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Gill and intestinal Na^+-K^+ ATPase activity, and estimated maximal osmoregulatory costs, in three high-energy-demand teleosts: yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*), and dolphin fish (*Coryphaena hippurus*)

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Abstract We hypothesize that the morpho-physiological adaptations that permit tunas to achieve maximum metabolic rates (MMR) that are more than double those of other active fishes should result in high water and ion flux rates across the gills and concomitant high osmoregulatory costs. The high standard metabolic rates (SMR) of tunas and dolphin fish may, therefore, be due to the elevated rates of energy expenditure for osmoregulation (i.e. teleosts capable of achieving exceptionally high MMR necessarily have SMR). Previous investigators have suggested a link between activity patterns and osmoregulatory costs based on Na^+-K^+ ATPase activity in the gills of active epipelagic and sluggish deep-sea fishes. Based on these observations, we conclude that high-energy-demand fishes (i.e. tunas and dolphin fish) should have exceptionally elevated gill and intestinal Na^+-K^+ ATPase activity reflecting their elevated rates of salt and water transfer. To test this idea and estimate osmoregulatory costs, we measured Na^+-K^+ ATPase activity (V_{max}) in homogenates of frozen samples taken from the gills and intestines of skipjack and yellowfin tunas, and the gills of dolphin fish. As a check of our procedures, we made similar measurements using tissues from hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). Contrary to our supposition, we found no difference in Na^+-K^+ ATPase activity per unit mass of gill or intestine in these four species. We estimate the cost of osmoregulation to be at most 9% and 13% of the SMR in skipjack tuna and yellowfin tuna, respectively. Our results, therefore, do not support either of our original suppositions, and the cause(s) underlying the high SMR of tunas and dolphin fish remain unexplained.

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Introduction

A suite of morpho-physiological adaptations provides skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albcares*) with the ability to achieve routine O_2 transfer factors (TO_2, the rate of O_2 transfer from water to blood per unit partial pressure difference between inhalant water and venous blood) at least an order of magnitude above those of other fishes (Bushnell and Brill 1992), and maximum metabolic rates (MMRs) more than double those of other active teleosts (Brett and Glass 1973; Gooding et al. 1981; Dewar and Graham 1994). Anatomical adaptations include gill surface areas approximately an order of magnitude larger and gill blood–water barriers up to approximately an order of magnitude thinner than other fishes (Muir and Hughes 1969; Hughes 1984; Perry 1992), and fusion of adjacent gill filaments or secondary lamella (Muir and Kendall 1968). Tunas also have routine cardiac outputs and ventilation volumes at least several times greater and up to more than an order of magnitude greater than those of other teleosts (Jones et al. 1990; Brill and Bushnell 1991, 2001; Bushnell and Jones 1994).

The advantages conferred on tunas and dolphin fish by their ability to achieve exceptionally high MMRs have been enumerated by Pauly (1981), Brill (1996), and Koresmeyer et al. (1996). Several authors (e.g. Priede 1985; Brill 1987) also hypothesized, however, that fish with the anatomical characteristics necessary to achieve high MMRs would also have high standard metabolic rates (SMRs) because of relatively high osmoregulatory

costs, the latter resulting from high passive water and ion flux rates occurring across their large thin gills. Subsequent studies confirmed that tunas and dolphin fish (*Coryphaena hippurus*) have comparable gill surface areas and SMRs (Brill 1987; Benetti et al. 1995). A similar connection between gill surface area and SMR has been established for aquatic salamanders (Ultsch 1973, 1976), although the problem of discerning "cause and effect" remains unsolved.

Support for the presumption of high osmoregulatory costs in tunas and dolphin fish comes from the well-described linkage of O_2 uptake rates and passive ion and water flux rates across the gills of freshwater fishes (Wood and Randall 1973a, b, c; Gonzalez and McDonald 1994). The dynamic conflict between gas exchange and passive ion and water movement (i.e. osmoregulatory burden) has long been recognized (e.g. Steen and Kruysse 1964; Randall et al. 1972) and has been formally termed the "osmorespiratory compromise" (Nilsson 1986; Gonzalez and McDonald 1992) or "respiratory-osmoregulatory compromise" (Wood and Randall 1973a; Nilsson and Sundin 1998). It is generally accepted to be the reason for the tight control of blood flow pathways (i.e. functional surface area) within the gills (Nilsson and Sundin 1998). Gonzalez and McDonald (1992, 1994), moreover, have shown that in several freshwater species elevated rates of O_2 uptake recorded following exhaustive exercise are accompanied by proportionally greater increases in rates of Na^+ efflux. They suggested that the disproportionate increase in osmoregulatory burden may be one of the factors limiting the maximum aerobic metabolic rates in fishes. Febry and Lutz (1987) estimated that osmoregulatory costs increase from essentially zero at rest to 12–16% of the metabolic rate at the upper range of sustainable swimming speeds in hybrid tilapia (*Oreochromis mossambicus* × *O. hornorum*). In contrast, however, they concluded that the energy costs of osmoregulation do not significantly limit the rate of O_2 delivery to the swimming muscles.

Attempts to quantify directly the energy devoted to osmoregulation in tunas by measuring whole animal metabolic rates at different salinities have failed (R.W. Brill, D.R. Jones, and P.G. Bushnell, unpublished observations), presumably because tunas lack the mechanisms to adjust osmoregulatory function that are available to euryhaline fishes (Maetz 1974). Morgan et al. (1996) have shown that juvenile (20–30 g) dolphins have 15% lower active metabolic rates when adapted to a salinity (20‰) that is nearly iso-osmotic with their plasma than they do in full-strength seawater (34‰). A significant number of studies, however, have shown that alterations in the metabolic rates of fishes often do not correlate with expected changes in osmoregulatory costs. In other words, decreases in metabolic rate do not necessarily occur as external salinities are made iso-osmotic with the plasma (e.g. Febry and Lutz 1987; Swanson 1998), and the suitability of estimating osmoregulatory costs by this method has been questioned (e.g. Nordlie 1978; Febry and Lutz 1987). Direct measurement of sodium and water flux rates in tunas has never been attempted. Indeed, these experiments would be extremely difficult (if not impossible) because of the large tank and respirometer volumes required when working with live tunas (e.g. Nakamura 1972; Dewar and Graham 1994).

An alternative method for estimating osmoregulatory costs is to quantify rates of ATP consumption devoted to active ion transport. This is accomplished by measuring, in vitro, the activity of sodium-potassium-activated adenosine triphosphatase (Na^+-K^+ ATPase, E.C.3.6.1.3) from gill and intestinal tissue samples (Kirschner 1995). This enzyme plays a critical role in sodium and water balance in both marine and freshwater fishes because it is involved in active sodium transport (Jampol and Epstein 1970; Epstein et al. 1980; Borgatti et al. 1992). In the gills, Na^+-K^+ ATPase drives the active ion transport processes of the "chloride" or "mitochondria-rich" cells (Epstein et al. 1980; Foskett and Scheffey 1982; Marshall and Bryson 1998). Na^+-K^+ ATPase similarly activates the ion pumps in the intestine. In seawater fish, the intestine actively absorbs Na^+ and Cl^- until intestinal fluid becomes hypo-osmotic to plasma, at which time water moves passively into the plasma, thereby maintaining overall osmotic balance (Kirsch et al. 1985; Karnaky 1998). In hypo-regulating marine fishes, drinking rates are relatively high and presumably would be exceptionally high for tunas and dolphin fish because their large gill surface areas and thin blood–water barriers should facilitate high rates of passive water loss.

Gibbs and Somero (1990) found that very inactive (i.e. low-energy-demand), deep-living fishes had gill Na^+-K^+ ATPase activity levels only approximately one-third those of more active, shallow-living species. They attributed this to differences in osmoregulatory costs and ultimately locomotor capacities. More specifically, they concluded that fish inhabiting the dark, energy-sparse environment of the pelagic deep sea evolved reduced locomotor energy expenditures and thereby relatively low metabolic rates. Lower metabolic rates require lower ventilation volumes, cardiac outputs, and functional gill surface areas. These should result in lower rates of passive ion and water movement across the gills, processes that must be counteracted by Na^+-K^+ ATPase-driven active ion transport. The observations of Gibbs and Somero (1990), therefore, provide additional (albeit indirect) evidence for the supposition that MMR, SMR, gill surface area, TO_2, and osmoregulatory costs are all linked.

We undertook our study to test the hypothesis that the specialized cardio-respiratory anatomy and physiology of tunas and dolphin fish, which permit their high-energy-demand lifestyle, also result in high osmoregulatory costs. Specifically, we propose that gill and intestinal Na^+-K^+ ATPase activity levels are elevated compared to other teleosts. We are thus re-examining the ideas of Gibbs and Somero (1990), that locomotor

capabilities and osmoregulatory costs are correlated, but by looking at fish at the opposite (i.e. high) end of the energy-demand spectrum. We therefore measured Na^+-K^+ ATPase activity in gills from skipjack tuna, yellowfin tuna, dolphin fish, and hybrid red tilapia (*O. mossambicus* × *O. niloticus*), and the intestine of skipjack and yellowfin tunas, and hybrid red tilapia. We included tilapia because of the extensive data already published on their metabolic rates, estimated osmoregulatory costs, and gill and intestinal Na^+-K^+ ATPase activity levels (e.g. Dange 1985; Febry and Lutz 1987; Hwang et al. 1989; Kültz et al. 1992; Morgan et al. 1997; Woo et al. 1997). These data provide a benchmark for our laboratory procedures. We also use our data to estimate the maximal fraction of the SMR accounted for by osmoregulatory costs by calculating the O_2 consumption attributable to maximal gill and intestinal Na^+-K^+ ATPase activities.

Materials and methods

Tissue collection and storage

Gill tissue was sampled from 25 yellowfin tuna (*Thunnus albacares*; 0.515–2.275 kg), 11 skipjack tuna (*Katsuwonus pelamis*; 0.645–1.745 kg), 14 hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*; 36–64 g), and 7 dolphin fish (*Coryphaena hippurus*; 6.5–14.5 kg). Intestinal tissue was sampled from 15 yellowfin tuna (1.351 ± 0.118 kg), 10 skipjack tuna (1.128 ± 0.690 kg), and 7 hybrid red tilapia (approximate body mass 100–120 g). The tunas and tilapia were maintained at the Kewalo Research Facility (National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Laboratory, Hawaii, USA). Fish were held in outdoor tanks supplied with running seawater (25° ± 1 °C and ≈34‰). Animal procurement, care, and handling procedures at the Kewalo Research Facility are described in Nakamura (1972). Dolphin fish were captured near the main Hawaiian Islands during research cruises aboard the NOAA vessel "Townsend Cromwell," and tissue samples were obtained at sea.

Fish were sacrificed either by a blow to the head, direct brain destruction (pithing), or an overdose of sodium phenobarbital. Gill filaments (1- to 2-g samples) were immediately trimmed from the first gill arch of tunas and dolphin fish, or from all the gill arches in tilapia, rinsed in ice cold SEI buffer (0.05 *M* imidazole, 0.02 *M* sodium EDTA, 0.3 *M* sucrose, pH 7.3) or 1.17% saline, and immediately frozen in liquid nitrogen. (The rinsing medium was not found to have any effect on measured ATPase activity.) To obtain estimates of gill filament mass, all four gill arches from one side of the branchial basket were removed and blotted dry. The filaments were then trimmed off and weighed. Total gill filament mass was estimated by doubling the measured mass.

For intestinal tissue samples from tuna, the entire organ was removed, blotted dry, the surrounding membranous tissue and blood vessels dissected away, and the contents evacuated. The gut was then weighed, divided into anterior and posterior sections according to locations described by Godsil and Byers (1944), opened longitudinally, rinsed in ice-cold SEI buffer, and immediately frozen in liquid nitrogen. For tilapia, portions (1–2 g) of anterior intestine were removed and treated as described above. All tissues were stored at −80 °C until analyzed.

Na^+-K^+ ATPase activity

Na^+-K^+ ATPase activities were measured in crude tissue homogenates or a suspension made from an isolated membrane fraction (gill tissue only). To prepare the former, approximately 250 mg of frozen gill or 400 mg frozen intestine was homogenized (using a Tissuemizer Model SDT-1810, Tekmar, Ohio, USA) in 4 ml of ice-cold SEID buffer (0.05 *M* imidazole, 0.02 *M* sodium EDTA, 0.3 *M* sucrose, 0.1% sodium desoxycholate, pH 7.3). The homogenate was centrifuged for 30 s in an Eppendorf centrifuge (≈16,000*g*) and the supernatant removed and stored on ice. Assays were then run immediately as described below. To prepare isolated membrane fractions, we followed the techniques of Gibbs and Somero (1990). Approximately 250 mg of gill tissue was homogenized and the crude homogenate centrifuged for 15 min at 2,000*g* to remove cellular debris. The supernatant was decanted and centrifuged for 90 min at 19,000*g*. (Both centrifugations were carried out at 4 °C.) The resultant membrane fraction pellet was re-suspended in 1 ml of homogenization buffer and analyzed immediately.

Na^+-K^+ ATPase activity was determined following the methods of Kültz and Somero (1995) with only minor modifications. The assay is based on the equimolar coupling of ATPase activity to the conversion of NADH to NAD, the latter being measured spectrophotometrically at 340 nm. The assay buffer contained 30 m*M* imidazole, 45 m*M* NaCl, 15 m*M* KCl, 3 m*M* $MgCl_2 \cdot 6H_2O$, 0.4 m*M* KCN, with pH adjusted appropriately. The reaction mixture, made fresh daily using the assay buffer, contained 1 m*M* Na_2ATP, 0.2 m*M* Na_2NADH, 2 m*M* PEP, 3 IU ml^{-1} pyruvate kinase, 2 IU ml^{-1} lactate dehydrogenase. All assays for gill tissue samples were performed at 25 °C (± 0.2 °C) and pH 7.5, except for those conducted at 15 °C and 30 °C and various pH specifically to measure the effects of those variables. All assays of intestinal Na^+-K^+ ATPase activity were likewise performed at 25 °C (± 0.2 °C). Salt and pH conditions were optimized, and final values were nearly identical to optimal conditions for salmonid intestinal Na^+-K^+ ATPase reported by Gjevre and Næss (1996). All reagents were obtained from Sigma, St, Louis, Mo., USA.

To measure total ATPase activity, 1,000 µl reagent mixture, 100 µl distilled water, and 20 µl tissue homogenate or membrane fraction suspension were combined in a spectrophotometer cuvette, mixed by inverting, and placed in a spectrophotometer (Shimadzu Bio-Spec 1601, Shimadzu Scientific Instruments Inc., Columbia, Md., USA) equipped with UVPC Spectroscopy software (revision 3.7). Na^+-K^+ ATPase activity was determined as the difference between total ATPase activity and that measured when the distilled water was replaced with 100 µl of 11.25-m*M* ouabain (a specific inhibitor of Na^+-K^+ ATPase). All assays were run in at least duplicate. Total protein content was measured following Bradford (1976), using bovine albumin as the standard. ATPase activities were expressed as the change in µmoles of ATP · mg^{-1} protein · h^{-1} and the change in µmoles of ATP · mg^{-1} (wet tissue weight) · h^{-1}.

Comparisons of means among the three species of fish were performed using one-way analysis of variance (ANOVA, SigmaStat, version 2.30, SPSS Inc.) with $P < 0.05$ taken to indicate significant differences.

Results

Na^+-K^+ ATPase activity: gill tissue

The Na^+-K^+ ATPase activity in the crude homogenate of gill tissue, and the membrane fractions produced therefrom, are given in Table 1. The Na^+-K^+ ATPase activities per milligram protein were not different ($P > 0.10$) in gill tissue homogenate from yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*), or in gill tissue from dolphin fish (*Coryphaena hippurus*) and hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). The Na^+-K^+ ATPase activity per milligram protein was significantly higher in the gills from dolphin fish and tilapia than in tuna gills, although

Table 1 *Thunnus albacares*, *Katsuwonus pelamis*, *Coryphaena hippurus*, *Oreochromis mossambicus* × *O. niloticus*. Mean (±SEM) Na^+-K^+ ATPase activity (measured at 25 °C) in crude homogenate and membrane fractions prepared from frozen gill tissue for three species of high-energy-demand fishes and hybrid red tilapia

Na^+-K^+ ATPase activity	Yellowfin tuna	Skipjack tuna	Dolphin fish	Tilapia
Homogenate	4.6 ± 0.5	4.2 ± 0.8	9.7 ± 1.1	7.3 ± 0.4
μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$	n = 14	n = 12	n = 12	n = 13
Homogenate	245 ± 20	217 ± 36	342 ± 39	341 ± 41
μmol ATP $^{-1}$ h^{-1} g gill $filament^{-1}$	n = 8	n = 10	n = 7	n = 7
Membrane fraction	20.6 ± 3.0	18.0 ± 3.7	23.3 ± 6.4	46.9 ± 5.3
μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$	n = 8	n = 10	n = 12	n = 9
Membrane fraction	50.0 ± 6.3	72.0 ± 14.0	60.1 ± 16.8	158 ± 11
μmol ATP $^{-1}$ h^{-1} g gill $filament^{-1}$	n = 8	n = 10	n = 12	n = 9

the absolute differences were relatively small compared to the range of values (≈5–10 μmol ATP^{-1} h^{-1} mg protein) reported for seawater-adapted teleosts (e.g. De Renzis and Bornancin 1984; Dange 1985; Kültz et al. 1992). When expressed per gram of gill filament, however, there were no significant differences among species. Likewise, there were no differences in the Na^+-K^+ ATPase activity in the membrane fractions isolated from skipjack tuna, yellowfin tuna, or dolphin fish gills, although activity in the membrane fraction of tilapia gill was higher ($P < 0.05$) whether expressed as per milligram protein or per gram gill filament. In homogenates from yellowfin tuna gills, pH had no significant effect on Na^+-K^+ ATPase activity over the range 7.4–7.7; the temperature effect (Q_{10}) was 2.09 when calculated from data taken at 15 and 30 °C.

Steps taken to purify the enzyme by isolating membrane fractions were clearly effective in that Na^+-K^+ ATPase activity per milligram protein increased by ≈2–6 times. In our laboratory, however, the losses of activity resulting from procedures to isolate the membrane fractions were higher (≈50–80% when activity is expressed per gram of gill filament) than reported by Gibbs and Somero (1990) (20–40%), even though we followed essentially identical procedures.

Na^+-K^+ ATPase activity: intestine

Enzyme activities in intestinal tissues are given in Table 2. When expressed per milligram protein, Na^+-K^+ ATPase activity was not significantly different in anterior intestines from yellowfin and skipjack tunas, whereas the activity in tilapia intestine was clearly higher ($P < 0.001$). There were significant differences in enzyme activity in the anterior and posterior intestines from skipjack tuna, but not yellowfin tuna. Values for both anterior and posterior intestines were averaged to calculate activity per gram intestine in tunas.

Gill filament and intestinal mass

Data on gill filament mass are shown in Fig. 1. Data were fitted to the power function gill mass = $a \cdot$ body $mass^b$, with both expressed in grams. For comparison, equivalent data from porgy (*Pagrus major*, Oikawa and Itazawa 1984) and carp (*Cyprinus carpio*, Oikawa et al. 1992) are also shown. Predicted gill filament mass for 1-kg carp, porgy, tilapia, yellowfin tuna, and skipjack tuna is 12.2 g, 10.1 g, 10.3 g, 26.6 g, and 21.4 g, respectively. In other words, tunas have a gill filament mass about twice those of the other species. The size range of tilapia available for our study did not overlap that of yellowfin and skipjack tunas (the regression lines in Fig. 1 show the range in body mass over which gill filament mass data were obtained). The relationship of gill filament mass to body mass in tilapia, however, is close to that for carp and porgy where data from 1-kg fish are available. Extrapolation of tilapia data to1 kg body mass appears to yield a reasonable estimate of gill filament mass (Fig. 1).

Data on intestinal mass are shown in Fig. 2. Data were fitted to the power function intestinal mass = $a \cdot$ body $mass^b$, with both expressed in grams.

Table 2 *Thunnus albacares*, *Katsuwonus pelamis*, *Oreochromis mossambicus* × *O. niloticus*. Mean (±SEM) intestinal Na^+-K^+ ATPase activity (measured at 25 °C) in crude homogenates prepared from frozen intestinal tissue for two species of high-energy-demand fishes and hybrid red tilapia

Na^+-K^+ ATPase activity	Yellowfin tuna	Skipjack tuna	Tilapia
Anterior intestine	2.7 ± 0.3	2.6 ± 0.5	5.2 ± 0.5
μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$	n = 15	n = 10	n = 7
Posterior intestine	4.1 ± 0.5	7.3 ± 0.7	–
μmol ATP $^{-1}$ h^{-1} mg protein	n = 14	n = 10	
Whole intestine	165 ± 16	227 ± 41	439 ± 42
μmol ATP $^{-1}$ h^{-1} g gill wet $weight^{-1}$	n = 14	n = 10	n = 7

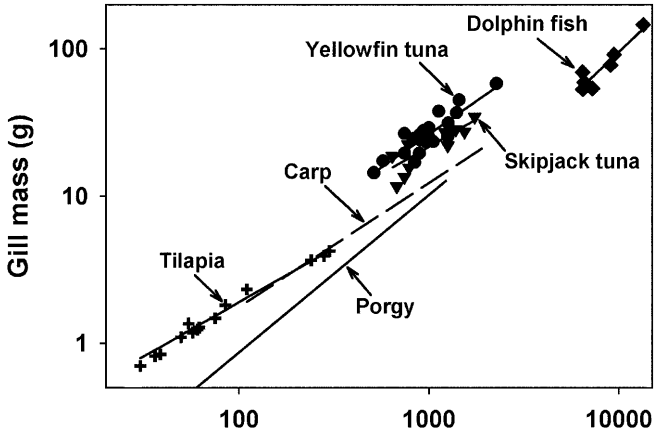

Fig. 1 *Thunnus albacares*, *Katsuwonus pelamis*, *Coryphaena hippurus*, *Oreochromis mossambicus* × *O. niloticus*, *Pagrus major*, *Cyprinus carpio*. Relationship between body mass and gill filament mass in yellowfin tuna, skipjack tuna, dolphin fish, hybrid red tilapia, porgy, and carp. Data for porgy and carp were taken from Oikawa and Itazawa (1984) and Oikawa et al. (1992). In all cases, *lines* show the range of body mass over which data were obtained. Regression lines were constructed by fitting data to the power function gill mass = $a \cdot$ body mass^b. The resultant parameters (±SE) were *yellowfin tuna* ($n = 24$, $r^2 = 0.83$) $a = 0.0371 \pm 0.0208$ and $b = 0.952 \pm 0.079$; *skipjack tuna* ($n = 11$, $r^2 = 0.74$) $a = 0.128 \pm 0.138$ and $b = 0.743 \pm 0.151$; *dolphin fish* ($n = 7$, $r^2 = 0.87$) $a = 3.83 \times 10^{-5} \pm 1.09 \times 10^{-4}$ and $b = 1.61 \pm 0.87$; *tilapia* ($n = 14$, $r^2 = 0.99$) $a = 0.0650 \pm 0.008$ and $b = 0.733 \pm 0.023$

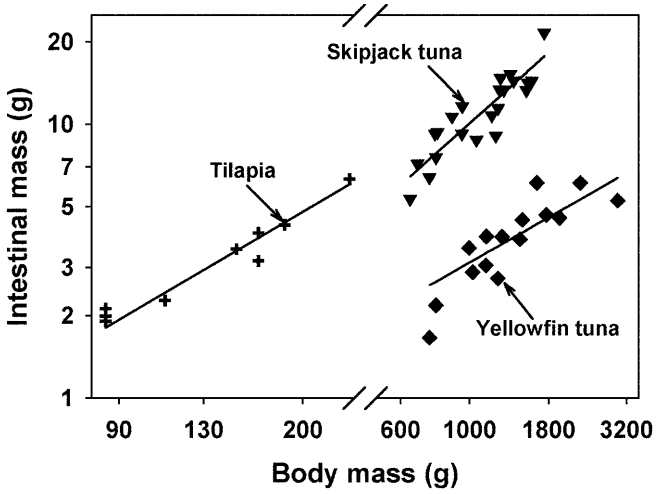

Fig. 2 *Thunnus albacares*, *Katsuwonus pelamis*, *Oreochromis mossambicus* × *O. niloticus*. Relationship between body mass and intestinal mass in yellowfin tuna, skipjack tuna, and hybrid red tilapia. *Regression lines* were constructed by fitting data to the power function gill mass = $a \cdot$ body mass^b. The resultant parameters (±SE) were *yellowfin tuna* ($n = 15$, $r^2 = 0.67$) $a = 0.0361 \pm 0.0341$ and $b = 0.646 \pm 0.128$; *skipjack tuna* ($n = 22$, $r^2 = 0.78$) $a = 0.0091 \pm 0.0083$ and $b = 1.01 \pm 0.13$; *tilapia* ($n = 9$, $r^2 = 0.96$) $a = 0.0115 \pm 0.0058$ and $b = 1.41 \pm 0.10$

O_2 consumption due to Na^+-K^+ ATPase activity

Because the Na^+-K^+ ATPase activity per gram of gill filament is relatively invariant, the maximum O_2 consumption attributable to activity of this enzyme is directly proportional to gill filament mass. Based on predicted gill filament mass and Na^+-K^+ ATPase activity per gram measured in the homogenate, ATP turnover would account for a maximum of 1,074 μmol O_2 h^{-1} and 775 μmol O_2 h^{-1} (assuming 6 mol ATP are generated per mole of O_2 consumed) in 1-kg yellowfin and skipjack tunas, respectively. The SMRs of 1-kg yellowfin and skipjack tunas (at 25 °C) are 8,983 μmol O_2 h^{-1} and 12,895 μmol O_2 h^{-1}, respectively (Brill 1987). Therefore, O_2 consumption due to gill Na^+-K^+ ATPase activity could account for at most 12% and 6% of the SMRs (respectively) of these two species. For a 10-kg dolphin fish, gill Na^+-K^+ ATPase activity would account for a maximum of 5.75 m*M* O_2 h^{-1} or 21% of the SMR (27.2 m*M* O_2 h^{-1}, Benetti et al. 1995). The SMR of 100-g tilapia (*Oreochromis niloticus niloticus*) acclimated to 25 °C and salinity 30‰ is 419 μmol O_2 h^{-1} (Farmer and Beamish 1969). From our data, tilapia gill Na^+-K^+ ATPase activity would result in a maximal O_2 consumption of 108 μmol h^{-1} or 26% of the SMR.

Based on Na^+-K^+ ATPase per gram intestine, ATP turnover would account for a maximum of 86 μmol O_2 h^{-1} and 382 μmol O_2 h^{-1} in a 1-kg yellowfin tuna and skipjack tuna, respectively, and 159 μmol O_2 h^{-1} in a 100-g tilapia. These represent 1%, 3%, and 37% of the SMR in yellowfin tuna, skipjack tuna, and hybrid red tilapia, respectively. By adding the energetic costs of operating Na^+-K^+ ATPase pumps in both gills and intestine, we calculate that maximal O_2 consumption devoted to osmoregulation could account for 13%, 9%, and 63% of the SMR for 1-kg yellowfin, 1-kg skipjack tuna, and 100-g tilapia, respectively.

Discussion and conclusions

Na^+-K^+ ATPase activities in teleost gill tissue differ significantly among published reports (summarized in Gjevre and Næss 1996), the highest values being approximately five times the lowest (when corrected for measurement temperatures). Similarly, intestinal Na^+-K^+ ATPase activities also vary significantly (Table 3). These discrepancies likely result from the use of different substrate and ion concentrations, pH, and specific buffers (Rey et al. 1991; Gjevre and Næss 1996), thereby rendering direct comparisons of our results from tunas and dolphin fish to data from other laboratories difficult. For this reason, and to check our methods directly, we measured Na^+-K^+ ATPase activity in gills and intestine from the well-studied tilapia. The Na^+-K^+ ATPase activity we found in gills from hybrid red tilapia (7.33–0.4 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$) is within the range reported for tilapia species by Dange (1985), Hwang et al. (1989), and Kültz et al. (1992) (≈5–10 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$, corrected to 25 °C when necessary assuming a Q_{10} of 2). Our value, however, exceeds that reported for Mozambique tilapia

Table 3 Mean Na^+-K^+ ATPase activity (μmol ATP h^{-1} mg $protein^{-1}$) in intestinal homogenates of fish in freshwater (FW) and seawater (SW) reported in the literature. When necessary, values have been corrected to 25 °C assuming $Q_{10} = 2$

Species	Na^+-K^+ ATPase activity	Reference
European eel (*Anguilla anguilla*)		
Whole intestine (SW)	10.5	Lionetto et al. 1998
Freshwater eel (*Anguilla rostrata*)		
Whole intestine (FW)	≈3.5	Jampol and Epstein 1970
Whole intestine (SW)	7.8	Jampol and Epstein 1970
Striped bass (*Morone saxatilis*)		
Middle intestine (SW)	≈12	Madsen et al. 1994
Posterior intestine (SW)	≈5.5	Madsen et al. 1994
Middle intestine (FW)	≈8.5	Madsen et al. 1994
Posterior intestine (FW)	≈5	Madsen et al. 1994
Atlantic salmon (*Salmo salar*)		
Whole intestine (FW)	≈1	Gjevre and Næss 1996
Rainbow trout (*Oncorhynchus mykiss*)		
Whole intestine (FW)	≈1	Gjevre and Næss 1996
Middle intestine (FW)	≈0.2	Fuentes et al. 1997
Middle intestine (SW)	≈0.6	Fuentes et al. 1997
Middle intestine (FW)	2.2	Colin et al. 1985
Posterior intestine (6) (FW)	1.2	Colin et al. 1985
Middle intestine (6) (SW)	4.0[a]	Colin et al. 1985
Posterior intestine (6) (SW)	2.4[a]	Colin et al. 1985
Anterior intestine (7) (FW)	range: ≈0.3–2.6[b]	Rey et al. 1991
Middle intestine (7) (FW)	range: ≈0.06–3.4[b]	Rey et al. 1991
Posterior intestine (7) (FW)	range: ≈0.06–1.1[b]	Rey et al. 1991
Tilapia (*Oreochromis mossambicus*)		
Anterior intestine (SW)	4.2	Reshkin et al. 1989
Posterior intestine (SW)	4.7	Reshkin et al. 1989
Anterior intestine (6) (SW)	15.9	Reshkin and Ahearn 1987
Posterior intestine (6) (SW)	19.3	Reshkin and Ahearn 1987
Black seabream (*Mylio macrocephalus*)		
Stomach (6) (SW)	5.1	Kelly et al. 1999
Stomach (6) (FW)	8.3	Kelly et al. 1999
Midgut (6) (SW)	7.9	Kelly et al. 1999
Midgut (6) (FW)	3.5	Kelly et al. 1999
Rectum (6) (SW)	8.8	Kelly et al. 1999
Rectum (6) (FW)	2.4	Kelly et al. 1999

[a] Assuming temperature at 25 °C
[b] Investigated over a wide range of temperatures, photoperiods, and pH

(*Oreochromis mossambicus*) acclimated to seawater (≈1–2 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$) by Vijayan et al. (1996) and Morgan et al. (1997). The Na^+-K^+ ATPase activity we measured in homogenates of hybrid red tilapia anterior intestine (5.2 ± 0.47 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$) is similar to values (4.2–4.7 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$) for epithelial homogenate from Mozambique tilapia acclimated to sea water (Reshkin et al. 1989). We conclude, therefore, that our laboratory procedures were suitable and our results comparable to those from other laboratories.

Gill Na^+-K^+ ATPase activity

Gibbs and Somero (1990) clearly showed that Na^+-K^+ ATPase activities in the gills of meso-pelagic fishes are reduced compared to those of active epipelagic species. The authors attribute this to the sluggish lifestyle, low metabolic rates, and consequently reduced osmoregulatory costs in the former. In support of their contention that deep-sea fishes are "sluggish" compared to epipelagic fishes, Gibbs and Somero (1990) cite the fact that muscle lactate dehydrogenase (LDH) levels in the former are one to three orders of magnitude lower than those of the latter (Sullivan and Somero 1980), indicating a reduced capacity for anaerobic ATP production in deep-sea fishes. In contrast, tuna white muscle LDH levels exceed those of other active epipelagic fishes by at least two- to three-fold (Dickson 1995). Tunas, moreover, produce some of the highest white muscle lactate levels of any vertebrate (up to 150 μmol g^{-1} white muscle (Guppy et al. 1979; Arthur et al. 1992). By these measures, elevated gill Na^+-K^+ ATPase activity in tunas would be expected. In our study, however, the linkage of locomotor activity or maximum aerobic metabolic rates and gill Na^+-K^+ ATPase activity is not seen. The Na^+-K^+ ATPase activity we measured in crude homogenates of tuna, dolphin fish, and hybrid red tilapia gills (217–342 μmol ATP $^{-1}$ h^{-1} g^{-1}) overlap the range Gibbs and Somero (1990) reported for six epipelagic marine fishes (144–336 μmol ATP $^{-1}$ h^{-1} g gill $filament^{-1}$, corrected to 25 °C, assuming a Q_{10} of 2). The level of Na^+-K^+ ATPase activity we measured in

dolphin fish gills (9.75 ± 1.12 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$) exceeds that reported by Morgan et al. (1996) for gills from juvenile (20–40 g body mass) dolphin fish (3.6 ± 0.2 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$ at 25 °C) but approaches that measured in gills from seawater-acclimated striped bass (*Morone saxatilis*) at 25 °C (≈11–12 μmol ATP $^{-1}$ h^{-1} mg $protein^{-1}$) (Madsen et al. 1994). We conclude therefore that, contrary to our predictions, high-energy-demand fishes such as tunas and dolphin fish do not have elevated gill Na^+-K^+ ATPase activity compared to other active marine fishes. In brief, although gill Na^+-K^+ ATPase activity is correlated with the metabolic rates of active and slugglish marine fishes, this correlation is not seen when looking in the opposite direction, at high-energy-demand fishes such as tunas and dolphin fish.

Because we did not have access to fish over a large range of body masses, we cannot account for effects of this variable (Packard and Boardman 1999). Gibbs and Somero (1990), however, found the mass exponent relating gill Na^+-K^+ ATPase activity to body mass to be approximately 0.8–1 (i.e. the effect of body mass is relatively minor). Therefore, although the ranges of body masses from the four species we sampled do not all overlap with each other nor with other species used for equivalent studies, we still feel justified in comparing gill Na^+-K^+.

ATPase activities

We find our results surprising for the following reasons. Tunas have a TO_2 (i.e. the rate of O_2 transfer from the water to the blood per unit O_2 partial pressure difference between the water and venous blood) at least an order of magnitude above those measured in other fishes (Bushnell and Brill 1992). Less is known about the cardiorespiratory physiology of dolphin fish. But, like tunas, they have a gill surface area up to more than an order of magnitude larger, and a gill blood–water barrier up to almost an order of magnitude thinner, than other active fishes (Hughes 1972, 1977; Perry 1992; Palzenberger and Pohla 1992). These should result in high rates of passive salt and water movement across the gills accompanying high rates of O_2 transfer. High levels of Na^+-K^+ ATPase activity in gills of tunas are likewise predicted from activities of citrate synthase (CS), a mitochondrial enzyme often used as an index of the aerobic metabolic potential. Mean CS level (per g tissue wet wt^{-1}, at 10 °C) in the gills from three species of tuna (1.92 ± 0.07) is over two times the values reported for three species of ecothermic scombrids (0.90 ± 0.09) and over six times those reported for six species of deep-sea fishes (0.27 ± 0.07) (Dickson 1995).

Intestinal Na^+-K^+ ATPase activity

The levels of Na^+-K^+ ATPase activity in yellowfin tuna and skipjack tuna anterior intestine are four times higher than values for middle intestine of rainbow trout (*Oncorhynchus mykiss*) acclimated to seawater (Fuentes et al. 1997), and almost three times higher than rainbow trout in freshwater (Gjevre and Næss 1996). Our values are, however, only slightly lower than those reported for rainbow trout in seawater (Colin et al. 1985). The Na^+-K^+ ATPase activity in the European eel (*Anguilla anguilla*) intestine (Lionetto et al. 1998) is approximately three times greater than in tuna and two times greater than in tilapia. Na^+-K^+ ATPase activity of intestinal tissue from striped bass (*Morone saxatilis*) (Madsen et al. 1994) is similar to our measurements. In general, our data for yellowfin and skipjack tuna intestine (Table 2) are at the low end of the range reported for other species (Table 3).

Na^+-K^+ ATPase activity and maximal osmoregulatory costs

Na^+-K^+ ATPase activity in the gills of skipjack tuna, yellowfin tuna, and dolphin fish can account for a maximum of 6%, 12%, and 21% of the SMR, respectively. These values are below that found for hybrid red tilapia (26% of the SMR) but are similar to the range (10–20% of the SMR) reported by Gibbs and Somero (1990) for ten other teleost species. These results imply that osmoregulatory costs are not exceptionally elevated in tunas. In contrast, Bushnell and Brill (1992) found that the metabolic rates of the gills alone can account for 68% and 54% of the metabolic rate of spinally blocked (i.e. non-swimming) skipjack and yellowfin tunas (respectively), well above the equivalent value reported for rainbow trout (27% of the SMR; Daxboeck et al. 1982). They attributed this difference (apparently incorrectly) to the higher osmoregulatory costs incurred by tunas. Bushnell and Brill (1992) also reported, however, the metabolic rates of skipjack and yellowfin tuna gills per unit surface area (6.70×10^{-6} and 4.42×10^{-6} ml min^{-1} mm^{-2}, respectively) to be somewhat below (when corrected for temperature differences) that reported for cod (*Gadus morhua*, 8.59×10^{-6} ml min^{-1} mm^{-2}; Johansen and Petterson 1981). This latter observation is somewhat surprising, given that (because of large gill surface areas) Na^+-K^+ ATPase activity per unit gill surface area in tunas must be well below those of other teleosts.

We are unaware of any study that has attempted to determine maximal osmoregulatory costs using Na^+-K^+ ATPase activity from both gills and intestine. Because reports on enzyme activity in the intestine do not include data on both enzyme activity per gram of intestine and intestinal mass, it is impossible to calculate maximal osmoregulatory costs based on Na^+-K^+ ATPase activity from the literature. In contrast, numerous investigators have attempted to use the metabolic rates of fishes either acutely exposed or adapted to various salinities to estimate osmoregulatory costs (e.g. Rao 1968, 1971; Farmer and Beamish 1969; Potts et al. 1973; Eddy 1975; Furspan et al. 1984; Febry and Lutz

1987; Perez-Pinzon and Lutz 1991; Kirschner 1993, 1995; Morgan et al. 1997; Swanson 1998). These experiments are based on the concept that fish in iso-osmotic media would have lower passive rates of salt and water fluxes and, therefore, a reduced energy expenditure on osmoregulation. Reductions in metabolic rate at iso-osmotic salinities are not always observed, however, even when they are accompanied by reductions in gill Na^+-K^+ ATPase activity (Morgan and Iwama 1998). These discrepancies notwithstanding, published estimates of osmoregulatory costs range from negligible up to approximately 30–50% of the active metabolic rate (Eddy 1975; Perez-Pinzon and Lutz 1991; Furspan et al. 1984). More importantly, osmoregulatory costs appear to increase with metabolic rate because of the linkage of O_2 transfer and passive ion and water flux rates across the gills. In hybrid tilapia (*Oreochromis mossambicus* × *O. hornorum*) osmoregulatory costs have been reported to increase from negligible, at rest, to 12–16% of the active metabolic rate during strenuous exercise (Febry and Lutz 1987).

Our calculated osmoregulatory costs based on maximal Na^+-K^+ ATPase activity for yellowfin and skipjack tuna (13% and 9% of the SMR, respectively) are approximately within the range for other fishes, whereas our calculated maximal osmoregulatory cost for tilapia (63% of the SMR) exceeds it. Without information on Na^+-K^+ ATPase activity in vivo, it is difficult to connect our data to whole animal osmoregulatory costs. However, even if Na^+-K^+ ATPase activity in vivo equals the maximal levels we measured in vitro, it could still not account for the elevated SMR of tunas (and most likely also dolphin fish). We conclude, therefore, that our data do not support our hypotheses, or the idea proposed by Brill (1987, 1996) that species capable of MMRs necessarily have high SMRs because of high osmoregulatory costs.

The reason(s) underlying the elevated SMRs of tunas and dolphin fish, therefore, remain to be explained. They cannot be due to ventilatory costs as these species are obligatory ram ventilators, and fish were force-ventilated when SMRs were measured (Brill 1987; Benetti et al. 1995). Tunas' elevated SMRs are also unlikely to be an artifact, as direct measurements of SMR in non-swimming tunas were identical to those quantified by the well-established method of estimating SMR by extrapolating swimming–metabolic rate curves back to zero swimming speed (Dewar and Graham 1994). They also cannot be due to elevated rates of protein synthesis (Brown and Cameron 1991), as fish were fasted prior to measurement of SMR. Skipjack and yellowfin tunas do have high cardiac output and ventral aortic pressures, so calculated cardiac energy outputs (22–25 mW kg^{-1}) are approximately an order above those of rainbow trout (1.5–2.9 mW kg^{-1}) (Brill and Bushnell 1991). Cardiac energy demand in tunas, however, is only 2–10% of the SMR, values comparable to those of rainbow trout (≈2–4% of the SMR).

The reasons underlying the elevated basal metabolic rates (BMRs) of endotherms compared to those of ectotherms has received considerable attention. Current hypotheses stress the importance of the energy required for maintaining trans-membrane gradients for setting BMR (e.g. Brand et al. 1991; Else and Wu 1999; Hulbert and Else 1999). Brand (1990) has specifically hypothesized that it is the rate of proton leak across the inner mitochondrial membrane that explains the linkage of the SMR and MMR in vertebrates. Alternatively, Coulson (1997) has suggested that it is the rate of metabolic substrate and O_2 delivered by the cardiovascular system that is the primary controller setting metabolic rates. Further investigation of the physiological and biochemical mechanisms responsible for tunas' elevated SMR may be a suitable avenue for testing these divergent concepts.

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