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The effects of temperature on peripheral neuronal function in eurythermal and stenothermal crustaceans

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Summary

To determine whether neuronal function in Antarctic crustaceans is adapted to the low and narrow range of temperatures at which these animals live, we have compared conduction velocities in the peripheral nervous systems of two temperate species, the decapod *Carcinus maenas* and the isopod *Ligia oceanica*, and two Antarctic species, the isopod *Glyptonotus antarcticus* and the amphipod *Paraceradocus gibber*.

Neuronal conduction velocity differs among the species in the order *C. maenas* > *G. antarcticus* > *P. gibber* > *L. oceanica*. When measured at the normal environmental temperatures characteristic of each species, conduction velocity of the Antarctic peracarid *P. gibber* is greater than that of its similar sized temperate relative *L. oceanica*, demonstrating complete thermal compensation.

The temperate decapod *C. maenas* has a higher thermal dependence of neuronal conduction velocity than either of the Antarctic species, *G. antarcticus* and *P. gibber*, but the temperate *L. oceanica* does not. These data, when collated with published values, indicate that peracarid crustaceans (*L. oceanica*, *G. antarcticus* and *P. gibber*) have lower neuronal conduction velocities and a lower thermal dependence of neuronal conduction velocity than do other arthropods, irrespective of habitat. There is a linear dependence of conduction velocity on temperature down to –1.8°C in all three species. Our data extend by more than 10° the lower range of temperatures at which conduction velocities have been tested systematically in previous studies.

The upper thermal block of neuronal conduction is similar in *C. maenas*, *G. antarcticus*, *P. gibber* and *L. oceanica* at 24.5, 19.5, 21.5 and 19.5°C, respectively. This suggests that failure to conduct action potentials is not what determines the mortality of Antarctic invertebrates at approximately 10°C.

The excitability of axons in the leg nerve of *G. antarcticus* is not affected by temperatures ranging from –1.8 to +18°C. The responses of sensory neurones activated by movements of spines on the leg, however, are strongly modulated by temperature, with maximal responses at 5–10°C; well above the normal environmental temperature range for the species. The responses fail at 20–22°C.

The number of large diameter axons (which produce the fast action potentials recorded in this study) is the same in *L. oceanica* and *G. antarcticus*, but the median axon diameter is greater in *L. oceanica* than *G. antarcticus*. In *G. antarcticus*, however, there are glial wrappings around some large (>5 μm diameter) axons that may increase their conduction velocity. Such wrappings are not found in *L. oceanica*.

Key words: neuronal conduction velocity, axon diameter, temperature compensation, crustacean, Antarctic, temperate, eurytherm, stenotherm.

Introduction

The ambient temperature range of the environment in which a poikilotherm lives governs almost completely the temperatures at which the nervous system must function. Temperate marine invertebrates experience large daily and seasonal fluctuations of temperature [maximum daily variation: 12°C, annual range: 2 to 23°C, rocky shore, NE England (Willows, 1987)]. In contrast, Antarctic marine invertebrates experience permanently low temperatures (annual range –1.9 to +1.7°C, at 15 m depth) (Barnes et al., in press). The evolutionary adaptations associated with life in such different thermal environments over the past 20 million years (Kennett, 1977) have been comprehensively studied for species of fish but, with few exceptions (e.g. Macdonald,

1981), have been neglected for marine invertebrates. We have compared the effects of temperature on the propagation of action potentials in the leg nerves of two temperate and two Antarctic crustaceans, and we have examined the responses of sensory neurones in one of the Antarctic species. Our choice of species permits comparisons of similarly sized peracarids from the two environments (*Ligia oceanica*, *Paraceradocus gibber*) and permits contrasts with a larger peracarid *Glyptonotus antarcticus*, and a similarly large decapod *Carcinus maenas*.

Propagation of action potentials in the peripheral nervous system represents a potential rate-limiting step in the relay of motor and sensory signals to and from the central nervous system, and can therefore constrain rates of behaviour. For example, the large Antarctic pycnogonid *Colossendeis robusta* has legs often in excess of 15 cm long, and neuronal conduction velocity is only $0.33\ m\ s^{-1}$ (Macdonald, 1981). This leads to an exceptionally long conduction delay of 450 ms, which must contribute to the animal's extremely sluggish behaviour.

A positive relationship between temperature and neuronal conduction velocity (Helmholtz, 1850) has been characterised in sensory transduction of the tactile spine afferents in the legs of the cockroach *P. americana* (Chapman and Pankhurst, 1967), sensilla involved in the detection of orientation in the crab *Carcinus maenas* (Fraser, 1990) and a slit sensillum of the Central American hunting spider *Cupiennius salei* (Höger and French, 1999). The effects of temperature on the conduction velocities of individual motor axons involved in jumping and kicking (Burrows, 1989) and flight (Xu and Robertson, 1994) have been assessed for two species of locust (*Schistocerca gregaria* and *Locusta migratoria*, respectively).

Only a few assessments of the effect of temperature on neuronal conduction velocity have been applied in the comparison of temperate and polar species, primarily in fish (Macdonald, 1981; Harper et al., 1990; Moran and Melani, 2001). If the polar species are physiologically compensated (Prosser, 1958), then the conduction velocities of different species measured at their respective environmental temperatures (e.g. –1.8°C for Antarctic, +5°C for Arctic, and +20°C for Mediterranean fish) should be the same. Perfect compensation is not achieved in either Antarctic fish [rates are 2–3 times slower than temperate fish measured at 16°C (Macdonald, 1981)] or Arctic fish [rates are 2–2.5 times slower than Mediterranean fish at 12°C (Melani and Moran, 1988)].

We show how temperature affects neuronal conduction velocity in four marine crustaceans: the amphipod *Paraceradocus gibber* and isopod *Glyptonotus antarcticus* from Antarctica; and the decapod *Carcinus maenas* and isopod *Ligia oceanica* from the British coast. For the first time in an Antarctic invertebrate we describe the responses of mechanosensory neurones, their conduction velocities and the thermal dependence of receptor responsiveness. The diameter of axons in the leg nerves of *L. oceanica* and *G. antarcticus* is related to conduction velocity.

Materials and methods

Animal maintenance

Carcinus maenas Linnaeus (35–50 mm in length) were obtained from Wells-next-the-Sea (Norfolk, UK) using baited traps. They were housed in 40 cm×80 cm×16 cm aquaria containing stones and artificial seawater (in $mmol\ l^{-1}$: Cl^-, 541.6; Na^+, 465.2; Mg^{2+}, 54.3; SO_4^-, 28.1; Ca^{2+}, 11.9; K^+, 10.5; CO_2^-, 4.4; B^{3+}, 0.5; Br^-, 0.03; Kent Sea Salt, Kent Marine Inc., Acworth, USA), which was aerated and filtered. They were fed on dead fish and maintained at +4°C.

Ligia oceanica Linnaeus (22–28 mm in length) were obtained from a tidal wall at Wells-next-the-Sea. They were housed individually in containers 8.5 cm diameter × 6.5 cm high lined with seawater-moistened tissue paper. They were fed on vegetable peelings and were maintained at +4°C.

Glyptonotus antarcticus Eights (60–110 mm in length) and *Paraceradocus gibber* Andres (33–51 mm in length) were taken from baited traps at Rothera (67°34′S, 68°08′W) and Palmer (64°46′S, 64°03′W) stations (Antarctica) and transported by ship to the UK where they were maintained in a recirculating natural seawater aquarium at 0 to +1°C with a 12 h:12 h light:dark regime. They were fed on dead fish and krill. Salinity was maintained at 33–36 psu (practical salinity units; the conductivity ratio relative to a standard KCl solution, approximately equivalent to 33-36‰.

Preparation of tissue

Experiments were performed on isolated legs that were cut from the rest of the animal at the coxa with a sharp pair of dissecting scissors. Following removal of a leg, a slow flow of haemolymph from the wound on the animal ceased within 30 s.

A leg from *C. maenas* was prepared with the ventral surface facing upwards. The merus was dissected open and the carpus flexor muscle removed to reveal the leg nerve. For *L. oceanica* and *G. antarcticus*, legs were mounted with the ventral surface facing upwards. The basis and ischium were dissected open, and the merus extensor, ischium extensor and ischium flexor muscles removed to expose the leg nerve. For *P. gibber*, a leg was fixed with the posterior surface facing upwards. The basis was dissected open to reveal the merus extensor, ischium extensor and flexor muscles, which were removed to reveal the leg nerve.

Electrophysiological recordings

Experiments on the Antarctic species, *G. antarcticus* and *P. gibber*, were conducted in a controlled-temperature room, maintained at 5°C. Experiments on the temperate species, *C. maenas* and *L. oceanica*, were conducted in a laboratory at 20°C.

Two pairs of 50 μm (for *C. maenas* and *G. antarcticus*) or 25 μm (for *L. oceanica* and *P. gibber*) silver bipolar hook electrodes were placed as far apart as possible around the leg nerve which was lifted clear of the saline, and the distance between the electrodes was measured. A 75:25% Vaseline: paraffin mixture was applied to the nerve around each pair of hook electrodes with a drawn out plastic syringe. The

preparation dish contained sufficient crustacean saline (Yamagishi and Ebara, 1985) to cover the preparation. The preparation was either cooled then warmed, or warmed then cooled, at approximately 0.25°C min^{-1} using a thermocirculator (Grant LTD 20G, Cambridge, UK) circulating a 50:50 mixture of ethylene glycol and water around a jacketed Perspex preparation dish (3 ml volume) lined with Sylgard (Dow Corning, Midland, USA).

A train of ten square pulses of 1 ms duration and 130% amplitude of the voltage threshold for eliciting action potentials, was delivered at 20 Hz once every 30 s (Master-8 stimulator, AMPI, Jerusalem, Israel) from the proximal pair of electrodes. Measurements of temperature were made using a thermocouple probe placed adjacent to the preparation.

Signals were amplified using a custom-built extracellular amplifier with a 50 Hz notch filter, digitised at 1 kHz (CED 1401 or Micro 1401, Cambridge Electronic Design, Cambridge, UK), and stored on a computer for later analysis.

Conduction velocity was calculated by dividing the distance between stimulating and recording electrodes by the latency of the first peak of the compound action potential (Fig. 1).

Neuronal conduction velocity was interpolated for temperatures of +2 and +10°C using a linear relationship fitted to data measured at temperatures between approximately –2 and +20°C. Values were calculated at 2°C because this temperature represents the overlap in thermal tolerances of the temperate and Antarctic species. Values were calculated at 10°C – the minimum experimental temperature used in many of the previous assessments – so that the neuronal conduction velocities of *C. maenas*, *L. oceanica*, *G. antarcticus* and *P. gibber*, could be compared directly with published data from other species. In the case of *C. maenas*, neuronal conduction velocity and its thermal dependence were calculated at temperatures between 0 and 15°C, where the relationship between temperature and neuronal conduction velocity was linear.

Sensory responses

In two *G. antarcticus*, we recorded the activity of leg tactile sensory neurones using paired hook electrodes as described above, with both channels set to record. One electrode was placed in the ischium, the other in the basis. Individual moveable spines on the merus or carpus were adducted by a standard brief (approximately 500 ms) deflection, delivered by hand. The number of action potentials elicited by the stimulus, and the conduction velocity in the axon, were recorded at temperatures ranging from –1.8°C to +25°C.

Axonal structure

Short (1–5 mm) lengths of leg nerve to be used for transmission electron microscopy (TEM) were taken from the segments used in the electrophysiological recordings. Tissue embedding for TEM followed a protocol modified from that used by Postel et al. (Postel et al., 2000). The section of nerve removed from the leg was immediately washed in 0.1 mol^{-1} phosphate buffer (9 mmol l^{-1} NaH_2PO_4 and 44 mmol l^{-1} Na_2HPO_4 in distilled water) at pH 7.0 and 4°C, before being fixed in 6% glutaraldehyde in phosphate buffer for 14 h at 4°C. Fixed nerves were washed in phosphate buffer and then post-fixed in 1% osmium tetroxide for 1 h at 4°C, before being dehydrated in an alcohol series, and then treated twice for 15 min in propylene oxide. Samples were then embedded overnight in Araldite. Semi-thin (10 μm) and ultra-thin (70 nm) sections were cut with an ultramicrotome (Reichert OmU2, Vienna, Austria) using glass and diamond knives, respectively. For light microscopic investigation, the semi-thin sections were stained with Toluidine Blue (0.8% Toluidine Blue, 0.8% borax, 0.2% pyronin in distilled water) for 30 s at 70°C. Ultra-thin sections placed on 100 Mesh grids were contrasted with uranyl acetate for 40 min, and lead citrate for 10 min, then viewed in a transmission electron microscope (Phillips, EM300).

Electron micrographs were taken at a magnification of approximately ×3300, and calibrated against a standard grid. Negatives were developed, then scanned at 1200 dpi, and the resulting images assembled to form a montage of each nerve (Canvas 7SE, Deneba).

Axon diameters were measured using the method described by Graham (Graham, 2003).

Results

Electrical stimulation of the leg nerve of *C. maenas*, *L. oceanica*, *G. antarcticus* and *P. gibber* yielded compound action potentials recorded at a second pair of hook electrodes placed distally on the nerve. The latency between electrical stimulation and the first peak of the action potential decreased with increasing temperature (Fig. 1A). At any given temperature there was a distinct voltage threshold for the excitation of axons in the leg nerve. In *G. antarcticus*, for example, the threshold was 1.25 V (Fig. 1B). Increasing the stimulation voltage above this threshold value caused a slight change in the compound action potential that led to a small increase in the maximum conduction velocity (Fig. 1B). In all experiments we stimulated at 130% of the threshold for each animal to ensure that we recorded the maximum conduction velocity (e.g. Fig. 1B).

Changing the temperature did not affect the threshold for stimulation of axons in the leg nerve in any species. In *G. antarcticus*, for example, an increase in temperature from –1.8°C to +16°C had no significant effect on threshold (Fig. 1C), although the conduction velocity was faster at higher temperatures (Fig. 1B). The upper temperature at which electrical stimulation failed to elicit a compound action potential, 'the upper thermal block', was similar in *C. maenas*, *L. oceanica*, *G. antarcticus* and *P. gibber*, occurring at +24.5, 19.5, 21.5 and 19.5°C, respectively (Fig. 2E).

Conduction velocity increased with temperature in all four species (Fig. 2). Action potentials were elicited by electrical stimulation over maximum ranges of –2.5 to +24.5°C in *C. maenas* (Fig. 2A), –3.5 to +21.5°C in *G. antarcticus* (Fig. 2B), –1.5 to +19.5°C in *L. oceanica* (Fig. 2C), and –2.5 to +19.5°C

in *P. gibber* (Fig. 2D). Neuronal conduction velocity increased linearly with temperature in *L. oceanica*, *G. antarcticus* and *P. gibber* (Fig 2, Fig 3A; Table 1). In three preparations of *C. maenas*, neuronal conduction velocity increased exponentially on warming. In the other three preparations, neuronal conduction increased sigmoidally with temperature (Fig. 2A,E). The difference between these two groups of recordings from *C. maenas* only became apparent at temperatures above 15°C (groups A and B in Fig. 2E).

The leg nerve contains the axons of both sensory and motor neurones. In *G. antarcticus* only we recorded the conduction velocity of individual sensory neurones activated by touching

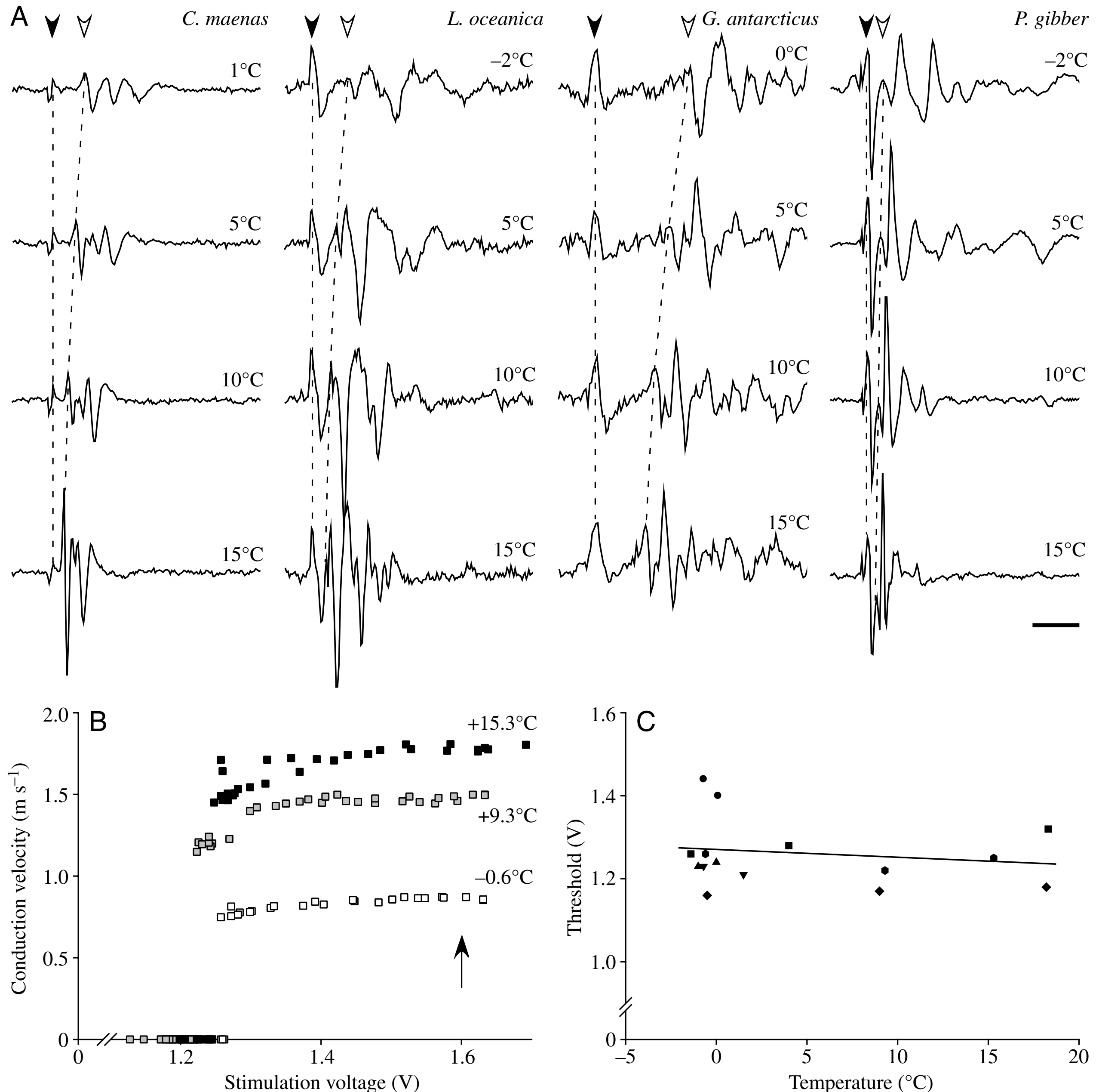

Fig. 1. (A) The neuronal conduction velocity of axons in the leg nerves of marine crustaceans increases with temperature. Electrical stimulation of the leg nerve at a proximal site produces a stimulus artefact (black arrowheads), and compound action potentials recorded at a distal site, the most rapid of which are indicated by open arrowheads. Traces are aligned to the stimulus artefact, indicated by the first broken line in each block. The first peak of the compound action potential occurs sooner as temperature increases (second broken line). Scale bar, 5 ms. (B,C) Increasing the stimulation voltage reveals a sharp threshold for eliciting a compound action potential (B), which does not change with temperature (C). Increasing the stimulus voltage further results in only a modest increase in the measured conduction velocity (B). The arrow indicates 130% of threshold, which is the stimulation level used throughout this study. Data in B are from one experiment in *G. antarcticus*. Data in C are from five experiments in *G. antarcticus,* denoted by different symbol types. Regression equation: y=–1.93×10^{-3}x+1.266, r^2=0.033, P=NS.

specific articulated spines on the leg. The conduction velocities and temperature dependence of conduction velocity were similar to the values obtained by electrical stimulation of the whole nerve (Fig 2F, Fig 3B; Table 1). Touching a single spine could elicit action potentials of more than one size, indicating that the spines are multiply innervated. The conduction velocities of the smaller amplitude action potentials were slower than those of larger action potentials, but their temperature dependence was similar (Fig. 3).

There was a greater effect of temperature on neuronal

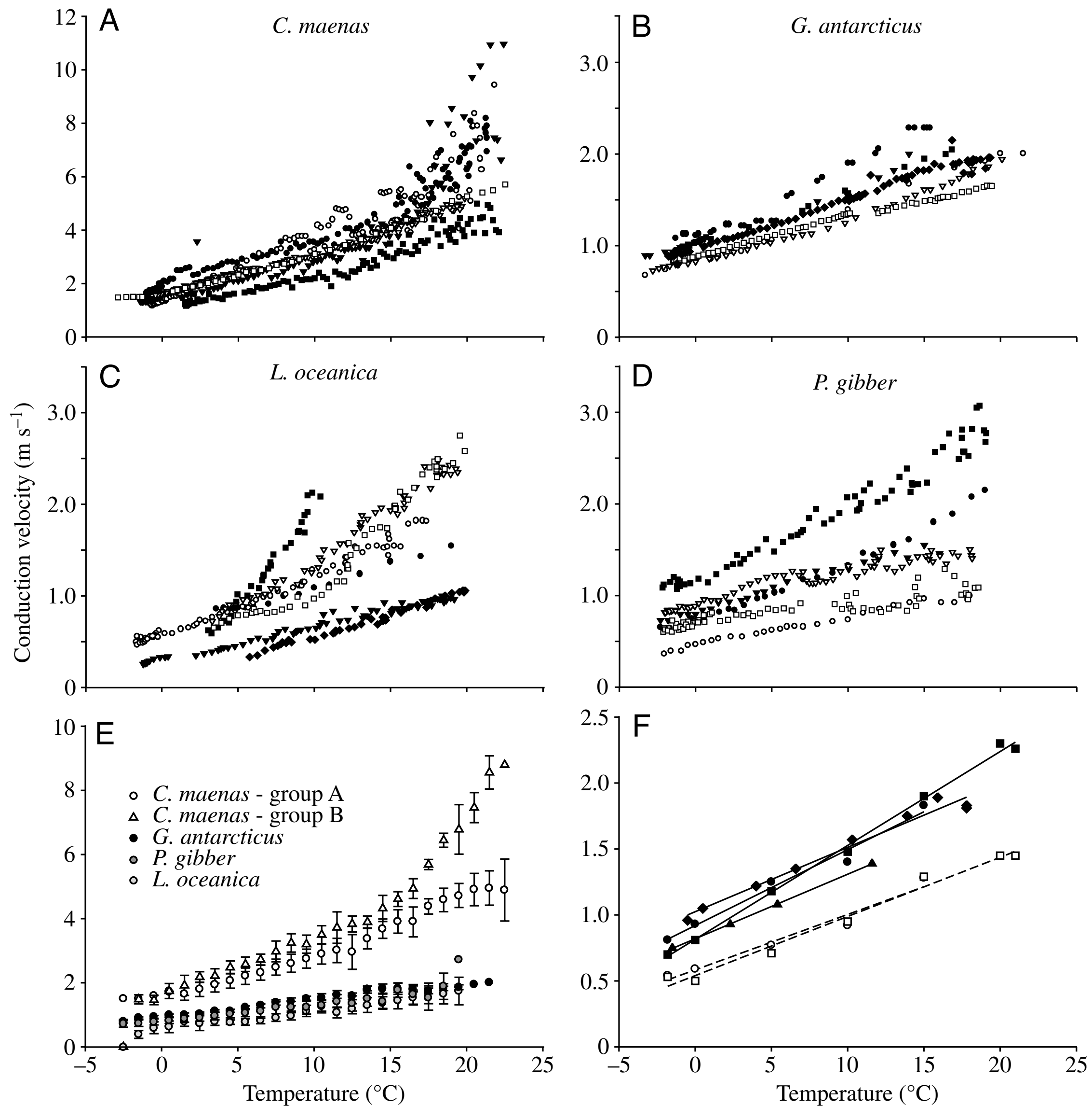

Fig. 2. (A–D) The effect of temperature on the neuronal conduction velocity of (A) *C. maenas* (N=6), (B) *G. antarcticus* (N=7), (C) *L. oceanica* (N=7) and (D) *P. gibber* (N=6). The different symbols denote different animals. Data extend across the full range of temperatures at which action potentials were elicited by electrical stimulation. Note the different scale in A. (E) There is a differential effect of temperature on the neuronal conduction velocity of four species of marine crustacean. Conduction velocity is derived from the mean conduction velocity per animal per 1°C temperature bin. Values are means ± s.e.m. For *L. oceanica*, *G. antarcticus* and *P. gibber*, the number of animals (N) was at least 5 except for the following temperature bins: *L. oceanica*; –1.5°C (N=2), –0.5 and +0.5°C (N=3), +1.5°C (N=2), +2.5°C (N=3), and +19.5°C (N=3). *G. antarcticus*; –2.5°C (N=1), +8.5°C (N=4), +10.5°C (N=4), +12.5°C (N=4), +15.5°C (N=4), +17.5°C (N=3), +18.5 and +19.5°C (N=4), +20.5 and +21.5°C (N=1). *P. gibber*; +6.5°C (N=4), +15.5°C (N=4), +17.5 and +18.5°C (N=4), and +19.5°C (N=1). For *C. maenas*, the number of animals was at least 3 except for the following temperature bins: Group A; –2.5 to –0.5°C (N=1), +0.5°C (N=2), +12.5°C (N=2), +16.5°C (N=2), and +20.5°C to +22.5°C (N=2). Group B; –1.5°C (N=2), +18.5°C (N=2), and +22.5°C (N=1). (F) Conduction velocity of sensory action potentials, elicited by moving single spines on the carpus or merus of *G. antarcticus*. Larger amplitude action potentials conducted more rapidly (solid symbols and solid lines) than the smaller amplitude ones (open symbols and broken lines), but their temperature dependence was similar to one another and to that of the compound potential recorded from the whole nerve. These data come from experiments on four spines in two animals. Animal 1: spine A, merus (circles), spine B, merus (squares) and spine C, carpus (triangles). Animal 2: spine D, merus (solid diamonds).

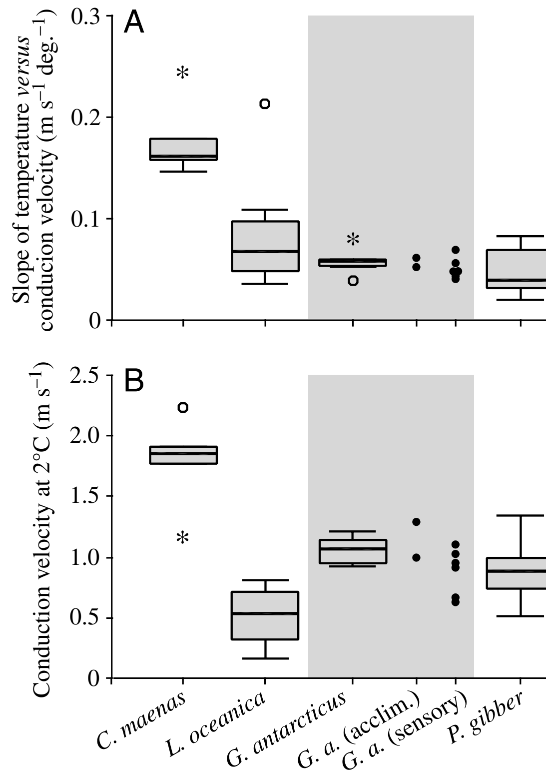

Fig. 3. (A) Changes of temperature have a greater effect on the neuronal conduction velocity of axons in the leg nerve of *C. maenas* than in the other three crustacean species (Kruskal–Wallis, χ^2=13.36, P<0.01). (B) Conduction velocity at 2°C is greater in *C. maenas* (Kruskal–Wallis, χ^2=19.55, P<0.0001) than in the other species. Slopes and intercepts were calculated by performing a linear regression on the temperature *vs* conduction velocity for each animal of each species. The *N* values are the number of animals per species used. Each boxplot shows the median value, the upper and lower quartiles and the minimum and maximum values. Outliers, which are either 1.5–3 times or >3 times the interquartile range from the quartiles, are denoted by a circle or an asterisk, respectively. The conduction velocity and thermal dependence of conduction velocity of sensory neurones in two *G. antarcticus* (*G. a.* sensory) were similar to values for the whole nerve. We acclimated two additional *G. antarcticus* to 4°C for 7 days before measuring leg nerve conduction velocity (*G. a.* acclim.). These values (solid circles) lie within the range for animals acclimated to 0–1°C (A,B). The light grey shading groups together all the values from *G. antarcticus*.

conduction velocity in *C. maenas* (0.162 m s^{-1} deg.$^{-1}$, N=6) than *L. oceanica* (0.067 m s^{-1} deg.$^{-1}$, N=7), *G. antarcticus* (0.058 m s^{-1} deg.$^{-1}$, N=7) or *P. gibber* (0.039 m s^{-1} deg.$^{-1}$, N=6) (Kruskal–Wallis, χ^2=13.36, P<0.01) (Fig. 3A). There was no significant difference between the thermal dependence of neuronal conduction velocity in the three peracarid crustaceans, *L. oceanica*, *G. antarcticus* and *P. gibber* (Kruskal–Wallis, χ^2=2.86, P=NS).

Neuronal conduction velocity at 2°C was significantly greater in *C. maenas* than in the other species (Fig. 3B; Table 2; Kruskal–Wallis, χ^2=19.55, P<0.01). It was significantly lower in *L. oceanica* than in the other peracarid species *G. antarcticus* and *P. gibber* (Kruskal–Wallis, performed on 2°C data, χ^2=12.78, P<0.01).

Table 1. *The slopes and intercepts of linear regression lines fitted to conduction velocity versus temperature relationships for individual animals from each species*

Species	ID	Slope (m s^{-1} deg.$^{-1}$)	Intercept (m s^{-1})
C. maenas	■	0.158	0.852
	□	0.179	1.528
	●*	0.159	1.913
	○*	0.245	1.331
	▼*	0.146	1.477
	▽	0.165	1.577
L. oceanica	■	0.213	–0.153
	□	0.085	0.637
	●	0.047	0.630
	○	0.067	0.569
	▼	0.036	0.305
	▽	0.109	0.321
	◆	0.049	0.069
G. antarcticus	■	0.060	1.021
	□	0.039	0.901
	●	0.081	1.051
	○	0.058	0.803
	▼	0.060	1.018
	▽	0.052	0.816
	◆	0.054	0.963
G. antarcticus	Fig. 3	0.053	0.900
(acclimated to +4°C)	Fig. 3	0.063	1.170
G. antarcticus	■	0.071	0.82
(sensory)	□	0.045	0.54
	●	0.057	0.91
	○	0.050	0.58
	▲	0.049	0.82
	◆	0.049	1.02
P. gibber	■	0.083	1.177
	□	0.020	0.699
	●	0.069	0.700
	○	0.031	0.456
	▼	0.046	0.833
	▽	0.032	0.929

In all cases, the regression lines fitted with an r^2 value greater than 0.76, and a probability of P<0.0001. In three animals of *C. maenas* (asterisks), in which conduction velocity showed exponential growth with increasing temperature (exponential growth, single, 2 parameter, F=314.0, r^2=0.93, P<0.001), regression lines were fitted to data at temperatures between 0 and 15°C, at which the relationship was linear.

The different symbols, corresponding to those used in Fig. 2, denote different animals of each species.

Sensory responses

In *G. antarcticus*, moving individual articulated spines on the leg in a repeatable way led to bursts of action potentials

Table 2. *Median conduction velocities of leg nerves measured at different temperatures*

Temperature (°C)	Conduction velocity (m s^{-1})			
	C. maenas	*L. oceanica*	*P. gibber*	*G. antarcticus*
–1.8	1.21	0.24	**0.71**	**0.87**
+1.5	1.70	0.48	**0.85**	**1.04**
+2.0	**1.82**	**0.54**	0.88	1.07
+10.0	**3.23**	**1.24**	1.27	1.50
+23.0	**5.37**	**2.11**	1.78	2.21

Bold values indicate velocities at species-specific temperatures. –1.8°C and +1.5°C represent the lower and upper environmental limits for the Antarctic species, whereas +2.0°C and +23°C represent the corresponding limits for the temperate species.

recorded in the leg nerve (Fig. 4A). The number of action potentials elicited by each touch depended on the temperature. At low temperatures relatively few action potentials were elicited, but as temperatures rose to 5–10°C the number increased to a maximum before declining again to fail at 20–22°C (Fig. 4B).

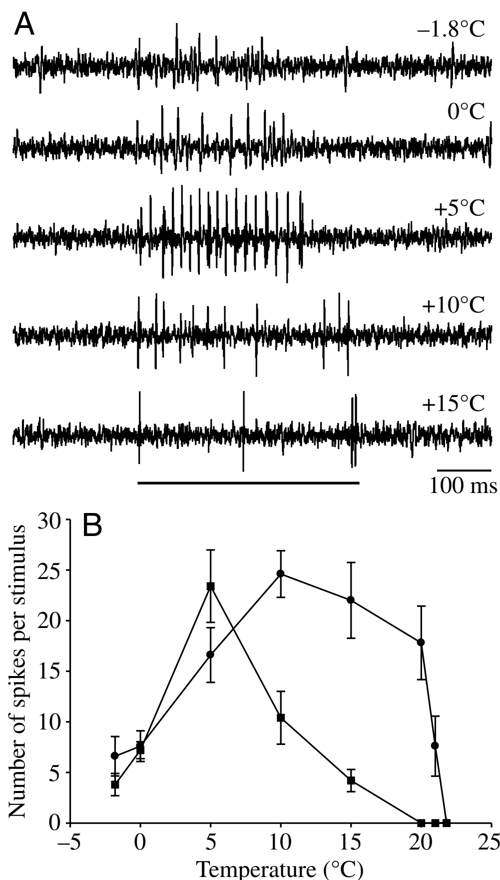

Fig. 4. (A) In *G. antarcticus* (N=2), moving individual articulated spines on the leg (black bar) produced bursts of action potentials recorded in the leg nerve. (B) The number of action potentials elicited by each touch depended on the temperature. Values are mean ±1 s.d.

Axon diameters

The number and diameter of axons within the main nerve at the recording site in the basis of the leg were assessed for *L. oceanica* and *G. antarcticus* (N=2 for each species). In both species, there were a large number of small diameter (<1 μm) axons and fewer large (>5 μm) diameter axons (e.g. *G. antarcticus*; Fig. 5A,B). The median axon diameters from nerves of both specimens of *L. oceanica* (0.43 μm and 0.52 μm; Fig. 6) were significantly different (Mann–Whitney U test, Z=–3.89, P<0.001), and the distribution of axon diameters was different (Kolmogorov–Smirnov, Z=6.03, P<0.001). Similarly, the median axon diameters from nerves

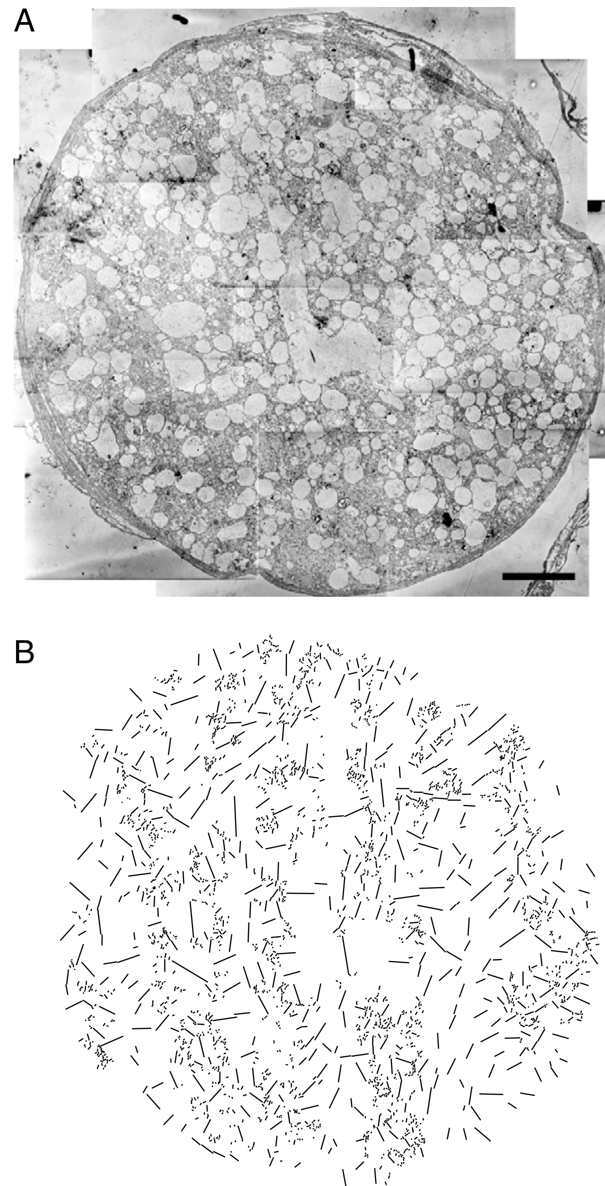

Fig. 5. (A) Composite transmission electron micrograph of a transverse section through one nerve bundle of the leg nerve within the basis of *G. antarcticus*. (B) The lines show the measured diameter of each axon. Scale bar, 10 μm.

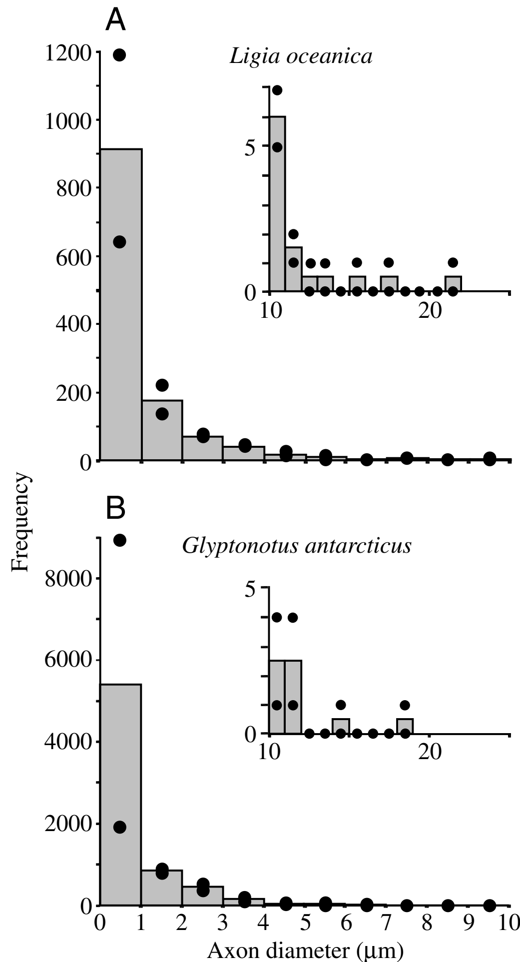

Fig. 6. The frequency distribution of axons of 0–10 μm diameter within the basal leg nerve of *L. oceanica* (A) and *G. antarcticus* (B). Insets show the frequency distributions for axons of 10–30 μm diameter. The filled circles are values for *N*=2 animals of each species; the grey bars are the mean frequencies for each species.

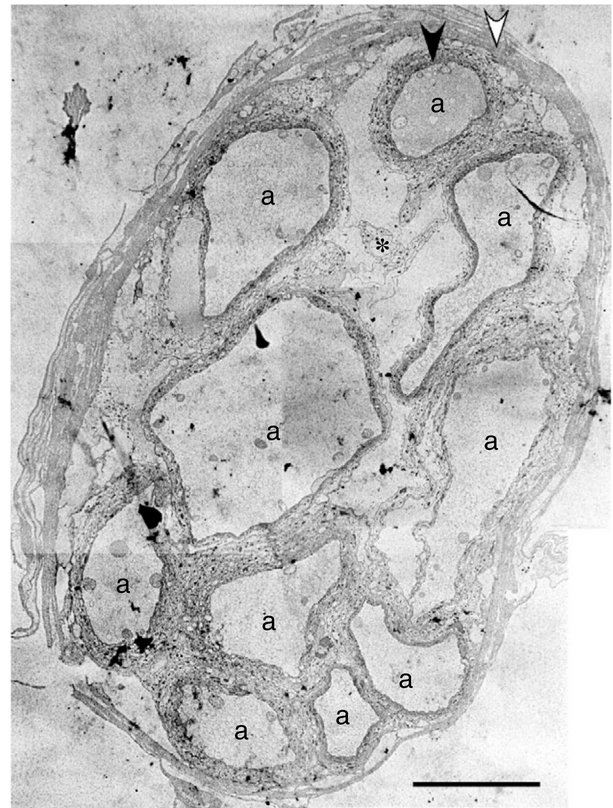

Fig. 7. A nerve bundle of *G. antarcticus* containing axons (a) surrounded by a wrapping (black arrowhead). The appearance of the axonal wrapping is very different from the sheath that surrounds the nerve bundle (white arrowhead). The nerve bundle contains substantial interstitial spaces (*). The dark blotches are staining artefacts. Scale bar, 10 μm.

of both specimens of *G. antarcticus* (0.89 μm and 0.28 μm; Fig. 6B) were significantly different (Mann–Whitney *U* test, Z=–61.09, P<0.001), and the distribution of axon diameters was different (Kolmogorov–Smirnov, Z=31.54, P<0.001).

The number of axons was also different between animals of the same species. For *L. oceanica*, the two values were 929 and 1570 axons. In *G. antarcticus*, the values were 3638 and 10313 axons.

When the data from pairs of animals were pooled, the median axon diameters of *L. oceanica* and *G. antarcticus* were significantly different (Mann–Whitney *U* test, Z=–15.87, P<0.001), and the distribution of axon diameters was different (Kolmogorov-Smirnov, Z=10.77, P<0.001) (Fig. 6).

The number and frequency distribution of large (>5 μm) diameter axons was similar for the two species; there were 20 axons between 10 and 30 μm in two specimens of *L. oceanica*, and 12 in two specimens of *G. antarcticus* (Fig. 6A,B insets).

Each *G. antarcticus* possessed a small nerve bundle, as part of the main leg nerve, containing fewer than 10 large (>5 μm) axons, surrounded by a wrapping, typically 0.5–3 μm in thickness (Fig. 7). The sheath surrounding the entire nerve bundle was loosely bound; the membrane was convoluted, and in places, gaps were present between adjacent layers. Similarly wrapped axons were not observed in the leg nerve tissue of *L. oceanica* (*N*=2).

Discussion

The conduction velocity of axons in the leg nerve differed between species in the order *C. maenas* > *G. antarcticus* > *P. gibber* > *L. oceanica* (Fig. 2E). For the temperate species, the difference between conduction velocities at +2°C and +23°C (i.e. spanning their normal environmental range) was 3-fold in *C. maenas*, and 3.9-fold in *L. oceanica*. For both of the Antarctic species, there was only a 1.2-fold difference between conduction velocities at the extremes of their environmental range (–1.8°C *vs* +1.5°C; see Table 2).

The lower neuronal conduction velocity of *L. oceanica* than *C. maenas* coincides with a large difference in leg size. Leg lengths are an order of magnitude greater in *C. maenas* than in

L. oceanica (approximately 10 cm for a 6 cm long *C. maenas*, approximately 1 cm for a 2.3 cm long *L. oceanica*). At 10°C the conduction velocity of axons in *L. oceanica* was 1.24 m s^{-1} so it takes 7 ms for an action potential to travel 1 cm along the leg. If the conduction velocity were exactly the same in *C. maenas*, it would take an action potential 70 ms to travel down its leg. The more rapid conduction velocity in *C. maenas* (3.2 m s^{-1} at 10°C), means that it takes only 31 ms to travel the length of the leg.

Neuronal conduction velocities in similar sized peracarid crustaceans from Antarctic (*P. gibber*) and temperate (*L. oceanica*) environments were very similar when measured at the appropriate species-specific environmental temperatures. At the minimum Antarctic environmental temperature of –1.8°C, conduction velocity in *P. gibber* was 0.71 m s^{-1}. Conduction velocity in its temperate relative *L. oceanica* was 24% slower than this, at 0.54 m s^{-1}, when measured at the higher environmental minimum temperature for that species of +2°C (Table 2). Even at 10°C, approximately the mid point of the temperature range for *L. oceanica*, and well above the maximum environmental temperature for *P. gibber*, the Antarctic species maintained a faster conduction velocity than its temperate relative. Although partial compensation (Prosser, 1958) has been observed in both Antarctic fish (Macdonald, 1981) and Arctic fish (Melani and Moran, 1998), our data provide evidence for perfect compensation of neuronal conduction velocity in temperate and Antarctic peracarid crustaceans. Conduction velocity in *L. oceanica* only became faster than that in *P. gibber* at temperatures above 10°C (Table 2). This means that, when compared at their respective upper environmental temperature limits of +1.5°C and +23°C, conduction velocity in the Antarctic species (0.85 m s^{-1}) was 60% slower than in the temperate one (2.11 m s^{-1}).

Neuronal conduction velocity was much more rapid in our recordings from *C. maenas* than in a previous study (Fig. 8) (Fraser, 1990), perhaps because previous data were from a *C. maenas* sensory nerve. The neuronal conduction velocity of *G. antarcticus* in our study was slightly slower than reported in a previous study (Fig. 8) (Macdonald, 1981), although the thermal dependence of neuronal conduction velocity of *G. antarcticus* was similar [0.058 m s^{-1} $deg.^{-1}$, this study; 0.052 m s^{-1} $deg.^{-1}$ (Macdonald, 1981)].

The thermal dependence of neuronal conduction velocity

Neuronal conduction velocity had a higher temperature dependence in temperate *C. maenas* than in the Antarctic species *G. antarcticus* and *P. gibber* (Fig. 3A). In contrast, the other temperate species *L. oceanica*, did not, so eurythermality alone cannot explain the different thermal dependencies of the different species. *L. oceanica* has a similar thermal dependence to that of Antarctic species tested in the present and previous studies (Fig. 8), but the thermal dependence of conduction velocity in *C. maenas* is more similar to those of the primarily tropical insects studied to date (Fig. 8). It may be beneficial to *C. maenas*, and perhaps tropical insects, to have a higher thermal dependence of neuronal conduction velocity, so that behavioural rates can be modified strongly to utilise resources that fluctuate with temperature.

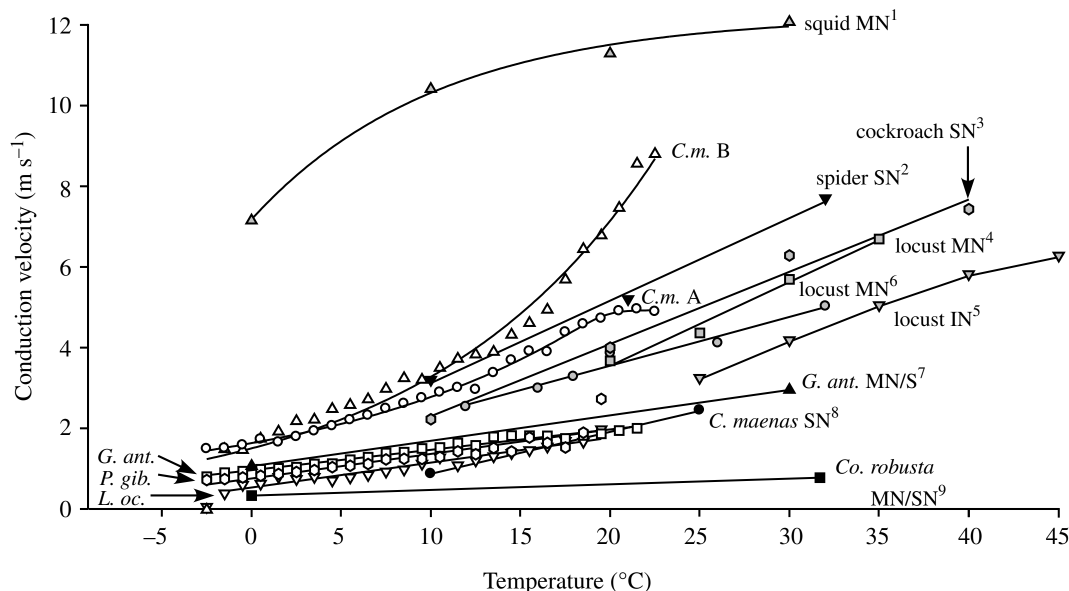

Fig. 8. The effect of temperature on the conduction velocity of identified neurones or nerves of invertebrates, from this study [*C. maenas* (*C.m.* A, *C.m.* B), *L. oceanica* (*L.o.*), *G. antarcticus* (*G.a.*) and *P. gibber* (*P.g.*); open symbols) and published data (solid and grey symbols]. Key: [1]squid *Loligo vulgaris*, motor neurone (MN) (Chapman, 1967); [2]spider *Cupiennius salei*, sensory neurone (SN) (Höger and French, 1999); [3]cockroach *Periplaneta americana*, SN (Chapman and Pankhurst, 1967); [4]locust *Locusta migratoria* MN (Xu and Robertson, 1994); [5]locust *Locusta migratoria* interneurone (IN) (Money et al., 2005); [6]locust *Schistocerca gregaria* (MN) (Burrows, 1989); [7]*G. antarcticus*, ventral nerve cord and leg nerve recordings combined (Macdonald, 1981), [8]*C. maenas*, SN (Fraser, 1990) and [9]*Colossendeis robusta*, leg nerve recordings (Macdonald, 1981).

The tropical arthropods studied to date are relatively inactive at low temperatures (e.g. Uvarov, 1977). Our study, therefore, broadens considerably the diversity of animals for which the temperature dependence of neuronal function has been studied. Moreover, our data extend by more than 10°C the lowest temperatures that have been analysed in most previous studies, and include for the first time temperatures significantly below 0°C. The linear temperature dependence of velocity reported in a range of species (Fig. 8) is shown to continue to –1.8°C in all four species of crustacean we studied. The high thermal dependence of conduction velocity in tropical species (Fig. 8) presumably contributes to the high thermal dependence of behavioural functions, such as wing-beat frequency in flying locusts (Xu and Robertson, 1994).

Of previous studies, only that of the squid *Loligo vulgaris* (Chapman, 1967) demonstrated a non-linear temperature dependence of conduction velocity (Fig. 8). Here, the rate of conduction dropped markedly at temperatures below 0°C. Our data show that this low-temperature non-linearity is not a general feature across species. Our data for *C. maenas*, in contrast, reveal a positive non-linearity at high temperatures (>10°C) in some animals and a weak plateau in others (Fig. 2E). It is unusual for a physiological process to have a linear temperature dependence, and yet with very few exceptions (e.g. *C. maenas*, this study), neuronal conduction does (Fig. 2) (Macdonald, 1981), so it is not clear whether this linearity results from the interaction of many non-linear relationships, or is due to one single underlying factor that has a positive linear relationship with temperature.

Upper thermal block of neuronal conduction

All four species studied have similar temperatures of upper thermal block (Fig. 2). Although temperate species survive temperatures exceeding 10°C, which cause high mortality in Antarctic invertebrates (Wells, 1979; Peck and Conway, 2000), there is no corresponding difference in their upper limit for action potential propagation. This suggests that the failure to conduct action potentials is not what determines mortality at 10°C in the Antarctic species. Moreover, it suggests that there is little difference in the thermal stability of the membrane ion channels involved in action potential propagation between temperate and Antarctic species.

For *G. antarcticus* and *C. robusta* Macdonald (Macdonald, 1981) reported the upper thermal block in leg nerves at 31°C and at 28°C, i.e. around 10°C higher than the values reported in the present study. This difference may result from different rates of imposed temperature change or from a differing thermal dose between the two studies.

Tropical insects and a temperate squid have higher temperatures of upper thermal block, propagating action potentials at temperatures greater than 30°C (Fig. 8) (Chapman, 1967; Chapman and Pankhurst, 1967; Burrows, 1989; Xu and Robertson, 1994; Höger and French, 1999), which is clearly an essential adaptation to carrying out behaviour at these high temperatures, to which the marine crustaceans are never naturally exposed.

The lowest temperature at which action potentials can be conducted is poorly documented because most studies have not tested temperatures below 10°C (e.g. Burrows, 1989; Höger and French, 1999), and in other cases, the minimum experimental temperature was not given (e.g. Fraser, 1990). The squid *L. vulgaris* has a lower thermal block of –0.5°C (Chapman, 1967). Our data demonstrate that in both stenothermal Antarctic species and eurythermal temperate species of crustacean, the lower limit for action potential conduction is close to the freezing point of seawater, –1.8°C. Terrestrial species of insects that continue to behave at even lower temperatures [e.g. the Himalayan midge *Diamesa* Meigen sp., active at –16°C (Kohshima, 1984)] must conduct action potentials at these extreme temperatures, but this has not been demonstrated explicitly.

For the Antarctic stenotherm *G. antarcticus*, we demonstrate that some sensory receptors produce more action potentials per stimulus at temperatures exceeding the normal environmental range (i.e. 5–10°C), than within it. A consequence of this must be that the dynamic range of the output firing of the receptors is not optimal under normal conditions. Perhaps the energy costs of generating higher frequencies of activity are prohibitive at low temperatures. Alternatively it may be that the output effects (e.g. post-synaptic effects in down-stream neurones) saturate at even low frequencies of firing when temperature is low, meaning that higher rates would simply waste energy to no effect.

The number and diameter of axons in the leg nerve

Our measure of conduction velocity was based on latency of the fastest axons contributing to the compound action potential (Fig. 1). Conduction velocities were faster for *G. antarcticus* than *L. oceanica* (Fig. 2E)*,* suggesting the presence of larger diameter axons in the former. Our transmission electron microscope data demonstrate, however, that the number of large diameter axons is the same in both species and that the median axon diameter is smaller in *G. antarcticus* than *L. oceanica* (Fig. 6).

The median diameters of axons from two individuals of *G. antarcticus* (0.89 and 0.28 μm) were greater than the average of 0.1 μm reported by Macdonald (Macdonald, 1981). A reason for this difference is not apparent, but Macdonald may have sectioned sensory nerves, which generally have smaller diameter axons than motor nerves (e.g. Xu and Robertson, 1994) or may have used particularly small or young animals. Axons of 0.1 μm diameter and smaller were resolved in the present study, but they represented only a small fraction of the axons within the leg nerves.

The conduction velocity of an axon is proportional to its length constant (Hodgkin, 1954), and both can be increased through an increase in axon diameter or membrane resistance. As the difference in absolute conduction velocity between *G. antarcticus* and *L. oceanica* is not explained by axon diameter, this suggests that the layers of wrapping around some large diameter axons in *G. antarcticus* increase membrane resistance and therefore conduction velocity. Some calanoid

copepods belonging to the superfamilies Megacalanoidea and Clausocalanoidea have axons and nerve bundles surrounded by myelin sheaths (Davis et al., 1999). Myelination enhances conduction velocity by increasing the membrane resistance and therefore the length constant of the axon (Eckert and Randall, 1983). The wrappings are responsible for the extremely rapid escape responses of the copepods *Undinula vulgaris* and *Neocalanus gracilis* (Lenz et al., 2000; Weatherby et al., 2000), which have latencies of around one quarter of those found in closely related species that do not have myelin-like wrappings. These more rapid reaction times apparently permit *U. vulgaris* and *N. gracilis* to colonise open water, whereas species without myelin are restricted to deep water. Despite the apparent advantages, myelin-like wrappings are surprisingly rare in invertebrates. The wrappings in *G. antarcticus* appear less dense than those of the copepods, but if they act to enhance conduction as we hypothesise, then this could provide a mechanism for cold adaptation in this large animal.

There was considerable variation in both the number and diameter of axons present in individuals of the same species. The largest difference was in the smallest axons (those less than 1 μm in diameter) with a fourfold difference between two individuals of *G. antarcticus*. There was no difference in the number of axons greater than 1 μm in diameter. The small sample size precludes a definitive explanation for this observation, but one possibility is that the animals were of different ages. As an arthropod ages and grows, the number of sensory sensilla increases (Jander and Jander, 1994; Sandeman and Sandeman, 1996; Brézot et al., 1997; Steullet et al., 2000). This proliferation would produce a difference in the number of primarily small diameter axons between two age groups. Such differences would not have resulted in intra-specific variation in neuronal conduction velocity, because our measure of velocity is based on the fastest action potentials, representing activity of the largest diameter axons.

We have demonstrated that neuronal conduction in the leg nerves of two species of Antarctic crustacean continues to temperatures at least 10°C higher than those that cause the animals to die. The upper thermal block for neuronal conduction is the same in temperate and Antarctic species, despite the temperate species surviving at higher temperatures. The temperature dependence of neuronal conduction velocity is the same in three peracarid species – the Antarctic *G. antarcticus* and *P. gibber*, and the temperate *L. oceanica* – but is greater in the temperate decapod *C. maenas*. There is no evidence to support an overriding effect of habitat (i.e. temperate *vs* Antarctic) on this aspect of neuronal function.

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References

Barnes, D. K. A., Fuentes, V., Clarke, A. and Schloss, I. R. (in press). Spatial and temporal variation in shallow seawater temperatures around Antarctica. *Deep Sea Res. II*.

Brézot, P., Tauban, D. and Renou, M. (1997). Sense organs on the antennal flagellum of the green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae): sensillum types and numerical growth during the post-embryonic development. *Int. J. Insect Morphol. Embryol.* **25**, 427-441.

Burrows, M. (1989). Effects of temperature on a central synapse between identified motor neurons in the locust. *J. Comp. Physiol.* **165**, 687-695.

Chapman, K. M. and Pankhurst, J. H. (1967). Conduction velocities and their temperature coefficients in sensory nerve fibres of cockroach legs. *J. Exp. Biol.* **46**, 63-84.

Chapman, R. A. (1967). Dependence of temperature of the conduction velocity of the action potential of the squid giant axon. *Nature* **213**, 1143-1144.

Davis, A. D., Weatherby, T. M., Hartline, D. K. and Lenz, P. H. (1999). Myelin-like sheaths in copepod axons. *Nature* **398**, 571.

Eckert, R. and Randall, D. J. (1983). *Animal Physiology*, 558pp. New York: Freeman.

Fraser, P. J. (1990). Effects of temperature on statocyst afferents of the crab *Carcinus maenas*. *J. Therm. Biol.* **15**, 25-31.

Graham, C. M. (2003). *Processes Determining Neuromere Position and Patterns of Neuromere Fusion in the Insect Nerve Cord.* PhD thesis, University of Cambridge, Cambridge, UK.

Harper, A. A., Watt, P. W., Hancock, N. A. and Macdonald, A. G. (1990). Temperature acclimation effects on carp nerve: a comparison of nerve conduction, membrane fluidity and lipid composition. *J. Exp. Biol.* **154**, 305-320.

Helmholtz, H. (1850). Messungen über den zeitlichen Verlauf der Zuckung animalischer Muskeln und die Fortpflanzungsgeschwindigkeit der Reizung in den Nerven. *Arch. Anat. Physiol. Wissensch. Med.*, 276-364.

Hodgkin, A. L. (1954). A note on conduction velocity. *J. Physiol.* **125**, 221-224.

Höger, U. and French, A. S. (1999). Temperature sensitivity of transduction and action potential conduction in a spider mechanoreceptor. *Eur. J. Physiol.* **438**, 837-842.

Jander, U. and Jander, R. (1994). Numerical allometric growth of the ommatidia, antennal sensilla, and teeth of the foretibial combs in the milkweed bug *Oncopeltus fasciatus* Dallas (Heteroptera: Lygaeidae). *Int. J. Insect Morphol. Embryol.* **23**, 329-344.

Kennett, J. P. (1977). Cenozoic evolution of Antarctic glaciation, the circum-Antarctic ocean and their impact on global paleoceanography. *J. Geophys. Res.* **82**, 3843-3876.

Kohshima, S. (1984). A novel cold-tolerant insect found in a Himalayan glacier. *Nature* **310**, 225-227.

Lenz, P. H., Hartline, D. K. and Davis, A. D. (2000). The need for speed. I. Fast reactions and myelinated axons in copepods. *J. Comp. Physiol. A* **186**, 337-345.

Macdonald, J. A. (1981). Temperature compensation in the peripheral nervous system: Antarctic vs temperate poikilotherms. *J. Comp. Physiol. A* **142**, 411-418.

Melani, R. and Moran, O. (1998). Characterization of the conduction properties in Mediterranean fish peripheral nerves. *Ital. J. Zool.* **65**, 155-158.

Money, T. G. A., Anstey, M. L. and Robertson, R. M. (2005). Heat stress-mediated plasticity in a locust looming-sensitive visual interneuron. *J. Neurophysiol.* **93**, 1908-1919.

Moran, O. and Melani, R. (2001). Temperature-dependent conduction properties in Arctic fish peripheral nerves. *Polar Biol.* **24**, 9-15.

Peck, L. S. and Conway, L. Z. (2000). The myth of metabolic cold adaptation: oxygen consumption in stenothermal Antarctic bivalves. In *Evolutionary Biology of the Bivalvia*. The Geological Society Special Publication No. 177 (ed. E. M. Harper, J. D. Taylor and J. A. Crame), pp. 441-450. Cambridge: Cambridge University Press.

Postel, U., Becker, W., Brandt, A., Luck-Kopp, S., Riestenpatt, S., Weihrauch, D. and Siebers, D. (2000). Active osmoregulatory ion uptake across the pleopods of the isopod *Idotea baltica* (Pallas): Electrophysiological measurements on isolated split endo- and exopodites mounted in a micro-Ussing chamber. *J. Exp. Biol.* **203**, 1141-1152.

Prosser, C. L. (1958). General Summary: the nature of physiological adaptation. In *Physiological Adaptation* (ed. C. L. Prosser), pp. 167-180. Washington, DC: Society of General Physiologists, American Physiology Society.

Sandeman, R. E. and Sandeman, D. C. (1996). Pre-embryonic and postembryonic development, growth and turnover of olfactory receptor neurons in crayfish antennules. *J. Exp. Biol.* **199**, 2409-2418.

Steullet, P., Cate, H. S. and Charles, C. D. (2000). A spatiotemporal wave of turnover and functional maturation of olfactory receptor neurons in the spiny lobster *Panulirus argus. J. Neurosci.* **20**, 3282-3294.

Uvarov, B. (1977). *Grasshoppers and locusts. A Handbook of General Acridology. Volume 2. Behaviour, Ecology, Biogeography and Population Dynamics.* Centre for Overseas Pest Research. Cambridge: Cambridge University Press.

Weatherby, T. M., Davis, A. D., Hartline, D. K. and Lenz, P. H. (2000). The need for speed. II. Myelin in calanoid copepods. *J. Comp. Physiol*. **186**, 347-357.

Wells, R. M. (1979). The lethal temperatures of Antarctic marine invertebrates. *N. Z. Antartic Record* **1**, 9-13.

Willows, R. I. (1987). Population and individual energetics of *Ligia oceanica* (L.) (Crustacea: Isopoda) in the rocky supralittoral. *J. Exp. Mar. Biol. Ecol.* **56**, 315-330.

Xu, H. and Robertson, R. M. (1994). Effects of temperature on properties of flight neurons in the locust. *J. Comp. Physiol. A* **175**, 193-202.

Yamagishi, H. and Ebara, A. (1985). Spontaneous activity and pacemaker property of neurons in the cardiac ganglion of an isopod, *Ligia exotica. Comp. Biochem. Physiol.* **81A**, 55-62.