Somero and Childress 1980, Sullivan and Somero 1980, Siebenaller et al. 1982). For example, the activities of lactate dehydrogenase g^{-1} muscle, an index of the muscle's capacity for anaerobic ATP production, are 1 to 3 orders of magnitude lower in sluggish, deep-living fishes compared to highly active shallow-living species. Enzymatic activities of brain, however, exhibit no depth-related decreases (Somero and Childress 1980, Sullivan and Somero 1980), indicating that the low metabolic rates of deep-sea fishes are not a reflection of selection for organism-wide reductions in metabolism.

Lower activities of the important osmoregulatory enzyme Na^+-K^+-ATPase in gills of deep-sea fishes suggest that osmoregulatory requirements also decrease in these species. In fact, the data presented here probably underestimate the *in situ* differences between deep- and shallow-living species, since the data presented in Tables 1 and 2 were obtained at a common measurement temperature (10 °C) and at 1 atm. If the data are corrected for the normal habitat temperatures and pressures of each species, differences in Na^+-K^+-ATPase activities between shallow- and deep-living fishes would be substantially greater. Typical deep-sea temperatures of 2° to 4 °C would reduce activities by up to 50%, and pressure inhibition would reduce enzymatic activities by as much as 30% under physiological conditions (Gibbs and Somero 1989). Conversely, for the shallow-living species, which were collected from waters having temperatures between 10° and 18 °C, activities at *in situ* temperatures would be up to twice the 10 °C values given in Tables 1 and 2. Thus, under physiological temperature and pressure conditions, Na^+-K^+-ATPase activities g^{-1} gill filament of shallow- and deep-living fishes are likely to differ by at least one order of magnitude.

Furthermore, the differences in total branchial Na^+-K^+-ATPase activities between these two groups of fishes may be even larger. Deep-sea fishes tend to have smaller gills and reduced gill surface area compared to shallow-living species (Hughes and Iwai 1978). Similar differences between the gills of shallow-living fishes have been correlated with interspecific differences in metabolic rates (Hughes 1970, 1984).

Intraspecific, capture depth-related decreases in gill Na^+-K^+-ATPase activities were also observed in fishes occurring over a wide depth range (Fig. 1; also *Sebastolobus altivelis,* data not shown). Na^+-K^+-ATPase activities of shallow-occurring sablefish resembled those of other shallow-living species, whereas deeper-occurring individuals had much lower Na^+-K^+-ATPase activities which were similar to those of solely deep-living fishes. Thus, osmoregulatory requirements reflect the physical and biological environment of a particular individual, rather than a fixed level of activity for an entire species. These results also suggest that the interspecific trends are not merely an artifact of the choice of species studied, but reflect real differences in osmoregulatory requirements between shallow- and deep-living species.

These depth-related trends are not influenced by size (scaling effects). No size dependence of Na^+-K^+-ATPase activity g^{-1} gill filament was observed, and the average scaling exponent of total enzymatic activity in the branchial apparatus (0.83; Table 3) was similar to the scaling exponents of standard metabolic rate (0.8 to 0.9, Schmidt-Nielsen 1984), gill surface area (0.8 to 0.9, Hughes 1984, Schmidt-Nielsen 1984), whole-fish unidirectional water flux (0.8 to 0.9; Evans 1969) and gill mass as a function of total mass (0.86; Table 4). For four species, individuals differed in mass by at least an order of magnitude (species marked with asterisk in Table 3). This subset of the data includes two of the three species for which b values are significantly different from 1; the average b value for these species is 0.76. We predict that, had we been able to obtain a similar size range for the other species examined, stronger scaling relationships would have been determined, and less intraspecific variation in b values would have been found.

We conclude that the size-related differences in total branchial Na^+-K^+-ATPase activity are due to size-related differences in gill mass (as a fraction of total mass), and not scaling of specific enzymatic activity with body size. Furthermore, the absence of scaling in the specific activity of Na^+-K^+-ATPase indicates that the depth-related trends shown in Tables 1 and 2 are not a reflection of differences in size among the species or individuals compared. The differences are due to lower Na^+-K^+-ATPase activities in the gills of deep-sea fishes, with depth-related differences in gill mass possibly leading to even greater differences in total branchial Na^+-K^+-ATPase activity.

Because of the large differences between shallow- and deep-living fishes in activities of Na^+-K^+-ATPase and metabolic rates, we sought to compare the total cost of osmoregulation, as a fraction of overall metabolism (ATP turnover), in these two groups of fishes. If the dominant share of osmoregulatory cost involves monovalent-ion transport at the gills (see below), and if the gill Na^+-K^+-ATPase were to function at maximal velocity (the condition employed in our assays), then a maximal cost of osmoregulation can be obtained. We tested the hypothesis that, despite the strong depth-related changes in these two traits, the fraction of ATP turnover devoted to osmoregulation was not different among species occurring at different depths.

Our estimates of maximal osmoregulatory costs for ten marine teleosts (Table 5) are lower than those obtained by Rao (1968) and Febry and Lutz (1987), who based their analyses on euryhaline species acclimated to different salinities. Their data suggest that the cost of osmoregulation may reach 25 to 30% of metabolism, depending on the osmotic gradient and activity level of the fish. Our lower estimates of osmoregulatory costs could be due to several factors. First, marine species may differ from the euryhaline freshwater species examined by the other investigators. Second, monovalent regulation may be underestimated using only branchial Na^+-K^+-ATPase activity. Third, regulation of ions other than Na^+ and K^+ may require a substantial fraction of total osmoregulatory energy.

The actual cost of branchial monovalent-ion regulation will be lower than our estimated values based on maximal Na^+-K^+-ATPase activities. The main factor regulating the activity of Na^+-K^+-ATPase is thought to be the intracellular concentration of Na^+, $[Na]_i$ (Yamamoto et al. 1979). At *in vivo* $[Na]_i$ (30 to 70 mM; Shelton et al. 1985), the activity of teleost gill Na^+-K^+-ATPase would be about three-fourths of maximal velocity (Gibbs and Somero 1989). Thus, the estimated fraction of total ATP turnover that is required for meeting the demands of osmoregulation in these ten species would average somewhat less than 10%. This fraction of ATP turnover is in the range estimated for gill metabolism relative to overall metabolic rate (Itazawa and Oikawa 1983, McCormick et al. 1989).

The large interspecific variation in the estimated osmoregulatory cost is undoubtedly due to several factors. As indicated, the scaling relationships for Na^+-K^+-ATPase activity are not very precise in most instances, so our size correction may reduce the reliability of our estimates. The oxygen-consumption measurements were made under a variety of conditions, using specimens that no doubt differed in physiological state and activity level. Within the limits of resolution afforded by our analysis, we conclude that there is no apparent change in osmoregulatory cost as a fraction of total metabolism with depth, despite a large decrease in total ATP expenditure for osmoregulation in deep-living fishes.

The link between metabolic costs for locomotion and those for osmoregulation appears direct. Vigorous swimming entails high flows of water over the gill surface, and the accompanying high oxygen demands, both during swimming and subsequent repayment of the incurred oxygen debt, dictate a high flow of blood through the gill lamellae. Thus, a fish that has high locomotory capability will have high amounts of passive ion and water flux, i.e., high osmoregulatory costs. The increase in osmoregulatory cost for a given species with swimming speed (Rao 1968, Febry and Lutz 1987) is consistent with this hypothesis. We propose, then, that lower Na^+-K^+-ATPase activities in the gills of deep-living species are a consequence of reduced osmoregulatory costs due to these fishes' sluggish locomotory habits. We view the low Na^+-K^+-ATPase activities in the gills of sluggish deep-sea species not to be a result of primary selection for reduced osmoregulatory costs, but rather a beneficial (in terms of

energy needs) concomitant of reduced locomotory activity. The relatively high activities of Na^+-K^+-ATPase in the gills of the two fishes endemic to the hydrothermal vents, the zoarcid *Thermarces andersoni* from 13 °N on the East Pacific Rise, and the bythitid *Bythites hollisi* from the Galápagos site, are proposed to reflect higher metabolic and locomotory capacities in these species relative to typical, i.e., non-vent, deep-sea fishes.

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