Supporting Information

Cziko et al. 10.1073/pnas.1410256111

SI Methods

Melting and Freezing Points. Fluids were sampled from anesthetized (100 mg tricaine methane sulphonate per liter of seawater) adult fishes collected by baited hook and line from McMurdo Sound, including shallow living *Trematomus pennellii*, *Trematomus bernacchii*, *Trematomus hansoni*, and *Pagothenia borchgrevinki*, and the deeper living *Dissostichus mawsoni.* Serum was isolated by centrifugation (14,000 × *g*, 10 min) of clotted blood samples. Total antifreeze glycoprotein (AFGP) were quantitatively isolated from a 0.5-mL sample of *T. pennellii* serum by addition of an equal volume of 5% (wt/vol) trichloroacetic acid (TCA), heating at 100 °C (10 min), and centrifugation (14,000 × *g*, 10 min) to pellet TCA-labile proteins (1). The initial supernatant and those from two additional washes of the pellet with 2.5% (wt/vol) TCA were combined and the TCA-soluble AFGPs were desalted by size-exclusion (BioGel P-2, 0.5 × 30 cm; Bio-Rad) column chromatography using buffer (50 mM ammonium bicarbonate, pH 7.8). Fractions exhibiting freezing hysteresis (FH) were combined, lyophilized, and reconstituted to physiological concentration (32 mg/mL) in the original serum volume with buffer for use in FH and melting hysteresis (MH) determinations. Antifreeze potentiating protein (AFPP) was isolated from *P. borchgrevinki* serum as previously described (2), by four precipitations (14,000 × *g*, 10 min) in 40% (vol/vol) saturated ammonium sulfate, resuspension in buffer, and fractionation of active peaks on a Sephacryl HR-100 (GE Healthcare) size-exclusion column (2 × 120 cm). AFPP was used as a 1-mg/mL solution in buffer, the approximate concentration found in *T. bernacchii* serum (1). We created a solution of antifreeze protein (AFPs) representative of concentrations in serum of McMurdo Sound trematomin fishes by mixing equal proportions of 2× stocks of purified AFGPs and AFPP in buffer.

The equilibrium freezing/melting point (eqFMP) is an effectively linear function of the concentration of colligatively acting solutes in solution (3). We determined the osmotic concentration of samples (Osm/kg; means of individual samples run three times) with a vapor pressure osmometer (Vapro 5520; Wescor), and calculated eqFMPs by multiplying the osmotic concentrations by the Cryoscopic Constant for water (−1.858 °C Osm/kg). The nonequilibrium hysteresis melting points (hMPs) and hysteresis freezing points (hFPs) were determined under 200× total magnification with a Clifton nanoliter freezing-point osmometer (Clifton Technical Physics) that we modified to allow computer control of ramp rates and a precise (0.002 °C resolution) determination of the sample temperature using a second microthermistor embedded in the stage. For instrument calibration, we used six temperature calibration points that encompassed the range of sample measurements; for points below 0 °C we used standard solutions of NaCl in H_2O (4) and for those above 0 °C we used solutions containing D_2O (eqFMP = +3.82 °C) and H_2O (5). Ice crystals were formed by freezing a submicroliter droplet of the sample suspended in immersion oil at <−20 °C followed by warming to melt most of the ice. Rapid warming and cooling within ±0.04 °C of the eqFMP was often used to overcome MH caused by AFPs, because the magnitude of the MH appears to be somewhat warming-rate dependent. For FH measurements, warming was stopped when only a single crystal (2–10 μm long) remained; for MH, when ∼20–200 crystals (approximately 2–100 μm long) remained (estimated at <1% of the volume of the droplet). Crystals were then allowed to anneal for 10 min at 0.32 °C below the eqFMP of the solution to allow the AFP–ice interaction to stabilize as it would during wintertime conditions in McMurdo Sound. For FH, the temperature was then lowered at 0.11 °C/min until rapid growth of the ice crystal was observed at the hFP (FH = eqFMP − hFP). For MH, the sample was warmed at 0.03 °C/min until the last visible crystal disappeared at the hMP (MH = hMP − eqFMP). A single sample was assayed three to four times for each MH and FH. An example of the melting behavior of ice in *T. pennellii* serum is presented in Movie S1.

In Vivo Superheating of Ice. Fishes were collected in early November 2011 from bottom depths of 15–30 m (*Trematomus* spp.) or from directly beneath the approximately 2-m-thick sea ice (*P. borchgrevinki*) using baited hook and line through 30-cm–diameter holes drilled within 3 km of McMurdo Station. Ice chips were removed from the hole with a dip net before fishing. Captured fishes were immediately (within 2 s) transferred from the fishing hole to insulated containers of seawater at −1.8 to −1.5 °C (within the FH interval), remained submerged while the hook was removed, and were then transported to ice-free aquaria at the research station or, for field experiments, in a tent that was erected on location, appropriately equipped, and powered by a generator.

The presence of internal ice can be detected with a simple assay that tests the ability of fishes to maintain their body fluids in a metastable undercooled state at low temperatures (6, 7). When submerged in a refrigerated 25% (by volume) glycerol-seawater solution (freezing point = −9 °C), fishes possessing internal ice begin visibly freezing as the temperature is lowered below the hFP of body fluids (approximately −3.5° to −2.5 °C), because the growth of internal ice crystals overwhelms adsorbed AFPs (6). However, if fishes are first warmed to 4 °C (1 h) to melt internal ice, they can be subsequently undercooled to −7 °C for at least 1 h, recovering completely upon their return to seawater (6, 8). Such extensive undercooling indicates that internal ice can be melted at 4 °C and confirms the absence of other nucleators in these fishes. The reintroduction of ice, by briefly spray-freezing a patch of integument with liquefied refrigerant or by maintaining fishes in cages in their native icy habitat for a few hours, once again precludes undercooling and leads to freezing at temperatures just below the hFP of body fluids (6).

Experiments were designed to determine whether internal ice remained in fishes following an experimental warming treatment in a temperature-controlled aquarium. For these experiments, fishes were divided into two groups to test whether naturally acquired and experimentally introduced internal ice differ in their superheating ability. The first group was maintained in ice-free seawater within the FH interval so that any internal ice naturally present would neither melt nor grow (Fig. S1). For the second group, internal ice was first melted by placing the fish in 4 °C seawater for 1 h, followed by a 1 h recovery period at −1.5 °C (within the FH interval). Ice was then reintroduced by briefly exposing one flank of the fish above the surface of the seawater and freezing a small patch of skin (approximately 0.5-cm diameter) with a spray (approximately 0.25-s duration) of canned liquefied refrigerant. The frozen patch disappeared within a few seconds upon return to seawater and fishes survived normally thereafter. For both treatments, fishes were maintained within the FH interval for 12 h to allow internal ice to disperse throughout the body before proceeding with warming treatments. Subgroups (n = 8–15 for each datapoint) from the two treatments were then maintained in a 400-L temperature-controlled aquarium for a set period at a set temperature as

recorded by a high-resolution SBE56 temperature logger (2-s interval; Sea-Bird Electronics) in close proximity to the fishes (within 30 cm). Vigorous aeration ensured a homogeneous temperature throughout the aquarium.

We used the above-described undercooling assay to determine whether internal ice remained following experimental warming. Individual fishes were quickly (within 2 s) transferred from the warming treatment to an enclosed jacketed 25% (by volume) glycerol-seawater bath, maintained at −6 °C by a circulating refrigeration unit, and then monitored over the following 10 min. Fishes that did not freeze within 10 min were deemed ice-free, returned to the aquarium, and recovered fully. Fishes collected from shallow sites (<30 m deep) in McMurdo Sound when the seawater temperature was near its freezing point (approximately −1.9 °C) and those with experimentally introduced ice that were maintained under conditions that did not melt internal ice always tested positive in this assay within the first 30 s. Negative control fishes (n = 10–30 individuals for each datapoint) that were warmed to 4 °C and then maintained and assayed alongside experimental fishes never tested positive for internal ice and could remain undercooled at −6 °C for at least 1 h, surviving normally upon return to seawater. We increased the number of negative controls for higher treatment temperatures; we performed 30 negative controls for the experiment in which we observed the persistence of ice inside a single individual *T. bernacchii* exposed for 24 h to +0.08 °C (mean aquarium temperature); no controls tested positive for ice in any experiment. Animal care and use was performed as per approved institutional guidelines (University of Illinois).

We performed field experiments to determine the fraction of fishes maintaining internal ice during natural warming episodes, because transport of the fishes to the laboratory would introduce additional variables. Following collection, fishes were held in ice-free flowing seawater in ambient seawater pumped from 5-m below the sea ice for <10 min and then assayed for internal ice on site using undercooling assays, as described above. Ice-free control fishes that were suspended in the water column at 10 m for 15 min (n = 7 for each sampling date) did not test positive for ice.

Oceanographic Data. Long-term benthic temperature records were obtained with SBE39 or SBE56 loggers (Sea-Bird Electronics; initial accuracy ±0.002 °C, maximum drift 0.002 °C per year) deployed by divers at 25- or 40-m depth within 100 m of the McMurdo Station seawater intake jetty (Table S3). The loggers were weighted and the probe positioned within 10 cm of the bottom in locales where benthic trematomin fishes are prevalent. Temperature (ITS-90) was recorded as point measurements at 15 min (1999–2009) or 5-min intervals (2011–2012). All dates and times are presented in coordinated universal time (UTC). Loggers were recovered at least once per year for download and battery replacement, and returned for factory calibration at ≤2-y intervals. Data were analyzed using custom-written routines in MATLAB (MathWorks), dividing the data into 1-y periods that began before the onset of the annual warming event in each year (October 1 of the calendar year). In 1999 and 2005 the date of the onset of the annual warming event was obtained from a logger at 9-m depth at Cape Armitage (S 77°51.688, E 166°40.606), 1.5-km away, because the McMurdo Logger was not deployed at those times. The record from Cape Armitage is practically identical to that from McMurdo (9), and is not reported here. For calculation of annual mean temperature (oceanographic year delineated as October 1 to September 30) and the long-term mean temperature of the McMurdo site, small gaps in the data were linearly interpolated using available data (Fig. S4 and Table S1); years with larger gaps in the data were omitted from this analysis. The long-term mean temperature of the McMurdo site was thus calculated from nine complete oceanographic years of temperature data. The long-term temperature record is publicly available at the Integrated Earth Data Applications repository (www.iedadata.org, doi:10.1594/IEDA/321474).

Vertical conductivity (salinity), temperature, and depth (CTD) profiles were obtained with a SBE25 through holes drilled in sea ice or with a SBE9Plus aboard the research vessel *Nathaniel B. Palmer*. The pressure and salinity dependent in situ seawater FP was calculated according to ref. 10 and this correction was also applied to fish eqFMP and greatest observed superheating in Fig. 5*F*. GPS coordinates of study sites are presented in Table S3.

Relationship Between Fish Body Temperature and Ambient Seawater Temperature. To assess the physiological relevance of our long-term temperature record and to accurately determine the magnitude of in vivo superheating occurring in our experiments, we performed a set of experiments to establish the relationship between fish body temperature and ambient seawater temperature. Experiments were designed to determine: (*i*) whether small-bodied notothenioid fishes retain sufficient metabolic heat to raise body temperature above ambient seawater temperature, and (*ii*) the time required for fish body temperature to equilibrate to the ambient temperature following a change in ambient temperature. For these experiments, we monitored deep body temperature of variously sized individuals of *T. bernacchii* every 10 s, using a 0.1 °C resolution microthermocouple thermometer probe inserted, under anesthesia, into the muscle between the dorsal fins until it touched the vertebrae. For both experiments, fish body temperature was first allowed to equilibrate over several hours in a temperature-controlled aquarium at −1.5 °C. Then, for (*i*), body temperature was monitored over 10 min while the aquarium temperature was maintained at a constant −1.5 °C and fishes were induced to swim intermittently by chasing them with a stick. For (*ii*), body temperature was monitored every 10 s following transfer of fishes directly from the −1.5 °C aquarium to an adjacent 0.5 °C tank with a small net. We measured seven fishes in this manner, repeating measurements with the smallest fish twice. We ceased measurements 2 min after body temperature reached the new, elevated ambient temperature. From these data, we derived the coefficient of heating (k) for each fish by fitting the data to the equation of Newton's Law of Temperature Change, $\Delta T = \Delta T_0\ e^{-kt}$ (11), where ΔT_0 is the initial difference between body temperature and ambient and ΔT is the difference in temperature at time (t). We then used nonlinear regression analysis to determine the relationship between the coefficient of heating (k) vs. mass or total length (TL) of the fish, under the assumption that the relationship was $k = aX^b$ (12), where X is either mass (g) or TL (mm). Using the empirically derived k for each fish, we then estimated the relationship between fish body size and the amount of time it would take for the body temperature change to achieve 95% of ΔT_0 following an instantaneous change in ambient temperature, over the size range of adult trematomin fishes commonly encountered in McMurdo Sound. Finally, we simulated fish body temperature over 1 y (2011) of the long-term temperature record at the McMurdo site for a 20-g and a 300-g fish. To do this, using custom-written routines in MATLAB, we iteratively calculated fish body temperature every 5 min (the time resolution of the temperature record) using Newton's Law of Temperature Change, the empirically derived k, and assuming that the coefficients of heating and cooling are identical. From these simulated data, we determined the maximum deviation of the modeled body temperature from the ambient environmental temperature over the course of the year, and the maximum temperatures that fishes of these sizes would have experienced during the highest summer temperature excursion in the decadal record, recorded on January 29, 2012.

SI Results

We found that notothenioid fish body temperature effectively tracks ambient seawater temperature. When held at a constant temperature of −1.5 °C for 10 min, the deep body temperature of *T. bernacchii* individuals (n = 3; 81, 116, and 308 g) did not deviate more than 0.1 °C from the ambient aquarium temperature despite intermittent burst swimming by the fish. This result suggests that the small-bodied notothenioid fishes are incapable of retaining metabolic heat that could melt internal ice, and that fish body temperature will equilibrate to the ambient environmental temperature given sufficient time. Thus, the use of seawater temperature as a proxy for the internal fish body temperature for calculating the magnitude of in vivo superheating of ice is justified for the range of fish sizes and the duration of experiments used herein.

The deep body temperature of fishes transferred directly from −1.5 to 0.5 °C equilibrated quickly with the new ambient temperature. The smallest adult fish tested (20 g, 104 mm TL) equilibrated within 10 min to within 0.1 °C of ambient temperature (the resolution of the temperature probe); the largest fish tested (308 g, 242-mm TL) required less than 30 min to achieve effective equilibrium (Fig. S2*A*). From these data, we calculated the coefficient of heating (k) over the range of fish sizes and masses commonly encountered for adult McMurdo Sound trematomin fishes (Fig. S2*B*), and estimated the relationship between fish size and the time required for fish body temperature to achieve 95% of an instantaneous change in ambient temperature (Fig. S2*C*).

Using the empirically derived coefficient of heating for *T. bernacchii* and Newton's Law of Temperature Change, we simulated the body temperature of 20-g and 300-g fishes experiencing the temperatures recorded at the McMurdo Site over the 2011 oceanographic year. Over this period, we calculated that the absolute maximum deviation in fish body temperature from the ambient seawater temperature would have been only 0.11 °C for a 20-g fish and 0.30 °C for a 300-g fish. Furthermore, the body temperature of a 20-g fish during the highest summer temperature excursion in the decadal record (−0.10 °C, January 29, 2012) would have essentially equaled that of the environment, while the internal temperature of a 300-g fish would have been only very slightly colder (body temperature = −0.11) (Fig. S2*D*). Because fish body temperature lags ambient temperature during increasing seawater temperature, fish body temperature could never be warmer than recorded peak seawater temperatures. Our simulations indicate that, for most practical purposes, the body temperature of adult, small-bodied notothenioid fishes (≤300 g) effectively tracks the ambient environmental temperature during natural changes in seawater temperature.

1. Jin Y, DeVries AL (2006) Antifreeze glycoprotein levels in Antarctic notothenioid fishes inhabiting different thermal environments and the effect of warm acclimation. *Comp Biochem Physiol B Biochem Mol Biol* 144(3):290–300.
2. Jin Y (2003) Freezing avoidance of Antarctic fishes: The role of a novel antifreeze potentiating protein and the antifreeze glycoproteins. PhD dissertation. (Univ of Illinois–Urbana, Champaign, IL).
3. DeVries AL (1988) The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. *Comp Biochem Physiol B* 90(3):611–621.
4. Scatchard G, Prentiss SS (1933) The freezing points of aqueous solutions. IV. Potassium, sodium and lithium chlorides and bromides. *J Am Chem Soc* 55(11): 4355–4362.
5. Kiyosawa K (1991) Freezing-point of mixtures of $H_2{}^{16}O$ and $H_2{}^{18}O$. *J Solution Chem* 20(6):583–588.
6. Praebel K, Hunt B, Hunt LH, DeVries AL (2009) The presence and quantification of splenic ice in the McMurdo Sound notothenioid fish, *Pagothenia borchgrevinki* (Boulenger, 1902). *Comp Biochem Physiol A Mol Integr Physiol* 154(4):564–569.
7. DeVries AL, Cheng C-HC (2005) in *The Physiology of Polar Fishes*, eds Farrell AP, Steffensen JF (Elsevier, San Diego), pp 155–201.
8. DeVries AL, Cheng C-HC (1992) *Water and Life*, eds Somero GN, Osmond CB, Bolis CL (Springer, Berlin), pp 301–315.
9. Hunt BM, Hoefling K, Cheng CHC (2003) Annual warming episodes in seawater temperatures in McMurdo Sound in relationship to endogenous ice in notothenioid fish. *Antarct Sci* 15(3):333–338.
10. Fofonoff NP, Millard RC (1983) Algorithms for computation of fundamental properties of seawater. *UNESCO Tech Pap Mar Sci* 44:1–53.
11. Stevens ED, Fry FEJ (1970) The rate of thermal exchange in a teleost, *Tilapia mossambica*. *Can J Zool* 48(2):221–226.
12. Stevens ED, Fry FEJ (1974) Heat transfer and body temperatures in non-thermoregulatory teleosts. *Can J Zool* 52(9):1137.

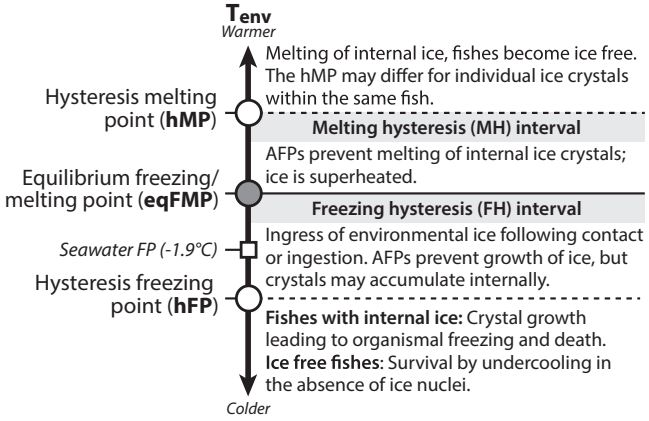


Fig. S1. Conceptual framework for the effects of AFPs and temperature on the fate of internal ice in polar teleost fishes. The acquisition and loss of internal ice depends upon the environmental temperature (T_{env}) in relation to the melting and freezing points of AFP-containing body fluids. In the absence of AFPs, the melting-to-freezing transition occurs precisely at the eqFMP (shaded circle), a colligative property dependent on the osmotic concentration of their body fluids. Teleost fishes are hypoosmotic to (less salty than) seawater thus their eqFMP is higher than the freezing point of seawater. Because most body fluids are essentially isosmotic with blood (Fig. 2*A*), the serum eqFMP provides a good proxy for the eqFMP of the entire fish. The presence of AFPs shifts the freezing and melting points of ice to nonequilibrium hysteresis values (white circles). To melt internal ice crystals, the ambient temperature must surpass the hMP of the body fluid in which the ice is bathed. In this study, we primarily address the magnitude of the MH interval and whether summer warming is sufficient to melt internal ice in McMurdo Sound notothenioid fishes.

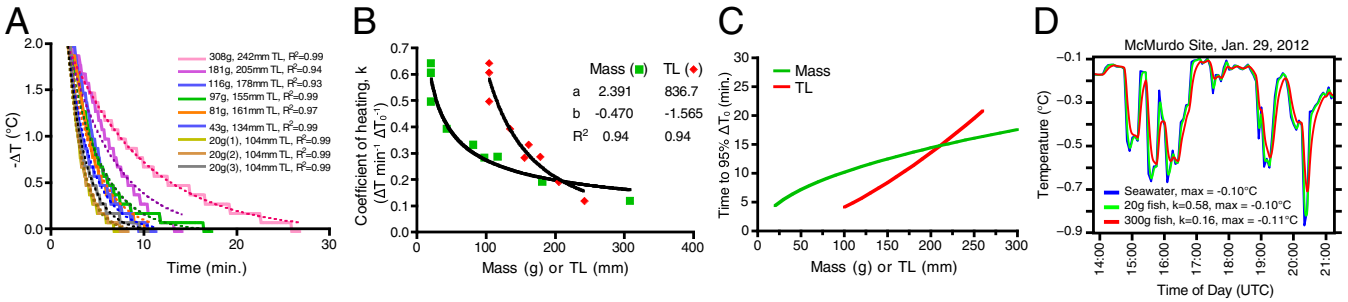


Fig. S2. Notothenioid fish body temperature effectively tracks ambient temperature. We performed a series of experiments to determine the time dependence of equilibration between fish internal temperature and ambient seawater temperature, and simulations to assess the degree to which the measured environmental temperature record reflects the internal body temperature of the small-bodied notothenioid fishes at the McMurdo logging site. (*A*) After several hours in an aquarium at −1.5 °C, individuals of *T. bernacchii* (104- to 242-mm total length, 20–308 g) were transferred directly to an adjacent 0.5 °C tank. Deep body temperature was monitored every 10 s as the fish warmed (solid lines) using a 0.1 °C resolution microthermocouple thermometer probe inserted into the muscle between the dorsal fins until it touched the vertebrae. The coefficient of heating (*k*) for each fish was derived by fitting the data to the equation of Newton's Law of Temperature Change, $\Delta T = \Delta T_0\, e^{-kt}$ (dotted line), where ΔT_0 is the initial difference between body temperature and ambient and ΔT is the difference in temperature at time *t*. (*B*) We used nonlinear regression analysis to determine the relationship between the coefficient of heating, *k*, and the mass or total length (TL) of the fish, under the assumption that the relationship was $k = aX^b$, where *X* is either mass (g) or TL (mm). (*C*) We then estimated the relationship between mass or TL and the amount of time it would take for fish body temperature to achieve 95% of ΔT_0 following an instantaneous change in ambient temperature, over the range of adult trematomin fish sizes commonly encountered in McMurdo Sound. The results show that small-bodied fishes (≤300 g) equilibrate quickly as ambient temperature changes. Regressions were calculated with Prism 4 (GraphPad). (*D*) Simulated internal body temperature for fishes with masses of 20 g and 300 g were calculated using Newton's Law of Temperature Change given the ambient temperature recorded by the logger at the McMurdo site for oceanographic year 2011. These simulations show that ambient temperature effectively reflects fish internal body temperature for the small-bodied trematomin fishes, with the maximum deviation over the course of the year being only 0.11 °C for a 20-g fish and 0.30 °C for a 300-g fish. During an episode on January 29, 2012, in which the decadal maximum seawater temperature was recorded (−0.10 °C), the simulations reveal that the internal body temperature of a 20-g fish would essentially equal the ambient temperature, and the body temperature of a 300-g fish would be only slightly colder (−0.11 °C).

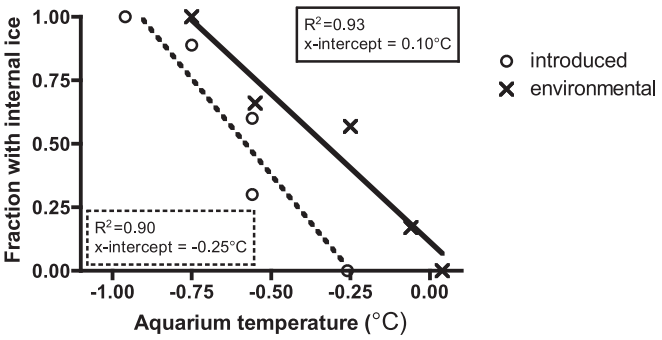


Fig. S3. Difference in the superheating ability of experimentally introduced and naturally acquired internal ice. Linear regression of the fraction of fishes maintaining internal ice vs. aquarium temperature during a 24-h warming treatment for experimentally introduced internal ice (circles) and naturally occurring environmentally acquired ice (crosses). Datapoints, for *T. pennellii* only, as shown in Fig. 3*A*, represent the mean temperature of the aquarium in which the fishes were held for 24 h. The proportion of fishes harboring ice inside their bodies declined as a function of increasing temperature. However, when exposed to similar warming treatments, a higher proportion of fishes with naturally acquired ice retained it compared with those whose ice had been experimentally introduced (elevations and intercepts of regression lines are significantly different, ANCOVA, $P < 0.02$) (1). This difference indicates that ice crystals experimentally introduced systemically by briefly spray-freezing the integument are quantitatively different from those internalized in the native environment. Our assay tests for the presence of ice only; we did not determine the number of ice crystals remaining inside fishes. Statistics were calculated with Prism 4 (GraphPad).

1. Zar JH (1984) *Biostatistical Analysis* (Prentice-Hall, Englewood Cliffs, NJ), 2nd Ed.

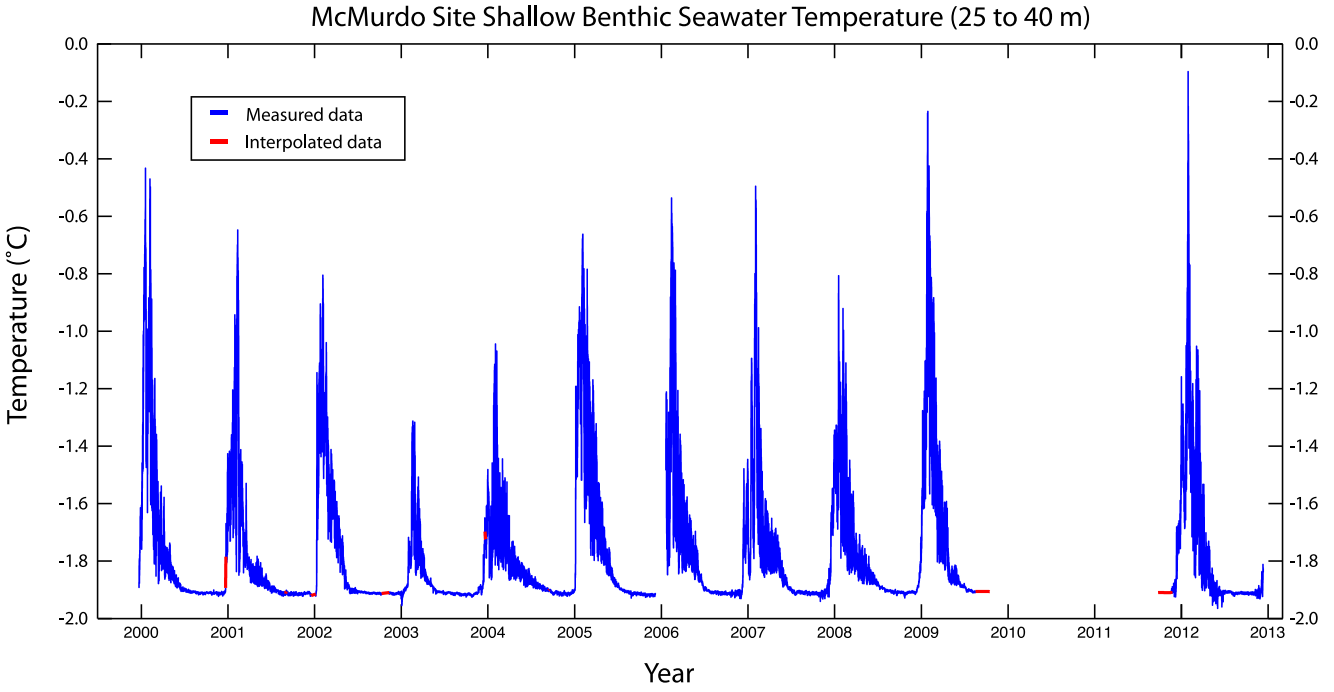


Fig. S4. Interpolated data in the long-term McMurdo temperature record. To calculate mean annual temperature (oceanographic year delineated as October 1 of one year to September 30 of the following year) (Tables S1 and S2) and the long-term mean temperature for the McMurdo site, small gaps in the record were linearly interpolated from the available data at the time interval of the dataset from the year of interest (5 or 15 min). For oceanographic years 2008 and 2011, we used −1.91 °C as the missing endpoint. Mean annual temperature was not calculated for oceanographic years 1999 and 2005 because the timing of the onset of summer warming was not precisely known. In addition to 2010 and 2011, when the logger was not deployed, these 2 y with incomplete data were excluded from the calculation of the long-term mean McMurdo site seawater temperature.

Table S1. Summary of long-term temperature logging data from the McMurdo site, adjacent to McMurdo Station

Oceanographic year	Logger depth = bottom depth (m)	Sample interval (min)	Annual mean temp. ± SD (°C)	Minimum recorded temperature (°C)	First day above −1.90 °C (7-d running mean)	Last day above −1.90 °C (7-d running mean)	Annual maximum temperature (°C)	Date of annual maximum temperature
1999	40	15	Not calculated*	−1.92	December 20, 1999†	June 25, 2000	−0.43	January 19, 2000
2000	38	15	−1.80 ± 0.21‡	−1.92	December 21, 2000	July 15, 2001	−0.65	February 11, 2001
2001	39	15	−1.78 ± 0.23‡	−1.92	January 8, 2002	May 30, 2002	−0.81	February 4, 2002
2002	39	15	−1.87 ± 0.10‡	−1.95	January 14, 2003	May 23, 2003	−1.31	February 18, 2003
2003	33	15	−1.81 ± 0.13‡	−1.93	November 23, 2003	September 8, 2004	−1.05	February 2, 2004
2004	33	15	−1.72 ± 0.29	−1.93	December 30, 2004	July 20, 2005	−0.66	February 4, 2005
2005	25	15	Not calculated*	−1.93	January 5, 2006†	July 10, 2006	−0.54	February 12, 2006
2006	25	15	−1.79 ± 0.20	−1.93	December 6, 2006	July 3, 2007	−0.50	February 2, 2007
2007	25	15	−1.79 ± 0.18	−1.94	November 29, 2007	July 31, 2008	−0.81	February 18, 2008
2008	25	15	−1.77 ± 0.28‡	−1.93	December 12, 2008	July 9, 2009	−0.24	January 27, 2009
2009§								
2010§								
2011	25	5	−1.76 ± 0.26‡	−1.96	November 27, 2010	May 16, 2012	−0.10	January 29, 2012
Mean	—	—	−1.79‡,¶	−1.93	December 18	July 3	−0.64	February 2
SD	—	—	0.22‡,¶	0.01	17 d	33 d	0.35	10 d
Range	25–40	5–15	−1.87 to −1.72‡	−1.96 to −1.92	November 23 to January 8	May 23 to September 8	−1.31 to −0.10	January 18 to Febuary 18

For these calculations, the oceanographic year begins October 1 of the indicated calendar year. Each year begins with temperatures near the freezing point of seawater (≤−1.90 °C), encompasses a full annual warming event, and ends with temperature near the freezing point of seawater.

*Mean annual temperature was not calculated for oceanographic years 1999 and 2005 because of missing data (Fig. S4).

†Data from the McMurdo logger are missing because of logistical issues; indicated datapoints are from a complementary logger at 9-m depth at Cape Armitage, 1.5-km away.

‡Annual mean temperature and SD calculated from data that includes a small amount of interpolated data (Fig. S4).

§The logger was not deployed in years when we did not have a field season at McMurdo Station or Scott Base.

¶Mean temperature and SD for entire dataset includes only the 9 y of the record for which annual means could be calculated.

Table S2. Summary of long-term temperature logging data from the McMurdo site, adjacent to McMurdo Station in relation to fish eqFMP

Oceanographic year	Start of first event above eqFMP*	End of last event above eqFMP*	No. of warming events above eqFMP*	Median length of events above eqFMP (h)*	Longest event above eqFMP (d)*	(Melting °C) × (d) of longest event†	Total (melting °C) × (d) for year†	Cumulative time above eqFMP (d)*	Maximum warming rate at temperatures above eqFMP (°C/min;°C/h)*
1999	January 9, 2000	February 14, 2000	47	1.00	9.79	2.46	3.86	16.24	0.03;2.04
2000	January 29, 2001	February 14, 2001	29	0.75	3.89	0.86	1.06	6.27	0.02;1.12
2001	January 24, 2002	February 19, 2002	15	0.50	2.10	0.25	0.34	3.56	0.01;0.32
2002	—	—	0	—	—	—	0.00	0.00	—
2003	—	—	0	—	—	—	0.00	0.00	—
2004	January 14, 2005	February 23, 2005	91	1.00	5.71	1.34	1.93	16.36	0.02;1.49
2005	February 8, 2006	March 1, 2006	102	1.00	0.83	0.27	2.03	11.16	0.04;2.44
2006	January 28, 2007	February 14, 2007	31	0.75	3.34	1.15	1.52	6.92	0.01;0.85
2007	January 17, 2008	February 7, 2008	9	3.25	0.44	0.04	0.11	1.58	0.02;1.29
2008	January 22, 2009	February 24, 2009	108	1.00	3.40	1.79	4.85	18.76	0.03;1.63
2009‡									
2010‡									
2011	January 24, 2012	February 5, 2012	34	0.42	4.77	2.46	3.29	10.47	0.09;5.52
Mean	January 22	February 16	42.36	1.07	3.81	1.18	1.73	8.30	0.03;1.85
SD	9 d	8 d	40.10	0.85	2.83	0.92	1.67	6.80	0.03;1.51
Range	January 9 to February 8	February 5 to March 1	0–108	0.42–3.25	0.44–9.79	0.04–2.46	0–4.85	0–18.76	0.01–0.09;0.32–5.52

For these calculations, the oceanographic year begins Oct.1 of the indicated calendar year. Each year begins with temperatures near the freezing point of seawater (≤−1.90 °C), encompasses a full annual warming event, and ends with temperature near the freezing point of seawater.

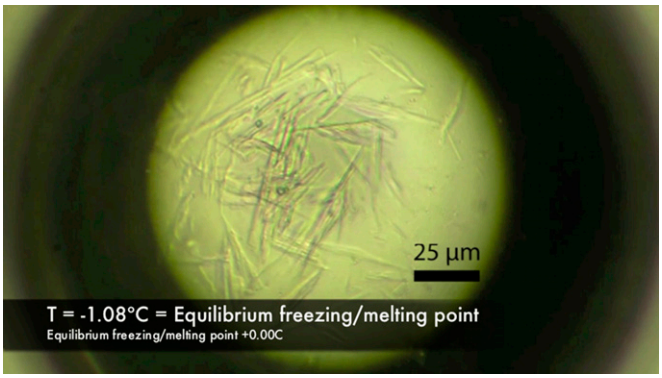
*For all calculations, eqFMP = −1.04 °C, the mean serum equilibrium freezing/melting point for all notothenioid fish species in this study.

†(Melting °C) = (seawater temperature) − (fish eqFMP), for temperatures greater than the fish eqFMP.

‡The logger was not deployed in years when we did not have a field season at McMurdo Station or Scott Base.

Table S3. GPS coordinates of temperature loggers, field experiments, and CTD casts

Site name	Data collected	Date (UTC)	GPS coordinates
Cape Evans	Field experiments and CTD casts	January 3 and 10, 2013	S 77°38.061, E 166°24.913 ± 100 m
Cape Evans	Summer CTD cast	January 17, 2013	S 77°42.017, E 166°15.114
Dailey Islands	Summer CTD cast	January 17, 2013	S 77°47.645, E 165°02.213
Granite Harbor	Summer CTD cast	January 17, 2013	S 76°56.994, E 163°20.686
McMurdo	Winter CTD cast	September 23, 2002	S 77°51.927, E 166°33.172
McMurdo	Long-term temperature record	1999–2012	S 77°51.061, E 166°39.868 ± 50 m
McMurdo	Summer CTD cast	January 17, 2013	S 77°52.972, E 166°43.136
Mid-Sound	Summer CTD cast	January 11, 2013	S 77°35.831, E 165°43.801
Mid-Sound	Summer CTD cast	February 12, 2013	S 77°31.674, E 165°43.941
New Harbor	Summer CTD cast	January 17, 2013	S 77°34.129, E 164°15.358



Movie S1. Direct observation of superheated ice crystals and the determination of the hMP of *T. pennellii* serum. We used a modified Clifton freezing point osmometer for these assays, monitoring samples at 200× magnification with a light microscope. Ice crystals were first formed by freezing a submicroliter volume droplet of serum embedded in oil at <−20 °C. Samples were then warmed until 20–200 lanceolate crystals of ∼2–100 µm in length remained (estimated at <1% of the total volume of the droplet). These ice crystals were then allowed to stabilize in the FH interval at a temperature 0.32 °C below the eqFMP of the solution for 10 min before warming. Ice crystals were observed to melt intermittently as the sample was warmed at a linear rate of 0.03 °C/min. The temperature of the sample stage was recorded, separately from the control thermistor, with an embedded microthermistor at 0.002 °C resolution. The hMP was taken as the temperature at which the last visible crystal was observed to melt. In most cases, small particles, air bubbles and oil droplets could be easily discerned from ice crystals. However, nonvisible crystals sometimes remained at temperatures above the apparent hMP, indicated by the inability to undercool the solution below the hysteresis freezing point following the disappearance of the last visible crystal. Thus, MH determinations performed in this way underestimate the maximum MH possible. Each sample was assayed three to four times. The video is real-time except where noted.

Movie S1