Supporting Information

Rosa and Seibel 10.1073/pnas.0806886105

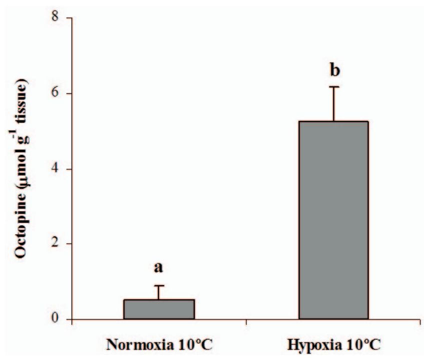

Fig. S1. Octopine levels (μmol g^{-1} wet tissue) at normoxic (21% O_2, $n = 5$) and hypoxic (1% O_2, $n = 7$) conditions (10°C) in the muscle of *Dosidicus gigas*. Values are means ± SE and different letters represent significant differences (*t* test, $P < 0.05$). Octopine was quantified in the mantle muscle of *D. gigas* according to standard procedures (1). The measurement principle is the oxidation of octopine to pyruvate and arginine by octopine dehydrogenase in the presence of NAD^+. The increase in NADH concentration, measured by the change in absorbance, is proportional to the amount of octopine. Measurement entailed the preparation of perchloric acid extracts from frozen tissues and the determinations were made immediately after neutralization with $KHCO_3$. Octopine was quantified spectrophotometrically (Shimadzu UV160U spectrophotometer, Shimadzu Scientific Instruments) at 339 nm using octopine (Sigma) standards.

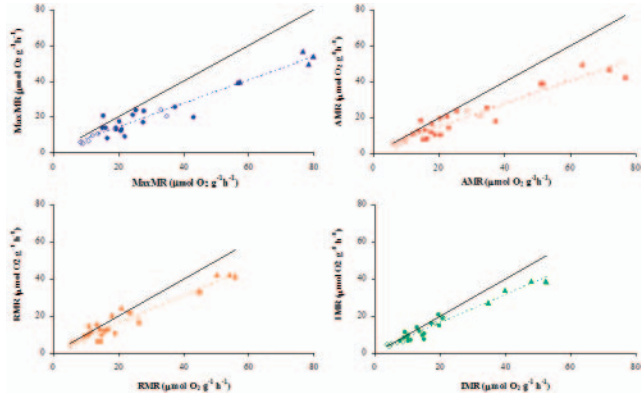

Fig. S2. (Source of Fig. 4*a*, individual data.) Inactive (IMR, green symbols), routine (RMR, orange symbols), active (AMR, red symbols), and maximum (MaxRM, blue symbols) metabolic rates (μmol O_2 g^{-1} h^{-1}), of every individual analyzed, under control conditions plotted against themselves (black solid line) and against rates of those same individuals under elevated (0.1%) CO_2 (open diamonds, 10°C, n = 7; solid circles, 20°C, n = 16; solid triangles, 25°C, n = 5).

1. Bergmeyer HU, Bergmeyer J, Grabl M (1985) *Methods of Enzymatic Analysis* (Verlag Chemie, Weinheim, Germany), Vol VIII.

Table S1. Effect of pH on hemocyanin oxygen binding (Bohr effect, $\Delta \log P_{50}/\Delta$ pH, where P_{50} is the oxygen partial pressure that results in 50% oxygen saturation of the respiratory protein) in coastal (Loliginidae) and epipelagic (Ommastrephidae) squids [he pH sensitivity is very high (Bohr coefficients < -1) as required to support the release of oxygen to the tissues at high metabolic rates]

Species	Temperature (°C)	Bohr Coefficient ($\Delta \log P_{50}/\Delta$ pH)	Source
Ommastrephidae			
Todarodes sagitattus	6	−1.09	(1)
	10	−1.17	(1)
	15	−1.13	(1)
	20	−1.09	(1)
	25	−0.92	(1)
Nototodarus sloanii	25	−1.16	(2)
Illex illecebrosus	15	−1.07	(3)
Loliginidae			
Doryteuthis vulgaris	10	−0.81	(1)
	20	−0.38	(1)
Doryteuthis pealei	15	−1.15	(3)
	25	−1.56	(4)
Doryteuthis forbesi	17	−1.14	(5)
Sepioteuthis lessoniana	25	−1.09	(2)

Sources:

1. Brix O, *et al.* (1989) Oxygen-binding properties of cephalopod blood with special reference to environmental temperatures and ecological distribution. *J Exp Zool* 252:32–42.
2. Lykkeboe G, Johansen K (1982) A cephalopod approach to rethinking about the importance of the Bohr and Haldane effects. *Pac Sci* 36:305–313.
3. Pörtner HO (1990) An analysis of the effects of pH on oxygen binding by squid (*Illex illecebrosus*, *Loligo pealei*) haemocyanin. *J Exp Biol* 150:407–424.
4. Redfield AC, Goodkind R (1929) The significance of the Bohr effect in the respiration and asphyxiation of the squid *Loligo pealei. J Exp Biol* 6:340–349.
5. Brix O, Lykkeboe G, Johansen K (1981) The significance of the linkage between the Bohr and Haldane effects in cephalopod bloods. *Resp Physiol* 44:177–186.

Table S2. Data in Fig. 2*a*

		Hypoxia, 10°C	Normoxia, 10°C	Normoxia, 20°C	Normoxia, 25°C
Inactive Metabolic Rate (IMR $= aM^b$)	Mass (g)	2.35–37.77	2.23–50.76	3.59–21.93	1.23–12.57
	Rate range (μmol O_2 g^{-1} h^{-1})	0.15–4.22	2.80–20.93	3.96–26.52	34.72–52.64
	a	3.09	8.91	11.73	53.66
	b	−0.51	−0.16	0.04	−0.15
	N	20	34	32	5
	r^2	0.32	NS	NS	0.82

Inactive mass-specific oxygen consumption rates (μmol O_2 g^{-1} h^{-1}) as a function of body size in *Dosidicus gigas*, under hypoxic (1% O_2 at 10°C) and normoxic (21% O_2 at 10°, 20°, and 25°C) conditions are shown. Rates (Y) are expressed as a function of mass (M) in power equations of the form $Y = aM^b$, *where* a is a normalization constant independent of size and b is a scaling coefficient that indicates the slope of the relationship. NS, not significant ($P > 0.05$).

Table S3. Data source in Fig. 1*b*

Species	Mass (kg)	*T* (°C)	SMR (mg O_2 kg^{-1} h^{-1})	Source
Shortfin Mako shark (*Isurus oxyrinchus*)	6.1	18	124	(1)
Hammerhead shark (*Sphyrna lewini*)	1.0	25	210	(2)
Lemon shark (*Negaprion brevirostris*)	1.6	25	153	(3)
Bonnethead shark (*S. tiburo*)	1.0	28	168	(4)
White shark (*Carcharodon carcharias*)	0.9	15	60	(5)
Bluefin tuna (*Thunnus orientalis*)	8.3	20	120	(6)
Yellowfin tuna (*T. albacares*)	5.4	20	91	(6)
Bonito (*Sarda chiliensis*)	1.2	18	107	(7)

References

1. Sepulveda CA, Graham JB, Bernal D (2007) Aerobic metabolic rates of swimming juvenile mako sharks, *Isurus oxyrinchus. Mar Biol* 152:1087–1094.
2. Brill RW (1996) Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. *Comp Biochem Physiol A* 113:3–15.
3. Scharold J, Gruber SH (1991) Telemetered heart rate as a measure of metabolic rate in the lemon shark, *Negaprion brevirostris*. *Copeia* 1991:942–953.
4. Carlson JK, Parsons GR (2003) Respiratory and hematological responses of the bonnethead shark, *Sphyrna tiburo*, to acute changes in dissolved oxygen. *J Exp Mar Biol Ecol* 294:15–26.
5. Carey FG, *et al.* (1982) Temperature and activities of a white shark, *Carcharodon carcharias*. *Copeia* 1982:254–260.
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7. Sepulveda CA, Dickson KA, Graham JB (2003) Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family Scombridae). I. Energetics. *J Exp Biol* 206:2739–2748.

Table S4. Data source of Fig. 1*d*

Species	Hypoxic threshold		Source
	mg l^{-1}	ml l^{-1}	
Bigeye tuna (*Thunnus obesus*)	1.3	1.0	(1)
Yellowfin tuna (*T. albacares*)	2.1	1.6	(2,3)
Skipjack tuna (*Katsuwonus pelamis*)	3.9	3.0	(2,3)

1. Hanamoto E (1987) Effect of oceanographic environment on bigeye tuna distribution. *Bull Jap Soc Fish Oceanogr* 51:203–216.
2. Bushnell PG, Brill RW (1991) Responses of swimming skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas to acute hypoxia, and a model of their cardio-respiratory function. *Physiol Zool* 64:787—911.
3. Brill RW (1994) A review of temperature and oxygen tolerance studies of tunas pertinent to fisheries oceanography, movement models and stock assessments. *Fish Oceanogr* 3:204–216.