#  Thermal acclimation experiment and Sampling

## Collection of TB and PB fish samples for the thermal acclimation experiment

Fish were primarily caught within McMurdo Sound, Antarctica, or the wider Ross Sea area. Fish collection was carried out during the austral summer of 2007 (October-November). Fish were caught using small fishing rods, baited with either small fish pieces or soft bait on barbless hooks. Typically at the fishing sites, 25 cm diameter holes were drilled through the sea ice (which ranged from 2-8 metres thick) using a motorised jiffy drill and fish were accessed through these holes. Alternatively, a hole-melter was used to create a 1 metre wide hole in the sea ice through which fish were accessed, sometimes with a heated wannagin over top. Holes were kept clear of sea ice by regular use of small dip-nets. Individuals of the benthic species, *T. bernacchii*, were caught in inshore waters at a water depth of 15-40 m. Individuals of the pelagic and schooling species, *P. borchgrevinki,* were collected from deeper water locations (up to 300m deep), near the ice runway in McMurdo Sound with sea ice cover less than 4m thick (typically 2 m) and away from seal activity. Following capture, and quick and careful hook removal, fish were immediately placed in an insulated container of seawater and transported to laboratory facilities at Scott Base, where they were held in flow–through aquaria with sea water temperature maintained at -1.0 ± 0.3 °C. Fish were held post-catching for a minimum period of three days to allow them to recover from handling stress. During the recovery period, fish were not fed and were maintained in a 24 hour light regime to replicate the austral summer conditions of Antarctica. Following the recovery period, and when sufficient fish had been caught for the experiment, fish were pre-acclimated to a temperature of -1 °C for a period of 15 days and were fed *ad libitum* twice weekly.

### Thermal acclimation experimental design and sampling

Following the pre-acclimation period of 15 days, five randomly chosen individuals of each fish species were euthanised before the thermal acclimation experiment started and their tissues were harvested as an initial control prior to temperature treatment. The remaining fish of each species were placed in either static or flow-through tanks (limitations of the aquaria facilities meant some treatments were in static tanks) and kept in groups of no more than 10 fish per tank. For all acclimation temperature treatments, the water temperature was then gradually increased from -1 °C to 4 °C, or 6 °C. The experimental temperature regime consisted of stepwise increases in temperature to the target temperature over a 24h period (except in the case of the 6 °C treatment, in which case slower acclimation over three days was employed). The tanks were maintained at the treatment temperature ± 0.3 °C using two heat exchangers connected to a feeder tank that contained thermostatically-controlled water heater. Where possible, flow-through tanks were used, but by necessity some of the heat treatments required the use of static tanks with oxygen bubblers. For static tanks, daily replacement of 25% of the tank capacity was done to avoid both (1) the accumulation of waste products and (2) decreases in oxygen concentration. A cohort of fish held at as close to environmental temperature as possible was used as a control treatment; in which case fish remained at -1 °C for the duration of the experiment. Further, they were fed *ad libitum* twice weekly during the acclimation period.

## Collection and transport of tissue and plasma samples

Fish (n=5) of each treatment, including controls) were euthanised, blood samples collected and tissues harvested at 1, 2, 3, 7, 14 and (in the case of PB only) 28 days post-acclimation, for 6 °C thermal acclimation tissues were harvested after 7 days post acclimation for both the species PB and TB. All routine procedures were followed as per the Animal Ethics approval and sampling procedures were performed at the Scott Base Wet Laboratory, with air temperature below 5 °C. Routine anaesthetic exposure via transfer to seawater containing MS-222 (ethyl m-amino benzoate methane-sulfonate) was performed. Fish were anaesthetised for five minutes in a 0.1 g/L solution of MS222 dissolved in sea water. Details of the individual fish were then recorded. Blood was collected from the caudal vein with a needled syringe (22 guage) and the fish was euthanised by severing the spinal cord. Fish were dissected using standard dissection procedures under sterile conditions and tissue samples were transferred into labelled vials and immediately frozen in liquid nitrogen. Tissues (liver, brain, heart, kidney, white muscle, red muscle, subcutaneous fat and adipose tissue) were rapidly removed, snap frozen in liquid nitrogen and stored at -80 °C for later biochemical and genetic analyses.