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RESEARCH ARTICLE

Temperature compensation of aerobic capacity and performance in the Antarctic pteropod, *Clione antarctica*, compared with its northern congener, *C. limacina*

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SUMMARY

In ectotherms living in cold waters, locomotory performance is constrained by a slower generation of the ATP that is needed to fuel muscle contraction. Both polar and temperate pteropods of the genus *Clione*, however, are able to swim continuously by flapping their parapodia (wings) at comparable frequencies at their respective habitat temperatures. Therefore, we expected polar species to have increased aerobic capacities in their wing muscles when measured at common temperatures. We investigated muscle and mitochondrial ultrastructure of *Clione antarctica* from the Southern Ocean (–1.8°C) and populations of a sister species, *Clione limacina*, from the Arctic (–0.5 to 3°C) and from the North Atlantic (10°C). We also measured oxygen consumption and the activity of the mitochondrial enzyme citrate synthase (CS) in isolated wings of the two species. The Antarctic species showed a substantial up-regulation of the density of oxidative muscle fibers, but at the expense of fast-twitch muscle fibers. Mitochondrial capacity was also substantially increased in the Antarctic species, with the cristae surface density (58.2±1.3 $\mu m^2 \mu m^{-3}$) more than twice that found in temperate species (34.3±0.8 $\mu m^2 \mu m^{-3}$). Arctic *C. limacina* was intermediate between these two populations (43.7±0.5 $\mu m^2 \mu m^{-3}$). The values for cold-adapted populations are on par with those found in high-performance vertebrates. As a result of oxidative muscle proliferation, CS activity was 4-fold greater in *C. antarctica* wings than in temperate *C. limacina* when measured at a common temperature (20°C). Oxygen consumption of isolated wing preparations was comparable in the two species when measured at their respective habitat temperatures. These findings indicate complete compensation of ATP generation in wing muscles across a 10°C temperature range, which supports similar wing-beat frequencies during locomotion at each species' respective temperature. The elevated capacity in the wing muscles is reflected in the partial compensation of whole-animal oxygen consumption and feeding rates.

Key words: mitochondria, cristae, citrate synthase, cold, temperature, Antarctica.

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INTRODUCTION

In ectotherms inhabiting cold waters, rates of substrate diffusion and enzymatic activity are constrained by the low temperature (for a review, see Guderley and St-Pierre, 2002). This may result in a decreased capacity for the synthesis of ATP that is used for force generation in locomotory muscles (Clark, 1984). However, in terms of ATP, the cost of muscle contraction is believed to be independent of temperature, being dependent, primarily, on the frequency of muscle contraction (cf. Suarez, 2003). Therefore, locomotory muscles of ectotherms that stay active in cold temperatures may be expected to undergo physiological modifications to increase the aerobic capacity for ATP production (Egginton and Sidell, 1989; Johnston et al., 1998; St-Pierre et al., 1998). Fish and some invertebrates exposed to seasonal or latitudinal reductions in temperature show proliferation of aerobic muscle fibers (Sidell, 1980; Johnston and Maitland, 1980; Tyler and Sidell, 1984) as well as an enhanced metabolic capacity of those fibers *via* (1) elevated mitochondrial densities (Johnston et al., 1998; Sommer and Pörtner, 2002), (2) increased surface density of folded membranes inside a mitochondrion (cristae) (St-Pierre et al., 1998; Kilarski et al., 1996) and/or (3) mitochondrial enzymatic capacities (Crockett and Sidell, 1990; Kawall et al., 2002). Such modifications presumably enhance aerobic capacity because mitochondria are the primary site of aerobic ATP production. However, mitochondria may also facilitate intracellular oxygen diffusion, which is also constrained in cold water (Sidell, 1998).

Enhanced aerobic capacity as a response to low temperatures is well documented in temperate eurytherms and some Arctic stenotherms (for reviews, see Guderley, 2004; Pörtner, 2002; Pörtner et al., 2009). Similar findings have been reported for Antarctic species (Clarke, 1980; Johnston and Harrison, 1985; Torres and Somero, 1988; Crockett and Sidell, 1990). However, investigations in Antarctic animals have been hampered by the lack of an appropriate comparative system in warmer waters (Weinstein and Somero, 1998). To date, most comparative studies on temperature adaptation have focused on the Notothenioidae (Crockett and Sidell, 1990; Weinstein and Somero, 1998; Hardewig et al., 1999), an abundant group of fishes that is highly endemic to the Southern Ocean, and some benthic invertebrates (Pörtner et al., 1999; Morley et al., 2009; Galarza-Muñoz et al., 2011). Although the studies uncovered substantial evidence of temperature adaptation, the models they used focused on sluggish,

bottom-dwelling species with little need to up-regulate aerobic capacity (Peck, 2002).

In contrast, gymnosomatous pteropods of the genus *Clione* are active swimmers with an accessible, well-studied locomotory system (Seibel et al., 2007; Borrell et al., 2005; Satterlie and Spencer, 1985) and a distribution range extending from polar to temperate waters (Gilmer and Lalli, 1990). As such, they are suitable for investigating cold-temperature compensation and adaptation (Seibel et al., 2007; Rosenthal et al., 2009). *Clione antarctica* in the cold waters of the Southern Ocean is ecologically similar to, and has been described historically as a subspecies of, *Clione limacina*, which lives in temperate and polar waters in the northern hemisphere. In addition to morphological similarities, both species have similar life histories. They feed exclusively on the thecosomatous pteropod *Limacina helicina* (Conover and Lalli, 1972; Gilmer and Lalli, 1990), although the northern and southern *L. helicina* populations are now known to be genetically distinct (Hunt et al., 2010). Both *Clione* populations swim continuously in the water column by sweeping their highly muscularized parapodia (wings) through an arc of approximately 180 deg at frequencies of 1–2 Hz (Satterlie et al., 1985; Borrell et al., 2005). *Clione* spp. are also a unique model in that when the wings and ganglia are dissected free from the rest of the body they maintain rhythmic contractions that approximate those exhibited by adult animals. Thus, the physiological performance of the swim system can be studied independently from the whole animal (Satterlie and Spencer, 1985; Rosenthal et al., 2009).

Locomotor musculature in *C. limacina*, as investigated previously, includes four layers of muscle bundles consisting of both mitochondria-rich (oxidative) and mitochondria-poor (anaerobic) muscle fibers (Satterlie et al., 1990; Rosenthal et al., 2009). These two types of muscle fibers occupy distinct regions in the muscle bundles, with anaerobic fibers occupying ~50% of the bundle (Rosenthal et al., 2009). In stark contrast, muscle bundles in the swim musculature of *C. antarctica* consist entirely of oxidative, mitochondria-rich fibers. The anaerobic fibers, as well as the giant swim motoneurons that control contraction of anaerobic fibers in *C. limacina*, are absent in *C. antarctica* (Rosenthal et al., 2009). The proliferation of oxidative, mitochondria-rich fibers displaces mitochondria-poor fibers in muscle bundles of *C. antarctica* and presumably offsets the temperature-induced decrease in aerobic capacity (Rosenthal et al., 2009). This increase in oxidative capacity allows *C. antarctica* to achieve activity levels equivalent to those of *C. limacina* in temperate waters (Seibel et al., 2007). This difference in tissue organization between temperate and Antarctic *Clione* spp. influences swimming patterns and behavior of both species. Whereas locomotion in *C. limacina* consists of two distinct 'gears' supported by both oxidative and anaerobic muscle fibers (Satterlie et al., 1990), only a single speed has been observed in *C. antarctica* with no detectable escape response (Gilmer and Lalli, 1990; Borrell et al., 2005; Seibel et al., 2007). When disturbed, *C. antarctica* displays a whole-body withdrawal reflex rather than an increased swimming speed (Norekian and Satterlie, 1996). These different responses to mechanical stimuli are likely related to the differences in locomotory musculature.

In the present study, we further investigate the ultrastructure and energetics of locomotory muscles in *C. antarctica* from the Southern Ocean in comparison with those in its sister species, *C. limacina*, from two different locations: one from relatively temperate waters in the North Atlantic and the other from the cold Arctic waters that approximate conditions found in Antarctica. Our previous study revealed that metabolic rates and wing-beat frequencies of *C. antarctica* at –2°C are comparable with those of *C. limacina* at 5°C (Seibel et al., 2007). We hypothesize that this is made possible by increased aerobic capacity in the locomotory muscles and enhanced feeding activity. To test our hypothesis, we measured mitochondrial volume densities, mitochondrial cristae surface densities and enzyme activities in isolated wing muscles. We also measured feeding and oxygen consumption rates to determine if the compensation is apparent at the whole-animal level. In parallel, we investigated the effect of laboratory cold-acclimation on locomotory muscle structure in *C. limacina*. This study presents a thorough comparison of whole-animal, muscle and mitochondrial energetics, ultrastructure and morphometrics in two highly active ectotherms living across a wide temperature range.

MATERIALS AND METHODS

Animals

Temperate specimens of *Clione limacina* (Phipps 1774) were collected by hand from the shore in Logy Bay, Newfoundland, Canada during early to mid summer (June and July). The temperature ranged from 5 to 15°C depending on local currents and upwelling in the bay. Arctic specimens of *C. limacina* were collected during a research cruise in mid to late summer (July and August) using a plankton net (0–240 m depth) in the Hornsund region of the Svalbard archipelago (76°59′N, 15°45′E). The sea surface temperature ranged between –0.5 and 3°C, depending on depth. Specimens of *Clione antarctica* (Smith 1902) were collected by hand from shore in the Ross Sea, near McMurdo Station (77°51′S, 166°40′E) and by net-tow in the Weddell Sea (60°56′S, 52°81′E) in December, during the austral summer [the seawater temperature during the austral summer in McMurdo Sound varies from –1.87 to –0.5°C but is typically near –1.7°C in January (Hunt et al., 2003)]. Upon collection, wings of animals with intact locomotory muscles were dissected and either preserved in 2% glutaraldehyde in 0.1 mol l^{-1} cacodylic acid for electron microscopy (some specimens from all regions) or immediately frozen in liquid nitrogen for subsequent citrate synthase (CS) activity analysis.

Live specimens of temperate *C. limacina* from Newfoundland were incubated at two acclimation temperatures (acclimated specimens in Table 1), 10°C or 0°C, for four weeks in temperature-controlled rooms at the University of Rhode Island, USA. Throughout incubation, animals were maintained individually in 1-liter plastic containers filled with filtered seawater that included streptomycin and ampicillin (25 mg l^{-1} each) to control bacterial respiration. Water was changed every second day. Animals were fasted due to the difficulties keeping their exclusive prey, *Limacina retroversa* and *L. helicina*, in captivity. *Clione* spp. from both hemispheres are known to have high lipid content, which may help maintain a neutral buoyancy (Phleger et al., 1997) but also facilitates extended fasting periods (up to 260 days) when their exclusive prey are absent (Böer et al., 2005; Böer et al., 2006; Seibel and Dierssen, 2003). Following the incubation period, wings were dissected and preserved as described above.

Quantitative ultrastructural analysis

Dissected and preserved parapodia of *C. limacina* and *C. antarctica* were processed for electron microscopy at JFE Enterprises (Brookeville, MD, USA). Specimen sample sizes were 3 for acclimatized specimens of *C. limacina* and 5 for all other groups (Table 1). Spurr's embedded cross sections of the center of each wing were thin sectioned (60–90 nm), placed on grids and stained with uranyl acetate and lead citrate. Grids were viewed at 80 kV with a JOEL 12EX Transmission Electron Microscope equipped with a TVIPS high-resolution digital camera (TemCam-F224).

Table 1. Morphometrics of wing muscles and mitochondria in *Clione limacina* and *Clione antarctica* at different temperatures

Species	*N*	Temperature (°C)	V_{mf} (%)	V_{mit} (%)	S_c (μm^2 μm^{-3})
Clione limacina					
Acclimatized	3	10	55.3±3.4	35.5±1.4	34.3±0.8
Acclimated	5	10	49.2±2.1	33.3±1.0	33.7±1.1
Acclimated	5	0	50.2±3.9	39.6±2.1	36.2±0.2
Acclimatized	5	–0.5 to 3.0	48.4±3.4	33.3±3.6	43.7±0.5[a]
Clione antarctica					
Acclimatized	5	–1.7	100±0.0[a]	36.5±0.7	58.2±1.3[b]

Values are means ± s.e.m. Dissimilar letters indicate statistically significant difference from other groups in a column (*t*-tests, $P<0.05$).
V_{mf}, volume fractions of oxidative muscle fibers in a muscle bundle; V_{mit}, volume fractions of mitochondria in oxidative muscle fibers; S_c, surface density of the mitochondrial cristae.

Micrographs of individual bundles of fibers from oxidative (mitochondria-rich) and anaerobic (mitochondria-poor) muscle were taken at magnifications ranging from 1200 to 1500×. Single oxidative muscle fibers and mitochondria were captured and analyzed at magnifications of 2000–2500× and 20,000×, respectively, and analyzed according to standard guidelines of stereology (Weibel, 1979). Micrographs of 4–6 representative muscle bundles were obtained for each animal. Within one muscle bundle, micrographs of 4–6 representative oxidative muscle fibers and of 4 representative mitochondria were captured. The micrographs were analyzed with Image-J software (National Institutes of Health, USA), with the use of grid, and cell-counter plugins. Using the point-counting method of Weibel (Weibel, 1979), a grid system was superimposed over each micrograph, and the intersections (points, *P*) that hit the microstructure of interest were counted. Volume fractions or densities (*V*) of oxidative muscle fibers (mf) in a muscle bundle (V_{mf}) and mitochondria (mit) in oxidative muscle fibers (V_{mit}) were calculated by dividing the number of points that hit the microstructure of interest (*P*) by the total number of test points (*T*):

$$V = P / T. \quad (1)$$

Surface density (*S*) of the mitochondrial inner membrane, or cristae (c), (S_c; μm^2 μm^{-3}) was calculated using a parallel line test system (Elias and Hyde, 1980):

$$S_c = 2P / L, \quad (2)$$

where *L* is the total length of lines contained within the mitochondrion.

Citrate synthase activity

Sets of wings were dissected from experimental animals, weighed on a microbalance while frozen and then homogenized in 1:10 parts weight/volume dilution of 0.01 mol l^{-1} Tris grinding buffer (pH 7.5 at 10°C) in hand-held homogenizers (Wheaton, Millville, NJ, USA) kept on ice. Homogenate was centrifuged at 3800 ***g*** for 10 min at 5°C. Activity of CS was assayed under non-limiting substrate conditions as previously described (Thuesen and Childress, 1994; Seibel et al., 1998).

25 μl aliquots of homogenate supernatant were placed in quartz cuvettes in a medium containing a 1 ml solution of 50 mmol l^{-1} imidazole/HCl buffer (pH 8 at 20°C), 0.1 mmol l^{-1} acetyl-CoA, 0.1 mmol l^{-1} 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), 1.5 mmol l^{-1} $MgCl_2$ and 0.5 mmol l^{-1} oxaloacetate. Citrate synthase (E.C. 2.3.3.1) activity was measured as the increase in absorbance at 412 nm, which results from the interaction of reduced CoA with DTNB, using a Shimadsu UV 1700 spectrophotometer (Columbia, MD, USA) equipped with a water-jacketed cuvette holder connected to a recirculating water bath. Measurements were carried out at 20°C. This temperature was previously shown to be below the denaturation temperature for this enzyme in pteropods (B.A.S., unpublished). Background activities were measured without the presence of oxaloacetate and subtracted from total activities to compute true CS activities in the samples. Enzyme activities are expressed as units (micromoles of substrate converted to product per min) per gram wet mass of tissue.

Oxygen consumption in isolated wing nerve-muscle preparation

Reduced preparations were made by dissecting specimens of each species in filtered seawater such that only the wings, wing nerves and the central ring ganglia remained. These structures were pinned in a dish coated with Sylgard (Dow Corning, Midland, MI, USA) using cactus spines. A section of the Sylgard® to which the preparation was pinned was excised and placed in a water-jacketed 300 μl chamber with an integrated Clark-type oxygen electrode (Strathkelvin Instruments, North Lanarkshire, UK). The chamber was filled with filtered seawater. We recorded the oxygen consumption rates of reduced preparations from each species near their respective habitat temperatures (–1.5°C and 5°C). We have shown previously that the swim system of both species spontaneously shifts between slow and fast modes (Rosenthal et al., 2009), presumably due to the intermittent release of endogenous serotonin. Experiments were performed with or without 50 μmol l^{-1} serotonin (5′-hydroxytryptophan, 5-HT). The application of exogenous serotonin forces the preparation exclusively into the fast mode, resulting in higher rates of oxygen consumption. Additional measurements were made with the wing nerves cut as a control. The contraction of pteropod wings depends on hydrostatic pressure generated by the entire body during locomotion (Arshavsky et al., 1990). Thus, in the reduced preparation, complete wing-beat cycles were not possible. Our measurements therefore cannot correspond to a real measure of performance in nature but rather provide an additional comparison of aerobic performance under identical conditions at different temperatures. The wing nerve-muscle preparations consumed oxygen in proportion to the amount of available oxygen in the chamber (i.e. oxyconformation). Therefore, only data at oxygen concentrations greater than 75% saturation were used in the analysis of temperature compensation. The runs typically lasted 1.5 h.

Rates of prey consumption

Individual *C. antarctica* were placed in filtered seawater in sealed 1-liter BOD bottles with five individual prey (*Limacina helicina forma antarctica*). The bottles were placed in a static water table connected to the flow-through seawater system at McMurdo Station, Antarctica. The temperature was maintained at –1.8°C.

Each day, the numbers of prey consumed were noted, and empty prey shells were removed and measured. Approximately 50% of the water was exchanged daily to maintain seawater quality. The relationships reported by Seibel et al. between shell diameter and wet, dry and ash-free dry mass (Seibel et al., 2007) were used to estimate the mass of consumed prey each day. The animals used to develop those relationships were collected at the same time, from the same population as those used in the present feeding experiments. Ash-free dry masses allowed subtraction of the shell mass from the consumed prey mass. The feeding rates for similarly sized specimens of *C. limacina* at 5°C were calculated from relationships that were developed by Conover and Lalli (Conover and Lalli, 1972) using a similar experimental protocol.

Statistics

All results are expressed as means ± s.e.m. or s.d. (feeding experiments). Statistically significant differences in ultrastructure of muscle bundles, fibers and mitochondria, as well as in citrate synthase activity between species, were determined using one-way analysis of variance (ANOVA) tests using the statistical program, Statview v.5 (SAS Institute Inc., San Francisco, CA, USA). In all instances, $P<0.05$ was used as the level of significance, and multiple comparisons were performed using Student–Newman–Keuls tests.

RESULTS

Ultrastructure of locomotory muscles

The results of quantitative measurements of volume density of oxidative, mitochondria-rich locomotory muscle fibers ($V_{mf,}$), volume density of mitochondria (V_{mit}) and surface density of mitochondrial inner membrane (S_c) are shown in Table 1. V_{mf} did not differ significantly between muscle bundles of *C. limacina* species, regardless of acclimation or acclimatization temperature (Fig. 1). The oxidative muscle fibers comprised roughly half of the entire bundle (from 48% to 55%) in all four examined groups. Four-week exposure of *C. limacina* to 0°C in the laboratory, as well as seasonal acclimation to cold temperature in the wild, did not result in proliferation of oxidative muscle fibers in the muscle bundles (Fig. 1). This is in contrast to C. *antarctica,* chronically exposed to the cold (–1.86°C), which have muscle bundles composed entirely of mitochondria-rich muscle fibers (Rosenthal et al., 2009).

Mitochondrial abundances in muscle fibers were not affected by exposure to cold temperature in any of the studied animals. No significant difference in V_{mit} existed among the different acclimation and acclimatization temperature groups of *C. limacina*. Similarly, no significant difference in V_{mit} existed between *C. limacina* and *C. antarctica*. V_{mit} for all tested animals was approximately 36% (Table 1).

In contrast, long-term exposure to cold results in proliferation of the inner mitochondrial membrane (Fig. 2). S_c was similar for *C. limacina* acclimated to 10°C and 0°C as well as for *C. limacina* acclimatized to 10°C and it ranged from 34.3±0.8 to 36.2±0.2 $\mu m^2 \mu m^{-3}$. However, in *C. limacina* acclimatized to –0.5 to 3°C, S_c was approximately 27% higher compared with 10°C-acclimatized animals. In *C. antarctica*, S_c was 58.2±1.3 $\mu m^2/\mu m^3$, a value 170% greater than in *C. limacina* at 10°C. The morphometric data of each species is presented in comparison to Antarctic fishes, mammals and active fish predators in Fig. 3.

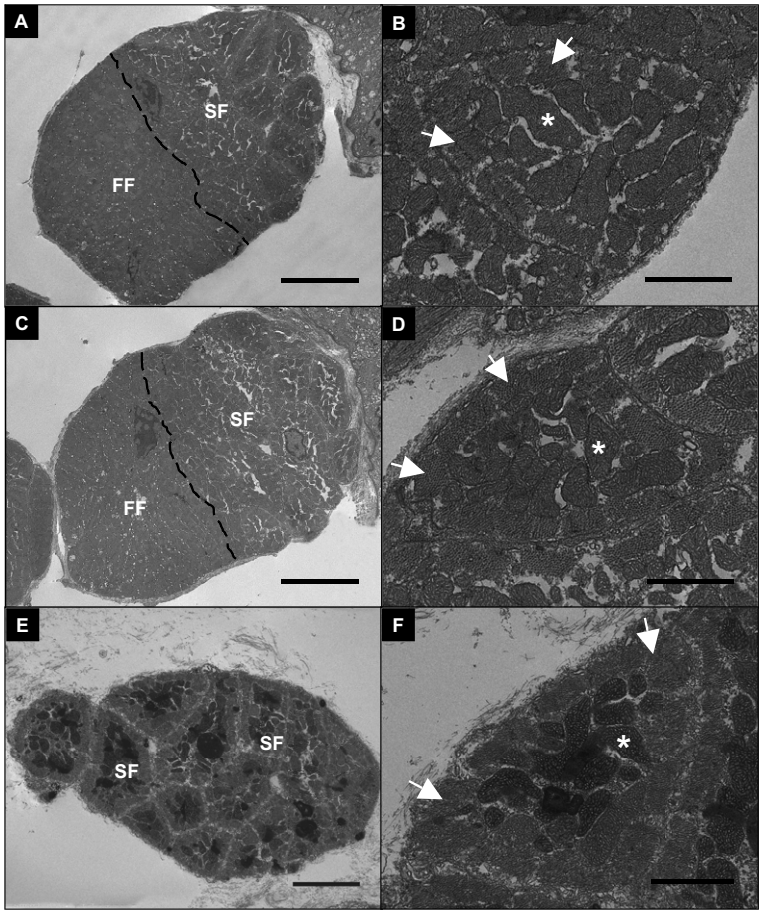

Fig. 1. Representative transmission electron micrographs showing muscle ultrastructure of *Clione* wings. Panels A, C and E show muscle bundle ultrastructure: (A) *C. limacina* from Newfoundland acclimatized to 10°C; (C) *C. limacina* from the Arctic acclimatized to –0.5 to 3°C; (E) *C. antarctica* from the Antarctic acclimatized to –1.86°C. Slow (aerobic) muscle fibers (SF) occupy roughly half of the muscle bundle in *C. limacina* from Newfoundland and the Arctic. The remaining area consists of fast (anaerobic) muscle fibers (FF). In *C. antarctica*, fast muscle fibers are absent. Panels B, D and F show slow muscle fiber ultrastructure: (B) *C. limacina* from Newfoundland; (D) *C. limacina* from the Arctic; (F) *C. antarctica*. The central core of the slow fibers is comprised of mitochondria (asterisk), and is surrounded by myofibrils (arrows). The amount of mitochondria in the slow muscle cells is equivalent regardless of the acclimation temperature or the species. Scale bar: 10 μm (A,C, E); 2 μm (B,D,F). Panel E is reproduced from Rosenthal et al. (Rosenthal et al., 2009) with permission.

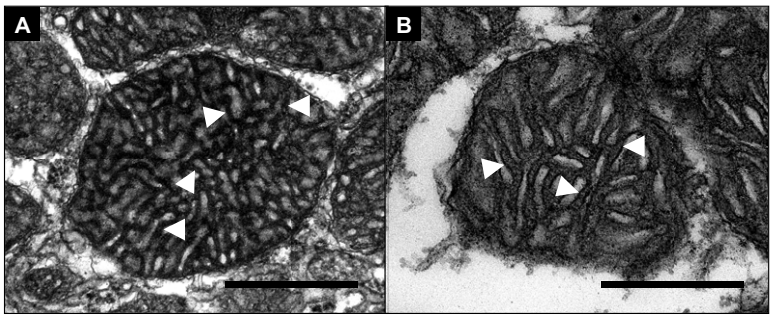

Fig. 2. Representative transmission electron micrographs showing ultrastructure of mitochondria in slow muscle fibers in the wings of *Clione antarctica* from the Antarctic acclimatized to –1.86°C (A) and *Clione limacina* from Newfoundland acclimatized to 10°C (B). Note the increased cristae of the inner membrane (indicated by arrows) in *C. antarctica*. Scale bars: 0.5 μm.

Citrate synthase assay

The activity of CS measured in the wing tissue of *C. limacina* acclimatized to 10°C and *C. antarctica* acclimatized to –1.8°C is presented in Fig. 4. CS activity (units g^{-1} wet mass) was approximately four times higher in *C. antarctica* than in acclimatized and acclimated *C. limacina*. This result is consistent with differences in the muscle ultrastructure between the two species. No significant difference in CS activity was observed between 0°C-acclimated and 10°C-acclimated and 10°C-acclimatized *C. limacina*, which indicates no effect of short-term exposure to cold on CS activity in wing muscle. The activity of CS was not measured in the Arctic *C. limacina*, as we obtained only preserved specimens from that region.

Oxygen consumption in isolated wing nerve-muscle preparation

Oxygen consumption rates of isolated wing-nerve preparations without addition of serotonin ranged from 0.64 to 1.31 μmoles $O_2\,g^{-1}\,h^{-1}$ for *C. antarctica* (mean=0.779±0.29 μmoles $O_2\,g^{-1}\,h^{-1}$; wing mass 3.5-9.1 mg, N=5, at –1.5°C) and from 0.86 to 1.28 μmoles $O_2\,g^{-1}\,h^{-1}$ for *C. limacina* (mean=1.087±0.21 μmoles $O_2\,g^{-1}\,h^{-1}$; wing mass 3.0–5.0 mg, N=3, at 5.0°C; Fig. 5A). Application of 50 mmol l^{-1} serotonin in seawater increased oxygen consumption by about 2-fold for *C. antarctica* (mean=1.68±0.30 μmoles $O_2\,g^{-1}\,h^{-1}$, N=3) but much less in *C. limacina* (1.5 and 1.34 μmoles $O_2\,g^{-1}\,h^{-1}$, N=2). The oxygen consumption rates of *C. limacina* at 5°C are not significantly higher than those of *C. antarctica* at –1.5°C, in agreement with measurements for the whole animal (Fig. 5B) (Seibel et al., 2007). Limited sample size restricts statistical power for experiments with serotonin.

Rates of prey consumption

In *L. helicina forma antarctica* (2–16 mg wet mass, N=25), dry mass (DM) was related to wet mass (M) as $\ln(DM)=-1.26+0.87\ln(M)$ (R^2=0.92), ash-free dry mass is equal to $0.38\ln(M)+0.16$ (R^2=0.65), and wet mass is related to shell diameter (D) as $M=-0.53+1.91\ln(D)$ (R^2=0.86) (Seibel et al., 2007). The relationships were all significant (P<0.05). *Limacina helicina* of intermediate sizes (~5–12 mg wet mass) were used as prey in feeding experiments (uneaten prey were not measured). Large *C. antarctica* (10.5±0.13 mg dry mass, N=13) were chosen for feeding experiments. They consumed 0.28±0.08 mg dry mass (mean ± s.d.) of *L. helicina* per day at –1.8°C (Fig. 6). Conover and Lalli (Conover and Lalli, 1972) reported a feeding rate on *L. helicina forma rangi* of about 50% less at 5°C for *C. limacina* (10 mg dry mass) (Fig. 6).

DISCUSSION

We observed increased cristae surface density and elevated activity of the mitochondrial enzyme, citrate synthase, in locomotory muscles of *C. antarctica* compared with *C. limacina*. In combination with the previously reported proliferation of oxidative muscle fibers, this clearly shows that aerobic capacities are up-regulated in *C. antarctica*, relative to warmer acclimatized *C. limacina*. Moreover, the difference between the polar and subpolar populations of *C. limacina* suggests a continuum of aerobic capacity related to temperature. The differences in muscle and mitochondrial structure were reflected in oxygen consumption rates of isolated wing-nerve preparations that were higher

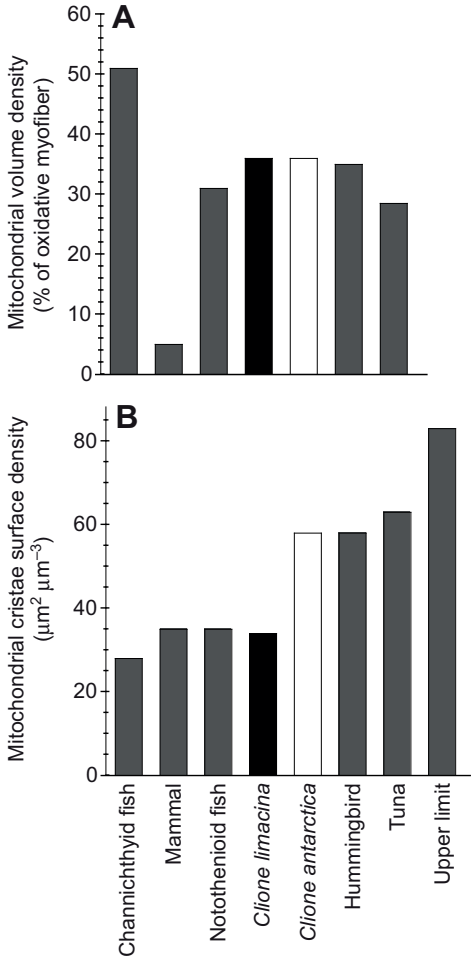

Fig. 3. (A) Volume density of mitochondria within a muscle fiber (%) and (B) surface density of mitochondrial inner membrane (μm² μm⁻³), or cristae, in wing muscles of *Clione antarctica* (open bars) and *Clione limacina* (black bars) in comparison to locomotory muscles of polar and 'high-performance' animals (grey bars). Included are polar fishes [Chanychthyid and Notothenioid (O'Brien et al., 2003)], mammals (Hoppeler and Lindstedt, 1985), hummingbirds (Suarez et al., 1991) and tuna (Mathieu-Costello et al., 1992). The upper limit to mitochondrial surface density, hypothesized by Srere (Srere, 1985), is based on a constraint imposed by the space between cristae for mitochondrial matrix enzymes.

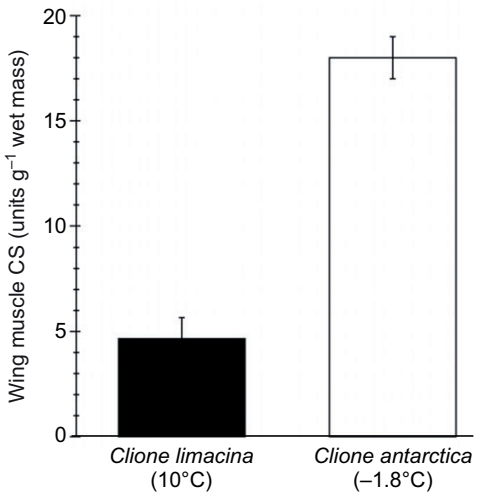

Fig. 4. Citrate synthase (CS) activity in wing muscle tissue of *Clione limacina* acclimatized to 10°C (filled bar) and *Clione antarctica* (open bar) acclimatized to –1.8°C. Acclimation of *C. limacina* to either 10 or 0°C for one month had no significant effect on CS activity (data not shown). Values are means ± s.e.m.

in the Antarctic species despite a much lower measurement temperature. However, laboratory acclimation of temperate *C. limacina* to cold did not result in ultrastructural changes in locomotory muscles, suggesting that modifications in *C. antarctica* are not a result of temperature-induced phenotypical plasticity but rather reflect permanent adaptation of this population to cold.

Muscle ultrastructure

In most eurythermal fish, increased mitochondrial volume densities in muscle fibers presumably compensate for a reduction in rates of aerobic ATP synthesis during exposure to cold (for reviews, see Pörtner, 2002; Guderley, 2004). For instance, striped bass, *Morone saxatilis*, acclimated to 5°C (for 8 weeks) show a 57% increase in V_{mit} in oxidative muscles compared with 25°C-acclimated fish (Egginton and Sidell, 1989). Similar results have also been observed for cold-acclimated crucian carp, *Carassius carassius*, and goldfish, *Carassius auratus* (Johnston and Maitland, 1980; Tyler and Sidell, 1984). Elevated mitochondrial densities are also found in some stenothermal organisms such as Antarctic fishes (Johnston et al., 1998; O'Brien et al., 2003) and in Sub-Arctic lugworm, *Arenicola marinara* (Sommer and Pörtner, 2002). These findings suggest that mitochondrial proliferation is a common strategy to compensate for cold. Thus, we were initially surprised not to find significant differences in mitochondrial volume densities in oxidative muscle fibers of polar and temperate pteropods (*Clione* spp.). In all investigated populations, mitochondria occupy approximately 36% of a muscle fiber volume regardless of temperature (Table 1). Since a theoretical upper limit to mitochondrial volume density in locomotory muscles may exist, beyond which force generation and contractile function of muscle fibers would be impaired (Weibel, 1985; Suarez et al., 1991; Rome and Lindstedt, 1998), *Clione* spp. might not be able to increase their aerobic capacity *via* further mitochondrial proliferation. This is supported by the fact that mitochondrial volume densities found in *Clione* spp. (Fig. 3) are on par with those found in the flight muscle of hummingbirds (~35%), honeybee (~43%) (Suarez et al., 1991; Suarez et al., 2000) and blowfly (~43%) (Smith, 1963). Mitochondrial densities in the fibers of *Clione* spp. may be close to maximal values where further increases would result in decreased sustained swimming and foraging performance (Rome and Lindstedt, 1998). However, the increase in the proportion of oxidative muscle fibers [100% in *C. antarctica* and 55% in *C. limacine* (Rosenthal et al., 2009)] in the Antarctic congener means that the total mitochondrial abundance in wing muscle is much greater than in the temperate species.

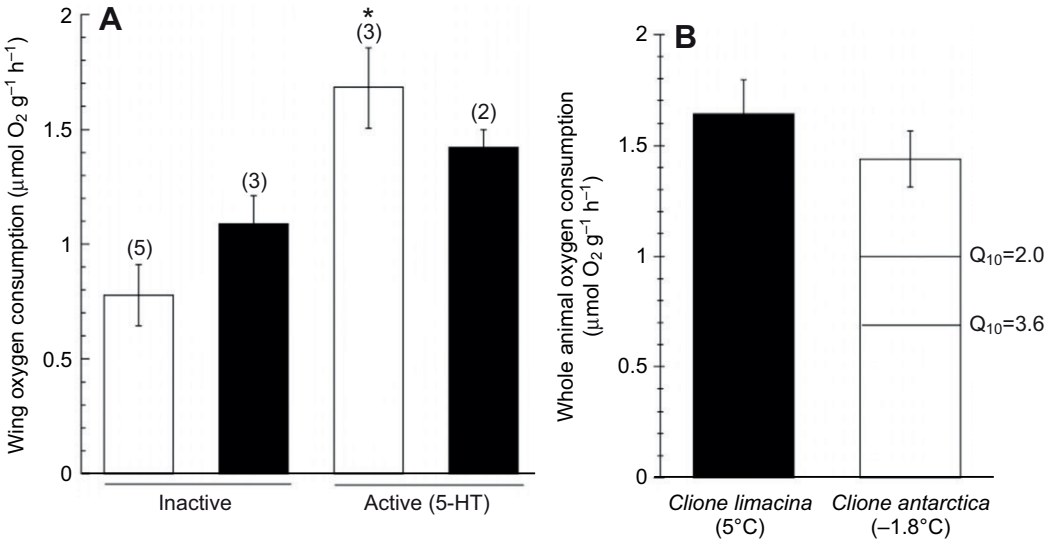

Fig. 5. Oxygen consumption rates for *Clione antarctica* at –1.8°C (open bars) and *Clione limacina* at 5°C (filled bars). *Clione limacina* specimens were those acclimatized to 10°C although environmental temperature variation for the population includes 5°C. (A) Isolated wing nerve-muscle preparations during routine activity and during high activity induced with bath application of serotonin (50 mmol l^{-1} 5-HT). Values are means ± 1 s.e.m., and sample sizes are indicated in parentheses over each bar for isolated wing nerve-muscle preparations. An asterisk indicates statistical difference between the two species in the 5-HT experiment. (B) Whole-animal rates normalized to a common body mass using significant scaling relationships measured for each species (data from Seibel and Dierssen, 2003; Seibel et al., 2007). Error bars represent the range of values calculated using the standard error for the slope of the scaling relationships. The black lines across the open bar represent the rate for *C. limacina* normalized to –1.8°C assuming a typical temperature coefficient (Q_{10}) of 2.0 and using the measured Q_{10} of 3.6 (Seibel et al., 2007).

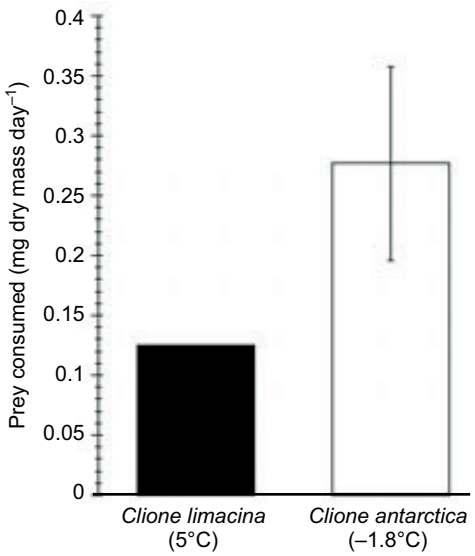

Fig. 6. The rate of consumption (mg dry mass day^{-1}) of the sole prey, *Limacina helicina* (thecosomatous pteropod), by *Clione limacina* at 5°C (filled bar) (calculated from Conover and Lalli, 1974) and *C. antarctica* (open bar) at –1.8°C. Prey tissue mass was estimated by measurement of the prey's shell diameter, which correlates with dry mass and ash-free dry mass according to relationships reported previously (Seibel et al., 2007). The error bar represents the standard deviation (*N*=13).

Interestingly, the proportion of oxidative muscle fibers in locomotory muscle bundles of Arctic *C. limacina* did not differ from that in temperate *C. limacina* (Table 1). Also, proliferation of oxidative muscle fibers did not occur during laboratory acclimation (4 weeks) of temperate *C. limacina* to 0°C (Table 1). These findings suggest that, in contrast to *C. antarctica*, there may exist strong selection for maintenance of the 2-geared swimming system in *C. limacina* during cold exposure. Although not yet described, preliminary observations reveal a latitudinal dichotomy of swimming behaviors in the prey species, *L. helicina*, similar to that reported here for *Clione* spp. (B.A.S., unpublished observations). Thus, we believe that a regressed locomotory system (i.e. lack of burst swimming ability) in *C. antarctica* is an example of a temperature-mediated 'disarmament' of predator and prey in a highly co-evolved predator–prey 'arms race'.

Mitochondrial ultrastructure

It seems that in both *C. antarctica* and Arctic *C. limacina*, compensation of muscle aerobic capacity is achieved by an increase in mitochondrial cristae surface density rather than mitochondrial proliferation. Although this strategy has been demonstrated during cold acclimation of crucian carp (Kilarski et al., 1996) and rainbow trout (St-Pierre et al., 1998), it has not been found in Antarctic and Sub-Antarctic fishes (Johnston et al., 1998; Guderley and St-Pierre, 2002). However, it is difficult to confirm the finding in the Antarctic fishes as the temperate species to which they are compared often have a different body-form, mode of swimming and activity pattern (Johnston and Harrison, 1985). Cristae surface density was 70% higher in *C. antarctica* and 27% higher in the polar *C. limacina* when compared to the temperate *C. limacina* (Table 1). The cristae surface densities in *C. antarctica* and Arctic *C. limacina* are higher than values reported for mammalian skeletal muscle (20–40 $\mu m^2 \mu m^{-3}$) (Hoppeler and Lindstedt, 1985) but lower than the values found in tuna red muscle (63–70 $\mu m^2 \mu m^{-3}$) (Mathieu-Costello et al., 1992). Interestingly, the value obtained for *C. antarctica* is identical to those previously reported for hummingbird flight muscle (58 $\mu m^2 \mu m^{-3}$) (Suarez et al., 1991). It appears that cristae surface density in mitochondria of *C. antarctica* is approaching the theoretical upper limit of 83 $\mu m^2 \mu m^{-3}$ (Srere, 1985). Higher values may leave insufficient room for Kreb's cycle enzymes in the mitochondrial matrix.

Although some studies have found no obvious correlation between mitochondrial cristae surface density and mitochondrial oxidative capacity (Moyes et al., 1992; Johnston et al., 1998), more cristae provide more surface area for electron transport to occur within a mitochondrion (St-Pierre et al., 1998), which should provide greater total capacity for ATP synthesis. This interpretation is consistent with the high cristae surface densities reported for high-performance animals mentioned above. O'Brien et al. found higher mitochondrial volume densities in icefishes lacking hemoglobin compared to their red-blooded counterparts (O'Brien et al., 2003). However, the mitochondria themselves had lower cristae surface density such that the total surface density per unit mass was relatively constant between these groups. They concluded that the mitochondrial proliferation served to enhance oxygen diffusion rather than aerobic capacity. This contrasts sharply with our finding of substantial elevations in both muscle fiber and cristae surface density.

Citrate synthase

Active polar ectotherms are often characterized by high activity of mitochondrial enzymes such as CS and cytochrome-*c* oxidase (CCO) (i.e. Torres and Somero, 1988; Crockett and Sidell, 1990; Kawall et al., 2002; Lucassen, 2003; Lucassen, 2006). For instance, Kawall et al. report over three times higher CS activity in muscles and almost two times higher activity in brain tissue of Antarctic fish than in tropical species (Kawall et al., 2002). In agreement with these previous findings, we find that CS activity in the locomotory muscles of *C. antarctica* was almost four times higher than in locomotory muscles of temperate *C. limacina* (Fig. 4). In part, the increased activity is accounted for by the 2-fold increase in mitochondria-rich muscle fibers in muscle bundles of *C. antarctica* and thus a higher total enzyme concentration. CS is a matrix enzyme so the elevated cristae surface density should not affect the concentration of enzymes in the mitochondria themselves. Further, the elevated cristae surface density may actually limit the space available for matrix enzymes. It may also be that CS in the polar pteropod has an increased catalytic capacity. It has been reported that some enzymes from Antarctic ectotherms are more efficient than those of warmer species (Kawall et al., 2002; Galarza-Muñoz et al., 2011). Therefore, the 4-fold difference in CS enzymatic activity between *C. limacina* and *C. antarctica* may be a result of both quantitative and qualitative changes to the enzyme. These ideas await further testing.

Although increased CS activity has been observed during cold acclimation of some eurythermal fish and invertebrates (i.e. Rodnick and Sidell, 1994; St-Pierre et al., 1998; Guderley and Leroy, 2001; Sommer and Pörtner, 2004), none was found with acclimation of *C. limacina* to 0°C. There are several possible reasons for this finding. In most studies on cold acclimation in fish, the acclimation period was longer than the month-long regimen employed here, normally lasting from 6 to 8 weeks. It is possible that a longer exposure to the cold would have induced changes similar to those observed in *C. limacina* from the Arctic. Moreover, animals were fasted throughout the incubation, a process that may have had a negative effect on the proliferation of mitochondrial structures and proteins.

Temperature compensation at the organ and whole-animal levels

The elevation of mitochondria and oxidative enzymes appears to result in an increased aerobic capacity for *C. antarctica*. Isolated wing-nerve preparations stimulated to contract by the addition of serotonin consumed more oxygen at –1.8°C than did those from *C. limacina* at 5°C (Fig. 5A) (N=2 for serotonin experiments). Contraction of wing muscles in whole animals is dependent on hydrostatic pressure and is linked to contraction of the heart (Arshavsky et al., 1990). Thus, muscle contractions in isolated wing-nerve preparations are incomplete and presumably consume far less energy than those in actively swimming whole animals. Nevertheless, our finding of elevated oxygen consumption in cold-adapted Antarctic pteropod wings is consistent with the upregulation of oxidative capacity observed at the cellular and subcellular levels. Although not yet measured in *Clione* spp., the cost of locomotion in some pteropods is a dominant fraction of total energy consumption in the whole animal (Davenport and Trueman, 1985). Our previous observations of elevated whole-animal oxygen consumption rates are thus not surprising (Fig. 5B) (Seibel et al., 2007). There is now wide agreement that temperature compensation does not exist at the whole-animal level in fishes (Kawall et al., 2002 and references therein). However, we hypothesize that this simply reflects the fact that most physiological processes do not require rate compensation and those that do (e.g. locomotion) are a minor component of the overall energy budget in sluggish, benthic fishes.

Maintenance of similar rates of ATP demand across a temperature gradient requires similar rates of energy consumption. Both *C. antarctica* and *C. limacina* feed exclusively on the thecosomatous pteropod genus *Limacina*. In fact, both species feed on *L. helicina* where the prey and predator populations overlap (Lalli and Gilmer, 1984). However, recent data suggest that *L. helicina* is not in fact a bipolar species but that substantial genetic differences exist between the northern and southern populations (Hunt et al., 2010) and we cannot rule out the possibility of different temperature sensitivities of predator–prey relationships in each hemisphere. Nevertheless, we have demonstrated here that *C. antarctica* consumes its sole prey, *L. helicina*, at a rate equivalent or higher than that reported previously for *C. limacina* (Fig. 6) (Conover and Lalli, 1972).

Concluding remarks

The observed changes in muscle and mitochondrial ultrastructure in *C. antarctica* and Arctic *C. limacina* clearly show up-regulation of aerobic capacity with decreasing temperature. This additional capacity appears to facilitate the maintenance of similar routine swimming rates across a temperature range and is reflected in the metabolic rates of whole animals (Seibel et al., 2007) and isolated wing muscles. In Arctic *C. limacina*, adaptation to cold is apparent in an increase in cristae surface densities; while in *C. antarctica*, compensation for cold is achieved by both the proliferation of oxidative muscle fibers (Rosenthal et al., 2009) and higher cristae surface densities. The fact that mitochondrial volume densities within oxidative muscle fibers do not change between species suggests that the observed adaptation does not result from a facilitation of oxygen diffusion between mitochondria. While in some instances the reduction of mean diffusion path length for oxygen through the tissue is advantageous for cold adaptation (Sidell, 1998), it does not seem to be a primary driver of adaptation to cold in *C. antarctica*. While increased cristae surface density may speed the diffusion of oxygen, we present a case where the need for increased aerobic capacity is clear. Thus, we hypothesize that benefit of mitochondrial enhancement for oxygen diffusion is secondary to the compensation of locomotory performance.

The difference in response to low temperature between Arctic and Antarctic *Clione* species may be due to differences in the temperature experienced throughout the year and the thermal history of the Arctic and Southern Oceans. In the Hornsund region, sea temperature varies from –1.86°C in the winter months to 3°C in the summer (Weslawski et al., 1988; Walczkowski et al., 2005) whereas in McMurdo Sound water temperature never warms above –0.5°C (Littlepage, 1965; Hunt et al., 2003). Although both *Clione* species occupy identical ecological niches in cold waters (Hunt et al., 2008), millions of years of isolation and thermal stability of Antarctic waters have led to the development of features of permanent cold adaptation in the Antarctic congener. The values we report here for mitochondrial abundance and cristae surface density in *C. antarctica* are close to theoretical upper limits and are comparable with those found in high-performance animals such as hummingbirds and tuna (Fig. 3). However, taking aerobic design to the extreme is not without a cost. For example, the high energy demand for synthesis and maintenance of mitochondria with a high cristae surface density may be associated with increased proton leak and hence higher metabolic rate and oxygen demand (Pörtner et al., 2009). More importantly, *C. antarctica* has lost the ability to swim in bursts and can maintain only the slow swimming speed of routine swimming (Rosenthal et al., 2009). We hypothesize that this loss is a direct displacement of the fast-twitch muscle fibers by oxidative muscle fibers in *C. antarctica*. While it may be that the ability to generate fast swimming is limited by the differing temperature sensitivities of fast- and slow-swimming systems (Rosenthal et al., 2009) or by the kinematics of thrust propulsion at low Reynolds numbers (Borrell et al., 2005), we have demonstrated a clear need for enhanced aerobic capacity that can, on its own, explain the loss of burst swimming.

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REFERENCES

Arshavsky, Y. I., Deliagina, T. G., Gelfand, I. M., Orlovsky, G. N., Panchin, Y. V., Pavlova, G. A., and Popova, L. B. (1990). Neural control of heart beat in the pteropod mollusk *Clione limacina*: coordination of the circulatory and locomotory systems. *J. Exp. Biol.* **148**, 461-475.

Böer, M., Gannefors, C., Kattner, G., Graeve, M., Hop, H. and Falk-Petersen, S. (2005). The Arctic pteropod *Clione limacina*: seasonal lipid dynamics and life strategy. *Mar. Biol.* **147**, 707-717.

Böer, M., Graeve, M. and Kattner, G. (2006). Impact of feeding and starvation on the lipid metabolism of the Arctic pteropod *Clione limacina*. *J. Exp. Mar. Biol. Ecol.* **328**, 98-112.

Borrell, B. J., Goldbogen, J. A. and Dudley, R. (2005). Aquatic wing flapping at low Reynolds numbers: swimming kinematics of the Antarctic pteropod, *Clione antarctica*. *J. Exp. Biol.* **208**, 2939-2949.

Clarke, A. (1980). A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. *Biol. J. Linn. Soc. Lond.* **14**, 77-92.

Clarke, A. (1983). Life in cold water: the physiological ecology of polar marine ectotherms. *Oceanogr. Mar. Biol.* **21**, 341-453.

Conover, R. J. and Lalli, C. M. (1972). Feeding and growth in *Clione limacina* (Phipps), a pteropod mollusk. *J. Mar. Exp. Biol. Ecol.* **9**, 279-302.

Conover, R. J. and Lalli, C. M. (1974). Feeding and growth in *Clione limacina* (Phipps), a pteropod mollusc. II. Assimilation, metabolism, and growth efficiency. *J. Exp. Mar. Biol. Ecol.* **16**, 131-154.

Crockett, E. L. and Sidell, B. D. (1990). Some pathways of energy metabolism are cold adapted in Antarctic fishes. *Physiol. Zool.* **63**, 472-488.

Davenport, J. and Trueman, E. R. (1985). Oxygen uptake and buoyancy in zooplanktonic organisms from the tropical eastern Atlantic. *Comp. Biochem. Physiol. A* **81**, 857-863.
Egginton, S. and Sidell, B. D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* **256**, R1-R9.
Elias, H. and Hyde, D. M. (1980). An elementary introduction to stereology (quantitative microscopy). *Am. J. Anat.* **159**, 412-446.
Galarza-Muñoz, G., Soto-Morales, S. I., Holmgren, M. and Rosenthal, J. J. C. (2011). Physiological adaptation of an Antarctic Na^+/K^+-ATPase to the cold. *J. Exp. Biol.* **214**, 2164-2174.
Gilmer, R. W. and Lalli, C. M. (1990). Bipolar variation in *Clione*, a gymnosomatous pteropod. *Am. Malacol. Bull.* **8**, 67-75.
Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev. Camb. Philos. Soc.* **79**, 409-427.
Guderley, H. and Leroy, P. H. (2001). Family origin and the response of threespine stickleback, *Gasterosteus aculeatus*, to thermal acclimation. *J. Comp. Physiol. B* **171**, 91-101.
Guderley, H. and St-Pierre, J. (2002). Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *J. Exp. Biol.* **205**, 2237-2249.
Hardewig, I., van Dijk, P. L. K., Moyes, C. D. and Pörtner, H. O. (1999). Temperature-dependent expression of cytochrome-c oxidase in Antarctic and temperate fish. *Am. J. Physiol.* **277**, R508-R516.
Hoppeler, H. and Lindstedt, S. L. (1985). Malleability of skeletal muscle in overcoming limitations: structural elements. *J. Exp. Biol.* **115**, 355-364.
Hunt, B., Strugnell, J., Bednarsek, N., Linse, K., Nelson, R. J., Pakhomov, E., Seibel, B., Steinke, D. and Würzberg, L. (2010). Poles apart: the "bipolar" pteropod species *Limacina helicina* is genetically distinct between the Arctic and Antarctic oceans. *PLoS ONE* **5**, e9835.
Hunt, B. M., Hoefling, K. and Cheng, C.-H. C. (2003). Annual warming episodes in seawater temperatures in McMurdo Sound in relationship to endogenous ice in notothenoid fish. *Antarct. Sci.* **15**, 333-338.
Hunt, B. P. V., Pakhomov, E. A., Hosie, G. W., Siegel, V., Ward, P. and Bernard, K. (2008). Pteropods in Southern Ocean ecosystems. *Prog. Oceanogr.* **78**, 193-221.
Johnston, I. A. and Harrison, P. (1985). Contractile and metabolic characteristics of muscle fibers from Antarctic fish. *J. Exp. Biol.* **116**, 223-236.
Johnston, I. A. and Maitland, B. (1980). Temperature acclimation in crucian carp *Carassius carassius* L., morphometric analyses of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113-125.
Johnston, I. A., Calvo, J., Guderley, H., Fernandez, D. and Palmer, L. (1998). Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *J. Exp. Biol.* **201**, 1-12.
Kawall, H. G., Torres, J. J., Sidell, B. D. and Somero, G. N. (2002). Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. *Mar. Biol.* **140**, 279-286.
Kilarski, W. M., Romek, M., Kozlowska, M. and Goerlich, A. (1996). Short-term thermal acclimation induces adaptive changes in the inner mitochondrial membranes of fish skeletal muscle. *J. Fish Biol.* **49**, 1280-1290.
Lalli, C. M. and Gilmer, R. W. (1989). *Pelagic Snail: The Biology of the Planktonic Gastropod Molluscs*. Stanford, CA: Stanford University Press.
Littlepage, J. L. (1965). Oceanographic investigations in McMurdo Sound, Antarctica. *Ant. Res. Ser.* **5**, 1-37.
Lucassen, M., Schmidt, A., Eckerle, L. G. and Pörtner, H. O. (2003). Mitochondrial proliferation in the permanent vs. temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. *Am. J. Physiol.* **285**, R1410-R1420.
Lucassen, M., Koschnick, N., Eckerle, L. G. and Pörtner, H. O. (2006). Mitochondrial mechanisms of cold adaptation in cod (*Gadus morhua* L.) populations from different climatic zones. *J. Exp. Biol.* **209**, 2462-2471.
Mathieu-Costello, O., Agey, P. J. and Logemann, R. B. (1992). Capillary-fiber geometrical relationships in tuna red muscle. *Can. J. Zool.* **70**, 1218-1229.
Morley, S. A., Lurman, G. J., Skepper, J. N., Pörtner, H. O. and Peck, L. S. (2009). Thermal plasticity of mitochondria: A latitudinal comparisom between Southern Ocean mollusks. *Comp. Biochem. Physiol. A* **152**, 423-430.
Moyes, C. D., Mathieu-Costello, O. A., Brill, R. W. and Hochachka, P. W. (1992). Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonas pelamis*) and slow (*Cyprinus cyprio*) fish. *Can. J. Zool.* **70**, 1246-1253.
Norekian, T. P. and Satterlie, R. A. (1996). Whole body withdrawal circuit and its involvement in the behavioral hierarchy of the mollusk *Clione limacina*. *J. Neurophysiol.* **75**, 529-537.
O'Brien, K. M., Skilbeck, C., Sidell, B. D. and Egginton, S. (2003). Muscle fine structure may maintain the function of oxidative fibres in haemoglobinless Antarctic fishes. *J. Exp. Biol.* **206**, 411-421.
Peck, L. S. (2002). Ecophysiology of marine Antarctic ectotherms: limits to life. *Polar Biol.* **25**, 31-40.
Phleger, C. F., Nichols, P. D. and Virtue, P. (1997). Lipids and buoyancy in Southern ocean pteropods. *Lipids* **32**, 1093-1100.
Pörtner, H. O. (2002). Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *J. Exp. Biol.* **205**, 2217-2230.
Pörtner, H. O., Hardewig, I. and Peck, L. S. (1999). Mitochondrial function and critical temperature in the Antarctic bivalve *Laternula elliptica*. *Comp. Biochem. Physiol. A* **124**, 179-189.
Pörtner, H. O, Peck, L. and Somero G. (2009). Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. *Phil. Trans. R. Soc. B* **362**, 2233-2258.
Rodnick, K. J. and Sidell, B. D. (1994). Cold acclimation increases carnitine palmitoyltransferase I activity in oxidative muscle of striped bass. *Am. J. Physiol.* **266**, R405-R412.
Rome, L. C. and Lindstedt, S. L. (1998). The quest for speed: muscles built for high-frequency contractions. *News Physiol. Sci.* **13**, 261-268.
Rosenthal, J. J., Seibel, B. A., Dymowska, A. and Bezanilla, F. (2009). Trade-off between aerobic capacity and locomotor capability in an Antarctic pteropod. *Proc. Natl. Acad. Sci. USA* **106**, 6192-6196.
Satterlie, R. A. and Spencer, A. N. (1985). Swimming in the pteropod mollusc, *Clione limacina*: II. Physiology. *J. Exp. Biol.* **116**, 205-222.
Satterlie, R. A., LaBarbera, M. and Spencer, A. N. (1985). Swimming in the pteropod mollusk *Clione limacina*. I. Behaviour and morphology. *J. Exp. Biol.* **166**, 189-204.
Satterlie, R. A., Goslow, G. E. and Reyes, A. (1990). Two types of striated muscle suggests two-geared swimming in the pteropod mollusk *Clione limacina*. *J. Exp. Zool.* **255**, 131-140.
Seibel, B. A. and Dierssen, H. M. (2003). Cascading trophic impacts of reduced biomass in the Ross Sea, Antarctica: just the tip of the iceberg? *Biol. Bull.* **205**, 93-97.
Seibel, B. A., Thuesen, E. V. and Childress, J. J. (1998). Flight of the vampire: ontogenetic gait-transition in *Vampyroteuthis infernalis* (Cephalopoda: vampyromorpha). *J. Exp. Biol.* **201**, 2413-2424.
Seibel, B. A., Dymowska, A. and Rosenthal, J. (2007). Metabolic temperature compensation and coevolution of locomotory performance in pteropod mollusks. *Integr. Comp. Biol.* **47**, 880-891.
Sidell, B. D. (1980). Responses of goldfish (*Carassius auratus*, L.) muscle to acclimation temperature: alterations on biochemistry and proportions of different fiber types. *Physiol. Zool.* **53**, 98-107.
Sidell, B. D. (1998). Intracellular oxygen diffusion: the roles of myoglobin and lipid at cold body temperature. *J. Exp. Biol.* **201**, 1119-1128.
Smith, D. S. (1963). The structure of flight muscle sarcosomes in the blowfly *Calliphora erythrocephala* (Diptera). *J. Cell Biol.* **19**, 115-138.
Sommer, A. M. and Pörtner, H. O. (2002). Metabolic cold adaptation in the lugworm *Arenicola marinara*: comparison of a North Sea and White Sea population. *Mar. Ecol. Prog. Ser.* **240**, 171-182.
Sommer, A. M. and Pörtner, H. O. (2004). Mitochondrial function in seasonal acclimatization versus latitudinal adaptation to cold in the lugworm Arenicola marina (L.). *Physiol. Biochem. Zool.* **77**, 174-186.
Srere, P. A. (1985). Organization of proteins within the mitochondrion. In *Organized Multienzyme Systems: Catalytic Properties* (ed. G. R. Welch), pp. 1-61. London, UK: Academic Press.
St-Pierre, J., Charest, P.-M. and Guderley, H. (1998). Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimatization of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **201**, 2961-2970.
Suarez, R. K. (2003). Shaken and stirred: muscle structure and metabolism. *J. Exp. Biol.* **206**, 2021-2029.
Suarez, R. K., Lighton, J. R. B., Brown, G. S. and Mathieu-Costello, O. (1991). Mitochondrial respiration in hummingbird flight muscles. *Proc. Natl. Acad. Sci. USA* **88**, 4870-4873.
Suarez, R. K., Staples, J. F., Lighton, J. R. B. and Mathieu-Costello, O. (2000). Mitochondrial function in flying honeybees (*Apis mellifera*): respiratory chain enzymes and electron flow from complex III to oxygen. *J. Exp. Biol.* **203**, 905-911.
Thuesen, E. V. and Childress, J. J. (1994). Respiratory rates and metabolic enzyme activities of oceanic California medusae in relation to body size and habitat depth. *Biol. Bull. Mar Biol. Lab.* **187**, 84-98.
Torres, J. J. and Somero, G. N. (1988). Metabolism, enzymatic activities and cold adaptation in Antarctic mesopelagic fishes. *Mar. Biol.* **98**, 169-180.
Tyler, S. and Sidell, B. D. (1984). Changes in mitochondrial distribution and diffusion distances in muscle of goldfish upon acclimation to warm and cold temperatures. *J. Exp. Zool.* **232**, 1-9.
Walczkowski, W., Piechura, J., Osinski, R. and Wieczorek, P. (2005). The West Spitsbergen Current volume and heat transport from synoptic observations in summer. *Deep-Sea Res.* **52**, 1374-1391.
Weibel, E. R. (1979). Stereological methods. *Practical Methods for Biological Morphology*, Vol. 1. London, UK: Academic Press.
Weibel, E. R. (1985). Design and performance of muscular systems: an overview. *J. Exp. Biol.* **115**, 405-412.
Weinstein, R. B. and Somero, G. N. (1998). Effects of temperature on mitochondrial function in the Antarctic fish *Trematomus bernacchii*. *J. Comp. Physiol. B* **168**, 190-196.
Weslawski, J. M., Zajaczkowski, M., Kwaśniewski, S., Jezierski, J. and Moskal, W. (1988). Seasonality in an Arctic fjord ecosystem: Hornsund, Spitsbergen. *Polar Res.* **6**, 185-189.